Synthesis and biological activity of monocyclic Spiro Azetidine-2- one.

Athraa Hameed Mekky* **Zainab Abed Ali Mohammad****

Sanaa Mahdi Auribi**

*Collage of Science, Chemistry Department.

**Collage of Education, Biology Department.

Abstract:

The present work include the synthesis of 3-(4-chlorophenyl)-2-[4-(diethylamino)phenyl]-5,8-dithia-2- [3.4]octan-1-one as potent bactericidal agents are described. The compound prepared by r 4eacting 1,3—dithiane-2-carbonyl chloride with N-(4–diethylamino) phenyl -4-chloro benzylidin in the presence of triethylamine in dry dichloromethane at room temperature characterized by IR , H-NMR and Mass spectral . This compound was screened for this antibacterial activity against *S. aureus, E. coli, B. subtilis* and *P.aeruginosa.* This compound carrying 3- (4-chlorophenyl)-2- [4-(diethyl amino) phenyl]-5,8-dithia-2-aspiro[3.4]octan-1-one moiety showed significant biological

الخلاصة

تم في هذه الدراسة تحضير 3-(4-كلوروفنيل)2-[4-(داي اثيل امينو) فنيل] 5,8-داي ثايا-2-[3.4] -1-اون **و وصفة كمركب مضاد** للبكتريا . **تم** تحضير **من تفاعل حامض** 3,1- **داي** ثايان -2- كاربونايل كلورايد مع مركب الايمين الناتج من تفاعل 4-كلوروبنزالديهايد و4- داي اثيل امين انيلين بوجود ثلاثي اثيل امين في ثنائي كلورو ميثان **الجاف عند درجة حرارة الغرفة** شخص المركب المحضر بوساطة مطيافيات الأشعة **تحت** الحمراء والمرئية وطيف الكتله ومطيافية الرنين النووي المغناطسي البروتون . **وتم اختبار** الفعالية البايولوجية لهذا المركب ضد بكتريا *E. coli, B. subtilis S. aureus,* *P.aeruginosa*. واظهر المركب 3-(4-كلوروفنيل)2-[4-(داي اثيل امينو) فنيل] 5,8-داي ثايا-2-[3.4] -1-**اون** فعالية بايولوجية واضحة .

Introduction

Since the advent of penicillin, the □-lactam antibiotics ahave been the subject of much discussion and investigation, within the scientific as well as the public sectors [1–7]. □-Lactam antibiotics have occupied a central role in the fight against pathogenic bacteria and the subsequent rise in quality of life for the world population as a whole.

However, the extensive use of common □-lactam antibiotics such as penicillins and cephalosporins (Fig. 1) in medicine has resulted in an increasing number of resistant strains of bacteria through mutation and □-lactamase gene transfer. It is well known

that bacterial resistance to □-lactam antibiotics stems from the expression of a □-lactamase that catalyzes the hydrolytic cleavage of the substrate amide bond

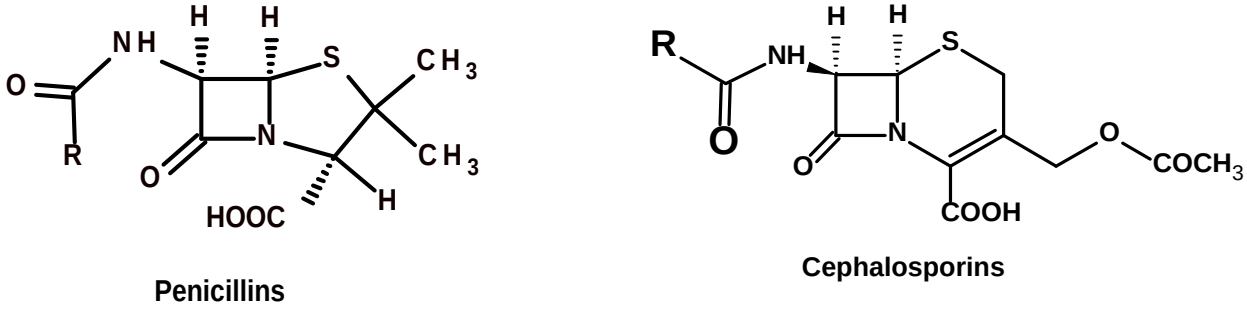


Fig .1 Common □-lactam antibiotics

□□□□□□□□-Lactamases can be classified into four different classes (A–D) according to structure. Class A, C, and D □-lactamases are serine enzymes, the serine residue acting as the nucleophile in the hydrolysis reaction.

Class A □-lactamases are also known as “penicillinases” on account of the ease with which they can hydrolyse penicillins, and class □□□lactamases as “cephalosporinases” by virtue of their increased activity against cephalosporins. Of the four structural classes of this enzyme, metallo-□-lactamases (class B) contain zinc and other divalent cations as cofactors.

Two main therapeutic strategies have been adopted to counteract bacterial resistance to □-lactam antibiotics. One strategy consists of modifying the structure of the □-lactam antibiotic, aiming to render it insensitive to the □-lactamase attack. Recently, trinems antibiotics (Fig. 2) have been the subject of considerable study owing to their broad spectrum of antibacterial activity, resistance to □-lactamases, and stability to renal dehydropeptidases [8–13].

As a result of their impressive biological activity, tricyclic □-lactams have become interesting targets for synthesis. A second approach uses a reagent, typically a □-lactam derivative, that incapacitates the □-lactamase, in synergy with the □-lactam antibiotic. Clavulanic acid (Fig. 2) is the archetype of □-lactamase inhibitors [14]: in synergistic mixture with amoxicillin (Fig. 2), under the name “augmentin”, it arrived in the field some years ago. Both

approaches have produced results and a new generation of antibiotics has been developed

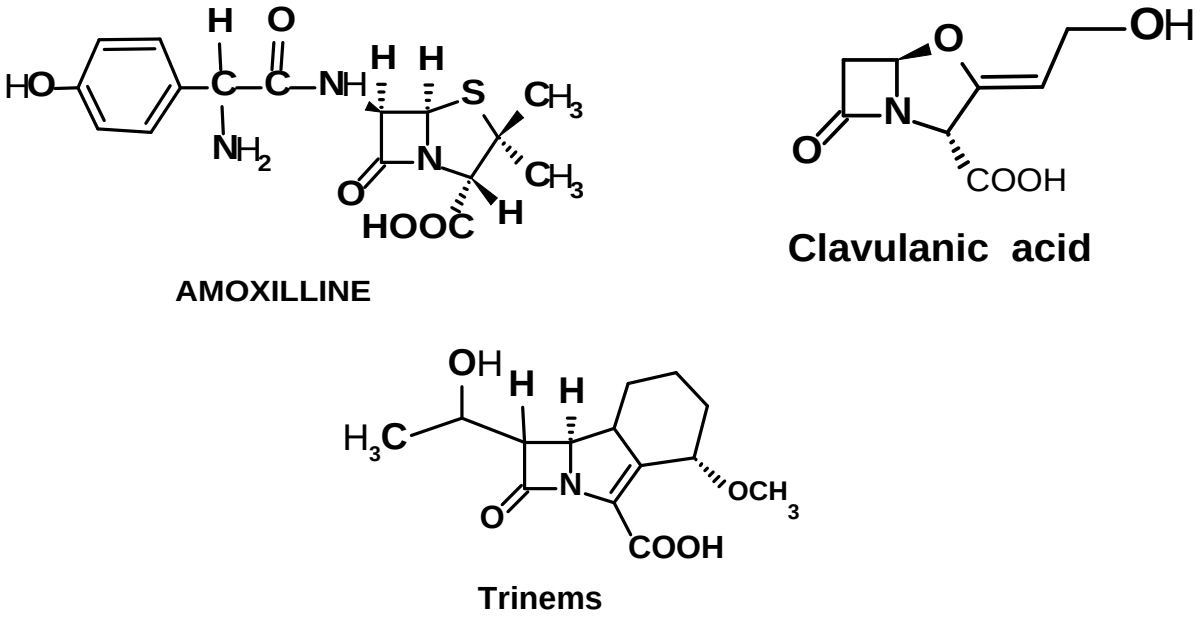


Fig. 2 □-Lactam antibiotics and □□-lactamase inhibitor

Experimental

All the melting points are uncorrected and are expressed in degree (°C), Using melting point \SMP$_3$1 in the Department of Chemistry, College of Science, University of Thi-Qar,Iraq.

IR spectra were recorded using shimadzu FT-IR 8400 spectrophotometer in the Department of Chemistry, College of Science, Basra University,Iraq, as KBr disks. Only principal absorption bands of interest are reported and expressed in cm^{-1}. **^{1}H NMR spectra** were recorded using Bruker system AL 300 (300 MHz) in the Department of Chemistry, AL-Bayt University, AL –Mafraqh , Jordan. The chemical shift values are expressed as δ (ppm) using tetramethylsilane (TMS) as internal standard. The coupling constant (*J*) are given in (Hz). While citing the ^{1}H NMR

data the following abbreviations are used: Singlet (s), Broad single (bs), Doublet (d), triplet (t), quartet (q) and Multiple(m). **Mass spectrum of compound** was recorded at 70 eV using GC-MS Spectrum in the Department of Chemistry, Al- Bayt Uinversity, AL –Mafraqh , Jordan. Thin layer chromatography (**TLC**) was performed using TLC grade silica gel 'G' (Acme Synthetic Chemicals). The spots were made visible by exposing plates to iodine vapour, and eluted with ethyl acetate : hexane mixtures unless otherwise stated. Column chromatography was performed with silica gel (Acme Synthetic Chemicals, 60-120 mesh).

preparation of Schiff base [56,57].

In R . B . F the reaction mixfrom N,N-diethyl -P-phenylenediamine o.o1 mole , 1.64gm and 4- chloro benzaldehyde 0.01mole , 1.405 gm ,10 mL in dry dichloromethane and one drop of glacial acetic acid) with constant stirring over night , progress of the reaction was checked by TLC ,after completion the solvent evaporated than recryastialized from a ethanol.

Yield: 84 % m.p

Preparation of 3- (4-chloro phenyl) - 2-(4-diethyl amino)phenyl -5,9 –dithia-2-azaspiro[3.5]nonan-1-one [58,59 ,60].

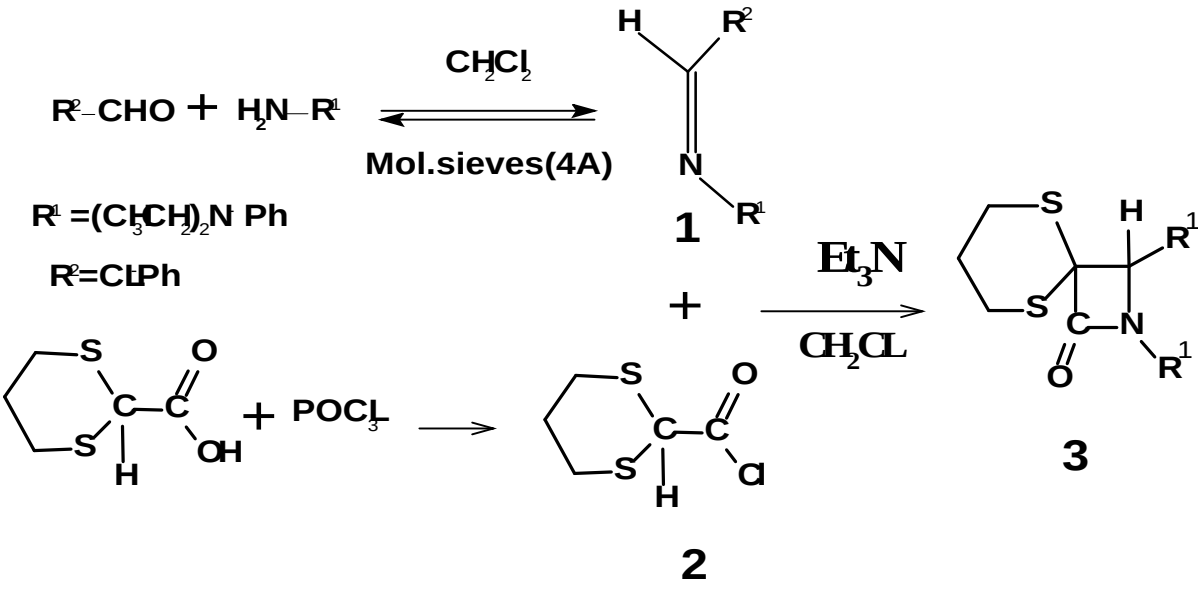
To a suspension of 1,3-dithiane-2-carboxylic acid (2.46 gm¸ 0.015 mole) added drop wise, , a solution of $POCl_3$ (2.301 gm ¸ 0.015 mole) in 20 mL of dry methylene chloride in around flask with constant stirring and check by TLC .The reaction mixture after completion was added drop wise to mixture of Schiff base (2.865gm¸ 0.01 mole) with triethylamine (3.03 gm ¸ 0.03 mole) in 10 ml of dry methylene chlor constant stirring at room temperature .The reactants were stirred overnight at room temperature . There after , the contents were washed successively with 1N HCL 20mL, water 2×20mL, 5% NaHCO3 20mL and brine 20mL .The organic layer was separated and dried over anhydrous Na2SO4 .The Solvent was removed under vacuum and the crude product was column chromatagraphed over silica gel using 7:3 ethyl acetate/ hexane as eluent .Solvent evaporation furnished pure compound

Yield :72% IR (KBr): 1737.74(s) υ C=O .^{1}H – NMR 5.31 C_{3}-H (s) , 3.35-3.52 (H_2)C6(m) , 1.68-2.11 (H_2)C7(m) , 2.6-3.04 (H_2)C8(m) 6.59-7.46 (dd) Ar-H . MS *m/z* (M+,432.1,75%) .

Results and Discussion

In the present work of the associated biological activity and its utility in organic synthesis, the synthesis of spiroazetidin-2-one**.**

The key step involves the treatment of imine **1** with 1,3-dithiane-2-carbony chloride acid **2** with triethylamine in dry methylene chloride at room temperature affords spiroazetidin-2one **3** as shown :.



Antibacterial activity

The Antibacterial activity of this compound was studied by employing filter paper disc method [9-12]. Representative organisms selected for evaluation of antibacterial activity were *S. aureus, P. Mirabilis, K. pneumonia, E. coli .* The antibacterial activity of this compound was evaluated at 1 µg mL^{-1}, 10 µg mL^{-1}, 50 µg mL^{-1}, 100 µg mL^{-1}, 500 µg mL^{-1}, 1000 µg mL^{-1} Concentration.An important and useful drug ampicillin was also tested under similar conditition, with view to compare, the results Ampicillin is a *beta*-lactam antibiotic13 that has been used extensively to treat bacterial infections since 1961. Ampicillin is designated chemically

as (2S, 5R, 6R)-6-([(2R)-2- amino-2-phenylacetyl] amino)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylicacid. Ampicillin is able to penetrate gram-positive and some gram-negative bacteria14 . Ampicillin acts as a competitive inhibitor of enzyme transpeptidase. The results indicate that all three compounds showed good activity. (Table 1) compounds showed very good activity against *S.aureus, P. Mirabilis, K. pneumonia, E. coli.*

Zone of Inhibition / mm						
Concentration of compound (control) Ampicillin	1 μg mL^{-1}	10 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}	500 μg mL^{-1}	1000 μg mL^{-1}
S. aureus	14 (5)	17 (10)	18 (12)	19 (16)	21 (18)	23 (22)
P. mirabilis	10 (4)	10 (6)	13 (11)	14 (16)	15 (17)	20 (19)
K. pneumonia	6 (3)	7 (7)	8 (10)	10 (14)	13 (14)	15 (19)
E. coli	11 (7)	12 (7)	13 (9)	15 (12)	15 (14)	18 (17)

Table 1. Evaluation of antibacterial activity of the compound

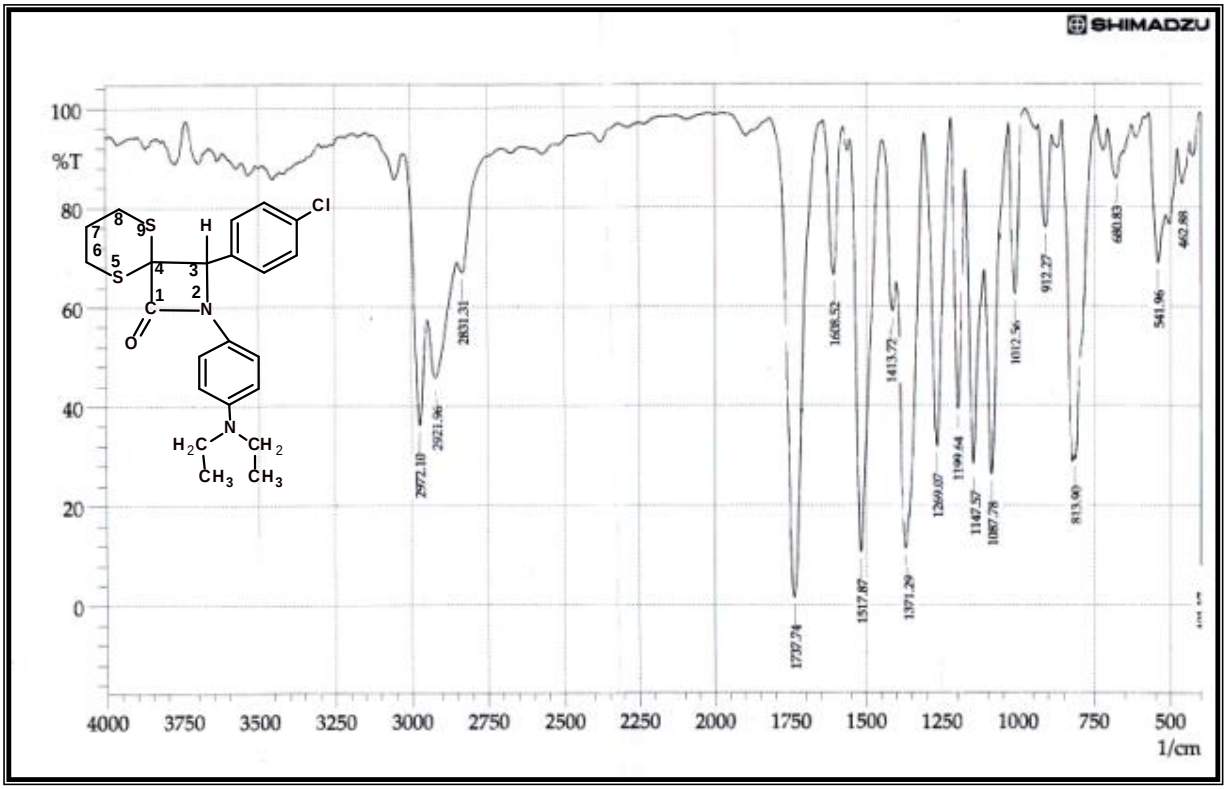


Figure (3) : IR spectra of 3- (4-chloro phenyl)- 2-(4-diethyl amino)phenyl-5,9-dithia-2- azaspiro [3.5]nonan-1-one .

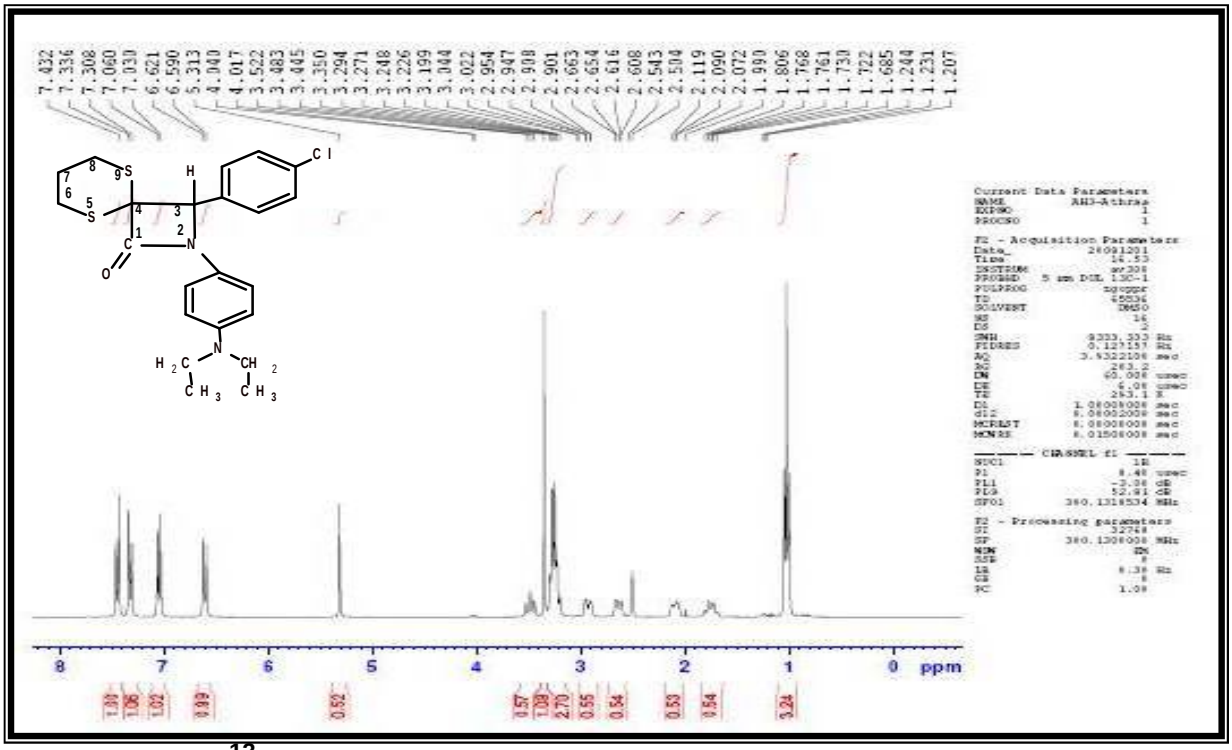


Figure (4) ^{13}C NMR spectra of 3-(4-chlorophenyl-2 -(4-diethylamino)phenyl)- 5,9-dithia-2-azaspiro[3.5]nonan-1-one

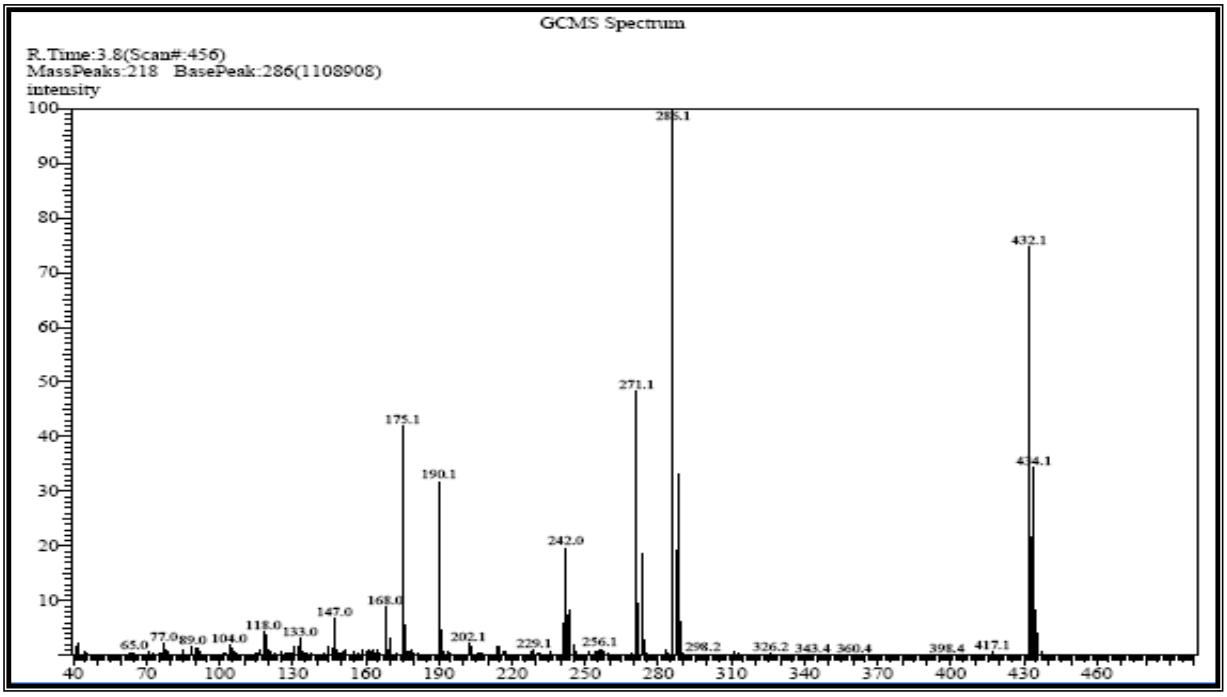


Figure (5) Mass spectra of **3- (4-chloro phenyl) 2-(4-diethyl amino)phenyl -5,9 dithia-2-azaspiro[3.5]nonan-1-one .**

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