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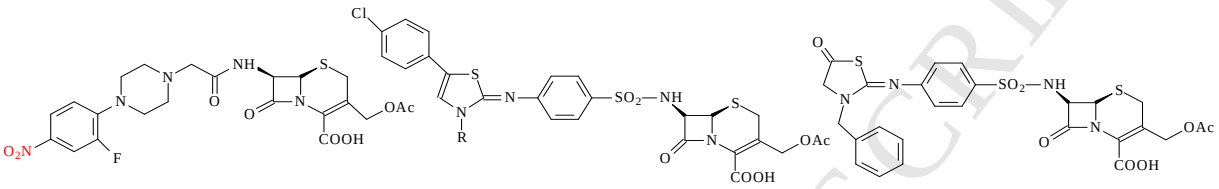

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Synthesis and biological activities of some cephalosporanic acid derivatives including 1,3-thiazol(idinon)e or piperazine ring were described.

Design, Synthesis and Biological Activities of Some 7-Amino Cephalosporanic Acid Derivatives

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Abstract- The treatment of 7-ACA with 4-substitutedbenzensulfonyl chlorides afforded the compounds containing 4-nitro/aminophenyl sulfonylamino moiety in the cephalosporanic acid skeleton (**2, 4**). The synthesis of the cephalosporanic acid derivatives containing 1,3-thiazole or 5-oxo-1,3-thiazolidine nucleus and sulfonamide function (**8a,8b, 10**) was performed starting from 7-ACA by several steps. The reaction of 7-ACA with [4-(2-fluoro-4-nitrophenyl)piperazin-1-yl]acetyl chloride afforded the corresponding 7-{[4-(2-fluoro-4-nitrophenyl)piperazin-1-yl]acetyl}amino derivative (**13**).

The synthesized compounds were screened for their antimicrobial and antiurease activities. Some of them were found to possess good-moderate antimicrobial activity against the test microorganisms. Compound **5d** was observed to have moderate anti-urease activity.

Keywords: 7-amino cephalosporanic acid, 1,3-oxazole, 1,3-thiazole, antimicrobial activity, anti-urease activity, anti β-lactamase activity.

1. Introduction

In recent decades, the growing incidence of bacterial resistance towards the present antibacterials has become the most serious clinical and socio-economical problem worldwide. Multidrug-resistant Gram-positive pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermis* (MRSE), vancomycin-resistant Enterococci (VRE), cephalosporin-resistant *Streptococcus pneumoniae* have been leading significant morbidity and mortality of infected patients [1-3]. Other pathogenic microorganism, *Streptococcus pneumoniae* have been reported responsible for approximately 3 million deaths each year worldwide due to pneumonia, meningitis and sepsis, and cause serious upper airway infections such as sinusitis and otitis media. Penicillin resistance on *S. pneumoniae* is another significant clinical problem with the resistance rate of 39 % [4-7]. Therefore, design and synthesis of new and potent antibacterial agents without cross-resistance with the present antibacterials is a crucial task for the effective treatment of bacterial infections.

Beside the development of completely new agents possessing different chemical properties than those of the existing ones, there is another approach containing to combine two or more pharmacophores into a single molecule. Therefore, a single molecule including more than one pharmacophore, each with different mode of action, could be beneficial for the treatment of microbial infectious. These synergistic antimicrobial combinations have several major advantages, including the potential to slow down the development of drug resistance, a broader antimicrobial spectrum, and a potential reduction in the dose and toxicity of each drug [5-11].

Cephalosporins and carbapenems belonging to a growing class of β-lactam antibiotics, continue to play an important role in antibacterial therapy due to their high efficacy and safety profile. They are the most versatile class of antibiotics used in whole world. Among

cephalosporins, cefotaxime, cephalothin, and cefazolin are well known antibiotics used currently. Since their discovery, many advances have been made in the synthesis, chemical modification and biology of these fascinating molecules [12-19], some of which contains different heterocyclic moieties in their structures [20, 21].

Other important pharmacophores, substituted piperazines constitute a molecular part of some important drugs such as Crixivan, a HIV protease inhibitor, piperazinyl linked Ciprofloxacin dimers which are potent antibacterial agents. Eperezolid that belongs oxazolidinone class antibacterials, can be given as another important example containing a piperazine nucleus [22, 23]. The drugs used as cardiovascular agents, Prazosin, Lidoflazine and Urapidil contain a piperazine nucleus in their structures [24, 25].

1,3-Thiazole and oxo-1,3-thiazolidine derivatives which are other important classes of pharmaceuticals have been reported to possess a broad spectrum of biological activities such as anti-mycobacterial [26], anti-fungal [26-28], anti-cancer [26, 28], anti-inflammatory [28, 29], anti-tuberculosis [26, 28, 30], anti-HIV [31], analgesic properties [28] and antibacterial [32, 33].

Bacterial urease enzymes, which accelerate hydrolysis of urea to ammonia gas with the reaction rate at least 10^{14} over the spontaneous reaction, have been reported as important virulent factors including several important pathogenesis such as pyelonephritis, hepatic coma, peptic ulceration, injection-induced urinary stones and stomach cancer [34-36]. The detrimental impact of ureases is not only on human health. As a result of urease activity, the NH_3 lost from fertilizers is an economic impact for farmers. Moreover, the interference of NH_3 to the atmosphere from urea will subsequently be deposited to land or water. The result of this is eutrophication and acidification of natural ecosystems on a regional scale [37].

Hydroxamic acids, phosphoramidates [38], polyphenols [34], 1,2,4-triazoles [39], 1,3,4-oxadiazoles and 1,3,4-thiadiazoles [40] have been reported as the compounds

possessing antiurease activity. Although some Schiff base-metal complexes have been found to display urease inhibitory effects along with other metal complexes, as well, the presence of heavy-metal atoms has restricted of their applications as drugs in the human body [41, 42].

It is known that the production of enzymes such as the serine- β-lactamases (SBLs) and metallo-β-lactamases (MBLs) in bacteria increasingly causes the resistance against a broader range of common β-lactam antibiotics such as penams, carbapenems and cephalosporins. Thus, the development of an inhibitor for SBLs and MBLs is an attractive approach to maintain the usefulness of existing antibiotics. Due to this reason, β-lactamase inhibitors have gained importance to overcome the antibacterial resistance [43, 44]. In this context, a number of natural and synthetic compounds have been reported to possess anti β-lactamase activity, which catalyzes the hydrolysis of the CO-N bond in the molecules of penicillins and cephalosporins. However, only a few of them has found field of use at clinical settings.

Motivated by these findings and in continuation of our ongoing efforts on the synthesis of new hybride molecules with potential chemotherapeutic activities, we would like to report here the synthesis, antiurease and antimicrobial activities of some new cephalosporanic acid derivatives incorporating also 1,3-oxazole, 1,3-thiazole, 5-oxo-1,3-oxazolidine and 5-oxo-1,3-thiazolidine moieties.

2. Results and Discussion

2.1. Chemistry

The main aim of the present study is to synthesize and investigate the antimicrobial and antiurease activities of some new cephalosporanic acid derivatives also containing 1,3-thiazole, 1,3-oxazole or piperazine nucleus and/or a sulfonamide function in the one

molecular structure. Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Scheme 1 and Scheme 2. The starting compound 4-nitrobenzensulfonyl chloride (**1**) was provided commercially. 4-Aminobenzensulfonyl chloride (**3**) obtained by the reduction of the nitro group of compound **1** is available commercially.

In the present study, compound **2** was obtained from the reaction of compound **1** with 7-ACA in the mild reaction conditions with the aim to introduce a sulfonamide function to the cephalosporanic acid skeleton. In addition, the presence of the phenyl ring in the structure of compound **2** is important to increase the lipophilicity of the molecule, because it is well known that the lipophilic character of a bioactive molecule facilitates the penetration of it into the cell [45, 46]. Moreover, the presence of nitro group on phenyl ring was necessary to obtain compounds **6a-d** via the formation of compound **4**. However, our efforts on the synthesis of compound **4** via the reduction of the nitro group of compound **2** did not succeed due to the decomposition of **2** at every turn, although a number of different reduction conditions and reagents were applied. The possible underlying reason of this is to contain the cephalosporanic acid moiety a *β*-lactam nucleus that is a strained and easy-decomposable ring in acidic and basic conditions [47].

We obtained compound **4** from the reaction of compound **3** with 7-ACA in the mild reaction conditions. Similarly, the treatment of compound **4** with phenyl/benzylisocyanates or phenyl/benzylisothiocyanates resulted in decomposition. The synthesis of compounds **6a-d** were achieved by the condensation of 7-ACA with compounds **5a-d,** which were obtained from the reaction of compound **3** with phenyl/benzylisocyanates or phenyl/benzylisothiocyanate. The synthesis of compounds **7a,b** was performed from the reaction of compounds **5a,b** with ethyl bromoacetate in ethanolic solution. With the aim to

merge the 1,3-thiazole nucleus and cephalosporanic acid skeleton via a sulfonamide linkage, compounds **8a,b** were synthesized by the treatment of **7a,b** with 7-ACA at room temperature.

The reaction of compound **5a** with ethyl bromoacetate generated the corresponding 1,3-thiazolidinone derivative (**9**); then, this compound was converted to (*6R,7R*)-3-[(acetyloxy)methyl]-7-{[(4-{[3-benzyl-5-oxo-1,3-thiazolidin-2-ylidene]amino}phenyl) sulfonyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**10**).

1-(2-Fluoro-4-nitrophenyl)piperazine (**11**), that was obtained from the reaction of piperazine with 3,4-difluoronitrobenzene is a commercially known compound. As a linker group between piperazine and cephalosporanic acid moieties, acetyl function was introduced to the structure of compound **12** by the reaction of compound **11** with chloroethanoyl chloride. Compound **13,** that is a hybrid molecule incorporating a cephalosporanic acid core linked to the (2-fluoro-4-nitrophenyl)piperazin-1-yl nucleus via an amide function, was obtained from the reaction of **12** with 7-ACA. It is well known that the presence of an amide group in the structure of β-lactam antibiotics is necessary for antimicrobial activity [47]. Moreover, it was reported that fluorophenylenpiperazinyl nucleus constitutes one of the active parts of Eperezolid that belongs to oxazolidinone class antibacterial agents [48].

The FT-IR and ^{1}H NMR spectra of compound **3** displayed signals due to amine function. When compound **3** was converted to compound **4**, additional signals derived from cephalosporanic acid moiety appeared at the related regions in the FT-IR and NMR spectra of compound **4**.

As different from compound **4,** ^{1}H and ^{13}C NMR spectra of compounds **5a-d** exhibited additional signals due to carbonothioyl- or carbonylamino moiety at the related chemical shift values, while the signal originated from any amino group was not recorded. Instead, new signals due to two NH groups appeared. In the FT-IR spectra of compounds **5a-d,** the

absorption bands belonging to the NH groups were observed. In addition, compounds **5a-c** gave Mass spectral data and elemental analysis consistent with the assigned structures.

The ^{1}H and ^{13}C NMR spectra of compounds **6a-d** exhibited additional signals representing the presence of a cephalosporanic acid moiety as a result of the condensation between compounds **5a-d** and 7-ACA. Compounds **6a-d** displayed ^{1}H and ^{13}C NMR spectra consistent with the assigned structures. The additional support for the formation of the targeted compounds was obtained by the appearance of $[M-1]^{+}$ ion peaks at corresponding *m/z* values confirming their molecular masses, besides elemental analysis data.

With the conversion of compound **5a-c** into 4-{[5-(4-chlorophenyl)-3-phenyl(benzyl)-1,3-thiazol-2(3*H*)-ylidene]amino}benzene sulfonyl chlorides (**7a-c**), the signals due to two NH function were disappeared, instead, new signal derived from chlorophenyl nucleus appeared at the aromatic region in the ^{1}H NMR and ^{13}C NMR spectra. Furthermore, in the FT-IR spectra, the absence of any signal due to NH absorption supported the ring closure. In the EI-MS spectra of these compounds, $[M]^{+}$, $[M+1]^{+}$, $[M+Na]^{+}$ and/or $[M+K]^{+}$ ion peaks are present at the related *m/z* values.

Similarly, compounds **8a-c** displayed FT-IR, ^{13}C NMR and ^{1}H NMR spectra representing the presence a cephalosporanic acid moiety in their structures. Moreover, elemental analysis results and mass spectral data supported the proposed structures.

The structure of compound **13** was elucidated on the basis of spectroscopic techniques. In the FT-IR spectrum, the $–NO_2$ stretching bands and the -OH vibration derived from carboxyl group were present. The absorption bands due to carbonyl groups were recorded as separate signals. The ^{1}H and ^{13}C NMR spectra and elemental analysis results of compound **13** supported the proposed structure. In addition, compound **13** gave relatively stable $[M+Na]^{+}$ ion peak in the EI-MS spectrum.

2.2. Biological activity

2.2.1. Antimicrobial activity

All the compounds were tested for their antimicrobial activities and the results were presented in Table1. Compound **2** that is a cephalosporanic acid derivative containing a 4-nitrophenylsulfonylamino moiety exhibited moderate activity selectively towards *Staphylococcus aureus* (*Sa*), *Enterococcus faecalis* (Ef) which are Gram positive cocci and *Bacillus cereus* (Bc) that is Gram positive spore bacillus. Whereas, compound **4** that is the amino derivative of nitro compound **3**, displayed good antimicrobial activities against the test microorganisms with the inhibition zones varying between 10-20 mm. For this compound (**4**), the highest activities were observed on enteric bacteria, *Escherichia coli* (Ec); Gram negative bacillus, *Pseudomonas aeruginosa* (Pa); Gram positive coccus, *Enterococcus faecalis* (Ef) and *Mycobacterium smegmatis* (Ms) that is an atipic tuberculosis factor.

When the activities were compared with each other, it can be seen that carbonothioylamino (**5a** and **5b**) derivatives demonstrated better activity than carbonylamino (**5c** and **5d**) compounds. Moreover, as can be seen in Table 1, compounds **5a,b** have better activity than the standard drug Ampicillin. When compounds **5a,b** were converted to the corresponding cephalosporanic acid derivatives (**6a,b**), the antibacterial activities decreased, surprisingly, while no important change were observed for antifungal activities of them. However nonetheless, when the inhibition zones were compared with Ampicillin, it can be concluded that these compounds (**6a,b**) possess better antibacterial activity against Ec and Ef. On the other hand, the conversion of **5c,d** to **6c,d** resulted in the increase in antimicrobial activities of compounds **6c,d**.

4-{[3-Alkyl-5-(4-chlorophenyl)-1,3-thia(oxa)zol-2(3*H*)-ylidene]amino}benzene sulfonyl chlorides (**7a-c)** displayed good-moderate activities against the test microorganisms excepted, Ec, Yp and Pa. On the other hand, as different from **7a** and **7b**, compounds **8a** and **8b** were

found to be active towards all the test microorganisms. The 5-oxo-1,3-thiazolidine compounds, **9** and **10** demonstrated similar antimicrobial activities with the corresponding 1,3-thiazole derivatives (**7a** and **8a**).

Compound **13**, that is a cephalosporanic acid derivative containing the 7-({[4-(2-fluoro-4-nitrophenyl)piperazin-1-yl]acetyl}amino) moiety was found to have activity towards the test microorganisms except Pa. For compounds **12** and **13,** the highest activity was observed on *Saccharomyces cerevisiae* (Sc) that is yeast like fungus.

2.2.2. Anti-urease activity

The synthesized compounds were assayed for their in vitro inhibitory activity against Jack Bean urease. Thiourea with IC_{50} value 51.62±7.28 µg/mL was used as standard inhibitor. Initially, all synthesized compounds were screened 250 µg/mL final concentration. The compounds that showed more than 80 % inhibition were assayed at different concentration for calculation IC_{50} values. Among the synthesized compounds, 4-[(anilinocarbonyl)amino]benzenesulfonyl chloride (**5d**) displayed the best inhibitory effect against urease with an IC_{50} value of 120.69±0.98 µg/mL. The other compounds show no significant inhibition (Table 2).

2.2.3. Anti-*β*-Lactamase activity

The synthesized compounds were assayed for their in vitro inhibitory activity against B. cereus *β*-lactamase. Three of those compounds showed *β*-lactamase inhibition. $HgCl_2$ with IC50 value 0.093 mM was used as standard inhibitor. Among tested compounds B7 was found to be the best inhibitory effect against *β*-lactamase with an IC50 value of 0.543 mM. Compounds B5 and B9 have moderate inhibitory activity (Table 3). These compounds might be considered as potential *β*-lactamase inhibitors. All compounds and $HgCl_2$ were assayed at final concentrations of 1 mM.

3. Experimental

3.1. General

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. The mobile phase was ethyl acetate:diethyl ether (1:1), and detection was made using UV light. FT-IR spectra were recorded using a *Perkin Elmer* 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a *BRUKER AVENE II* 400 MHz NMR Spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in ppm relative to Me_4Si as an internal reference, *J* values are given in Hz. The elemental analysis was performed on *a Costech Elemental Combustion System* CHNS-O elemental analyzer. All the compounds gave C, H and N analysis within ±0.4% of the theoretical values. The Mass spectra were obtained on a *Quattro LC-MS* (70 eV) Instrument.

3.1.1. (6R,7R)-3-[(Acetyloxy)methyl]-7-{[(4-nitrophenyl)sulfonyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (2)

7-Aminocephalosporanic acid (7-ACA) (10 mmol) was added to the solution of K_2CO_3 (11 mmol) in 4 mL of water and 3 mL of acetone cooled to -5 °C, and the resulting solution was stirred for 10 min. The solution of 4-nitrobenzenesulfonyl chloride (11 mmol) in acetone was added drop wise in a period of 2.5-3 h. Then, the temperature was allowed to reach to room temperature, and the reaction was continued for 7 hours by stirring. 2-3 mL of water was added, and the reaction mixture was stirred for additional 1 h. 2-3 Drop of ethyl acetate was added into it, and the reaction mixture was acidified to pH 3.0-3.5 with 10 % HCl. The solution was extracted with 5 mL of ethyl acetate there times, the combined organic

layers were dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The oily product obtained was recrystallized from petroleum ether. M.p.: 181 °C, yield: 34 %. FT-IR (υ_{max}, cm^{-1}): 3267 (NH + OH), 2972 (aliphatic CH), 1736 (C=O), 1528 and 1350 (NO_2). Elemental analysis for $C_{16}H_{15}N_3O_9S_2$, Calculated (%), C: 42.01; H: 3.31; N: 9.19. Found (%), C: 42.28; H: 3.43; N: 9.55. ^{1}H NMR (DMSO-d_6) δ ppm: 1.97 (s, 3H, CH_3), 2.48 (s, 2H, CH_2), 4.66 (brs, 1H, CH), 5.00-5.04 (m, 2H, CH_2), 5.68 (brs, 1H, CH), 8.10 (d, 2H, ar-H, *J*= 8.2 Hz), 8.44 (d, 2H, ar-H, *J*= 9.0 Hz), 9.54 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 16.88 (CH_3), 25.35 (CH_2), 60.57 (CH_2), 82.63 (2CH), arC:[125.25 (2CH), 128.89 (2CH), 140.57 (C), 151.36 (C)], 132.78 (2C), 158.71 (2C=O), 165.57 (C=O). EI-MS: 477.85 ($[M+Na]^+$, 58), 455.65 ($[M+1]^+$, 32), 312.02 (100).

3.1.2. 4-Aminobenzenesulfonyl chloride (3)

Pd–C (5 mmol) catalyst was added to solution of the 4-nitrobenzenesulfonyl chloride **1** (10 mmol) in ethanol, and the mixture was run under microwave irradiation at 100°C, 150 W for 20 min. in the presence of hydrazine hydrate (50 mmol). The progress of the reaction was monitored by TLC. After completion of the reaction, the catalyst was separated by filtration. Upon evaporating the reaction solvent under reduced pressure, a liquid product was obtained. This was used without further purification. FT-IR (υ_{max}, cm^{-1}): 3331 and 3286 (NH_2+OH), 1346 (S=O). ^{1}H NMR (DMSO-d_6) δ ppm: 7.26 (brs, 2H, NH_2), 7.68- 7.74 (m, 2H, ar-H), 8.16-8.22 (m, 2H, ar-H). ^{13}C NMR (DMSO-d_6) δ ppm: 120.25 (2CH), 134.26 (2CH), 137.98 (C), 145.10 (C).

3.1.3. (6R,7R)-3-[(Acetyloxy)methyl]-7-{[(4-aminophenyl)sulfonyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (4)

7-Aminocephalosporanic acid (7-ACA) (10 mmol) was added to the solution of K_2CO_3 (11 mmol) in 4 mL of water and 3 mL of acetone cooled to -5 °C, and the resulting solution was stirred at this temperature for 10 min. The solution of compound **3** (11 mmol) in acetone was added into it drop wise in a period of 2.5-3 h. After the addition was completed, 2 mL of water was added, and the reaction mixture was stirred at room temperature for another 1 h. The reaction mixture was acidified to pH 3.0-3.5 with 10% HCl and the acidified solution was extracted with 5 mL of ethyl acetate three times. The combined organic layers were dried over anhydrous Na_2SO_4, and filtered. Upon evaporating the solvent under reduced pressure, an oily product was obtained. This was recrystallized from dimethyl sulfoxide:water (1:2) to afford the desired compound. M.p.: 68 °C, yield: 12 %. FT-IR (υ_{max}, cm^{-1}): 3260 and 3102 (OH+NH+NH_2), 1723 (C=O), 1345 (S=O). Elemental analysis for $C_{16}H_{17}N_3O_7S_2$, Calculated (%), C: 44.96; H: 4.01; N: 9.83. Found (%), C: 44.88; H: 3.93; N: 9.55. ^{1}H NMR (DMSO-d_6) δ ppm: 2.02 (s, 3H, CH_3), 2.92 (s, 2H, CH_2), 4.22 (brs, 3H, CH_2+CH), 4.92 (s, 1H, CH), 7.81-7.85 (m, 2H, ar-H), 7.96-8.07 (m, 3H, NH_2+NH), 8.27-8.38 (m, 2H, ar-H). ^{13}C NMR (DMSO-d_6) δ ppm: 20.52 (CH_3), 25.12 (CH_2), 51.90 (CH_2), 63.46 (CH), 64.13 (CH), arC: [113.23 (2CH), 124.08 (2CH), 140.57 (C), 144.27 (C)], 128.11 (C), 129.94 (C), 151.39 (C=O), 156.34 (C=O), 179.67 (C=O). EI-MS: 427.45 ($[M]^+$, 28), 338.16 ($[M+2-(NH_2CH_2OAc]^+$, 15), 102.12 (100).

3.1.4. General Method for The Synthesis of Compounds 5a and 5b

The corresponding alkylisothiocyanate (10 mmol) was added to the solution of compound **3** (10 mmol) in ethanol, and the reaction was performed under microwave irradiation at 120°C, 150 W for 20 min. On cooling the reaction mixture to room temperature,

a solid appeared. This crude product was recrystallized from ethanol to give the target compound.

3.1.4.1. 4-{[(Benzylamino)carbonothioyl]amino}benzenesulfonyl chloride (5a)

M.p.: 103-105 °C, yield 75 %. FT-IR (υ_{max}, cm^{-1}): 3383 (NH+OH), 3304 (NH), 3059 (aromatic CH), 1278 (S=O), 1210 (C=S). Elemental analysis for $C_{14}H_{13}ClN_2O_2S_2$, Calculated (%), C: 49.33; H: 3.84; N: 8.22. Found (%), C: 49.68; H: 3.93; N: 8.55. ^{1}H NMR (DMSO-d_6) δ ppm: 4.69 (d, 2H, CH_2, *J*= 6.2 Hz), 7.19-7.29 (m, 9H, ar-H), 8.30 (s, 1H, NH), 8.75 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 46.43 (CH_2), arC: [125.49 (2CH), 126.60 (2CH), 127.27 (2CH), 128.05 (2CH), 128.09 (CH), 139.39 (C), 139.76 (C), 151.73 (C)], 181.44 (C=S). EI-MS: 340.87 ($[M]^+$, 48), 389.16 (56), 242.24 ($[M+1-SO_2Cl]^+$, 85), 212.07 (100).

3.1.4.2. 4-[(Anilinocarbonothioyl)amino]benzenesulfonyl chloride (5b)

M.p.: 158-160 °C, yield 75 %. FT-IR (υ_{max}, cm^{-1}): 3302 (NH+OH), 3241 (NH), 1310 (S=O), 1252 (C=S). Elemental analysis for $C_{13}H_{11}ClN_2O_2S_2$, Calculated (%), C: 47.77; H: 3.39; N: 8.57. Found (%), C: 47.68; H: 3.63; N: 8.55. ^{1}H NMR (DMSO-d_6) δ ppm: 6.91-6.96 (m, 1H, ar-H), 7.24- 7.33 (m, 2H, ar-H), 7.52-7.57 (m, 2H, ar-H), 7.77-7.81 (m, 1H, ar-H), 7.92- 7.97 (m, 2H, ar-H), 8.20-8.26 (m, 1H, ar-H), 9.87 (s, 2H, 2NH). ^{13}C NMR (DMSO-d_6) δ ppm: 117.42 (2CH), 121.92 (2CH), 125.21 (CH), 127.45 (2CH), 129.74 (2CH), 141.61 (C), 144.30 (C), 156.37 (C). EI-MS: 349.87 ($[M+Na]^+$, 67), 292.30 ($[M-Cl]^+$, 23), 269.08 (100).

3.1.5. General Method For The Synthesis of Compounds 5c and 5d

The corresponding alkylisocyanate (10 mmol) was added to the solution of compound **3** (10 mmol) in ethanol, and the reaction mixture was refluxed for 13 h (for **5c**) or 10 h (for **5d**). On cooling it to room temperature, a solid appeared. The crude product was

recrystallized from ethyl acetate (for **5c**) or acetone:diethyl ether (1:2) (for **5d**) to give the desired product.

3.1.5.1. 4-{[(Benzylamino)carbonyl]amino}benzenesulfonyl chloride (5c)

M.P.: 203-205 °C, yield 75 %. FT-IR (υ_{max}, cm^{-1}): 3285, 3195 (2NH+OH), 1657 (C=O), 1294 (S=O). Elemental analysis for $C_{14}H_{13}ClN_2O_3S$, Calculated (%), C: 51.77; H: 4.03; N: 8.63. Found (%), C: 51.68; H: 3.83; N: 8.55. ^{1}H NMR (DMSO-d_6) δ ppm: 4.21 (d, 2H, CH_2, J= 5.6 Hz), 6.67 (brs, 2H, arH), 6.99 (s, 2H, arH), 7.25 (brs, 5H, arH), 7.79 (s, 2H, 2NH). ^{13}C NMR (DMSO-d_6, δ ppm): 42.56 (CH_2), arC:[112.50 (2CH), 125.50 (CH), 126.42 (CH), 126.63 (CH), 128.03 (2CH), 128.75 (2CH), 149.08 (C), 150.01 (C), 153.91 (C)], 158.84 (C=O). EI-MS: 346.85 ($[M-1+Na]^+$, 65), 324.80 ($[M]^+$, 84), 271.00 (100).

3.1.5.2. 4-[(Anilinocarbonyl)amino]benzenesulfonyl chloride (5d)

M.p.: 226-227 °C, yield 47 %. FT-IR (υ_{max}, cm^{-1}): 3294, 3211 (2NH+OH), 1667 (C=O), 1309 (S=O). Elemental analysis for $C_{13}H_{11}ClN_2O_3S$, Calculated (%), C: 50.24; H: 3.57; N: 9.01. Found (%), C: 50.68; H: 3.63; N: 8.85. ^{1}H NMR (DMSO-d_6, δ ppm): 6.94 (t, 3H, arH, *J*= 7.2 Hz), 7.25 (t, 3H, arH, *J*= 7.6 Hz), 7.50 (d, 3H, arH, *J*= 7.9 Hz), 8.18 (s, 1H, NH), 8.98 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): arC:**[**119.02 (2CH), 122.34 (2CH), 126.04 (2CH), 129.26 (3CH), 136.87 (C), 140.56 (2C)], 156.82 (C=O). EI-MS: 359.41 ($[M+K]^+$, 68), 334.98 8[$M+1+Na]^+$, 78), 310.79 ($[M]^+$, 15), 165.48 (100).

3.1.6. General Method For The Synthesis of Compounds 6a and 6b

7-ACA (10 mmol) was added to the solution of K_2CO_3 (11 mmol) in 4 mL of water and 3 mL of acetone cooled to -5 °C, and the resulting solution was stirred at this temperature for 10 min. Then, the solution of the corresponding compound **5** (11 mmol) in acetone was

added into it drop wise in a period of 2,5-3 h. After the addition was completed, the temperature was allowed to reach to room temperature, and the reaction mixture was stirred for 14 h (for **6a**) or 5 h (for **6b**). Then, water was added, and the reaction mixture was stirred for an additional 1 h. 2-3 Drop of ethyl acetate was added, and the reaction mixture was acidified to pH 3.0-3.5 with 10% HCl. The acidified solution was extracted with 5 mL of ethyl acetate there times. The combined organic layers were dried over anhydrous Na_2SO_4, and filtered. Upon evaporating the solvent under reduced pressure, a solid obtained. The oily product was recrystallized from acetone (for **6a**) or petroleum ether (for **6b**).

3.1.6.1. (6R,7R)-3-[(Acetyloxy)methyl]-7-{[(4-{[(benzylamino)carbonothioyl]amino} phenyl) sulfonyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (6a)

M.p.: 128-129 °C, yield: 22 %. FT-IR (υ_{max}, cm^{-1}): 3675 (OH), 3294 and 3280 (3NH), 2971 (aliphatic CH), 1655 (3C=O), 1270 (S=O), 1248 (C=S). Elemental analysis for $C_{24}H_{24}N_4O_7S_3$ Calculated (%) C: 49.99; H. 4.19; N: 9.72. Found (%) C: 50.37; H: 4.57; N: 10.06. ^{1}H NMR (DMSO-d_6) δ ppm: 1.92 (s, 3H, CH_3), 2.48 (s, 2H CH_2+ DMSO), 3.34 (s, 2H, CH_2+H_2O), 4.76 (s, 4H, 2CH+CH_2), 7.23-7.30 (m, 9H, ar-H), 8.65 (s, 1H, NH), 10.03 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ ppm: 25.00 (CH_3), 28.50 (CH_2), 56.95 (CH_2), 60.00 (CH), 61.45 (CH_2), 64.20 (CH), arC: [117.50 (2CH), 120.83 (2CH), 126.68 (CH), 127.31 (2CH), 128.10 (2CH), 139.42 (C), 148.40 (C), 151.67 (C)], 132.34 (C), 134.68 (C), 164.78 (C=O), 167.68 (C=O), 178.11 (C=S + C=O). EI-MS: 575.37 ($[M-1]^+$, 34), 531.34 ($[(M- CO_2H]^+$, 40), 212.08 (100).

3.1.6.2. (6R,7R)-3-[(Acetyloxy)methyl]-7-[({4-[(anilinocarbonothioyl)amino]phenyl} sulfonyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (6b)

M.p.: 202-203 °C, yield 22 %. FT-IR (υ_{max}, cm^{-1}): 3524 (OH), 3184 and 3130 (3NH), 2972 (aliphatic CH), 1794, 1735 (3C=O), 1229 (C-O). Elemental analysis for $C_{23}H_{22}N_4O_7S_3$ Calculated (%) C: 49.10; H: 3.94; N: 9.96. Found (%) C: 49.17; H: 4.33; N: 10.06. ^{1}H NMR (DMSO-d_6) δ ppm: 2.03 (s, 3H, CH_3), 3.51 (q, 2H, CH_2, *J*= 20.0 Hz), 4.73 (d, 1H, CH, *J*= 12.6 Hz), 4.88 (d, 1H, CH, *J*= 5.2 Hz), 4.99 (t, 2H, CH_2, *J*= 7.7 Hz) 6.93 (t, 2H, ar-H, *J*= 8.0 Hz), 7.30 (t, 3H, ar-H, *J*= 8.0 Hz), 7.56 (d, 4H, ar-H, *J*= 7.7 Hz), 9.88 (brs, 2H, 2NH). ^{13}C NMR (DMSO-d_6) δ ppm: 20.55 (CH_3), 25.11 (CH_2), 58.81 (CH), 62.83 (CH_2), 63.48 (CH), arC: [116.73 (3CH), 120.96 (3CH), 128.93 (3CH), 141.15 (2C), 155.61 (C)], 125.56 (C), 126.54 (C), 163.16 (C=O), 169.82 (C=O), 170.21 (C=S+C=O). EI-MS: 561.32 ($[M-1]^+$, 13), 531.32 (33), 487.29 ($[M-Ph]^+$, 43), 212.07 (100).

3.1.7. General Method For The Synthesis of Compounds 6c and 6d

7-ACA (10 mmol) was added to the solution of K_2CO_3 (11 mmol) in 4 mL of water and 3 mL of acetone cooled to -5 °C, and the resulting solution was stirred at this temperature for 10 min. The solution of corresponding compound **5** (11 mmol) in acetone was added drop wise in a period of 2 hours. Then, temperature was allowed to reach to room temperature, and the mixture was stirred for 48 h (for **6c**) or 24 h (for **6d**). 2-3 mL of water was added into it and the mixture was stirred for an additional 1 h. The reaction content was acidified to pH 3 with 10 % HCl, and the acidified solution was extracted with 5 mL of ethyl acetate three times. The combined organic layers were dried on anhydrous $NaSO_4$ and filtered. On evaporating the solvent under reduced pressure, a solid obtained. This was recrystallized from acetone (for **6c**) or acetone:water (1:2) (for **6d**) to afford the desired compound.

3.1.7.1. (6R,7R)-3-[(Acetyloxy)methyl]-7-{[(4-{[(benzylamino)carbonyl]amino} phenyl)sulfonyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (6c)

M.p.: 229-230 ℃, yield: 20 %. FT-IR (υ_{max}, cm^{-1}): 3643 (OH), 3286, 3177 (3NH), 1798, 1737, 1658 (4C=O), 1335 (S=O). Elemental analysis for $C_{24}H_{24}N_4O_8S_2$ Calculated (%) C: 51.42; H: 4.32; N: 9.99. Found (%) C: 51.17; H: 4.33; N: 10.26. ^{1}H NMR (DMSO-d_6 δ ppm): 2.01 (s, 3H, CH_3), 3.50 (q, 2H, CH_2, *J*= 16.0 Hz), 4.22 (brs, 2H, CH_2), 4.67 (brs, 1H, CH), 4.81 (brs, 1H, CH), 4.93-4.99 (m, 2H, CH_2), 7.25 (brs, 9H, arH), 7.77 (s, 1H, NH), 7.93 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 21.05 (CH_3), 25.12 (CH_2), 62.56 (CH_2), 58.84 (CH), 62.85 (CH_2), 63.31 (CH), 122.47 (C), 126.69 (C), arC: [126.34 (CH), 126.41 (2CH), 126.92 (2CH), 127.97 (2CH), 128.02 (2CH), 140.56 (C), 158.75 (C), 158.79 (C)], 163.19 (C=O), 169.54 (C=O), 170.22 (C=O), 172.00 (C=O). EI-MS: 560.65 ($[M]^+$, 74), 583.79 ($[M+Na]^+$, 39), 325.70 (100).

3.1.7.2. (6R,7R)-3-[(Acetyloxy)methyl]-7-[({4-[(anilinocarbonyl)amino]phenyl} sulfonyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (6d)

M.p.: 235 ℃, yield 20 %. FT-IR (υ_{max}, cm^{-1}): 3676 (OH), 3296, 3218 (2NH), 3096 (aromatic CH), 2988, 2902 (aliphatic CH), 1799, 1737, 1668 (4C=O), 1333 (S=O). Elemental analysis for $C_{23}H_{22}N_4O_8S_2$ Calculated (%) C: 50.54; H: 4.06; N: 10.25. Found (%) C: 50.17; H: 4.33; N: 10.06. ^{1}H NMR (DMSO-d_6, δ ppm): 2.02 (s, 3H, CH_3) 3.54 (q, 2H, CH_2, *J*= 16.0 Hz), 4.67 (d, 1H, CH, *J*= 12.6 Hz), 4.79 (d, 1H, CH, *J*= 4.7 Hz), 4.92-5.10 (m, 2H, CH_2), 6.93 (t, 3H, arH, *J*= 7.2 Hz), 7.24 (t, 3H, arH, *J*= 7.4 Hz), 7.49 (d, 3H, arH, *J*= 7.6 Hz), 8.18 (s, 1H, NH), 9.10 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, δ ppm): 21.06 (CH_3), 25.10 (CH_2), 62.90 (CH_2), 63.30 (CH), 64.40 (CH), 122.10 (2C), arC: [118.19 (2CH), 121.58 (2CH), 128.54 (3CH), 128.75 (2CH), 139.85 (C), 156.07 (2C)], 163.23 (C=O), 169.51 (C=O), 170.22 (C=O), 171.98 (C=O). EI-MS: 570.14 ($[M+1+Na]^+$,45), 546.78 ($[M]^+$, 68), 236.15 (100).

3.1.8. General Method For The Synthesis of Compounds 7a-c

4- Chlorophenacylbromide (10 mmol) and dried sodium acetate (50 mmol) was added to the solution of the corresponding compound **5** in absolute ethanol, and the reaction mixture was refluxed for 15 h (for **7a**), 18 h (for **7b**) or 20 h (for **7c**). Then, the solvent was removed under reduced pressure and the solid formed was washed with water. The obtained oily product was extracted with 5 mL of ethyl acetate there times. The combined organic layers were dried over anhydrous Na_2SO_4, and filtered. Upon evaporating the solvent under reduced pressure, an oily product obtained. This was recrystallized from acetone:diethyl ether (1:2) (for **7a**) or ethanol:water (1:2) (for **7b** and **7c**) to afford the desired product.

3.1.8.1. 4-{[3-Benzyl-5-(4-chlorophenyl)-1,3-thiazol-2(3H)-ylidene]amino}benzene sulfonyl chloride (7a)

M.p.: 106-108 °C, yield: 28 %. FT-IR (υ_{max}, cm^{-1}): 3068, 3031 (aromatic CH), 1496 (C=N), 1396 (S=O). Elemental analysis for $C_{22}H_{16}Cl_2N_2O_2S_2$ Calculated (%) C: 55.58; H: 3.39; N: 5.89. Found (%) C: 55.17; H: 3.03; N: 6.06. ^{1}H NMR (DMSO-d_6, δ ppm): 4.78 (d, 2H, CH_2, J= 6.2 Hz), 6.75 (s, 1H, CH), 7.30-7.46 (m, 13H, ar-H). ^{13}C NMR (DMSO-d_6, δ ppm): 56.00 (CH_2), 126.35 (CH), 131.63 (C), arC: [127.21 (CH), 127.26 (CH), 127.31 (2CH), 127.47 (2CH), 127.55 (CH), 127.61 (CH), 128.69 (CH), 128.77 (2CH), 128.83 (2CH), 141.25 (2C), 149.00 (C), 153.45 (2C)], 156.72 (C=N). EI-MS: 476.48 ($[M+1]^+$, 87), 475.46 ($[M]^+$, 75), 125.54 (100).

3.1.8.2. 4-{[5-(4-Chlorophenyl)-3-phenyl-1,3-thiazol-2(3H)-ylidene]amino}benzene sulfonyl chloride (7b)

M.p.: 98-100 °C, yield 55 %. FT-IR (υ_{max}, cm^{-1}): 3093, 3028 (aromatic CH), 1543 (C=N), 1336 (S=O). Elemental analysis for $C_{21}H_{14}Cl_2N_2O_2S_2$ Calculated (%) C: 54.67; H:

3.06; N: 6.07. Found (%) C: 54.87; H: 3.13; N: 6.36. ^{1}H NMR (DMSO-d_6, δ ppm): 6.86-6.95 (m, 2H, CH+ar-H), 7.21 (t, 6H, ar-H, J= 8.0 Hz), 7.78-7.58 (m, 6H, ar-H). ^{13}C NMR (DMSO-d_6, δ ppm): 104. 99 (C), 124.67 (CH), arC: [117.46 (2CH), 122.74 (2CH), 125.16 (C), 125.60 (CH), 128.55 (2CH), 129.76 (2CH), 130.91 (2CH), 132.00 (2CH), 136.00 (2C), 141.52 (C), 143.95 (C)], 156.47 (C=N). EI-MS: 484.31 ([M+Na]$^{+}$, 16), 242.24 ([M+2-($C_{12}H_6Cl_2$)], 94), 212.08 (100).

3.1.8.3. 4-{[3-Benzyl-5-(4-chlorophenyl)-1,3-oxazol-2(3H)-ylidene]amino}benzene sulfonyl chloride (7c)

M.p.: 223-225 °C, yield: 44 %. FT-IR (υ_{max}, cm^{-1}): 3064 (Aromatic CH), 1489 (C=N), 1296 (S=O). Elemental analysis for $C_{22}H_{16}Cl_2N_2O_3S$ Calculated (%) C: 57.52; H: 3.51; N: 6.10. Found (%) C: 57.87; H: 3.13; N: 6.36. ^{1}H NMR (DMSO-d_6, δ ppm): 3.69 (brs, 2H, CH_2+ H_2O), 6.40-6.45 (m, 1H, CH), 7.23-7.29 (m, 2H, ar-H), 7.72 (s, 1H, ar-H), 7.80-7.86 (m, 4H, ar-H), 8.13-8.25 (m, 4H, ar-H), 8.26 (s, 2H, ar-H). ^{13}C NMR (DMSO-d_6, δ ppm): 53.53 (CH_2), 112.90 (CH), arC:[123.85 (3CH), 123.93 (2CH), 126.17 (CH), 127.35 (2CH), 127.61 (3CH), 128.90 (2CH), 148.00 (2C), 149.72 (2C), 154.44 (C)], 135.83 (C), 166.49 (C=N). EI-MS: 498. 68 ([M+K]$^{+}$, 89), 459.35 ([M]$^{+}$, 65), 234.81 (100).

3.1.9. General Method For The Synthesis of Compounds 8a and 8b

7-ACA (10 mmol) was added to the solution of K_2CO_3 (11 mmol) in 4 mL of water and 3 mL of acetone cooled to -5 °C and the resulting solution was stirred at this temperature for 10 min. The solution of the corresponding compound **7a, 7b** (11 mmol) in acetone was added drop wise in a period of 2,5-3 h. After the addition was completed, the reaction mixture was stirred at room temperature for 24 h (for **8a**) or 6 h (for **8b**). Then, water was added into it, and the mixture was stirred for another 1 h. The reaction mixture was acidified to pH 3

with 10% HCl and the acidified solution was extracted with 5 mL of ethyl acetate three times. The combined organic layers were dried over anhydrous Na_2SO_4, and filtered. After evaporating the solvent under reduced pressure, an oily product was obtained. This was recrystallized from acetone:diethyl ether (1:2) (for **8a**) or acetone: water (1:2) (for **8b**) to give the target compound.

3.1.9.1. (6R,7R)-3-[(Acetyloxy)methyl]-7-{[(4-{[3-benzyl-5-(4-chlorophenyl)-1,3-thiazol-2(3H)-ylidene]amino}phenyl)sulfonyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (8a)

M.p.: 158-160 ℃, yield 12 %. FT-IR (υ_{max}, cm^{-1}): 3275 (NH+OH), 2987, 2923 (aliphatic CH), 1801, 1735 (3C=O), 1535 (C=N), 1335 (S=O). Elemental analysis for $C_{32}H_{27}ClN_4O_7S_3$ Calculated (%) C: 54.04; H: 3.83; N: 7.88. Found (%) C: 54.27; H: 3.73; N: 7.46. ^{1}H NMR (DMSO-d_6, δ ppm): 2.03 (s, 3H, CH_3), 3.51 (q, 2H, CH_2, J= 8.0 Hz), 4.67 (d, 1H, CH, J= 12.7 Hz), 4.77 - 4.80 (m, 1H, CH), 4.96-5.01 (m, 4H, $2CH_2$), 6.77 (brs, 1H, CH), 7.27-7.41 (m, 5H, ar-H), 7.59-7.71 (m, 5H, ar-H), 7.87- 7.96 (m, 3H, ar-H). ^{13}C NMR (DMSO-d_6, δ ppm): 20.54 (CH_3), 25.61 (CH_2), 46.46 (CH_2), 58.64 (CH), 62.84 (CH_2), 63.29 (CH), 122.57 (2C), 126.61 (C), 126.68 (CH), arC: [126.89 (2CH), 127.25 (2CH), 128.16 (2CH), 128.30 (2CH), 128.58 (2CH), 128.77 (CH), 128.84 (CH), 131.10 (CH) 139.41 (2C), 151.66 (2C), 163.18 (C)] 169.47 (C=N), 170.20 (C=O), 172.00 (C=O), 178.10 (C=O). EI-MS: 734.45 ($[M+Na]^+$, 85), 713.21 ($[M+2]^+$, 35), 220.68 (100).

3.1.9.2. (6R,7R)-3-[(Acetyloxy)methyl]-7-{[(4-{[5-(4-chlorophenyl)-3-phenyl-1,3-thiazol-2(3H)-ylidene]amino}phenyl)sulfonyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (8b)

M.p.: 202-203 °C, yield 22 %. FT-IR (υ_{max}, cm^{-1}): 3183 (NH+OH), 3006 (aromatic CH), 2922, 2849 (aliphatic CH), 1801, 1736 (3C=O), 1540 (C=N), 1335 (S=O). Elemental analysis for $C_{31}H_{25}ClN_4O_7S_3$ Calculated (%) C: 53.41; H: 3.61; N: 8.04. Found (%) C: 53.77; H: 3.43; N: 8.36. ^{1}H NMR (DMSO-d_6) δ ppm: 2.03 (s, 3H, CH_3), 3.51 (q, 2H, CH_2, J= 20.0 Hz), 4.67 (d, 1H, CH, J= 12.7 Hz), 4.84 (d, 1H, CH, J= 4.7 Hz), 4.97-5.01 (m, 2H, CH_2), 6.83 (brs, 1H, CH), 6.91 (t, 1H, ar-H, J= 7.2 Hz), 7.22-7.38 (m, 4H, ar-H), 7.40-7.81 (m, 8H, ar-H). ^{13}C NMR (DMSO-d_6, δ ppm): 20.54 (CH_3), 25.17 (CH_2), 58.35 (CH), 62.78 (CH_2), 62.96 (CH), 114.73 (CH), 122.93 (C), arC: [116.26 (2CH), 116.72 (2CH), 120.90 (2CH), 123.75 (CH), 124.69 (2CH), 126.47 (2C), 128.89 (2CH), 131.29 (2CH), 133.14 (C), 141.19 (C), 142.32 (C)], 133.84 (2C), 155.63 (C=N), 163.09 (C=O), 168.93 (C=O), 170.20 (C=O). EI-MS: 720.01 ($[M+Na]^+$, 75), 697.28 ($[M]^+$, 95), 156.45 (100).

3.1.10. 4-{[3-Benzyl-5-oxo-1,3-thiazolidin-2-ylidene]amino}benzenesulfonyl chloride (9)

The solution of compound **5a** (10 mmol) in acetic acid was refluxed with etilbromo acetate (10 mmol) in the presence of dried sodium acetate (30 mmol) for 24 h. Then, the reaction mixture was poured into water, and a white solid appeared. This crude product was filtered and recrystallized from ethyl acetate:diethyl ether (1:2). M.p.: 213-215. Yield: 25 %. FT-IR (υ_{max}, cm^{-1}): 3033 (aromatic CH), 1705 (C=O), 1525 (C=N), 1341 (S=O). Elemental analysis for $C_{16}H_{13}ClN_2O_3S_2$ Calculated (%) C: 50.46; H: 3.44; N: 7.36. Found (%) C: 50.77; H: 3.73; N: 8.00. ^{1}H NMR (DMSO-d_6, δ ppm): 4.03 (s, 2H, CH_2), 4.79 (s, 2H, CH_2), 7.35 (brs, 9H, ar-H). ^{13}C-NMR (DMSO-d_6, δ ppm): 32.91 (CH_2), 46.52 (CH_2), arC: [128.26

(3CH), 128.97 (3CH), 129.27 (3CH), 136.57 (C), 157.35 (C), 160.40 (C)], 168.33 (C=N), 172.48 (C=O). EI-MS: 402.15 ($[M-1+Na]^+$, 56), 381.98 ($[M+1]^+$, 67), 178.02 (100).

3.1.11. (6R,7R)-3-[(Acetyloxy)methyl]-7-{[(4-{[3-benzyl-5-oxo-1,3-thiazolidin-2-ylidene]amino}phenyl)sulfonyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (10)

7-ACA (10 mmol) was added to the solution of K_2CO_3 (11 mmol) in 4 mL of water and 3 mL of acetone cooled to -5 ℃ and the resulting solution was stirred at this temperature for 10 min. The solution of the corresponding compound **9** (11 mmol) in acetone was added drop wise in a period of 2,5-3 h. After the addition was completed, the reaction mixture was stirred at room temperature for 9 h. Then, water was added into it, and the mixture was stirred for another 1 h. The reaction mixture was acidified to pH 3 with 10% HCl and the acidified solution was extracted with 5 mL of ethyl acetate three times. The combined organic layers were dried over anhydrous Na_2SO_4, and filtered. After evaporating the solvent under reduced pressure, an oily product was obtained. This crude product was crystallized from butyl acetate: diethyl ether (1:3). M.p.: 207-208 ℃, yield: 35 %. FT-IR (υ_{max}, cm^{-1}): 3450 (OH), 3065 (aromatic CH), 2985, 2875 (aliphatic CH), 1705, 1685, 1617 (4C=O), 1341 (S=O). Elementel analysis for $C_{26}H_{24}N_4O_8S_3$ Calculated (%) C: 50.64; H: 3.92; N: 9.09. Found: C: 50.25; H: 4.12; N: 9.28. ^{1}H-NMR (DMSO-d_6, δ ppm): 1.91 (s, 3H, CH_3), 3.38 (brs, 4H, $2CH_2$), 3.97 (s, 2H, CH_2), 4.39 (s, 2H, CH_2), 4.83 (s, 1H, CH), 5.49 (s, 1H, CH), 7.31 (brs, 9H, arH).). ^{13}C-NMR (DMSO-d_6, δ ppm): 14.68 (CH_3), 32.57 (CH_2), 46.45 (CH_2), 52.26 (2CH), 56.50 ($2CH_2$) 117.20 (2C), arC:[127.50 (CH), 127.95 (2CH), 128.18 (2CH), 128.59 (CH), 128.81 (CH), 129.05 (2CH), 134.65 (2C), 138.26 (C)], 155.65 (C=N), 166.72 (C=O), 168.25 (C=O), 174.67 (2C=O). EI-MS: 639.40 ($[M+Na]^+$ 25), 616.70 ($[M]^+$,72).

3.1.12. 1-(2-Fluoro-4-nitrophenyl)piperazine (11)

The solution of 3,4-difluoronitrobenzene (10 mmol) in acetonitrile was refluxed in the presence of piperazine (50 mmol) for 5 h. After evaporating the solvent under reduced pressure, an oily mass was obtained. The crude product was treated with water and extracted with 5 mL of ethyl acetate three times. The combined organic layers were dried on anhydrous $NaSO_4$ and filtered. On evaporating the solvent under reduced pressure, a crude product was obtained. This was crystallized from butyl acetate: diethyl ether (1:3). M.p.: 66-68°C [47].

3.1.13. [4-(2-Fluoro-4-nitrophenyl)piperazin-1-yl]acetyl chloride (12)

Chloroethanoylchloride (15 mmol) was added to the mixture of compound **11** (10 mmol) and triethylamine (30 mmol) in THF cooled to -5^{o}C drop wise over a 2-hour period. Then, the temperature was allowed to reach to room temperature and the mixture was stirred for 4 h. The precipitated salt was removed by filtration, the solvent was evaporated under reduced pressure and water was added into it. The resulting yellow oily product was recrystallized form ethyl acetate: hexane (1:3) to afford the desired compound. M.p.: 118-119 °C. 60 % yield. FT-IR (υ_{max}, cm^{-1}): 1661 (C=O), 1494 and 1330 (NO_2). Elemental analysis for $C_{12}H_{13}ClFN_3O_3$ Calculated (%) C: 47.77; H: 4.34; N: 13.93. Found (%) C: 47.57; H: 3.99; N: 13.66. ^{1}H NMR (DMSO-d_6) δ ppm: 3.31 (brs, H_2O+2CH_2), 3.61 (s, 4H, 2CH_2), 4.43 (s, 2H, CH_2), 7.18 (t, 1H, ar-H, *J*= 4.0 Hz), 7.98- 8.07 (m, 2H, ar-H). ^{13}C-NMR (DMSO-d_6) δ ppm: 45.59 (CH_2), 46.05 (CH_2), 49.49 (CH_2), 49.66 (CH_2), 60.83 (CH_2), arC: [112.71 and 113.23 (d, CH, *J*= 26.0 Hz), 118.83 (CH), 121.93 and 121.97 (d, CH, *J*= 2.2 Hz), 140.24 and 140.42 (d, C, *J*= 9.0 Hz), 145.59 and 145.74 (d, C, *J*= 7.5 Hz), 150.26 and 155.18 (d, C, *J*= 246 Hz)], 170.93 (C=O). EI-MS: 301.08 ([M]$^{+}$, 82), 217.05 (100).

3.1.14. (6R,7R)-3-[(Acetyloxy)methyl]-7-({[4-(2-fluoro-4-nitrophenyl)piperazin-1-yl]acetyl}amino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (13)

7-ACA (10 mmol) was added to the solution of K_2CO_3 (11 mmol) in 4 mL of water and 3 mL of acetone cooled to -5 °C and the resulting solution was stirred at this temperature for 10 min. Then, the solution of the corresponding compound **12** (11 mmol) in acetone was added drop wise in a period of 2,5-3 h. After the addition was completed, the reaction mixture was stirred at room temperature for 1h. Water was added into it, and the mixture was stirred for another 1 h. The reaction mixture was acidified to pH 3 with 10% HCl, the resulting precipitate was filtered off and recrystallized from acetone. M.p.: 178-180 °C, yield: 86 %. FT- IR (υ_{max}, cm^{-1}): 3145 (NH+ OH), 1794 (C=O), 1735 (C=O), 1661 (2C=O), 1511 and 1334 (NO_2), 1228 (C-O). Elementel analysis for $C_{22}H_{24}FN_5O_8S$ Calculated (%) C: 49.16; H: 4.50; N: 13.03. Found (%) C: 49.18; H: 4.40; N: 12.63. ^{1}H-NMR (DMSO-d_6) δ ppm: 2.01 (s, 3H, CH_3), 3.30 (brs, 4H, $2CH_2$), 3.54 (brs, 6H, $3CH_2$+H_2O), 4.43 (s, 2H, CH_2), 4.67 (s, 1H, CH), 4.82 (s, 1H, CH), 4.99 (s, 2H, CH_2), 7.17 (t, 1H, ar-H, *J*= 9.0 Hz), 8.0-8.05 (m, 2H, ar-H). ^{13}C-NMR (DMSO-d_6) δ ppm: 21.03 (CH_3), 25.18 (CH_2), 41.30 (CH_2), 41.88 (CH_2), 44.87 (CH_2), 48.96 (CH_2), 49.00 (CH_2), 58.21 (CH), 62.76 (CH_2), 62.81 (CH), 112.13 and 112.39 (d, CH, *J*= 26 Hz), 118.12 and 118.16 (d, CH, *J*= 4.0 Hz), 121.23 (CH), 123.04 (C), 126.43 (C), 139.58 and 139.66 (d, C, *J*= 8.0 Hz), 144.92 and 144.97 (d, C, *J*=5.0 Hz), 150.77 and 153.22 (d, C, *J*= 245.0 Hz), 163.05 (C=O), 164.78 (C=O), 168.68 (C=O), 170.20 (C=O). LC-MS: 560.10 ($[M+Na]^+$, 22), 491.17 ($[(M-1)-CO_2H]^+$, 22), 313.07 (100).

3.2. Biological activity

3.2.1. Antimicrobial activity

The test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* (*E. coli*) ATCC35218, *Yersinia*

pseudotuberculosis (Y. pseudotuberculosis) ATCC911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC43288, *Enterococcus faecalis* (*E. faecalis*) ATCC29212, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Bacillus cereus* (*B. cereus)* 709 Roma, *Mycobacterium smegmatis* (*M. smegmatis*) ATCC607, *Candida albicans* (*C. albicans*) ATCC60193 and *Saccharomyces cerevisiae* (*S. cerevisia*) RSKK 251, Ar: *Arthrobacter oxydans* (laboratory strain), Ct: *Candida tropicalis*, ATCC 13803, Pv: *Proteus vulgaris* ATCC 13315, Ac: *Acinetobacter sp* (laboratory strain), except *Serretia marcescens* (Sm), *Acinetobacter sp* (Ac) and *Klebsiella oxitoka* (Ko) which are laboratry strains. All the newly synthesized compounds were weighed and dissolved in hexane to prepare extract stock solution of 20.000 microgram/milliliter (µg/mL).

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double microdilution and the minimal inhibition concentration (MIC) values (µg/mL) were determined. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The micro dilution test plates were incubated for 18-24 h at 35 °C. Brain Heart Infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis*, and incubated for 48-72 h at 35 °C [50]. Ampicillin (10 µg) and fluconazole (5µg) were used as standard antibacterial and antifungal drugs, respectively. Dimethylsulphoxide with dilution of 1:10 was used as solvent control.

3.2.2. Urease inhibition assay

Reaction mixtures comprising 25 µL of Jack Bean Urease, 55 µL of buffer (100 mM urea, 0.01 M K_2HPO_4, 1 mM EDTA and 0.01 M LiCl, pH 8.2) and 100 mM urea were incubated with 5 µL of the test compounds at room temperature for 15 min in microtiter plates. The production of ammonia was measured by indophenol method and used to

determine the urease inhibitory activity. The phenol reagent (45 µL, 1% w/v phenol and 0.005 % w/v sodium nitroprusside) and alkali reagent (70 µL, 0.5 % w/v sodium hydroxide and 0.1 % v/v NaOCl) were added to each well and the increasing absorbance at 625 nm was measured after 20 min, using a microplate reader (Molecular Device, USA). The percentage inhibition was calculated from the formula 100 – (ODtestwell/ODcontrol) × 100. Thiourea was used as the standard inhibitor. In order to calculate IC_{50} values, different concentrations of synthesized compounds and standard were assayed at the same reaction conditions [51].

3.2.3. β-Lactamase assay

In vitro *β*-lactamase (*Bacillus cereus* metallo *β*-lactamase, Sigma) activity was determined by monitoring the hydrolysis of reporter substrate Nitrocefin (Calbiochem, Darmstadt, Germany) by *β*-lactamase, at 486 nm (Louie et al. 2012). Enzyme assays were performed in 25 mM piperazine-*N*,*N*'-bis (2-ethane sulfonic acid) (PIPES) buffer pH 7.0 with 100 µM $ZnSO_4$. 50 µL 1.5 µM enzyme solution, 50 µL 150 µM Nitrocefin and 50 µL 1 mM synthesized compound were recorded continuously for 20 minutes at 37 °C against the buffer alone by using microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Scientific) at 486 nm. The inhibitory activity of those compounds and $HgCl_2$, a positive control against *β*-lactamase were measured at various concentrations. Residual activities were calculated by comparing to control without inhibitor (T+). The assays were done in triplicate. The IC_{50} value was determined as the concentration of compound that give 50% inhibition of maximal activity [52].

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Scheme Captions

Scheme 1: Reaction and conditions: *i*: Pd-C, H_2NNH_2; *ii, iii, v, viii* and *x*: 7-aminocephalosporanic acid; *iv*: RNCS, MW (150W); *vi*: compound **5**; *vii*: (*4*-)$ClC_6H_4CH_2Br$; *ix*: $BrCH_2CO_2Et$.

Scheme 2: Synthetic Pathway for the preparation of compounds **11-13**. *i*: 3,4-difluoronitrobenzene, MW (150W); *ii*: chloroethanoyl chloride; *iii*: 7-ACA.

Table 1. Screening for antimicrobial activity of the compounds (μg/μl).

Comp.	**Microorganisms[a] and inhibition zone (mm)**								
No	Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
2	-	-		23	6	8	-	-	-
3	12	12	28	18	12	15	10	13	20
4	12	12	30	20	16	18	12	14	20
5a	28	25	20	30	16	18	17	8	8
5b	-	-	-	6	-	-	-	8	15
5c	28	25	20	30	16	18	15	18	20
5d	-	-	-	6	-	-	-	8	15
6a	12	8	6	15	10	8	16	8	10
6b	14	10	8	18	10	12	18	14	22
6c	14	10	8	18	10	10	14	10	10
6d	12	8	6	15	8	10	10	8	8
7a	-	-	-	16	18	6	10	10	20
7b	-	-	-	18	8	8	12	10	22
7c	-	-	-	18	8	6	12	8	10
8a	6	8	8	22	8	-	-	10	-
8b	12	6	6	-	-	8	16	10	14
9	-	-	-	18	10	10	12	10	18
10	10	8	8	22	10	8	16	10	18
12	6	-	-	15	8	8	-	15	30
13	10	6	-	18	8	10	22	15	30
Amp.	10	18	18	35	10	15			
Strp.							35		
Flu								25	>25

[a] Ec*: Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 43288, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Bc: *Bacillus cereus* 702 Roma , *Ms: M. smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, Sc: *Saccharomyces cerevisiae* RSKK 251, Amp.: Ampicillin, Strep.: Streptomycin, Flu.: Fluconazole, (—): no activity.

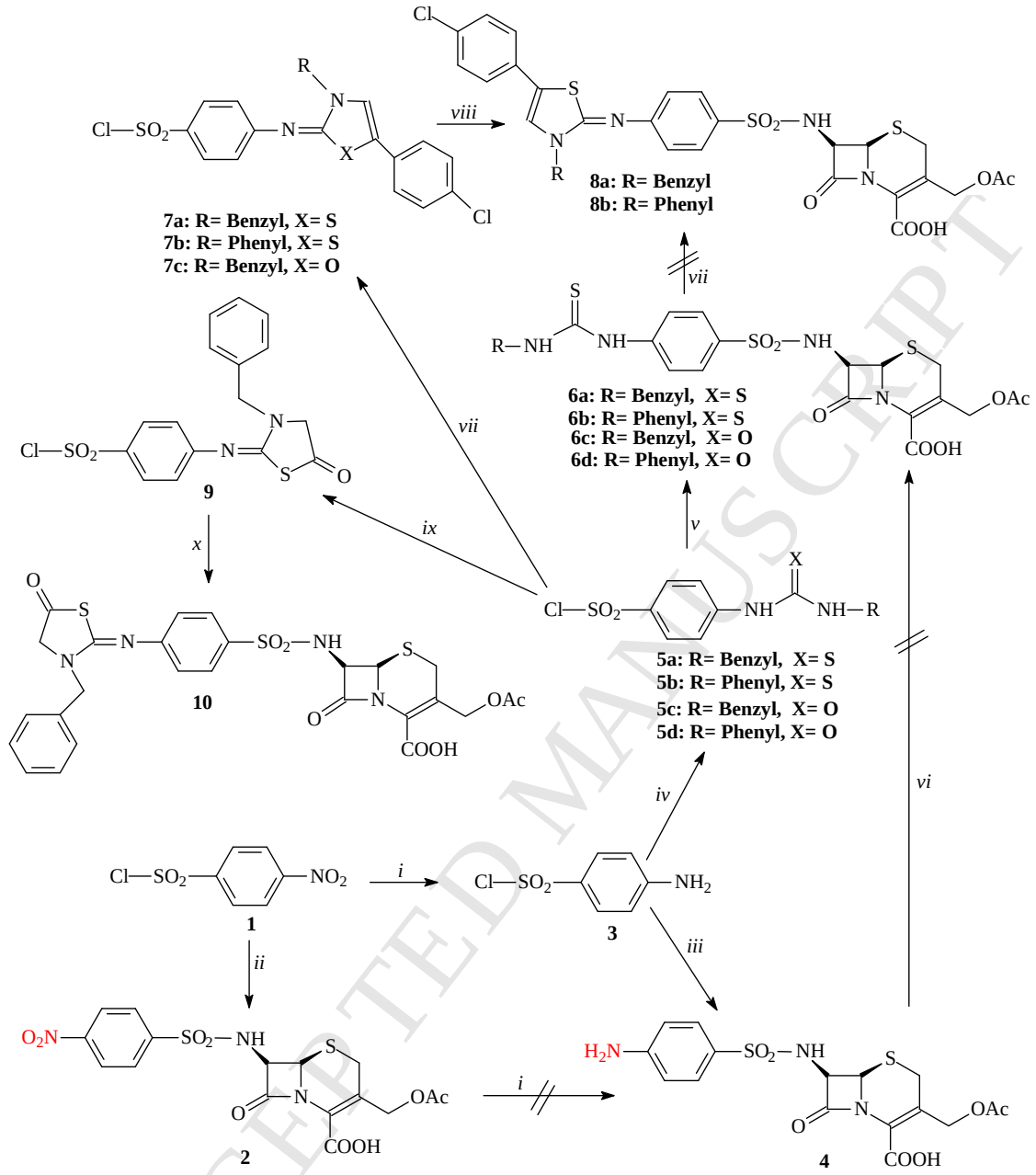
Table 2. Inhibitory activities and IC_{50} values of the synthesized compounds against Jack Bean urease[a].

Compound No	% Inbhibition		IC_{50} (µg/mL)
	250 µg/mL	100 µg/mL	
2	28%	-	-
4	1%	-	-
5b	21%	-	-
5c	23%	-	-
5d	100%	39.3%	120.69 ± 0.98
7b	3%	-	-
7c	21%	-	-
8b	15%	-	-
12	25%	-	-
13	-	-	-
Thiourea	100%	92.2%	51.62 ± 7.28

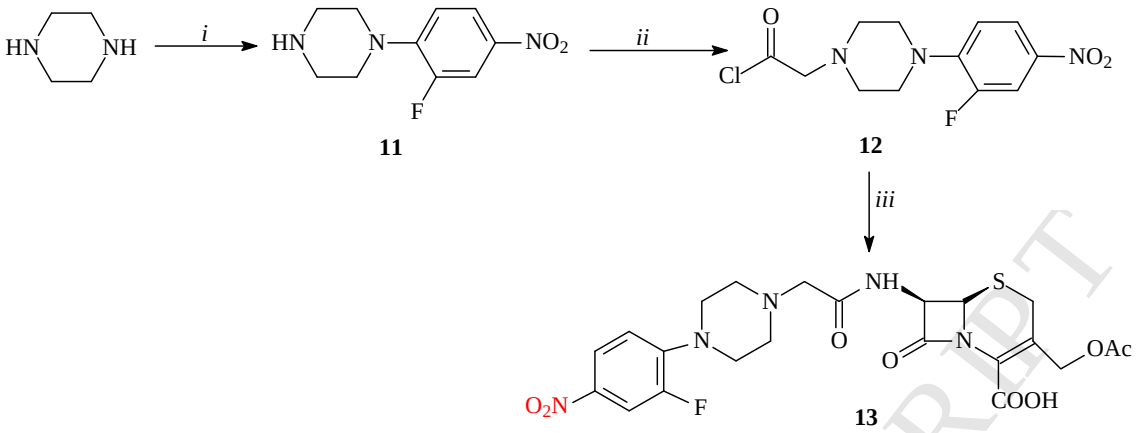
a: (-) not determined

Table 3. Inhibitory activities of the synthesized compounds against *B. cereus* β-lactamase. All compounds and $HgCl_2$ were assayed at final concentrations of 1 mM.

Compound	**% Inhibition**	**IC_{50} (mM)**
2	18%	nd
8a	64%	0.543
13	24%	1.166
$HgCl_2$	76%	0.093



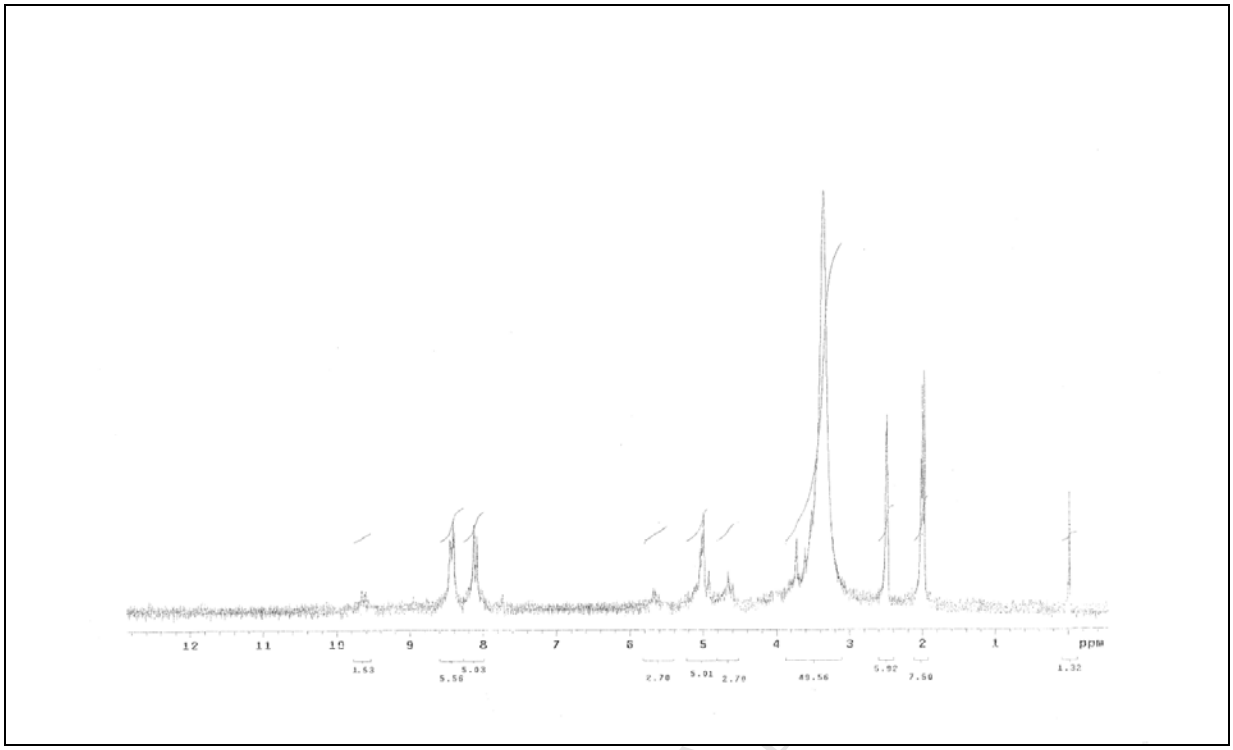
Scheme 1.



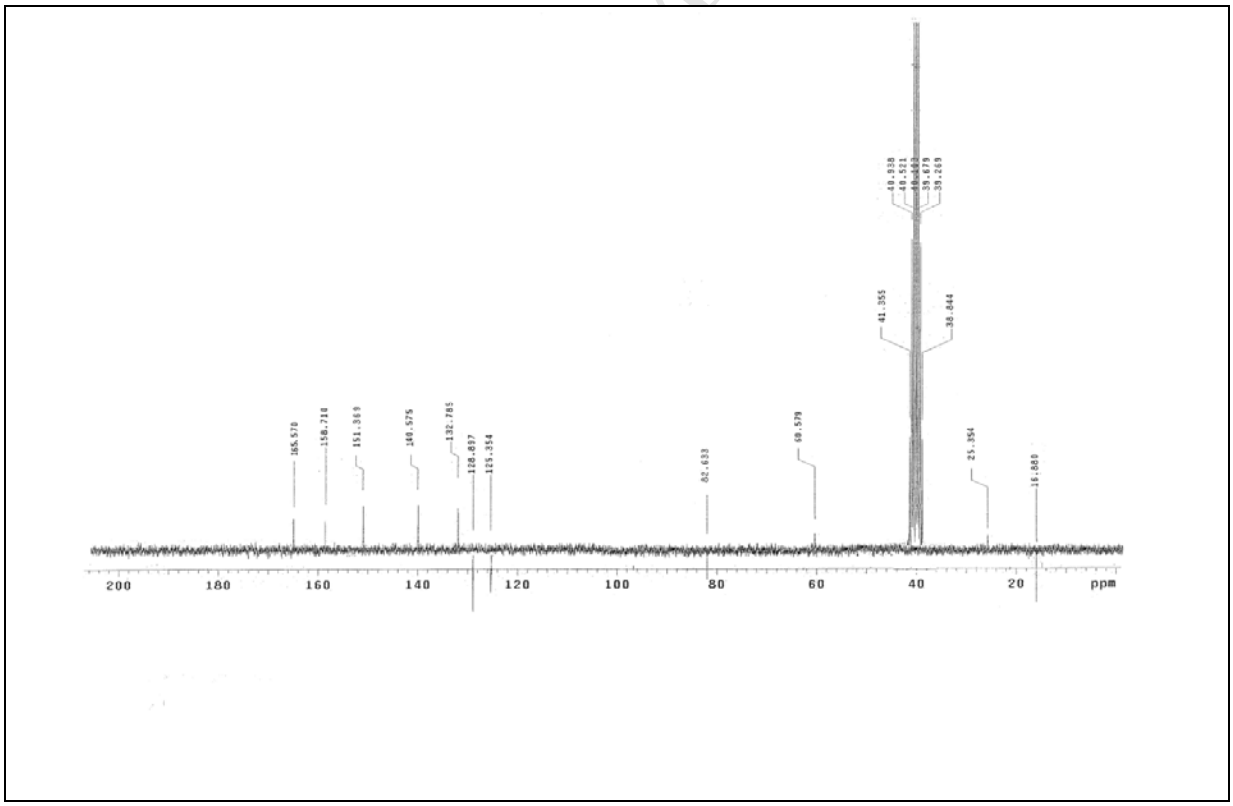
Scheme 2.

Highlights

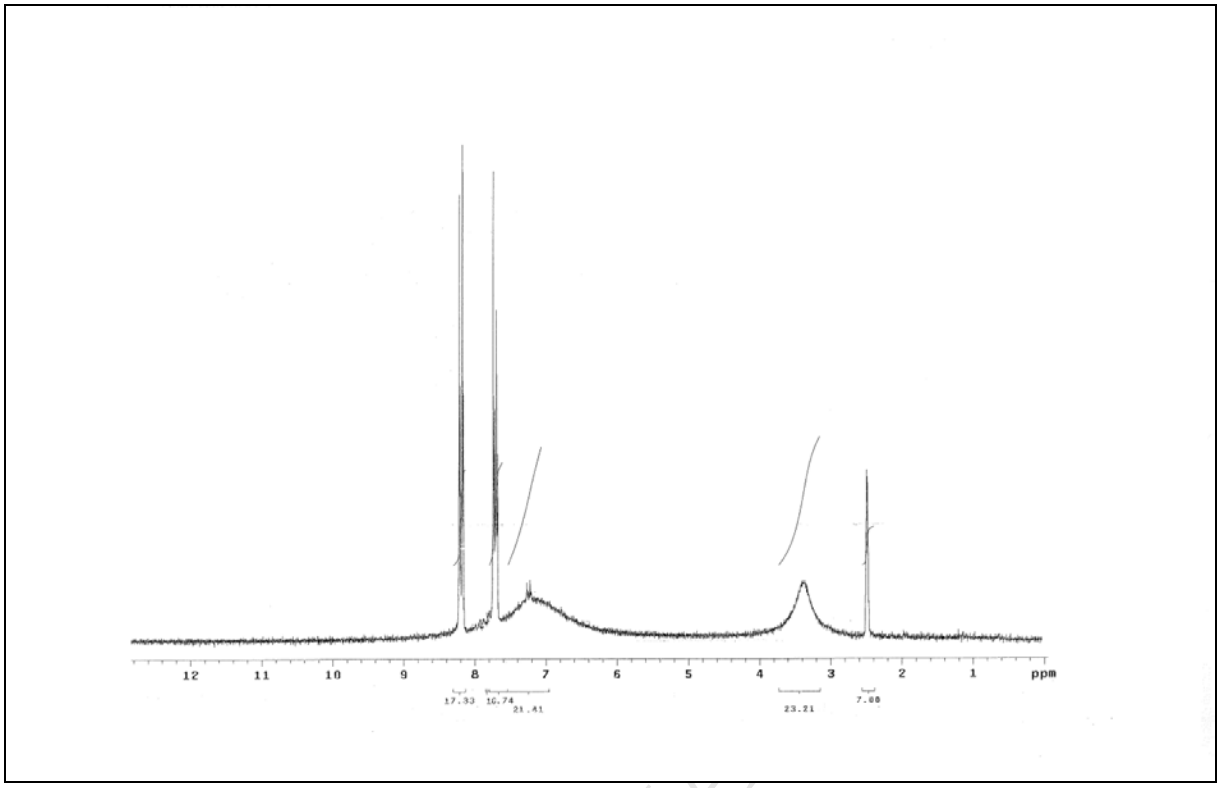
- Cephalosporanic acid derivatives including 1,3-thiazol(idinon)e or piperazine moiety have been synthesized.
- Most of the synthesized compounds display antimicrobial activity.
- One compound have been found to possess antiurease activity.



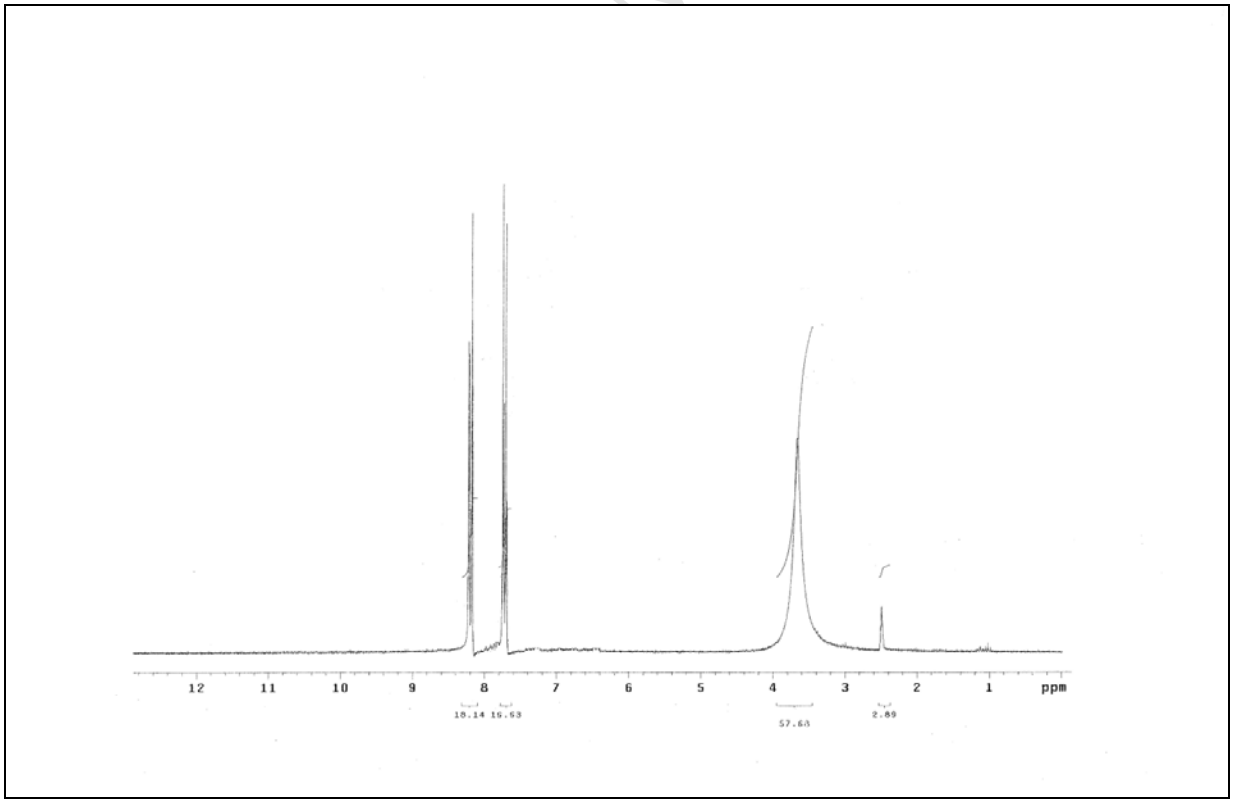
^{1}H-NMR Spectrum of compound 2



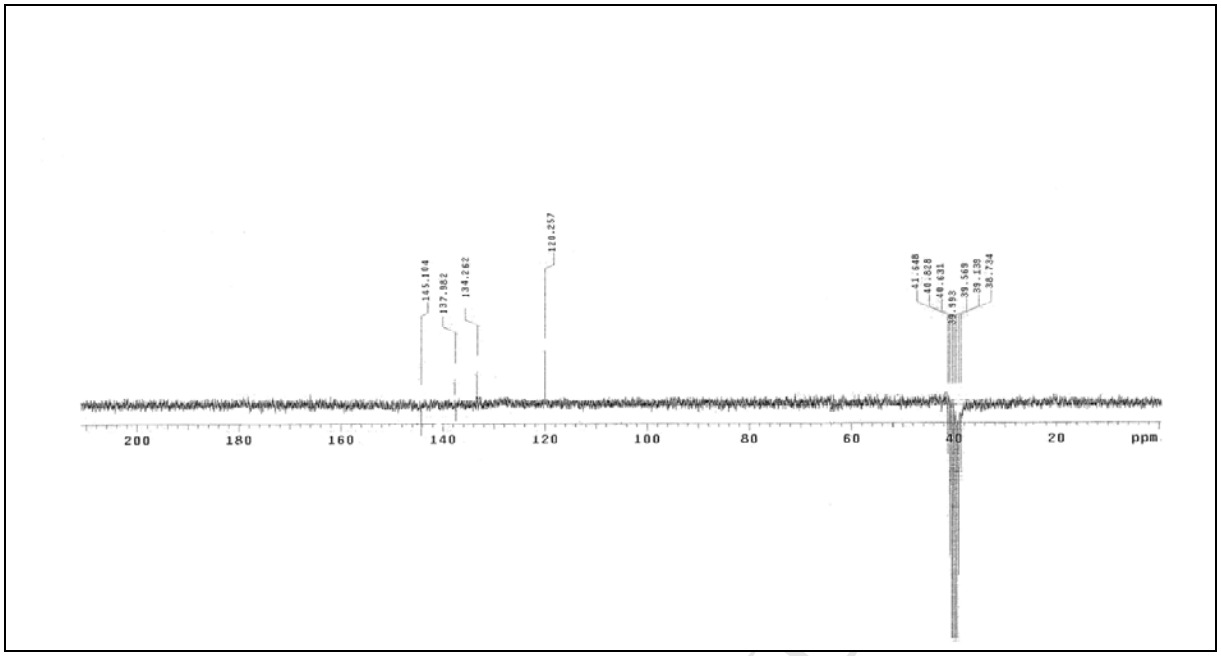
APT Spectrum of compound 2



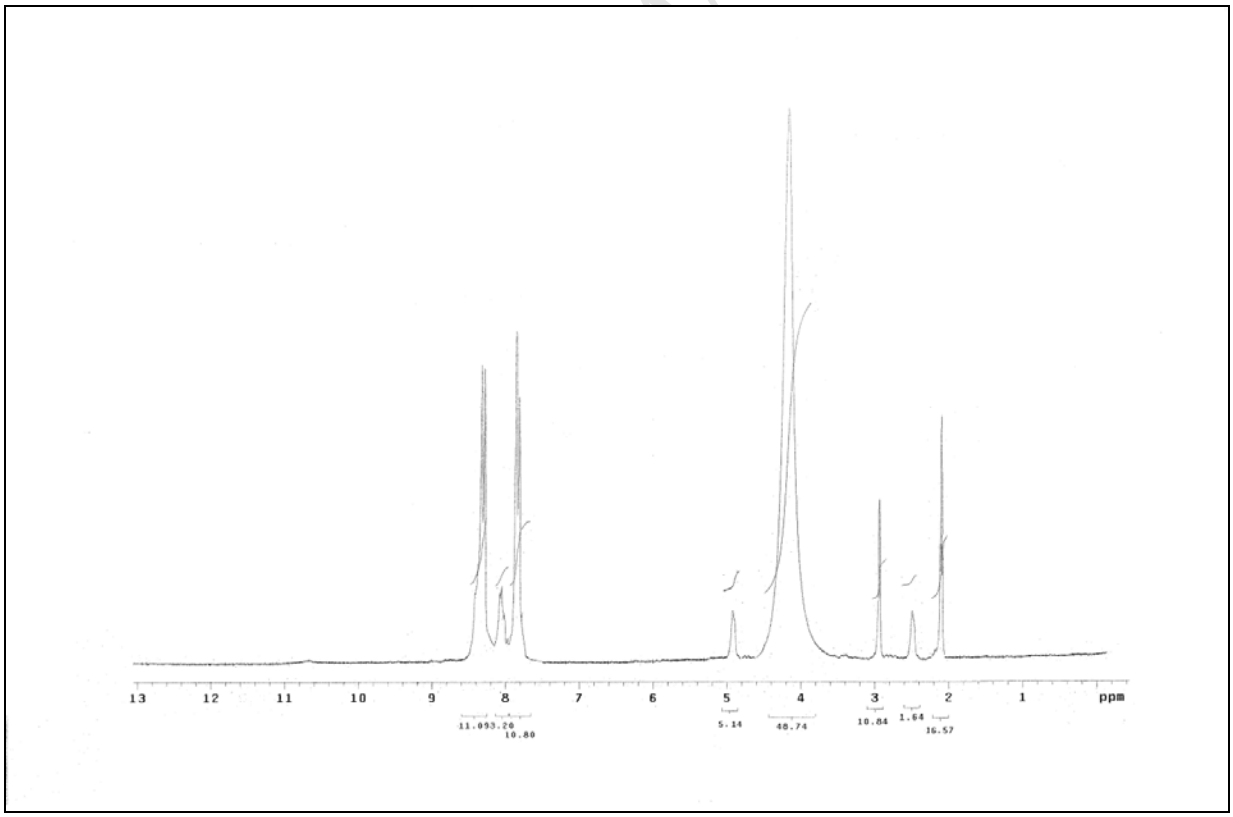
^{1}H-NMR Spectrum of compound 3



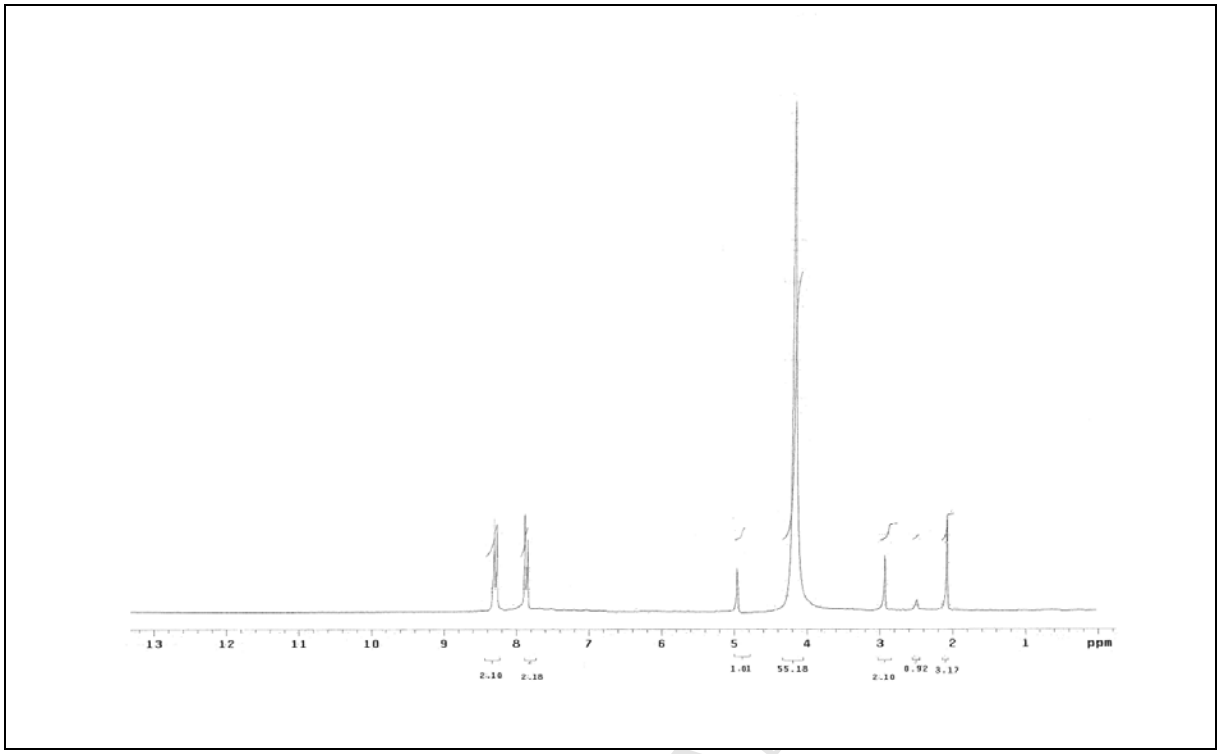
D_2O Exchange spectrum of compound 3



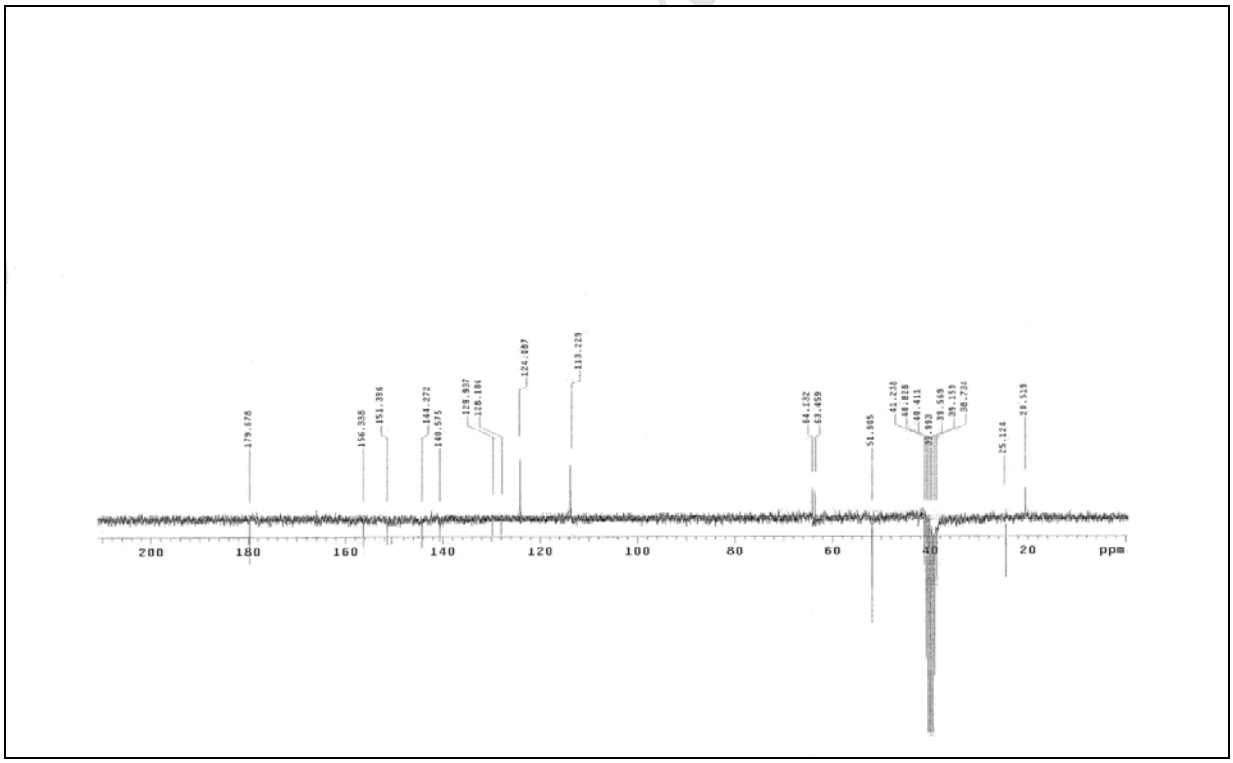
APT Spectrum of compound 3



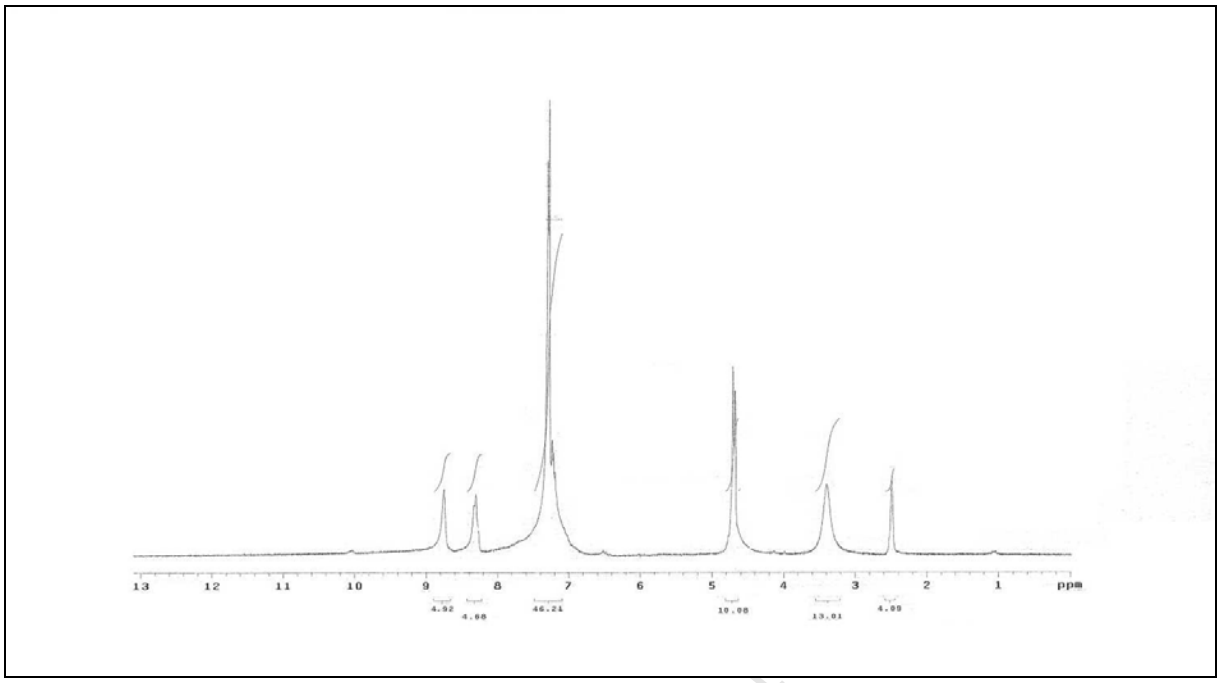
^{1}H-NMR Spectrum of compound 4



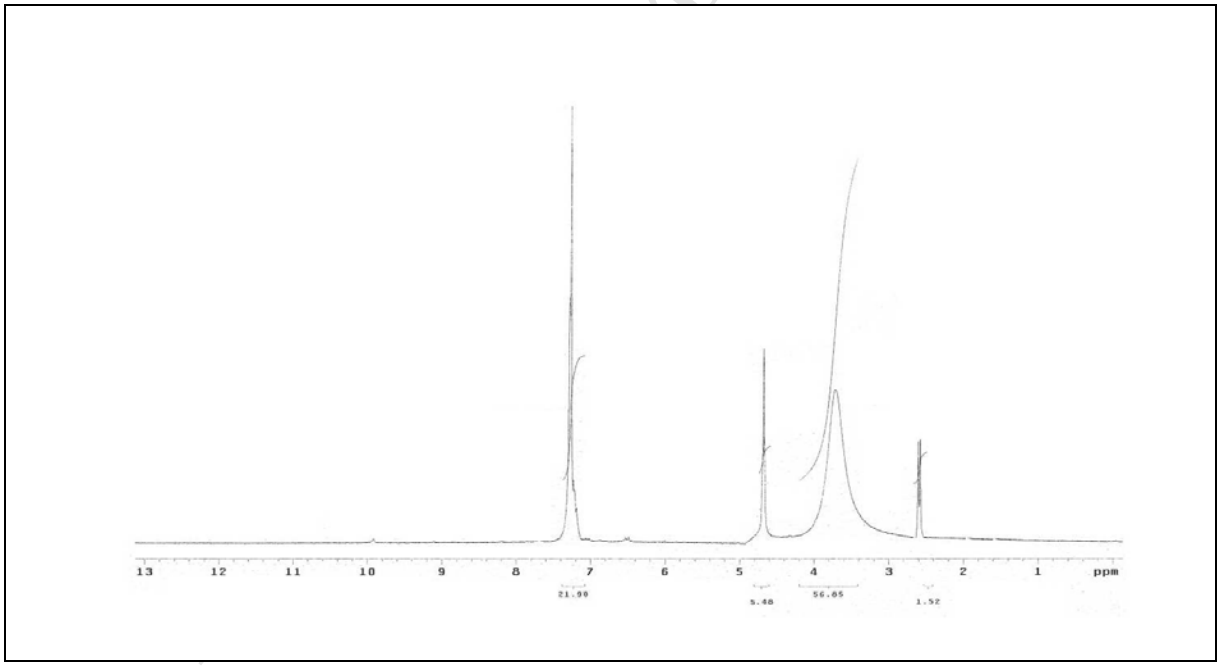
D_2O exchange spectrum of compound 4



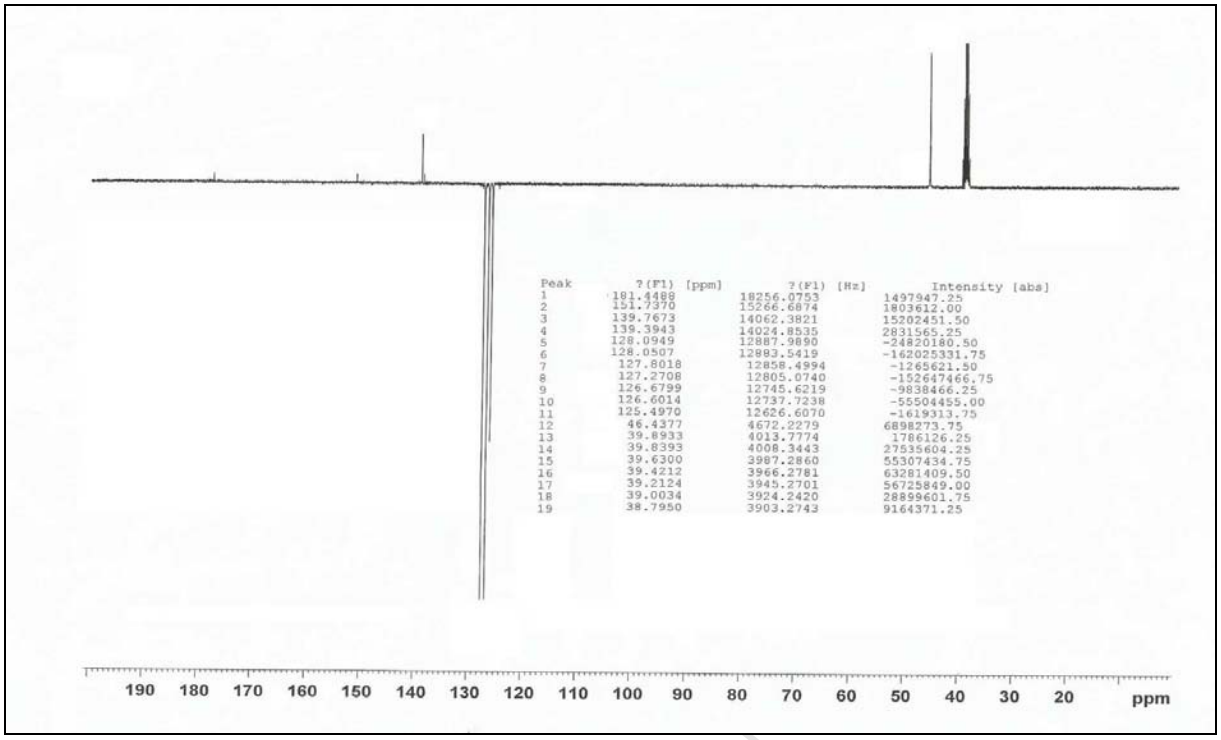
APT Spectrum of compound 4



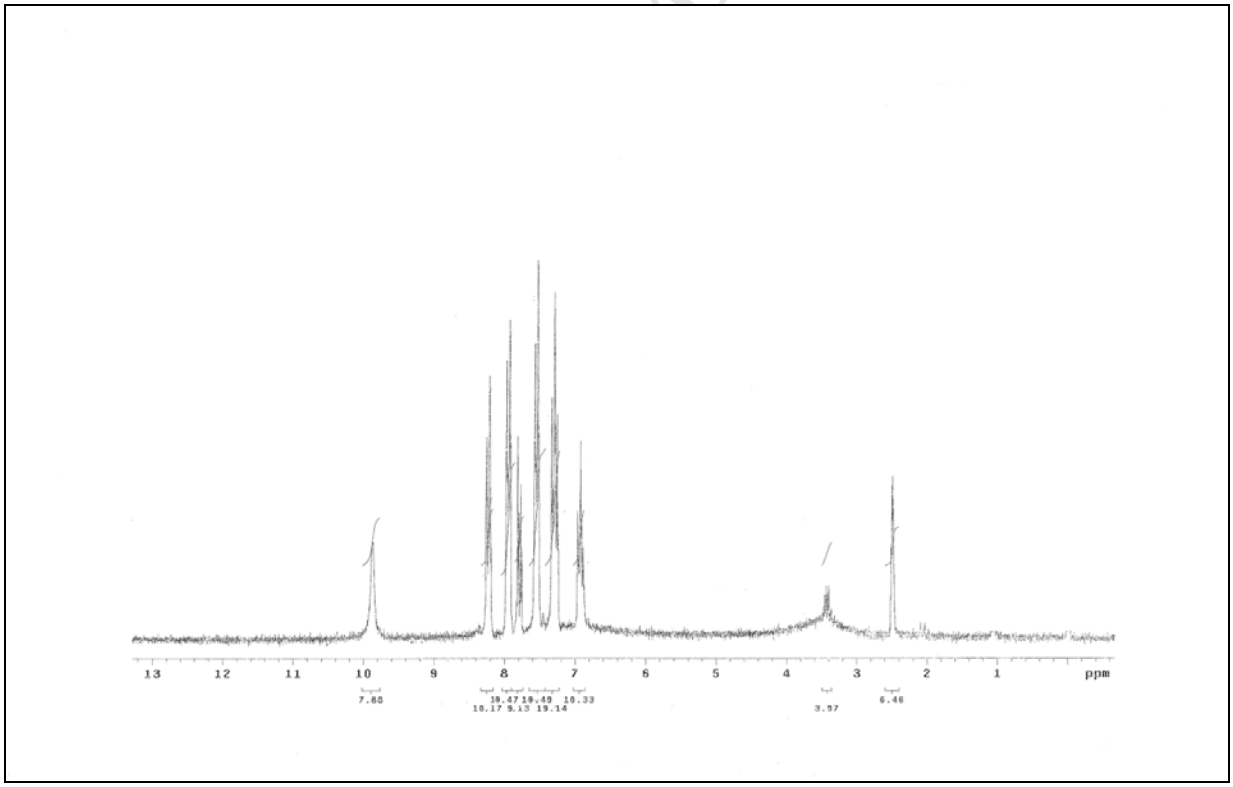
^{1}H-NMR Spectrum of compound 5a



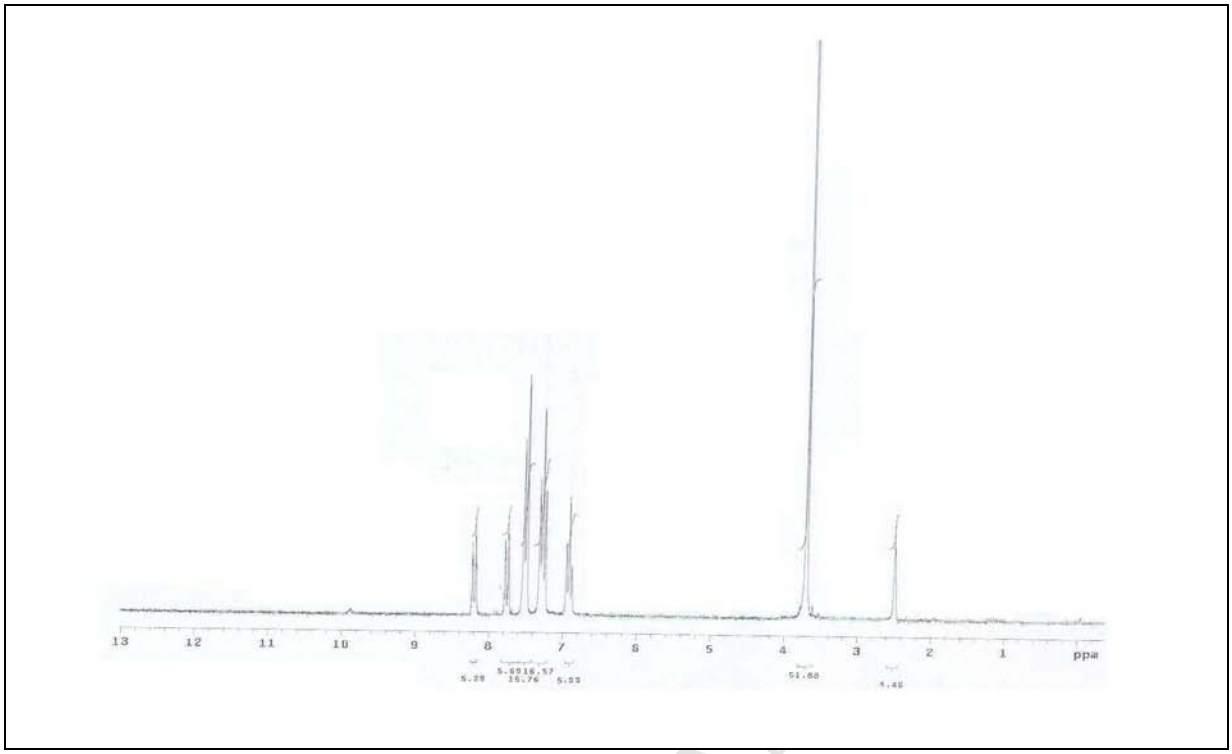
D_2O exchange spectrum of compound 5a



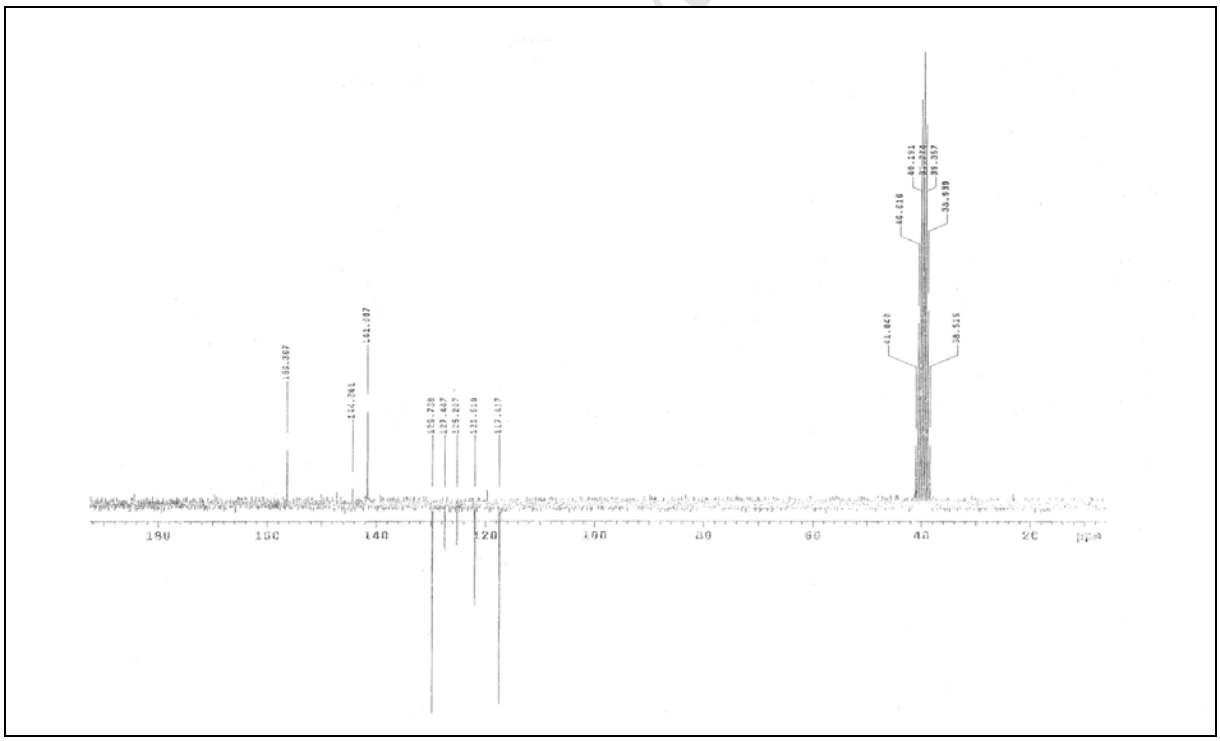
APT spectrum of compound 5a



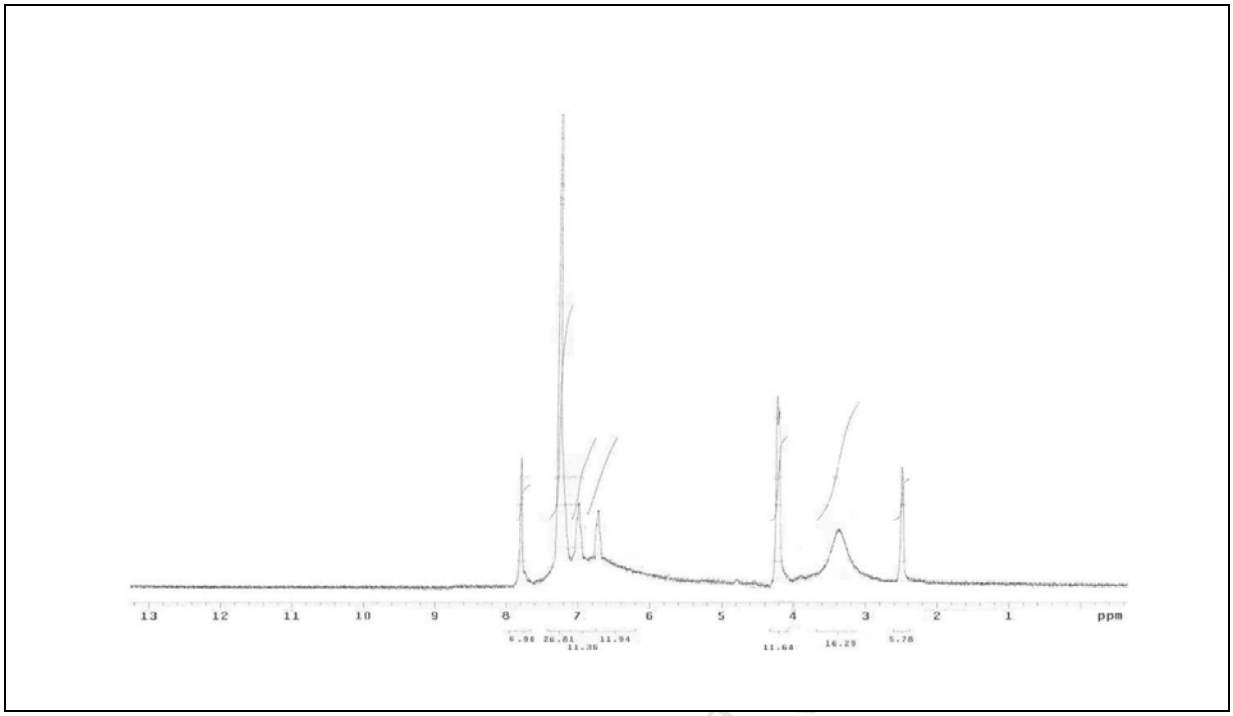
^{1}H-NMR Spectrum of compound 5b



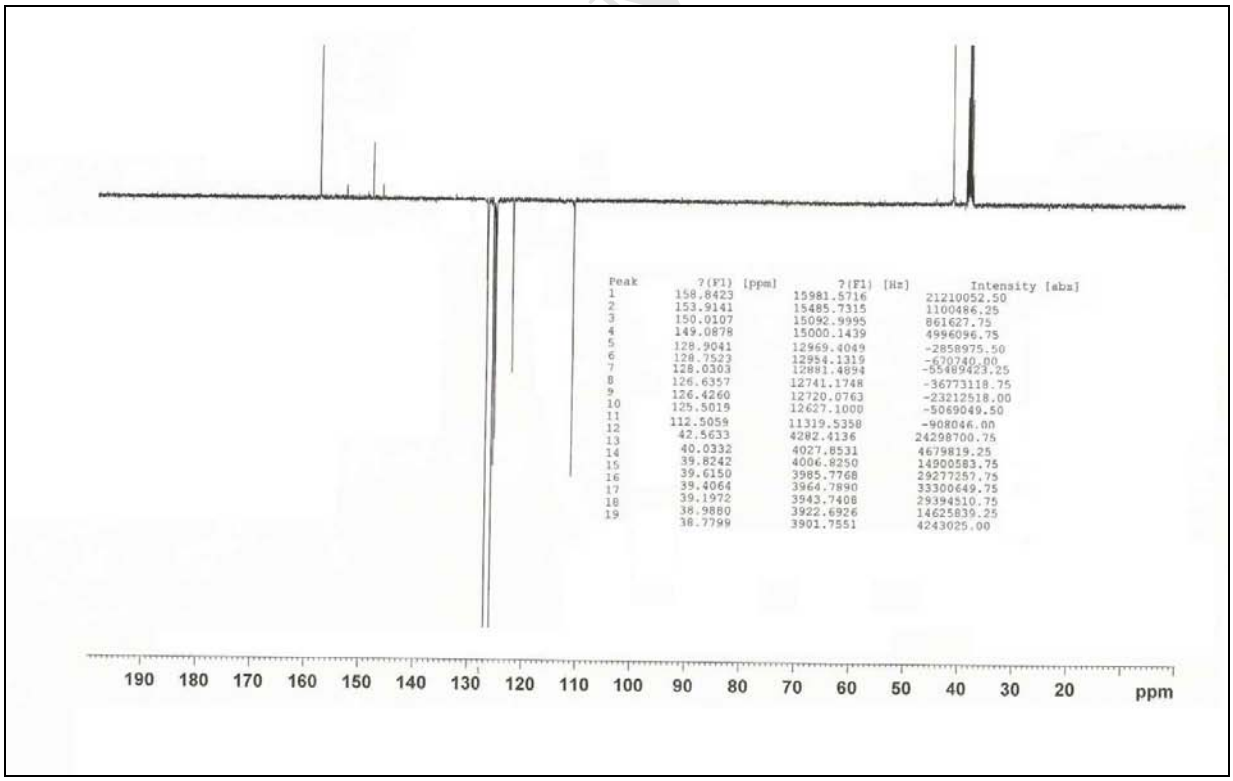
D_2O exchange spectrum of compound 5b



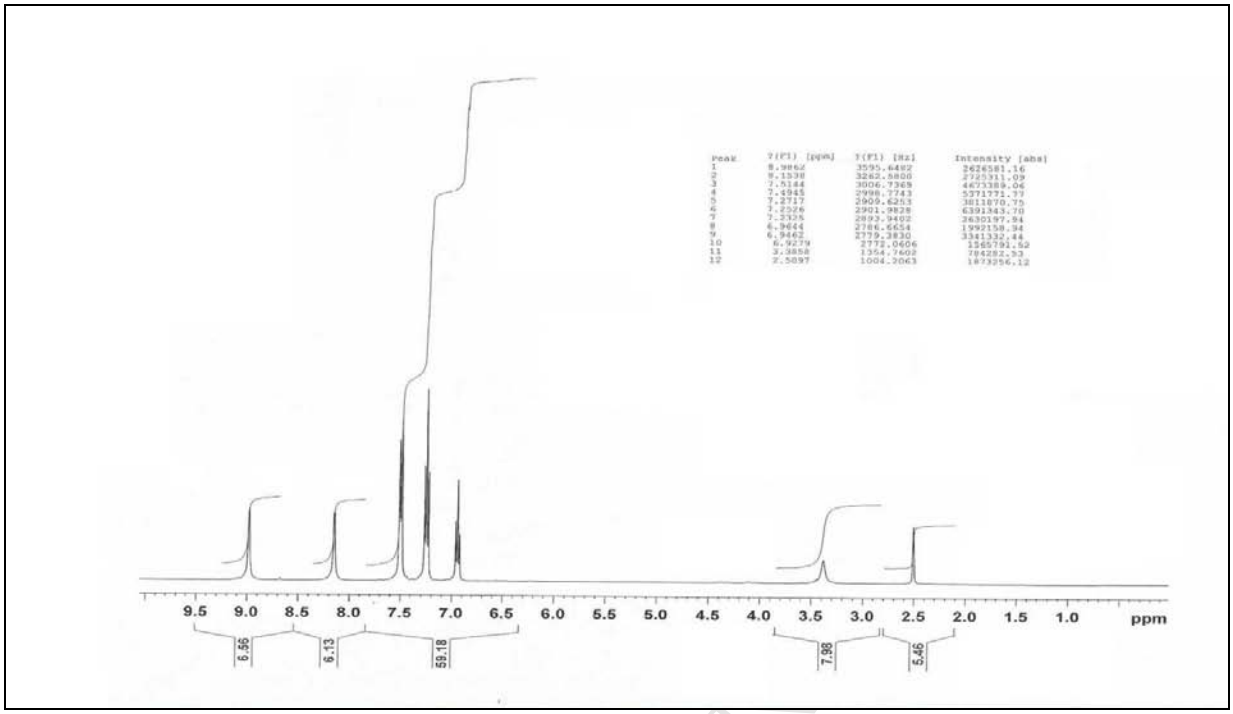
APT spectrum of compound 5b



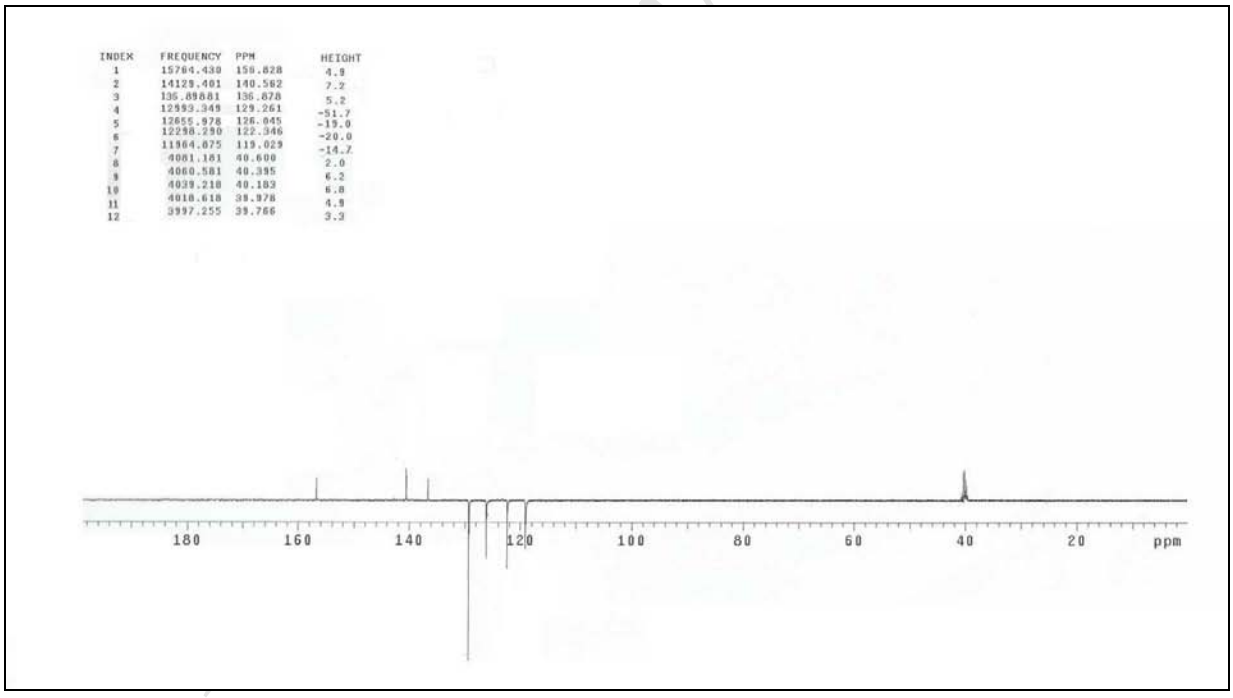
^{1}H-NMR Spectrum of compound 5c



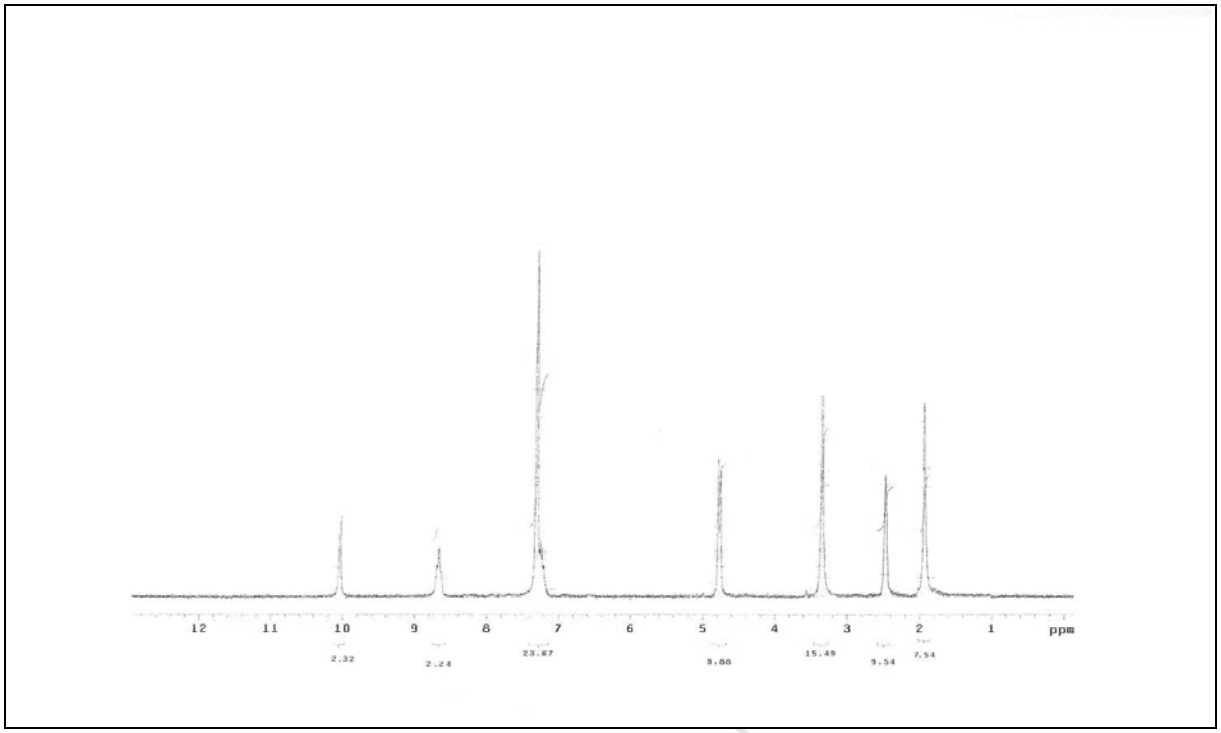
APT spectrum of compound 5c



^{1}H-NMR Spectrum of compound 5d

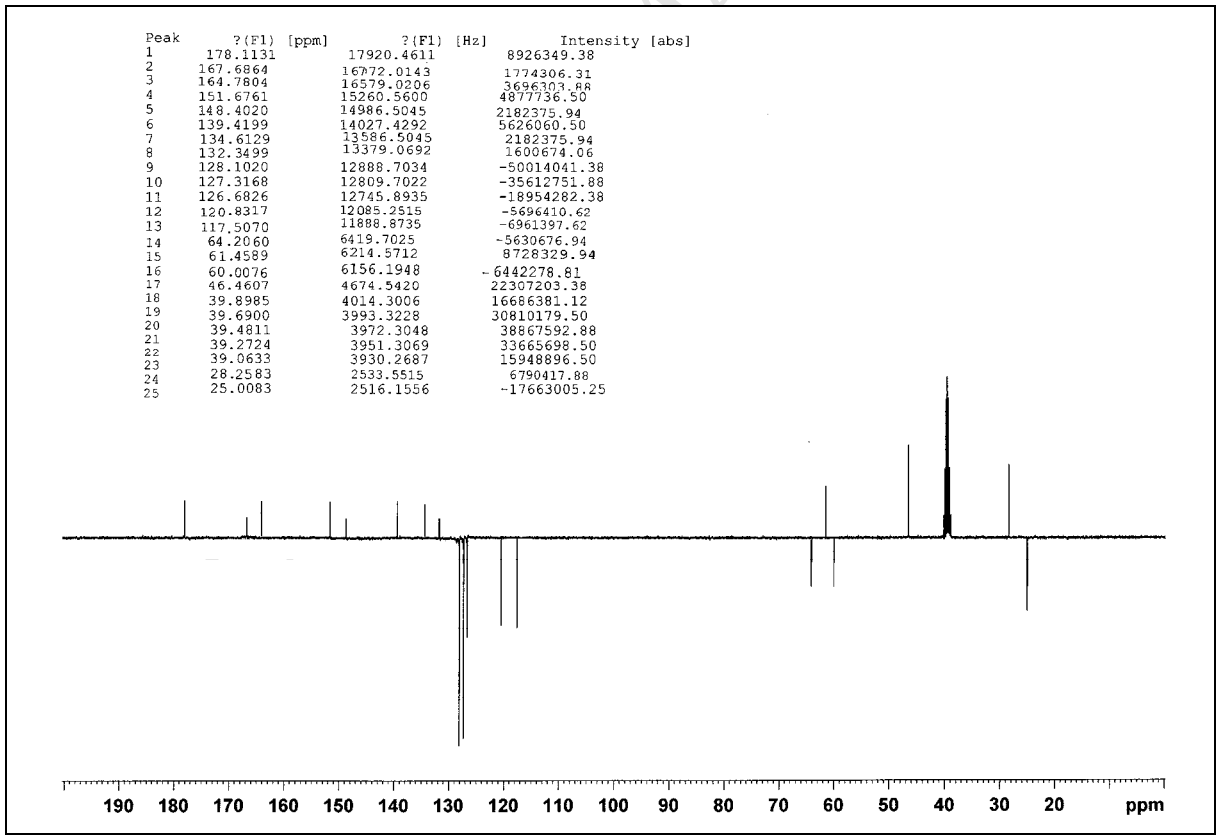


APT spectrum of compound 5d

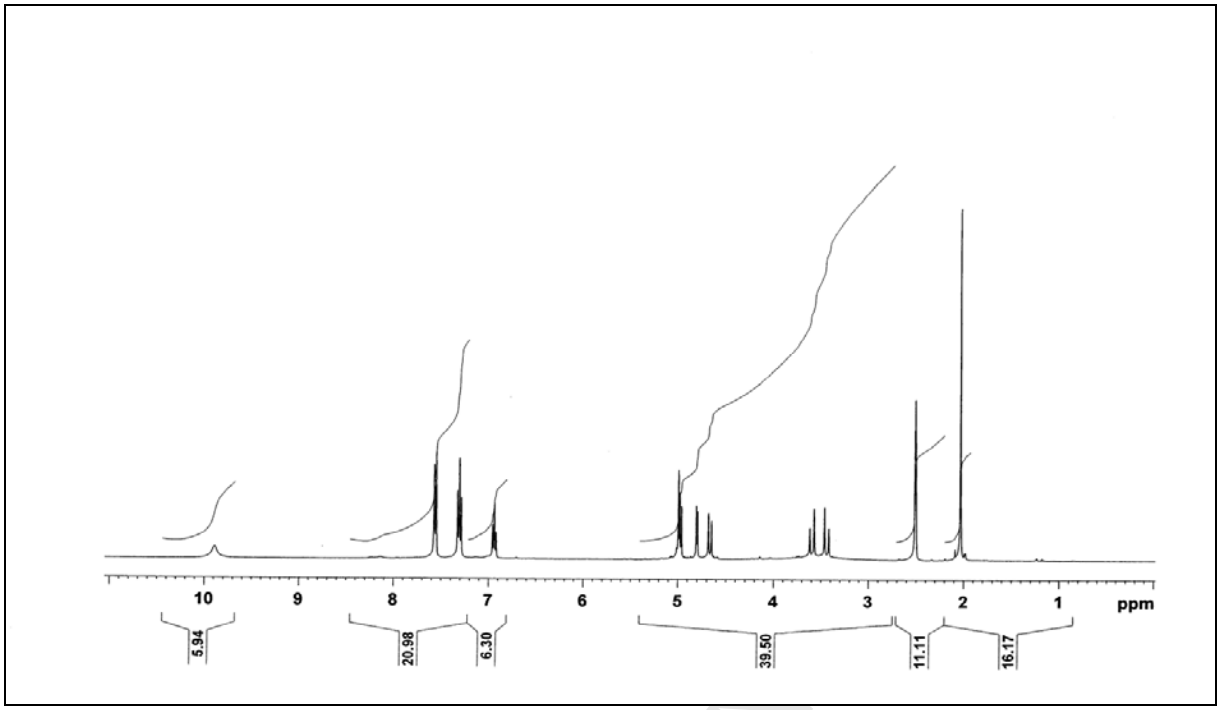


^{1}H-NMR Spectrum of compound 6a

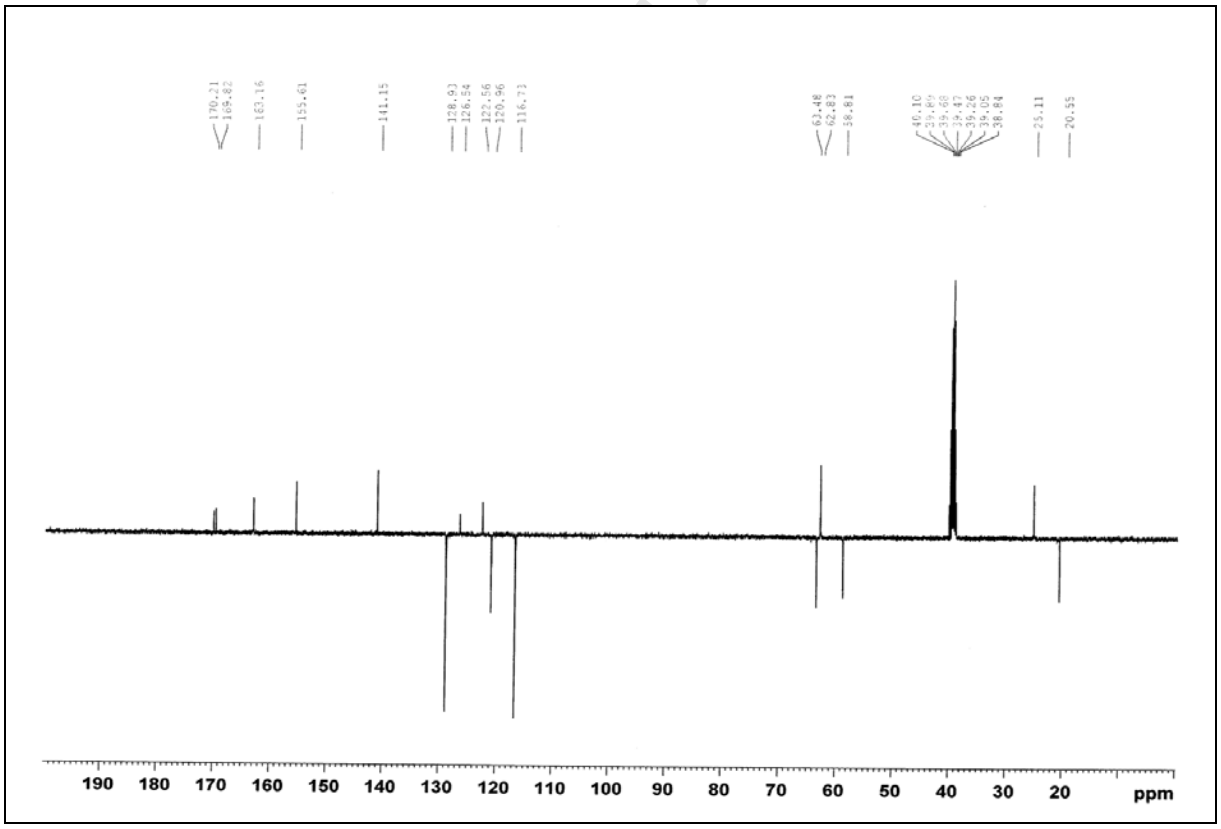
Peak	?(F1) [ppm]	?(F1) [Hz]	Intensity [abs]
1	178.1131	17920.4611	8926349.38
2	167.6864	16772.0143	1774306.31
3	164.7804	16579.0206	3696303.88
4	151.6761	15260.5600	4877736.50
5	148.4020	14986.5045	2182375.94
6	139.4199	14027.4292	5626060.50
7	134.6129	13586.5045	2182375.94
8	132.3499	13379.0692	1600674.06
9	128.1020	12888.7034	-50014041.38
10	127.3168	12809.7022	-35612751.88
11	126.6826	12745.8935	-18954282.38
12	120.8317	12085.2515	-5696410.62
13	117.5070	11888.8735	-6961397.62
14	64.2060	6419.7025	-5630676.94
15	61.4589	6214.5712	8728329.94
16	60.0076	6156.1948	-6442278.81
17	46.4607	4674.5420	22307203.38
18	39.8985	4014.3006	16686381.12
19	39.6900	3993.3228	30810179.50
20	39.4811	3972.3048	38867592.88
21	39.2724	3951.3069	33665698.50
22	39.0633	3930.2687	15948896.50
23	28.2583	2533.5515	6790417.88
24	25.0083	2516.1556	-17663005.25
25			



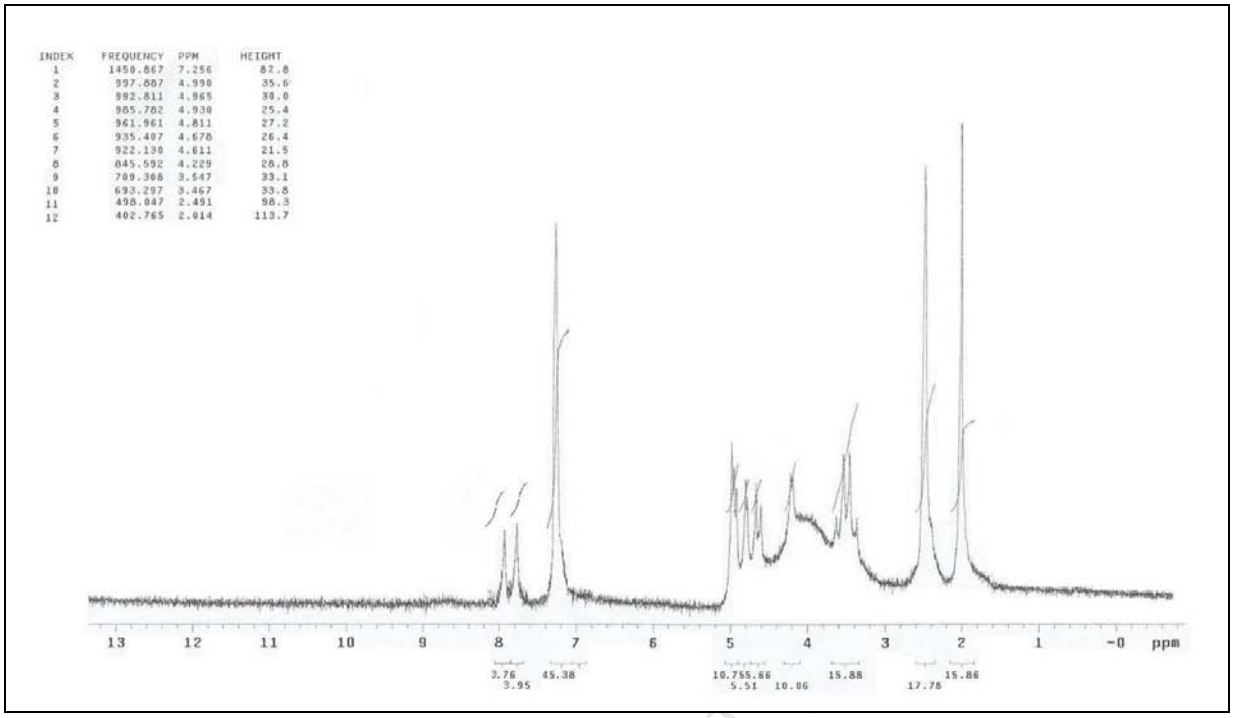
APT spectrum of compound 6a



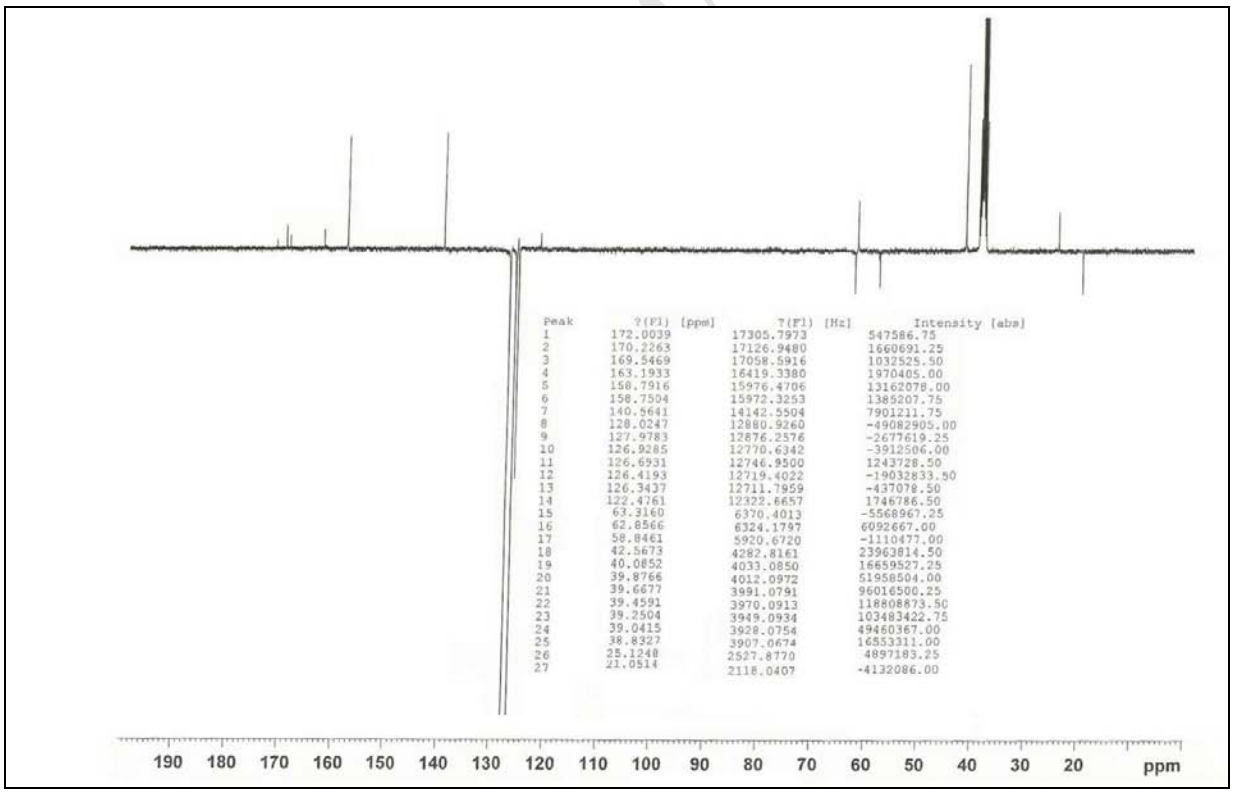
^{1}H-NMR Spectrum of compound 6b



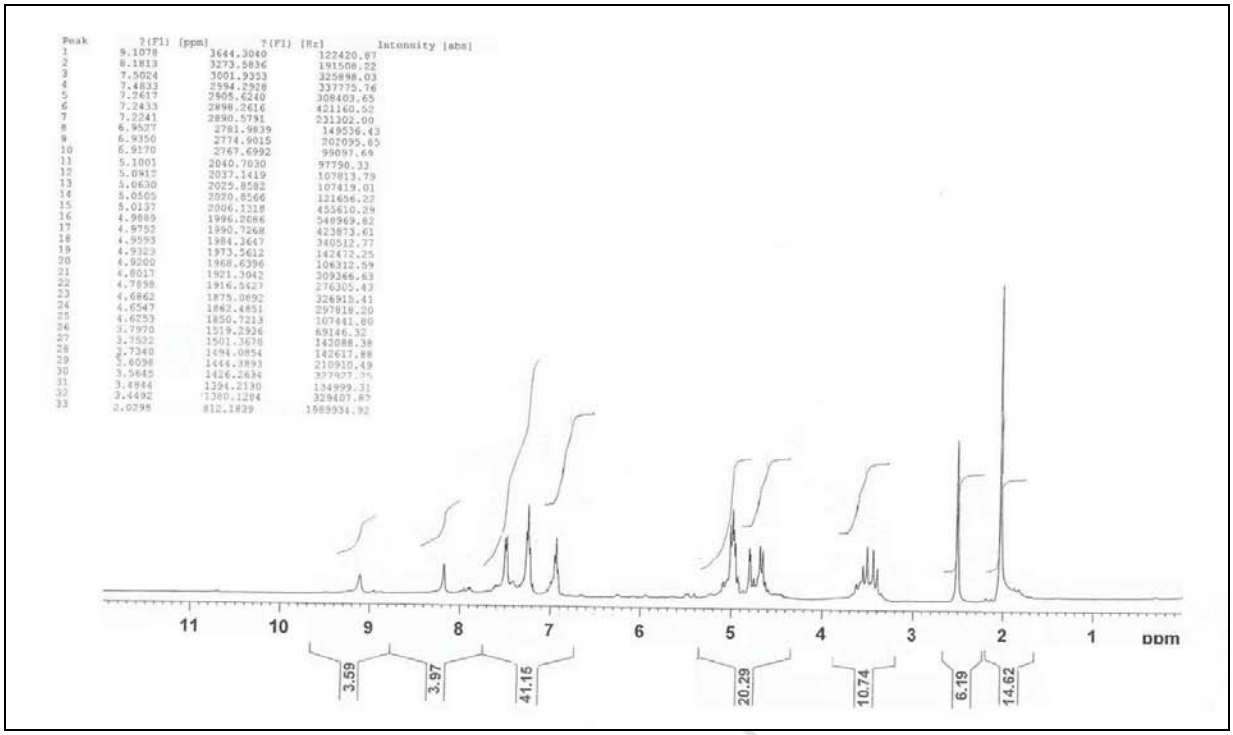
APT spectrum of compound 6b



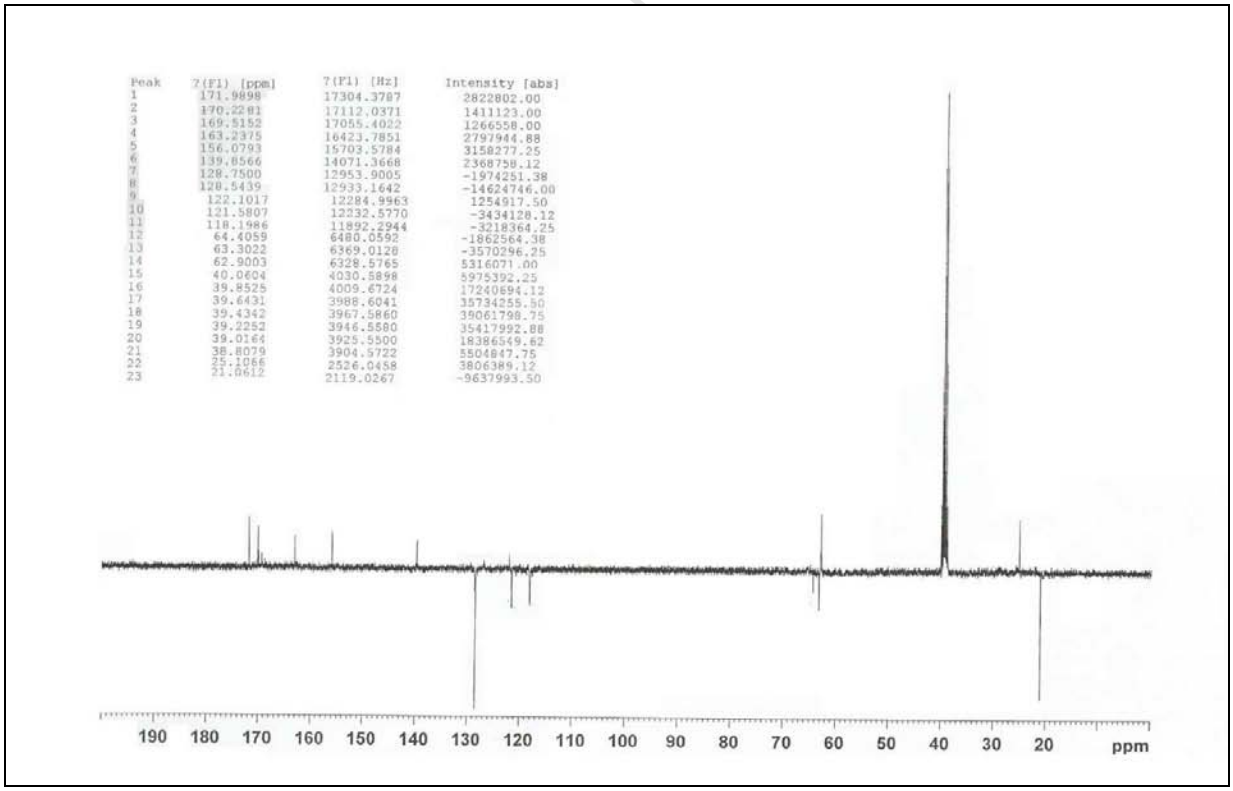
^{1}H-NMR Spectrum of compound 6c



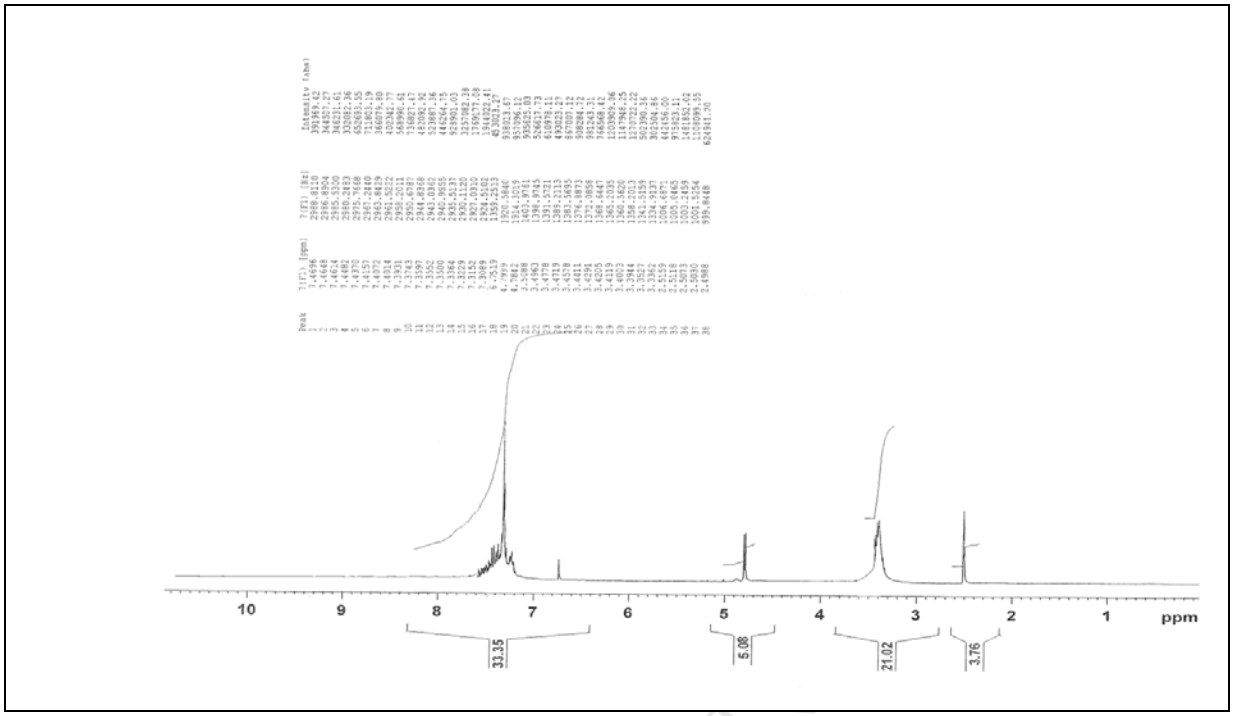
APT spectrum of compound 6c



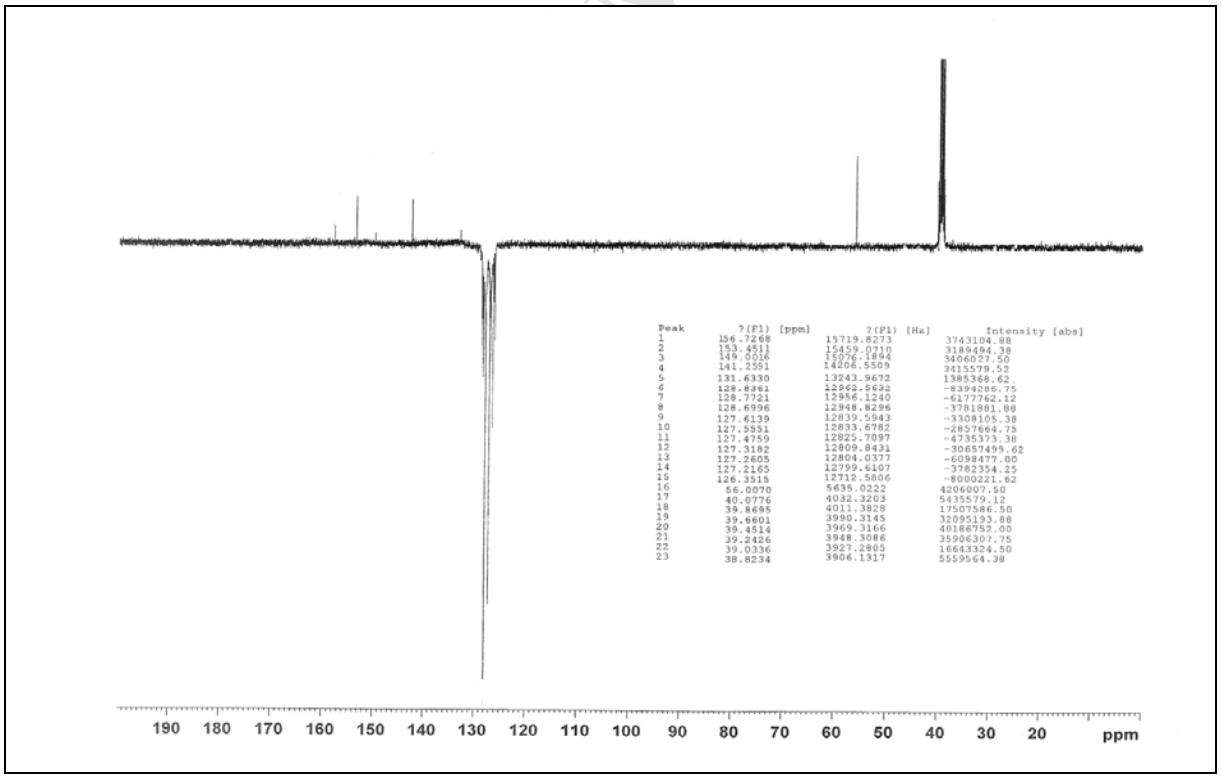
^{1}H-NMR Spectrum of compound 6d



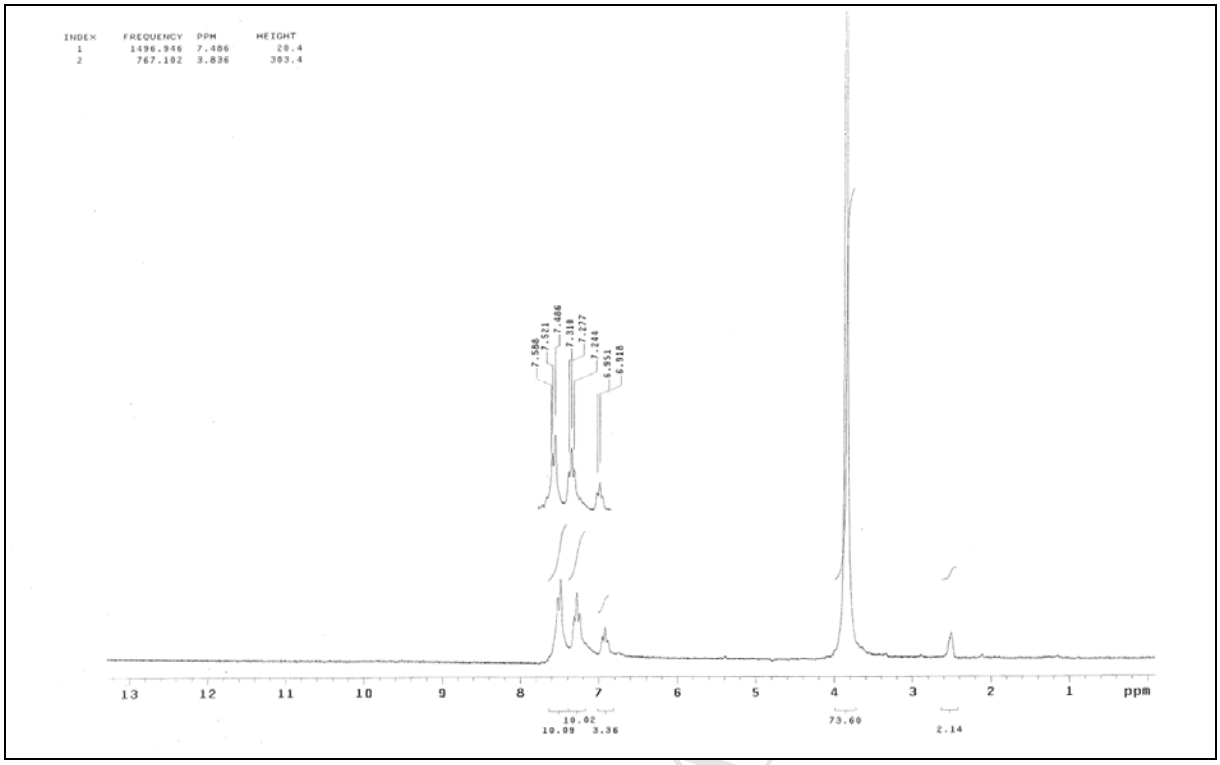
APT spectrum of compound 6d



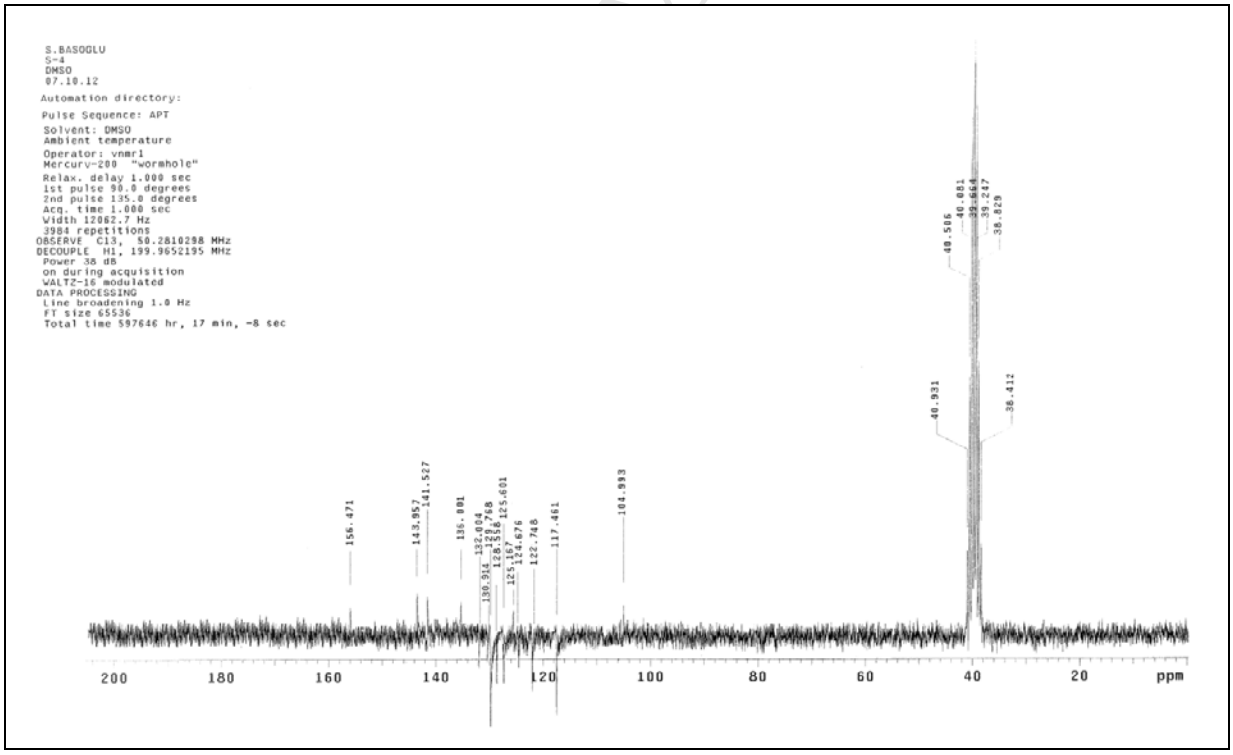
^{1}H-NMR Spectrum of compound 7a



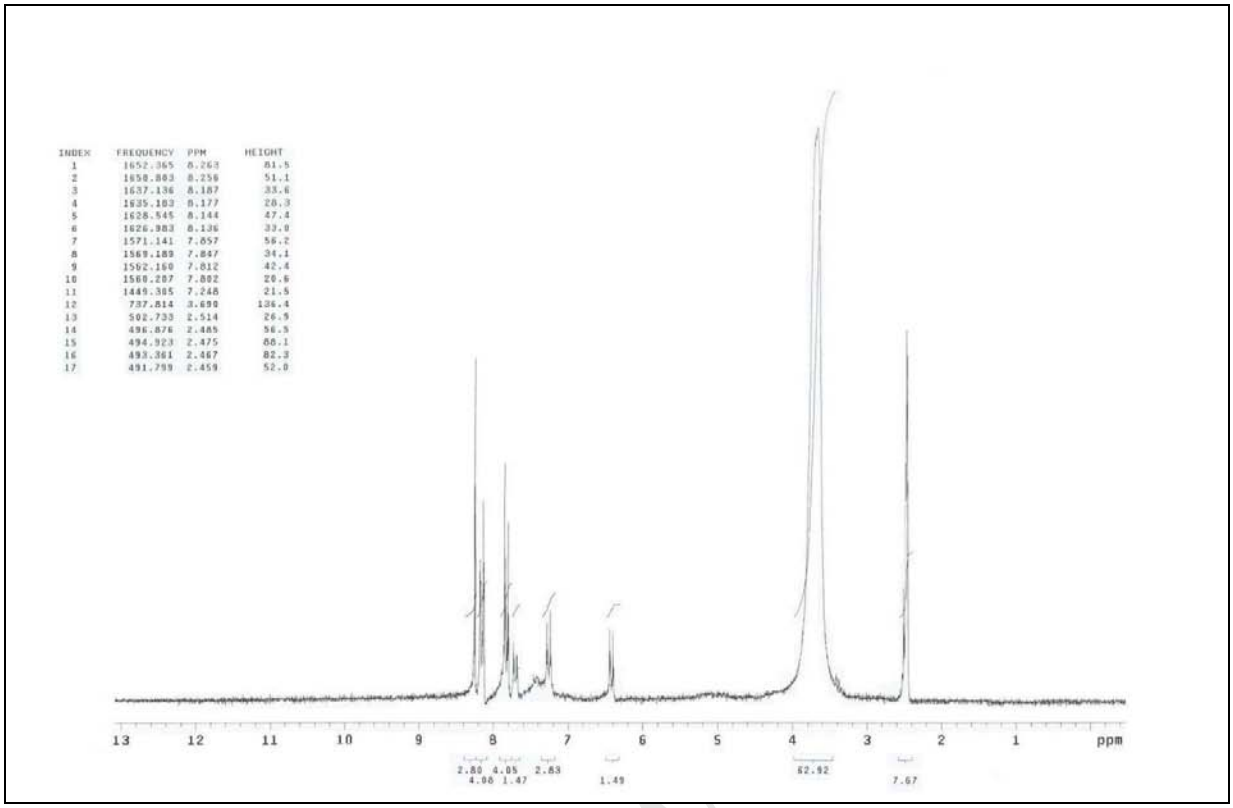
APT spectrum of compound 7a



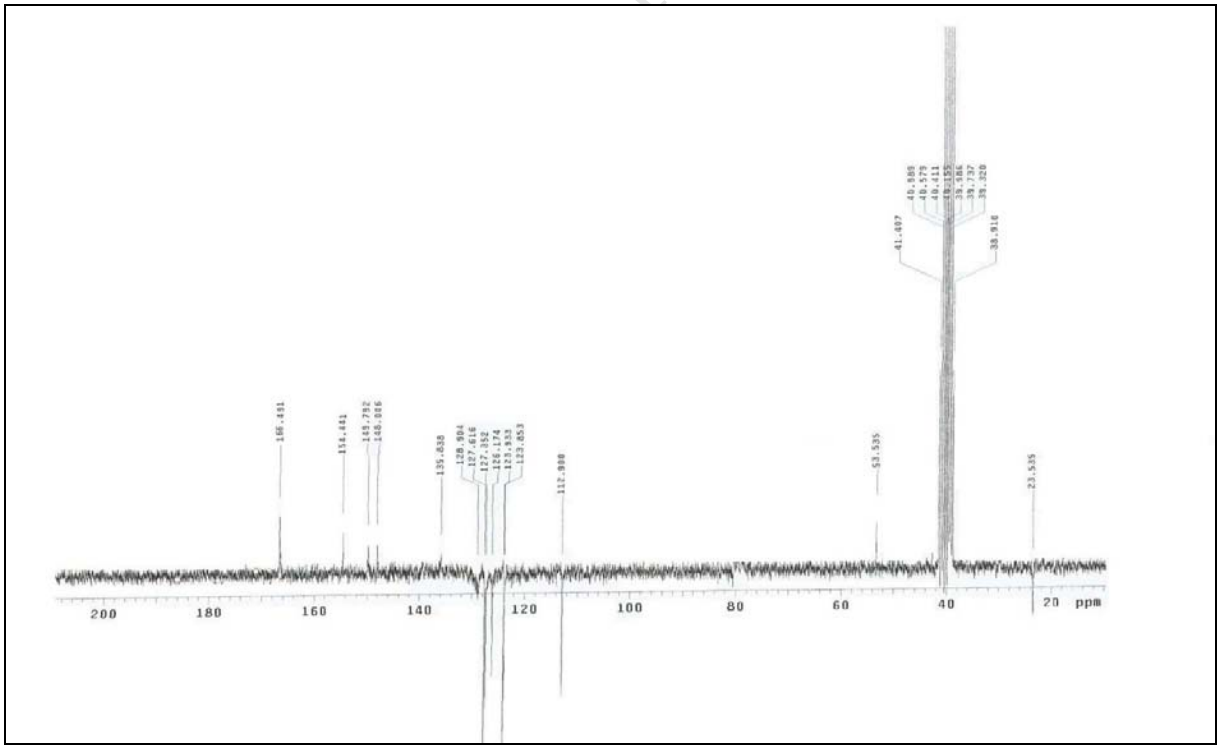
^{1}H-NMR Spectrum of compound 7b



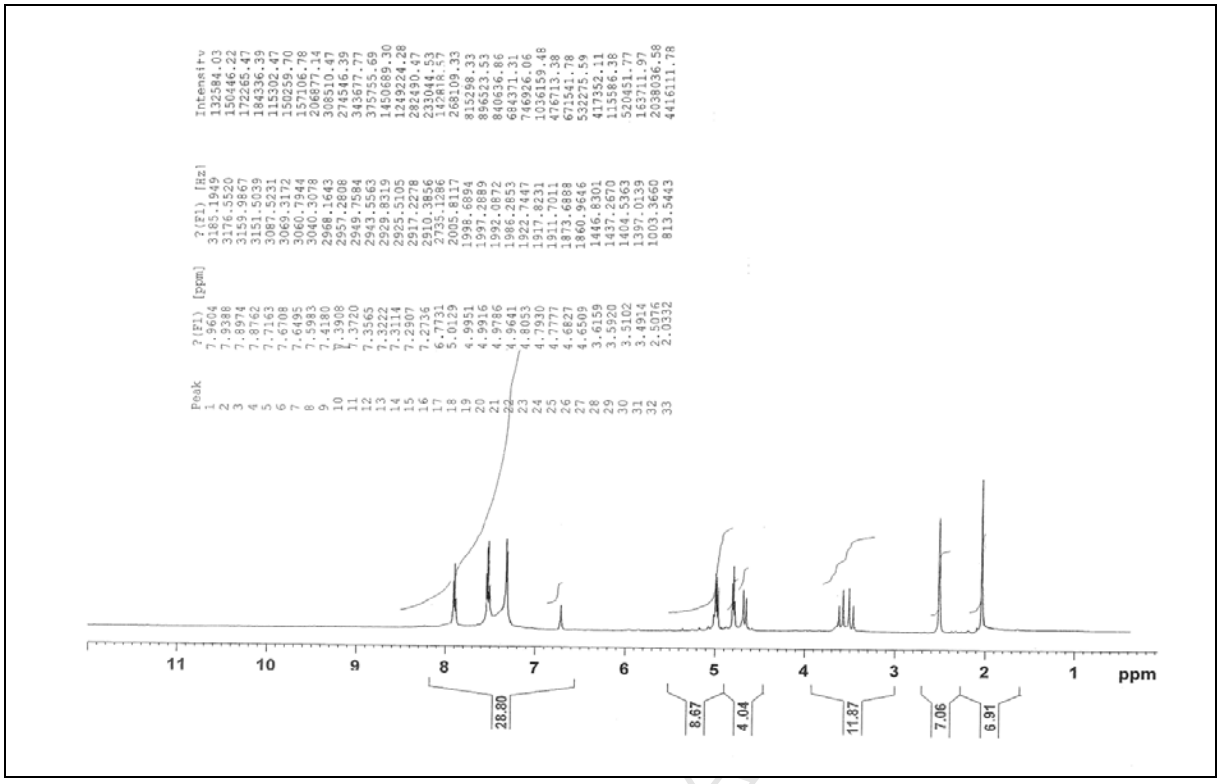
APT spectrum of compound 7b



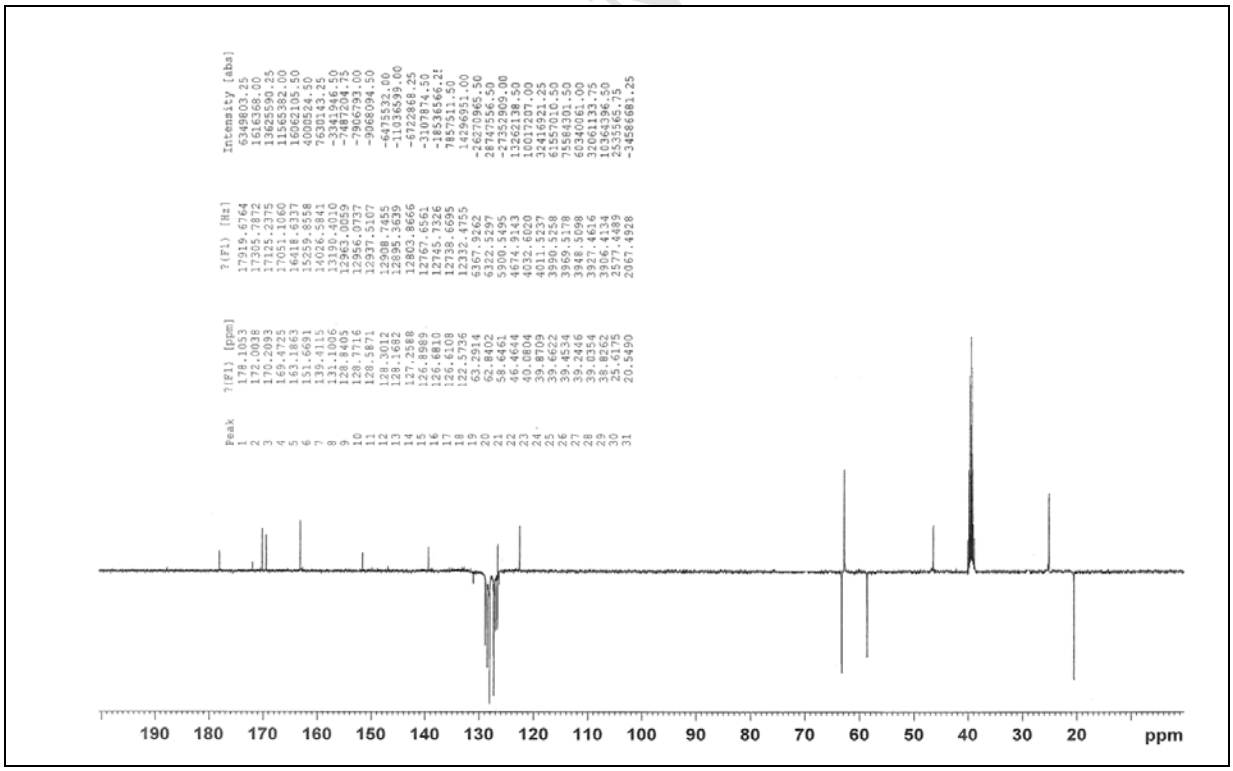
^{1}H-NMR Spectrum of compound 7c



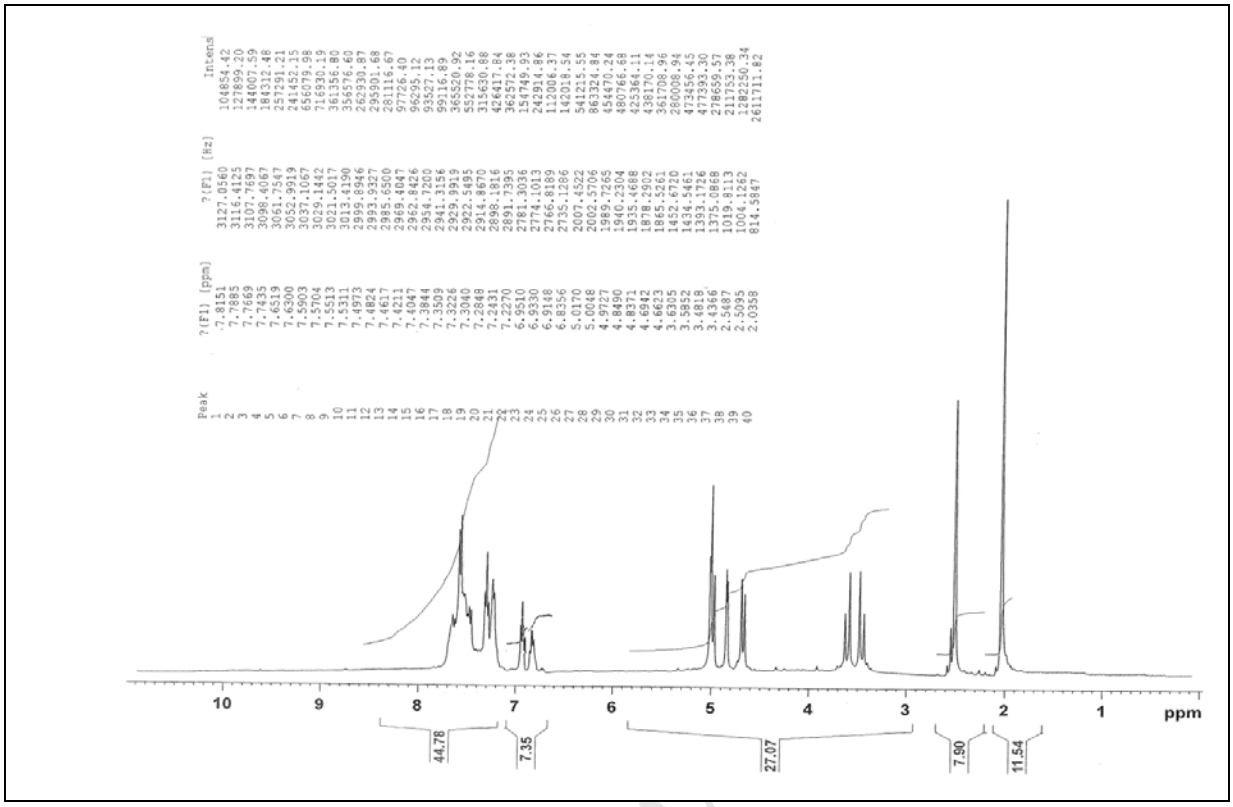
APT spectrum of compound 7c



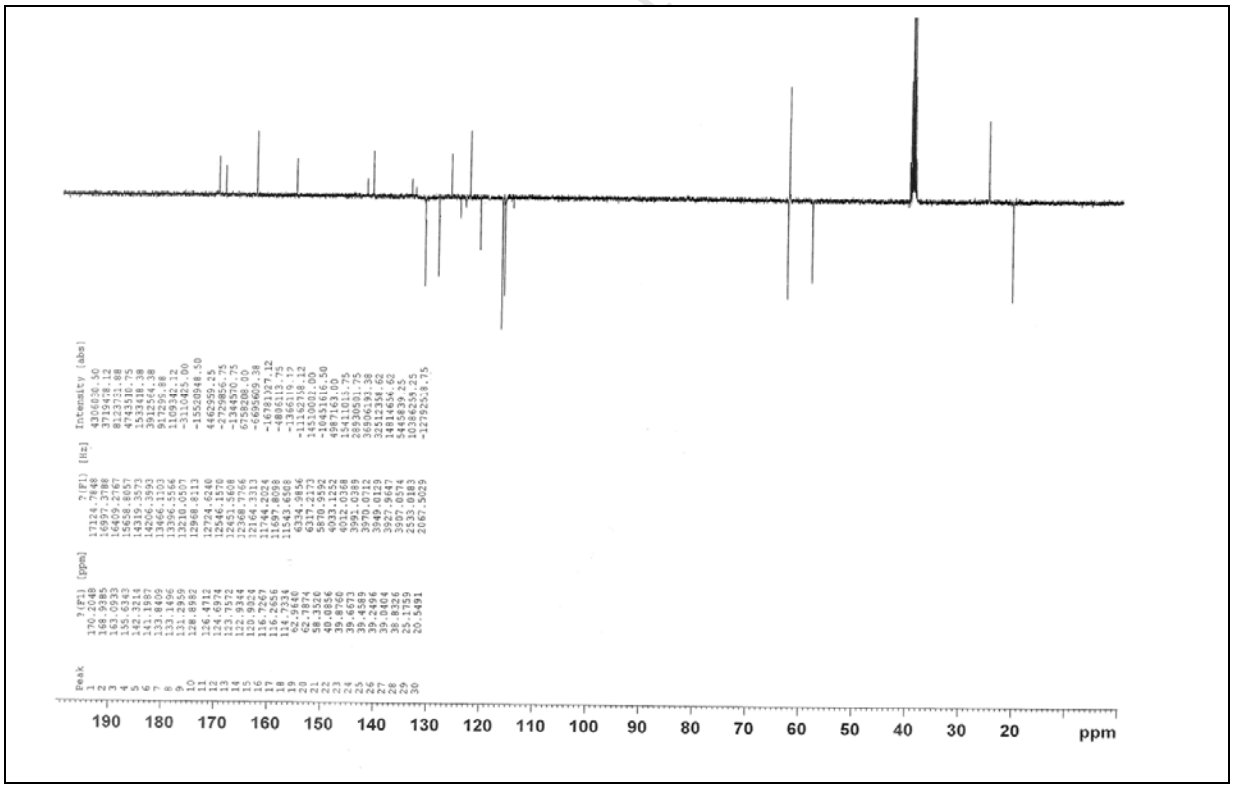
^{1}H-NMR Spectrum of compound 8a



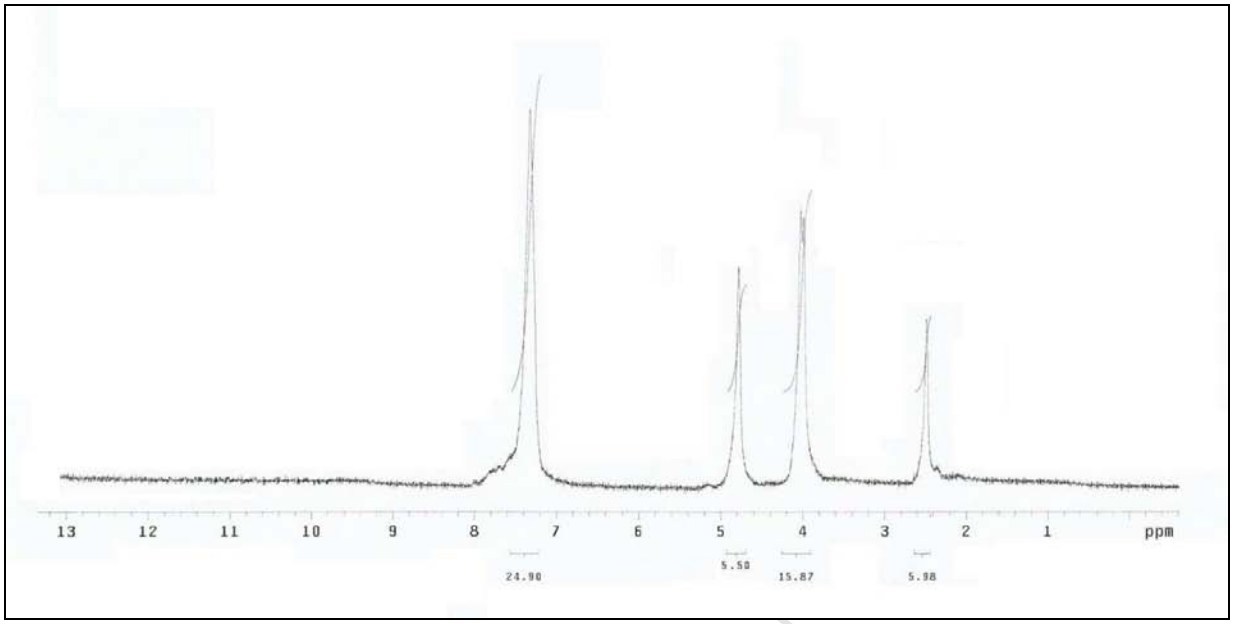
APT spectrum of compound 8a



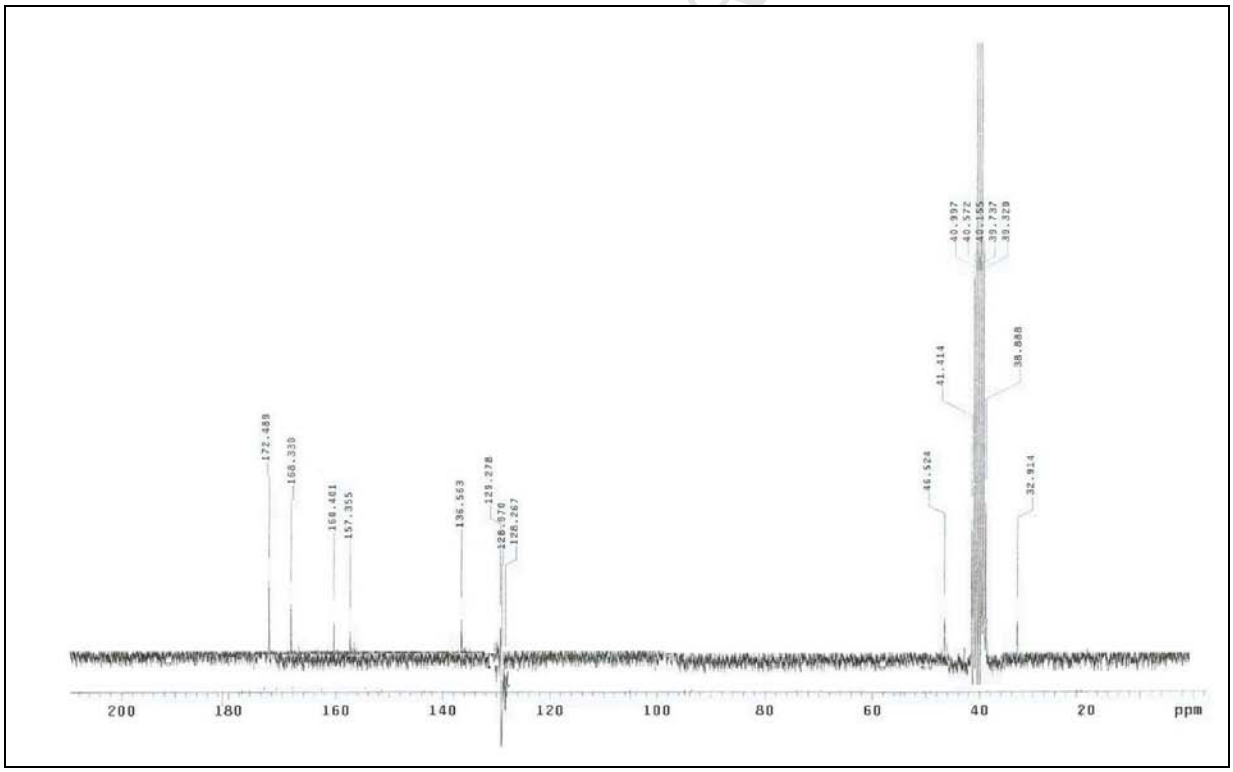
^{1}H-NMR Spectrum of compound 8b



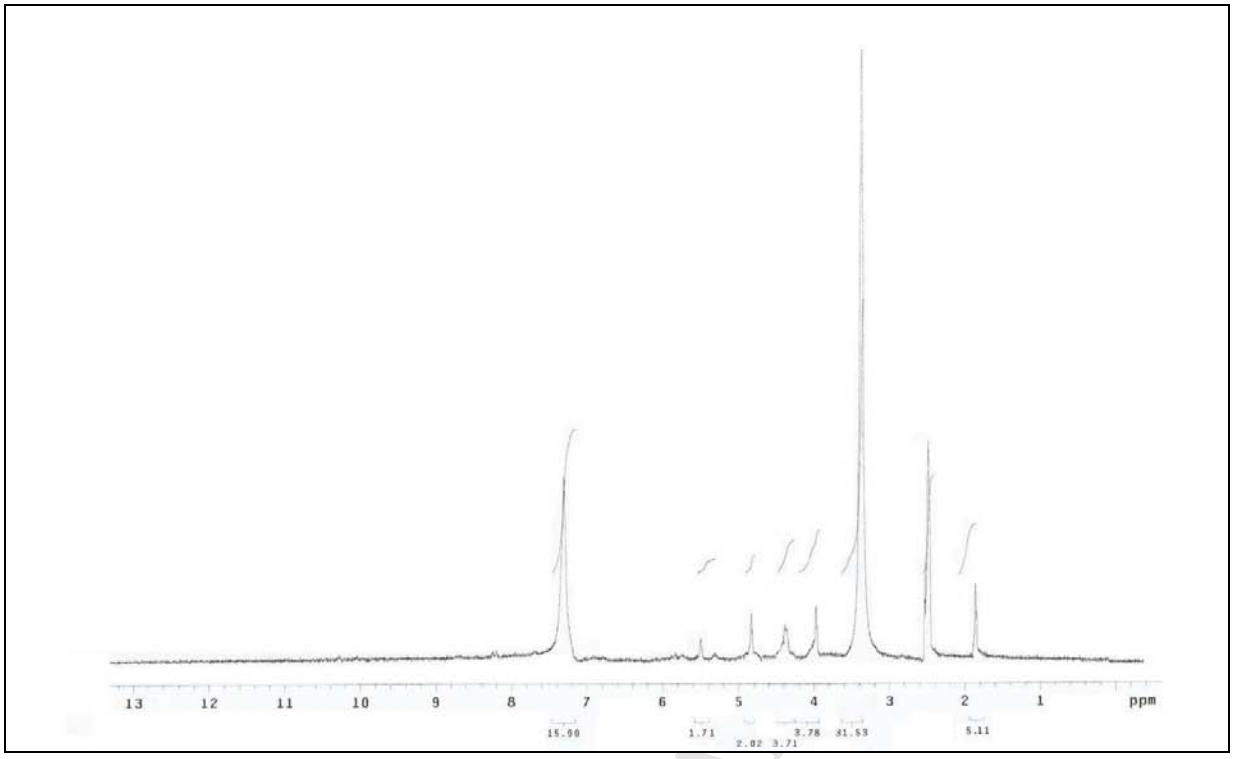
APT spectrum of compound 8b



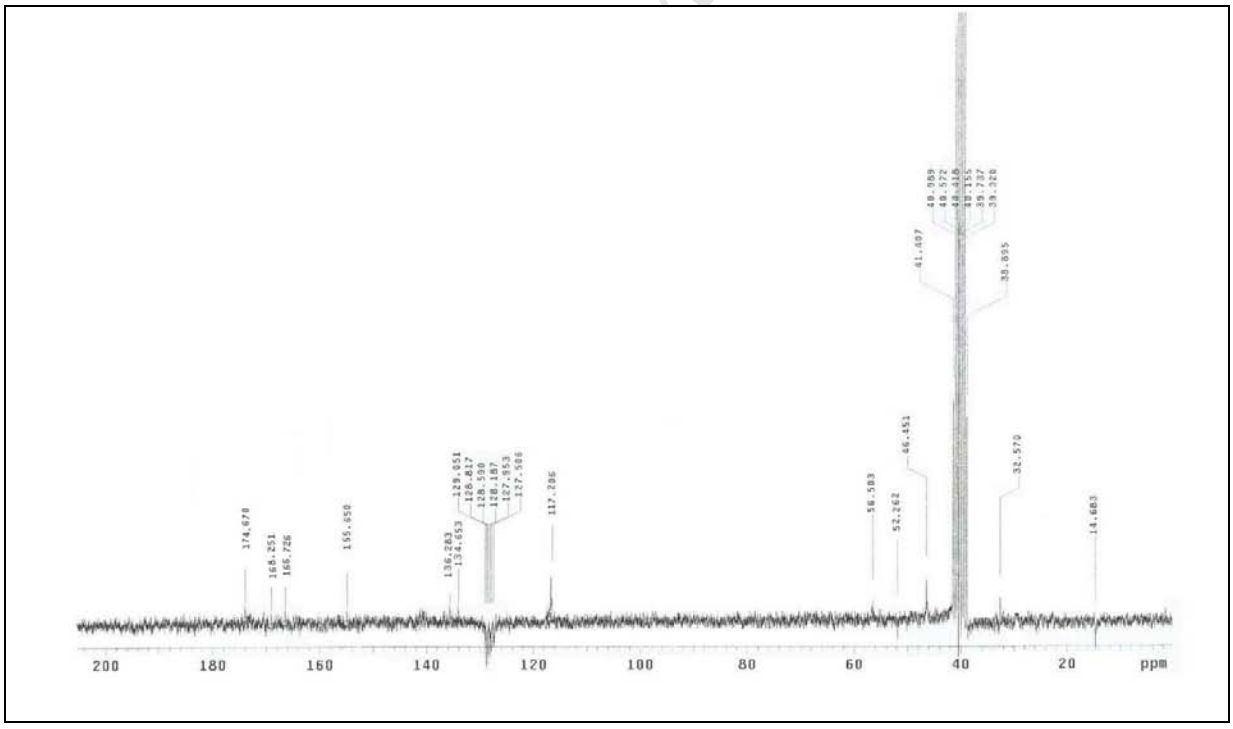
^{1}H-NMR Spectrum of compound 9



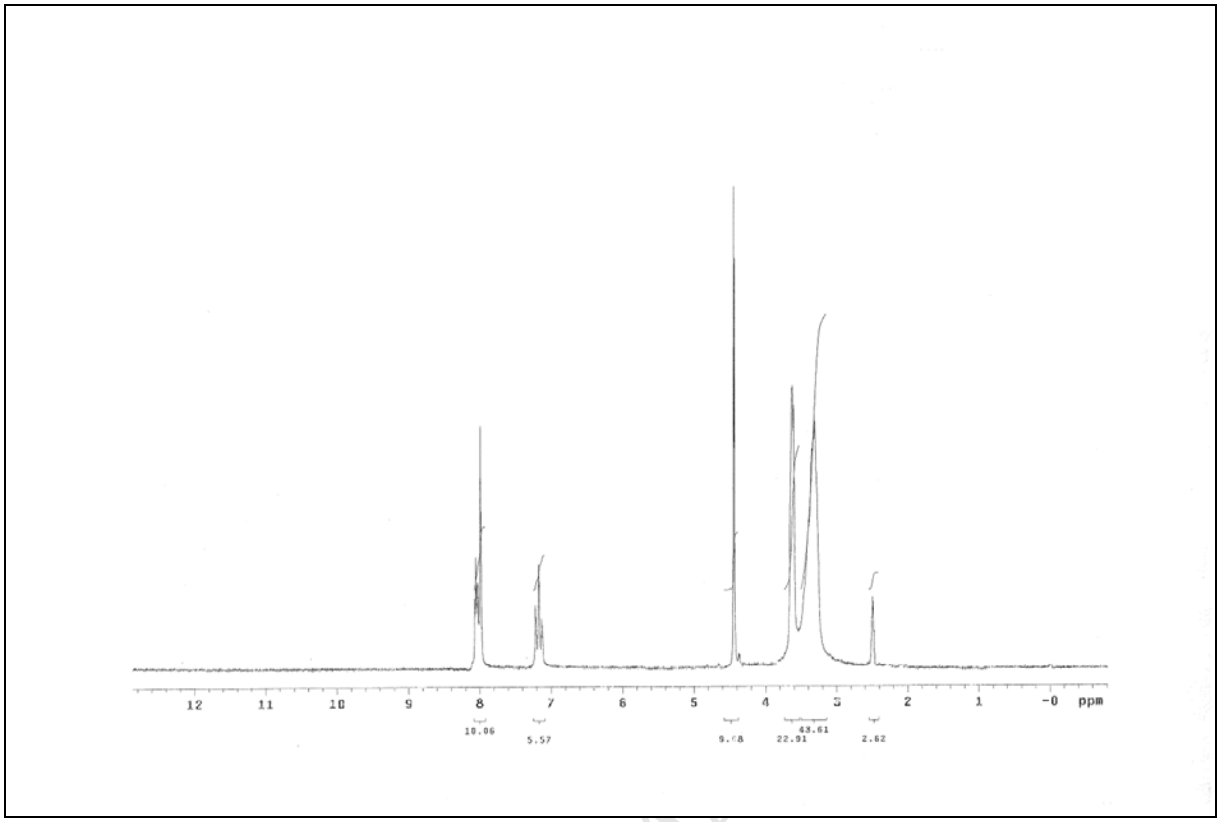
APT spectrum of compound 9



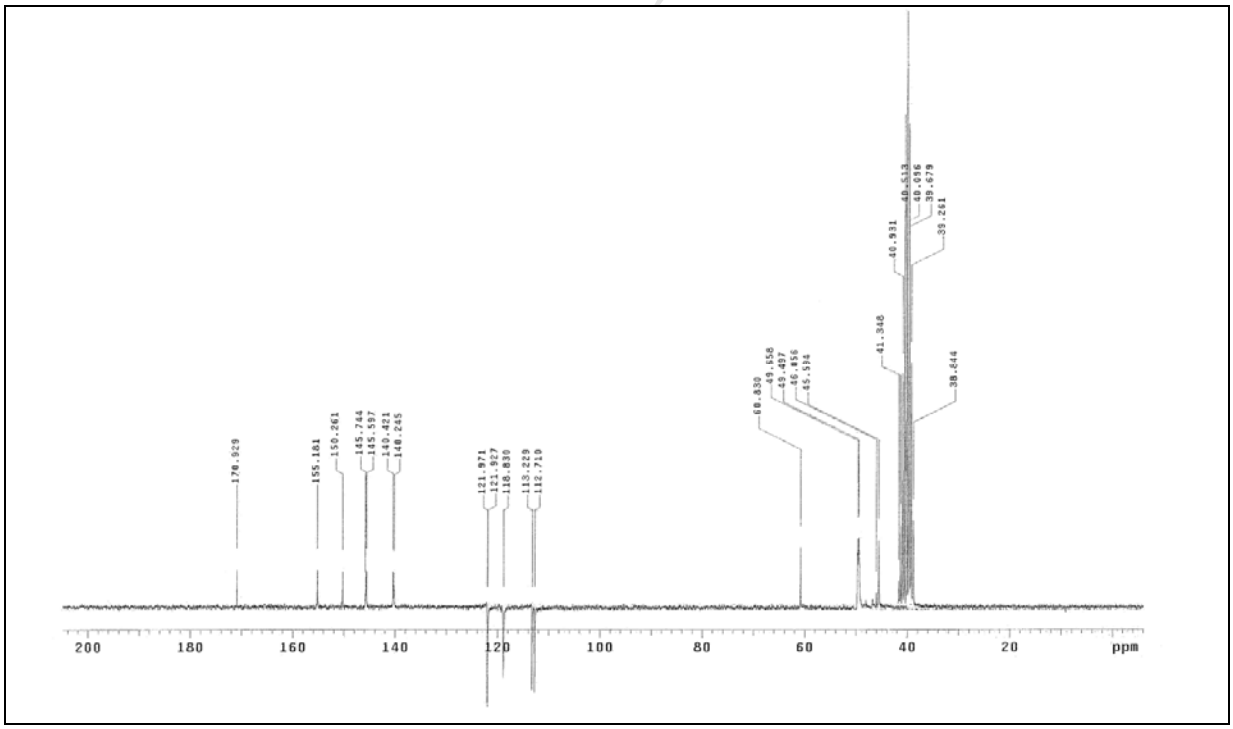
^{1}H-NMR Spectrum of compound 10



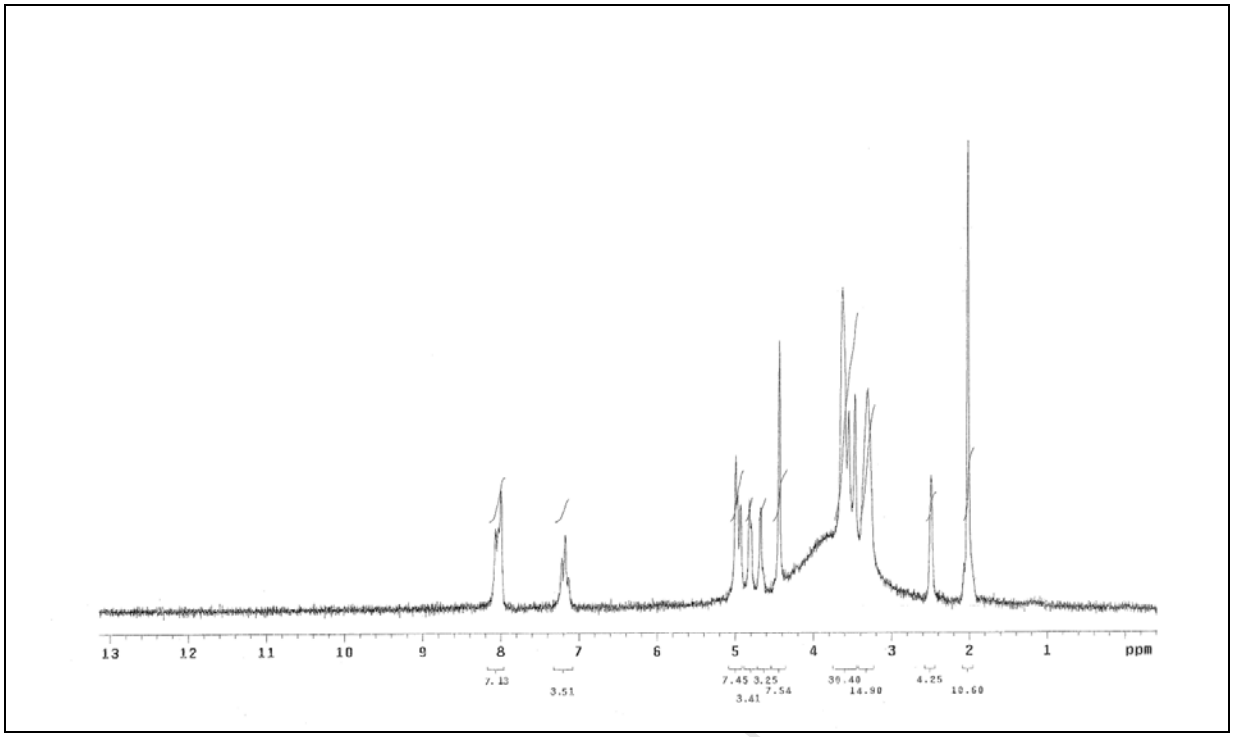
APT spectrum of compound 10



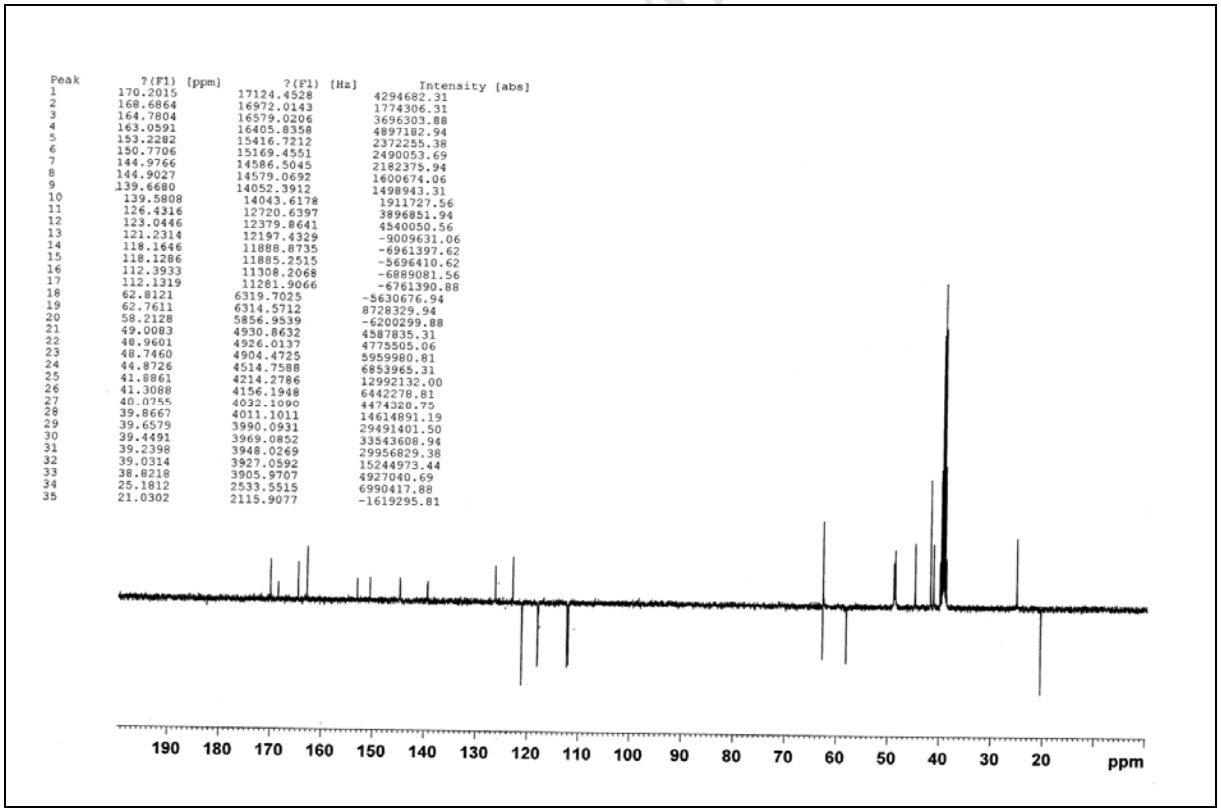
^{1}H-NMR Spectrum of compound 12



APT spectrum of compound 12



^{1}H-NMR Spectrum of compound 13



Peak	?(F1) [ppm]	?(F1) [Hz]	Intensity [abs]
1	170.2015	17124.4528	4294682.31
2	168.6864	16972.0143	1774306.31
3	164.7804	16579.0206	3696303.88
4	163.0591	16405.8358	4897182.94
5	153.2282	15416.7212	2372255.38
6	150.7706	15169.4551	2490053.69
7	144.9766	14586.5045	2182375.94
8	144.9027	14579.0692	1600674.06
9	139.6680	14052.3912	1498943.31
10	139.5808	14043.6178	1911727.56
11	126.4316	12720.6397	3896851.94
12	123.0446	12379.8641	4540050.56
13	121.2314	12197.4329	-9009631.06
14	118.1646	11888.8735	-6961397.62
15	118.1286	11885.2515	-5696410.62
16	112.3923	11308.2068	-6889081.56
17	112.1319	11281.9066	-6761390.88
18	62.8121	6319.7025	-5630676.94
19	62.7611	6314.5712	8728329.94
20	58.2128	5856.9539	-6200299.88
21	49.0083	4930.8632	4587835.31
22	48.9601	4926.0137	4775505.06
23	48.7460	4904.4725	5959980.81
24	44.8726	4514.7588	6853965.31
25	41.8861	4214.2786	12992132.00
26	41.3088	4156.1948	6442278.81
27	40.0755	4032.1090	4474320.75
28	39.8667	4011.1011	14614891.19
29	39.6579	3990.0931	29491401.50
30	39.4491	3969.0852	33543608.94
31	39.2398	3948.0269	29956829.38
32	39.0314	3927.0592	15244973.44
33	38.8218	3905.9707	4927040.69
34	25.1812	2533.5515	6990417.88
35	21.0302	2115.9077	-1619295.81

APT spectrum of compound 13