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# Synthesis, Characterization and Study the Biological Activity of New Morpholine Derivative

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## Abstract:

A new series of morpholine derivative were prepared by reacting the morpholine with ethyl chloro acetate in the presence triethylamine as an catalyst and benzene as a solvent gave the ethyl morpholin-4-ylacetate reaction with hydrazine hydrate and ethanol as a solvent gave the 2-(morpholin-4-yl)acetohydrazide gave series of Schiff base were prepared by reacting 2-(morpholin-4- yl)acetohydrazide with different aromatic aldehydes and ketons . The new series of (3-9 )were synthesis by reaction of Schiff base (10-14) with chloroacetyl chloride, triethyl amine as an catalyst and 1,4dioxane as a solvent .The chemical structures of the synthesis compound were identified by spectral methods their [ IR ,$^{1}$H-NMR and $^{13}$C-NMR ].The synthesised compounds were screened for antibacterial activity and antifungal activity promising by disc diffusion method by measuring the zone of inhibition and the results were compared to standard drugs ciprofloxacin .

**Key words:** Morpholine, Schiff bases, Anibacterial, Antifungal**.**

## Introduction:

Morpholine is a six-membered heterocyclic compound [1] and an organic chemical compound having the chemical formula O $(CH_2CH_2)_2NH$. This heterocyclic structure features both amine and either functional groups. Because of the amine group, morpholine is a base; its conjugate acid is called as morpholinium [2]. Morpholine derivative plays an important role in the treatment of several diseases. Heterocyclic ring systems having morpholine nucleus have aroused great interest in recent years due to their variety of biological activities and medicinal activities Substituted morpholine derivatives are the core of various natural products and biologically active compounds. Thus, morpholine has been used in the production of many types of therapeutic agents such as antibacterials, antimicrobials, anticancers, antitussives, antimalarials, anticonvulsants and analgesics [3]. Schiff base are condensation products of primary amines with carbonyl compounds also know as imins or Azo methine is nitrogen analogue of an aldehyde or ketone in which the

carbonyl group (C=O) is replaced by an imine or azomethine group[4,5,6]. Schiff base are associated with antibacterial, antifungal and antitubercular activities and have diverse biological activities [7].Schiff base possess diversified biological application[8]. the strategy of using a β- lactam inhibitor in combination with β- Lactamase -sensitive penicillin in the therapy for infections caused by β-Lactamase-producing bacterial strains [9]. A large number of 3-chloro monocyclic β- Lactams having substitution at position 1 and 4 posses powerful antimicrobial ,anticonvosant and anti-tubercular activity[10].

## Materials and Methods:

Chemicals used in this work are supplied from Merck, BDH, sigma Aldrich ND Fluka companies and are used without further purification. Melting points were recorded using digital Stuart scientific SMP3 melting point apparatus and are uncorrected. FT-IR spectra were recorded on SHIMAZU FT-IR-8400 Fourier transform Infrared spectrophotometer using KBr discs in the (4000 - 6000) $cm^{-1}$ spectral range. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on Burker 500M Hzistrument using DMSO-$d_6$ as solvent and TMS as internal reference.

**Experimental:**

**Synthesis of Ethyl morpholin-4-ylacetate (1)**

A mixture of morpholine (9ml ,0.1mol), ethylchloro acetate(12 ml ,0.1mol) and triethyl amine(10 ml, 0.1mol) with dry benzene in 100ml round bottom flask were refluxed for 6 hrs at $115^{0}$c.The resultant reaction mixture was cooled and filteredand recrystallized from aqueous
Ethanol. Physical properties are listed in Table (1) , FT-IR spectral are listed in Table (2) and in Figure(3) ,$^{1}$H-NMR spectral showed in table(3) and in Figure(2) ,$^{13}$C-NMR spectral showed in table(4) and in Figure(7).

## Synthesis of 2-(morpholin-4-yl) acetohydrazide (2)

A mixture of Ethyl morpholin-4-ylacetate (10ml.0.05mol), hydrazine hydrate 80% 2.8ml, 0.05mol) in ethanol (20ml) was refluxed for 6 hrs . The resultant mixture excess of ethanol was removed by distillation. On cooling, from the resultant mixture, white crystal of 2-(morpholin-4-yl) acetohydrazide, It was collected and then recrystallized from ethanol, Physical properties are listed in Table (1), FTIR spectral are listed in Table (2) .

## General Method for the synthesis of Schiff base [3-9](3)

A mixture of 2-(morpholin-4-yl) acetohydrazide (2ml, 0.01mol was dissolved in minimum quantity of ethanol), {A}(2 ml, 0.01mol) and (1ml) glacial acetic acid and heated on a steam bath for 7hrs . The resultant solution was cooled in ice bath. The separated solid was filtered and recrystallized from ethanol Physical properties are listed in Table (1) , FTIR spectral are listed in Table (2) and in Fig.(5,7) , $^{1}$HNMR spectral showed are listed in table(3) and in Figures(4,6) , $^{13}$CNMR spectral showed in table(4) and in Figures(8,9).

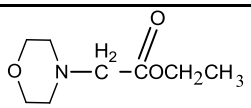
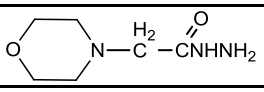
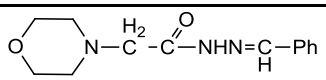
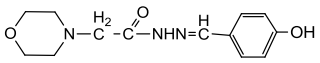
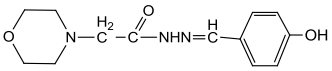
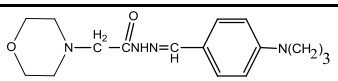
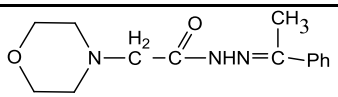
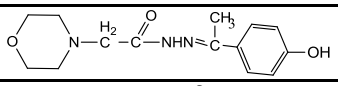
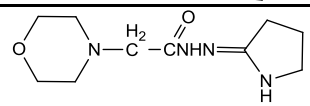
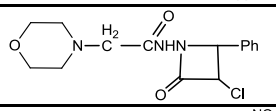
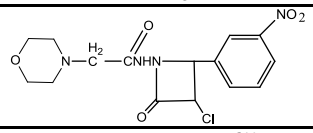
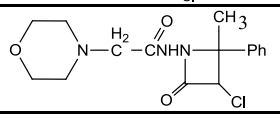
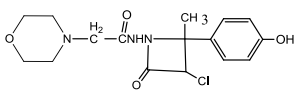
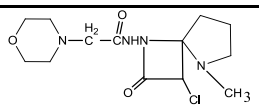
{A}=(benzaldehyde, 4-hydroxy benzaldehyde, 3- nitro benzaldehyde, 4- dimethylamino benzaldehyde, acetophenone, 4-hydroxy acetophenone, 1-methyl-2-pyrrolidone).

**General Method for the synthesis of β-Lactam (10-14) [4]**

A mixture of compounds (3-9) (0.002mol) and triethylamine (0.004mol) were dissolved in dry dioxane (25ml). Chloroacetyl chloride

(0.004mol) was add dropwise at $10^0$c. The mixture was stirred for 24 hrs. The triethyl amine hydrochloride precipitate, formed was filtered and washed several time with dry 1,4dioxan and filterete. Half of the solvent was removed by distillation .The residue was poured into crushed ice and the crude product obtained was recrystallized from ethanol , Physical properties are listed in Table (1) , FT-IR spectral are listed in Table (2) and shown in figures (10.11).

**Table (1):- The physical properties 0f prepared compound (1-14)**

| Compd. No. | Nomenclature | Structure formula | Yield % | Color | M. P. °C |
|---|---|---|---|---|---|
| 1 | morpholin-N-ethyl acetate |  | 70 | Deep - Brown | 232-233 |
| 2 | morpholin-N-acetohydrazide |  | 78 | White | 105-106 |
| 3 | N\-benzylidene-morpholin-N-acetohydrazide. |  | 82 | Yellow | 82-83 |
| 4 | N\-(4-hydroxybenzylidene)-morpholin-N- acetohydrazide |  | 55 | Light - Brown | 338-339 |
| 5 | N\-(3-nitro benzylidene)-morpholin-N-acetohydrazide |  | 59 | Light - Green | 221-222 |
| 6 | N\-(4-dimethylamino benzalidene)-morpholin-N-acetohydrazide |  | 66 | Dark Red | 229-230 |
| 7 | N\-(1-phenyl ethylidene)-morpholin-N-acetohydrazide |  | 93 | Off - White | 106-107 |
| 8 | N\-[1-(4-hdroxyphenyl)ethylidene ]--(morpholin-N-acetohydrazide |  | 76 | Yellow | Oil |
| 9 | N\ -(1-methylpyrrolidin-2-ylidene)-morpholin-N- acetohydrazide |  | 42 | Light-Yellow | 172-173 |
| 10 | N\-(3-chloro-2-oxo-4-phenylazetidin-1-yl)morpholin-N-acetamide |  | 45 | Brown | Oil |
| 11 | N\-[3-chloro-2-(3-nitrophenyl)-4-oxoazetidin -1-yl]morpholin-N-acetamide |  | 48 | Dark Brown | Oil |
| 12 | N\-3-chloro-2-methyl-4-oxoazetidin -1-yl]morpholin-N-acetamide |  | 39 | Brown | Oil |
| 13 | N\-[3-chloro-2-(4-hydroxyphenyl)-2-methyl-4-oxoazetidin -1-yl]morpholin-N- acetamide |  | 33 | Brown | Oil |
| 14 | N\-[3-chloro-2-oxo-4-(1-methyl pyrrolidine-2-yl)]morpholin-N-acetamide |  | 38 | Dark Brown | Oil |

## Antimicrobial Activity:

The compounds synthesis were screened for their antibacterial activity using was tested against geam (+) *(Staphylococcus aureus, Staphococcus epidermis, Bacillus)* and gram (-) (Escherichia coli, *Streptococcus pneumonia, Pseudomonas.* The activities of these compound were tested using disc diffusion method [3,5,8 , 11] at concentration using 5mm filter paper disc and filled with (100,50,25)mg/ml of compounds . The solvent (DMSO) was used as a negative control while ciprofloxacin (10µg/disc)was used as a positive control , plates were incubated at $37^0$C for 18-24 hr [12]. The area of inhibition of zone was measured .Compound (10-14) showed good antibacterial activity .The antifungal activity was tested against the fungal species *Candida* at 150ppm concentration. The antifungal data revealed the compounds (10-14) to be moderately active against the fungi. The antibacterial and antifungal activity data are given in Table 3. As shown in Fig.(1)

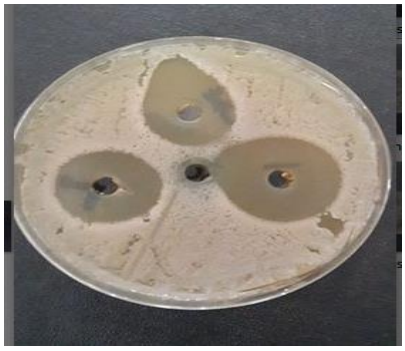
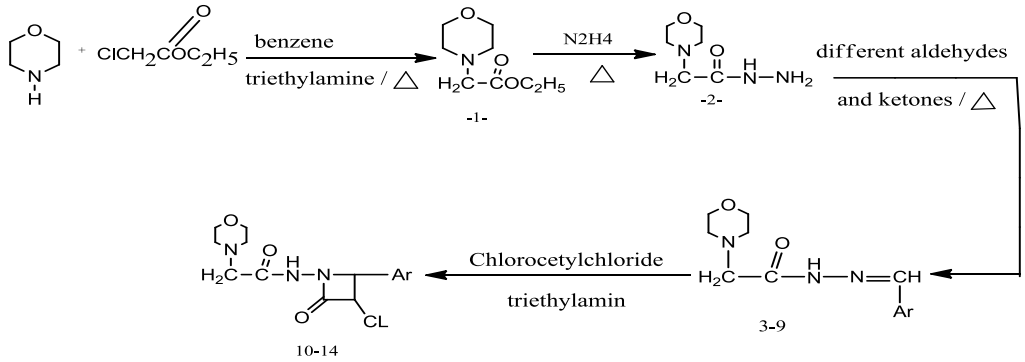


**Fig. (1):The Effect on *staphylococcus aureus***

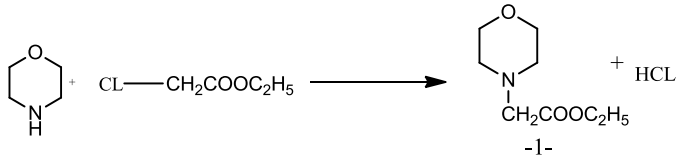
## Results and Discussion:

The synthesis sequences for preparation of series new morpholine derivatives are out lined in the following scheme (1,2 and 3):




**Scheme-1**

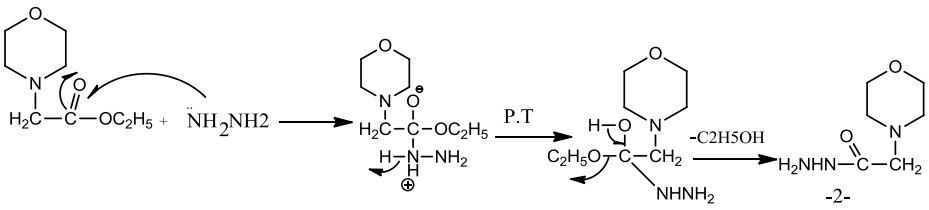
As starting material ethyl morpholin-4-yl acetate was prepared by reaction Morpholine with ethyl chloro acetate in the presence of triethyl amine and dry benzene as a solvent a according to the following equation :




**Scheme-2-**

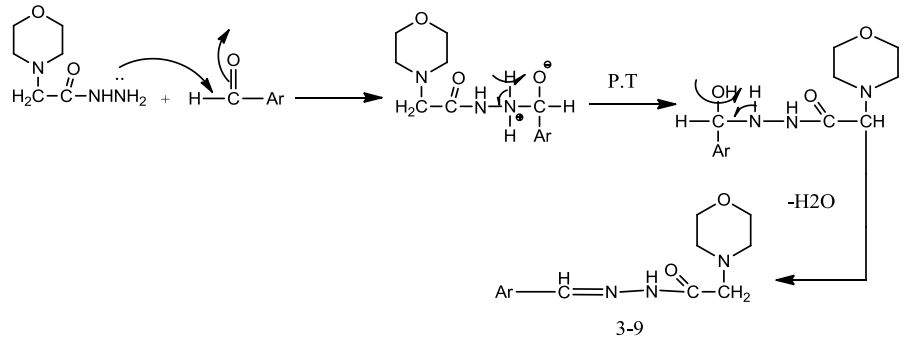
The physical properties are given in table(1) and FTIR spectral are given in table(2) .

The compound 1, on further treatment with hydrazine hydrate in ethanol, affords 2-(morpholin-4-yl) acetohydrazide [13] as shown in the following scheme3:




**Scheme-3-**

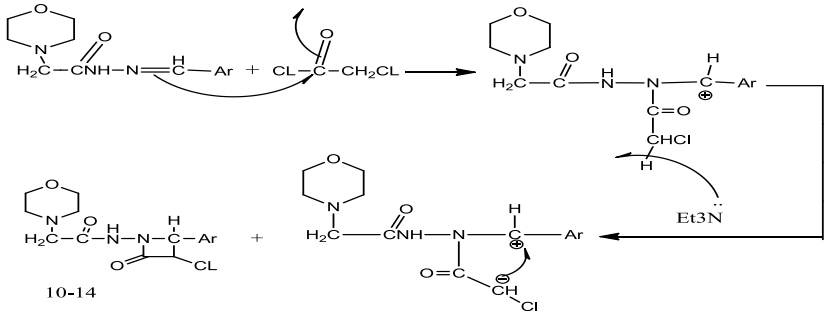
The compounds[3-9] were synthesized from the reaction between compound [2] and different substituted aromatic aldehydes/ketones in absolute ethanol and glacial acetic acid resulted in the formation of Schiff's bases[14,15] .as shown in following scheme 4:




**Scheme-4-**

The synthesis of Schiff's bases with different specific aldehydes in ethanol as a solvent and catalyst (glacial acetic acid) resulted in five new series of Schiff's bases with the general formula RHC =N-R1. Here R1= 2-( morpholin-4-yl)acetohydrazide R = benzaldehyde, 4-N,N-dimethyl benzaldehyde,4-hydroxybenzaldehyde, 3-nitrobenzaldehyde , acetophenone , 4-hydroxy acetophenone, 1-methyl-2-pyrrolidone . were synthesized by the reaction of 2-(morpholin-4--yl) acetohydrazide and substituted aldehydes in ethanol Such compounds were characterized by different physicochemical techniques like melting point, elemental analysis, FT-IR spectroscopy, and multinuclear NMR (1H), show scheme 4, the physical properties are given in table(1) and FT-IR spectral are given in table(2)

Schiff basses [3-9] in the next steps cyclized by treatment with chloro acetyl chloride followed by the addition of triethyl amine to give β-Lactam (10-14)[16] , as shown in the following scheme 5 :




**Scheme-5-**

The Synthesized compound were screened for antibacterial (*Escherichia coli, Staphylococcus aureus, Staphococcus epidermis, Streptococcus pneumonia, α strep, Bacillus, Pseudomonas),antifungal(Candida albicans)* and showed good antibacterial and antifungal activity

against all tested organisms by disc diffusion method . All the synthesized compound have shown to be more potent than ciprofloxacin ,show scheme 5, The physical properties are given in table(1) and FTIR spectral are given in table(2) and Antibacterial effect of β- Lactam are given in table 3.

**Table (2):- FTIR spectral data $cm^{-1}$ of the prepared compounds (1-14)**

| Com. No. | υ(C-H) $cm^{-1}$ Aromatic | υ(C-H) $cm^{-1}$ Aliphatic | υ(C=O) $cm^{-1}$ amide | υ(C=C) $cm^{-1}$ | υ(N-H) $cm^{-1}$ | υ(C-O-C) $cm^{-1}$ | υ(N-N) $cm^{-1}$ | Other Band $cm^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| 1 | _ | 2928 | 1743 ester | _ | _ | 1224 | _ | υ(C-O)1188 , υ(C-N)1307 |
| 2 | _ | 2992 | 1670 | _ | 3160 | 1220 | 1496 | υ($NH_2$) 3242 , υ(C-N)1410 |
| 3 | 3051 | 2955 | 1693 | 1572 | 3110 | 1288 | _ | υ(N=C)1622 , υ (C-N) 1369, υ(C-O)1211 |
| 4 | 3026 | 2945 | 1680 | 1514 | 3294 | 1257 | _ | υ(N=C)1680, υ(C-N)1388 υ(OH) 3483 |
| 5 | 3006 | 2970 | 1645 | 1479 | 3126 | 1296 | _ | υ(N=C)1610, υ(C-N)1257 υ C-NO21531 |
| 6 | 3055 | 2918 | 1653 | 1527 | 3120 | 1225 | 1435 | υ(C=N)1599, υ(C-O)1188 υ(N-C-O)1170 |
| 7 | 3112 | 2978 | 1690 | 1541 | 3192 | 1255 | _ | υ(C=N) 1650, υ(C-O)1116 υ(C-N)1338 |
| 8 | 3095 | 2992 | 1670 | 1566 | 3201 | 1286 | _ | υ(OH) 3450, υ(C=N) 1602 υ(C-O) 1124, υ(C-N)1363 |
| 9 | 2780 | 1683 | _ | _ | 3188 | 1278 | _ | υ(C=N) 1606, υ(C-O)1226 υ(C-N-O) cyclic1371, υ(C-N) 1512 |
| 10 | 3063 | 2960 | 1678 | 1604 | 3194 | 1236 | 1452 | υ(C-N) 1313, υ(C-Cl) 756 υ(N-C-O)1207 |
| 11 | 3030 | 2992 | 1694 | 1621 | 3102 | 1212 | 1491 | υ(C-N) 1410, υ(C-O) 1188, υ(N-C-O) 1201, υ(C-NO2) 1530, υ(C-Cl) 750 |
| 12 | 3033 | 2992 | 1690 | 1504 | 3120 | 1360 | 1430 | υ(C-N) 1380, υ(N-C-O)1207, υ(C=0)cyclic amide 1530, υ(C-Cl)730 |
| 13 | 3040 | 2980 | 1635 | 1510 | 3105 | 1203 | 1408 | υ(C-N) 1390, υ(N-C-O)1124, υ(C-Cl)790 |
| 14 | - | 2985 | 1630 | - | 3184 | 1222 | 1404 | υ(C-Cl)740, υ(N-C-O)1124, υ(C-N)340 |

**Table 3: $^{1}$H-NMR spectral data data ($^{\delta}$ppm) for selected compounds**

| Com. NO | $^{1}$H-NMR Spectral data ($^{\delta}$ ppm) |
|---|---|
| 1 | 1.2(S,2H,CH2-O);2.5(S,2H,CH2-N);3.7(S,3H,CH2-O);9.7(S,2H,CH2-C=O) |
| 4 | 2.2(S,2H,CH2-O); 3.2(S,2H,CH2-N);6.8(S,H,CH=);7.4(S,H,CHaromatic);8.3(S,H,NH-C=O);9.6(S,2H,CH2-C=O);11.8(S,H,OH) |
| 6 | 1.6(S,2H,CH2-O); 2.4(S,2H,CH2-N);2.9(S,3H,CH3);4.1(S,H,CH=)6.6(S,H,CHaromatic);7.6(S,H,NH-C=O);8.6(S,2H,CH2-C=O) |

**Table 4: $^{13}$C-NMR spectral data data ($^{\delta}$ppm) for selected compounds**

| Com. NO | $^{13}$C-NMR Spectral data ($^{\delta}$ ppm) |
|---|---|
| 1 | 14.1(CH3);45(CH2-N);63(CH2-O);163(CH2-C=O) |
| 4 | 65(CH2-N);74(CH2-O);116-132(6C aromatic);148(CH=N);160(NH-C=O) |
| 6 | 41(CH3);53(CH2-N); 56(CH2-N) ;63(CH2-C=O);111-153(CH aromatic);169(NH-C=O);145(CH=N) |

**Table-5(A)-: Antibacterial effect of β- Lactam**

| No of com. | Nam of β-lactam | *Staphylococcus aureus* | | | | | *Staphococcus epidermis* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Conc. Of the extract in mg / ml | | | DMSO | Ciprofloxacin 10 µ /disc | Conc. Of the extract in | | | DMSO | Ciproflo-xacin 10 µ /disc |
| | | 100 | 50 | 25 | | | 100 | 50 | 25 | | |
| 11 | N\-[3-chloro-2-(3-nitrophenyl)-4-oxoazetidin -1-yl]morpholin-N-acetamide | 13 | 10 | 8 | negative | 25 | 12 | 11 | 10 | negative | 31 |
| 12 | N\-3-chloro-2-methyl-4-oxoazetidin -1-yl]morpholin-N-acetamide | 28 | 21 | 20 | negative | 25 | 20 | 17 | 14 | negative | 31 |
| 14 | N\-[3-chloro-2-oxo-4-(1-methyl pyrrolidine-2-yl)]morpholin-N-acetamide | 29 | 25 | 19 | negative | 25 | 30 | 29 | 22 | negative | 31 |

## Table-5(B)-: Antibacterial effect of β- Lactam

| NO OF COM. | Nam of β-lactam | *Staphylococcus aureus* | | | | | *Bacillus* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Conc. Of the extract in mg / ml | | | DMSO | Ciprofloxacin 10 µ /disc | Conc. Of the extract in | | | DMSO | Ciprofloxacin 10 µ /disc |
| | | 100 | 50 | 25 | | | 100 | 50 | 25 | | |
| 10 | N\-(3-chloro-2-oxo-4-phenylazetidin-1-yl)morpholin-N-acetamide | 19 | 15 | 10 | negative | 25 | 17 | 14 | 12 | negative | 31 |
| 13 | N\-[3-chloro-2-(4-hydroxyphenyl)-2-methyl-4-oxoazetidin -1-yl]morpholin-N-acetamide | 11 | 10 | 9 | negative | 25 | 17 | 13 | 10 | negative | 31 |

## Table-5(C)-: Antibacterial effect of β- Lactam

| NO OF COM. | Nam of β-lactam | *Candida* | | | | | *Streptococcus pneumoniae* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Conc. Of the extract in | | | DMSO | Ciprofloxacin 10 µ /disc | Conc. Of the extract in | | | DMSO | Ciprofloxacin 10 µ /disc |
| | | 100 | 50 | 25 | | | 100 | 50 | 25 | | |
| 11 | N\-[3-chloro-2-(3-nitrophenyl)-4-oxoazetidin -1-yl]morpholin-N-acetamide | 12 | 11 | 10 | negative | negative | 25 | 20 | 15 | negative | 20 |
| 12 | N\-3-chloro-2-methyl-4-oxoazetidin -1-yl]morpholin-N-acetamide | 35 | 25 | 20 | negative | negative | 28 | 21 | 20 | negative | 25 |
| 14 | N\-[3-chloro-2-oxo-4-(1-methyl pyrrolidine-2-yl)]morpholin-N-acetamide | 29 | 25 | 20 | negative | negative | 28 | 24 | 22 | negative | 22 |

## Table-5(D)-: Antibacterial effect of β- Lactam

| NO.OF COM. | Nam of β-lactam | *Pseudomonas* | | | | | *E.Coli* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Conc. Of the extract in mg / ml | | | DMSO | Ciprofloxacin 10 µ /disc | Conc. Of the extract in | | | DMSO | Ciprofloxacin 10 µ /disc |
| | | 100 | 50 | 25 | | | 100 | 50 | 25 | | |
| 10 | N\-(3-chloro-2-oxo-4-phenylazetidin-1-yl)morpholin-N-acetamide | 19 | 17 | 16 | negative | 20 | 21 | 17 | 13 | negative | negative |
| 13 | N\-[3-chloro-2-(4-hydroxyphenyl)-2-methyl-4-oxoazetidin -1-yl]morpholin-N-acetamide | 19 | 17 | 14 | negative | 20 | 20 | 17 | 15 | negative | negative |

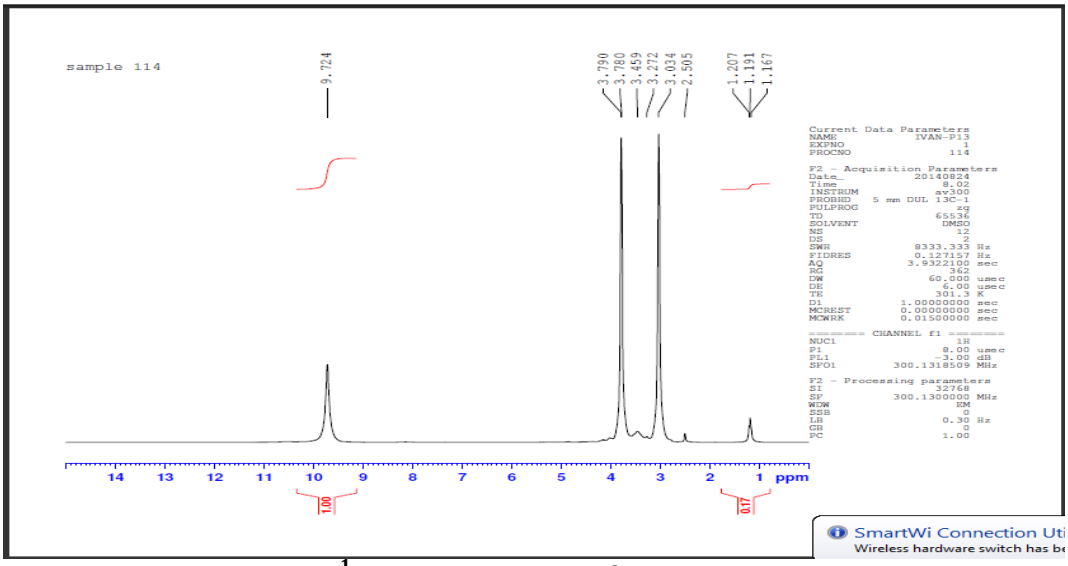



**Fig. 1-[1] HNMR Spectral of compound (1)**

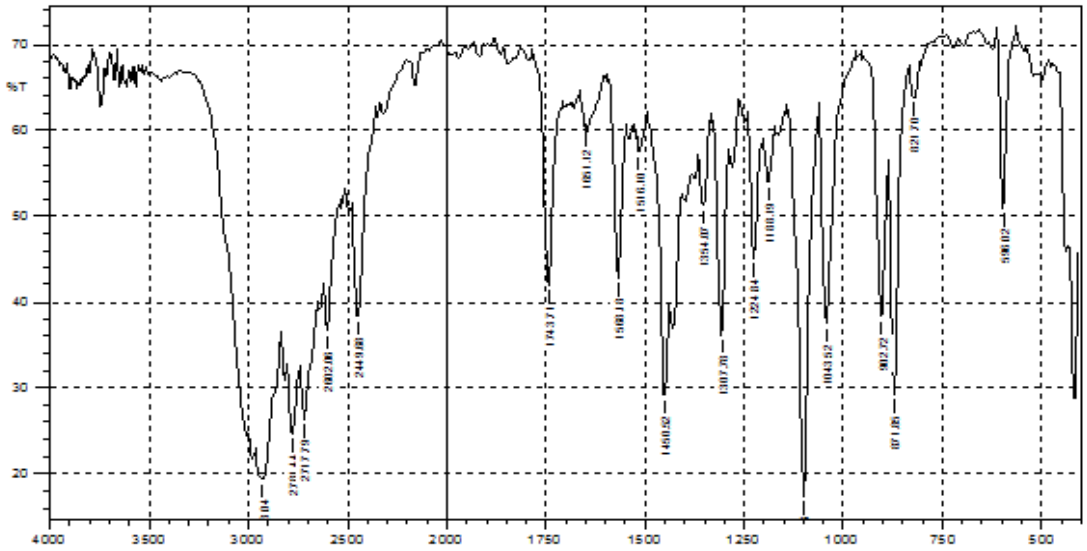



**Fig. 2- FT-IR Spectral of compound (1)**

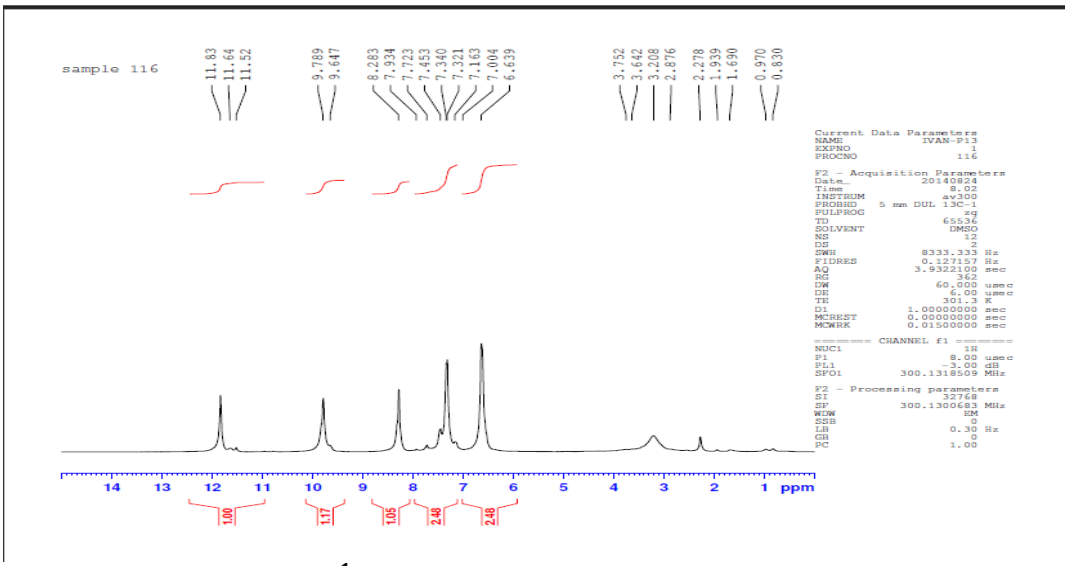



**Fig. 3-[1] HNMR Spectral of compound (4)**

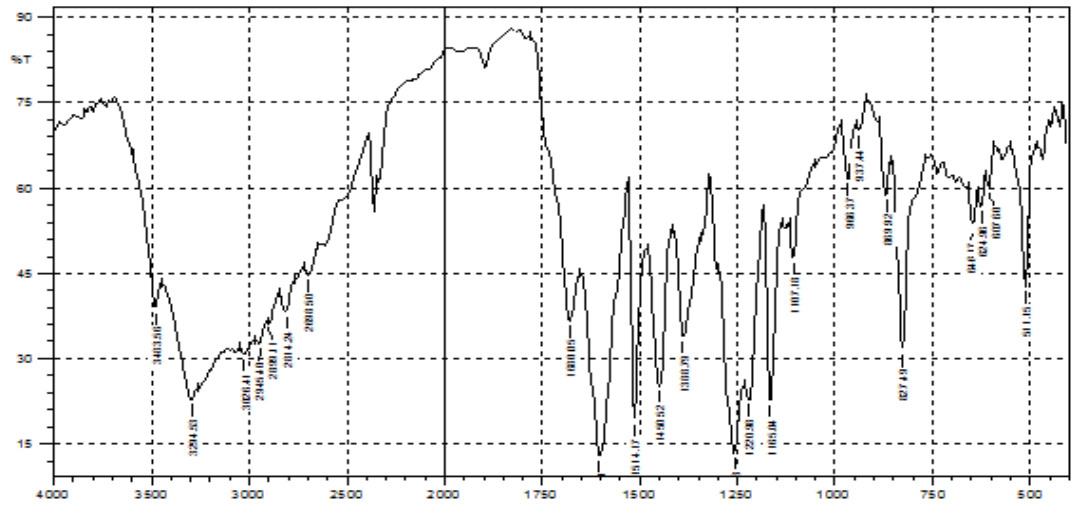


**Fig. 4- FT-IR Spectral of compound (4)**

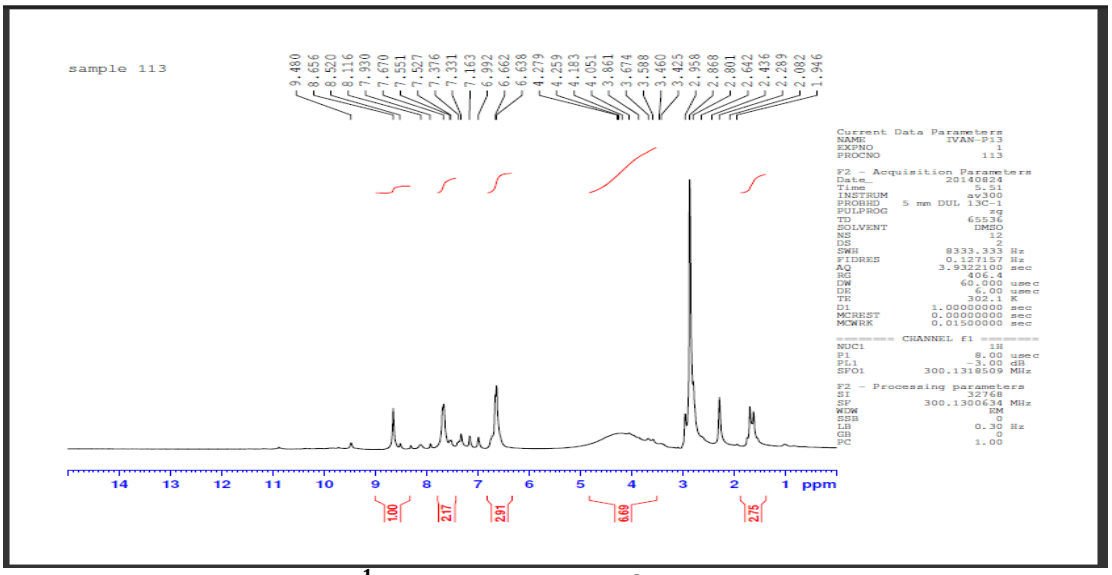



**Fig. 5-[1] HNMR Spectral of compound (6)**

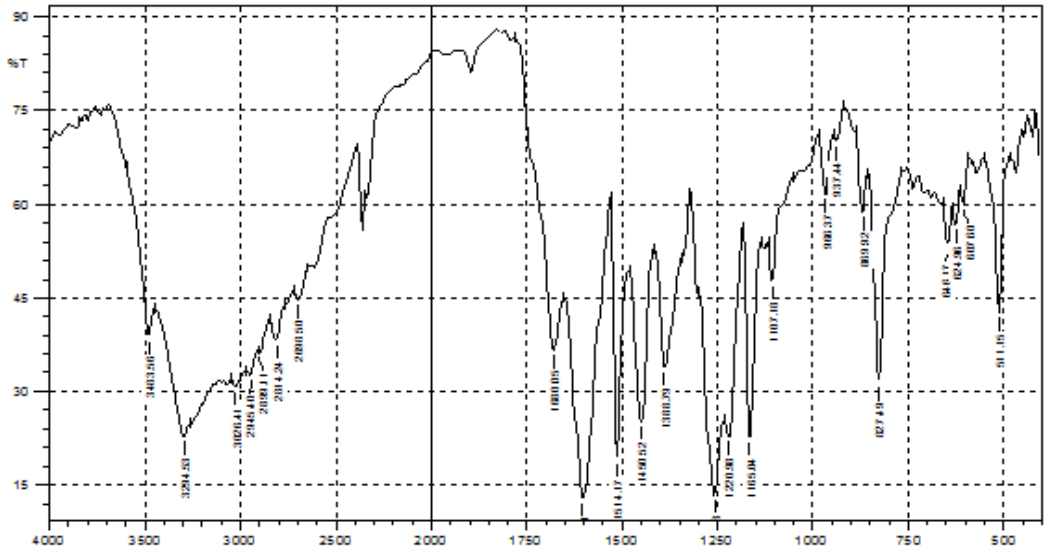


**Fig. 6- FT-IR Spectral of compound (6)**

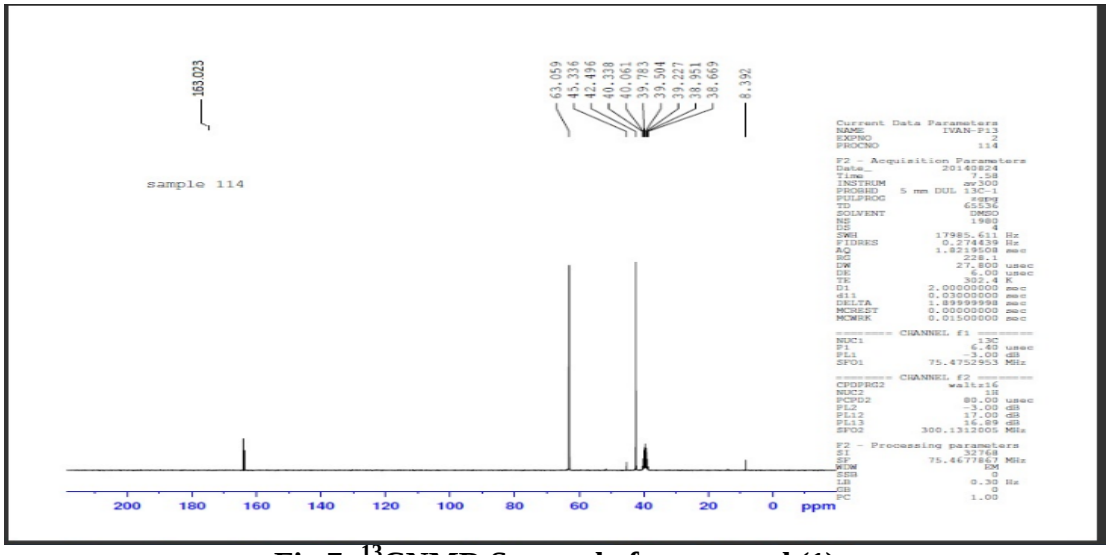



**Fig.7- $^{13}$CNMR Spectral of compound (1)**

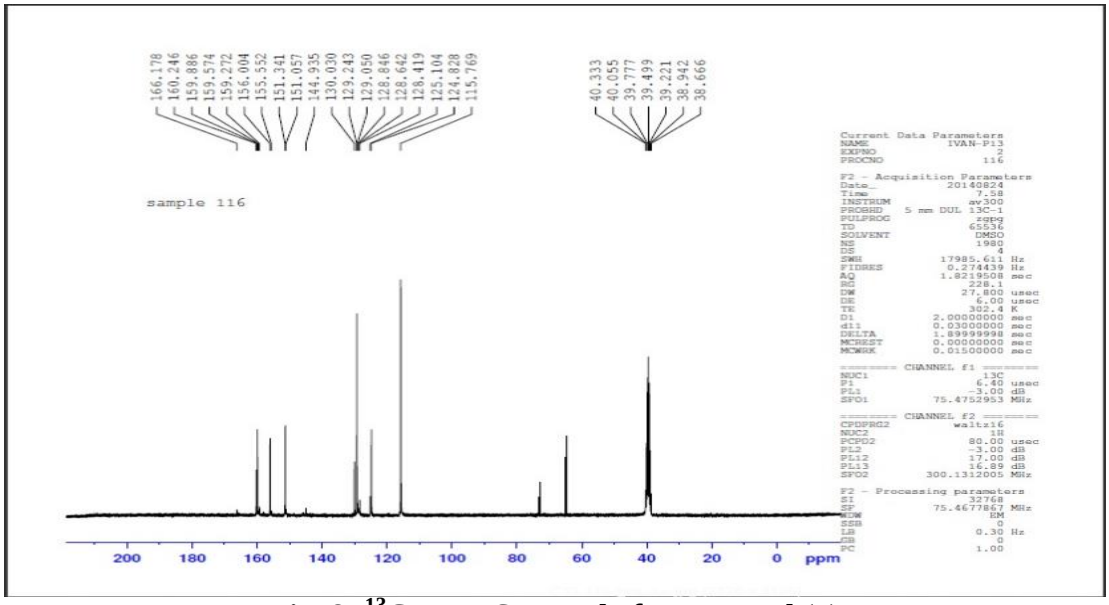



**Fig. 8- $^{13}$CNMR Spectral of compound (4)**

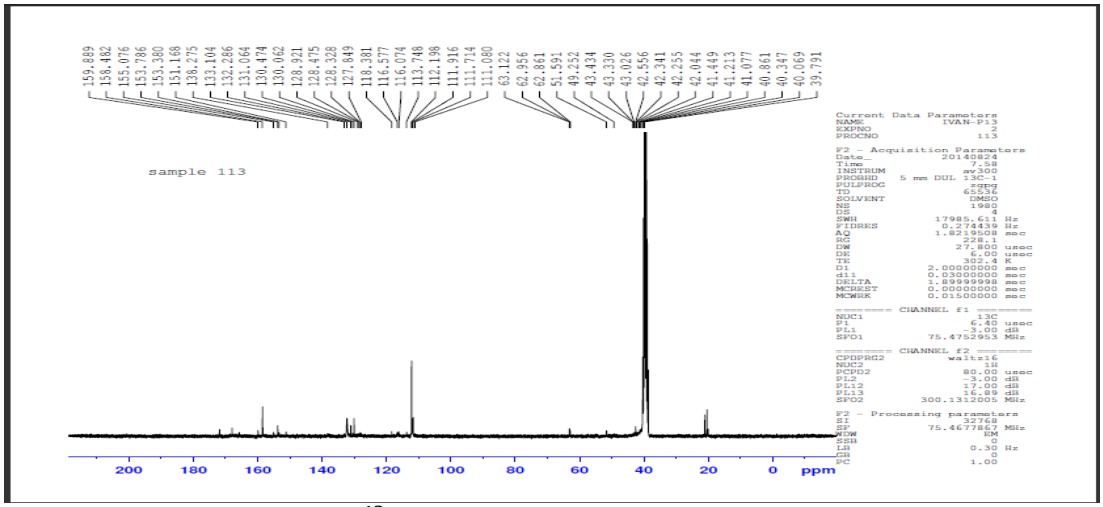



**Fig. 9- $^{13}$CNMR Spectral of compound (5)**

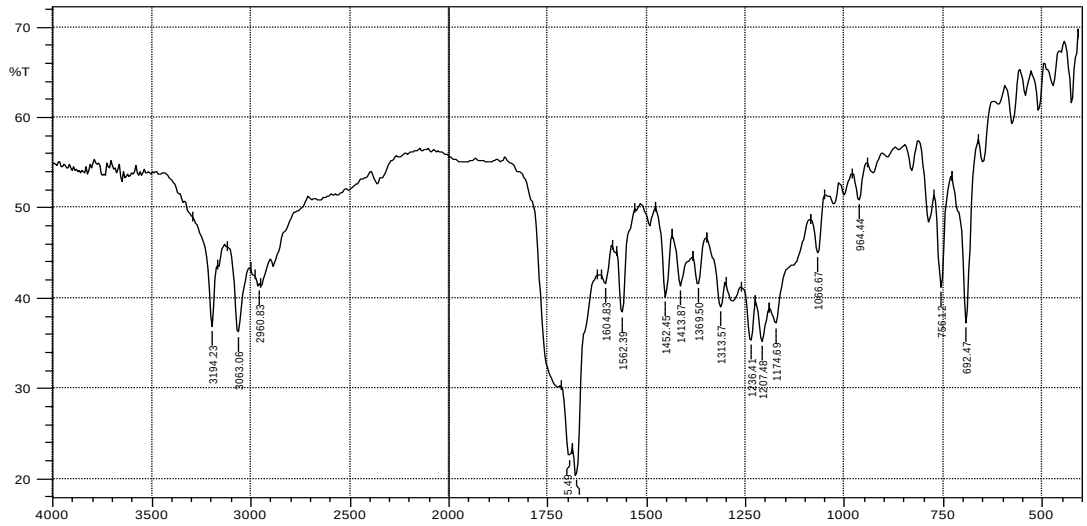



**Fig. 10- FT-IR Spectral of compound (10)**

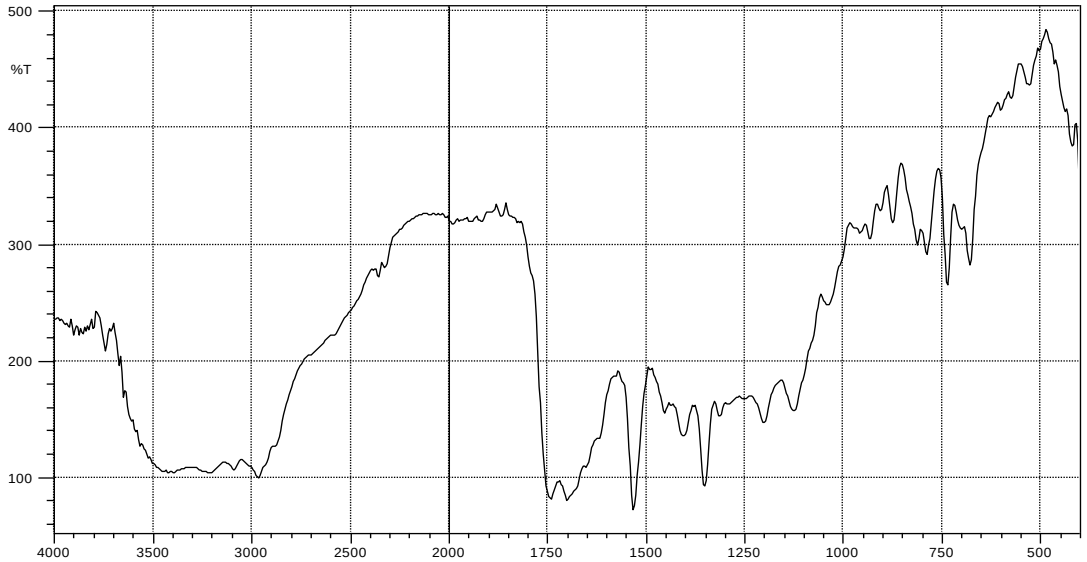



**Fig. 11- FT-IR Spectral of compound (11)**

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# تحضير وتشخيص مشتقات جديدة للمورفولين ودراسة الفعالية البايولوجية لها

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**الخلاصه:**
البحث يتضمن تحضير سلسلة جديدة من مشتقات المورفولين المحضرة من خلال تفاعل المورفولين مع الاثيل كلورواسيتيت وتراي اثيل امين كعامل مساعد والبنزين كمذيب حيث تم الحصول على المركب اثيل مورفولين-4-يل خلات الذي فوعل مع الهايدرازين هيدريت بوجود الايثانول كمذيب ليعطي مركب2-(مورفولين -4-يل ) خلات الهيدرازايد . الذي اعطى سلسلة من قواعد شيف حضرت من خلال تفاعل 2-(مورفولين -4-يل)خلات الهيدرازايد مع الديهايدات اورماتية مختلفة وكيتونات اورماتية .سلسلة جديدة من (1-5) تم تخليقها من خلال تفاعل قواعد شيف (1-5) مع كلورواسيتايل كلورايد و تراي اثيل امين كعامل مساعد 1و4- دايوكسان كمذيب .حيث شخصت تراكيب المركبات الكيميائية المحضرة طيفيا من خلال $^{13}$C-NMR, ثم فحصت مضادات البكتريا ومضادات الفطريات للمركبات المحضرة بواسطة طريقة الانتشار .FT-IR and $^{1}$H-NMR .

**الكلمات المفتاحية:** مورفولين، قواعد شف، مضادات بكتريا، مضادات فطريات.