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Faculty of Graduate Studies

Synthesis and Biological Activities of Distamicin A Analogues

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Dedication

Submitted with grateful love, recognition and appreciatıon to my great husbund Nasry Yasin.

To my father, mother, brothers and sisters.

To all teachers in my entire life.

To my dearest friends.

I dedicate this work.

Acknowledgements

After thanking God, who granted me the ability to finish this work, I would like to express my sincere gratitude to my supervisors: Dr. Waheed Jondi for all his guidance, understanding, support and advices in all of my research work and Dr. Hassan Alnees for his scientific support and constructive advice.

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Thanks to my family with all my love, especially my husbund, who stood with me throughout my study and provided me with psychological support and encouragement.

الإقرار

أنا الموقعة أدناه مقدمة الرسالة التي تحمل العنوان:

Synthesis and Biological Activities of Distamicin A Analogues

أقر بأن ما اشتملت عليه هذه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة اليه حيثما ورد، وأن هذ الرسالة ككل أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى

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Student's name: **اسم الطالبة: صمود محمد ياسين**

Signature: **التوقيع:**

Date: **التاريخ: 22/12/2015**

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Synthesis and Biological Activities of Distamicin A Analogues

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Abstract

Distamycin A is a natural product possessing amide group and three N-methyl pyrrole rings terminated with neutral formamide and positively charged propylamidinium.

It has been discovered and investigated as DNA minor groove binder, it acts as anti bacterial agent.

Distamycin A binds to the minor groove of double- stranded DNA at Adinine-Thymine rich regions by forming hydrogen bond and hydrophobic interaction.

Terminal amidine group of molecule is basic and have positive charge, this serve the molecule to be attracted to negatively charged DNA phosphodiester backbone.

DNA is changed when it is bind with distamycin A. This is recognized from the chemical structure of minor groove.

Distamycin A analogues have been synthesized to increase its binding with minor groove. In this research a new distamycin A analogues were synthesized by changing N-terminal alkyl group which have lower molecular weight and higher lipophilicity than previous analogues .This will increase the binding with minor groove, and may increase biological activity as anti cancer, in addition to its anti bacterial activity.

We have synthesized five new analogues of Distamycin A in this research, the synthesized analogues were characterized by ^{1}HNMR, ^{13}C NMR and IR.

Synthesized compounds were tested for antioxidant ,antibacterial and antifungal activities.

According to antibacterial activities the most active one was N-(1-Methyl-5-(2-(4-sulfamoyl-phenyl)-ethylcarbamoyl)-1H-pyrrol-3-yl)-nicotinamide and Methanesulfonic acid 4-(2-((4-benzoylamino-1-methyl-1H-pyrrole-2-carbonyl)amino)-ethyl)-phynel ester. They reveled about 10% of *Gentamicin*, whihl 4-benzoylamino-1-methyl-1H-pyrrole-2-carboxylic acid (3-dimethylamino-propyl)-amide revealed the highest activity against *Microsproum Canis* (72% inhibition).

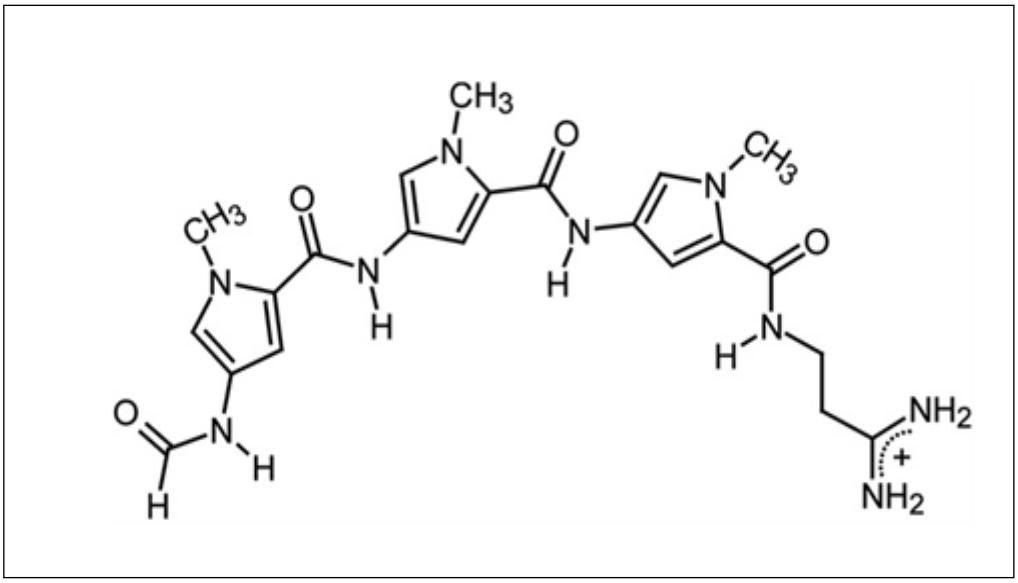


Fig (1): distamycin A structure.

Chapter One

Introduction

1.1 Distamycin A

Distamycin A is a natural antibiotic product with anticancer activity discovered in 1964. It was isolated from Bacterium Streptomyces Distallicus [1].

Distamycin A is one of the most extensively studied compounds which can bind reversibly in the minor groove of duplex DNA, by hydrogen bonds, Vander Waals contacts and electrostatic interactions, with high specificity for regions containing Adinine-Thymine (A-T) nitrogen base pairs [2]. Distamycin A acts as antibiotic with anticancer activity but it is too toxic to find application in cancer therapy[3]. It exhibits a high binding specificity and inhibits selectively initiation of transcription directed by certain prokaryotic promoters. Distamycin A displays antiviral and antibiotic activity, and active against gram-positive and gram-negative bacteria [3]. It shows antiprotozoal activity, which related to the ability of binding reversibly to the minor groove of DNA with a high selectivity for T-A rich sequences. At the cellular level, studies on Distamycin A show that, it inhibits the pathogenesis of functional complex formation at the promoter, by activating transcription initiation, and re-complex , box binding protine (TBP) and basal in vitro transcription[4]. There are other compounds like Distamycin A that have similar properties such as Netropsin.

Due to those reasons many of distamycin A analogues and conjugates have been synthesized.

1.2. Distamycin A Structure and Binding

Distamycin A is oligopeptide constructed from 4-amino-l-methyl pyrrole acid moieties and strong basic side chain possessing isohelical shape to minor groove of DNA. The chemical structure of Distamycin A characterized by chain of three N-methyl pyrrole rings interconnected through with neutral formamide and positively charged propylamidinium Figure (1.1) [5].

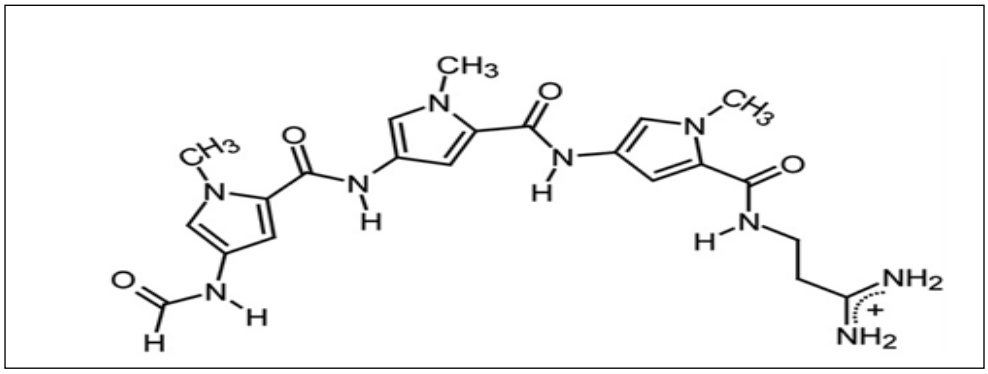


Figure (1.1): Structure of Distamycin A.

Distamycin A -like minor groove binders reach the cell nucleus hardly because of their poor membrane permeability[6].

The manner by which Distamycin A binds to DNA has inspired the various search for compounds with similar mode of action; some of these are being tested in clinical trials [7].

The binding of Distamycin A with minor groove takes place due to favorable Vander Waals interactions between C-H's of DNA minor groove along with hydrogen bond formation between NH groups of the pyrrole

carboxamide ring complex which stabilized negatively charged phosphate backbone binding of Distamycin A to DNA minor groove, widens the minor groove by unbending the helix axis and increases its length by nearly at cellular level [8].

The topology of the groove can be considered a smooth curve, however introducing a Guanine-Cytocin (G-C) nitrogen base pair with the exocyclic N-2 of guanine which points up into the groove causing a steric hindrance (clash) with the proton on C-3 of the pyrrole rings, this preventing the essential hydrogen bonds from being formed. So that the effective hydrogen bonds are possible in A-T regions ,Figure (1.2).

Two Distamycin A molecules can be packed in minor groove with peptide group of one drug molecule stacked on the aromatic ring of the other.

Anti-parallel hairpin motifs containing different sequences of N-methyl pyrrole and N-methylimidazole residues can recognize the broad category of nucleotide sequences in the minor groove .It has been reported that two parallel oligopyrrole carboxamide strands can be sandwiched in the minor groove of DNA[9].

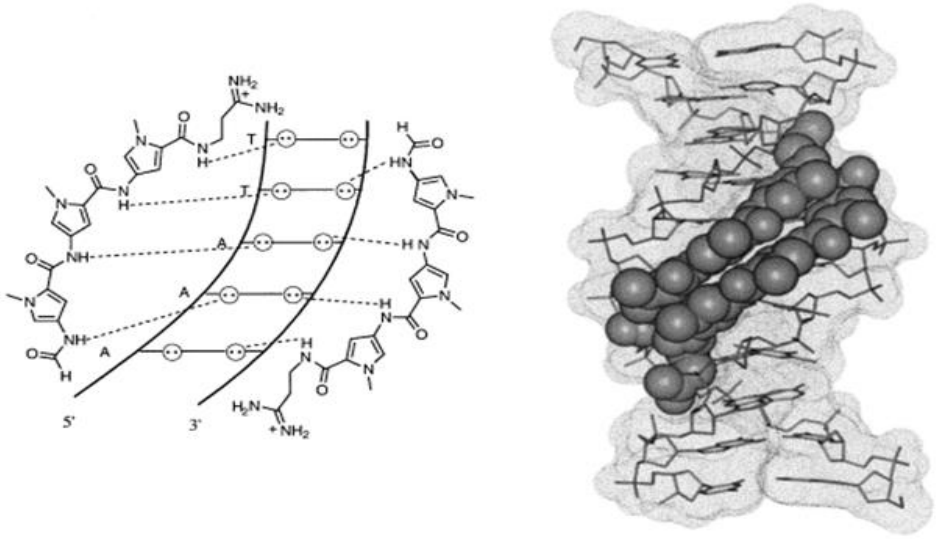


Figure (1.2): Binding of Distamycin in the Minor Groove 2:1

1.3 Distamycin A Analogues

Designing new molecules which are capable for recognizing specific sequences in DNA may be useful for achieving selective inhibition of the expression of certain oncogenes [10].

This may be useful in control of the development and proliferation of the tumor cells. Chemical biologists approach to this goal by using low molecular weight ligands that can bind specific sequences in DNA.

Distamycin A which is, a natural product, one of these molecules remains a principle candidate for spear heading such design due to its manner in which it is binds to DNA which has inspired the search for compounds with similar mode of action. Distamycin A can bind to five consecutive A-T base pair, and its site of binding coincides with that of the TA-TA-box binding protein (TBP), which is a general transcription factor for RNA polymerase II [11].

Many of Distamycin Analogues were synthesized and its ability for DNA-binding has been tested. Studies showed DNA-binding properties for these compounds are reversible minor groove binders with high selectivity for A-T rich regions in DNA [12].

There is a group of synthetic oligopeptides, designed based on the pattern of described antibiotic and selective to specific base pairs of DNA, obtained the name “lexitropsins” which have both antiviral and antibiotic activity .Studies such as thermodynamic, structural and spectroscopic techniques have established the molecular basis for DNA –binding affinity and specificity of Distamycin A. Baird and Dervan (2006)described solid phase synthesis of polyamides which containing imidazole and pyrrole amino acids using tert-butyloxycarbonyl-protection strategy [13].

Boger and co-workers (2001)used the previous strategy to design many compounds inspired by the structure of Distamycin A. They applied solution-phase synthesis and acid/base liquid – liquid extraction techniques in order to isolate the compounds [14].

Brucoli and others(2009) used SynPhase Lanterns to prepare Distamycin A analogues, where one of pyrrole rings was replaced by biaryl motifs.

Bhattacharya and Thomas(2001) reported the first example of cholesterol-conjugated Distamycin A analogues, which retain their strong binding capacity to double-stranded (ds)- DNA[15].

Fluorescent Distamycin A was also synthesized, which enable to study kinetics of polyamide-DNA interactions and the monitoring of their cellular distribution [16].These Fluorescent molecules report the physical details of

DNA binding sites, e.g. polyamide part of the Distamycin A-porphyrin conjugates was found to bind to the DNA minor groove with preference for A-T rich sequences, while the fluorescent porphyrin fragment exhibited intercalation and the non-specific electrostatic interaction with the DNA phosphate groups [17].

Synthesis of Distamycin A analogues without leading amide unit at the N-terminus shown that a hydrogen bond donors or acceptors atom at N-terminus is not necessory for their DNA binding, a minimum of three pyrrole carboxamide units is necessary for the onset of DNA binding [18].

Replacement of heterocyclic rings in Distamycin A by carbocyclic rings yields minor –groove binders which have a reduced affinity to DNA rich regions A-T and an increased affinity to G-C DNA region, which increases antibacterial and antiviral activity, these analogues exhibit lower toxicity in comparison with Distamycin A[19].

Carbocyclic analogues of Distamycin A are available and stable under experimental conditions. Intense induced circular dichroism (ICD) spectra were obtained with A-T rich DNA and with poly ds (GC), ds (GC) exist in the Z conformation.

These result confirmed the importance of poly amide – based minor groove binders in gene regulation processes, and showed the ability of modification in the ligands molecular structure that have effect on the DNA binding properties.

Valike and other (2006)synthesized 'head to head' oligo –N-methyl pyrrole peptide dimmers linked by methanol to improved DNA recognition [20].

DNA binding study in racemic, as well as chiral fusion, showed that novel dimmer prefer A-T sequences , higher affinity to play (dC- dG) than Distamycin A.

Ghosh and co –authors(2008) designed photoisomerizable azobenzene-Distamycin A conjugates in which two Distamycin A units linked via electron- rich alkoxy or electron withdrawing carboxyamide moieties with azobenzene core duplex. DNA binding abilities for these conjugates depend upon nature and length of the spacer , the location of proton table residue and isomeric state of conjugate[21].

Distamycin A has poor membrane permeability, so synthetic pyrrole octa-arginie conjugates are capable of rapid localization in the cell nuclei with low nano-molecular affinity , targeting specific sites in DNA that contain A-T rich tracts .

Significant synergistic transport effect was observed between the minor-groove binding tripyrrole unit and the octa-arginine peptide, which cooperate in localizing the hybrid molecular in the cell nuclei [22].

In the drug delivery field using Distamycin A derivatives, selected sequences of oligodeoxyribonucleotides (ODNs) were conjugated efficiently with Distamycin A based peptides containing reactive cysteine and oxyamine functionalities at C- terminus.

This method is time-saving and allows investigating the DNA based diagnostic properties of antigens or therapeutic ODNs by improvement it's target binding properties, cellular uptake, exo-nuclease stability and can be used in situ hybridization probe[23].

Distamycin A is known to bind to A-T rich region of duplex DNA; it has been shown to interact with four-stranded parallel DNA quadraplex, this has stimulated syntheses of Distamycin A polyamides targeting G-quadruplex DNA.

Moore and co-workers(2006) for example have synthesized a number of oligopyrrole by solid-state methods and investigated their interactions with human intramolecular G- quadraplex [24].

Different applications for Distamycin A and its analogues found to be important in biological field, so that new synthesis methods are still in demand. Most approaches to solid phase synthesis of Distamycin A analogues are based on the traditional peptide strategy. Also different solid phase synthesis inspired by investigation in the field of carbocyclic lexitropsin synthesis of carbocyclic minor groove binders [25].

These methods use aromatic nitro compounds –amines and acyl chlorides to build carbocyclic oligoamides. The precursor nitro group of first substrate is reduced to obtain amine, and then undergoes acylation with acyl chloride containing the next nitro group in the presence of DMAP at room temperature. Synthesis and biological evaluation of derivatives containing benzene in place of N –methyl pyrrole rings have been reported, these compounds show sequence selectivity and these compounds bind to A-T sequences less strongly than Distamycin A [26].

All tested carbocyclic minor groove binders showed the antiproliferative and cytotoxic effects against breast cancer cell .

The carbocyclic analogues of Distamycin A with un-substituted N-terminal group NH_2 inhibited in vitro activity of topoisomerase I and II.

The derivatives with N-terminal chlorambucyl group also exhibited activity in cultured breast cancer [27]. Using solid phase strategy in synthesis of Distamycin A analogues increases both these new compounds, which can be synthesized.

New method of solid – phase synthesis of Distamycin A derivatives, was performed with p-nitrophenyl carbonate Wang resin, and in continuation of searching more selective DNA-binding molecules, based on Distamycin A as a lead molecule [28].

1.4 Discovery and Properties of DNA

Deoxyribonucleic acids (DNA) are polymer known as "polynucleotide", its monomer units are nucleotides. DNA is one of the three major macromolecules that are essential for all known forms of life. DNA was isolated by Fridrich Miescher and the double helix structure of DNA was first discovered by James D. Watson and Francis Crick [29]. DNA can replicate, or make copies of it. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. This is important when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell [30].

The strand backbones are closer together on one side of the helix than on the other. The major groove occurs where the backbones are far apart, the minor groove occurs where they are close together Figure (1.3). The grooves twist around the molecule on opposite sides [31]. Certain proteins

bind to DNA to regulate transcription (copying DNA to RNA) or replication (copying DNA to DNA). The bases of DNA are hydrophobic, succeeding pairs attract each other, causing the structure to twist into a right-handed double helix [32].

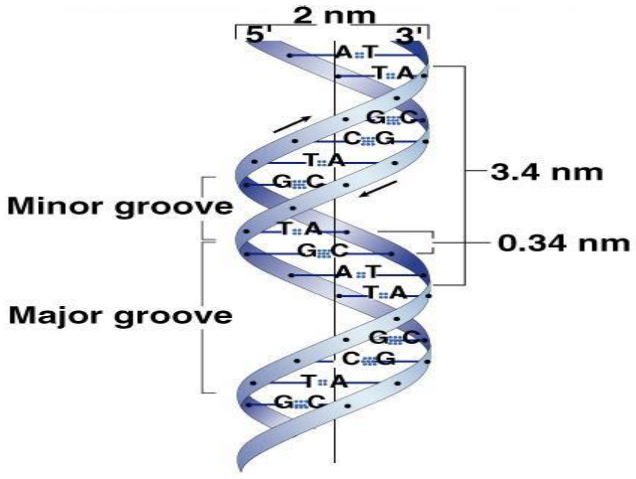


Figure (1.3): DNA Structure.

Each nucleotide in DNA consist of a 5 carbon sugar (deoxyribose) , a nitrogen containing base attached to the sugar , and phosphate group. Four types of nucleotides found in DNA ,differing in nitrogenous base, only, and each one is given one letter abbreviation as shorthand for the four bases: A is for adenine, G is for guanine, C is for cytosine, and T is for thymine, which are important for genetic information which encoded as a sequence of nucleotides Figure (1.4) [33].

Adenine and guanine are purines, which are the two larger types. The two smaller types are pyrimidines Cytosine and thymine Figure (1.5) [34].

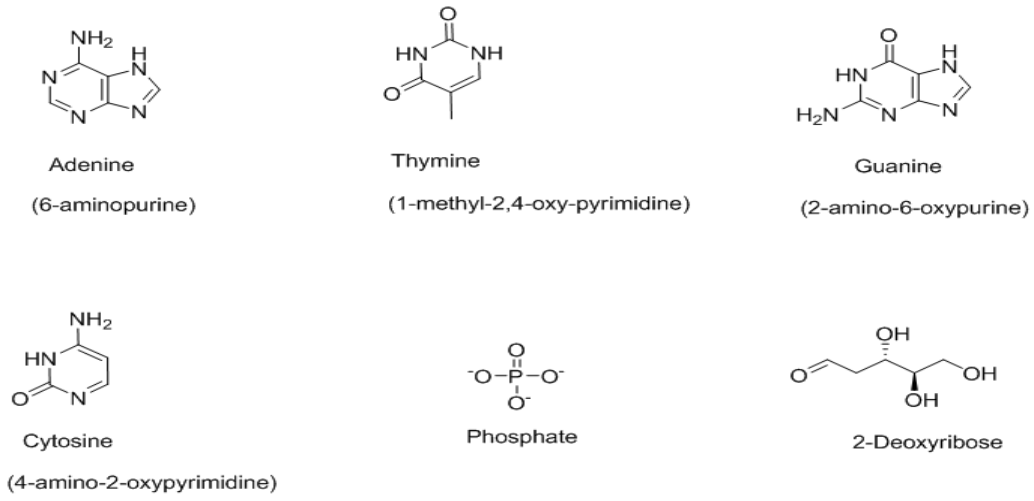


Figure (1.4): Structural Formulas of the Constituents of DNA.

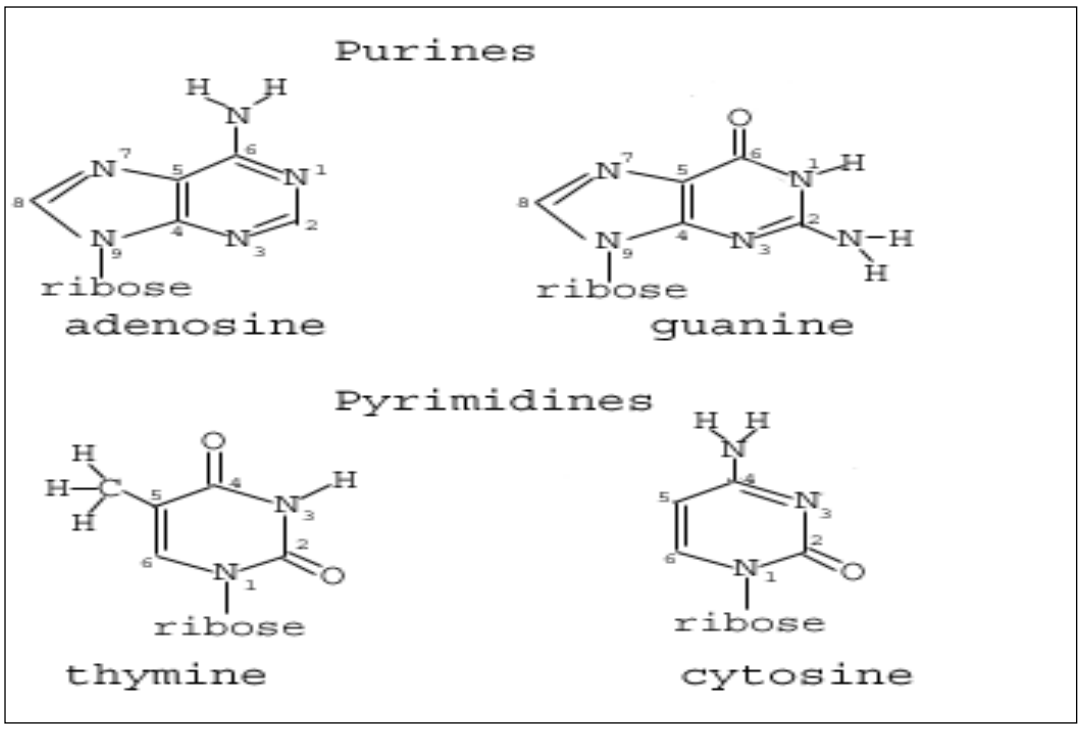


Figure (1.5): Chemical Constituents of DNA.

Deoxyribose sugar of DNA backbone has 5 carbons and 3 oxygens, the Carbon numbered 1', 2', 3', 4', and 5'. The hydroxyl group on the 5'- and 3'-carbons link to phosphate groups to form DNA back bone.

Nucleosides one of the four DNA bases covalently attached to C'1 position of the sugar; nucleosides differ from nucleotides in the lacking phosphate groups, nucleotides is nucleoside with one more phosphate group covalently to the 3'- and/or 5'-hydroxyl groups Figure (1.6) [35] .

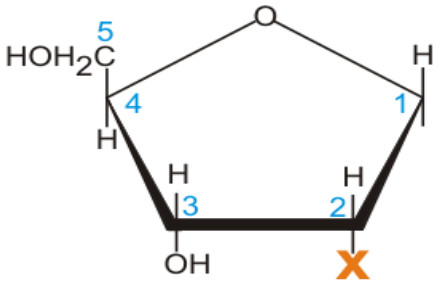


Figure (1.6): Deoxyribose and Ribose Sugars, Deoxyribose=H, Ribose =X

DNA backbone is a polymer with alternating sugar –phosphate sequence and is normally double stranded macromolecule, the two polynucleotide chains, held together by weak thermodynamic forces.

Two DNA strands form helical spiral, winding around a helix axis, the two polynucleotides chains run in opposite directions, the sugar- phosphate backbones of the two DNA strands wind around the helix axis like the railing of a spiral staircase. The bases of individual nucleotides are on the inside of the helix [36].

Within the DNA double helix, adinine form 2 hydrogen bonds with thymine on opposite strand, and guanine forms 3 hydrogen bonds with cytosine on opposite strand

Base pairs are of the same length Figure (1.7), occupy same space d(A)-d (T) and d(G)- d(C) Within a DNA double helix, and it's can occur in any order within DNA [37].

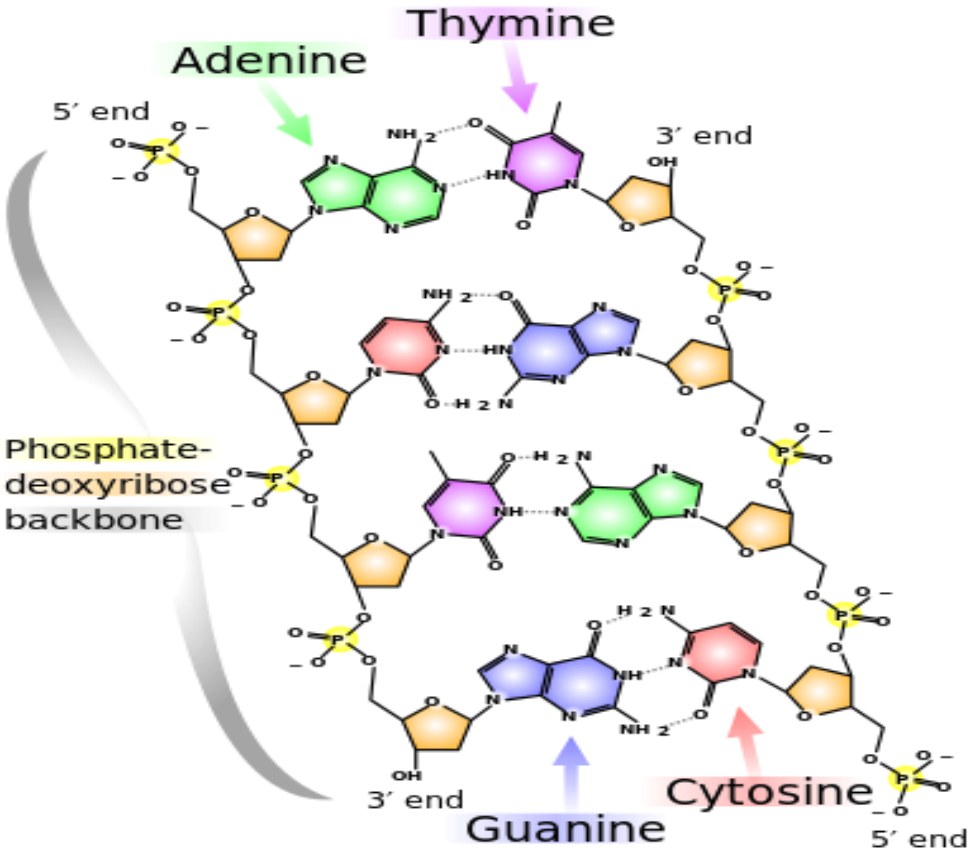


Figure (1.7): Hydrogen Bonds Formed in Base Pairing

1.5. Natural Compounds That Bind to the Minor Groove

There has been a lot of work done on the design and synthesis of biologically active compounds that can bind to DNA with high affinity and specifity.

Cell-permeable sequence-specific DNA – binding ligands can control gene expression and serve as pro-types of gene-selective drugs.

Netropsin and Distamycin A are among the best characterized sequence-specific ligands due to their mode of interaction with DNA.

Studies show that these antibiotics bind in the minor groove of double-helical DNA [38].

1.5.1 Netropsin

Netropsin is an oligopeptides was discovered by Finalay et al.

Netropsin has other names which are Congocidin and Sinanomysin.

It was isolated from the *Actinobacterium Streptomyces Netropsis* Figure (1.8). Netropsin have antibiotic and antiviral activity. It has an activity against gram –positive bacteria and gram-negative bacteria and used in cancer research because of its binding ability to specific sequences within minor groove of double –helical DNA [39]. Netropsin inhibits DNA and RNA tumor viruses in mammalian cells. It belongs to the class of pyrrole amidine antibiotics.

Netropsin interacts with the deep and narrow minor groove of B-DNA and binds to the minor groove of A-T rich sequences of double stranded DNA. Netropsin binds exclusively in a 1:1 complex with DNA due to the repulsive force which occur between two positively charged groups side by side Figure (1.9)[40].

Netropsin makes hydrogen bonding interactions with four subsequent base pair of the DNA duplex. H-bonding occurs between the proton donors of Netropsin and acceptors N-3 of adinine and O-2 of thymine .Binding of the DNA increases the twist per base by similar to 9̊0 per molecule bound.

Therefore, it removes super coils when interacting with positively super-coiled DNA and introduces negative super coils when binding to relaxed or negatively super-coiled DNA [41].

Crystallographic structure of DNA – bound Netropsin exposed the details of how the drug binds in the minor groove [42].

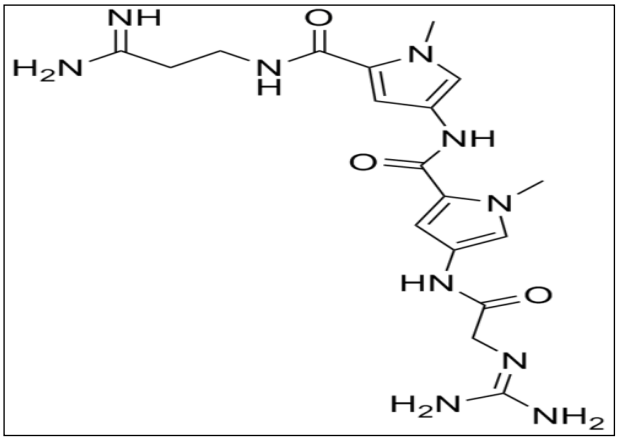


Figure (1.8): Netropsin Structure.

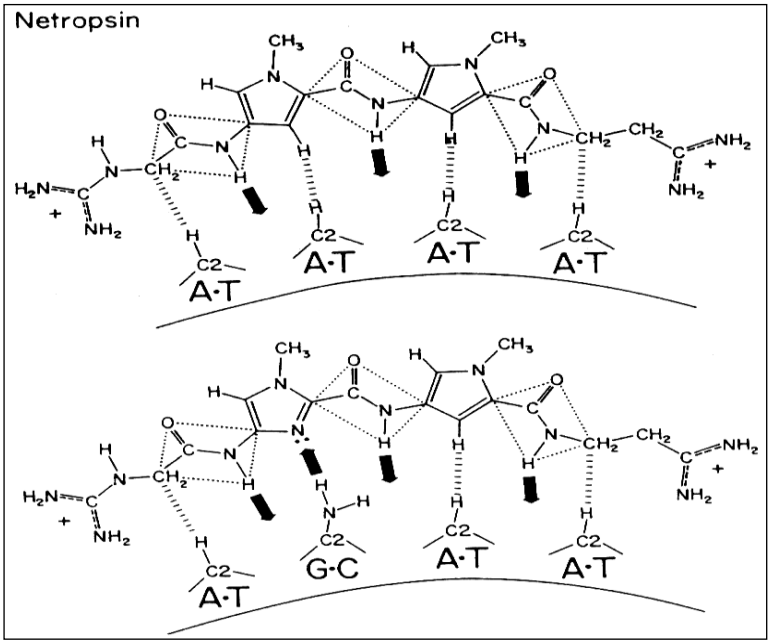
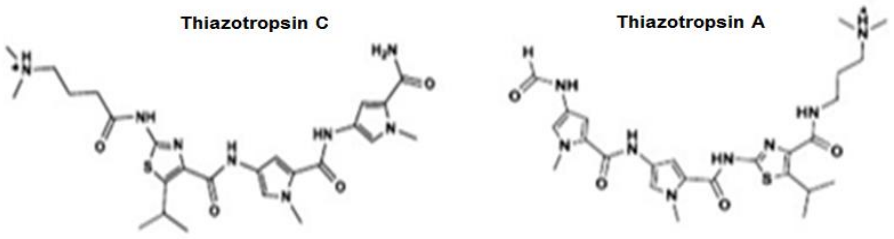


Figure (1.9): Netropsin Bound in the Minor Groove 1:1

1.5.2 Thiazotropsin C

It is one of the cationic lexitropsins analogues of Distamycin A. It is bind non-covalently to the minor groove of DNA. It has shown therapeutic activity in the treatment of cancer, viral, fungal and bacterial illness. The ring slippage of the side-by-side minor groove binders enabled the extension of DNA sequences with six pairs to these ligands. Thiazotropsin

C based on a four-pairs based on the size of the ligands Figure (1.10). The lexitropsins have shown ability to recognize a DNA sequence. The importance of Thiazotropsin appears in gene targeting. These ligands with small molecular weight and enhanced lipophilicity able to disrupt the binding of transcription factors to the response element of the target gene which is composed of six base pair sequences [43].



Figure(1.10): Thiazotropsin A and its analogue thiazotropsin C

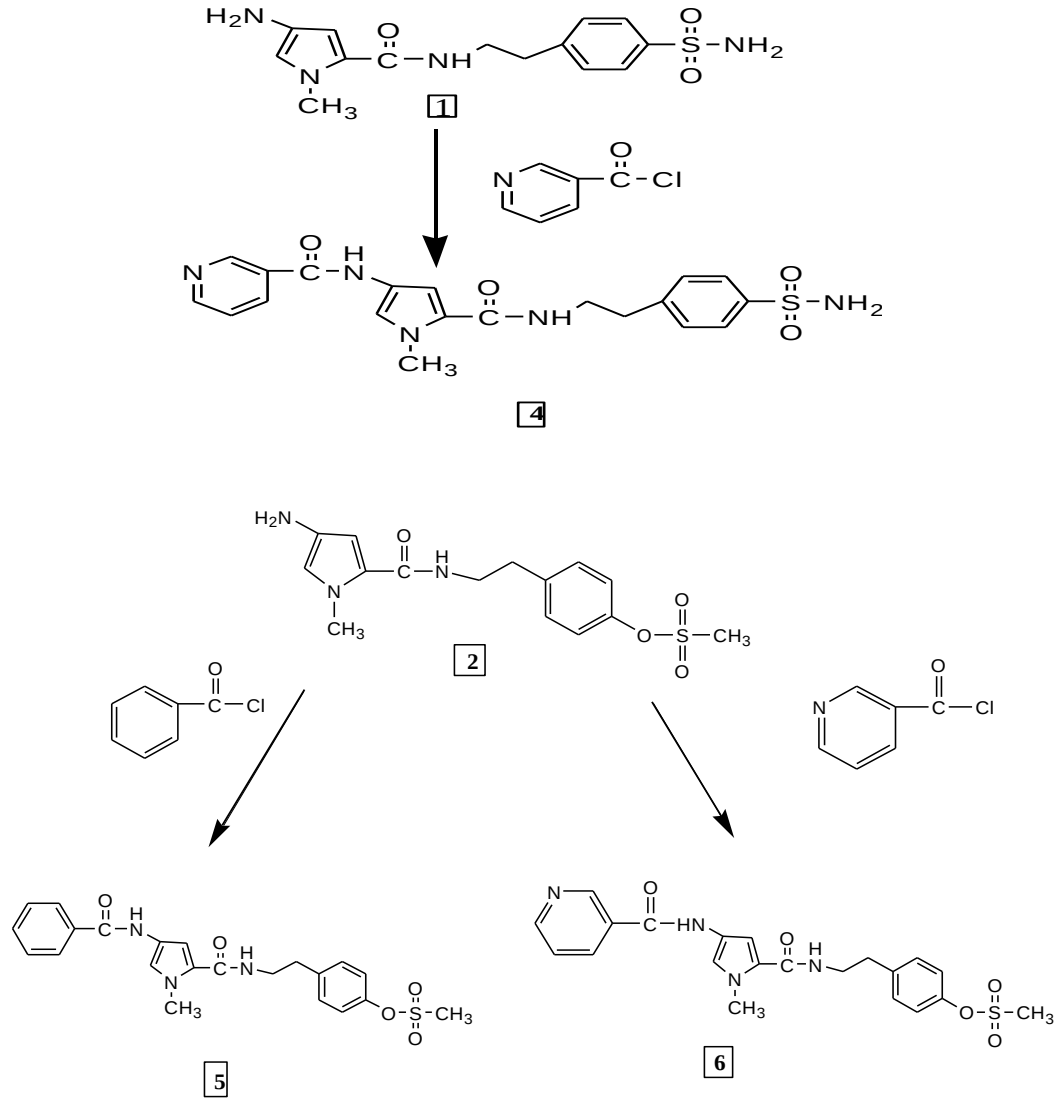
1.6 Antimicrobial Activity of Distamycin A Analogues

Forty-eight heterocyclic amino acid trimers analogues of Distamycin A, with a number of features that enhance lipophilicity are described. They contain alkyl or cycloalkyl groups larger than methyl; some are N-terminated by Acetamide or Methoxy-benzamide and are C-terminated by Dimethylaminopropyl or aliphatic heterocyclic Aminopropyl substituents. The ability of these compounds to bind principally to A-T tracts of DNA has been evaluated using capillary zone electrophoresis .Significant antimicrobial activity against key organisms such as MRSA and Candida Albicans is shown by several compounds, especially those containing a

Thiazole. Moreover, these compounds have low toxicity with respect to several mammalian cell lines [44].

1.7 Proposed Pathway for Synthesis of Distamycin A Analogues:

From the retro-synthetic analysis shown below (Figure 12), the final products which are the proposed analogues (4, 5, 6, 7, 8) will be synthesized by using a convergent synthesis through coupling the tail of each one of (1, 2, 3) compounds with Benzoyl chloride and Nicotinoyl chloride after the reduction of the nitro group.



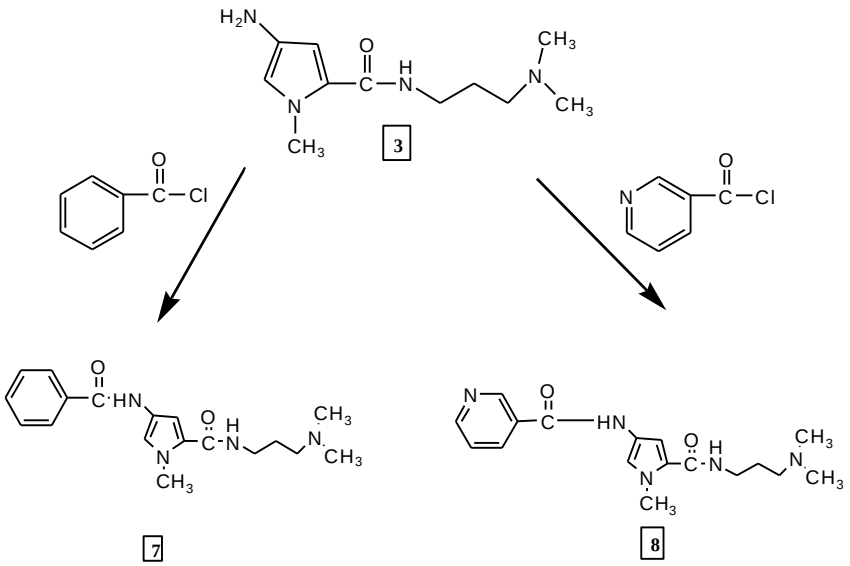


Figure (1.11): Retro synthetic Analysis of Distamycin A Aanalogues.

1.8 Aim of the Project

The main aim of this project is to synthesize and characterize new analogues of Distamycin A as potential anti-bacterial agents.

The proposed analogues of Distamycin A will have small molecular weight and enhanced lipophilicity in order to improve their binding with minor groove of DNA and increase the absorption and cell permeability of these compounds. In second part of this project the biological activity of these compounds will be examined. The consequences of our study would be of great importance in terms of both the development of new analogues of Distamycin A as potential anticancer and antibacterial agents.

Chapter Two

Chemical Synthesis

2.1 Materials and Chemicals

All chemicals used in this study were purchased from Sigma Aldrich Chemical Company (Trichloroacetyl chloride, N- methylpyrrole, 4-(2-aminoethyl) phenol, 3,3-dimethylaminopropylamine, 4-(2-Aminoethyl) benzene sulfonamide, Pd/C, Nicotinoyl chloride). All other reagents and chemicals are of analytical grade.

2.2 Equipments and Devices

Melting points of the compounds were measured by a Stuart Scientific Melting Point SMP1, while Infrared Spectra were run on Mattson Genesis Series FTIR spectrometer using KBr disc. Proton NMR was measured using BRUKER nuc1 400 MHz.

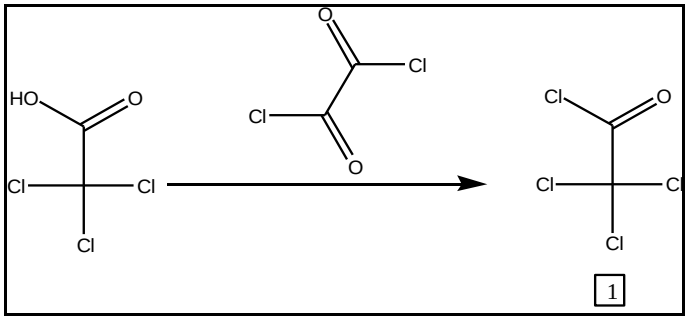
2.3 Chemical Synthesis

2.3.1 Preparation of Primary Compounds

2.3.1.1 Preparation of trichloroacetyl chloride 1.

Trichloroacetic acid (40.2g, 246mmol.) was dissolved in (152 ml) Dichloromethane (DCM) in a round bottom flask, and Oxalyl chloride (41.3g, 325mmol) was dissolved in (108.7ml) DCM in a dropping funnel. The final solution was added to the first one dropwise at 0 ˚C within two

hours, and the mixture was then refluxed for 3hours. The product was collected and purified by fractional distillation at boiling point 115-117˚C. Yield; 37g (83%), B.p. =115-117°C (Scheme 1).



Scheme 1: Preparation of trichloroacetyl chloride

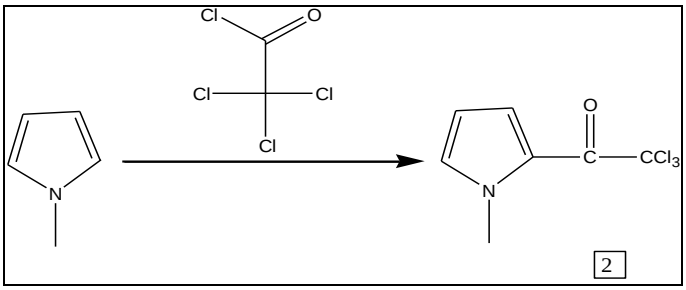
2.3.1.2 Preparation of 2,2,2-trichloro-1-(1-methyl-1H-pyrrol-2-yl) ethanone 2.

Trichloroacetyl chloride (36.2g, 200.65mmol), was dissolved in (130 ml) DCM in 250 ml round bottom flask under nitrogen atmosphere.

N- methylpyrrole (16.2g , 200.32mmol) was dissolved in (70 ml)DCM. The later one was added drop wise to the former within 2.5 hours at room temperature. The solution was left overnight with stirring; solvent was then removed under vacuum (Scheme 2). The product was purified with a column chromatography packed with silica gel as white- yellow solid crystals.

Yield; 9g ,75%, m.p. = 62-64°C.

IR (KBr): 3121, 2941, 2439, 1652, 1242, 1090, 740 cm^{-1}.



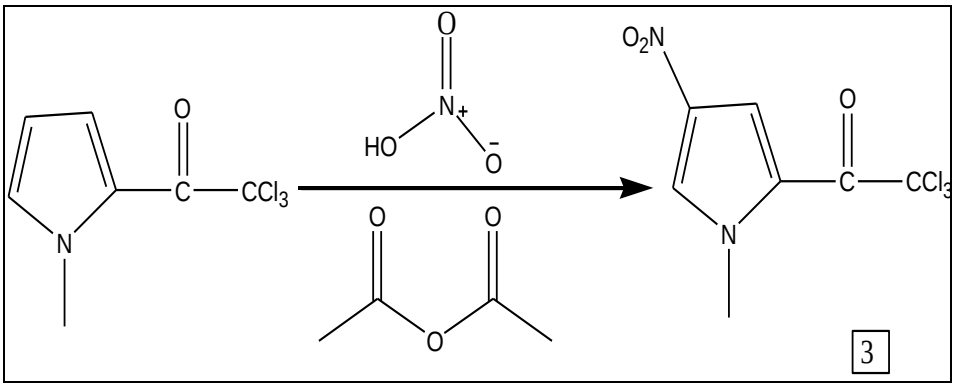
Scheme 2: preparation of 2,2,2-trichloro-1-(1-methyl-1H-pyrrol-2-yl)ethanone .

2.3.1.3 Preparation of 2,2,2-trichloro-1-(1-methyl-4-nitrio-1H- pyrrole-2- yl)ethanone 3.

Acetic anhydride (60 ml) was placed in a round-bottomed flask, then nitric acid (70%, v/v, 8 ml) was added drop-wise at -30 °C. The solution was left for 20 min with stirring. This solution was then added drop-wise to another round-bottomed flask containing 2-Trichloroacetyl -N-methylpyrrole (10.0 g, 44.64 mmol), in Acetic anhydride (40 ml) at –30 °C and allowed to warm up to 0 °C. The solution was cooled to -40 °C and water was added slowly at which point the product precipitated as an off-white-yellow solid. The solid was collected and washed with Hexane, before being dried under vacuum (Scheme3).

Yield; 9g, 75%, m.p. =133-135°C (Scheme 3). (Litt = 137-140°C)

IR (KBr): 3130, 1688, 1308, 1107 cm^{-1}.



Scheme 3: Preparation of 2,2,2-trichloro-1-(1-methyl-4-nitrio-1H- pyrrole-2-yl)ethanone .

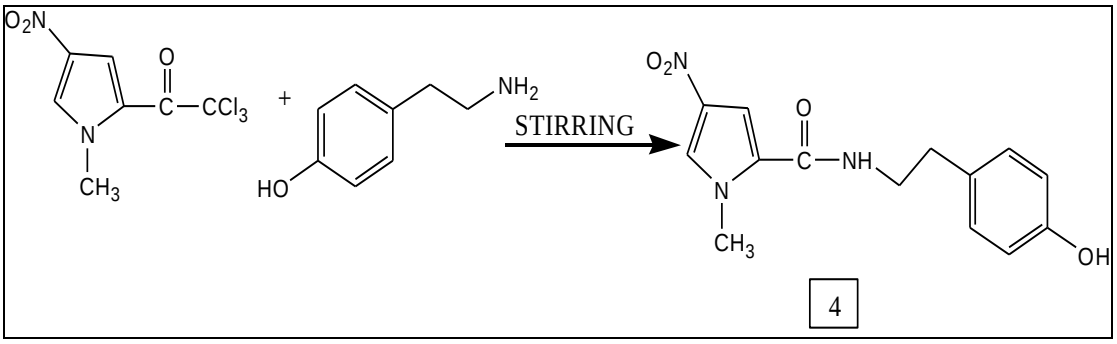
2.3.1.4 Preparation of 1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid (2-(4-hydroxy-phenyl)-ethyl)-amide 4

4-(2-aminoethyl) phenol (0.237 g, 1.792 mmol) was dissolved in lowest amount of DCM, dissopropyl amine (0.98ml) was added.

2,2,2-trichloro-1-(l-methyl-4-nitro-1H-pyrrol-2-y1) ethanone (0.467 g, 1.9 mmol) was dissolved in lowest amount of DCM, and then added to 4-(2-aminoethyl) phenol solution. The mixture was left for 24 hours with stirring.

Purification was done by flash chromatography. Yield; 0.389g, 77.8%, m.p. =197.4-199.5°C (Scheme 4).

IR (KBr): 3345.37, 3146.38, 1642.59,1547.58,1355.93, 1302.7,1259.73, 824.05, cm^{-1}.

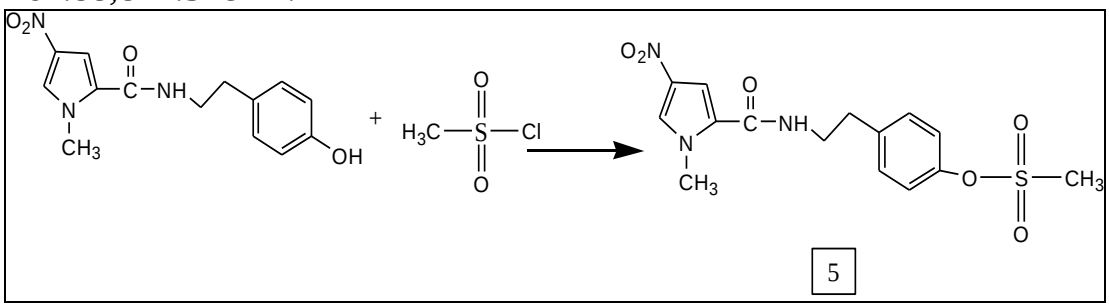


Scheme 4: Preparation of 1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid(2-(4-hydroxy-phenyl)-ethyl)-amide

2.3.1.5 Preparation of Methanesulfonic cid4-(2-((1-methyl-4-nitro-1H-pyrrole-2- carbonyl)-ethyl)-phenyl ester 5

1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid (2-(4-hydroxy-phenyl)-ethyl)-amide (0.1g,0.346mmol) was dissolved in lowest amount of THF. (1ml) of pyridine was added. A solution of (0.04g,3.492mmol) Methanesulfonyl chloride (0.04g,3.492mmol) in THF(1ml) was added to previous mixture and kept for 10 min. Pyridinium salt was precipitated (Scheme 5). Yield; 0.07g, 60.15%, m.p. =130.5-135.5°C.

IR (KBr): 3328.55, 1640.35, 1546.86,1357.94, 1312.1, 1257.55 1167.88,872.51cm^{-1}.

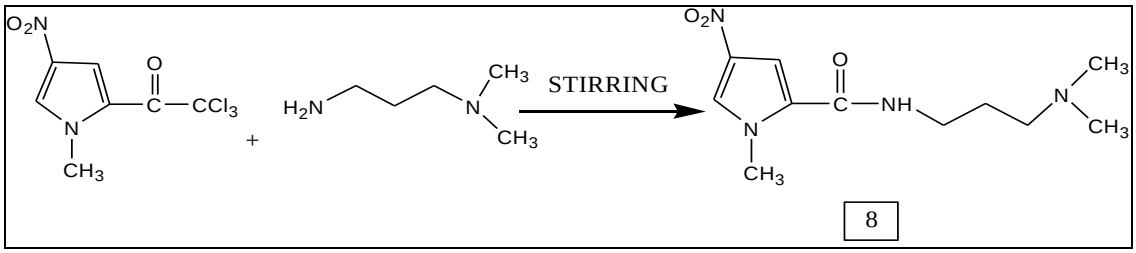


Scheme 5: Preparation of Methanesulfonic cid4-(2-((1-methyl-4-nıtro-1H-pyrrole-2-carbonyl)-ethyl)-phenyl ester.

2.3.1.6 Preparation of 1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid(3-dımethylamino-propyl)-amide 8.

2,2,2-trichloro-1-(l-methyl-4-nitro-1H-pyrrol-2-yl) ethanone (0.531 g ,2.18 mmol) was dissolved in lowest amount of DCM. 3-dimethyl amino propylamine(0.201 g, 1.967 mmol) was dissolved in the lowest amount of THF, then added to the previous reaction mixture, the solution was kept for 24 hours with continuous stirring. Purification was made by extraction with ethyl acetate. Yield; 0.402g, 80.4%, m.p. =123.5-127°C.

IR (KBr):3237.56, 1664.33,1544.09,1340.05, 1137.1 cm^{-1}.



Scheme 6: Preparation of 1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid(3-dımethylamino-propyl)-amide

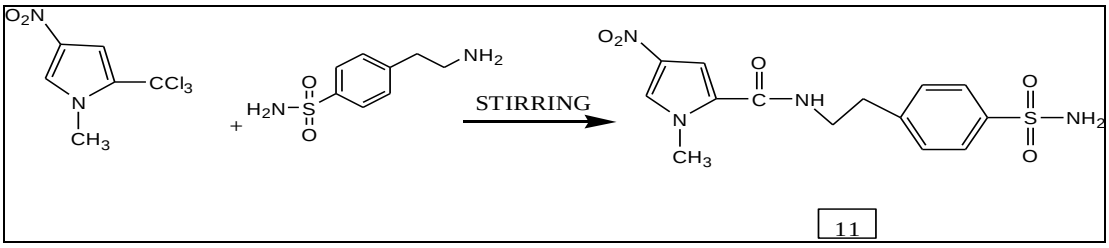
2.3.1.7 Preparation of 1-Methyl-4-nitro-1H-pyrrole-2-carboxylicacid (2-(sulfonyl-phenyl)-ethyl)-amide 11

2,2,2-trichloro-1-(l-methyl-4-nitro-1H-pyrrol-2-yl) ethanone (0.383 g, 1.57 mmol) was dissolved in lowest amount of DCM.

4-(2-Aminoethyl) benzene sulfonamide (0.2844 g, 1.42 mmol) was dissolved in lowest amount of THF, and (1.18 ml) of diisopropyl amine was added, then the solution was added to the former one, and kept for 24 hours with stirring. Purification was done by flash chromatography Scheme7.

(Yield; 0.344g, 68.8%, m.p. =236.3-242.1°C.

IR (KBr): 3384.16, 3143.82, 1640.71,1549.41, 1307.24, 1259.41 683.96 cm^{-1}.



Scheme 7: 5-Preparation of 1-Methyl-4-nitro-1H-pyrrole-2-carboxylıc acid (2-(sulfonyl-phenyl)-ethyl)-amide

2.3.2 Preparation of Distamycin A Analogues

2.3.2.1 Preparation of Methanesulfonic acid 4-(2-((1-methyl-4-(pyridin-3- carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenyl ester6

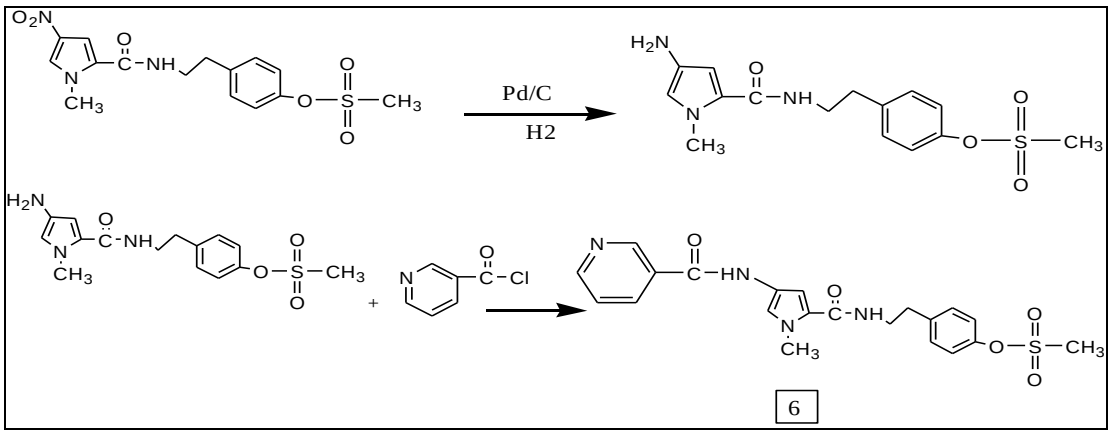
Methanesulfonic acid 4-(2-((1-methyl-4-nitro-1H-pyrrole-2-carbonyl)-ethyl)-phenyl ester (0.2 g, 0.692 mmol) was dissolved in methanol (25 ml). Pd/C (10%, 95 mg) was added slowly to the solution at zero temperature. The suspension was left for 4 hours with stirring, and then was filtrated through (5 g) Celite. The solvent removed under reduced pressure to yield Methanesulfonıc acid 4-(2-((4-amino-1-methyl-1H-pyrrole-2-carbonyl)-amıno)-ethyl)-phenyl ester.

This product was dissolved in DCM (20 ml) and Diisoprpylamine (0.6 g, 0.605 ml). Nicotinoyl chloride (0.108 g, 0.772 mmol) was dissolved in DCM (5 ml) was added drop wise. Solution was left over night with stirring. The product was collected as yellow precipitate and purified by

filtration. The solvent was removed under reduced pressure (Scheme 8). Yield; 0.1g, 55.5%, m.p. =254.3-257.6°C.

IR (KBr): 3385.3,1599.14,1504.1, 1395.39.1290.93,1101.96,869.53, 704.07cm $^{-1}$.

^{1}H-NMR(δ): H3:7.46, H5:6.73, 3H6:3.52, 2H9,12:10.67, 2H13:3.68, 3H14:2.88, 2H16,20:7.22, 2H17,19:7.24,H22:9.35, H24:8.86, H25:7.66, H26:8.5, 3H31:2.8 ppm.



Scheme 8: Preparation of Methanesulfonicacid 4-(2-((1-methyl-4-(pyridin-3- carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenylester

2.3.2.2 Preparation of Methanesulfonic acid 4-(2-((4-benzoylamıno-1-methyl-1H- pyrrole-2-carbonyl)amino)-ethyl)-phenyl ester 7

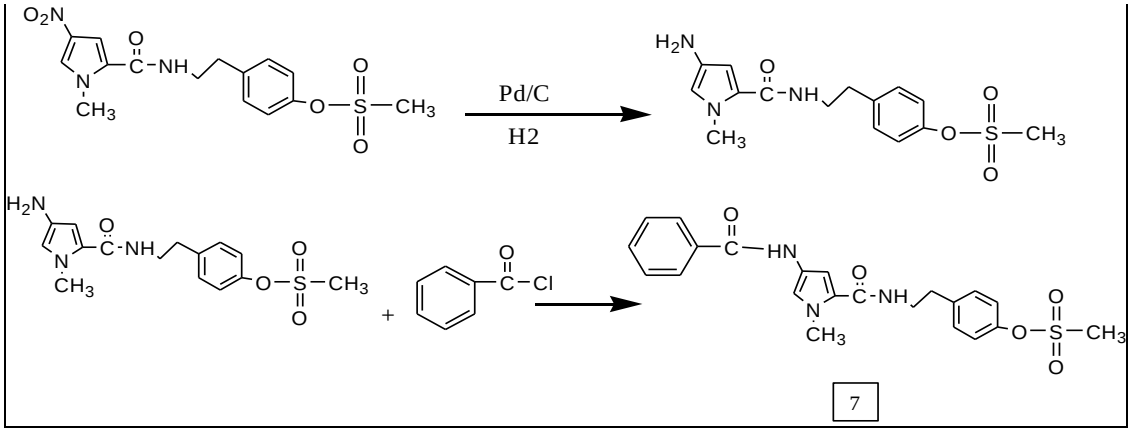
Methanesulfonicacid4-(2-((1-methyl-4-nıtro-1H-pyrrole-2-carbonyl)-ethyl)- phenylester (0.2 , 0.692 mmol) was dissolved in(25 ml) Ethanol (25 ml) Pd/C (10% , 95 mg) was added slowly at zero °C the suspension then was put under hydrogen and allowed to stir for 4 hours.The suspension then filtrated through Celite (5 g) The solvent removed under reduced pressure to yield Methanesulfonicacid 4-(2-((1-methyl-4-nitro-1H-pyrrole-2-carbonyl)-ethyl)-phenyl ester This product was dissolved in DCM 20 ml

diisoprpylamine (0.326 g , 0.3292 ml) added followed by the drop wise addition of Benzoyl chloride(0.108g,0.766 mmol) in DCM (5 ml) , and left over night with stirring.

(Scheme 9) A yellow ppt formed which filtrated and removed under reduced pressure Yield; 0.8g, 44.4%, m.p. =260.0-264.6°C

IR (KBr): 3300.14,1614.47, 1576.1,1395.89,1148.31,1102.3,694.49 cm^{-1}.

^{1}H-NMR (MeOD) δ; H3:7.39, H5:6.66, 3H6:3.52, 2H12,9:10.27, 2H13:3.68, 2H14:2.88,4H:7.23, 2H22,26:7.96,3H:7.57, 3H31:2.8 ppm.



Scheme 9: Preparation of Methanesulfonicacid 4-(2-((4-benzoylamino-1-methyl-1H-pyrrole-2-carbonyl)amino)-ethyl)-phenylester

2.3.2.3 Preparation of N(-((3-(dimethylamino)propyl)amino)carbonyl)-1-methyl-1-H-pyrrole-3-yl)nicotinamide 9

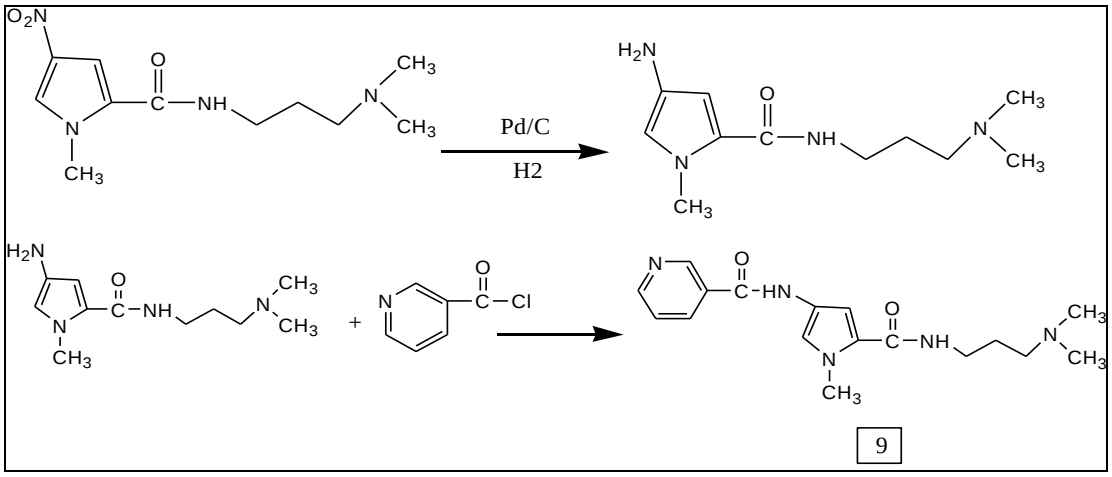
1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid (3-dimethylamino-propyl)-amid (0.200 g, 0.786 mmol) was dissolved in methanol (25ml). Pd/C ((10%, 95 mg) was added slowly at zero temp. The suspension was kept under hydrogen with stirring for 4 hours. The suspension was filtrated

through Celite (5g), and the solvent was removed under reduced pressure. The product was dissolved in DCM(20 ml) and Diisoprpylamine(0.600 , 0.6650 ml).This solution was added drop wise to the solution of Nicotinoyl chloride(0.1257 g , 0.892 mmol) in DCM(5 ml). The solution was kept over night during with stirring. The product was collected as yellow precipitate, filtrated and the solvent was removed under reduced pressure (Scheme 10).

(Yield; 0.09g, 53%, m.p. =195.7-201.2°C

IR (KBr): 3350.85,2969.35,1586.48,1553.57, 1375.24,1261.23,1150.83, 696.19cm^{-1}.

^{1}H-NMR (MeOD) δ; H3:7.41, H5:6.73, 3H6:3.52, 2H12,9:9.26, 2H13:3.51, 2H14:1.41,2 H15:2.44, 6H:2.26, H19:9.35, H21:8.86, H22:7.66, H23:8.5 ppm.



Scheme 10: Preparation of N(-((3-(dimethylamino)propyl)amino)carbonyl)-1-methyl-1-H-pyrrole-3-yl)nicotinamide 9

2.3.2.4Preparation of 4-((benzoylamino)methyl)-N-(3-(dimethylamino) propyl)-N,1dimethyl-1H-pyrrole-2-carboxamide10

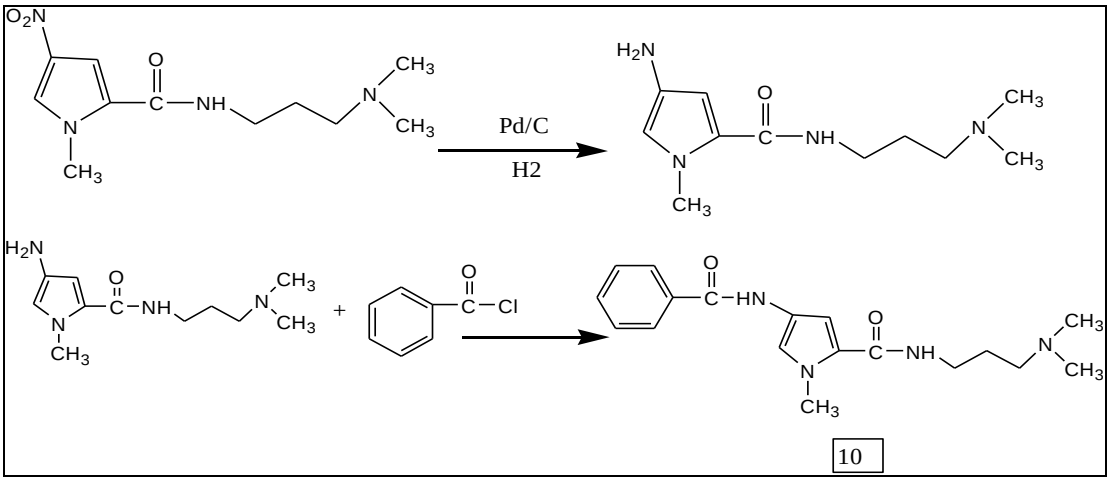
1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid(3-dimethylamino-propyl)-amide (0.2g , 0.787 mmol) was dissolved in Methanol (25 ml),Pd/C (10%, 95 mg) was added slowly at zero temperature. The suspension then was left under hydrogen with steiring for 4 hours The suspension filtrated through celite(5 g) and solvent removed under reduced pressure to yield 4-Amıno-1-methyl-1H-pyrrole-2-carboxylic acid (3-dimethylamino-propyl)-amide. This product was dissolved in DCM (20 ml) Diisoprpylamine (0.377 g , 0.38 ml)was added, followed by drop wise addition of Benzoyl chloride (0.1258 g , 0.9 mmol) in DCM(5 ml) .The solution was left over night with stirring.A yellow precipitate formed which filtrated and removed under reduced pressure (Scheme 11).

Yield; 0.07g, 48%, m.p. =205.1-210.3°C

IR (KBr):

3092.85,962.18,1495.43,1454.14,1264.84,1209,1027.65,765.06cm^{-1}.

H-NMR (MeOD):δ;H3:6.77, H5:6.14, 3H6:3.65, 2H9:5.02, H10,13:7.35, 2H14:3.51, 2H15:1.41, 2H16:2.44, 6H:2.26, 2H20,24:7.64,3H:7.35, ppm.



Scheme 11: 4-((benzoylamino)methyl)-N-(3-(dimethylamino)propyl)-N,1dimethyl-1H-pyrrole-2-carboxamide

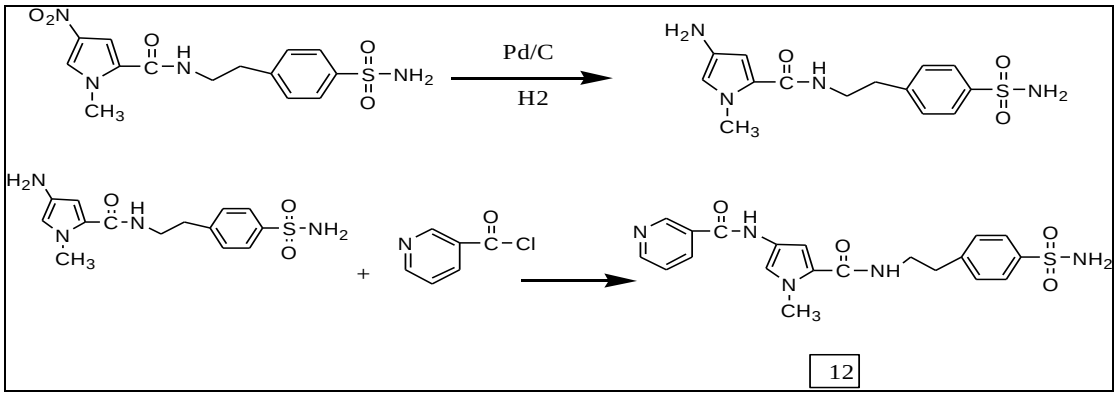
2.3.2.5 Preparation of N - (1 - Methy l – 5 - (2 - (4 – sulfamoyl - phenyl)-ethylcarbamoyl)-1H-pyrrol-3-yl)-nicotinamide 12

1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid (2-(sulfonyl-phenyl)-ethyl)-amide (0.2 g, 0.5665 mmol) was dissolved in ethanol (25 ml), Pd/C (10%, 95 mg) was added slowly at zero temp, the suspension then was left under Hydrogen with stirring for 4 hours. The suspension was filtrated through Celite (5g) then solvent was removed under reduced pressure to yield 4-amino-1-methyl-1H-pyrrole-2-carboxylic acid(2-(4-sulfonyl-phenyl)-ethyl)-amide. This product was dissolved in DCM (20 ml) Diisoprpylamine (0.6 g, 0.605 ml) and added drop wise to Nicotinoyl chloride (0.087g, 0.62mmol) in DCM (5 ml). Solution was left over night with stirring. The product was collected as yellow precipitate and purified by filtration. The solvent was removed under reduced pressure (Scheme 12).

(Yield; 0.11g, 60%, m.p. =295.3-299.1°C.

IR (KBr): 3281.2, 2969.63,1618.77,1565.74,1339.37,1132.42, 1023. 56, 721.21cm^{-1}.

^{1}H-NMR (MeOD):δ;H2:8.85,H3:7.61, H4:8.39, H6:9.24, 2H10:5.18, 5H:7.1, H13:7.31, H15:6.95, 2H19:3.57, 2H20:2.88,2H22,26:7.61, 2H23,25:7.68 ppm



Scheme 12: Preparation of N-(1-Methyl-5-(2-(4-sulfamoyl-phenyl)-ethylcarbamoyl) -1H-pyrrol-3-yl)-nicotinamide

Chapter Three

Biological Activities

3.1 Antioxidant activity

3.1.1 Chemicals:

1, 1–Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid were purchased from Sigma,(Sigma, Aldrich GmbH, Sternhheim, Germany), while sodium carbonate, ethanol, chloroform and other chemicals and reagents were purchased from Merck (Darmstat, Germany), chloramphenicol, peptone, agar, dextrose, ethanol, Muller–Hinton agar (Fluka), Sabouraud dextrose agar (Difco), gentamicin, ampicilline, amphotricine B, econazole, ethanol, DMSO and all chemicals and reagents were of analytical grade.

3.1.2 DPPH Assay

The hydrogen atom or electron donation abilities of the corresponding compounds were measured from the bleaching of purple-colored methanolic solution of 1, 1-diphenly-2-picrylhydrazyl-hydrate (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent. 200 µl of various concentrations of the compounds in (3ml ethyl acetate+ 7ml ethanol) were added to 3 ml of 0.004% methanol solution of DPPH. After 30 minutes, incubation period at room temperature, the absorbance was read against a blank at 517 nm. The percent Inhibition I (%) of free radical by DPPH was calculated as follows:

$I\ (\%) = ((A_{blank} - A_{sample})/ A_{blank}) \times 100$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Compounds concentration providing 50% inhibitions (IC_{50}) are calculated from the plot of inhibition (%) against compound concentration. Tests were carried out in triplicates.

3.2 Antibacterial activity

3.2.1 Microorganisms used and growth conditions:

Antimicrobial activity of compounds was determined through agar disc diffusion method.[45] All the cultures and isolates investigated in this study were obtained from Biodiversity and Environmental Research Center (BERC) Til Village Palestine, and experiments were performed in triplicate (Table 1).

Table 3.1: Types and names of investigated microorganisms

Micro-organism	Names	Accession No.	Source
Bacteria	*Staphylococcus aureus* *Escherichia coli* *Klebsiella pneumoniae* *Proteus vulgaris* *Pseudomonas aeruginosa* *Salmonella enteica*	(ATCC 25923) (ATCC 25922) (ATCC 13883) (ATCC 13315) (ATCC 27853) (ATCC 14028)	Biological and Enviromental Research Center (BERC)

3.2.3 Disk diffusion method

Disc diffusion method was made according to (Zongo *et al.* 2009) [45].Fifty mg of each compound dissolved in a mixture of two solvents (3ml ethyl acetate and 7 ml ethanol) to a final concentration (10 mg/ml) and sterilized by filtration through a 0.45 mm membrane filter. Inoculums (10^6 bacterial cells/ ml) were spread on Muller–Hinton agar plates (1 ml

inoculum/ plate). Filter paper discs (6 mm in diameter) were individually impregnated with 50 µl of each compound. The disks were left for 10 minutes to evaporate the solvent and then placed onto the agar plates. Before incubation, all Petri dishes were kept in the refrigerator (4 C) for 2 h and incubated after at 37 C for 24 h for bacteria growth. After incubation, the diameters (mm) of the inhibition zones were measured including the diameter of discs. The antimicrobial potentials were estimated according to index reported by (Rodriguez *et al.* 2007) [46]. Gentamicin concentration and DMSO (µg/disc) served as a positive and negative control, respectively.

3.3 Antifungal Activity

3.3.1 Microorganisms:

All the fungi tested were from BERC center

Table 3.2: Types of investigated fungi

Fungi	*Trichophyton rubrum* *Microsporum canis* *Epidermaphyton Floccosum var Floccosum*	(CBS 398.58) (CBS 132.88) (CBS 358.93)

3.3.2Antifungal testing

Compounds were tested at different concentrations for their antifungal activity against the test pathogens using a modified poisoned food technique *(Husein et al. 2012)* [47]. Different amounts of each extract were incorporated in pre-sterilized SDA medium to prepare a series of concentrations of the compound 375, 750, 1500 µg/ml). A mycelial agar

disk of 5 mm diameter was cut out of 12 days old culture of the test fungus and inoculated on to the freshly prepared agar plates. In controls, sterile distilled water was used in place of the tested sample. Three replicate plates were used for each treatment (concentration).The inoculated plates were incubated in the dark at 24˚C and the observations were recorded after 10 days. Percentage of mycelial inhibition was calculated using the following formula:

% mycelial inhibition = (dc- ds / dc) x100%

dc: colony diameter of the control , ds: colony diameter of the sample

Chapter Four

General Discussion

4.1 Synthesis of Distamycin A Analogue

The main aim of this project, as mentioned, is to synthesize Distamycin A analogue to target the androgen response element sequence (WGWWCW where W is A or T), then characterize the biological activities of these new analogues of Distamycin A. In this project, we propose to synthesize an analogue of Distamycin A by changing N-terminal alkyl group which have lower molecular weight and higher lipophilicity than previous analogues in order to improve their binding with minor groove of DNA and increase the absorption and cell permeability of these compounds. These compounds, which bind non–covalently to the minor groove of DNA, have shown therapeutic potential in the treatment of cancer, viral, fungal and bacterial diseases.

Changing N-terminal alkyl group which have lower molecular weight and higher lipophilicity than previous analogues.

From the retrosynthetic analysis shown previously (**Figure** 1.11), the proposed analogues can be prepared by using a convergent synthesis mainly through coupling the acid chloride with an amine to form the amide link.

4.2 Synthesis of the Minor Groove Binders Monomers

2,2,2-Trichloro-1-(1-methyl-4-nitro-1H-pyrrole-2-yl) ethanone **3** (Figure 4.1) was prepared by the nitration of 2-trichloroacetyl-N-methylpyrrole

with nitric acid in acetic anhydride , in a good yield (75%). 2-Trichloro-acetyl-N-methylpyrrole **2** was also prepared in a good yield (75%) by the reaction of Trichloroacetylchloride **1**and N-methylpyrrole.

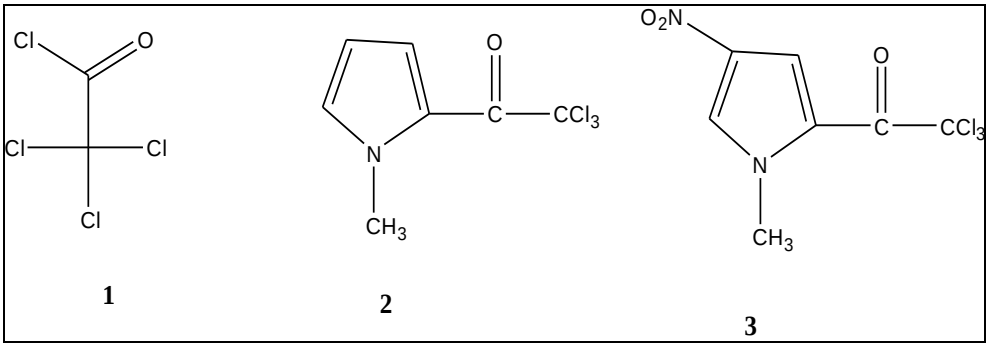


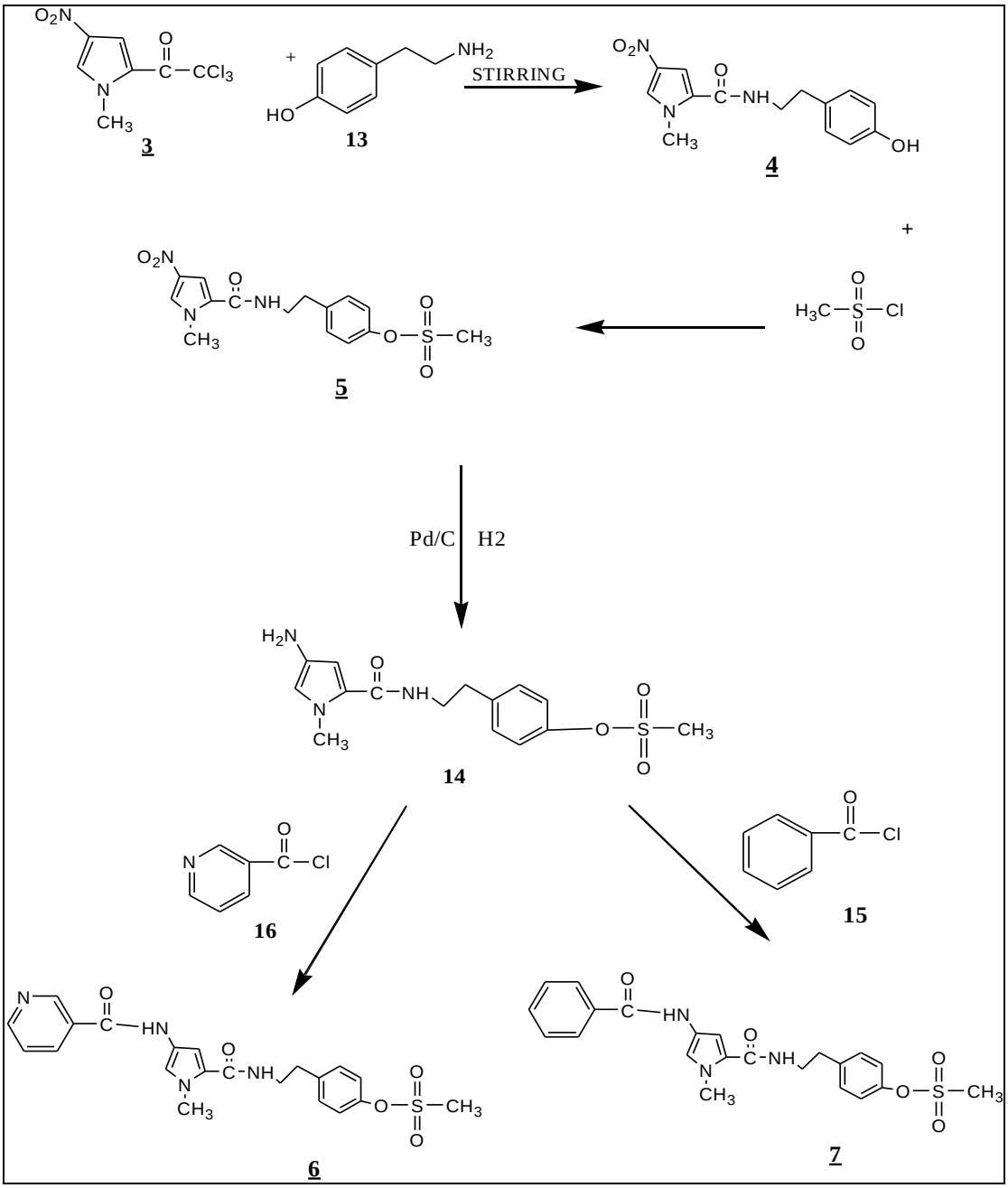
Figure (4.1): Structure of Some Monomers Used in the Synthesis of Distamycine A Analogues.

Also trichloroacetylchloride**1** was prepared in a good yield (82.84%) by the reaction of trichloroacetic acid with oxalyl chloride by reflux.

4.3 Synthesis of of Methanesulfonic acid 4-(2-((1-methyl-4-(pyridin-3-carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenyl ester 6. and synthesis of Methanesulfonic acid 4-(2-((4-benzoylamino-1-methyl-1H-pyrrole-2-carbonyl)amino)-ethyl)-phenyl ester 7.

N-(4-hydroxyphenethyl)-1-methyl-4-nitro- 1H-pyrrole-2-carboxamide 4 was prepared by the coupling between the two compounds 3 and 13this followed by protection the hydroxyl group to prevent its hydrogenation in the next step with methanesulfonyl chloride to produce compound 5 .Then followed by catalytic hydrogenation using Pd-C/H_2 to yield product 14 (Scheme 13). The amino compound 14 was used directly in the next step of synthesis due to the lack of stability of the amino group attached to the pyrrole ring. Methanesulfonic acid 4-(2-((1-methyl-4-(pyridin-3-carbonyl)-

amino)-1H-pyrrol-2-carbonyl)-amıno)-ethyl)-phenyl ester 6 was prepared by the coupling between the two compounds 16 and 14 , with yield 55.5%. Methanesulfonic acid 4-(2-((4-benzoylamino-1-methyl-1H-pyrrole-2-carbonyl)amino)-ethyl)-phynel ester 7 was prepared by the coupling between 15 and 14 , with yield 44.4% (Scheme 13).

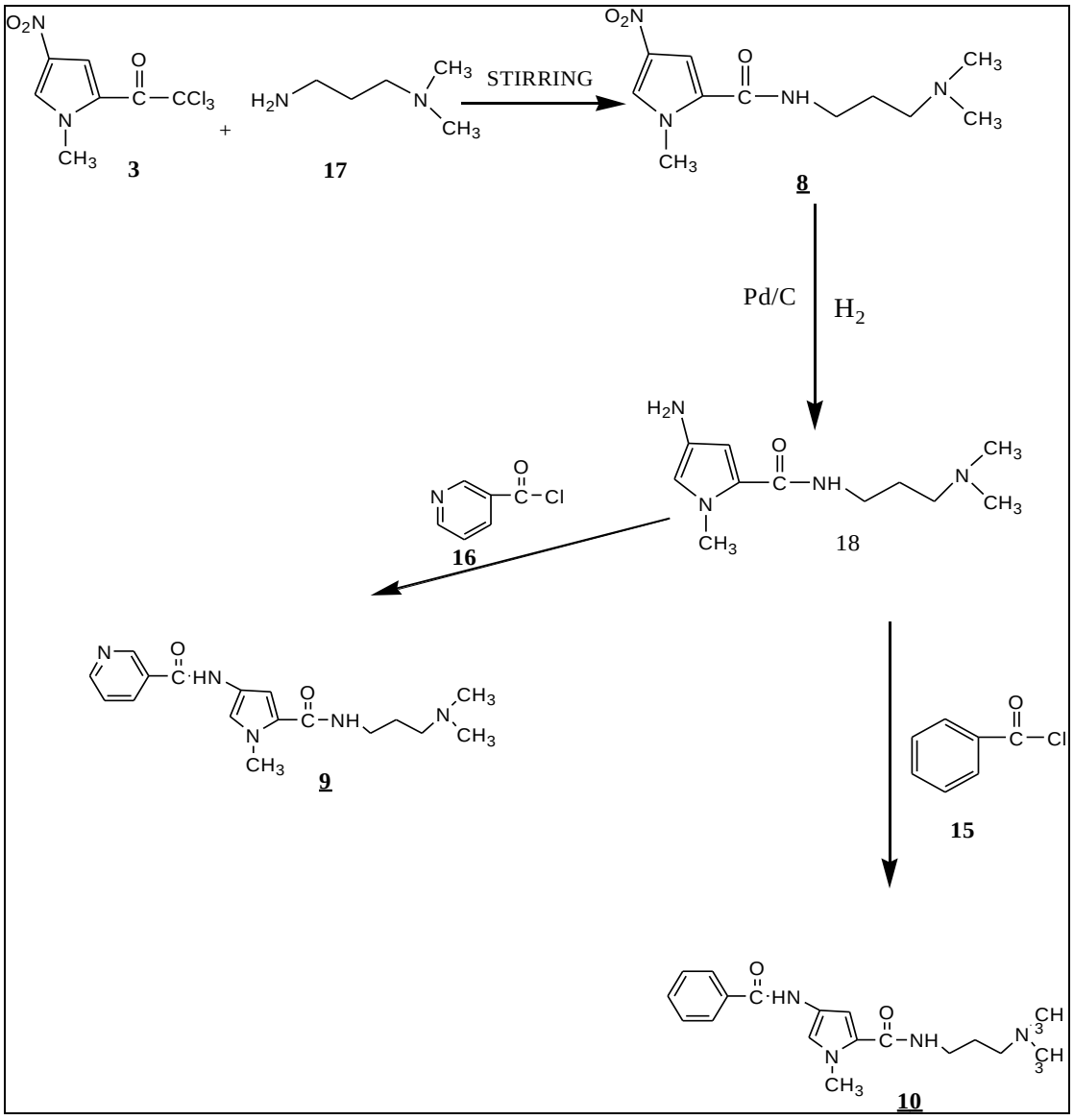


Scheme13: Distamycin A analogues 6,7.

4.4 Synthesis of N (- ((3- (dimethylamino) propyl) amino) carbonyl) - 1-methy l - 1 - H - pyrrole - 3 - yl)nicotinamide 9 and Synthesis of 4 - ((benzoylamino) methyl) - N - (3- (dimethylamino) propyl) - N, 1 dimethy l-1H-pyrrole-2-carboxamide 10.

1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acıd(3-dimethylamino-propyl)-amide 8 was prepared by the coupling between compound 3 and compound 17.Then followed by catalytic hydrogenation using Pd-C/H_2 to yield product 18. The amino compound 18 was used directly in the next step of synthesis due to the lack of stability of the amino group attached to the pyrrole ring. N(-((3-(dimethylamino)propyl)amino)carbonyl)-1-methyl-1-H-pyrrole-3-yl)nicotinamide 9 was prepared by the coupling between the two compounds 16 and 18 , with yield 53% (Scheme 14).

4-((benzoylamino)methyl)-N-(3-(dimethylamino)propyl)-N,1dimethyl-1H-pyrrole-2-carboxamide 10 was prepared by the coupling between the two compounds 15 and 18, with yield 48%.

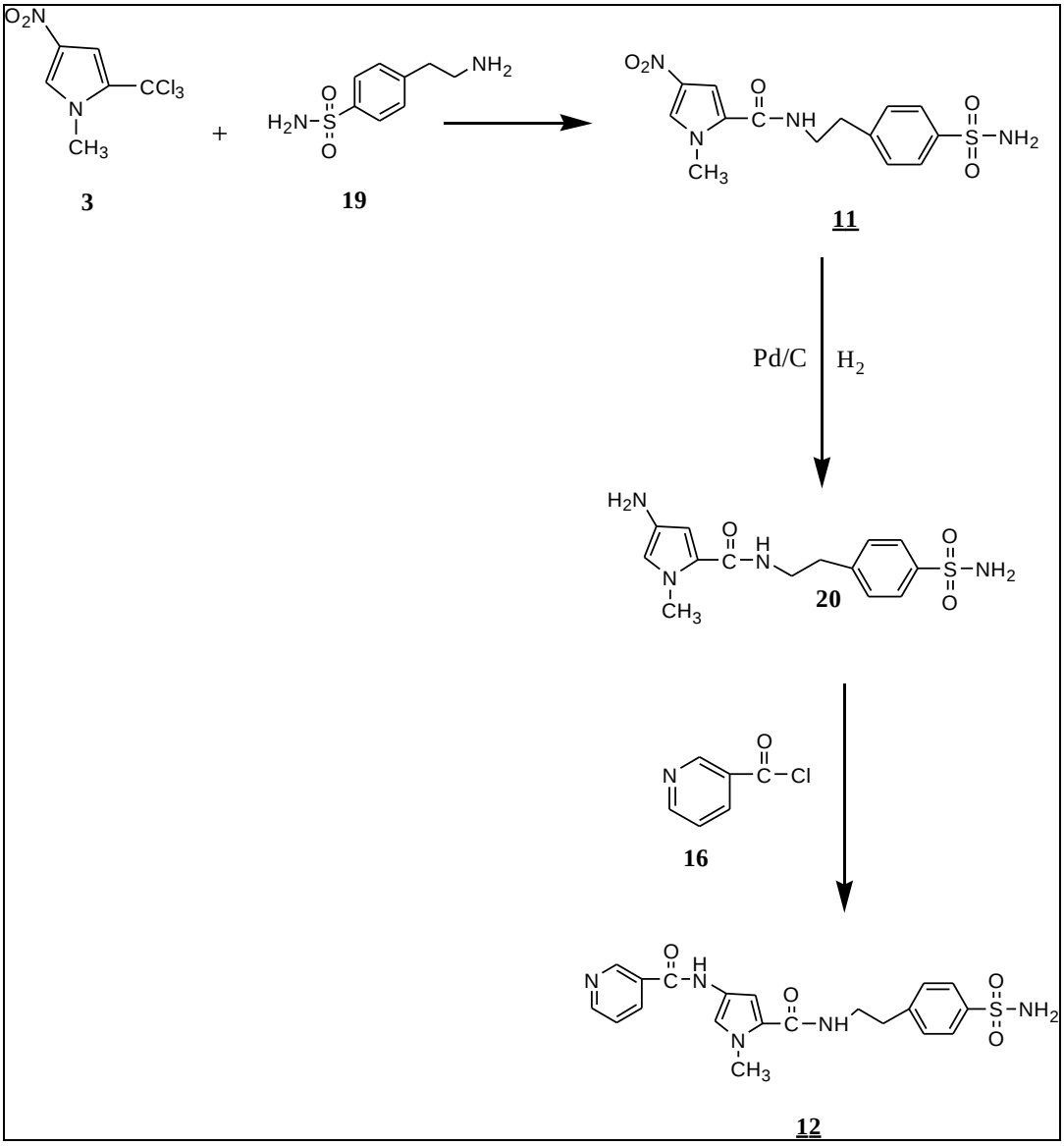


Scheme14: Structure of Distamycine A Analogues 9,10

4.5 Preparation of N- (1 – Methyl – 5 - (2 - (4 – sulfamoyl – phenyl) – ethylcarbamoyl) -1H-pyrrol – 3 – yl) - nicotinamide 12

1-Methyl-4-nitro-1H-pyrrole-2-carboxylıc acid(2-(sulfonyl-phenyl)-ethyl)-amide 12 was prepared by the coupling between 3 and 19.Then followed by catalytic hydrogenation using Pd-C/H_2 to yield product 20. The amino compound 20 was used directly in the next step of synthesis due to the lack of stability of the amino group attached to the pyrrole ring.

N-(1-Methyl-5-(2-(4-sulfamoyl-phenyl)-ethylcarbamoyl)-1H-pyrrol-3-yl)-nicotinamide 12 was prepared by the coupling between 16 and 20 , with yield 60% (Scheme 15).



Scheme15: Structure of Distamycin A Analogues 12

Table 4.1 Frequencies of main functional groups of Distamycine A analogues in cm^{-1}

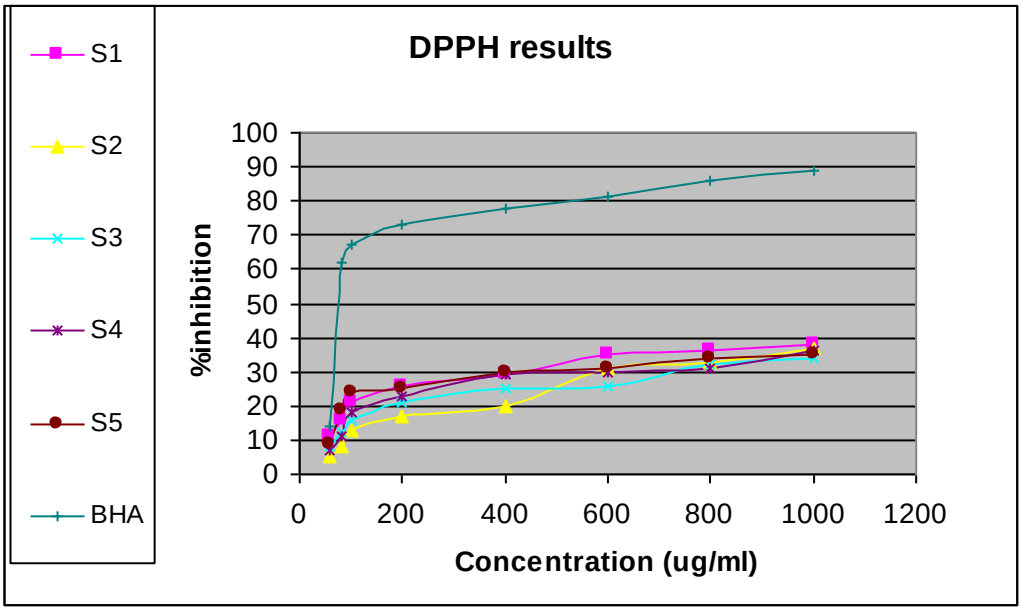
Others	**C-H** Aromatic bending	**C=C** Aromatic stretching	**C-N** stretching of 2^0 amide	**C=O** stretching of 2^0 amide	**N-H** stretching of 2^0 amide	**Cpd No**
S=O 1101.96,1290.93	704.07	1395.39	1101.96	1599.14	3385.30	**6**
S=O 1395.89,1148.31	694.49	1576.1	1102.30	1614.47	3300.14	**7**
CH2 stretching 2969.35 **C-N** of 3^0aliphatic amine 1261.23	696.19	1553.57	1150.83	1586.48	3350.85	**9**
CH2 stretching 2962.18 **C-N** of 3^0aliphatic amine 1209	765.06	1454.14	1027.65	1495.43	3392.85	**10**
S=O 1339.37,1132.42 **CH2** stretching 2969.63	721.20	1561.74	1023.56	1618.77	3281.2	**12**

4.6 Biological Activity Result

4.6.1 Antioxidant Activity Test.

Ethanolic solutions (10 mg/ml) of each compound were prepared to study their antioxidant activities. DPPH is one of the methods used to evaluate the antioxidative activity of antioxidants. As shown in (Figure 4.5) Distamycin A analogues gave various degrees of free radical scavenging, but the values were lower than that of BHA. The concentrations of the tested compounds needed to reduce DPPH absorption by 50 % at 517 nm were more than 1200 µg/ml. While for BHA was 100 µg /ml. These results

didn’t show significant difference in the antioxidant activity between those. It has been shown that the antioxidant activities of the compounds are slightly affected by changing the moiety of the compound. This is consistent with studies done on polyphenolic compounds and showed that the structure is not required for the antioxidant activity [23, 24].



Figure(4.2): Antioxidant effect of all synthesized compounds

4.6.2 Ntibacterial Activity

All the tested compounds didn’t show distinguishable results. The most active one was S2 and S8. They reveled about 10% of *Gentamicin*

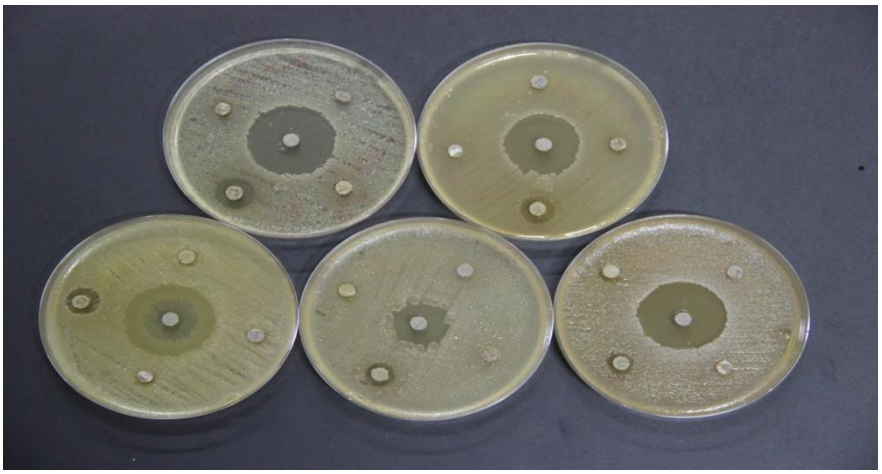


Figure 4.3: Antibacterial activities of synthesized compound

4.6.3 Antifungal Activity.

Tables 1,2,3 and Figure 4.7 showed the nature static activity of the synthetic compounds. S3 revealed the highest activity against *M. canis* (72% inhibition) and E. floccosum (61%). S2 was least active one which showed less activity against T. rubrum (40%), E. floccosum (21%) and M. canis (24%).

Table 4.2:Antifungal activity of the compounds against *Trychophyton Rubrum*

Compound	% inhibition Concentration		
	C1=375ug/ml	C2=750ug/ml	C3=1500ug/ml
S1	17.6 ±4.2	35.2 ±4.2	67± 2.5
S2	8.79 ±5.5	25.3 ±3.6	39.5± 18
S3	17.6 ±4.2	30.8 ±4.2	47.2± 3.5
S4	25.3 ±3.6	40.7±5.7	59.3± 4.2
S7	27.5 ±2.5	29.7±3.6	39.5± 12
S8	34.1± 3.6	41.8±4.2	59.3± 4.2
S9	34.1± 3.6	38.5±3.6	58.2± 5.7

Table 4.3: Antifungal activity of the compounds against *Microsporum canis*

Compound	% inhibition Concentration		
	C1=375ug/ml	C2=750ug/ml	C3=1500ug/ml
S1	9.68± 2.6	32.3±2.6	49.2± 3.1
S2	4.84± 4.2	21± 4.2	22.6± 2.6
S3	29± 2.6	40.3±4.2	71± 2.6
S4	14.5± 1.9	33.1±3.1	54± 5.5
S7	21± 4.2	23.4±3.1	54.8± 2.6
S8	29.8± 3.1	39.5± 2.6	64.5± 2.6
S9	30.6± 4.2	39.5±3.1	46.8± 4.2

Table 4.4: **Antifungal activity against *Epidermophyton floccosum var flaccosum*.**

Compound	% inhibition Concentration		
	C1=375ug/ml	C2=750ug/ml	C3=1500ug/ml
S1	12.2± 2.2	32.2±4.3	42.2± 3.6
S2	11.1± 3.6	16.7±4.3	21.1± 2.2
S3	20± 3.6	37.8±3.6	61± 4.3
S4	8.89± 2.6	23.3±4.3	35.6± 2.6
S7	15.6± 3.6	20±3.6	34.4± 4.3
S8	20± 3.6	24.4±3.6	31.1± 2.6

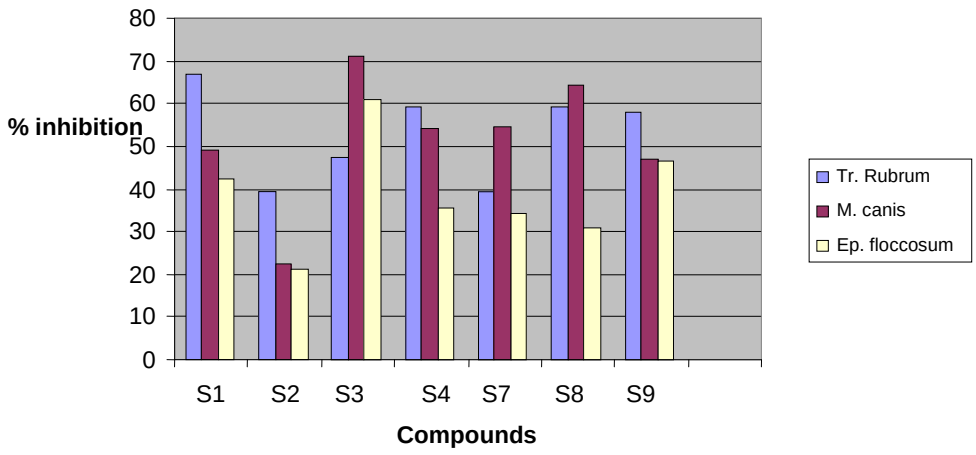


Figure (4.4): Antifungal Activity of tested compound

Table 4.5:Structure of Distamycin A analogues and other compounds used in biological activity test.

Compound Name	Compound Symbol
1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid(3-dimethylamino-propyl)-amide	S1
N-(1-Methyl-5-(2-(4-sulfamoyl-phenyl)-ethylcarbamoyl)-1H-pyrrol-3-yl)-nicotinamide	S2
4-Benzoylamino-1-methyl-1H-pyrrole-2-carboxylic acid(3-dimethylamino-propyl)-amide	S3
N-(5-((3-dimethylamino-propylcarbamoyl)-1-methyl-1H-pyrrol-3-yl)nicotinamide	S4
1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid(2-(4-hydroxy-phenyl)-ethyl)-amide	S5
1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid(2-(sulfonyl-phenyl)-ethyl)-amide	S6
Methanesulfonic acıd 4-(2-((1-methyl-4-(pyridin-3-carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenyl ester	S7
Methanesulfonic acid 4-(2-((4-benzoylamino-1-methyl-1H-pyrrole-2-carbonyl)amino)-ethyl)-phynel ester	S8
Methanesulfonic cid4-(2-((1-methyl-4-nitro-1H-pyrrole-2-carbonyl)-ethyl)-phenyl ester	S9

4.7 Conclusion and Future Work

In conclusion, we provided a successful synthetic pathway for preparing a new distamycin A analogues, using a wet lab synthesis. Also we study the biological activity of these compounds. Some of these analogues showed biological activities, but these molecules will be taken as a lead compounds and various modifications can be done at the head and tail positions of these molecules for enhance there biological activities.

Future work will be concerned with developing the possible analytical uses for these minor groove binders. These compounds can be sent abroad for foot-printing and isothermal titration calorimetry (ITC) studies to

determine their exact binding site, and to determine whether such modifications in the structure of Distamycin A Analogues has any effect on the preferred sequence for binding.

Finally, we faced the absence of some crutial analytical instruments at An-Najah University while carrying out our project, which are required for purification and identification of organic compounds, such as preparative HPLC, mass spectrometer (MS), and NMR spectrometer. The lack of these instruments has slowed our progress due that we used the old fashion methods for purification and identification of organic compounds.

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Appendix

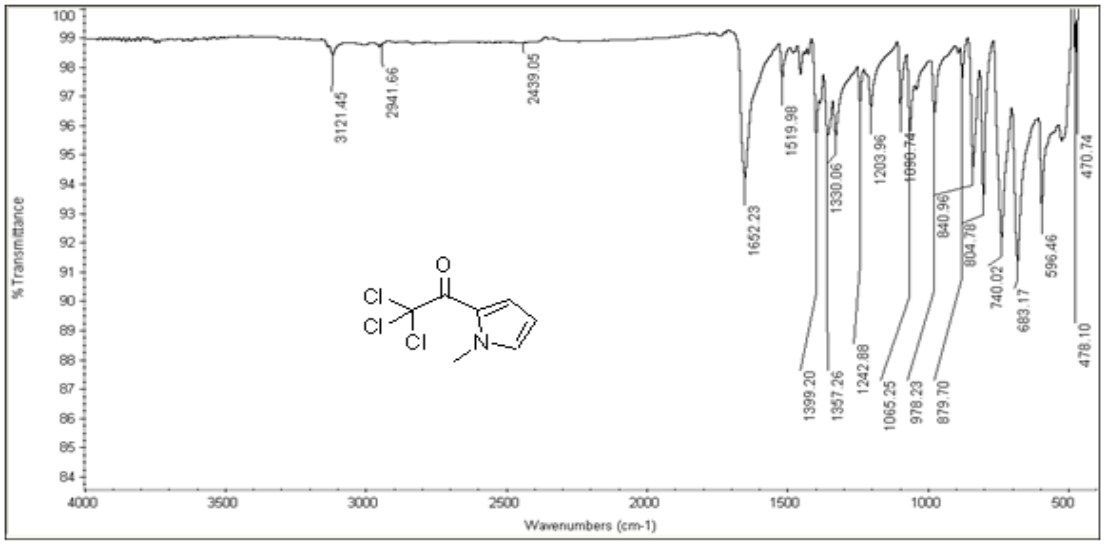

Figure 1: FT-IR spectrum of 2-Trichloroacetyl-N-methylpyrrole **2** .

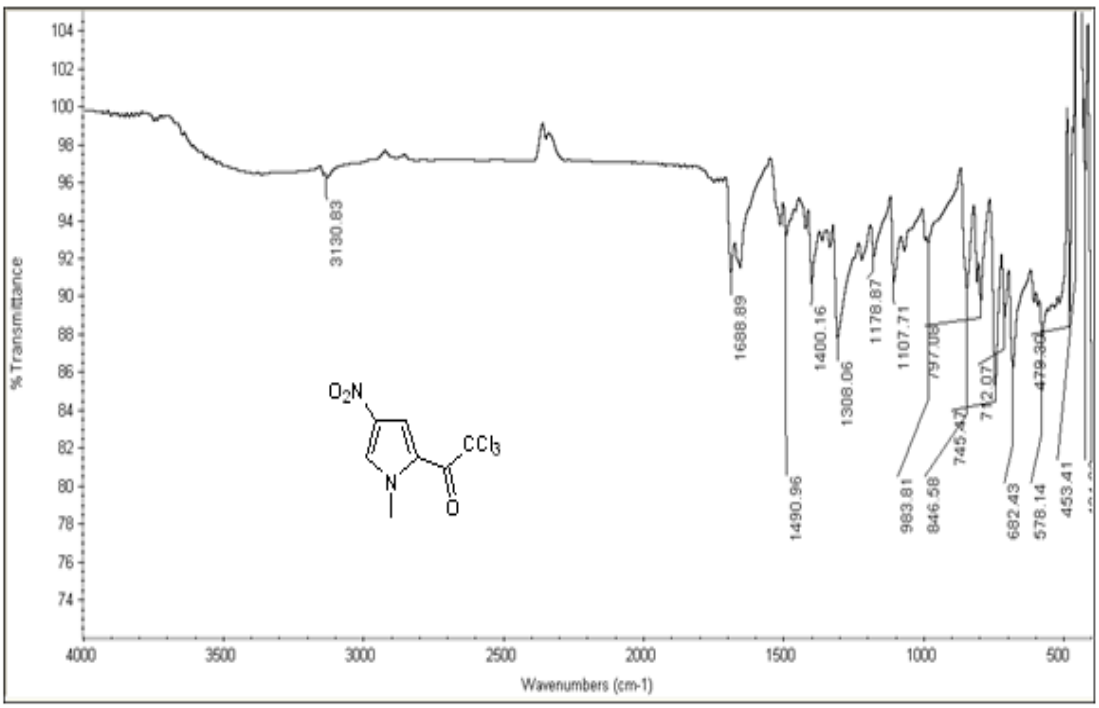

Figure 2 : FT-IR spectrum of 2,2,2-Trichloro-1-(1-methyl-4-nitro-1*H*-pyrrol-2-yl) ethanone **3**

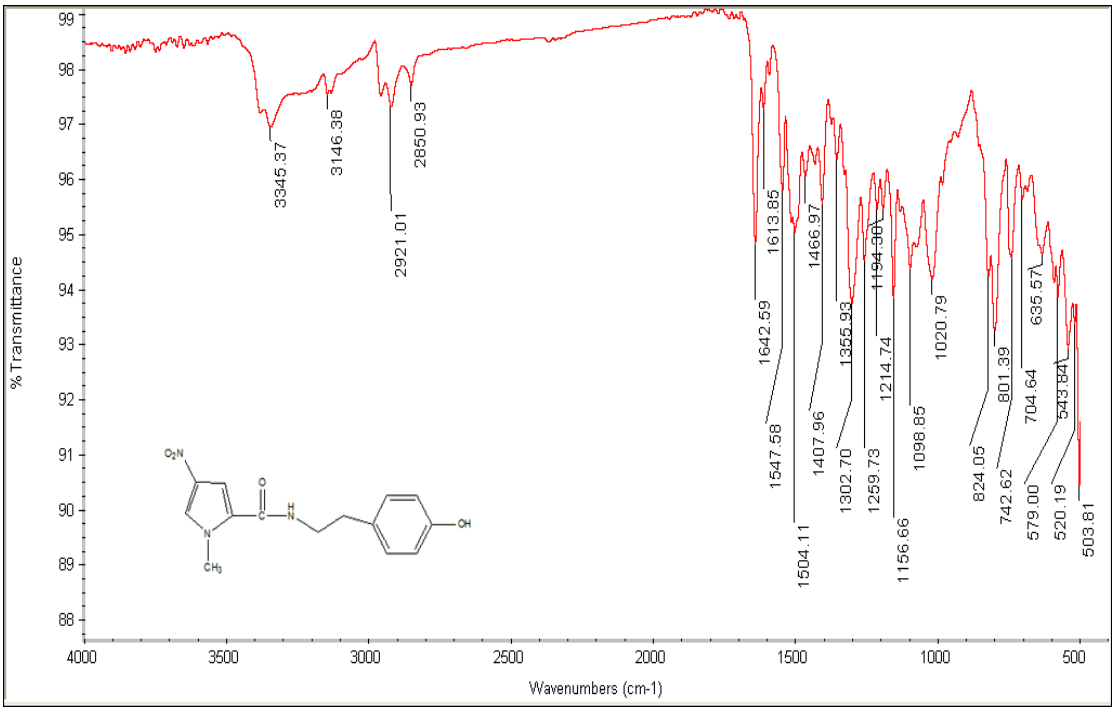

Figure 3 : FT-IR spectrum of 1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid(2-(4-hydroxy-phenyl)-ethyl)-amide **4**.

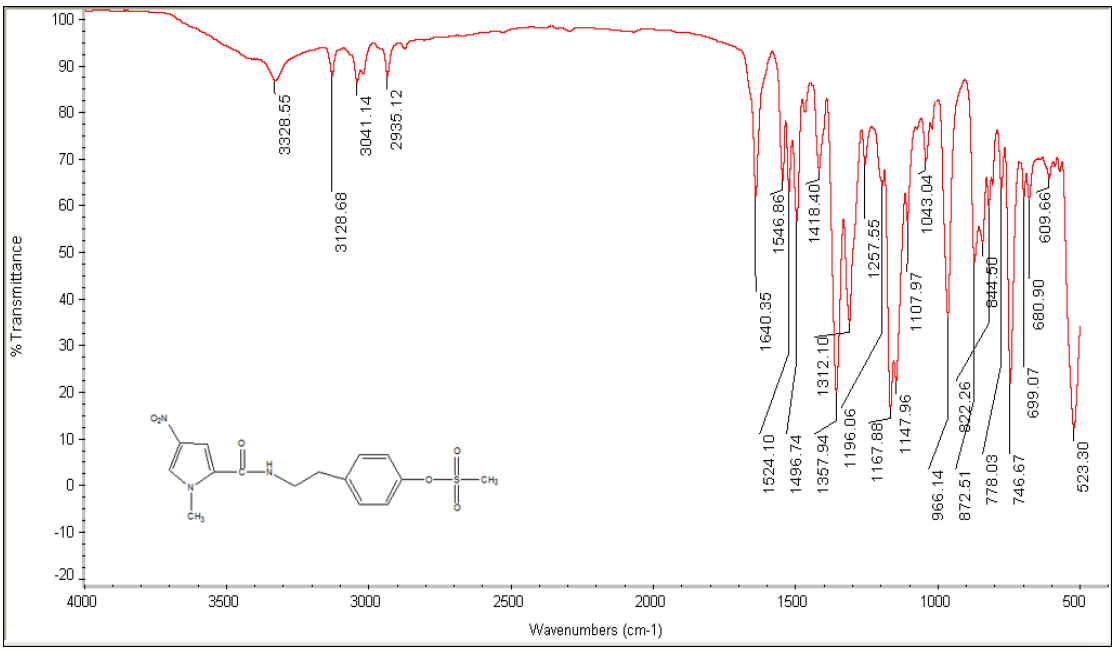

Figure 4 : FT-IR spectrum of Methanesulfonic cid4-(2-((1-methyl-4-nitro-1H-pyrrole-2-carbonyl)-ethyl)-phenyl ester **5.**

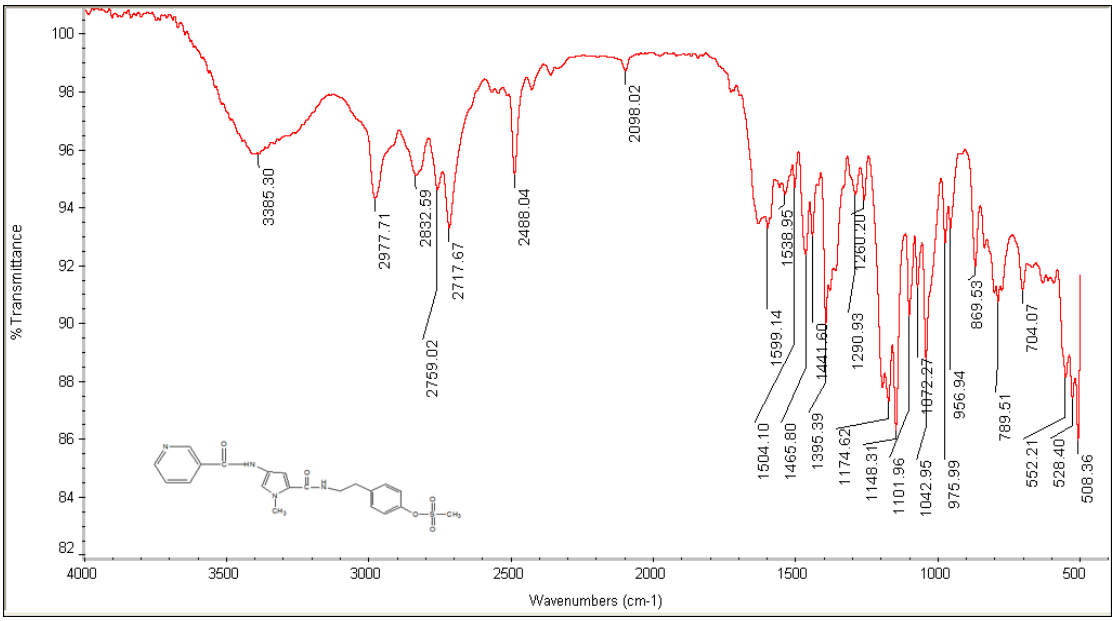

Figure 5: FT-IR spectrum of Methanesulfonic acid 4-(2-((1-methyl-4-(pyrİdİn-3-carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenyl ester **6**.

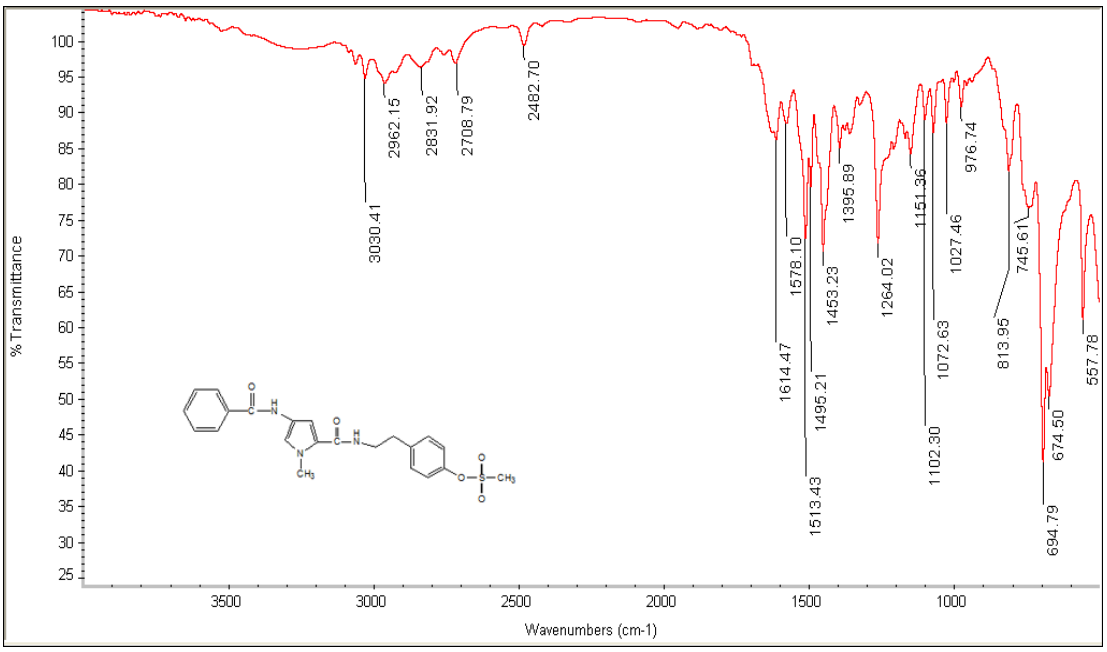

Figure 6: FT-IR spectrum of Methanesulfonıc acid 4-(2-((4-benzoylamino-1-methyl-1H-pyrrole-2-carbonyl)amino)-ethyl)-phynel ester **7**.

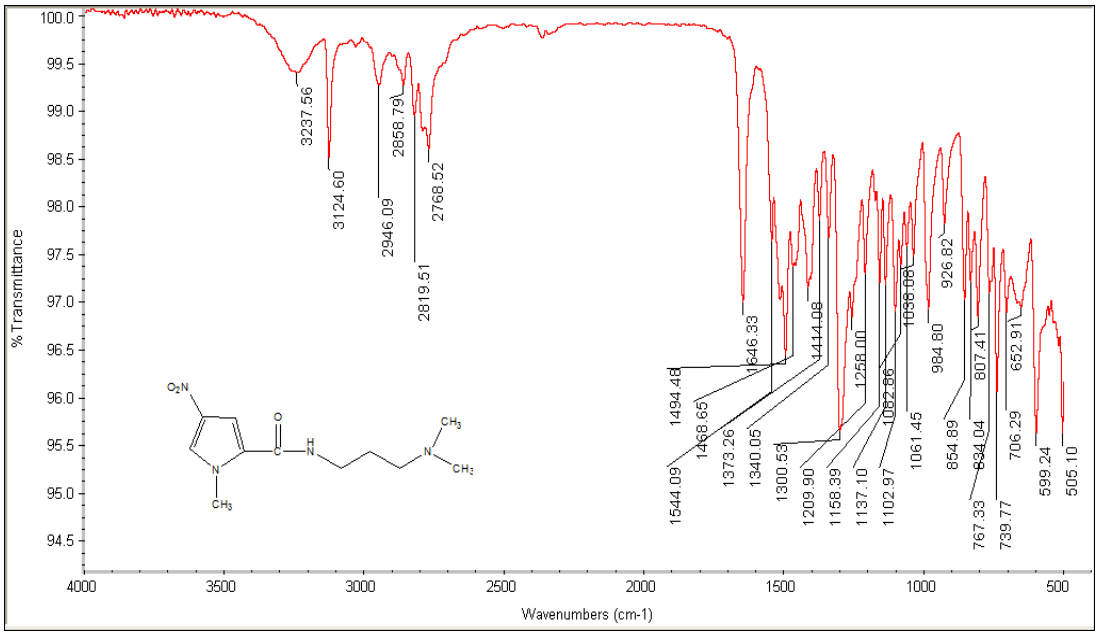

Figure 7: FT-IR spectrum of 1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid(3-dımethylamino-propyl)-amide **8**.

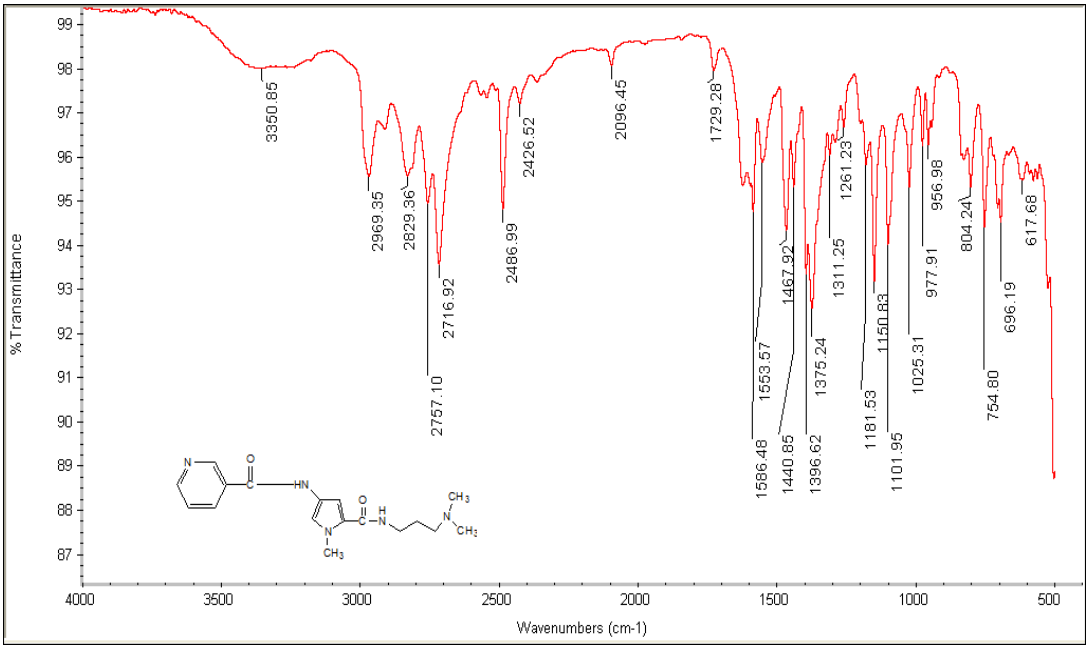

Figure 8: FT-IR spectrum of N(-((3-(dimethylamino)propyl)amino)carbonyl)-1-methyl-1-H-pyrrole-3-yl)nicotinamide **9**.

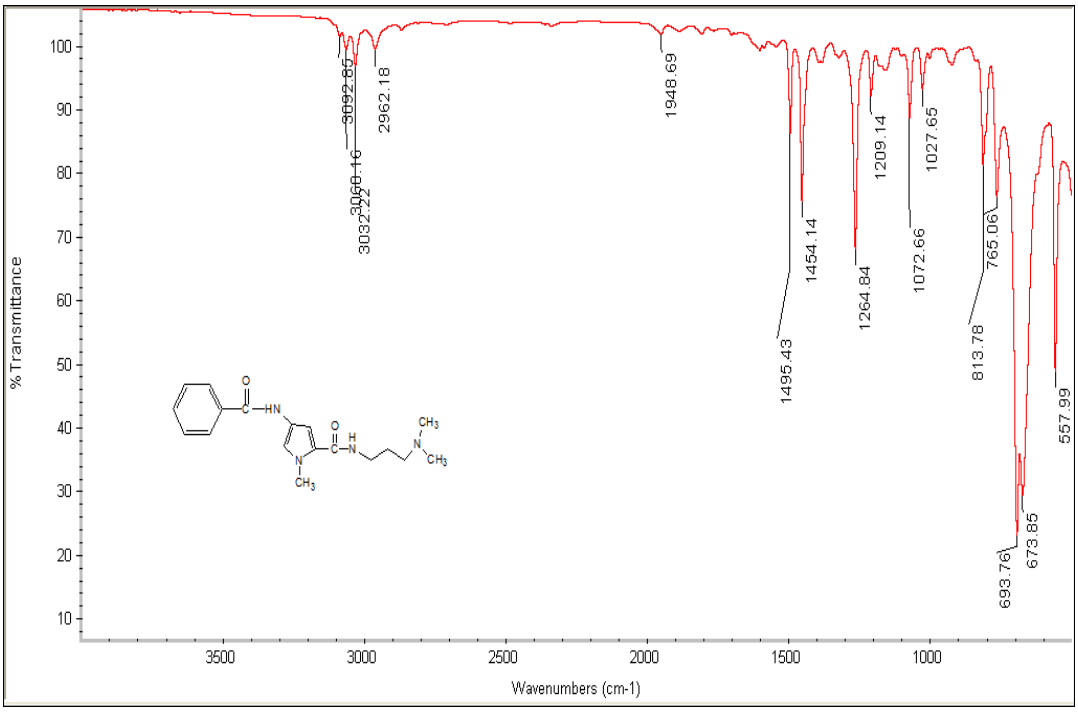

Figure 9: FT-IR spectrum of4-((Benzoylamino)methyl)-N-(3-(dimethylamino)propyl)-N,1dimethyl-1H-pyrrole-2-carboxamide **10**.

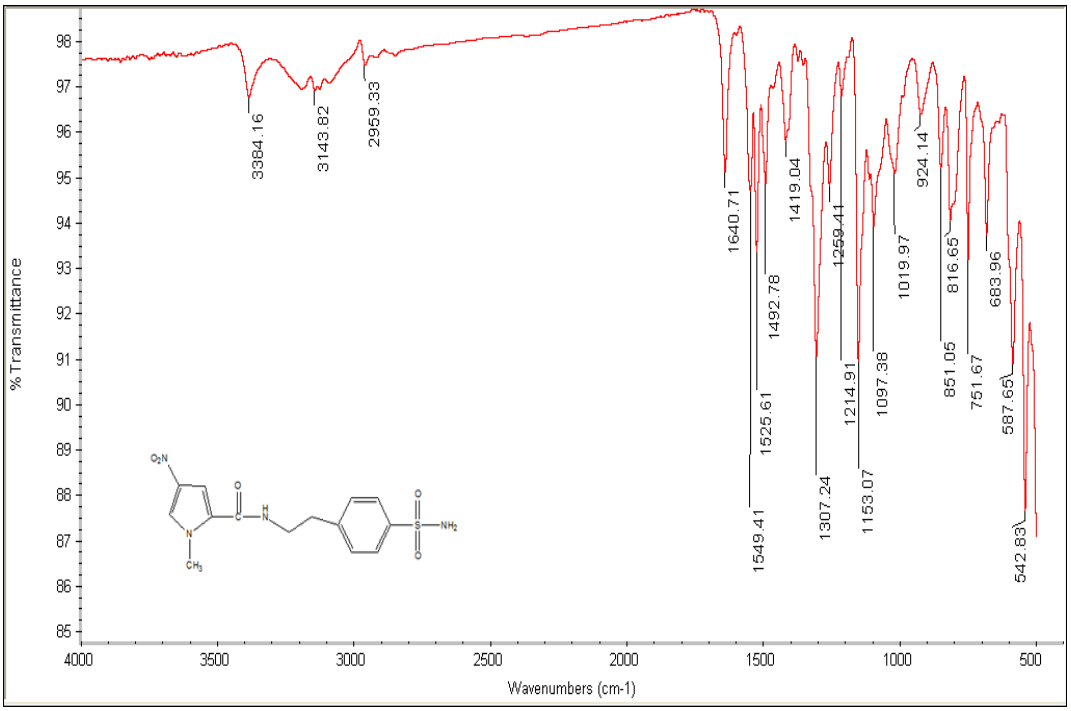

Figure 10: FT-IR spectrum of 1-Methyl-4-nitro-1H-pyrrole-2-carboxylıc acid(2-(sulfonyl-phenyl)-ethyl)-amide **11**.

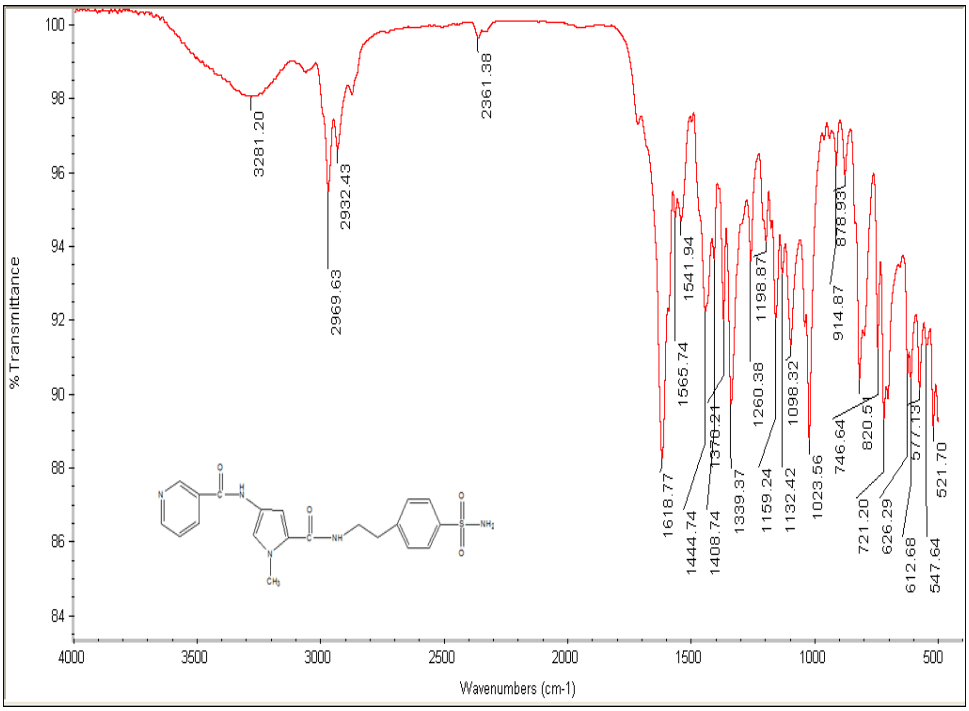

Figure 11: FT-IR spectrum of N-(1-Methyl-5-(2-(4-sulfamoyl-phenyl)ethylcarbamoyl)-1H-pyrrol-3-yl)-nicotinamıde **12**.

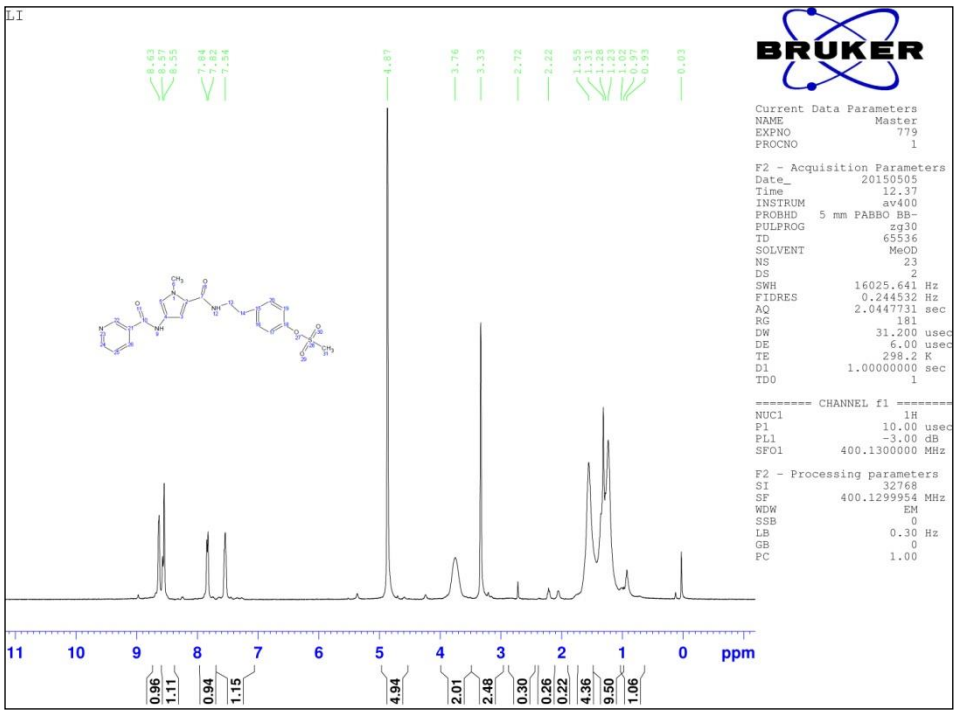

Figure (12): [1]HNMR spectra of Methanesulfonic acid 4-(2-((1-methyl-4-(pyridin-3-carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenyl ester **6**.

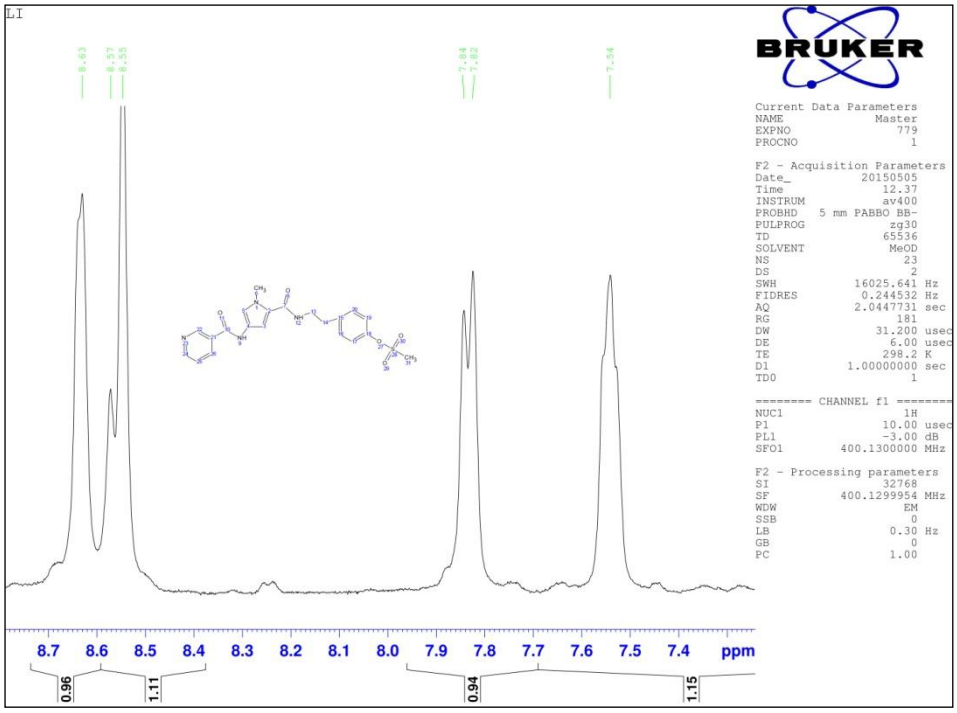

Figure (12.1): [1]HNMR spectra of Methanesulfonic acid 4-(2-((1-methyl-4-(pyridin-3-carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenyl ester **6**.

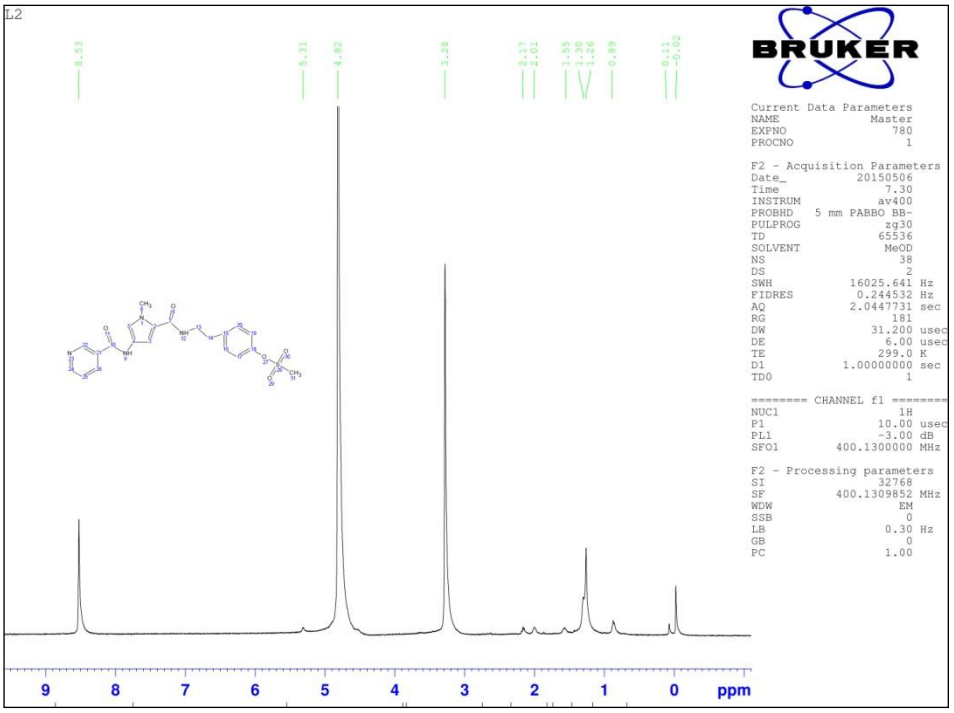

Figure (12.2): [1]HNMR spectra of Methanesulfonic acid 4-(2-((1-methyl-4-(pyridin-3-carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenyl ester **6** .

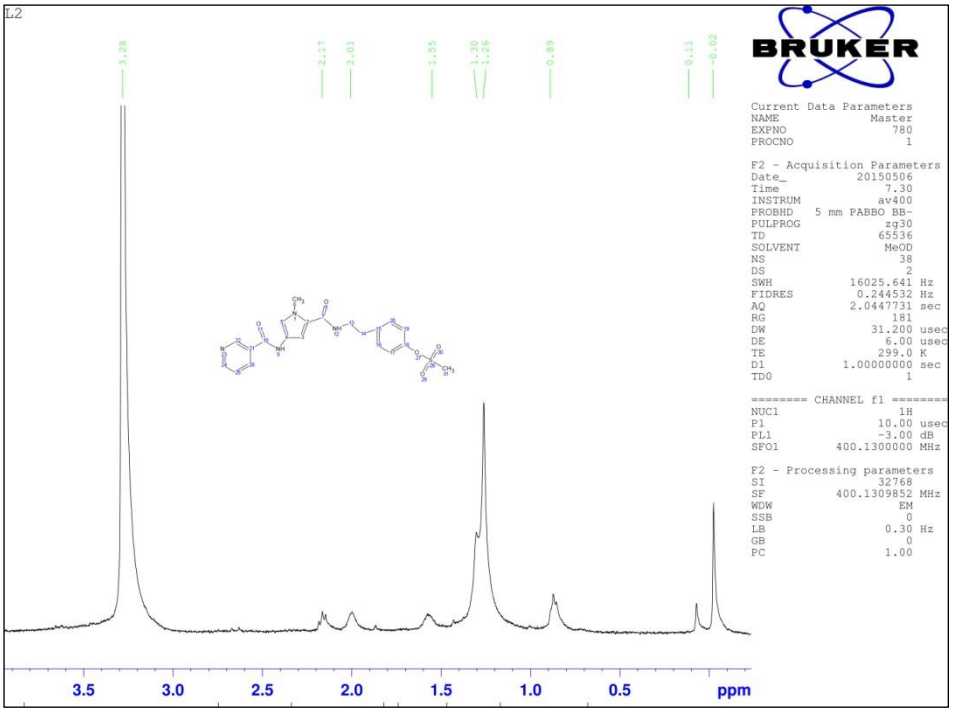

Figure (12.3): [1]HNMR spectra of Methanesulfonic acid 4-(2-((1-methyl-4-(pyridin-3-carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenyl ester **6** .

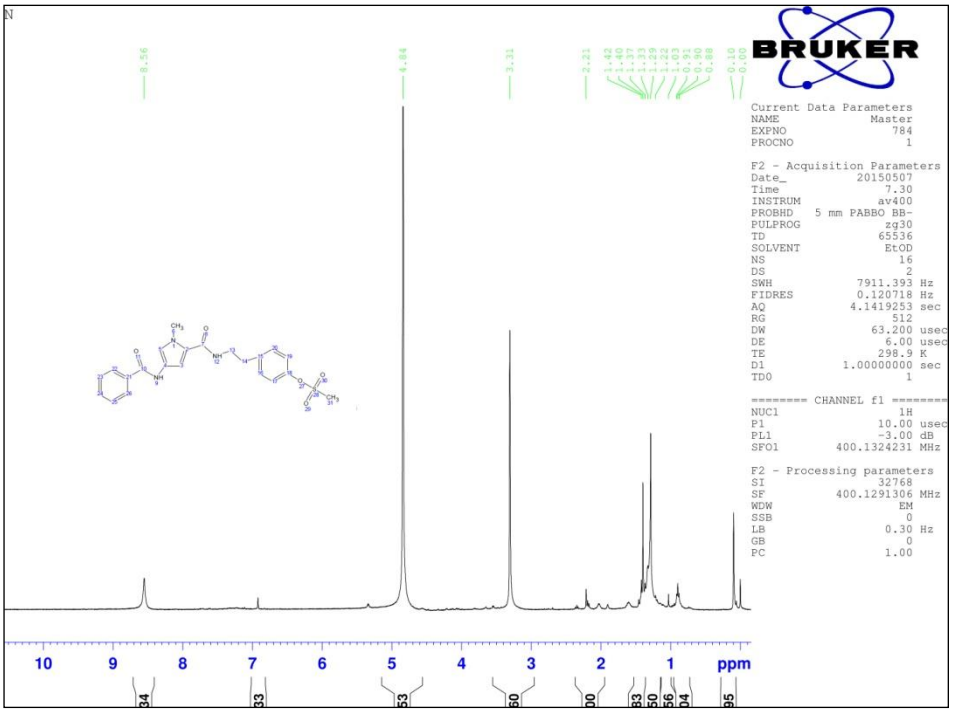

Figure 13: [1]HNMR spectrum of Methanesulfonic acid 4-(2-((4-benzoylamino-1-methyl-1H-pyrrole-2-carbonyl)amino)-ethyl)-phynel ester **7**.

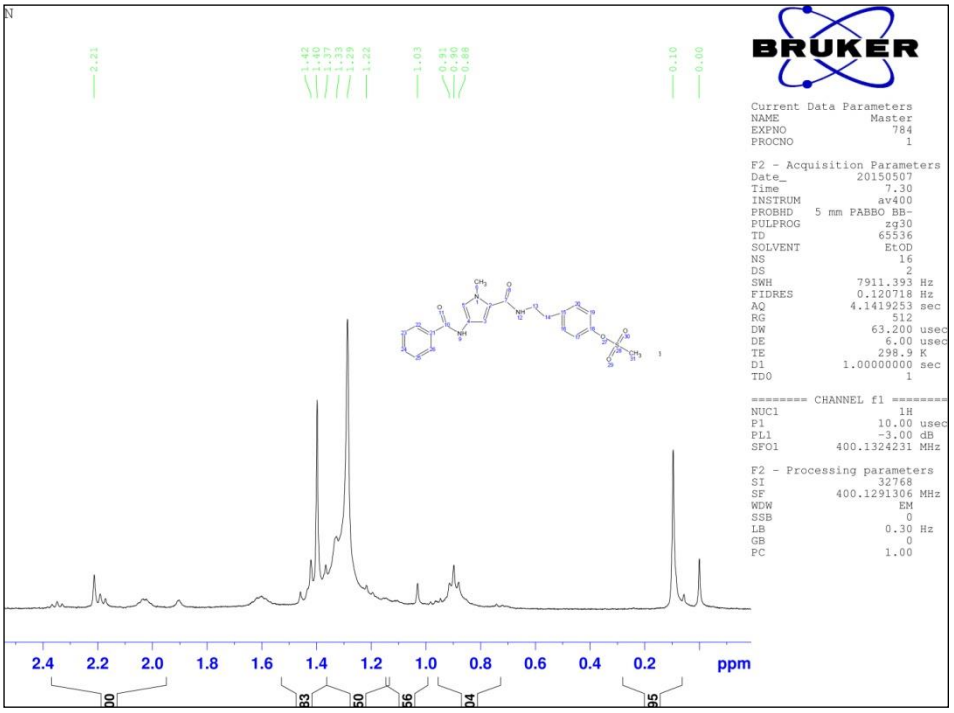

Figure 13.1: [1]HNMR spectrum of Methanesulfonic acid 4-(2-((4-benzoylamino-1-methyl-1H-pyrrole-2-carbonyl)amino)-ethyl)-phynel ester **7**.

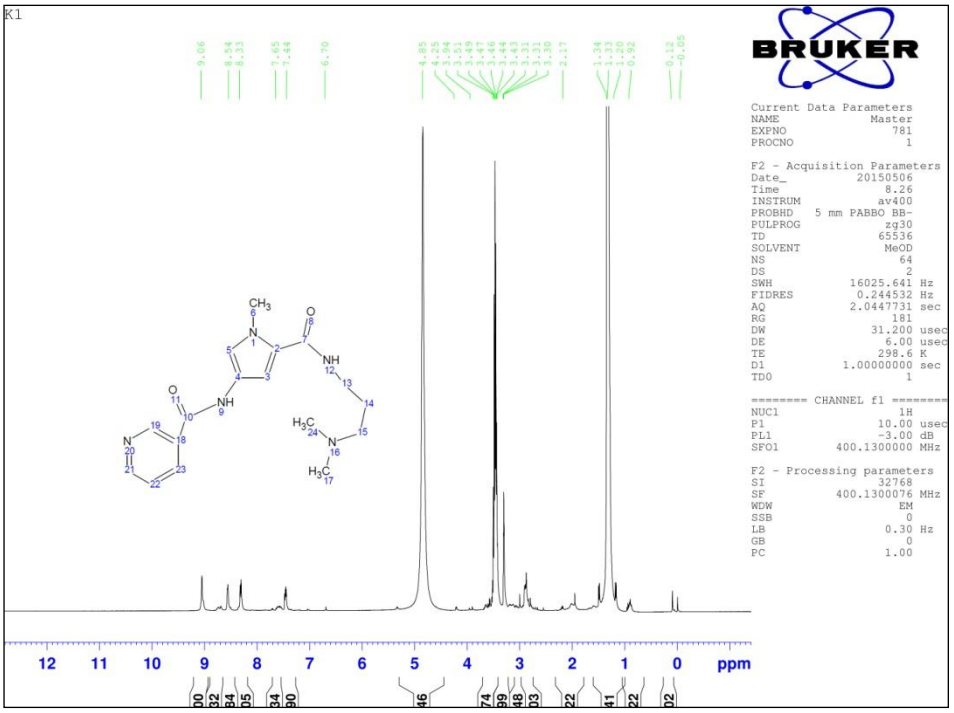

Figure 14: [1]HNMR spectrum of N(-((3-(dimethylamino)propyl)amino)carbonyl)-1-methyl-1-H-pyrrole-3-yl)nicotinamide **9.**

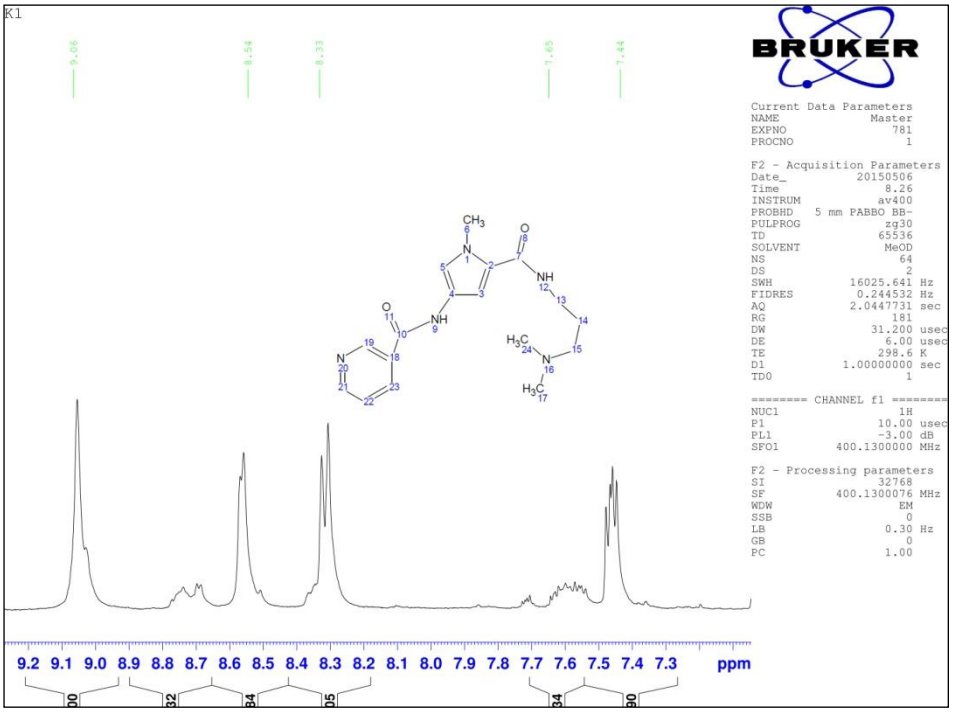

Figure 14.1: [1]HNMR spectrum of N(-((3-(dimethylamino)propyl)amino)carbonyl)-1-methyl-1-H-pyrrole-3-yl)nicotinamide **9**.

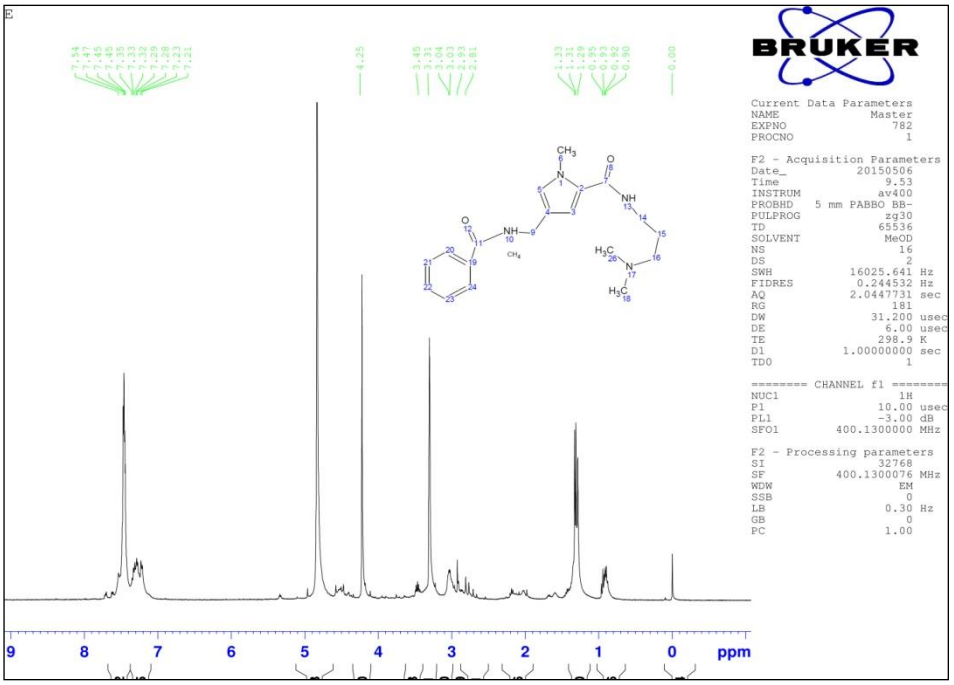

Figure 15: [1]HNMR spectrum of 4-((Benzoylamino)methyl)-N-(3-(dimethylamino) propyl)-N,1dimethyl-1H-pyrrole-2-carboxamide **10**.

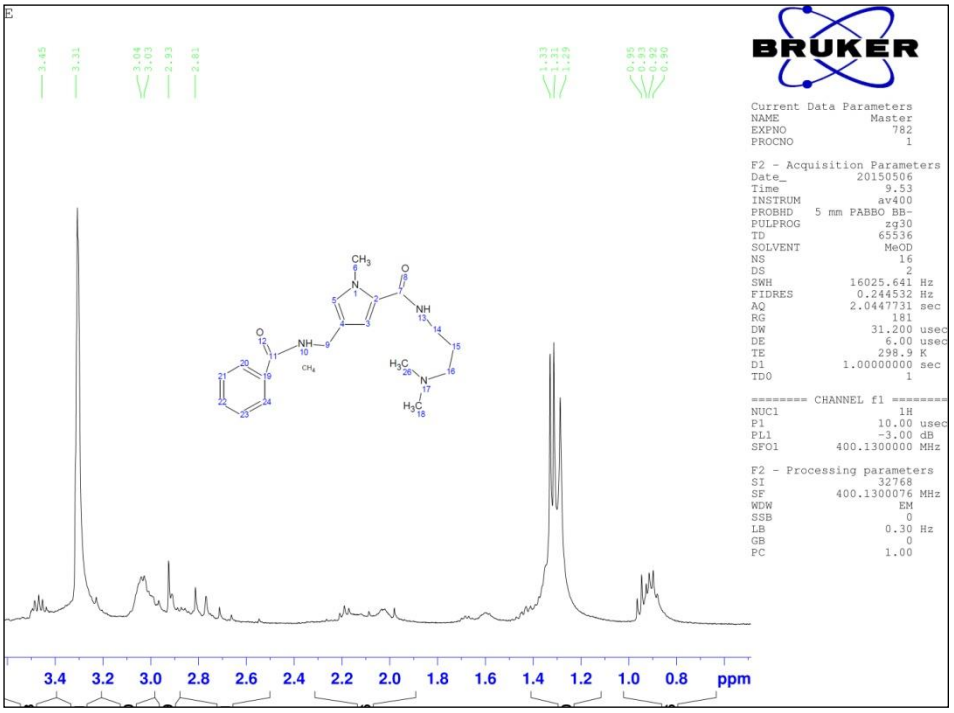

Figure 15.1: [1]HNMR spectrum of 4-((benzoylamino)methyl)-N-(3-(dimethylamino) propyl)-N,1dimethyl-1H-pyrrole-2-carboxamide **10**

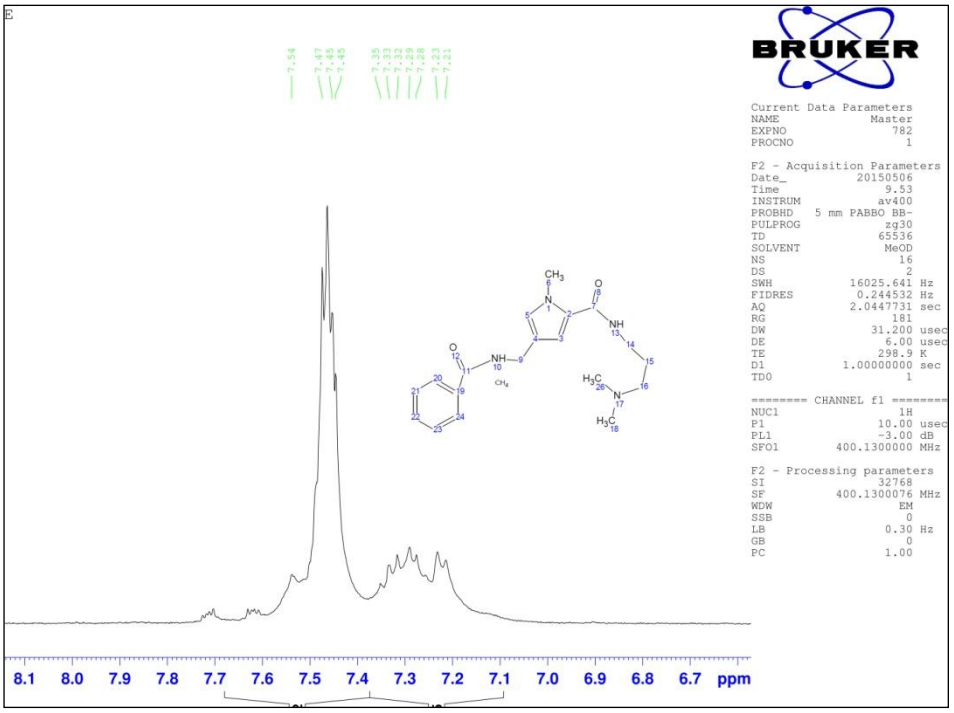

Figure 15.2: [1]HNMR spectrum of 4-((benzoylamino)methyl)-N-(3-(dimethylamino) propyl)-N,1dimethyl-1H-pyrrole-2-carboxamide **10**

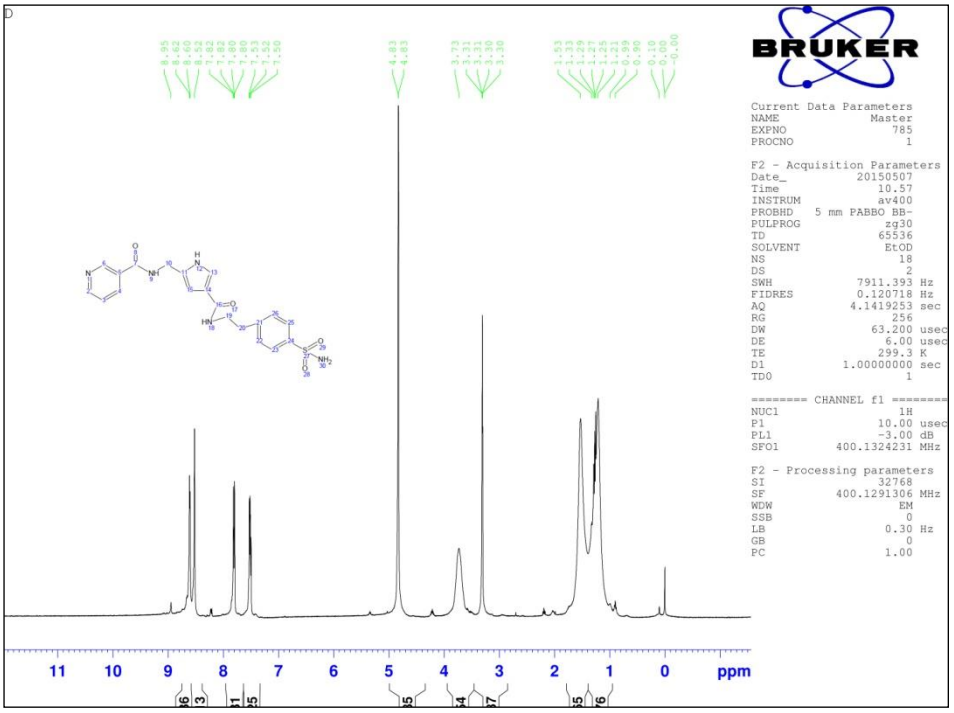

Figure 16: [1]HNMR spectrum of N-(1- Methyl- 5- (2- (4- sulfamoyl- phenyl)-ethylcarbamoyl)-1H-pyrrol-3-yl)-nicotinamide **12**.

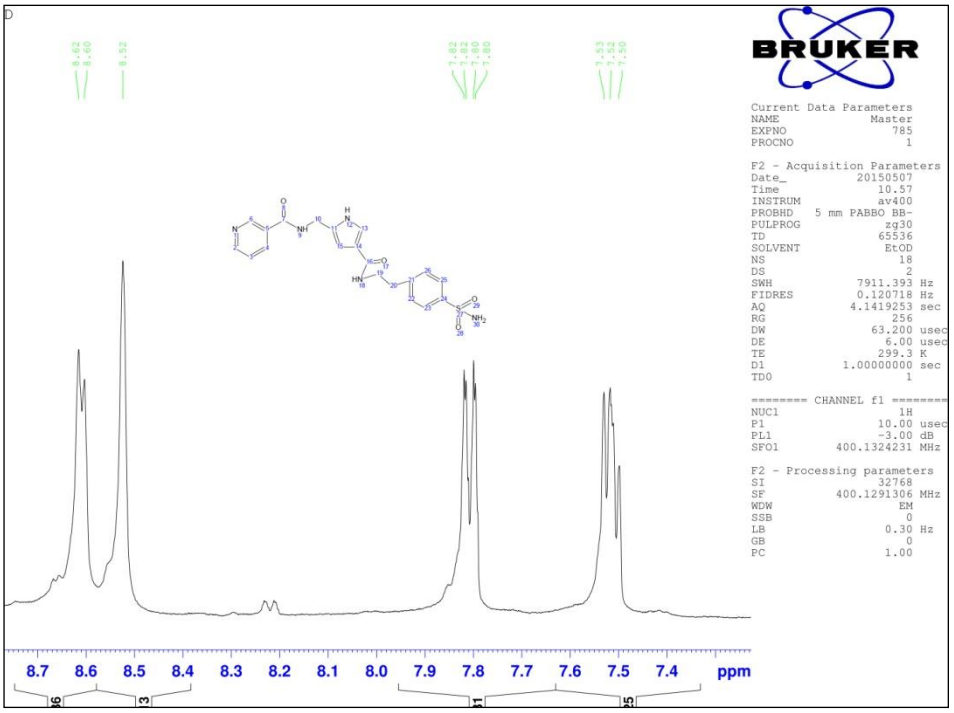

Figure 16.1: [1]HNMR spectrum of N- (1- Methyl-5- (2-(4- sulfamoyl- phenyl)-ethylcarbamoyl)-1H-pyrrol-3-yl)-nicotinamıde **12** .

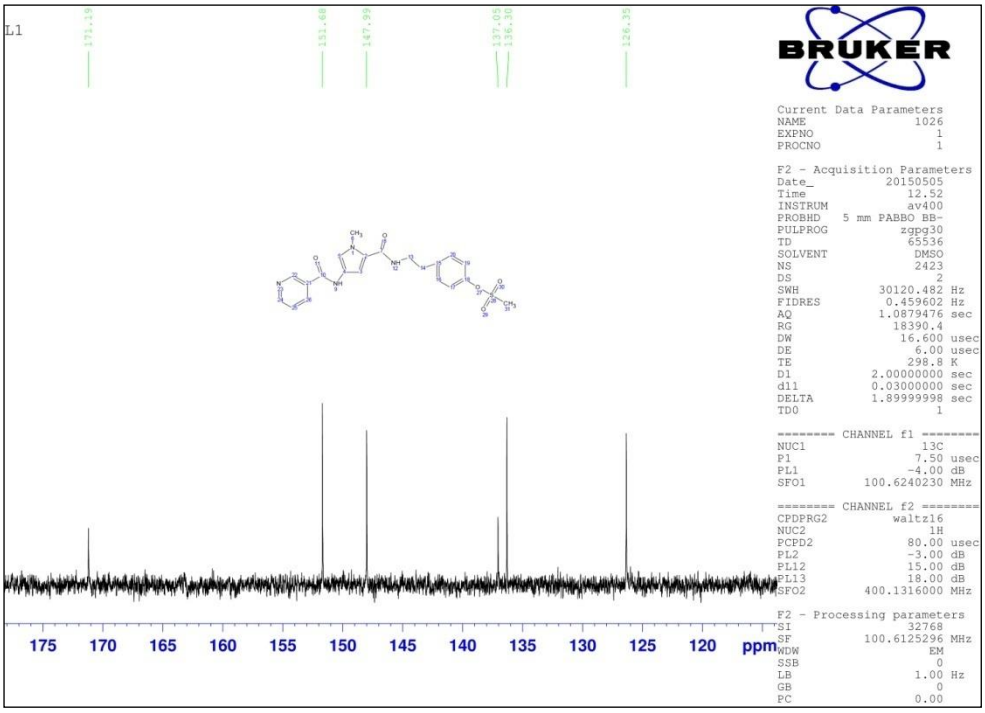

Figure (17):13C NMR spectra of Methanesulfonic acid 4-(2-((1-methyl-4-(pyridin-3-carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenyl ester **6** .

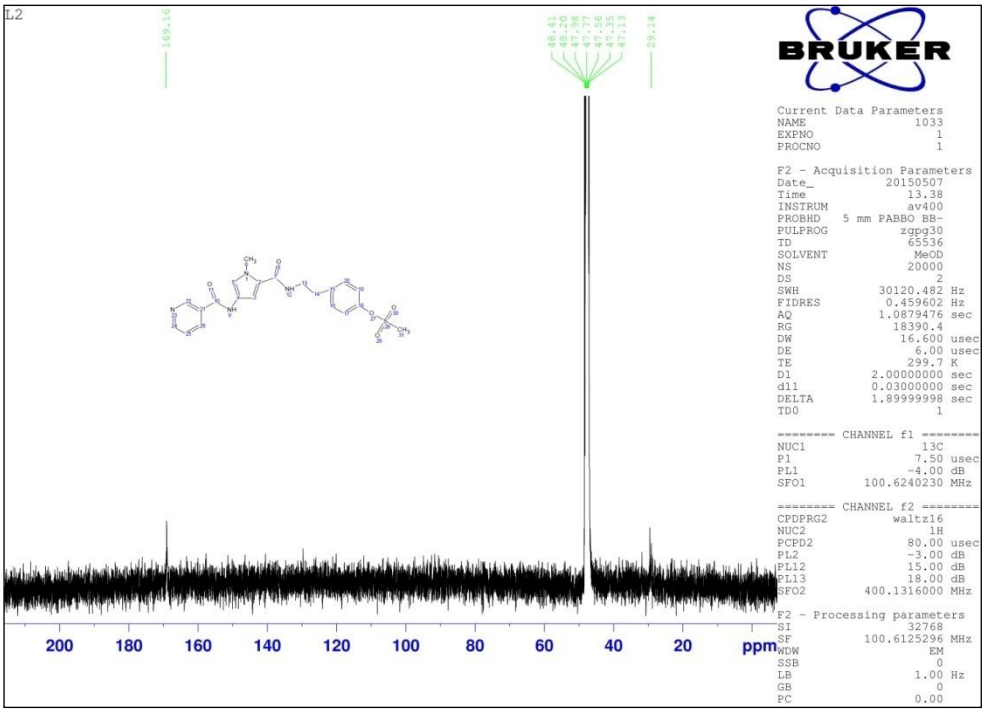

Figure (17.1): 13C NMR spectra of Methanesulfonic acid 4-(2-((1-methyl-4-(pyridin-3-carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenyl ester **6** .

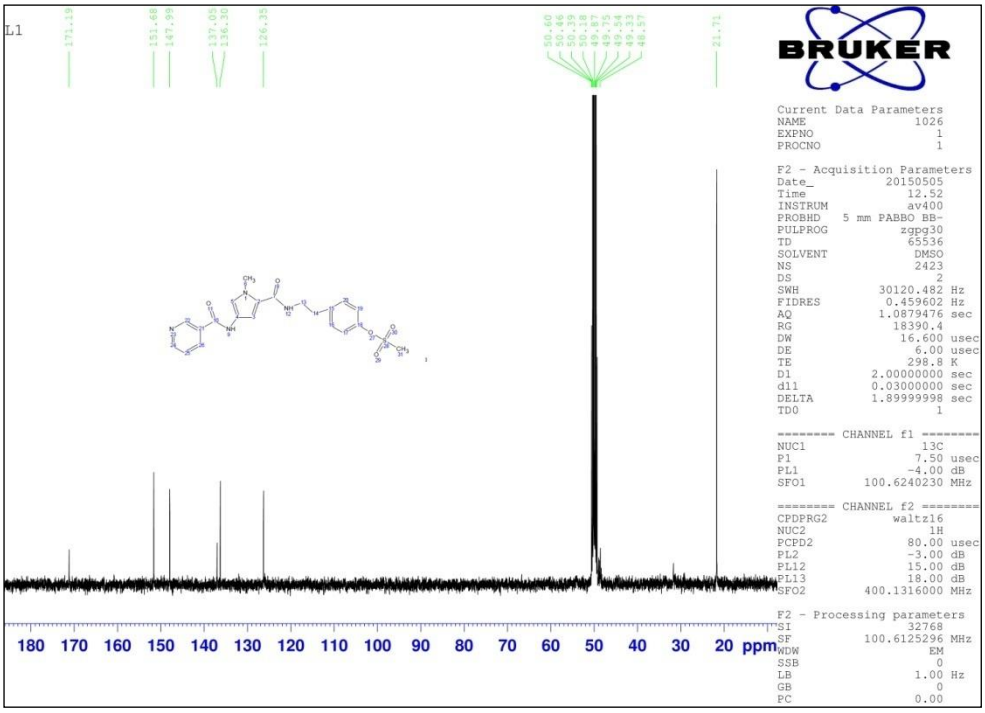

Figure (17.2): 13C NMR spectra of Methanesulfonic acid 4-(2-((1-methyl-4-(pyridin-3-carbonyl)-amino)-1H-pyrrol-2-carbonyl)-amino)-ethyl)-phenyl ester **6** .

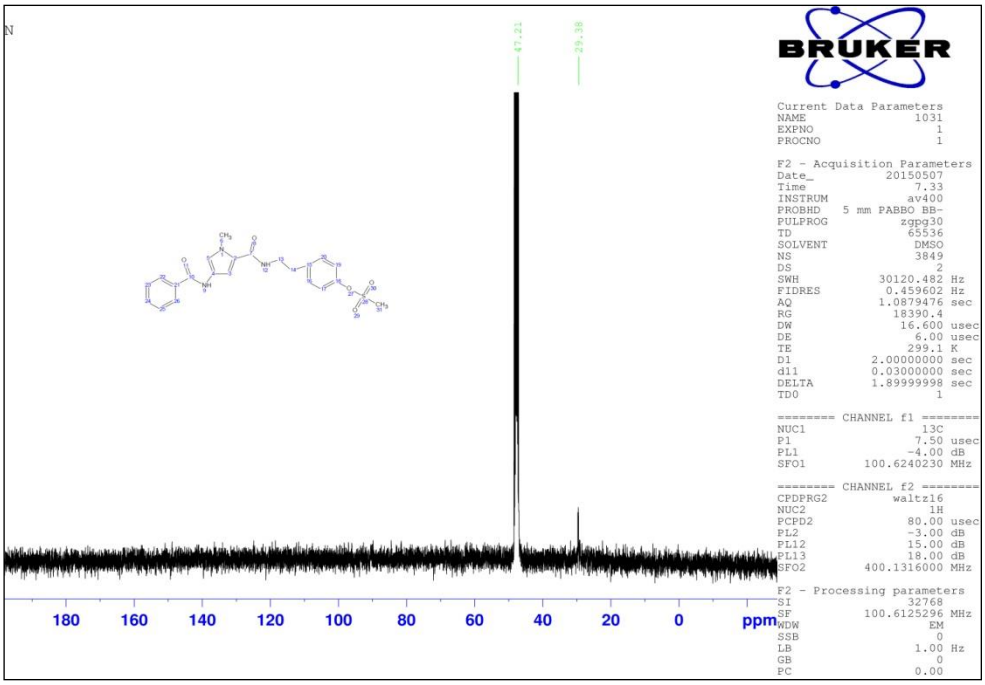

Figure 18: 13C NMR spectrum of Methanesulfonic acid 4-(2-((4-benzoylamino-1-methyl-1H-pyrrole-2-carbonyl)amino)-ethyl)-phynel ester **7**.

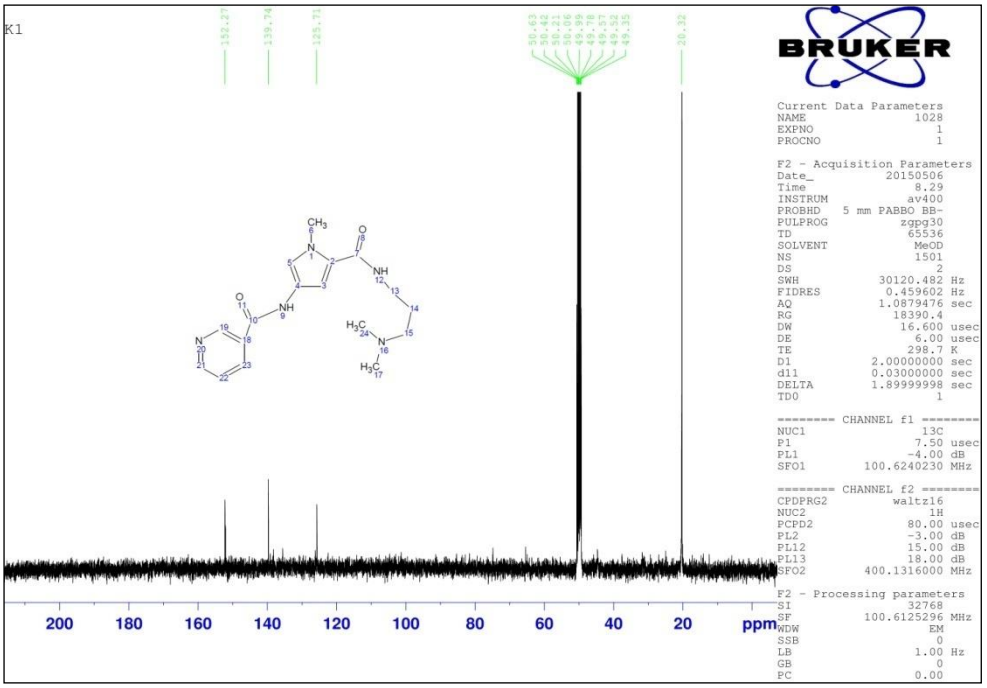

Figure 19: 13C NMR spectrum of N(-((3-(dimethylamino)propyl)amino)carbonyl)-1-methyl-1-H-pyrrole-3-yl)nicotinamide **9.**

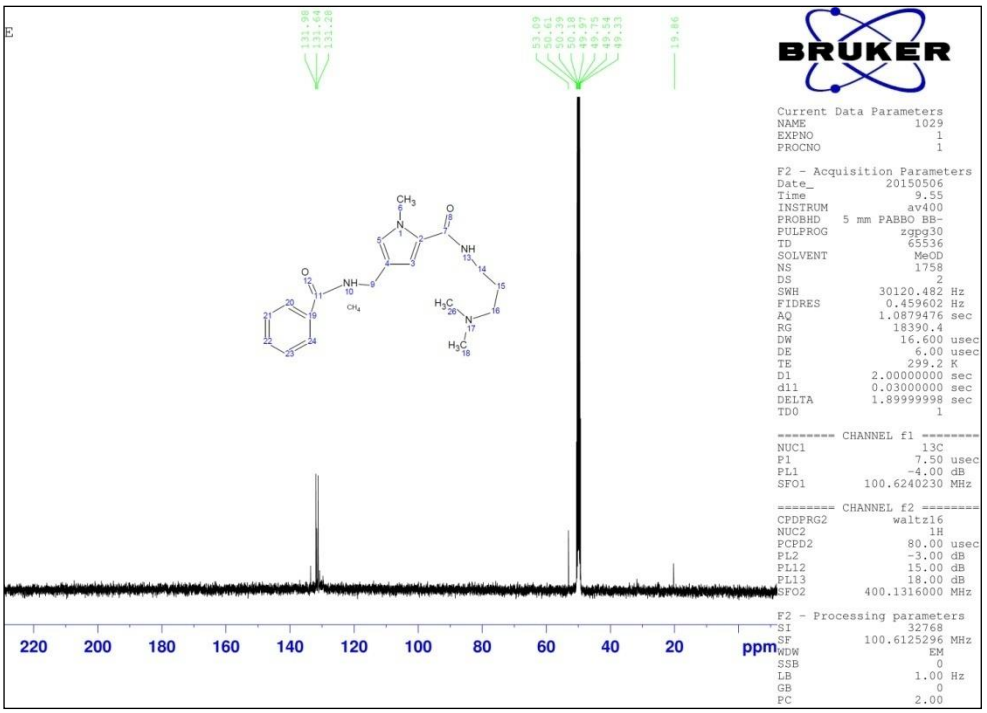

Figure 20: 13C NMR spectrum of 4-((benzoylamino)methyl)-N-(3-(dimethylamino)propyl)-N,1dimethyl-1H-pyrrole-2-carboxamide **10**

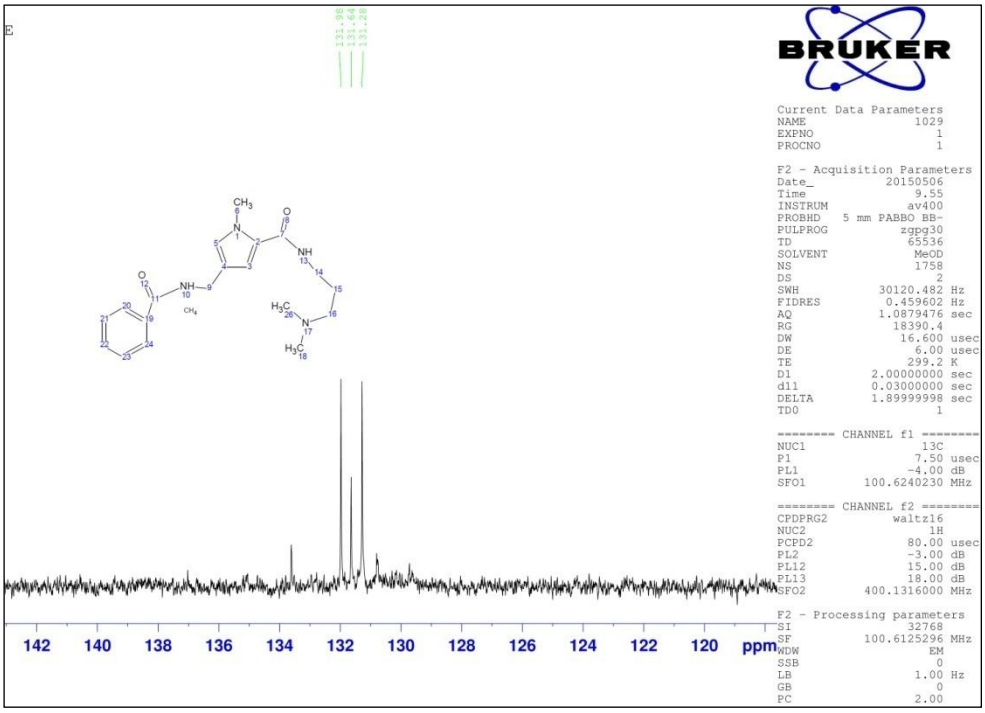

Figure 20.1: 13C NMR spectrum of 4-((benzoylamino)methyl)-N-(3-(dimethylamino)propyl)-N,1dimethyl-1H-pyrrole-2-carboxamide **10.**

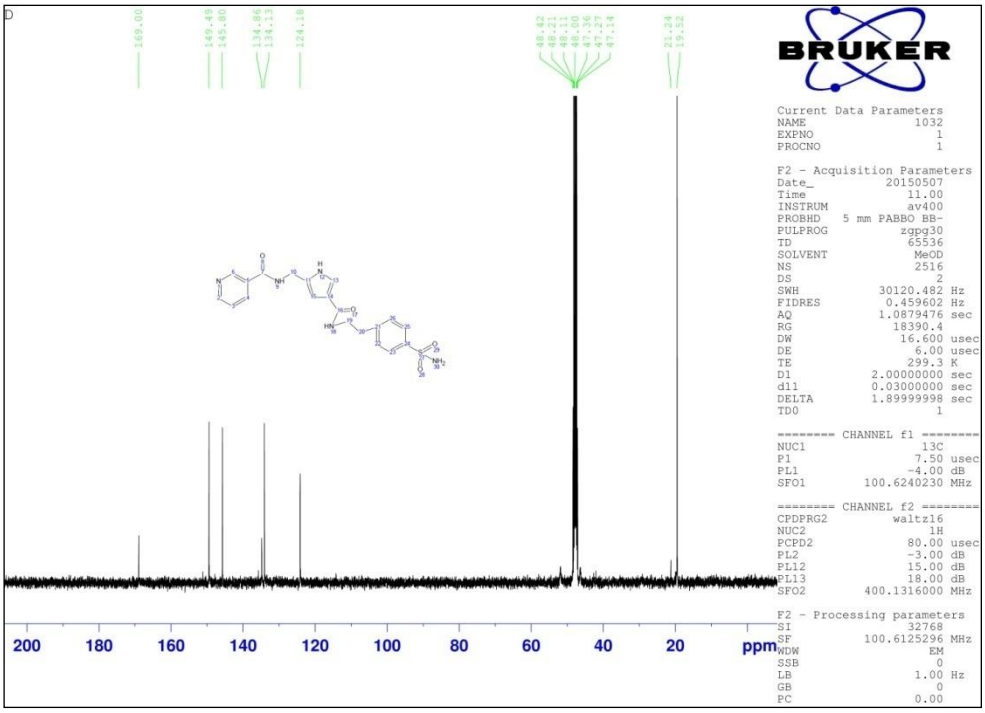

Figure 21: 13C NMR spectrum of N-(1-Methyl-5-(2-(4-sulfamoyl-phenyl)-ethylcarbamoyl)-1H-pyrrol-3-yl)-nicotinamide **12**

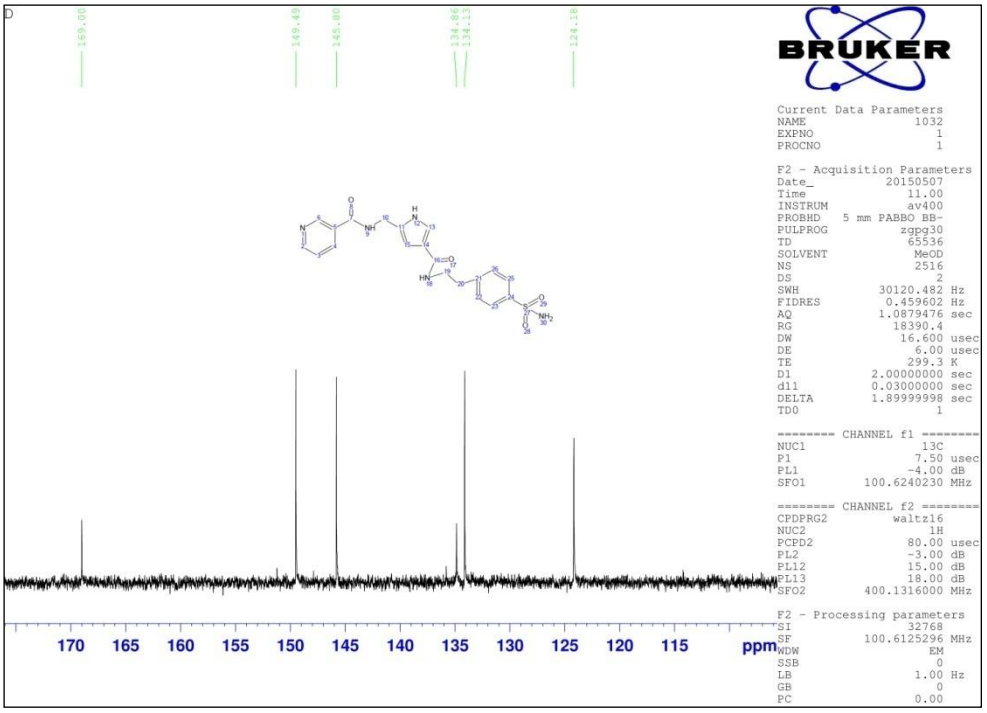

Figure 21.1: 13C NMR spectrum of N-(1-Methyl-5-(2-(4-sulfamoyl-phenyl)-ethylcarbamoyl)-1H-pyrrol-3-yl)-nicotinamide **12** .

كلية الدراسات العليا
جامعة النجاح الوطنية

تحضير مركبات شبيهة الدستاميسين وفحص الفعالية الحيوية لها

اعداد
صمود محمد ياسين

اشراف
د.وحيد الجندي
د.حسن النيص

قدمت هذه الاطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية

2015

تحضير مركبات شبيهة الدستاميسين وفحص الفعالية الحيوية لها

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الملخص

الدستاميسين A هو عباره عن مضاد حيوي طبيعي تم استخلاصه سنة 1964م من نبات يسمى ستبرتوميسس ديستيليكس .

يعتبر الدستاميسين A من اكثر المركبات التي يتم دراستها بكثافه وذلك لما لها من فعاليه كبيرة ضد بعض انواع الفيروسات،البكتيريا،الفطريات وبعض انواع من الخلايا السرطانية.

يتركب الدستاميسين من ثلاثة حلقات N-ميثل بيرول مرتبطة بفورم اميد وبليدينيوم مشحون بشحه موجبة والذي يساعده في الارتباط ب DNA المشحون بشحنة سالبة .

يرتبط الدستاميسين ب DNA بواسطة روابط هيدروجينية ،فاندروالز وروابط الكتروستاتيكية مع المناطق التي يوجد الادنين(A)والثايمين (T) بحيث يرتبط بشكل متقطع ومزدوج مع كل من الادنين(A)والثايمين (T) .

هذه الطريقه التي يرتبط بها الدستاميسين ب DNA شجعت الدراسات والعديد من الابحاث لايجاد مركبات شبيهة بالدستاميسين من حيث الروابط وطريقة الارتباط وبعض هذه المركبات اظهرت نتائج افضل من الدستاميسين.

الارتباط يحدث عن طريق ارتباط C-H الموجودة في DNA وال (NH) الموجودة في البيرول المكون للدستاميسين والمركبات الشبيهة له.

في هذا البحث تم تصنيع خمسة من المركبات شبيهة الدستاميسين الجديدة وذلك عن طريق تغيير N-terminal alkyl group بحيث يكون لها وزن جزيئي اقل و لها قابليهة اكثر على الذوبان

في الدهون(high lipophilicity)، وهذا من شأنه ان يزيد من ارتباط المركبات المصنعة مع الDNA وبالتالي زيادة الفعالية الحيوية لها .

تم التاكد من الصيغه البنائية للمركبات التي صنعت عن طريق تحليلIR , ^{1}H NMR و ^{13}C NMR.

وقد تم فحص هذه المركبات على قدرتها في منع عمليات الاكسدة. وكذالك اثرها على كل من نمو البكتيريا والفطريات واظهرت النتائج ان بعض المركبات كان لها فعالية على نمو البكتيريا وبعضها لم يظهر اي فعالية بشكل واضح . اذ اظهر كل من المركبين-4)-2)-5-(1-Methyl-N-(1-sulfamoyl-phenyl)-ethylcarbamoyl)-1H-pyrrol-3-yl)-nicotinamide: و Methanesulfonic acid 4-(2-((4-benzoylamino-1-methyl-1H-pyrrole-2-carbonyl)amino)-ethyl)-phynel ester فعالية جيدة على نمو البكتيريا. فحص منع عمليات الاكسدة لم يظهر شئ واضح ،اما بالنسبه لفحص الفطريات فقد اظهر المركب 4-benzoylamino-1-methyl-1H-pyrrole-2-carboxylic acid(3-dimethylamino-propyl)-amide نتائج واضحه على كل من النوع *Microsproum Canis*