

SYNTHESIS, CHARACTERIZATION AND STUDY BIOLOGICAL SCREENING OF SOME NEW AZETIDINONE DERIVATIVES FROM AZO- SULPHADIAZINE

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ABSTRACT

This research Involved synthesis of some new azetidinone Derivatives from Azo Compound[4-Amino-3--((4-methoxy-2-nitrophenyl)diazanyl)-N-(Pyrimidine-2-yl)benzen-esulfonamide](N₁) by two routsone of them Conversion the free amino group in an azo comp. to Schiff base, then formation of azetidinone derivatives by ketene-imine reaction or Staudinger's Cyclo addition .The other rout is the cyclization by reaction of the free amino group with Chloro acetyl chloride and Et₃N to give N-Chloroacetamide derivative then reaction with hydrazine hydrate to produce the hydrazinyl acetamide derivative. Then conversion to Schiff base and finally formation azetidinone derivative. Most of these derivatives were confirmed by "FT-IR,¹HNMR and ¹³CNMR" spectra and by Mass spectrum for two prepared derivatives. the prepared compounds are tested as anticancer and antimicrobial activity against of bacteria and fungi.

Keywords: Staudinger reaction, Anticancer activity, Azetidinone, Sulphadiazine.

INTRODUCTION:

The Staudinger [2+2] cycloaddition is a reaction between ketene and imine which Represent as one of the most essential and flexible strategies for the synthesis of structurally varied derivatives of 2-azetidinone, although numerous manufactured strategies have been created to date [Jiaxi Xu, 2009]. The Staudinger reaction gets thermally or photochemically by using acid chlorides in the presence of (Et₃N) triethylamine or α -diazoketones as precursors for ketene, Singh [2003].The ketenes are unlooked for new family of reactive intermediates which that reported by Staudinger In 1905,the first example is diphenyl ketene (ph)₂=C=C=O. dimethyl ketene CH₂C=O also prepared and found to undergo dimerization [2+2] to produce the symmetrical cyclobutanedione in addition to participate in [2+2] cycloaddition with the (C=N)imine group to form B-lactam [Tidwell, 2005]. ketenes, that which participate in this reaction may be afford amino-, oxy-, or halo groups [Palomo et al., 1999]. β -lactam or Azetidinone is a four-membered cyclic has been known as a beneficial building block for the preparation of numerous of organic compounds by take advantage of the strain energy that linked with it [Rajasekaran, 2010]. is a part of the antibiotic structure that known to offer interesting biological activities such as antimicrobial, anti-bacterial, anti-fungal, anti-tubercular, anti-inflammatory, enzyme inhibition, anti-convulsant, in addition to central nervous system activities [Abbas, 2017]. There are

various synthetic methods of azetidinone derivatives like ketene-imine Cycloaddition As previously mentioned, by utilizing microwave irradiation and from cyclization [Jubie et al., 2009, Vijay et a l., 2009, Devprakash et al., 2011].

MATERIALS AND METHODS

Chemical part: The melting points were recorded and expressed in degree (^oC) by using the electro thermal 9300 melting point LTD, UK. Thin layer chromatography T.L.C was performed on aluminum and glass plates coated with 0.25mm layer of silica-gel (Fluka). Some of the derivativess were detected by iodine vapor. FT-IR spectra, Fourier transform infrared (SHIMADZU, 8400) spectrophotometer, Japan the rang 4000-600cm⁻¹. The samples were run in KBr disc. ¹³C & H¹-NMR spectra in (ppm) unit were operating in DMSO -d₆ as solvent using (Bruker- Ultra Shield 300 MHz Switzerland). And Mass Spectra were recorded on AB Sciex 3200 QTRAP LC/MS/MS , (Mass range - m/z 5-2000-quad mode and 50-1700- linear ion trap mode).

Synthesis of Basic compound(Azo)[4-Amino-3--((4-methoxy-2-nitrophenyl) diazenyl)-N-(Pyrimidine-2-yl)benzenesulfonamide] (N₁) [yaqoob et al., [2016].

Nitro-4-methoxy aniline (1.68 gm, 0.01mole) was dissolved in (4ml) of concentrated acid HCl and (15 ml) of distillation water. The mixture was cooled at (0-5 ^oC) in ice-water bath ,Then a solution of NaNO₂ (0.01mole)(0.69gm) was dissolved in (10 ml) of distilled water then it will be cooled at (0-5

$^{\circ}\text{C}$). This solution was added a drop wise to the mixture with stirring in the same temperature. The diazonium salt solution was added portion wise to solution of (0.01mol, 2.5gm) sulfadiazine in distilled water with sodium hydroxide (5 gm) dissolved in (100ml) distilled water. The basicity was neutralize by adding drops of (HCl) until the P_H become (7) and temperature was maintained at (0-5 $^{\circ}\text{C}$).The mixture was stirred for 30 mint and was left over night. The yield was precipitated and filtered, washed with distilled water and recrystallized in absolute ethanol: yield, (Fire brick), (74%), m.p. 110-112 $^{\circ}\text{C}$.

^1H NMR spectrum, (δ ppm), (DMSO- d_6 MHz), (OCH₃)(s 3.731), (NH₂) (6.048), 6.61 (1H, t, J = 5.3 Hz), 6.98-7.08 (2H, 7.05 (dd, J = 8.3, 0.5 Hz), 7.00 (dd, J = 7.9, 0.5 Hz)), 7.18 (1H, dd, J = 8.3, 1.6 Hz), 7.70 (1H, dd, J = 1.6, 0.5 Hz), 8.04 (1H, dd, J = 7.9, 1.7 Hz), 8.52 (2H, dd, J = 5.3, 1.9 Hz), 8.69 (1H, dd, J = 1.7, 0.5 Hz), (N-H) Sulfone (11.381). ^{13}C -NMR-spectrum, (δ ppm), (DMSO d_6 , MHz), 55.913, 105.177, 112.572, 115.990, 121.271, 125.239, 127.701, 129.432, 130.305, 142.469, 149.605, 153.514, 157.679, 158.730.

General procedure for synthesis Azetidinone and 3-Chloro Azetidinone Derivatives:

(1) Azetidinone Derivatives [Jetti et al., 2015]: (0.001mol) Schiff base, glacial acetic acid 0.06ml (0.001mole) and Et₃N 0.35ml (0.0025mol) were stirred in (20 ml) of anhydrous dichloromethane (DCM). A solution of POCl₃ 0.093ml (0.001mol) in 5ml (DCM) was added drop wise under (N₂) atm. at 0 $^{\circ}\text{C}$ with stirring. The reaction mixture was stirred overnight at room Temp. progress of the reaction was followed by T.L.C. After, the completion the mixture was washed with (1N) HCl (10ml), dis. water also solution of 5% NaHCO₃. The organic layer was dried by (Na₂SO₄). yielded solid was recrystallized from Absolute Ethanol (2)-3-Chloro Azetidinone Derivatives [Ezzat et al., 2012, Chavan et al., 2007]. A mixture of Schiff base (0.01 mol) in dioxane (25 ml) and Et₃N 3.49 ml (0.025 mole), was added chloro acetyl chloride 1.99 ml (0.025mole) dropwise at 5-10 $^{\circ}\text{C}$. The mixture was stirred for 6hrs and kept at room temperature for two days then poured into crushed ice. The solvent was evaporated, and the yield recrystallized in Absolute ethanol. All the reactions were monitored by T.L.C.

The first line: synthesis of Schiff Base [4-(4-Hydroxy-3-methoxybenzylideneamino)-3-((4-methoxy-2-nitrophenyl-diazenyl)-N-(pyrimidine-2-yl)-

benzenesulfonamide (B1) [Ezzat, 2014, Nabeel, 2018]. Azo Compound (N1) (2.15gm, 0.005mole) dissolved in hot CH₃COOH acid about 50ml then added to (0.76 gm, 0.005mol) of Vaniline was dissolved in 5ml CH₃COOH acid. The mixture was refluxed with the stirring at 100 $^{\circ}\text{C}$ for 40 hours. The progress of this reaction was followed by T.L.C. After the completion the mixture was poured on the ice crushed. The solid was filtered off, wash with 2% Sodium bicarbonate solution and distilled water then re-crystallized from abs. Ethanol.

^1H NMR spectrum, (δ ppm), (DMSO- d_6 MHz), (OCH₃)(s 3.731), 6.58-6.68 (2H, 6.61 (t, J = 5.3 Hz), 6.65 (dd, J = 8.5, 0.5 Hz)), 6.74-6.81 (2H, 6.78 (dd, J = 8.5, 2.8 Hz), 6.77 (dd, J = 2.8, 0.5 Hz)), 7.14-7.23 (2H, 7.16 (dd, J = 7.3, 0.5 Hz), 7.21 (dd, J = 7.3, 1.6 Hz)), 7.69 (1H, dd, J = 1.6, 0.5 Hz), 7.88-7.96 (2H, 7.93 (dd, J = 8.1, 0.5 Hz), 7.91 (dd, J = 8.1, 2.0 Hz)), 8.52 (2H, dd, J = 5.3, 1.9 Hz), 8.70 (1H, dd, J = 2.0, 0.5 Hz), (OH) (s 9.78).

^{13}C -NMR-spectrum, (δ ppm), (DMSO- d_6 MHz), 55.918, 105.199, 110.351, 111.038, 112.561, 115.827, 115.906, 121.204, 121. 266, 124.001, 125.958 ,126 .607, 127.684, 129.097, 129. 449, 130.253 ,142.362, 142.462 .148.414, 148.619, 149.612 ,150.312, 153.551, 158.682, 161.138.

Synthesis of 4-(2-(4-Hydroxy-3-methoxy phenyl)-4-oxoazetidine-1-yl)-3-(4-methoxy-2-nitrophenyl) diazenyl)-N-(pyrimidine-2-yl)-benzenesulfonamide (L1).

The derivative was prepared according to the procedure (1) from Schiff base (B1), acetic acid, Et₃N and POCl₃, MS (m/e) 605 (M+)

^{13}C -NMR-spectrum, (δ ppm), (CDCl₃, MHz), 55.9-41, 65.265, 105.265, 121.203, 121.265, 127.660, 129.486, 142.347, 142.445, 149. 630, 168.630, 172.445.

Synthesis of 4-(3-chloro-2-(4-Hydroxy-3-methoxy phenyl)-4-oxoazetidine-1-yl)-3-((4-methoxy-2-nitrophenyl) diazenyl)-N-(pyrimidine-2-yl) benzene sulfonamide (L11). The comp. was prepared according to the procedure (2) from Schiff base (B1), triethylamine and chloro acetyl chloride.

The second line: Synthesis of 2-Chloro-N-(2-((4-Methoxy-2-nitro phenyl) diazenyl)-4-(N-pyrimidine-2-yl sulfamoyl) phenyl)acetamide (N2) [Ezzat et al., 2016].

A mixture of Comp.(N1) (4.29 gm, 0.01mol) and tri ethylamine (1.41 ml, 0.01mol) in DMF, chloro acetyl chloride (0.8 ml, 0.0 1mol) was added dropwise. The mixture was stirred for(4hrs.) at room temp. The progress of the reaction was

monitored by T.L.C. at the end of the reaction; the solvent was evaporated. The precipitate obtained was filtered and washed with distilled water then recrystallized in Abs. ethanol. The product has been confirmed to be formed by the Sodium fusion process [16].

C^{13} -NMR-spectrum, (δ ppm), (DMSO- d_6 , MHz), 43.305, 44.015, 56.504, 109.695, 191.167, 120.88 0, 123.837, 127.775, 129.444, 135.126, 142.839, 143.748, 156.974, 157.335, 165.561, 165.

Synthesis of 2-Hydrazinyl-N-(2-((4-Methoxy-2-nitrophenyl) diazenyl)-4-(N-pyrimidine-2-yl sulfamoyl)-phenyl) acetamide (N3) [Sharma et al., 2012, Ali, 2013].

The comp. N2 1gm (0.002mole) in ice water bath and hydrazine hydrate (99%) were stirred on a magnetic stirrer for 30 minutes then stirred at room Temp. about 5.00 hours. by TLC plates the reaction was monitored. the mixture was left to evaporate excess hydrazine, then the mixture was washed by (0.1 M HCl) and distilled water. the product filtered and re-crystallized from absolute ethanol.

Synthesis of [N-(2-((4-methoxy-2-nitrophenyl) diazenyl)-4-(N-pyrimidin-2-ylsulfamoyl)-phenyl)-2-(2-(4-methylbenzylidene) hydrazinyl) acetamide](B2) and [2-((E)-2-(4chlorobenzylidene) hydrazinyl)-N-(2-((E)-(4-methoxy-2-nitrophenyl) diazenyl)-4-(N-pyrimidin-2-ylsulfamoyl)phenyl)acetamide] (B3) [Ramachandran et al., 2011, Lina, 2018].

These derivatives were prepared from p - methyl benzaldehyde (0.12gm, 0.001mol) and p-Chloro benzaldehyde (0.14gm, 0.001mol) respectively dissolved in 10ml abs. Ethanol with 3 drops glacial acetic acid then added Compound(N₃) 0.5gm (0.001 mol) in 20ml abs. Ethanol for each aldehyde. The reaction mixtures were refluxed at 80°C with stirring for 25 hours. The progress of the reactions followed by TLC. The solid separated after removal the solvent and then re-crystallized from abs. Ethanol.

(B3).

Synthesis of (N-(2-((4-methoxy-2-nitrophenyl) diazenyl)-4-(N-pyrimidin-2-ylsulfamoyl)-phenyl)-2-(2-oxo-4-p-tolylazetid-1-ylamino)acetamide)(L2) and (2-(2-(4-chlorophenyl)-4-oxoazetid-1-ylamino)-N-(2-((4-methoxy-2-nitrophenyl) diazenyl)-4-(N-pyrimidin-2-ylsulfamoyl)phenyl)acetamide) (L3).

These derivatives were prepared according to the procedure (1) from Schiff base (B2) and Schiff bas

(B3) respectively with acetic acid, Et₃N and POCl₃ for each Schiff Base(L2), MS (m/e) 644.9 (M+).

Biological part: All Chemicals and biological materials were supplied from Sigma, Difco, USA, Santacruz biotechnology Inc, Europe, BDH, Flow laboratories, GCC, UK, Merck, Germany were supplied from Arnold Sons, Genex, Beckman Model J2-21, Lab-TeK and Nunc, USA, Memmert, Hermle, Leica, Sartorius, Leitz Germany, Marubeni, Ogawa seiki, Japan, Gallen-kamp, UK, Eppendroff Oxford, LKB Sweden, Nunc Denmark.

Equipment's and instruments:

1- **Cell culture media:** Two type of cell culture media were used in this assay: Growth media (GM) and maintenance media (MM), The PH was checked and adjusted to about (6.8-7.1). The antibiotics were added to culture medium at final concentration of 100 IU/ml and 100 μ g/ml of penicillin G and streptomycin, respectively (1ml of antibiotic solution to 100 ml of culture medium), Nystatin was also added to give the final concentration of 25 IU/ml. Filtration of media was carried out in biohazard safety cabinet using 0.22 μ m Millipore filter. To prepare 100 ml GM and MM, the components were mixed up to prepare media necessary for LS-174 cell line (Colon Cancer) revealed in table 1.

Table 1: Showing the constituents of cell culture media for LS-174 cell line

Components	GM	MM
1X RPMI 1640	84.5 ml	91.5 ml
HEPES 1M	2 ml	2 ml
Fetal calf serum (FCS)	10 ml	2 ML
Penicillin/streptomycin solution (3-1-3-2-A)	1 ml	1 ml
Nystatin	1 ml	1 ml
NaHCO ₃ (7.5%) solution	1.5 ml	2.5 ml

Each bottle was sealed tightly, labeled with name, date and kept in incubator at 37°C. The bottles were examined after 2-3 days later. If no turbidity and no indication of bacterial growth, they would be transferred to refrigerator to stored till be used.

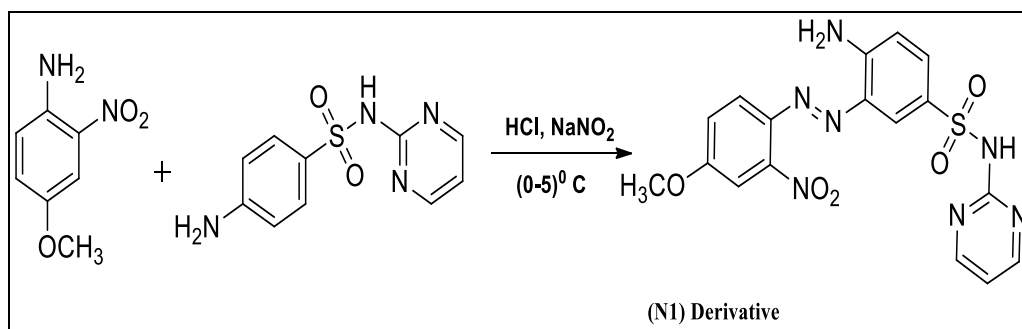
2-Cytotoxicity assay [Wang et al., 2004, Accardo et al., 2014]: Cell line were seeded on 96 well plates with a concentration of 1.0×10^5 cell/ml. After incubation at 37°C for 24 - 48 hr, when the confluent monolayer of LS-174 cells were complete 80-100% concentrations (0.5, 1, 10, 100, 1000, 2000,

3000,4000,5000 and 10000 $\mu\text{g/ml}$) of micro tittered composites were added to cultured wells at a final volume of 100 μl in each well except cells control in triplicate. After 24 hrs incubation at 37°C in 5% CO_2 , the micro titer 96 wells plates were marched out and transferred to biohazard safety cabinet by sterilized environments to avoid any contamination, all used wells media were discarded, The LS-174T cell mono layers were washed by PBS (Phosphate buffered saline) solution to remove any residual amount of composites or standard anticancer drugs used that may be interacts with MTT (Methyl thiazolyl tetrazolium) reagents. Then 100 μl of maintenance media was added to all wells included drugs treated cells, drugs untreated cells and blank wells, then, MTT reagent 20 μl was added to each well. After 4 hrs of incubation at 37°C, 5% CO_2 , the formazan particles were formed as a mitochondrial enzymatic process of the non-effected viable LS-174T cells, the dead or viral effected cells were didn't form formozan particles because it is mitochondria organelles were disrupted. The

formazan was solubilized by adding diluted DMSO (dimethylsulfoxide) (1:1) in isopropanol on each wells included blank wells, the absorbance was read at 490nm with a reference wavelength of 630 nm by an ELISA reader, this protocol of MTT assay measurement was mentioned by many reports [Accardo et al., 2014, Wang et al., 2004].

RESULTS AND DISCUSSION

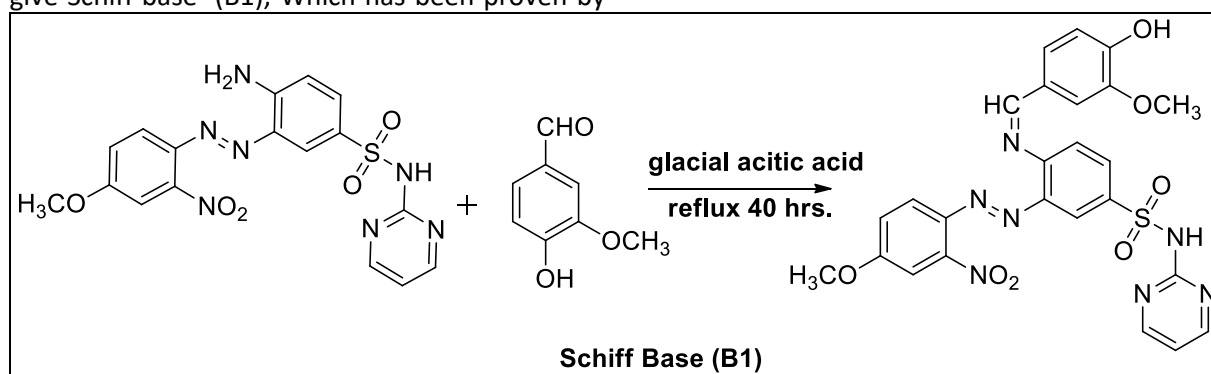
In this research new Azo compound was prepared from *O*-Nitro-*P*-Anisidine that which converted to the corresponding diazonium salt by reaction it with concentrated HCl and Sodium Nitrite NaNO_2 at 0°C then directly introduced to Sulfadiazine as coupling comp. to give Azo amine derivative(N1).In FT-IR observed Appearance of free aromatic amine stretching in 3487-3427 cm^{-1} as to clearly two bands and one band for NH Sulfon amide stretching at 3389 cm^{-1} and Azo group at 1417 cm^{-1} and showed- $^1\text{H-NMR}$ spectrum of this compound characteristic signals (δ 3.731) (3H,-OCH₃), (6.048) (2H,-NH₂) and (11.381) (1H-NH) Sulfon near attributed to the formation of Azo amine derivative.



Scheme 1: Derivative N1

Diazo-coupling reaction from this derivative was prepared different Azetidione derivatives by two lines. in the first line the free amino group in Comp. (N1) converted to Azomethine group by reaction it with Vaniline (*p*-hydroxy-*m*-methoxy benzaldehyde) in glacial acetic acid as solvent to give Schiff base (B1), Which has been proven by

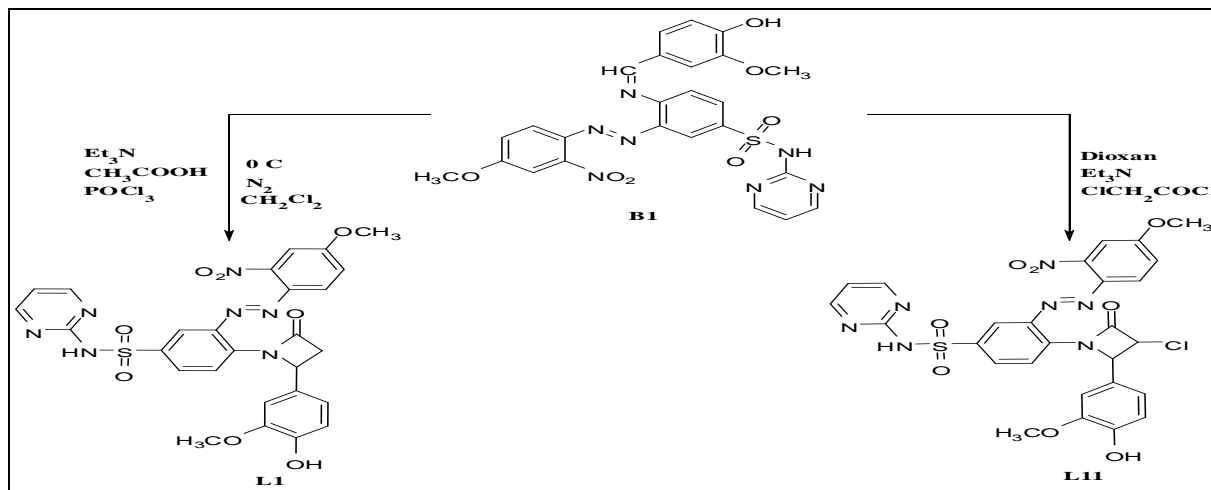
disappearance the free amino group and appearance Azomethine group at 1674 cm^{-1} in FT-IR and stretching vibration at 3373 cm^{-1} due to (NH sulfon) in FT-IR also disappearance the signal of amino group in H-NMR of (B1) and appearance the signal of (1H, -CH=N) Schiff base at (δ 8.59).



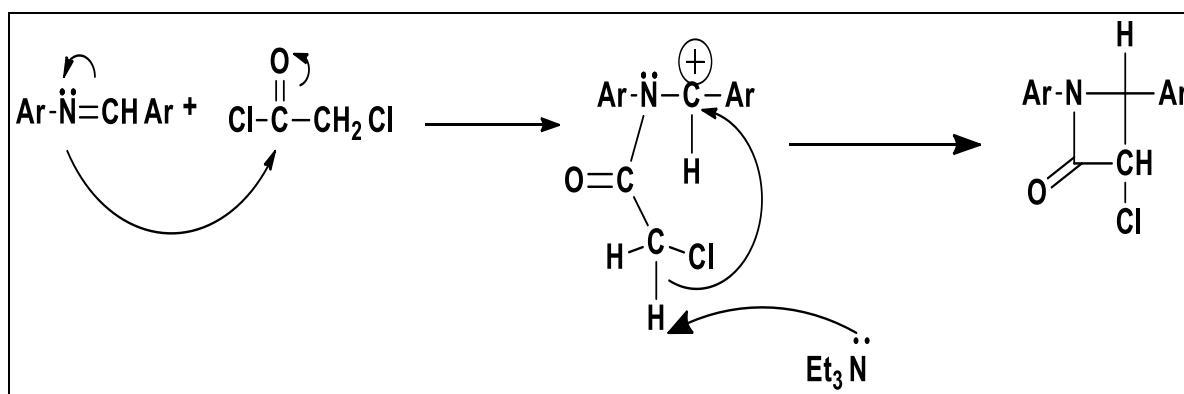
Scheme 2: preparation of Schiff Base (B1)

From this Schiff Base prepared two Azetidione derivatives by [2+2] cyclo addition once with glacial acetic acid(CH₃COOH), tri Ethyl amine(Et₃N) and Phosphoryl chloride POCl₃ in Di chloro methane as solvent with stirring under (N₂) gas at 0 °C

to give Azetidione ring derivative without any substitution and once with Chloro acetyl chloride (ClCH₂COCl), Et₃N in 1,4-Dioxane as solvent at 5-10°C with stirring to give 3-Chloro Azetidione derivative.



Scheme 3: preparation of Azetidione derivatives (L1, L11)



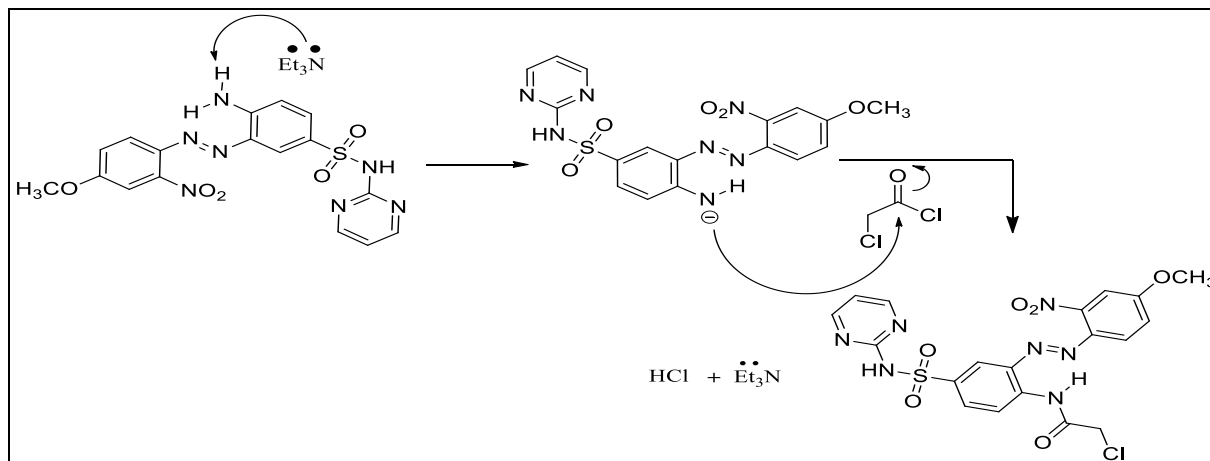
Scheme 4: mechanism of [2+2] cycloaddition, [Parmar et al., 2011]

The derivatives L1 and L11 has been proven by disappearance of Azomethine band in FT-IR 1674 cm^{-1} and appearance the band of carbonyl Azetidione ring in 1707, 1699 cm^{-1} respectively with a slight difference between them but in (L11) FT-IR spectrum observed sharp band at (673) cm^{-1} due to the stretching for (C-Cl) bond They do not exist in (L1) FT-IR spectrum. Moreover, they can be characterized in H-NMR by disappearance of Azomethine signal for (B1) at 8.59 and appearance the signal of (CO-CH, Azetidione ring) at δ 3.815 in (L1), but for (L11) at δ 4.319 due to the presence electron withdrawing group (Cl) that which shifts the proton toward downfield (higher ppm) or De shielding, Muhammad et al., [2014]. The signal of (N-CH-) appearance in these derivatives at δ 5.992, 3.833 respectively also the signal of (OH) group were Observed at different loca-

tions in B1, (L1) and (L11) at δ 9.78, 8.6, 10.1 respectively. The chemical shift of the OH proton is changing and furthermore relies upon the degree of association through hydrogen bonding. In general, the stronger the association, the lower the field strength required to induce resonance [Raymond et al., 2007]. In the second line comp. N1 was used to prepare the Azetidione derivatives in several steps Starting the reaction of (N1) with Chloroacetyl chloride, Et₃N in DMF as solvent with stirring at room temperature to produce the chloroacylated derivative compound (N2) that which confirmed by disappearance the aromatic amine band at 3487-3427 cm^{-1} in N1 and appearance the bands of (NH sulfon, NH amide) str. at (3371, 3321) cm^{-1} respectively in addition the appearance of carbonyl amide band (O=C-CH₂Cl) str. at 1697 cm^{-1} . This value is high

Attributed to the link Chloride atom, which are characterized as strong electron-withdrawing group Close to the carbonyl amide group. in H-NMR spectrum can confirmed comp. (N2) by disappearance the signal of amine group at δ 6.048 and appearance the signals at δ 4.338 due to the (2H, CH₂-Cl), δ 10.764 due to (N-H) amide and δ 11.814 due to (N-H) Sulfone amide. Further-

more it the signal of carbon (CH₂-Cl) observed at δ 43.3 and carbon carbonyl amide at δ 165.5 in C¹³-NMR spectrum. The proposed mechanism for this reaction in Scheme 5, analysis C¹³-NMR of (B1) observed the signal of C (N=CH) Schiff Base at δ 130.253 but disappearance it and Instead of appearance the signal of Carbonyl lactam ring at δ 172.445 for (L1) derivative.



Scheme 5: The proposed mechanism for preparation Comp. (N2)

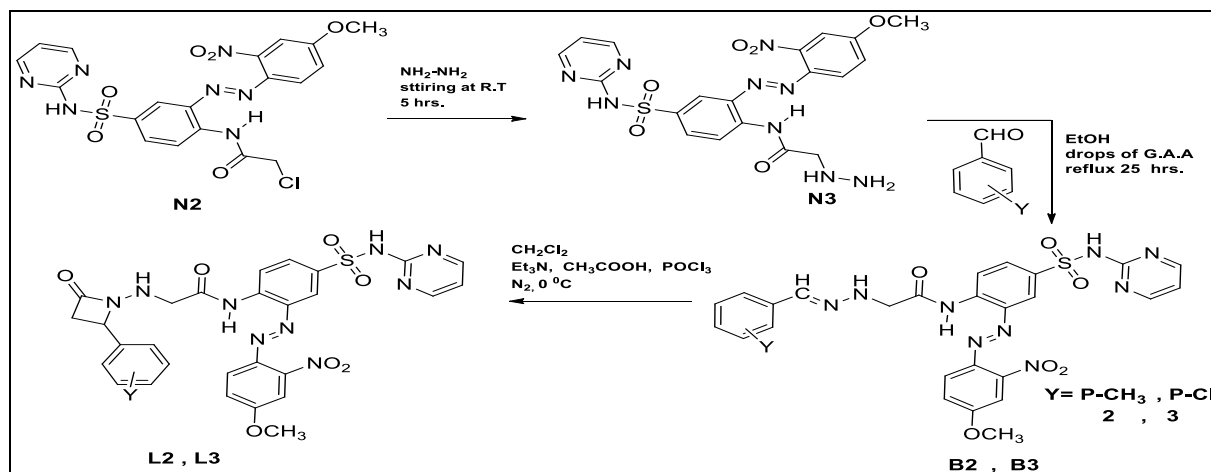
At another step the comp. (N2) was reacted with hydrazine hydrate(99%) at room temperature through SN₂ mechanism [Lafta et al., 2012] to give the comp. (N3) that was distinguished by appearance the two bands Which are attributed to the amine group at 3444-3435Cm⁻¹ and a decrease the wave number of carbonyl amide from 1697Cm⁻¹ in comp. (N2) to 1668Cm⁻¹ Due to the loss of the chloride group. appearance the bands of (N-H) str. Sulfone at 3377, (N-H) str. a mide at 3313 and (NH-NH₂) str. at 3230 Cm⁻¹. The comp. (N3) also can confirmed it in H-NMR spectrum by appearance the signals of NH₂, (NH-NH₂) at δ 4.072, 8.523 respectively [Mahdi et al., 2015] and signal of CH₂ for the acyl group was displace to the up field(shielding region)at δ 2.135 while it observed at downfield (de shielding region)due to its proximity of an electronegative atom(Cl) in comp. (N2) .Then two Schiff Bases (B2 , B3) were prepared from the comp. (N3) with (p- Methyl , p- Chloro Benzaldehyde) respectively in Absolute Ethanol as sol-vent and a few drops of Glacial Acetic Acid for protonation the carbonyl group in an aldehyde. Schiff Bases (B2, B3) was characterized by disappearance the stretching frequency of an amine group and appearance an Azomethine group band at 1608, 1625Cm⁻¹ in addition to appear the carbonyl amide 1668, 1680Cm⁻¹ respec-

tively. The wave number increasing for Imine and carbonyl amide in comp.(B3) than the comp. (B2) may be due to the effect of the electron-withdrawing by (Cl) in comp. (B3) by HNMR can characterization the comp (B3) via appearance the signal which back to (2H, CH₂) Nearby to the carbonyl group and (NH) as singlet at δ 3.71, singlet signal at 3.833 due to 3H,OCH₃.the proton of an Imine group (1H,CH=N)observed at 9.763 ppm [Abdulhamid et al., 2014] and the protons of different(N-H)groups (near to the Imine group, amide and sulfonamide) showed at 10.249 , 10.254, 10.290ppm respectively. Finally, Azetidione derivatives (L2, L3) were prepared from these Schiff Bases through [2+2] cyclo addition in a similar manner as the derivative (L1) under N₂ gas. The purpose of using inert nitrogen gas in some of the organic reactions to remove their components water and oxygen so this prevent the organic compounds from reacting with these components [Duward 1986] and also to raise the ratio of the product [Mori 2009]. Phosphoryl Tri chloride (POCl₃) is hydrolyzed quickly in water or moist air forming hydrochloric acid and phosphoric acid. to prevent this is used inert nitrogen Where it is a separating layer between the reactants and moist air [Michał et al., 2010].

$$\text{O}=\text{P}(\text{Cl})_3 + 3 \text{H}_2\text{O} \rightarrow \text{O}=\text{P}(\text{OH})_3 + 3 \text{HCl}$$

The FT-IR spectra of two Azetidinone derivatives (L2, L3) which showed the absorption of carbonyl Azetidinones (N-C=O) at (1741, 1739) cm^{-1} is an excellent evidence for the formation of these derivatives. By $^1\text{H-NMR}$ spectrum can also confirm the formation of Azetidinone derivative (L2) by appearance the signal protons [7] NH-Azetidinone ring at δ 9.139, CH_2 -acyl group at δ 4.580 and protons (N-CH-Ar, O=C- CH_2) Azetidinone ring

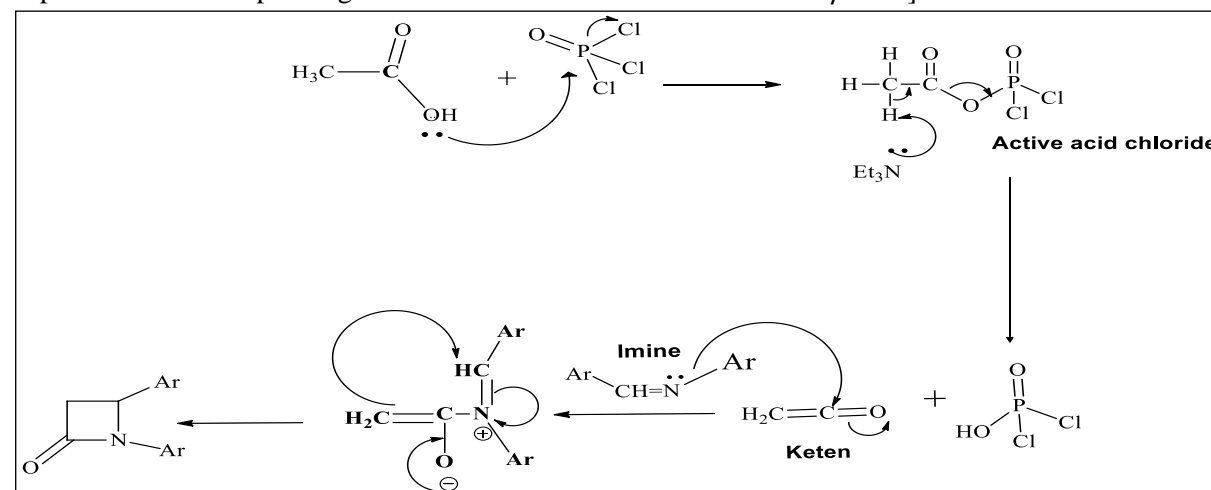
at δ 3.655-3.547, 3.257 respectively. The mass spectrum of (L2) showed the molecular ion peak corresponding to the particularly derivative $[\text{M}^+]$, $m/z = 644.9$. In all Azetidinone derivatives The reaction between an imine and ketene occur simultaneously by Mechanism of the pericyclic reaction which include The breaking and formation of bonds therefore the reaction occur via a single cyclic [Mahdi et al., 2015].



Scheme 6: preparation of Azetidinone derivatives from Comp. (N2)

The role of POCl_3 in the reaction is formation. The active acid chloride which reacted with Et_3N to produce the corresponding ketene *in situ* then

reacts with imine to furnish the corresponding Azetidinone derivative in Moderate ratio [Magt-oof Altamemey 2012].



Scheme 7: Mechanism of formation of the Azetidinone derivative

Table 2: The physical properties of prepared derivatives

Comp. No.	m.p. ^o C	Yield%	Color	M .Wt	M.F	Rf of T.L.C.
N ₁ AZO	110-112	74	Fire brick	429	C ₁₇ H ₁₅ N ₇ SO ₅	—
N ₂	81-84	50	Maroon	505.5	C ₁₉ H ₁₆ N ₇ SO ₆ Cl	0.67(Met: Tol)1:4
N ₃	120-123	40	Red	501	C ₁₉ H ₁₉ N ₉ O ₆ S	0.59(Met: Tol)2:3
B ₁	95-97	61	Dark red	563	C ₂₅ H ₂₁ N ₇ O ₇ S	0.82(Met:Tol) 2:3
L ₁	65-67	52.5	Maroon	605	C ₂₇ H ₂₃ N ₇ O ₈ S	0.82(Met: Tol)4: 1
L ₁₁	73-75	47	Maroon	639.5	C ₂₇ H ₂₂ N ₇ O ₈ SCI	0.80(Met: Tol) 2:3
B ₂	87-89	45	Sienna	603	C ₂₇ H ₂₅ N ₉ O ₆ S	0.6(Met: Tol)1:4
L ₂	Oily	46.5	Yellow	645	C ₂₉ H ₂₇ N ₉ O ₇ S	0.73(Met: Tol) 1:4
B ₃	114-116	48	sienna	623.5	C ₂₆ H ₂₂ ClN ₉ O ₆ S	0.67(Met: Tol) 1:4
L ₃	Oily	45	yellow	665.5	C ₂₈ H ₂₄ ClN ₉ O ₇ S	0.54(Met: Tol) 1:4

Table 3: FT-IR bands of prepared compounds.

Comp.	Structure	Table (3) FT-IR bands of prepared compounds FT-IR(Cm ⁻¹)
N1		(NH ₂) str. (3487-3427), AZO(1417),(C=C) str. Aromatic (1585), (N-H) str. Sulfone (3389),(C=N) str. Pyrimidine(1647),(NO ₂)(1508-1332) ,(C-O) 1217, (C-H) str. Aromatic(3039), (C-H) str Aliphatic (2873-2939), SO ₂ (1251) .
N2		(N-H) str. Sulfone amide (3371), (N-H) str. a mide (3321), (C-H) str. Aromatic(3037), (C-H) str Aliphatic (2939-2868), (C=O) amide str. (1697) , (C=N) str. Pyrimidine interaction with (C=C) str.Aromatic (1585) ,(C-NO ₂)(1516-1336) ,Azo(N=N)(1463-1438), SO ₂ (1251), (C-O) 1219,(C-Cl) 665.
N3		(NH ₂) str. (3444-3435), (N-H) str. Sulfone (3377), (N-H) str. a mide (3313), (NH-NH ₂) str.(3230), (C-H) str .Aromatic(3091-3039), (C-H) str. Aliphatic (2931-2848),(C=O) amide (1668) ,(C=N) str. Pyrimidine interaction with the (C=C) str.Aromatic (1581), (C-NO ₂)(1527-1340),Azo(1440-1411), SO ₂ (1249), (C-O) (1153)
B1		,(OH) str. (3489),(N-H) str. Sulfone amide (3373), (C=N) str.imine (1674), (C-H) str Aliphatic (2933-2850), (C=N) str. Pyrimidine str.(1643), (C=C) str.Aromatic (1595-1575), ,(C-NO ₂)(1512-1338),Azo(1463-1428), SO ₂ (1252), (C-O) (1213)
L1		,(OH) str. (3487),(N-H) str. Sulfone a mide (3371), (C=O) str. Azetidinone ring (1707), (C-H) str Aliphatic (2926-2856), (C=N) str. Pyrimidine str.(1645), (C=C) str.Aromatic (1593-1585), ,(C-NO ₂)(1512-1338),Azo(1462-1427), SO ₂ (1251) , (C-O) (1217).

L11		,(OH) str. (3431),(N-H) str. Sulfone a mide (3373), (C=O) str. Azetidinone ring (1699), (C-H) str Aliphatic (2972-2941), (C=N) str. Pyrimidine str. interaction with (C=C) str. Aromatic (1591), ,(C-NO ₂)(1517-1350) ,Azo(1465), SO ₂ (1276),(C-Cl) str. (673).
B2		(N-H) str. Sulfone (3448), (N-H) str. a mide (3421), (NH-N=CH-) str.(interact with(N-H) str. a mide), (C-H) str Aliphatic (2922-2852),(C=O) amide (1668) ,(C=N) str. Pyrimidine(1637),(C=N) Imine str. (1608), (NO ₂)(interact with (C=C) 1490-1377),(C=C)str. Aromatic(1490-1463),Azo(1440-1425), SO ₂ (1288), (C-O) (1222)
L2		(N-H) str. Sulfone (3412), (N-H) str. a mide (3388), (NH-N=CH-) str.(interact with(N-H) str. a mide), (C-H) str Aliphatic (S 2927-2856),(C=O)Azetidinone ring (S 1741) ,(C=O) amide (1668) ,(C=N) str. Pyrimidine(1637), (NO ₂)(1517,1354),Azo(1462), (C-O) (1176).
B3		(N-H) str. Sulfone (3373), (C-H) str Aliphatic (2918-2850),(C=O) amide (1680) ,(C=N) str. Pyrimidine(1643),(C=N) Imine str. (1625), (NO ₂ (1506-1338) ,(C=C)str. Aromatic(1593-1573),Azo(1487-1475), SO ₂ (1280), (C-O) (1251) .
L3		(N-H) str. Sulfone (3421), (C-H) str Aliphatic (2926-2858) , (C=O) str. Lactam(1739),[(C=O) amide str.,(C=N) str. Pyrimidine] interference with the carbonyl lactam, (C=C)str. Aromatic(1500) ,Azo(1458), SO ₂ (1288), (C-O) (1242) .

1-ANTICANCER ACTIVITY

Statistical analysis: Statistical analyses were performed using SPSS 21.0 for windows. Inc. Data were expressed as Mean \pm SEM, Two-tailed T test unless otherwise stated by ANOVA test. In all tests, $P < 0.05$ was considered statistically significant R^2 was calculated by Pearson Correlation Coefficient. The halve inhibitory concentration (IC_{50}) of different composites was

calculated by Graph Pad Prism 6. The anticancer activity of the synthesized derivative (L2) *in vitro* was determined against an *Homo sapiens*, human ,Colon cancer (Disease: Dukes' type B, colorectal adenocarcinoma) LS-174Tcell line using MTT reagents assay Compared to anti-cancer drugs which are Methotrexate(MTX)&Doxorubicin (DOX). The results were as follows

Table 4: The percentage of Inhibition and (IC_{50}) for the derivative(L2)

(x) =Conc.of (L2) $\mu\text{g} / \text{ml}$	0.5	1	10	100	1000	2000	3000	4000	5000	10000
Log x	0	0	1	2	3	3.3	3.5	3.6	3.7	4
Inhibition %	42.8	51.3	53.8	51.6	54.9	60.8	61.7	69.2	81.8	90.6
IC ₅₀ = 1386 $\mu\text{g} / \text{ml}$ = 1. 386mg/ ml										

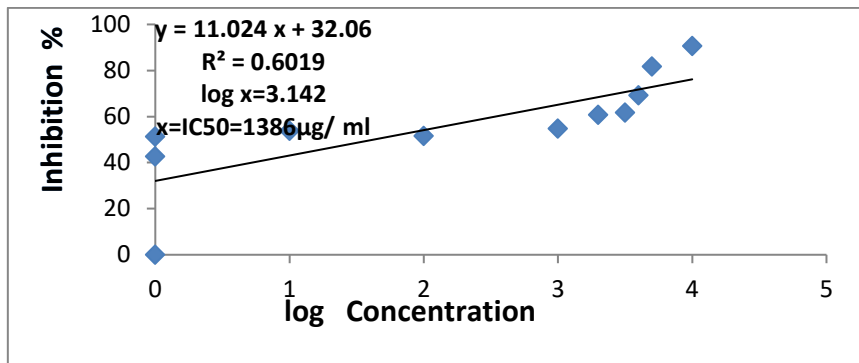


Figure 1: IC50 of derivative (L2)

Table 5: The percentage of Inhibition and (IC50) for the Drugs MTX & DOX

	MTX					DOX				
Conc. of MTX & DOX Drugs µg / ml (x)	0.5	1	10	50	100	0.5	1	10	50	100
Log (x)	0	0	1		2	0	0	1	1.698	2
Inhibition %	32.1	36.2	39.0	47.1	70.3	30.8	34.2	33.2	74.1	73.1
IC50	29.919 µg / ml = 0.03 mg / ml					15.634 µg / ml = 0.0156 mg / ml				

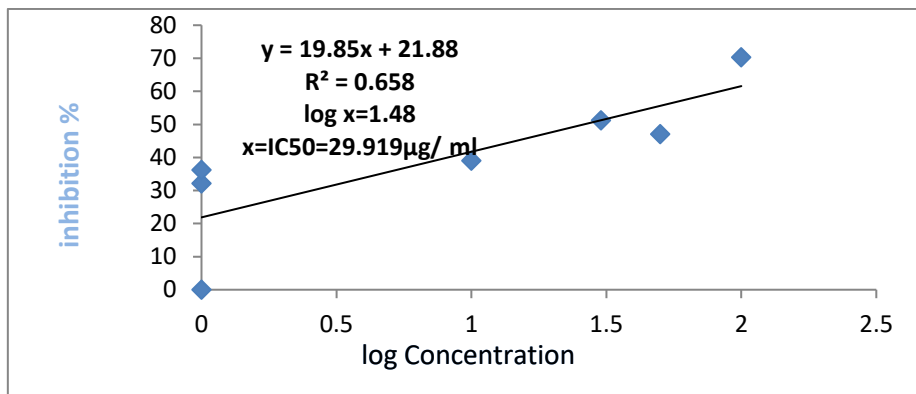


Figure 2: IC50 of MTX Drug

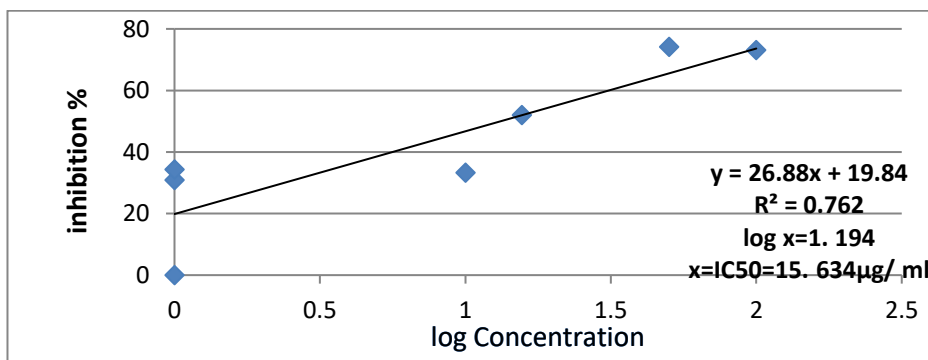


Figure 3: IC50 of DOX Drug

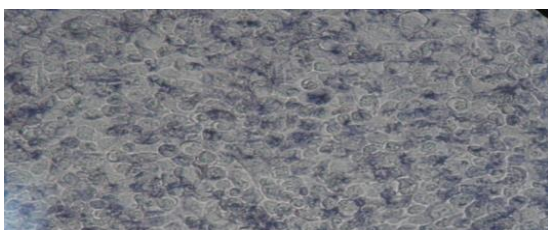


Figure 4: control untreated cell without MTT



Figure 5: control untreated cell with MTT



Figure 6: treated cell with sample after MTT addition

2-ANTIBACTERIAL ACTIVITY: Monobactams and carbapenams are monocyclic 2-azetidinones, which are possessing biological activity only

against Gram-negative bacteria [Accardo et al., 2014]. However, the synthesized derivatives exhibited activity against both Gram- negative and Gram- positive bacteria. The biological activity of some Azetidinone derivatives was examined in this work against four types of bacteria *Staphylococcus aureus*, *Bacillus* are Gram + whilst *Proteus mirabilis*, *Enterobacter* are Gram- compared with antibiotic Amoxicillin (25µg) that did not give any inhibition against the four types of bacteria used. Two concentrations of each derivative were prepared (1000, 250ppm) and the results were shown in the table 6.

Table 6: Inhibition zones

Conc. PP M Comp.	<i>Staphylococcus aureus</i> (Gram +)		<i>Bacillus subtilis</i> (Gram +)		<i>Proteus mirabilis</i> (Gram -)		<i>Enterobacter</i> (Gram -)	
	1000	250	1000	250	1000	250	1000	250
L1	1mm	4mm	-----	10mm	-----	3mm	-----	10mm
L11	2mm	4mm	---	----	5mm	4mm	5mm	5mm
Amoxicillin 25µg	Did not affect							

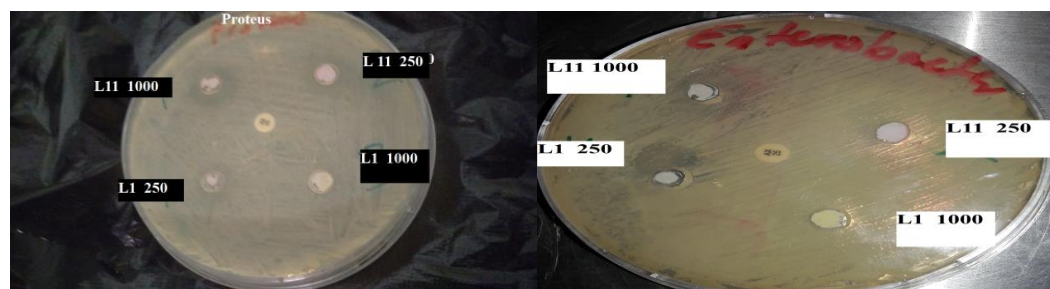


Figure 7: Antibacterial activity

Also the derivatives (L1, L11) were screened against the Fungal *Candida albicans* which is a type of yeast. These derivatives gave good inhibition at (1*10⁻⁴) Molar for each derivative Where the amount of inhibition zone was about (10.5 , 17) mm for (L1, L11) respectively.

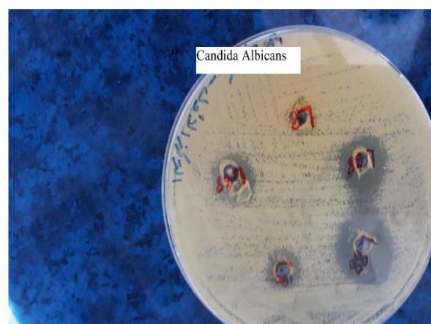


Figure 8: Antifungal activity

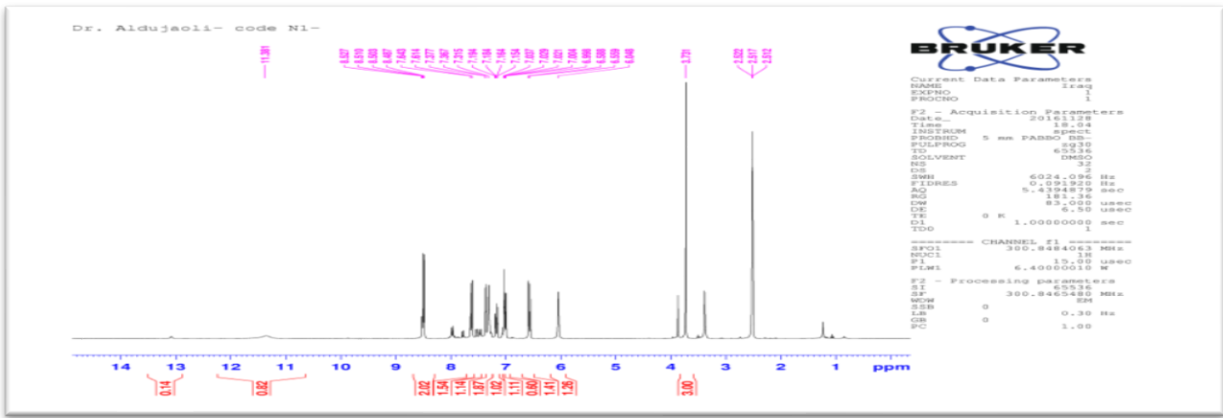


Figure 9: ¹H-NMR of Azo comp. (N1)

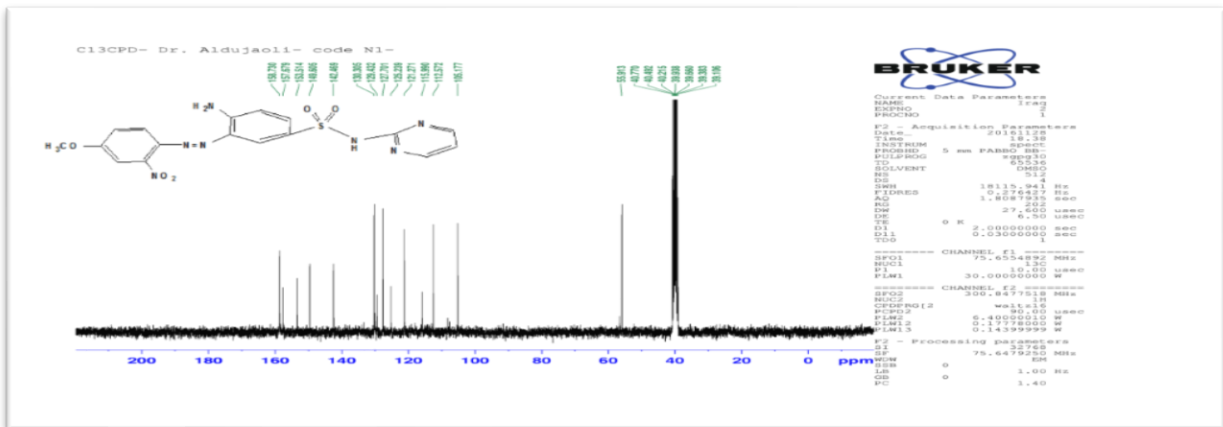


Figure 10: ¹³C-NMR of Azo comp. (N1)

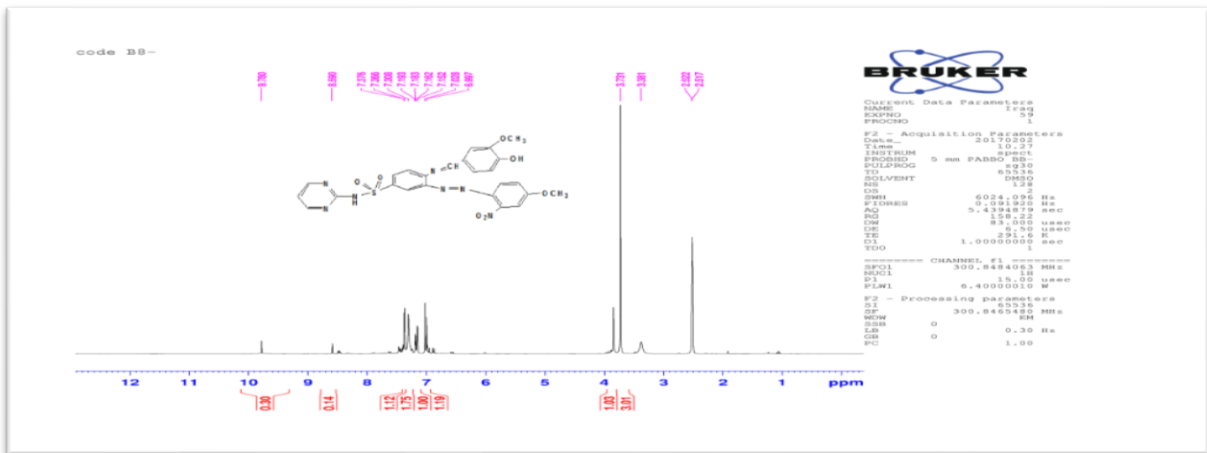


Figure 11: ¹H-NMR of Schiff base(B1)

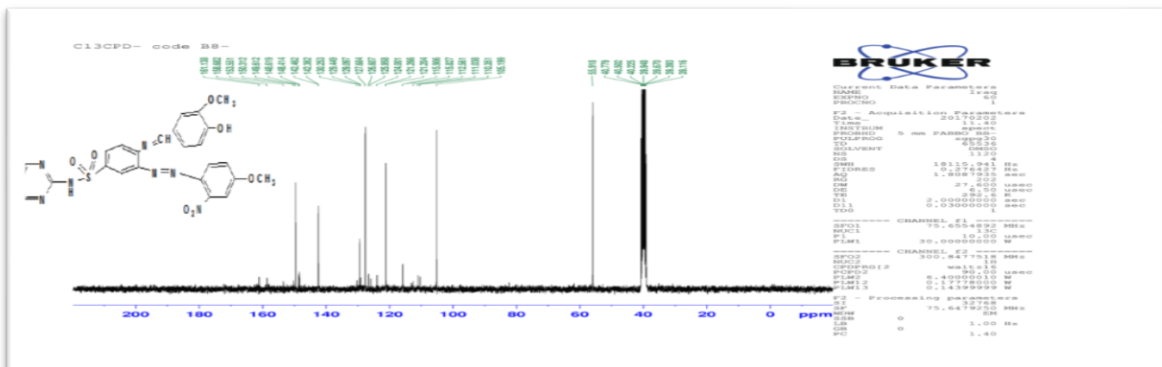


Figure12: C¹³-NMR of Schiff base (B1)

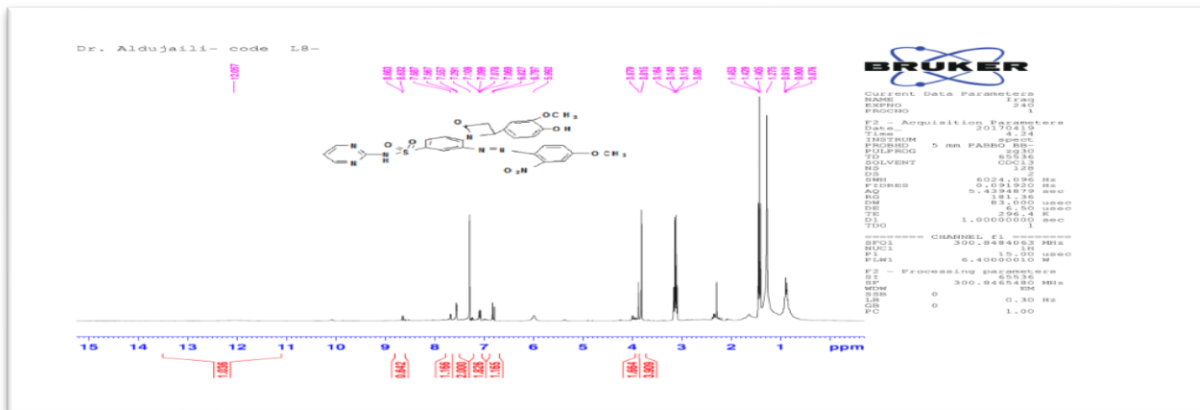


Figure13: H¹-NMR of Azetidinone derivative(L1)

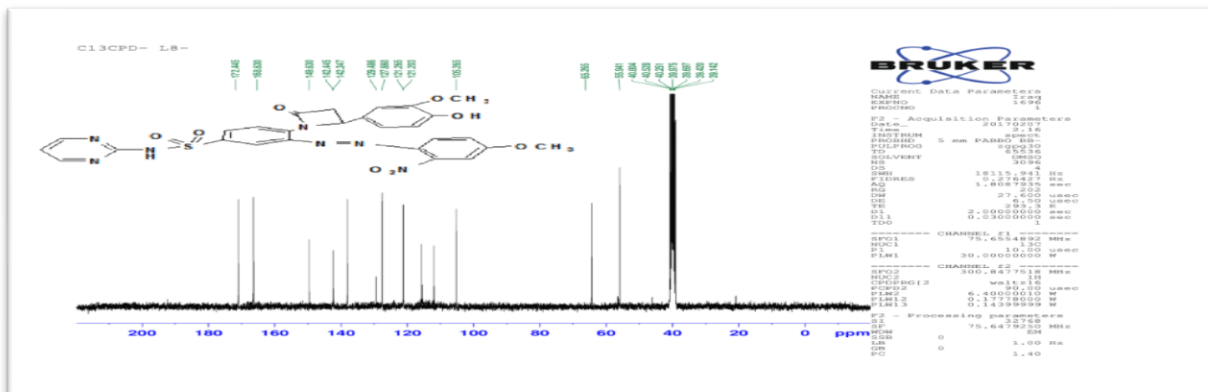


Figure 14: C¹³-NMR of Azetidinone derivative(L1)

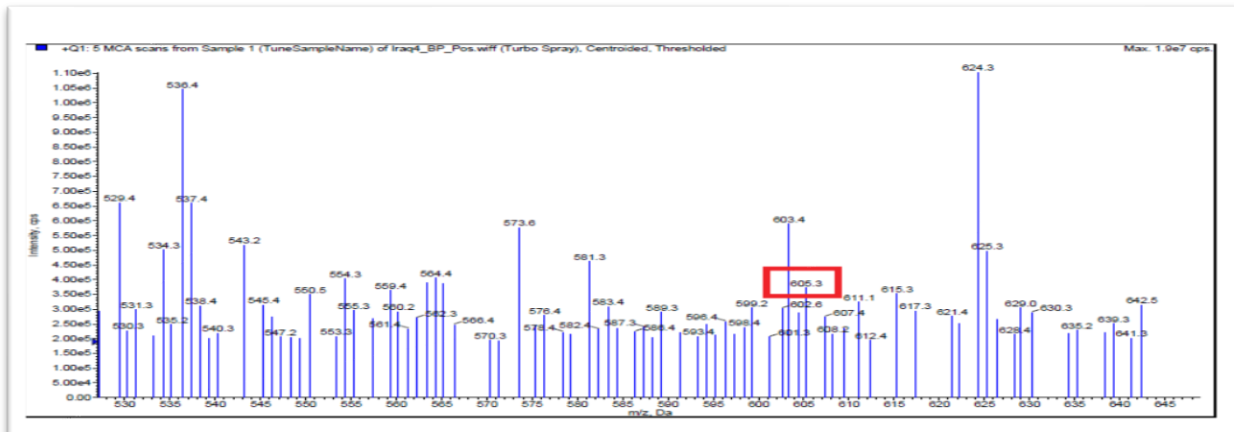


Figure 15: Mass spectrum of Azetidinone derivative(L1)

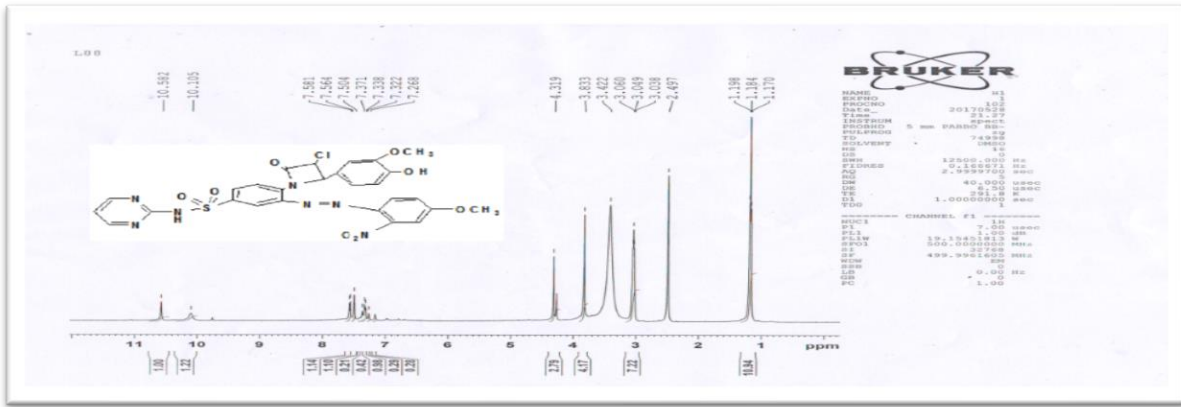


Figure 16: H-NMR of Azetidinone derivative (L11)

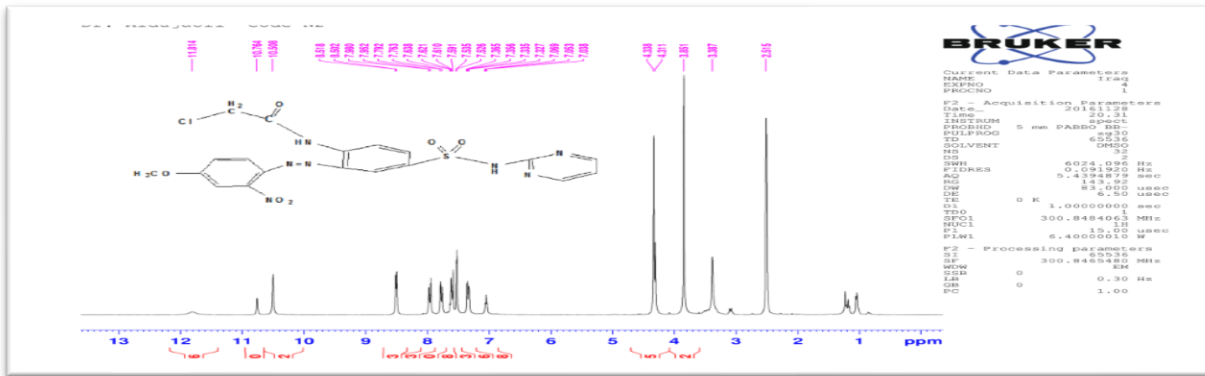


Figure17: H¹-NMR of comp.(N2)

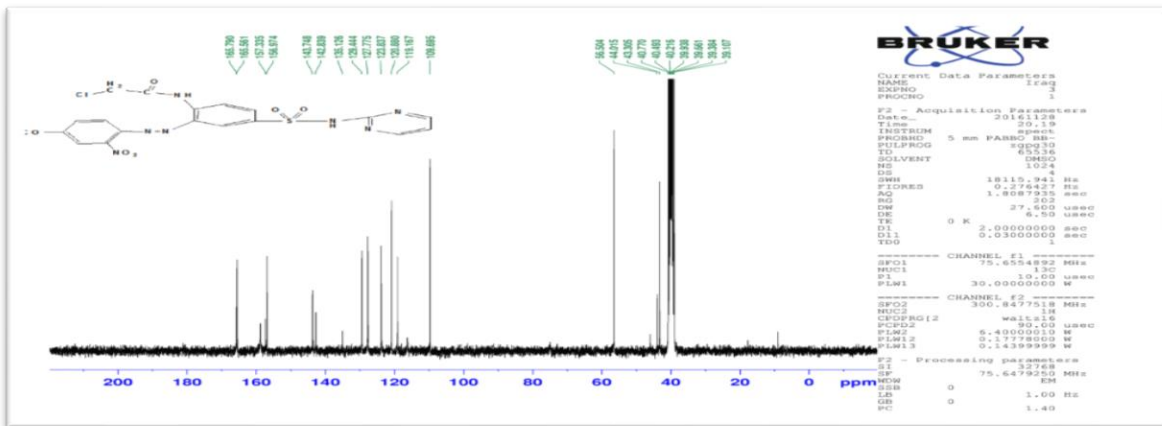


Figure18:C¹³-NMR of comp.(N2)

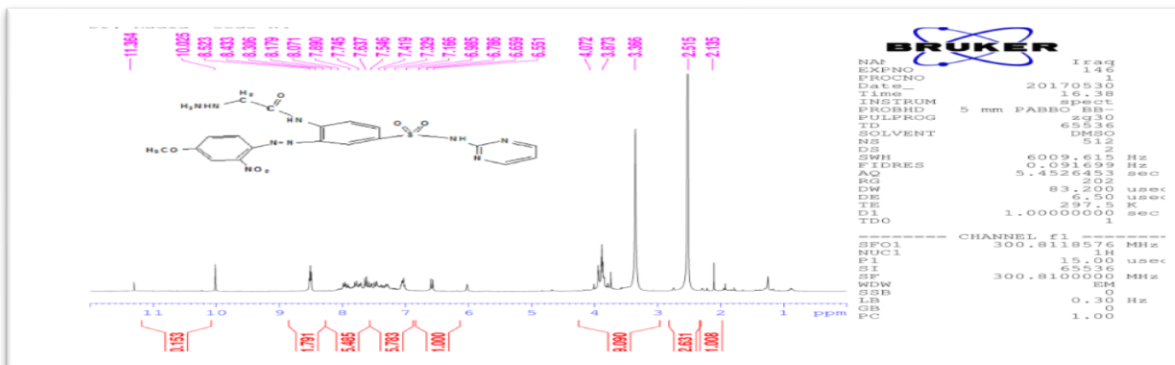


Figure 19: H¹-NMR of comp.(N3)

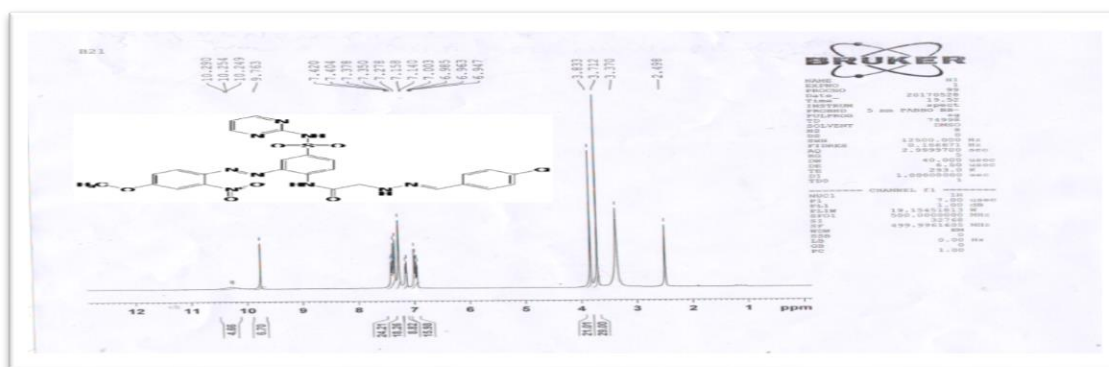
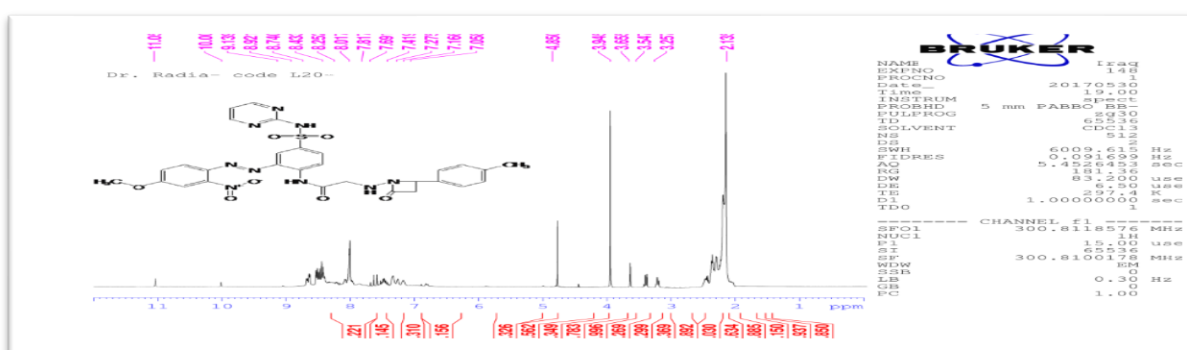
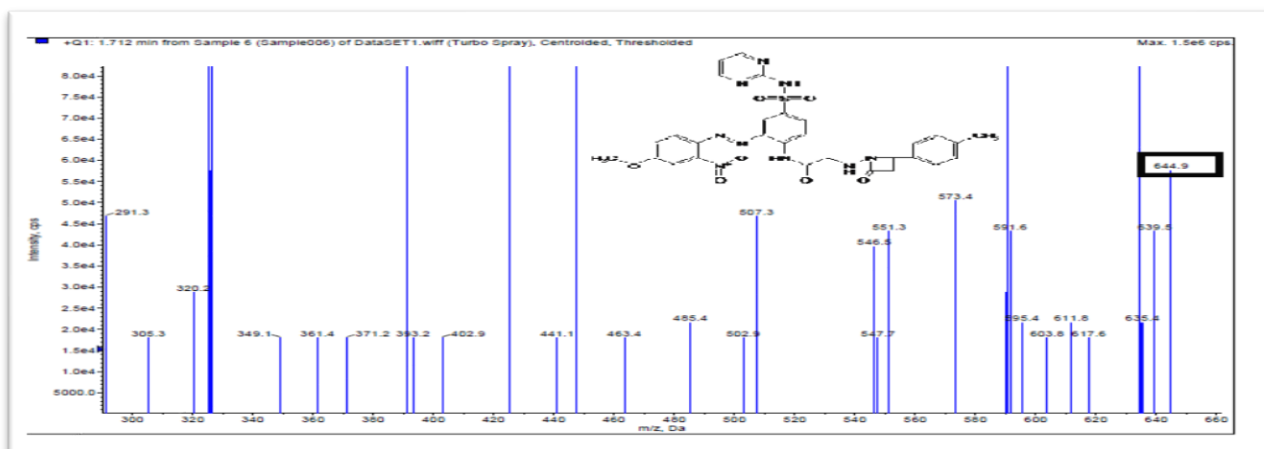
Figure 20: ^1H -NMR of comp.(B3)Figure 21: ^1H -NMR of Azetidinone derivative(L2)

Figure 22: Mass spectrum of Azetidinone derivative(L2)

CONCLUSION

In this work we report the synthesis of new Azetidinone derivatives via Staudinger Reaction [2+2] cyclo addition starting from sulfadiazine drug. the FT-IR, ^{13}C -NMR, ^1H -NMR and Mass Data for some of them gave good evidence for the formation of the prepared derivatives. These derivatives have relatively good stability due to high resonance in it, as well as acceptable the products ratios. The β -Lactam derivative (L1) Showed good antibacterial activity against *Enterobacter* and *Bacillus subtilis* bacteria but

the derivative (LII) Posses high inhibition as anti-fungal.

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