An-Najah National University

Faculty of Graduate Studies

Synthesis and Characterization of M-Aminobenzoic Acid Containing Distamycin Analogues Which Have Potential Biological Activity

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Dedication

To my parents for their encouragement, help, care and for their prayers.

To my husband Malik for his support, love, encouragement, and his moral support

To my husband's family who supported me and helped me by taking care of my daughter.

To my brother Ahmed for his love, sincere feelings and his moral support.

To my sisters for supporting me and sharing my worries.

To all who prayed for me.

To all whom I loved and knew.

Acknowledgments

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I would like to thank my supervisor Dr. Waheed J. Jondi for his support throughout the several months of work of my Master thesis, keeping me going when times were tough, asking insightful questions, and offering invaluable advice.

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Very great thank for BERC Centre Til Village- Nablus for the tests of the biological impact of my studied compounds.

Finally, it is my family, siblings, daughter and close friends who deserve my deep gratitude. Thank you for being in my life, what made this achievement and successpossible. Lastly and exclusively, my warmest thanks must be to my husband, Malik, for his continuous and unfailing love. With his support and understanding, he underpinned my persistence in my Master study and made the completion of my thesis possible.

الإقرار

أنا الموقعة أدناه مقدم الرسالة التي تحمل العنوان:

Synthesis and Characterization of M-Aminobenzoic Acid Containing Distamycin Analogues Which Have Potential Biological Activity

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو بحث علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

DNA	Deoxyribonucleic acid
A	Adenine
T	Thymine
G	Guanine
C	Cytocine
MGBs	Minor Groove Binders
DCM	Dichloromethane
THF	Tetrahydrofuran
DCFC	Dry Column Flash Chromatography
m.p	Melting point
NMR	Nuclear Magnetic Resonance
I.R	Infrared
ppm	Part per million
mol	Mole

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Abstract

Distamycin analogues were prepared. Through the synthesis of these compounds, some intermediates had to be prepared: 3-benzamido-N-(3-((4-sulfamonylphenethyl) carbamoyl) phenyl)benzamide (R_1), the intermediates of this analogues were:3-Benzoylamino-benzoic acid(1), 3-benzoylamino-benzoyl chloride(2), 3-(3-Benzoylamino-benzoylamino)-benzoic acid(3), 3-(3-Benzoylamino-benzoylamino)-benzoyl chloride(4).

The intermediates for 3-acetamido-*N*-(3-((4-sulfamoylphenethyl) carbamoyl) phenyl) benzamide(R_2) were: 3-Acetylamino-benzoic acid(5),3-Acetylamino-benzoyl chloride(6), 3-(3-Acetylamino-benzoylamino) -benzoic acid(7),3- (3-Acetylamino-benzoylamino)-benzoyl chloride(8).

The intermediates for *N*-(3-((3-((4-sulfamoylphenethyl)c Z\arbamoyl) phenyl) carbamoyl)phenyl)nicotinamide(R_3) were: 3-[(Pyridine-3-carbonyl)-amino]-benzoic acid(9), 3-[(Pyridine-3-carbonyl)-amino]-benzoyl chloride (10), 3-{3-[(Pyridine-3-carbonyl)-amino]-benzoylamino}-benzoic acid(11), 3-{3-[(Pyridine-3-carbonyl)-amino]-benzoylamino}-benzoyl chloride(12).

The intermediates for 3-benzamido-N-(3-((3-(dimethylamino) propyl) carbamoyl) phenyl) benzamide(R_4) were: 3-Benzoylamino-benzoic

acid(1), 3-Benzoylamino-benzoyl chloride(2), 3-(3-Benzoylamino-benzoylamino)-benzoic acid(3), 3-(3-Benzoylamino-benzoylamino)-benzoyl chloride(4).

The intermediates for N-(3-((3-((3-(dimethylamino) propyl) carbamoyl) phenyl)nicotinamide(R_5) were:3-[(Pyridine-3-carbonyl)-amino]-benzoic acid(9), 3-[(Pyridine-3-carbonyl)-amino]-benzoyl chloride(10), 3-{3-[(Pyridine-3-carbonyl)-amino]-benzoylamino}-benzoic acid(11), 3-{3-[(Pyridine-3-carbonyl)-amino]-benzoylamino}-benzoyl chloride(12).

The intermediates for N-(3-(dimethylamino) propyl)-3-(3-(methylsulfonamido) benzamido) benzamide(R_6) were: 3-Methanesulfonylamino-benzoic acid(13), 3-Methanesulfonylamino-benzoyl chloride(14), 3-(3-Methanesulfonylamino-benzoylamino)-benzoic acid(15), 3-(3-Methanesulfonylamino-benzoylamino)-benzoyl chloride(16).

The intermediates for 3-benzamido-N-(3-((3-((3-(dimethylamino) propyl)carbamoyl)phenyl)carbamoyl)phenyl)Benzamide(R_7) were: 3-Benzoylamino-benzoic acid(1), 3-Benzoylamino-benzoyl chloride(2), 3-(3-Benzoylamino-benzoylamino)-benzoic acid(3), 3-(3-Benzoylamino-benzoylamino)-benzoyl chloride(4), 3-[3-(3-Benzoylamino-benzoylamino) benzoylamino]-benzoic acid(17),3-[3-(3-Benzoylamino-benzoylamino)benzoylamino]-benzoyl chloride(18).

The intermediates for 3-(methylsulfonamido) -*N*-(3-((3- ((4-sulfamoylphenethyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide(R_8) were**:** 3-methanesulfonylamino- benzoic acid(13),3-methanesulfonylamino-

benzoyl chloride(14),3-(3-Methanesulfonylamino-benzoylamino)-benzoic acid(15),3-(3-methanesulfonylamino-benzoylamino)-benzoyl chloride(16), 3-[3-(3-methanesulfonylamino-benzoylamino)-benzoylamino]-benzoic acid(19), 3-[3-(3-methanesulfonylamino-benzoylamino)-benzoylamino]-benzoyl chloride(20).

The intermediates for 3-(methylsulfonamido)-*N*-(3-((3-((2-morpholinoethyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide(R_9) were: 3-Methanesulfonylamino-benzoic acid(13),3-methanesulfonylamino-benzoyl chloride(14),3-(3-Methanesulfonylamino-benzoylamino)-benzoic acid(15),3-(3-Methanesulfonylamino-benzoylamino)-benzoyl chloride(16), 3-[3-(3-Methanesulfonylamino-benzoylamino)-benzoy -lamino]-benzoic acid(19), 3-[3-(3-methanesulfonylamino-benzoylamino)-benzoylamino]-benzoyl chloride(20).

The structures of all the distamycin analogues above were established by spectral data (IR, ^{1}HNMR and C^{13}NMR).

Each of distamycin analogues wastested against oxidants, reductive potential, bacteria and fungi.

R_7 and R_4 showed strong antioxidant activity. R_1, R_2, R_3 showed strong reductive potential.Non of compounds showed antibacterial activity. Compounds R_1, R_2 and R_3 showed strong antifungal activity, against M. canis activity. Compounds R_4 and R_8 showed 84% activity against M.canis. R_3, R_8 revealed 100% activity against T.mentagrophytes. While R_1 and R_2 revealed more than 95% activity. R_1, R_2 and R_3 showed strong activity against T.rubrum.

Chapter One

Introduction

Introduction

1.1 Pharmaceutical Chemistry:

pharmaceutical chemistry is a discipline at the intersection of chemistry, especially synthetic organic chemistry, pharmacology and various other biological specialization, where they are involved with design, chemical synthesis and development for market of pharmaceutical agents, or bio-active molecules (drugs). Compounds used as medicines are most often organic compounds, which are often divided into the broad classes of small organic molecules (e.g., atorvastatin, fluticasone, clopidogrel) and "biologics" (infliximab,erythropoietin, insulin glargine), the latter of which are most often medicinal preparations of proteins (natural and recombinant antibodies, hormones, etc.) [1].

For ages, nature has been an excellent source of new drugs or precursors for drugs. Human beings have searched for cures of illnesses by chewing herbs, berries, roots, and barks. When a natural product is found to be active, its functional groups were modified to improve its properties. Greater than 60% of the anticancer and anti-infective agents that are on the market or in clinical trials are of natural product origin or derived from natural products. This is a result of the inherent nature of secondary metabolites of plants that act in defense of their producing organisms [2].

DNA is the molecular target for many of the drugs that are used in cancer therapeutics, and is viewed as a non-specific target of cytotoxic agents.

Although this is true for traditional chemotherapeutics, other agents that were discovered more recently have shown enhanced efficacy. Furthermore, a new generation of agents that target DNA-associated processes are anticipated to be far more specific and effective [3].

1.2 Properties and Composition of DNA Molecule:

Deoxyribonucleic acids (DNA) is a complex molecule which exists in all living organisms. The major function of DNA is to protect and carry genetic information that tells cells which protein to produce and when to produce it. DNA is one of the three major macromolecules that are fundamental for all known forms of life. DNA can replicate, or make copies of itself [4].

In 1953, James Watson and Francis Crick suggested a model for DNA molecule, that consisted of two nucleotide chains that wrap around each other to form a double spiral. This shape is called a double helix. Each nucleotide is composed of one of four nitrogen-containingnucleobases—either cytosine (C), guanine (G), adenine (A), or thymine (T), a sugar called deoxyribose and a phosphate group. The nucleotides are combined to one another in a chain by covalent bonds, between sugar of nucleotide and phosphate group of the next, resulting in an alternating sugar-phosphate backbone. The nitrogenous bases of the two detach polynucleotide strands are bound together (according to base pairing rules (A with T, and C with G) with hydrogen bonds to produce double-stranded DNA Fig.1.1 [5,6].

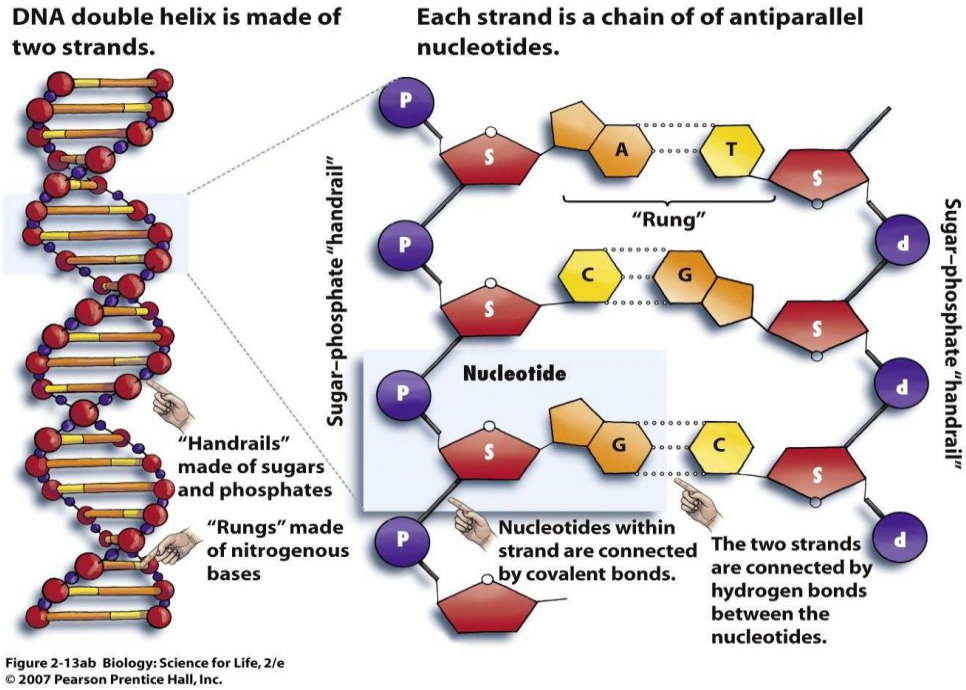


Figure1.1: Normal Segment of DNA and Detailed Attachment of Nucleotide, Sugar and Phosphate.

Deoxyribose sugar of DNA backbone has 5 carbons and 3 oxygen's, the carbons numbered 1',2',3',4',5'. The hydroxyl groups on the 5'- and 3'-carbons bonded to phosphate groups to form DNA backbone. Nucleosides one of the four DNA bases covalently linked to C'1position of sugar; nucleoside vary from nucleotides in lacking phosphate groups, nucleotides is nucleoside with one more phosphate group bonded covalently to the 3'- and/or 5'-hydroxyl groups Fig.1.2[6].

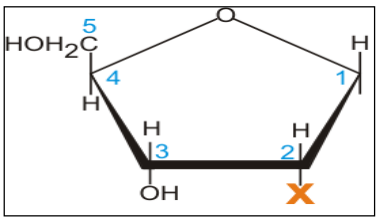


Figure (1.2): Deoxyribose and Ribose Sugars, Deoxyribose X=H, Ribose X=OH.

Each nucleotide in DNA consist of5 carbons sugar (deoxyribose), a nitrogen including base linked to the sugar and phosphate group. Four types of nucleotides set 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, these bases are essential for genetic information which encoded as a series of nucleotides Fig.1.3[7]**.**

1.3: Watson-Crick Base Pairing

Two of the bases (C and T) contain only one Pyrimidine ring. The other two bases (A and G) contain two rings, they are Purines Fig.1.4. Within DNA the bases pair by complementary base pairing, as in the Watson–Crick model, there are two hydrogen bonds for an A-T base pair, and three hydrogen bonds for a G-C base pair. Hydrogen bonds (H-bonds) are weak, and in DNA, the hydrogen bonds have only about 2 kcal mol^{-1} energy this is likely to be due, in part, to propeller twisting of the bases, which results in strain in H-bonds Fig.1.5 [8].

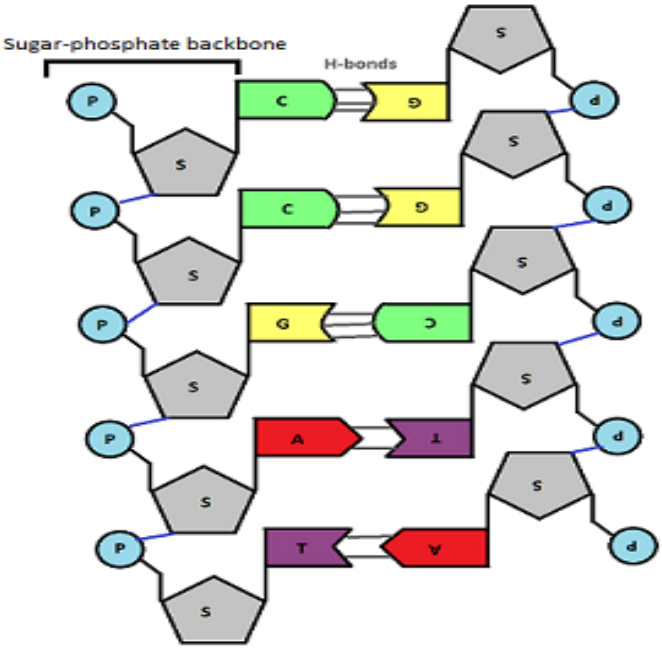


Figure1.3: Structural Formulas of the Constituents of DNA.

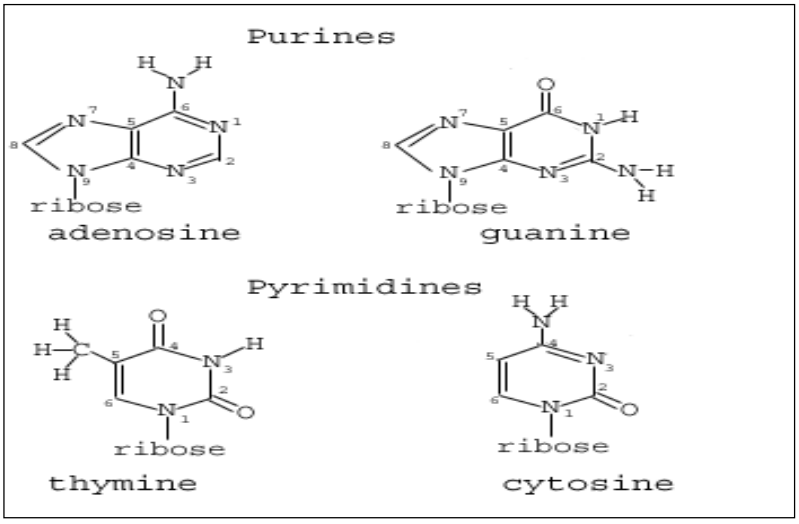


Figure1.4: Chemical Constituents of DNA(Purines and Pyrimidines)

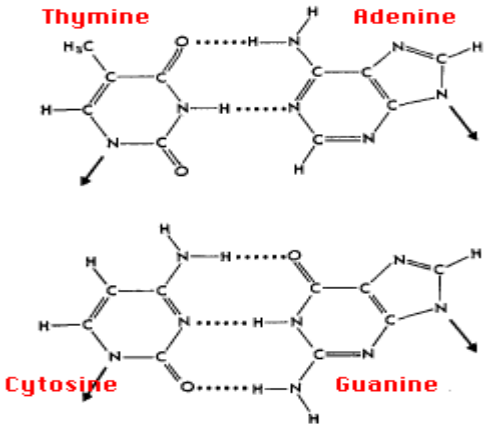


Figure1.5: Watson–Crick Base Pairs (A-T), (G-C).

Nucleotides are linked with each other by phosphodiester linkage made of two strands of nucleotides that are joined together by hydrogen bonding. Hydrogen bonding exists as a result of complementary base pairing. Adenine and thymine pair up; Cytosine and Guanine pair up, each pair attached through hydrogen bonds. Hydrogen bonding always occurs between one pyrimidine and one purine Fig.1.6 [9].

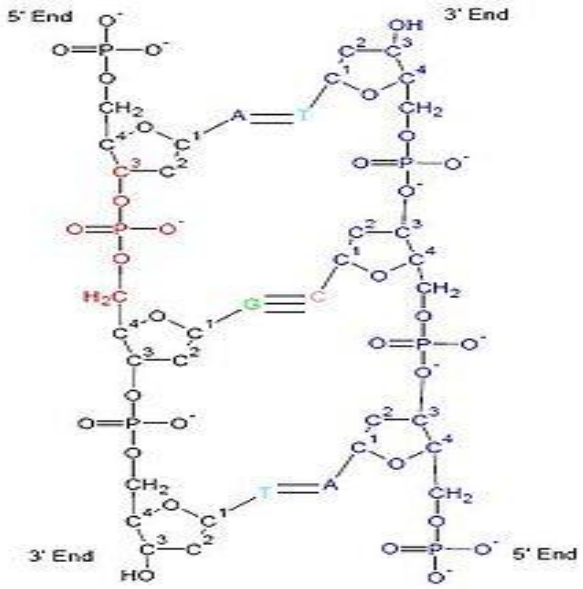
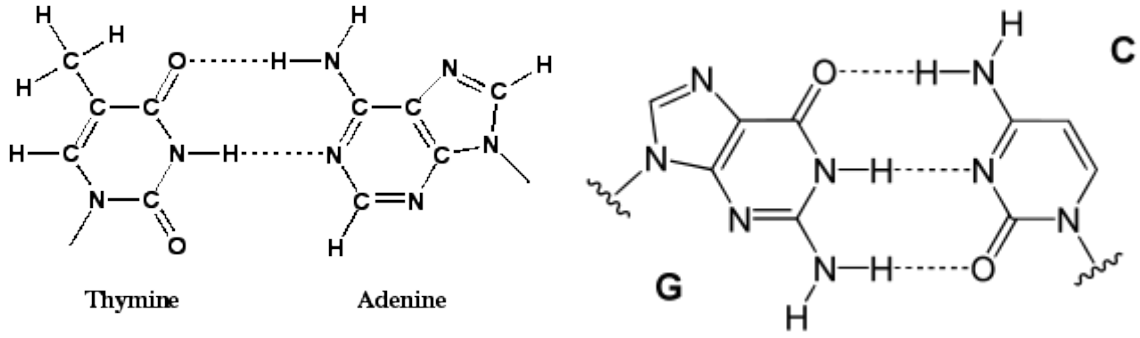


Figure1.6: Hydrogen Bonding Between One Pyrimidine and One Purine.

The G-C base pair offers two hydrogen accepter and one hydrogen-donor position. However, at A-T base pairs there are two hydrogen acceptor



(a) **(b)**

Figure1.7: Hydrogen Bonding Properties in the A-T (a) and G-C (b) Watson-Crick DNA Base.

DNA molecule contains of two grooves, minor groove and major groove, the protein which links to this groove is called minor or major groove binder(MGB).

1.4 Minor and Major Groove of DNA :

In a particular analysis of DNA structure, there are two kinds of grooves which are shown in Fig1.8. The major groove has the nitrogen and oxygen atoms of the base pairs pointing inward toward the helical axis, whereas in the minor groove, the nitrogen and oxygen atoms point outwards. Among both, the minor groove is more important because it can catch the drugs such as distamycin analogues; Since the major groove is dependent on base composition and may be the position for protein recognition of specific DNA sequence [10].

The double helix is a quite rigid and viscous molecule of an immense length and a small diameter. It presents a major groove and a minor

groove. The major groove is deep and wide, the minor groove is narrow and shallow [11].

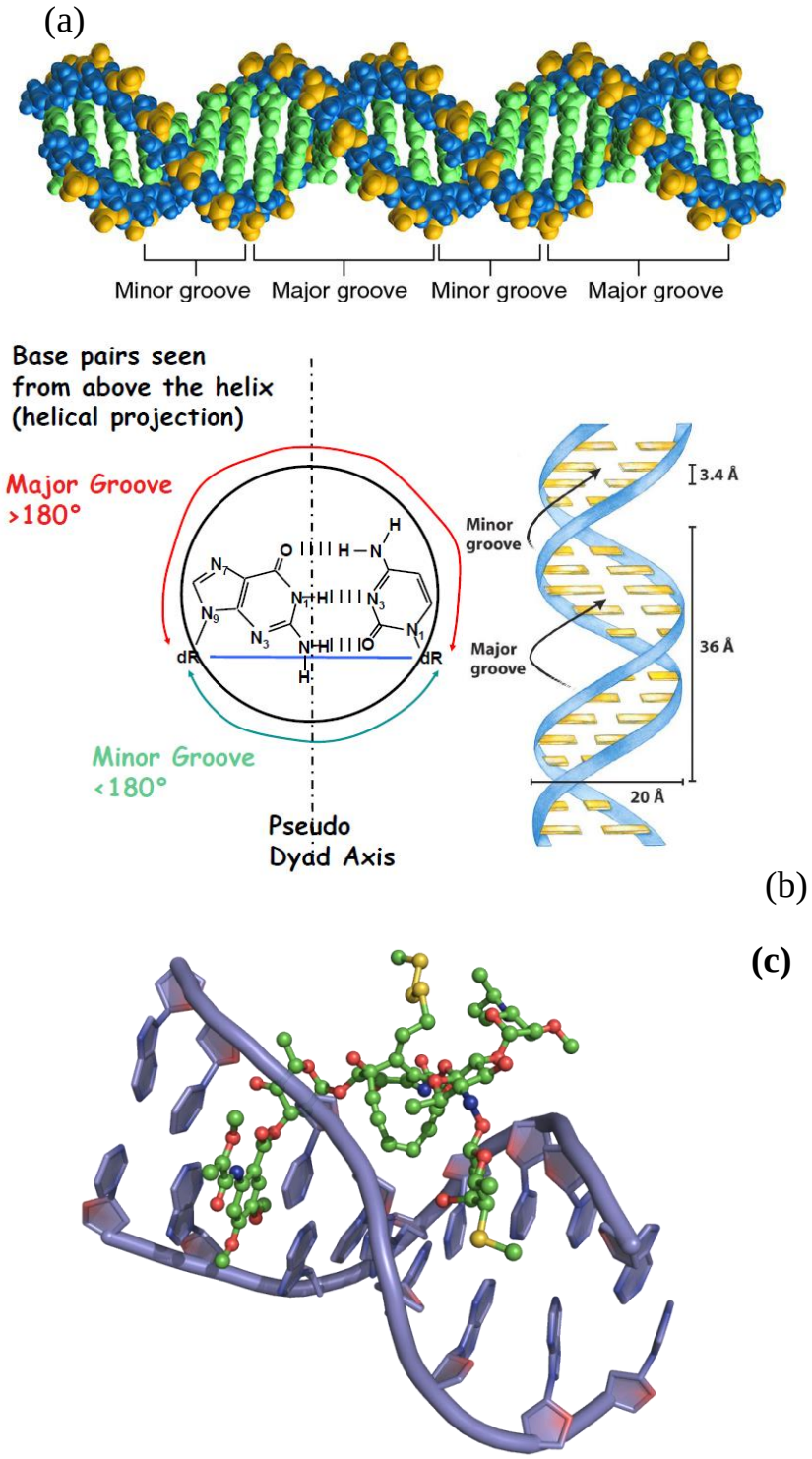


Figure 1.8: (a) Major and Minor groove of DNA. (b)Specification of Minor and Major Groove of DNA. (C) Distamycin in the Minor Groove of DNA.

Proteins exhibit binding specificity firstly through major groove binding , but small molecules characterize minor groove binding. Minor groove binding molecules generally have aromatic rings linked by single bonds that permit for torsional rotation in order to fit into the helical curvature of the groove with displacement of water molecules. Drugs bind to the DNA molecules in two possible sites, minor groove, major groove [12,13]. The grooves are unequally sized. One groove, the major groove, is 2.2nm (22Å) wide occurs where a backbones far apart and the other, the minor groove, is 1.2nm(12Å) wide occurs where they are close together. These grooves are the sites where proteins interact with DNA Fig.1.9 [14].

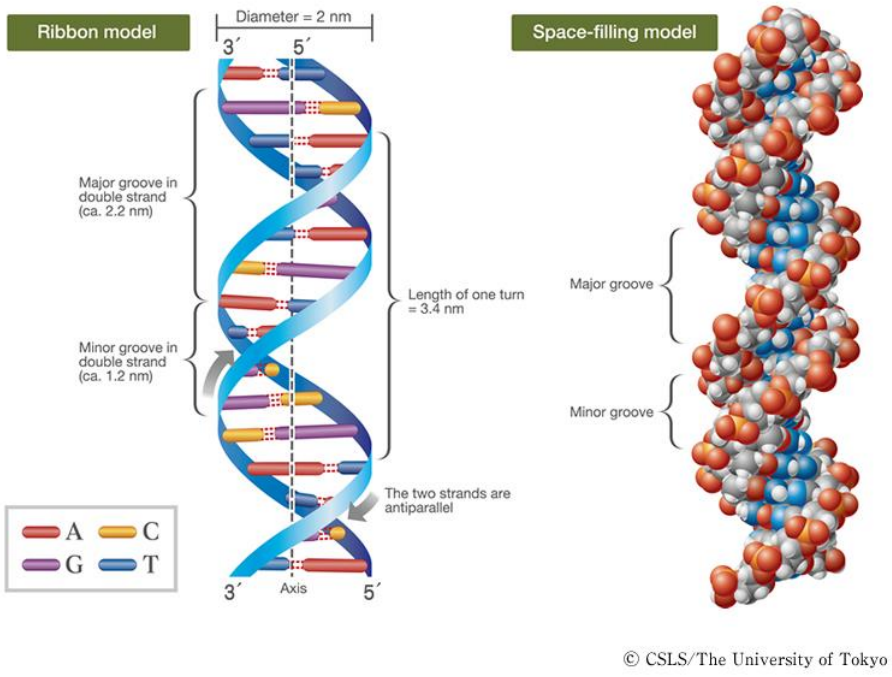


Figure1.9: Differentiate between Major and Minor Groove

The minor groove in A-T is not as wide as in G-C, therefore A-T rich regions may be more amenable to flat aromatic molecule binding than are G-C rich regions. The more narrow A-T region produces a more snug fit of

molecules into the minor groove and lead to van der Waals interactions with the DNA functional groups that define the groove. Binding also arises from interactions with the edges of the base pairs on the backbone of the DNA grooves [15].

DNA-protein interactions are essential processes in the cell life (transcription activation or repression, DNA replication and repair)[16].

Proteins bind at the backbone of the DNA grooves, using specific binding: hydrogen bonds, and non specific binding: van der Waals interactions and generalized electrostatic interactions [17].

Minor groove binders are small molecules that form strong complexes with the minor groove of DNA. There are different categories of compounds which have the properties of binding to minor groove of DNA. Like this compounds may be natural products or synthetic compounds, There are several species of natural products with minor groove binding properties and these have been known for many years. Among the simpler structures are the polyamides, netropsin and distamycin. These compounds bind to the minor groove principally at A-T segment [18].

Molecules that bind in the A-T regions of the minor groove, typically, crescent shape with hydrogen bonding NH groups on the interior of the crescent [19].

1.5 Natural Compounds that Bind to the Minor Groove of DNA:

Over the past twenty years, researchers have designed and examined a large number of minor groove binders. These ligands were found to bind

in the minor groove of duplex DNA. The majority of these were synthesized based on naturally occurring distamycin and netropsin. These are called lexitropsins. Distamycin and netropsin(also called Distamycin A) are antibiotics [20,21,22].

The naturally occurring polyamide antibiotics, Distamycin A bind within the minor groove of DNA at regions with four or five A-T base pairs. Not surprisingly, the cytotoxicity of these compounds can't be used as drugs[21]. Nature employs a diverse order of structural motifs for DNA recognition by proteins, using combinations of electrostatic interactions with the sugar phosphate backbone and van der Waals contacts with the nucleo bases within the helical grooves to facilitate specificity Fig. 1.11 [23,24].

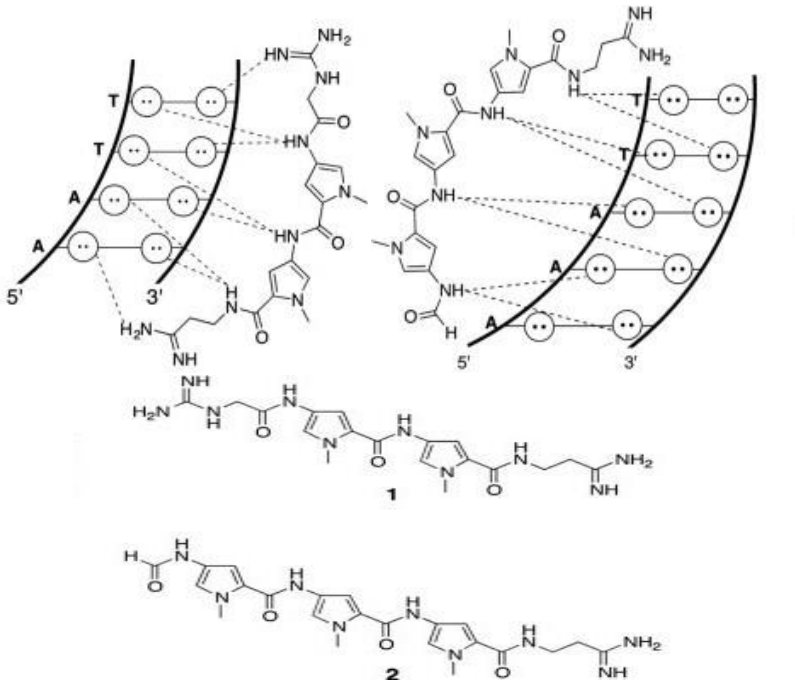


Figure 1.11: Netropsin(1) and Distamycin(2) Bind with DNA Molecule.

Among these natural products, netropsin and distamycin are particularly attractive leading compounds, owing to their modular nature, reduced size, and diminished conformational freedom with respect to native proteins.

DNA-binding polyamides composed of N-methylimidazole(Im), N-methylpyrrole(Py), and N-methyl-3-hydroxypyrrole(Hp) are crescent-shaped molecules that bind the minor groove as antiparallel dimmers [25]. These compounds represent prototypic DNA minor groove binders with a pronounced selectivity for A-T-rich sequences of DNA association and is driven by a combination of van der Waals and hydrogen bonding interactions [26,27].

Side by-side pairings of aromatic residues stack five-member heterocyclic against each other and the walls of the minor groove, positioning the polyamide backbone and aromatic 3-substituents for intimate contacts with the edges of nucleotide bases on the adjacent DNA strand [28].

1.6 Minor Groove Binders:

Minor groove binders(MGBs) are small molecules that form strong complexes with the minor groove of DNA. There are several structural types of which distamycin, netropsin analogues, oligoamides(built from heterocyclic and aromatic amino acids), and bis-amidines(isolated from aromatic and heterocyclic rings). These MGBs are of particular pharmaceutical use. Distamycin and netropsin compounds bind to the minor groove principally at A-T rich region. In the case of netropsin, a singlemolecule binds in the minor groove but in the case of distamycin and its analogues, it is more usual to find two molecules binding in a widened minor groove, face to face and antiparallel [29].

1.6.1 Distamycin:

Distamycin is an oligopeptide antibiotic, biosynthesized by *Streptomyces distallicus*. It is known to bind isohelically to the minor groove at A-T rich site. Distamycin A acts as antibiotic with anticancer activity but it is too toxic to be used in the cancer therapy. It also has similar properties to those observed with Netropsin. Distamycin displays antiviral and antibiotic activity, it shows antiprotozoal activity, and active against gram-positive and gram-negative bacteria. Due to these reasons, many of distamycin analogues and related conjugated systems have been synthesized [30].

1.6.2 Distamycin Chemical 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. Chemical structure of Distamycin consists of three methyl pyrrole monomers linked by amides, with a head to tail group also linked by amides. The compound has neutral formamide head and a positively charged propylamidinium tail. Distamycin A has a planner and crescent shape which helps the ligands to fit strongly into the minor groove of DNA Fig.1.12 [31,32].

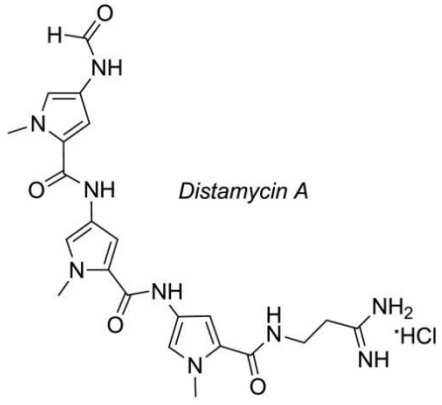


Figure 1.12: Structure of Distamycine A

Interaction of distamycin with DNA has been studied extensively, this molecule can bind reversibly in the minor groove of duplex DNA by hydrogen bonds, Van der Waals contacts and electrostatic interaction [33,34].

Ligand-DNA complex is further stabilized by electrostatic interactions between the negatively charged phosphate backbone of DNA and the positively charged terminus of the ligand. Binding of Distamycin A to DNA, widens the minor groove by unbending the helix axis and lengthening it by nearly 12–15% Fig.1.13[35].

Distamycin A probably interferes with transformation by competing with DNA for some unknown bacterial component involved in transport of DNA into the cell Fig.1.13[36].

Distamycin binds to ATATA and ATATAT sequences in a dimeric fashion with high affinity and positive cooperation, whereas it binds to ATAT both monomerically and dimerically, but binds dimerically with lower affinity and non-cooperatively[37].

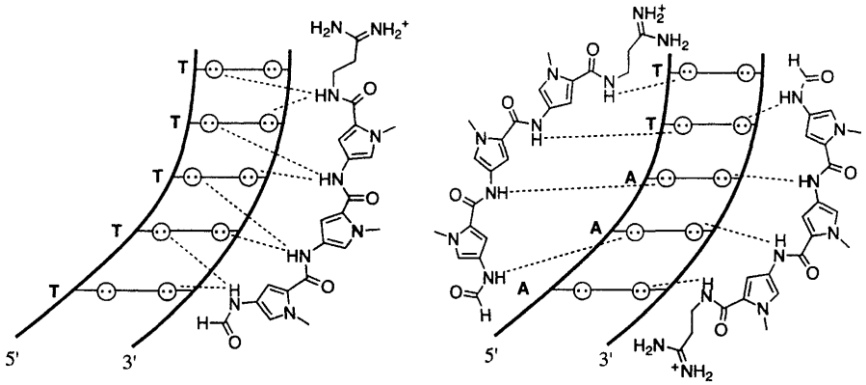


Figure 1.13: Distamycin A Binds to DNA at A-T base pair.

Distamycin A is immediately may take advantage for development of new pharmaceutical agents [38], artificial restriction enzymes [39], as well as DNA probes [40].

Distamycin A used as anti-bacterial, anti-cancer, antifungal and antiviral agent. It was found that distamycin binds to the minor groove of DNA and it inhibits, in various degrees, tumors in mice [41], and interferes with the process of cell division *in vitro*. It was also found that distamycin can inhibit plant pathogenic organisms, the growth of gram positive bacteria, gram negative bacteria, molds, and some viruses and has insecticidal activity [42,43].

1.7 Biological Activity of Some Modified Compound

Biological activity refers to substances having or producing an effect on the living tissue or its ability to affect a change in a biological process. The relation between the molecular entity and the biological activity can be tested by answering the following questions: (1) What is it? (2) What does it do? and (3) How much of it is present? These questions can express the activity of the compound. The importance of biological processes refers to the description of functional relationships between biological activities and the chemical substances that express them [44].

1.7.1 Anti-oxidants

Anti-oxidants “free radical scavengers” are substances that may prevent or delay some types of cell damage by reacting with and blocking the activity

of free radicals and preventing them from causing the damage of scavengers so as to prevent or delay different diseased states. These free radicals are considered as highly reactive species that have an odd number of electrons, which gives them high potentials to cause damage to cells called cellular pathologies. Some of these damages may lead to cancer. In the biological system, oxygen gives rise to a large number of free radicals and other reactive species collectively known as ‘reactive oxygen species’ (ROS).‘Reactive nitrogen species’ (RNS) are another group of reactive species that play a dual role as both deleterious and beneficial species [45,46,47].

Antioxidants are very important organic compounds especially in designing new novel drugs. Two types of free radicals exist. The first type is synthesized naturally by the body. The second type is introduced to our bodies through external sources. Sources of radicals are tobacco smoke, exposure to the sun, and other pollution forms of the body. This makes endogenous antioxidants, which are used to neutralize free radicals. However, the body also needs external sources of antioxidants called (exogenous) sources or dietary antioxidants like fruits and vegetables [48,49].

The high potential of free radicals gives them the high reactivity which harms the cells. They are formed by hemolytic cleavage of C-C bond and when an atom or a molecule either gains or loses an electron (a small negatively charged particle found in atoms) Fig.1.16 [50].

As the concentration of free radicals increases, their hazard on the body increases and causes the damage to all major components of cells, including proteins, DNA, and cell membranes. Many of these mutagens and carcinogens may act through the generation of oxygen radicals, as a result of the damage of DNA. Such conditions are suitable environments for the establishment and progression of cancer [51,52].

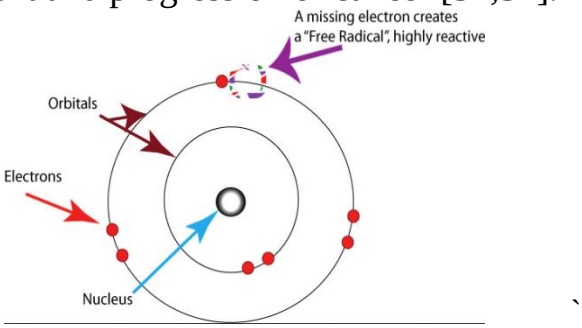


Figure 1.14: Configuration of free radical

Plants convert the solar energy into chemical energy so there's a hazard due to the excess energy and due to fear of oxidative damage of the plant cell. Nonetheless, the presence of antioxidant in plants will prevent the oxidative damage. Many of such compounds that protect plant cells are also found in human and protect human cells[53].

1.7.2 Anti-microbials (Antibacterial)

Microbes are tiny organisms seen by a microscope. These microbes are found in air, soil, rock, plants, bodies and water. Microbes are known to replicate and spread rapidly. Microbial organisms include bacteria, viruses, fungi, and protozoa. Some microbes cause disease and are called parasites. However, many others exist in the body as normal flora without causing harm and may be beneficial [54].

Antimicrobial drugs are synthesized to inhibit the microbe without any side effects on the patients [55]. Antibiotics are one of the most important weapons we have in the fight against bacterial infections, and the manufacture of these antibiotics has a strong relationship with the nature of life associated with human health. But recently, these health benefits have become limited because, and as a result of natural selection, bacterial resistance to these drugs is a major issue. In this respect, the development of medicines derived from natural sources play an important role in the prevention and treatment of human diseases [56].

1.7.3 Anti-microbials (Antifungal Activities)

An antifungal medicine is a drug that works selectively to eliminate fungal pathogens from a host with minimal toxicity to the host [57]. Unlike bacterial disease, fungal diseases are more difficult to treat. Topical and oral treatments are long term and partially successful in controlling the fungus. Many of these infections will be chronic and if you are fortunate enough to rid the infection from your body, there is always the possibility of recurrence of the disease [58].

Fungal infections of the skin are the most abundant and widespread group of all mycoses. Skin mycoses affect more than 20–25% of the world's population, which makes them one of the most frequent forms of infections [59,60].

1.8 Aim of the Study:

The main objective is the preparation new distamycin analogous by changing N-terminal alkyl groups which have lower molecular weight and higher lipophilicity than previous analogues. This will increase the binding with minor groove and decrease the toxicity of distamycin. Also the aim is detect the biological activities such as antimicrobial, the antiviral and anti-oxidant.

Chapter Two

Materials and Methods

2.1 Chemicals:

The following Chemicals were purchased from Sigma and Aldrich chemical companies and used without further purification:m-aminobenzoic acid, benzoyl chloride, tetrahydrofurane(THF), dichloromethane(DCM), oxalyl chloride, ethanol, n-hexane, methanol, ethyl acetate,N,N-Dimethyl-1,3-propanediamine, N-necotylchloride, methanesulfonyl chloride, dioxane, acetic anhydride,4-(2-aminoethyl)benzenesulfonamide, 2-(morpholin-4-yl) ethanamine.

As for biological activities, all tested microorganisms in this work were obtained from Biodiversity & Environmental Research Center (BERC) Til Village-Nablus.

2.2 Chromatography

Chromatography was established by the scientist Mikhail Tsvet in 1906, when he tried to separate pigments of a colored leaf such as chlorophyll, carotenes, and xanthophylls. The different colors of these compound gave the techniques its name [61,62].

2.2.1 Thin Layer Chromatography:

TLC is used for non-volatile mixtures. The stationary phase is a solid of silica gel. The mobile phase is eluent: methanol, hexane and ethanol.

2.2.2 Dry Column Flash Chromatography:

DCFC is a safe, powerful, and easily applied preparative chromatography technique. Similar to the column chromatography, the dry-column flash chromatography includes packing the column by TLC adsorbent grade,

loading the sample, and eluting the column with suction Fig.2.1**.**This will give the advantage of TLC in separation, and the advantage of column chromatography in quantities. It is similar to vacuum filtration that uses the same glassware. The column is a sintered glass funnel contain a “dry” bed of silica gel, and the elution occurs through suction. The column is then drained dry after each fraction, which makes it much easier to pack the column, and the person will not be worried about their columns going “dry” [63].

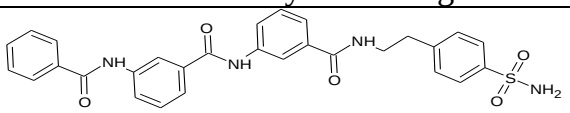
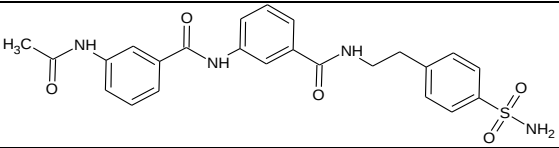
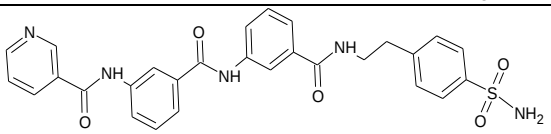
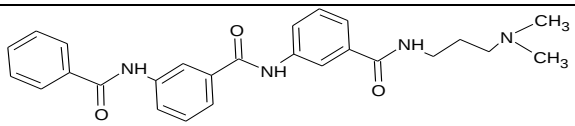
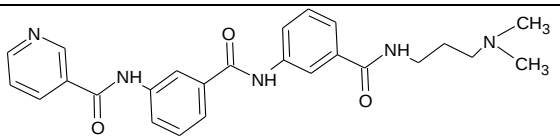
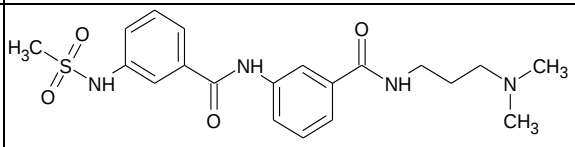
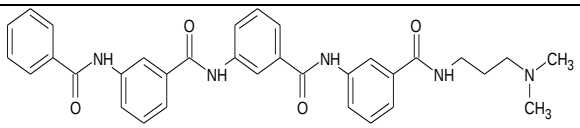
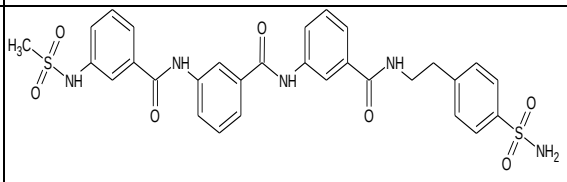
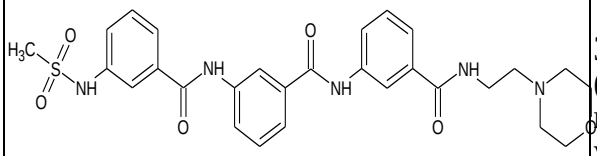
Figure 2.1: Dry Column Flash Chromatography Setup

2.3 Physical Measurements:

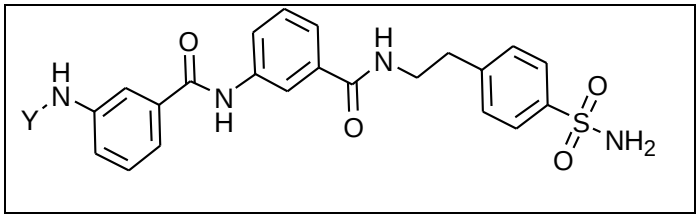
Melting point of each product was measured by Stuart meting point apparatus, SMP3, ^{1}H –NMR and C^{13}-NMR was determined in the Jordan University of Science and Technology (Bruker 400 MHz-Avance III).

IR was performed through Fourier transform spectrophotometer (Necolet Is 5-Id3).

Table 1.2:Structures of Distamycin Analogues

No.	Structures of Distamycin Analogues	Name of Structures
R_1		3-benzamido-N-(3-((4-sulfamonylphenethyl)carbamoyl) phenyl)benzamide
R_2		3-acetamido-*N*-(3-((4-sulfamoylphenethyl)carbamoyl)p henyl)benzamide
R_3		*N*-(3-((3-((4-sulfamoylphenethyl)carbamoyl)p henyl)carbamoyl)phenyl)nicotiNa mide
R_4		3-benzamido-N-(3-((3-(dimethylamino)propyl)carbamoy l)phenyl)benzamde
R_5		N-(3-((3-((3-(dimethylamino)propyl)carbamoy l)phenyl)nicotinamide
R_6		N-(3-(dimethylamino)propyl)-3-(3-(methylsulfonamido)benzamido) benzamide
R_7		3-benzamido-N-(3-((3-((3-(dimethylamino)propyl)carbamoy l)phenyl)carbamoyl)phenyl)Benz amide
R_8		3-(methylsulfonamido)-*N*-(3-((3-((4-sulfamoylphenethyl)carbamoyl)p henyl)carbamoyl)phenyl)benzami de
R_9		3-(methylsulfonamido)-*N*-(3-((3-((2-morpholinoethyl)carbamoyl)phen yl)carbamoyl)phenyl)benzamide

2.4General Procedure for the Preparation of Compounds Which Have Two 3-Amino Benzeamide Rings and Amine(4-(2-Amino-ethyl)-Benzenesulfonamide:



Where Y functional chemical group can be any of the following three entities:

R_1(Y= Benzoyl chloride)

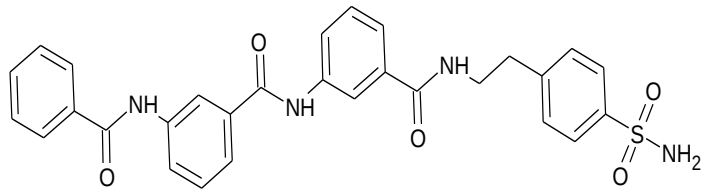
R_2 (Y=Acetic anhydride)

R_3(Y= N-nicotyl chloride)

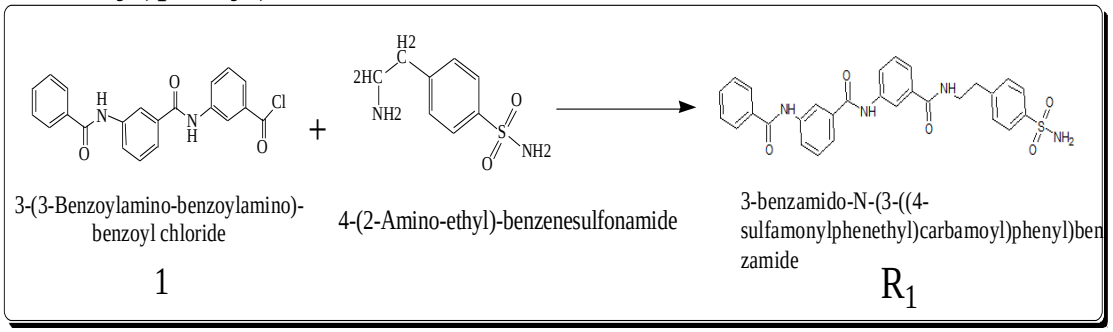
A proper amount of the corresponding substituted Y is dissolved in dichloromethane (DCM), was added to a proper amount of m-aminobenzoic acid, in THF, then, stirred overnight. The mixture was filtered by Buckner funnel, and the solid, oxalyl chloride was added followed by DCM (30mL). Then, the mixture was stirred for 2 hours. The mixture was filtered and the solid was collected. The solid was dissolved in DCM and retreated by m-aminobenzoic acid, in THF, as described above, followed by oxalyl chloride and 30 mL DCM, Then, The mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. This solid was added to the terminal amine (4-(2-Amino-ethyl)-benzenesulfonamide), the mixture was stirred over night, and then filtered by Buckner funnel. Finally, water (60ml) was added, and the product was

extracted with ethylacetate (80ml) and then the solvent was removed under reduced pressure to give the product which was purified by Dry Column Flash Chromatography (DCFC) and by recrystallization. The purity was then checked by Thin layer chromatography(TLC).

2.4.1 Preparation of 3-benzamido-N-(3-((4-sulfamoylphenethyl) carbamoyl)phenyl)benzamideWhen YI is Benzoyl chlori de(ph-C(=O)-Cl):

Structure of Compound R_1	Name of Compound
	3-benzamido-*N*-(3-((4-sulfamoylphenethyl)carbamoyl)phenyl)benzamide

Equation2.1: Preparation of3-benzamido-*N*-(3-((4-sulfamoylphenethyl) carbamoyl)phenyl)benzamide.



3-(3-Benzoylamino-benzoylamino)-benzoyl chloride (1g, .0026mol)[1], 4-(2-aminoethyleq)benzenesulfonamide-methane (1:1) (0.5g, 0.0024mol), the compound(R_1**)** produced. 3-benzamido-*N*-(3-((4-sulfamoylphenethyl) carbamoyl)phenyl)benzamide.

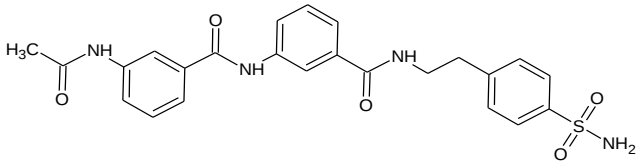
(0.5g, 0.0009mol), (yield=38.5%), (m.p= 211-215°C).

IR: 3295; 2921; 1682; 1643; 1592; 751; 673 cm^{-1} [Page69].

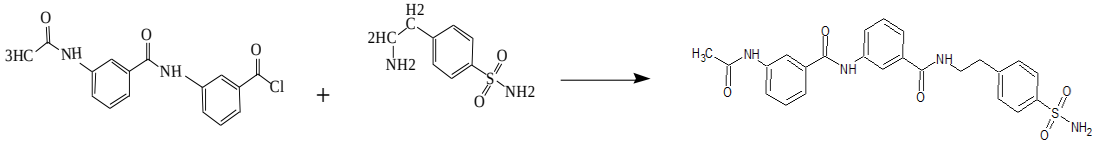
^{1}HNMR at δ: 2.884(2H, t); 3.572(2H, t, *J*=8Hz); 7.299(1H, s); 7.56(1H, s); 7.59(1H, s); 7.64(1H, s); 7.865(1H, s); 7.991(5H, s) ppm.

^{13}CNMR at δ: 27.63; 36.41; 57.32; 118.62; 119.55; 120.02; 125.07; 128.9; 129.49; 130.09; 133.00; 138.34; 163.24; 164.85; 167.3; 169.87 ppm.

2.4.2 Preparation of 3-acetamido-*N*-(3-((4-sulfamoylphenethyl) carbamoyl)phenyl)benzamideWhen Y_{II} is Acetic anhydride($CH_3C(=O)-O-C(=O)-CH_3$):

Structure of Compound R_2	Name of Compound
	3-acetamido-*N*-(3-((4-sulfamoylphenethyl)carbamoyl)phenyl)benzamide

Equation2.2: Preparation of 3-acetamido-*N*-(3-((4-sulfamoylphenethyl)carbamoyl)phenyl)benzamide.



2 **R_2**

3-(3-Acetylamino-benzoylamino)-benzoyl chloride(2.20g, 0.0069mol)[2],4-(2-aminoethyl)benzenesulfonamide - methane (1:1) (1.38g, 0.0068mol), the compound (R_2) are produce, 3-acetamido-*N*-(3-((4-sulfamoylphenethyl)carbamoyl)phenyl)benzamide.

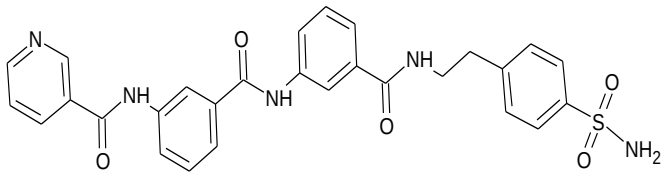
(0.95g, 0.0019mol), (yield= 29%), (m.p=342-346°C).

IR: 3322.26; 3276.23; 3054.13; 2935.43; 1659.96; 1602.37; 1528.62; 1438; 767.89; 659.91; 590.68 cm^{-1} [Page70].

^{1}H NMR at δ: 2.49(3H, S); 2.72(imp); 2.88(imp); 2.91(2H, t, *J*=8Hz); 3.81(2H, t, *J*=8Hz); 3.56,many H's, m(all amide H's and solvent residue); 7.37(1H, t, *J*=8Hz); 7.44(1H, d, *J*=8Hz); 7.69(1H, d); 7.75(1H, d); 7.86(1H, d); 7.93(1H, S); 8.40(1H, S) ppm [Page 78].

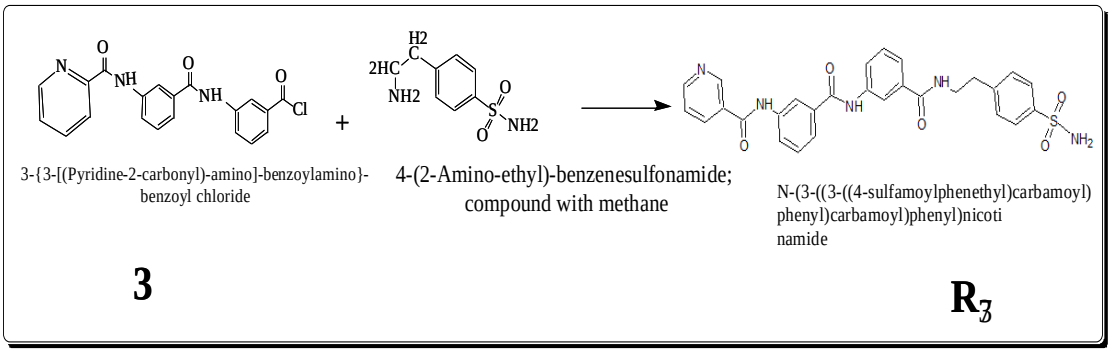
$^{13}CNMR$ at δ: 34.72, CH_2-ph; 39.79, CH_3; 66.40, CH_2-N; 121.54; 125.86; 125.90; 128.19; 128.20; 129.20; 129.22; 136; 137.24; 142.24; 142.52(all for aryl carbons); 158.75; 164.00; 168.00(all for amide carbons) ppm [Page80, 81].

2.4.3 Preparation of *N*-(3-((3-((4-sulfamoylphenethyl) carbamoyl) phenyl)carbamoyl)phenyl)nicotinamide When Y_{III} is N-necotyl chloride(py—C(=O)—Cl):

Structure of Compound R_3	Name of Compound
	*N*-(3-((3-((4-sulfamoylphenethyl)carbamoyl)phenyl)carbamoyl)phenyl)nicotinamide

Equation2.3: Preparation of *N*-(3-((3-((4-sulfamoylphenethyl) carbamoyl)phenyl) carbamoyl) phenyl) nicoti

namide.



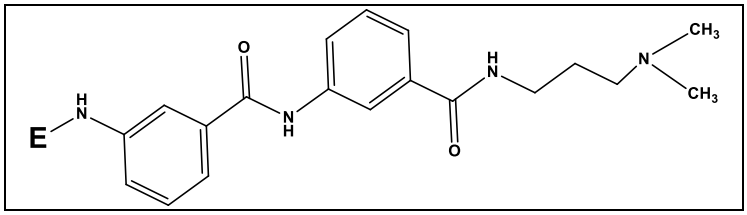
3-{3-[(Pyridine-3-carbonyl)-amino]-benzoylamino}-benzoyl chloride(1.7g, 0.00447mol) [3], , 4-(2-aminoethyl)benzenesulfonamide - methane (1:1) (0.89g, 0.0044mol), the compound (R_3) are produced

N-(3-((3-((4-sulfamoylphenethyl) carbamoyl) phenyl) carbamoyl) phenyl) nicotinamide.

(0.8g, 0.0015mol), (yield=33%), (m.p=240-244°C).

IR: 3356.55; 2996.5; 2920.43; 1701; 1594; 1556; 1486; 721; 676; 577; 539 cm^{-1} [Page 71].

2.5 General Procedure for the Preparation of Compounds which have Two Benzene Rings and Amine(N,N-Dimethyl-1,3-propanediamine):



Where **E** functional chemical group can be any of the following three entities:

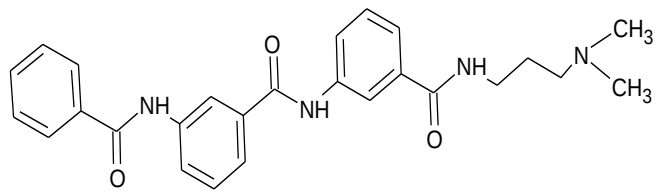
R_4(E= Benzoyl Cloride)

R_5(E=Pyridine-2-carbonyle chloride)

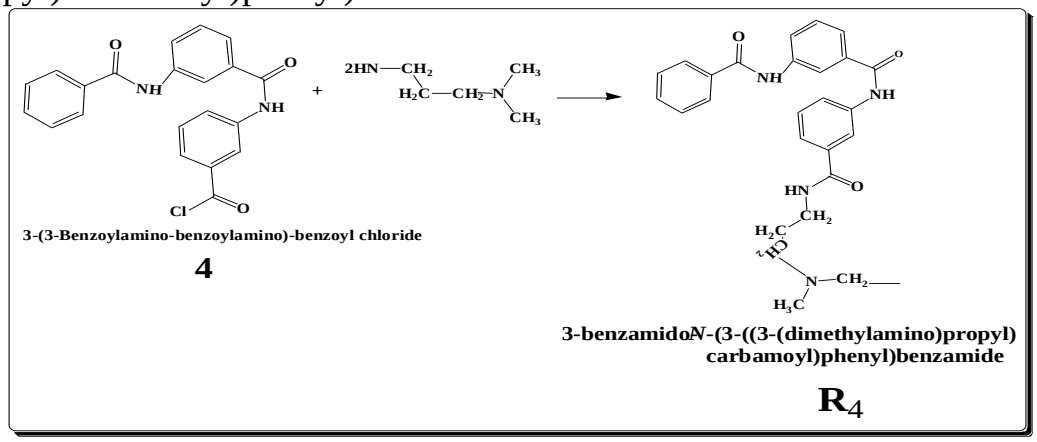
R_6(E= Methane sulphonyl chloride)

A proper amount of the corresponding substituted E dissolved in dichloromethane (DCM), was added to a proper amount of m-aminobenzoic acid ,in THF, then, stirred over night. The mixture was filtered by Buckner funnel. To the solid, oxalyl chloride was added followed by DCM (30mL), Then, The mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. The solid was dissolved in DCM and retreated in m-aminobenzoic acid, in THF, as described above, followed by oxalyl chloride and 30 mL DCM , Then, The mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. This solid was added to the terminal amine (Dimethylaminopropelamine), the mixture was stirred over night and then filtered by Buckner funnel. Finally, water (60ml) was added, and the product was extracted with ethylacetate (80ml) and then the solvent was removed under reduced pressure to give the product, purified by Dry Column Flash Chromatography (DCFC) and by recrystallization. The purity was then checked by Thin layer chromatography(TLC).

2.5.1Preparation of 3-benzamido-*N*-(3-((3-(dimethylamino) propyl) carbamoyl)phenyl)benzamide When E_I is benzoyl chloride(ph-C(=O)-Cl):

Structure of Compound R_4	Name of Compound
	3-benzamido-*N*-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)benzamide

Equation2.4: Preparation of 3-benzamido-*N*-(3-((3-(dimethylamino) propyl) carbamoyl)phenyl)benzamide

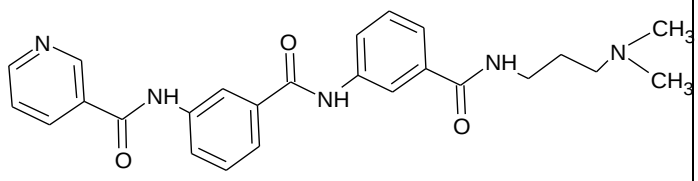


3-(3-Benzoylamino-benzoylamino)-benzoyl chloride(2g, 0.0053mol)[4],3-Dimethylaminopropylamine(0.5g, 0.0049 mol). The compound R_4 are produced,3-benzamido-*N*-(3-((3-(dimethylamino)propyl)carbamoyl) phenyl)benzamide. (0.9g, mol), (yield=41%), (m.p= 210-214°C).

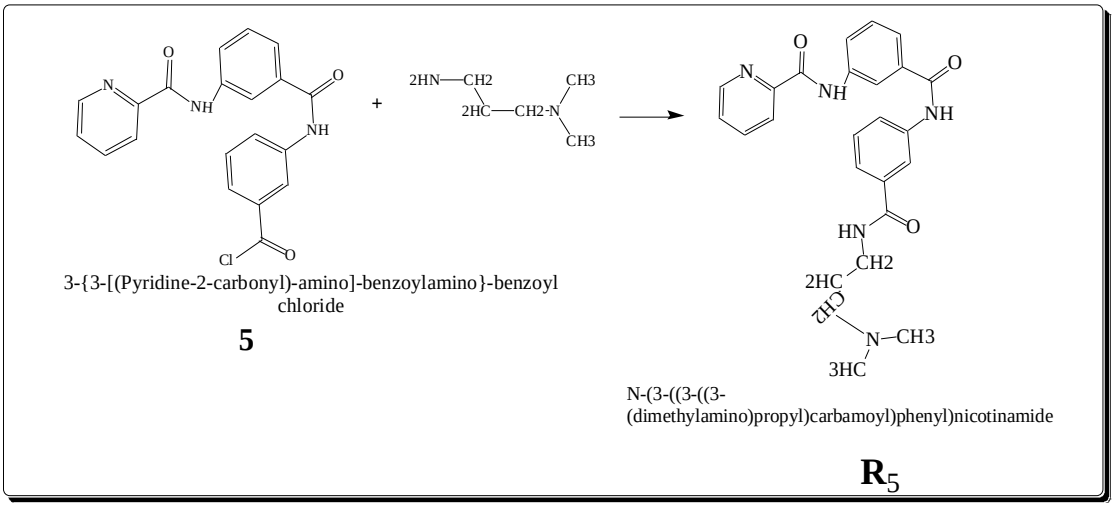
IR: 3278; 2960; 1677; 1587; 1453; 751; 705; 665; 534cm^{-1} [Page72].

2.5.2Preparation of *N*-(3-((3-((3-(dimethylamino) propyl) carbamoyl) phenyl)carbamoyl)phenyl)nicotinamide

When E_{II} is Pyridine-2-carbonyle chloride(py—C(=O)—Cl):

Structure of compound R_5	Name of compound
	*N*-(3-((3-((3-(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)phenyl)nicotinamide

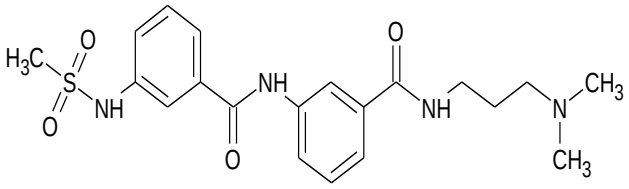
Equation2.5: Preparation of*N*-(3-((3-((3-(dimethylamino) propyl)carbamoyl)phenyl)carbamoyl)phenyl)nicotinamide



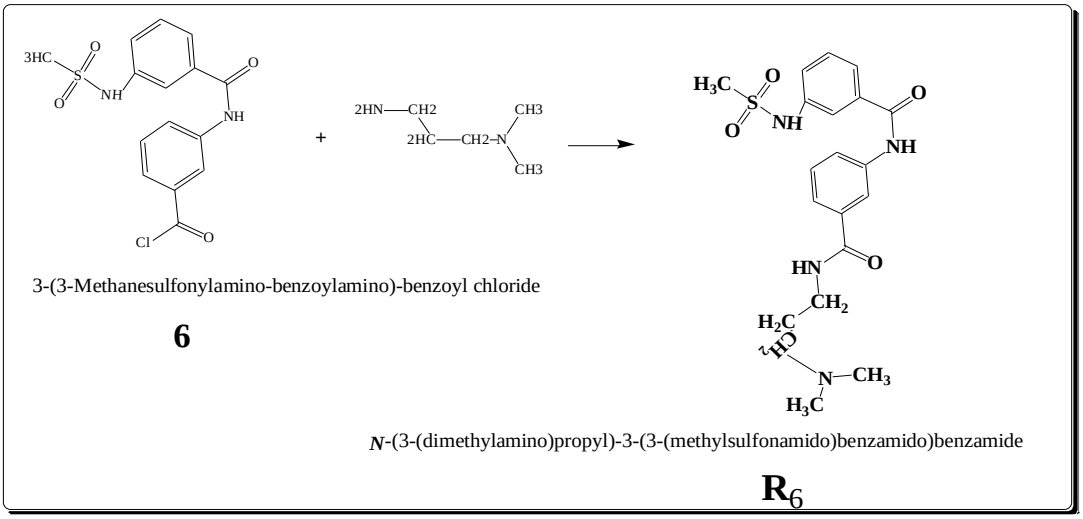
3-{3-[(Pyridine-2-carbonyl)-amino}-benzoylchloride(3g, 0.0079mol)[5**]**, 3-Dimethylaminopropylamine(0.8g, 0.0078mol).The compound $R_5$are produced ,*N*-(3-((3-((3-(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)phenyl)nicotinamie(. 2.11g, 0.0047mol), (yield=34%), (m.p= 250-253°C).

IR: 3271; 1689; 1662; 1642; 1593; 755; 698cm^{-1} [Page73].

2.5.3 Preparation of *N*-(3-(dimethylamino)propyl)-3-(3-(methylsulfonamido) benzamido)benzamideWhenE_{III} ismethanesul -fonylchloride(CH_3-SO_2-Cl):

Structure of Compound R_6	Name of Compound
	*N*-(3-(dimethylamino)propyl)-3-(3-(methylsulfonamido)benzamido)benzamide

Equation2.6: Equation for the preparation of *N*-(3-(dimethylamino)propyl)-3-(3-(methylsulfonamido)benzamido)benzamide



3-(3-Methansulphonylamino-benzoylamino)-benzoyl chloride(2.5g, 0.00708mol) (6), 3-Dimethylaminopropylamine(0.72g, 0.00704mol)**,**

The compound R_6 are produced, *N*-(3-(dimethylamino)propyl)-3-(3-(methylsulfonamido)benzamido)benzamide.

(0.68g, 0.0016mol), (yield=23%) , (m.p=208-212°C).

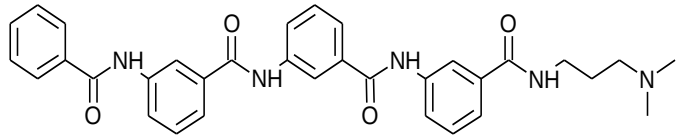
IR: 3374; 2970; 2711; 1701; 1631; 1594; 750; 674 cm^{-1} [Page74].

^{1}HNMR at δ:1.41(2H, q); 2.26(6H, S); 2.44(2H, t); 3.08(3H, S); 3.63(2H, t); 7.43(1, S); 7.53(1H, S); 7.78(1H, S); 8.96(3H, S) ppm.

^{13}CNMR at δ: 27.63; 36.41; 44.90; 57.32; 125.07; 129.62; 137.82; 149.3; 165.18; 169.87 ppm.

2.6 Preparation of 3-benzamido-*N*-(3-((3-((3-(dimethylamino) propyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide

Which have three benzeamide rings and N,N-Dimethyl-1,3-propanediamine.

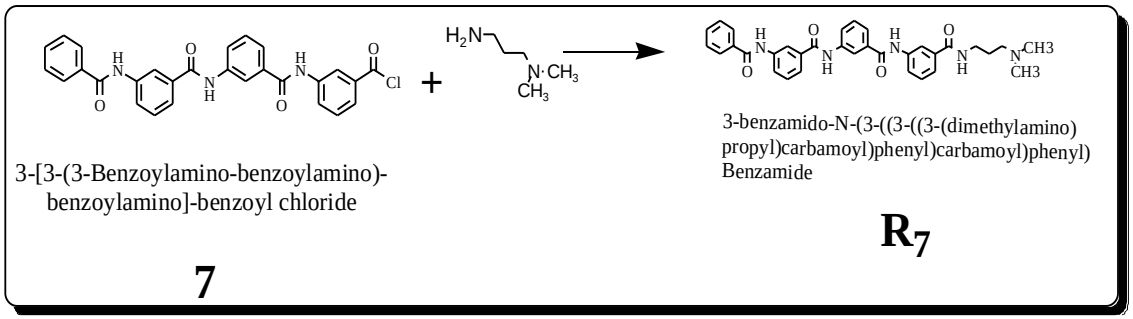
Structure of Compound R_7	Name of Compound
	3-benzamido-*N*-(3-((3-((3-(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide

2.6Procedure for the Preparation of 3-benzamido-*N*-(3-((3-((3(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide:

Benzoyl chloride (1.8g, 0.0132mole) dissolved in dichloromethane (DCM), was added to m-aminobenzoic acid (1.8g, .013mole) ,in THF, then, stirred over night. The mixture was filtered by Buckner funnel. To the solid, oxalyl chloride was added followed by DCM (30mL). Then, the mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. The solid was dissolved in DCM and retreated in m-aminobenzoic acid, in THF, as described above, followed by oxalyl chloride and 30 mL DCM, then, the mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. The solid was dissolved in DCM and retreated in m-aminobenzoic acid, in THF, as described above, followed by oxalyl chloride and 30 mL DCM, then, the mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. This solid was added to the terminal amine (Dimethylaminopropelamine), the mixture was stirred over night and then filtered by Buckner funnel. Finally, water (60ml) was added, and the product was extracted with ethylacetate (80ml) and then the

solvent was removed under reduced pressure to give the product which was purified by Dry Column Flash Chromatography (DCFC) and by recrystallization. The purity was then checked by Thin layer chromatography(TLC).

Equation 2.7: Preparation of 3-benzamido-*N*-(3-((3-((3(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide.



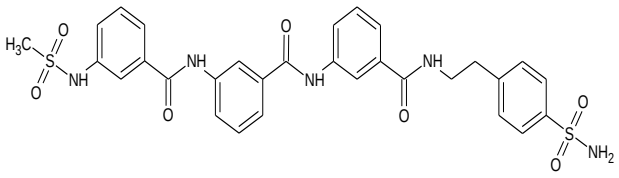
(1.5 g, 0.0026mol), (yield=53%), (m.p=345-349).

IR: 3273.86; 2824.32; 1662; 1589; 1522; 751; 737; 676; 570; 540 cm^{-1} [Page75].

^{1}HNMR at δ: 1.414(2H, q); 2.26(6H, S); 2.44(2H, t); 3.63(2H, t); 7.35(1H, s); 7.53(1H, s); 7.56(1H, s); 7.64(1H, s); 7.88(1H, s); 9.176(4H, S) ppm.

^{13}CNMR at δ: 27.63; 39.2; 44.9; 57.32; 118.59; 124.61; 129.62; 130.2; 142.16; 164.85; 169.87 ppm.

2.7: Preparation of3-(methylsulfonamido)-*N*-(3-((3-((4-Sulfamoylphenethyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide

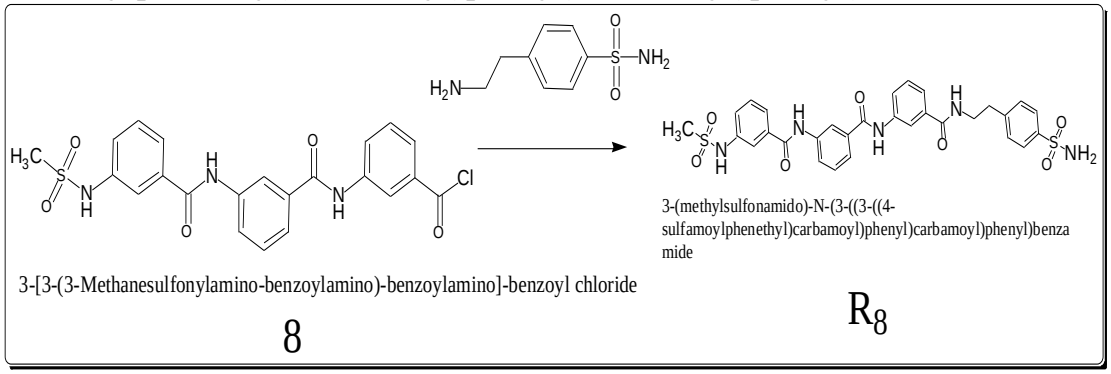
Structure of Compound R_8	Name of Compound
	3-(methylsulfonamido)-*N*-(3-((3-((4-sulfamoylphenethyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide

2.7 Procedure for the preparation of3-(methylsulfonamido)-*N*-(3-((3-((4sulfamoylphenethyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide:

Methanesulfonyl chloride (2.20g, 0.0192mole) dissolved in dichloromethane (DCM), was added to m-aminobenzoic acid(1.8g, 0.013mole) ,in THF, then, stirred over night. The mixture was filtered by Buckner funnel. To the solid, oxalyl chloride was added followed by DCM (30mL). Then, the mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. The solid was dissolved in DCM and retreated in m-aminobenzoic acid, in THF, as described above, followed by oxalyl chloride and 30 mL DCM, then, the mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. The solid was dissolved in DCM and retreated in m-aminobenzoic acid, in THF, as described above, followed by oxalyl chloride and 30 mL DCM, then, the mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. This solid was added to the terminal amine(4-(2-Amino-ethyl)-benzenesulfonamide), the mixture was stirred over night and then filtered by Buckner funnel. Finally, water (60ml) was added, and the product was extracted with

ethylacetate (80ml) and then the solvent was removed under reduced pressure to give the product which was purified by Dry Column Flash Chromatography (DCFC) and by recrystallization. The purity was then checked by Thin layer chromatography(TLC).

Equation2.8: Preparation of 3-(methylsulfonamido)-*N*-(3-((3-((4-sulfamoylphenethyl)carbamoyl)phenyl)carbamoyl)phenyl) benzamide



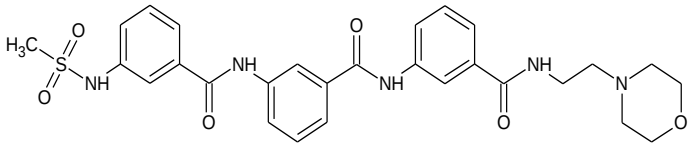
(1.35g, 0.002mole), (yield 45.3%), (m.p=310-313°C).

IR: 3274.30; 2832.49; 1664.03; 1590.71; 1523.96; 1455.84; 752.20; 676.92; 514 cm^{-1} [Page 76].

^{1}H NMR at δ: 2.49(3H, S); 2.71(2H, S); 2.87(2H, S); 3.21; 3.42; 3.55,many H's, m(all amide H's and solvent residue); 7.50(1H, t, *J*=8Hz); 7.73(1H, d, *J*=8Hz); 7.90, m; 8.04(1H, d, *J*=8Hz); 8.53(1H, S, for mobile H's) ppm [Page 79].

$^{13}CNMR$ at δ: 34.72, CH_2-ph; 39.79, CH_3; 66.40, CH_2-N; 121.54; 125.86; 125.90; 128.19; 128.20; 129.20; 129.22; 136; 137.24; 142.24; 142.52(all for aryl carbons); 158.75; 164.00; 168.00(all for amide carbons) ppm [Page 82, 83].

2.8 Preparation of 3-(methylsulfonamido)-*N*-(3-((3-((2-morpholinoethyl) carbamoyl)phenyl)carbamoyl)phenyl)benzamide:

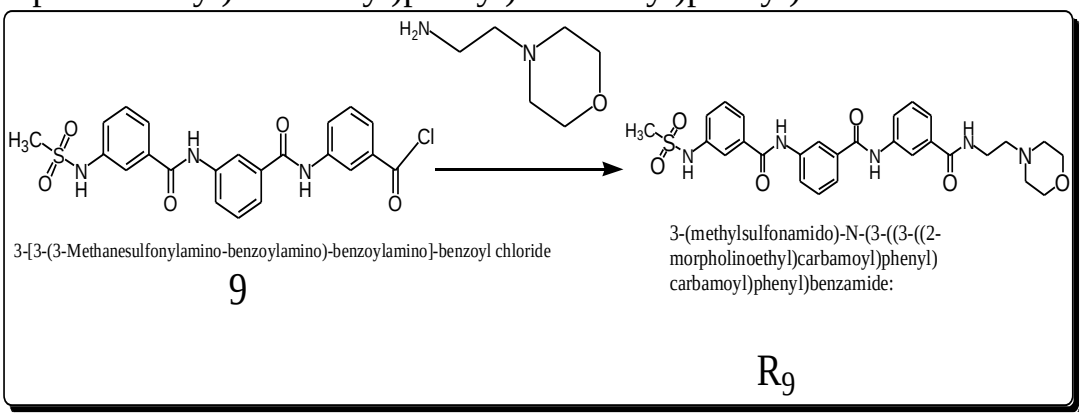
Structure of Compound R9	**Name of Compound**
	3-(methylsulfonamido)-*N*-(3-((3-((2-morpholinoethyl) carbamoyl)phenyl)carbamoyl) phenyl)benzamide

2.8 Procedure for preparation of 3-(methylsulfonamido)-*N*-(3-((3-((2 morpholinoethyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide:

Methanesulfonyl chloride (3g, 0.026mole) dissolved in dichloromethane (DCM), was added to m-aminobenzoic acid (3.56g, 0.026mole) ,in THF, then, stirred over night. The mixture was filtered by Buckner funnel. To the solid, oxalyl chloride was added followed by DCM (30mL). Then, the mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. The solid was dissolved in DCM and retreated in m-aminobenzoic acid, in THF, as described above, followed by oxalyl chloride and 30 mL DCM, then, the mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. The solid was dissolved in DCM and retreated in m-aminobenzoic acid, in THF, as described above, followed by oxalyl chloride and 30 mL DCM, then, the mixture was stirred for 2 hour. The mixture was filtered and the solid was collected. This solid was added to the terminal amine (2-Morpholin-4-yl-ethylamine)(.6g, .0046mole), the mixture was stirred over night and then filtered by Buckner

funnel. Finally, water (60ml) was added, and the product was extracted with ethylacetate (80ml) and then the solvent was removed under reduced pressure to give the product which was purified by Dry Column Flash Chromatography (DCFC) and by recrystallization. The purity was then checked by Thin layer chromatography(TLC).

Equation 2.9: Preparation of 3-(methylsulfonamido)-*N*-(3-((3-((2-morpholinoethyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide



(1.68g**,** 0.003mol) (Yeild=64.8%) (m.p= 260-265°C**).**

IR: 3274; 2832; 1664; 1590; 1523; 752; 676 cm^{-1} [Page77].

Chapter Three

Results and Discussion

-Results and Discussion

The synthesized compounds characterized by (^{1}HNMR, ^{13}CNMR, IR), and tested for biological activities.

3.1 Synthesis of Compounds

The following compounds were prepared**:**

3.1.1 3-**Benzamido-N-(3-((4-sulfamonylphenethyl) carbamoyl)phenyl) benzamide(R_1)**, from benzoyl chloride(1), 3-aminobenzoic acid(2), DCM(3), THF(4), oxalyl chloride(5), (4-(2-Amino-ethyl)-benzene -sulfonamide)(6).

3.1.23-Acetamido-N-(3-((4-sulfamoylphenethyl)carbamoyl) phenyl)benzamide(R_2), prepared from acetic anhydride(7), 3-aminobenzoic acid(2), DCM(3), THF(4), oxalyl chloride(5), (4-(2-Amino-ethyl)-benzenesulfonamide)(6).

3.1.3N-(3-((3-((4-Sulfamoylphenethyl)carbamoyl)phenyl) carbamoyl) phenyl)nicotiNamide(R3), prepared from nicotyl chloride(8), 3-aminobenzoic acid(2), DCM(3), THF(4), oxalyl chloride(5), (4-(2-Amino-ethyl)-benzenesulfonamide)(6).

3.1.43-Benzamido-N-(3-((3-(dimethylamino)propyl)carbamoyl) phenyl)benzamde(R4), prepared from benzoyl chloride(1), 3-aminobenzoic acid(2), DCM(3), THF(4), oxalyl chloride(5), N,N-Dimethyl-1,3-propanediamine(9).

3.1.5N-(3-((3-((3-(Dimethylamino)propyl)carbamoyl)phenyl) nicotinamide(R5),prepared from nicotyl chloride(8), 3-aminobenzoic acid(2), DCM(3), THF(4), oxalyl chloride(5), N,N-Dimethyl-1,3-propanediamine(9).

3.1.6N-(3-(Dimethylamino)propyl)-3-(3-(methylsulfonamido) benzamido) benzamide(R6),prepared from methanesulfonylchloride(10), 3-aminobenzoic acid(2), DCM(3), THF(4), oxalyl chloride(5), N,N-Dimethyl-1,3-propanediamine(6).

3.1.73-Benzamido-N-(3-((3-((3-(dimethylamino)propyl)carbamoyl) phenyl)carbamoyl)phenyl)Benzamide(R7), benzoyl chloride(1), 3-aminobenzoic acid(2), DCM(3), THF(4), oxalyl chloride(5), N,N-Dimethyl-1,3-propanediamine(9).

3.1.83-(Methylsulfonamido)-N-(3-((3-((4-sulfamoylphenethyl) carbamoyl)phenyl)carbamoyl)phenyl)benzamide(R8), prepared from methanesulfonylchloride(10), 3-aminobenzoic acid(2), DCM(3), THF(4), oxalyl chloride(5),(4-(2-Amino-ethyl)-benzenesulfonamide)(11).

3.1.93-(Methylsulfonamido)-N-(3-((3-((2-morpholinoethyl) carbamoyl) phenyl)carbamoyl)phenyl)benzamide(R9),prepared from methanesulfonylchloride(10), 3-aminobenzoic acid(2), DCM(3), THF(4), oxalyl chloride(5), 2-(morpholin-4-yl)ethanamine(12).

3.2 Establishment of Structures

The structures of these compounds are established as follows:

3.2.1 3-Acetamido-*N*-(3-((4-sulfamoylphenethyl) carbamoyl) phenyl) benzamide(R_2), was established by:

^{1}H NMR at δ: 2.49(3H, s); 2.72(imp); 2.88(imp); 2.91(2H, t, *J*=8Hz); 3.01(2H, t, *J*=8Hz); 3.56,many H's, m (all amide H's and solvent residue); 7.37(1H, t, *J*=8Hz); 7.44(1H, d, *J*=8Hz); 7.69(1H, d); 7.75(1H, d); 7.86(1H, d); 7.93(1H, S); 8.40(1H, S) ppm [Page75].

^{13}CNMR at δ: 34.72, CH_2-ph; 39.79, CH_3; 66.40, CH_2-N; 121.54; 125.86; 125.90; 128.19; 128.20; 129.20; 129.22; 136; 137.24; 142.24; 142.52 (all for aryl carbons); 158.75; 164.00; 168.00 (all for amide carbons) ppm [Page 77, 78].

IR: 3322.26; 3276.23; 3054.13; 2935.43; 1659.96; 1602.37; 1528.62; 1438; 767.89; 659.91; 590.68 cm^{-1} [Page 67].

3.2.2 3-(Methylsulfonamido) -*N*-(3- ((3- ((4-sulfamoylphenethyl) carbamoyl)phenyl)carbamoyl)phenyl)benzamide(R_8), was established by:

^{1}H NMR at δ: 2.49(3H, s); 2.71(2H, s); 2.87(2H, s); 3.21; 3.42; 3.55,many H's, m(all amide H's and solvent residue); 7.50(1H, t, *J*=8Hz); 7.73(1H, d, *J*=8Hz); 7.90, m; 8.04(1H, d, *J*=8Hz); 8.53(1H, S, for mobile H's) ppm [Page 76].

^{13}CNMR at δ: 36.93, CH_2-ph; 39.20, CH_3; 121.05, C=C-ph; 124.47, C-C-C=O; 128.73, C-H in ph, 131.10, CH; 137.65, C-NH; 158, C=N ppm [Page79, 80].

IR: 3274.30; 2832.49; 1664.03; 1590.71; 1523.96; 1455.84; 752.20; 676.92; 514 cm^{-1} [Page73].

3.2.1.1 The signal at 2.49 for 3H in CH3, the signal at 3.01 and 3.91 have been shown as two distorted triplets, that is because they are splitted by each other and the chemical shift difference relative (.1ppm, 40Hz) to the coupling constant is relatively small.

The sulphonamide is acidic and the NH_2protons enhance, proton exchange.

3.2.3 The theoretical analysis of R_1, R_2, R_3:

-3-Benzamido-N-(3-((4-sulfamonylphenethyl) carbamoyl)phenyl)benzamide(R_1) at δ:

^{1}HNMR 2.88(2H, t); 3.57(2H, t, *J*=8Hz); 7.29(1H, s); 7.53(1H, s); 7.56(1H, s); 7.59(1H, s); 7.64(1H, s); 7.86(1H, s); 7.99(5H, s) ppm.

^{13}CNMR at δ:27.63; 36.41; 57.32; 118.62; 119.55; 120.02; 125.07; 128.9; 129.49; 130.09; 133.00; 138.34; 163.24; 164.85; 167.3; 169.87 ppm.

-N-(3-(Dimethylamino)propyl)-3-(3-(methylsulfonamido) benzamido)benzamide(R_6):

^{1}HNMR at δ: 1.41(2H, q); 2.26(6H, S); 2.44(2H, t); 3.08(3H, S); 3.63(2H, t); 7.43(1, S); 7.53(1H, S); 7.78(1H, S); 8.96(3H, S) ppm.

^{13}CNMR at δ: 27.63; 36.41; 44.90; 57.32; 125.07; 129.62; 137.82; 149.3; 165.18; 169.87 ppm.

- 3-Benzamido-N-(3-((3-((3-(dimethylamino)propyl) carbamoyl) phenyl)carbamoyl)phenyl)benzamide(R_7):

^{1}HNMR at δ: 1.414(2H, q); 2.26(6H, S); 2.44(2H, t); 3.63(2H, t); 7.35(1H, s); 7.53(1H, s); 7.56(1H, s); 7.64(1H, s); 7.88(1H, s); 9.176(4H, S) ppm.

^{13}CNMR at δ: 27.63; 39.2; 44.9; 57.32; 118.59; 124.61; 129.62; 130.2; 142.16; 164.85; 169.87 ppm.

Chapter Four

Biological Activity

4.1 Introduction

This research is focused on the synthesis of molecules that possess unique biological activity, useful for biological and chemical biology research. Also It is interested in controlling post-translational protein modifications and elucidating the relationship between biological activity and the structures of compounds. The study was also looking into the behavior of molecules within the cell and their selectivity to the binding of proteins. Our purpose is to apply the developed molecules towards drug discovery and structural biological research.

Analogues of naturally occurring antitumor agents, such as Distamycin A, which bind in the minor groove of DNA, represent a new class of anticancer compounds currently under investigation[60]. Distamycin A has driven researcher's attention not only for the biological activity, but also for its non-intercalative binding to the minor groove of double stranded B-DNA, where it forms strong reversible complex preferentially at the nucleotide sequences consisting of 4-5 adjacent AT base pairs[61]. Different biological assays were done to test the activities of the synthesized compounds such as antioxidant activity, reductive potential, antibacterial and antifungal activities.

4.2 Materials and Methods

4.2.1 Chemicals

Chloramphenicol, peptone, agar, dextrose, ethanol, Muller–Hinton agar, gentamicin, ampicilline, chloramphenicol and econazole, 1,1- diphenly-2-picrylhydrazyl (DPPH), all chemicals and reagents were of analytical grade.

4.3 Antioxidant Activity

The hydrogen atom or electron donation abilities of the pure compounds were measured from the bleaching of the purple-colored methanol solution of 1,1- diphenly-2-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits and Bucar, 2000; Cuendet et al., 1997). One ml of various concentrations of the compounds in ethanol were added to 4 ml of 0.004% methanol solution of DPPH (OD= 1.1128). Gallic acid (0.25mg/ml) was used as standard. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I%) was calculated in the following way:

I(%)=((A blank–A sample)/A blank)x 100% Equation (1)

Compounds concentration providing 50% inhibition (IC50) was calculated from the graph plotted inhibition percentage against extract concentration.

4.4 Reductive Potential

Each sample (1 ml , 2.5mg/ml) or standard (1ml , 1mg/ml) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, centrifuged for 10 min at 3,000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl3 (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a Shimadzu 160-UV spectrophotometer.[64]

4.5 Antibacterial Activity

Antibacterial activity testing

The antibacterial activity of the synthesized compounds was determined against the following microorganisms: Staphylococcus aureus (ATCC 25923), Salmonella, (ATCC14028), Klebsiellapneumoniae(ATCC 13883), Proteus vulgaris (ATCC 13315), and Pseudomonas aeruginosa (ATCC 27853), all the isolates were purchased from BERC /Til Village. Solutions of each synthetic compound (5.0 mg/mL) in ethanol were sterilized by filtration through a 0.45 mm membrane filter. Antibacterial tests were then carried out by disc diffusion method. Compounds were investigated by disc diffusion using 6 mm filter discs prepared from Whatman paper 3. Bacteria were cultured overnight at 28°C in LB medium and then adjusted with sterile saline to a concentration of 1.0x105 CFU mL-1. The suspension was swapped on the top of Muller–

Hinton agar plates (20 mL agar/1 plate). Discs were flooded with the 10ul compounds (5.0 mg mL-1) are placed on the inoculated agar. (4 discs per agar plate).. After 24 h of incubation at 37°C for bacteria the diameter of the growth inhibition zones was measured. Gentamycin was used as a positive control and 10 μL was applied to the discs from stock solution (1 mg mL-1), . All tests were done in duplicate. (Sokovic et al., 2008).

4.6 Antifungal Activity

Antifungal activity testing

The antifungal activity test was done against the following dermatophytes: Trichophytonrubrum (CBS 392.58), Trichophytonmentagophytes (CBS 106.67 and Microsporumcanis (CBS 132.88). All the isolates were purchased from BERC/Til Village.

The synthesized compounds were tested for their antifungal activity against the test pathogens using a modified poisoned food technique. Each compound (5mg/ml) was mixed with the pre-sterilized SDA medium to concentrations (200, 100, 50, 25 ug/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 test sample. 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% Equation (2)

Where dc is colony diameter of the control, and ds is colony diameter of the sample. All tests were performed in triplicates.

4.7 Results and Discussion

4.7.1 Antioxidant Activity

Table 3.1 and Figure 3.1 showed the percent inhibition of the tested compounds in DPPH assay. None of the compounds showed significant activity compared with Gallic acid (percent inhibition =68 at the concentration 50 µg/ml and IC50= 32 µg/ml).The highest Values of percent inhibition for the compounds were (R7=29.27 and R4=28.51 at the concentration 50 µg/ml respectively.

Table 4.1: DPPH Assay for the Compounds

	% Inhibition							
Conc. µg/ml	Compounds*							
	R1	R2	R3	R4	R6	R7	R8	R9
0	0	0	0	0	0	0	0	0
10	-0.41	1.17	-5.91	19.27	8.61	11.87	5.83	12.47
20	-0.76	2.79	-4.11	24.13	12.89	18.75	8.97	14.89
30	0.81	2.8	-0.95	25.9	13.46	22.83	9.13	15.85
40	1.1	3.04	-0.23	28.71	13.52	26.23	12.12	17.07
50	2.17	3.73	-0.21	28.51	14.58	29.27	13.53	18.07

*Compounds are identified in table 3.1

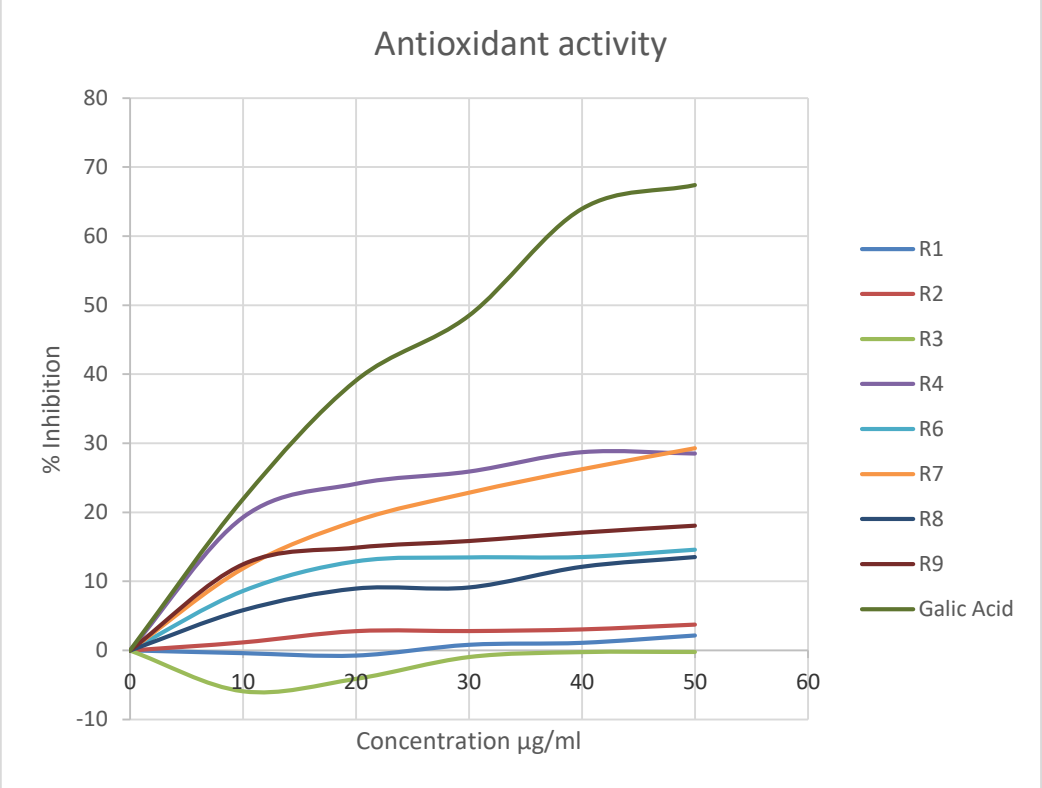


Figure4.1: Antioxidant Activity of the Compounds

4.7.2 Reductive Potential

Fe (III) reduction can be used as an indicator of electron-donating activity and therefore reflects an important mechanism of the synthesized compounds antioxidant action. In this study, the reducing power was evaluated by monitoring the ferric-ferrous transformation at 700 nm. The reducing ability generally increased with increasing sample concentration The following Table and Figure showed the reductive potential activity of the compounds compared with Gallic acid as a standard. Compounds (R1=361.4, R2=193.1, R3=110.3)revealed the highest values compared with (Gallic acid =474). The other compounds are relatively of very low activity compared to Gallic acid.

Table4. 2: Reductive Potential for Compounds and Gallic Acid

Compound	R1	R2	R3	R4	R5	R6	R7	R8	R9	**Gallic acid**
Red. Potential	361.4	193.1	110.3	37	32.3	27.7	41.8	33.1	47.3	**474**

*Compounds are identified in Table 3.2

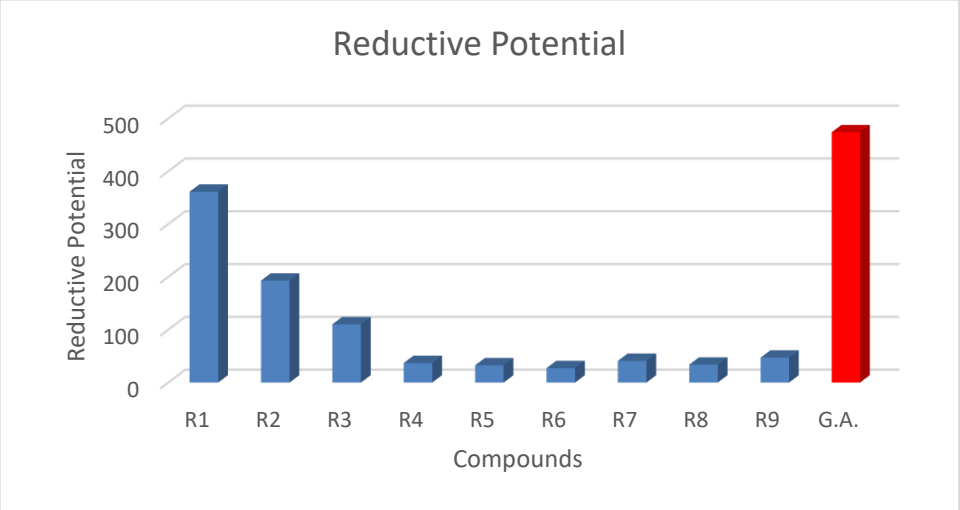


Figure4.2: Reductive Potential for the Compounds and Gallic Acid

4.7.3Antibacterial Activity

Figure 4.3 showed the activity of the tested compounds against six types of bacteria, using disk diffusion method. Non of them showed significant activity compared with gentamycin (50µg/disk). The inhibition zones for gentamycin are (Kleb. 20mm,Pro.22 mm, Staph. 22mm, Salm. 23mm, Psedo. 21mm).

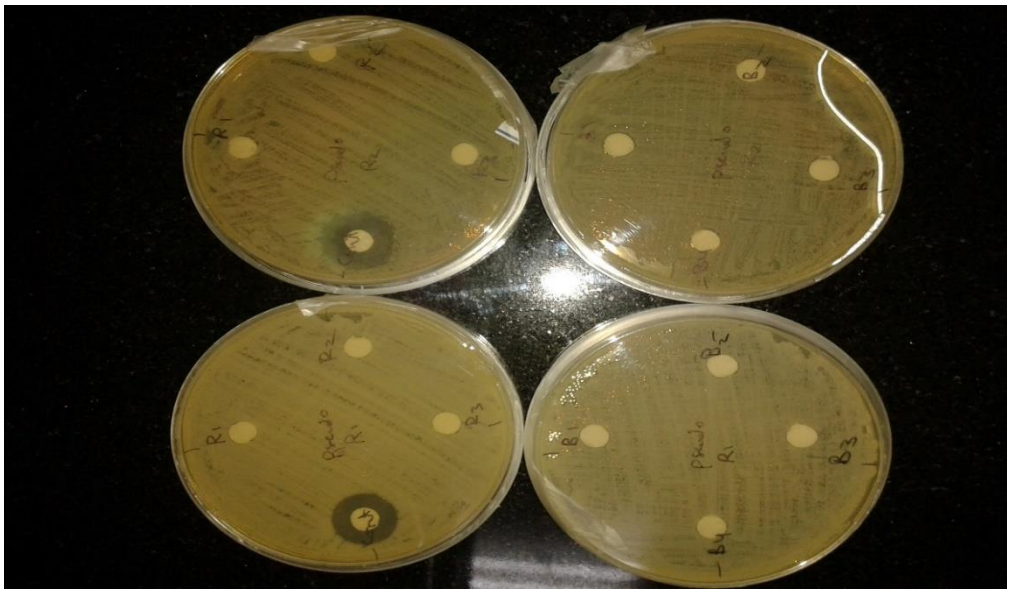


Figure4.3: Disk Diffusion Test Against Bacteria Strains

4.7.4 Antifungal Activity

Table4.3 and Figure4.4 showed the activity of the compounds at different concentrations against *M. canis* . Compounds R1c2, R2c2and R3c2 revealed 100 % inhibition at the concentration 200 µg/ml. Then compounds R4c2 and R8c2 showed nearly 84 % inhibition at the same concentration.

Table 4.3: Antifungal Activity of Compounds Against *M. canis*

Compd.	Conc .ug/ml								Compd	% Inhib.
R1c1	50	29	30	28	29	29	0.27	26.6	R1c1	26.6
R1c2	200	0	0	0	0	0	1	100	R1c2	100
R2c1	50	32	31	30	30	30.8	0.22	22.2	R2c1	22.2
R2c2	200	0	0	0	0	0	1	100	R2c2	100
R3c1	50	27	26	28	29	27.5	0.3	30.3	R3c1	30.3
R3c2	200	0	0	0	0	0	1	100	R3c2	100
R4c1	120	36	34	37	36	35.8	0.09	9.5	R4c1	9.5
R4c2	240	6	7	6	6	6.25	0.84	84.2	R4c2	84.2
R5c1	120	40	38	36	37	37.8	0.04	4.4	R5c1	4.4
R5c2	240	8	7	14	15	11	0.72	72.2	R5c2	72.2
R6c1	120	25	26	28	30	27.3	0.31	31	R6c1	31
R6c2	240	21	18	20	17	19	0.52	51.9	R6c2	51.9
R7c1	120	23	28	22	29	25.5	0.35	35.4	R7c1	35.4
R7c2	240	15	17	9	8	12.3	0.69	69	R7c2	69
R8c1	120	33	34	31	30	32	0.19	19	R8c1	19
R8c2	240	6	6	7	7	6.5	0.84	83.5	R8c2	83.5
R9c1	120	35	26	33	36	32.5	0.18	17.7	R9c1	17.7
R9c2	240	23	22	24	18	21.8	0.45	44.9	R9c2	44.9

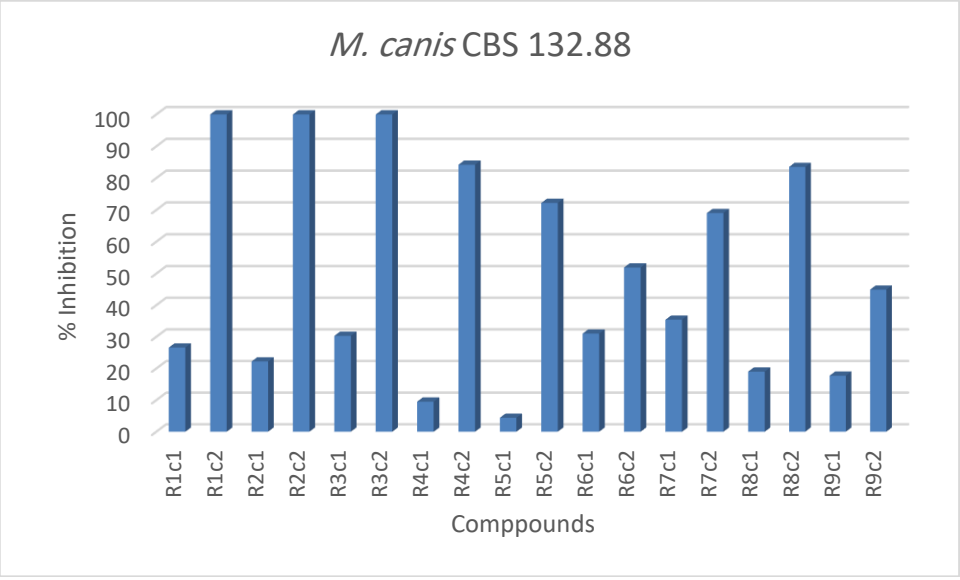


Figure4.4: Antifungal Activity of the Compounds Against *M. canis*

Table4.4 Figure4.5 showed the activity of the compounds against T. mentagrophytes. R3c2, R8c2 revealed 100% inhibition at 200µg/ml, while R1c2 and R2c2 revealed more than 95% inhibition at the same concentration.

Table4.4: Antifungal Activity of Compounds Against *T. mentagrophytes*

Control		33	34	37	34	34.5				
	Conc. ug/ml									
R1c1	50	24	25	24	26	24.8	0.37	37.3	R1c1	37.3
R1c2	200	1	1	2	1	1.25	0.97	96.8	R1c2	96.8
R2c1	50	26	25	26	25	25.5	0.35	35.4	R2c1	35.4
R2c2	200	1	1	0	0	0.5	0.99	98.7	R2c2	98.7
R3c1	50	26	27	24	24	25.3	0.36	36.1	R3c1	36.1
R3c2	200	0	0	0	0	0	1	100	R3c2	100
R4c1	120	28	27	29	26	27.5	0.3	30.1	R4c1	30.1
R4c2	240	9	10	8	11	9.5	0.76	76	R4c2	76
R5c1	120	24	25	23	24	24	0.39	39.2	R5c1	39.2
R5c2	240	6	7	6	7	6.5	0.84	83.5	R5c2	83.5
R6c1	120	22	23	21	23	22.3	0.44	43.7	R6c1	43.7
R6c2	240	12	13	11	13	12.3	0.69	69	R6c2	69
R7c1	120	23	21	20	24	22	0.44	44.3	R7c1	44.3
R7c2	240	10	11	13	14	12	0.7	69.6	R7c2	69.6
R8c1	120	22	21	23	20	21.5	0.46	45.6	R8c1	45.6
R8c2	240	0	0	0	0	0	1	100	R8c2	100
R9c1	120	20	21	23	20	21	0.47	46.8	R9c1	46.8
R9c2	240	18	19	17	18	18	0.54	54.4	R9c2	54.4

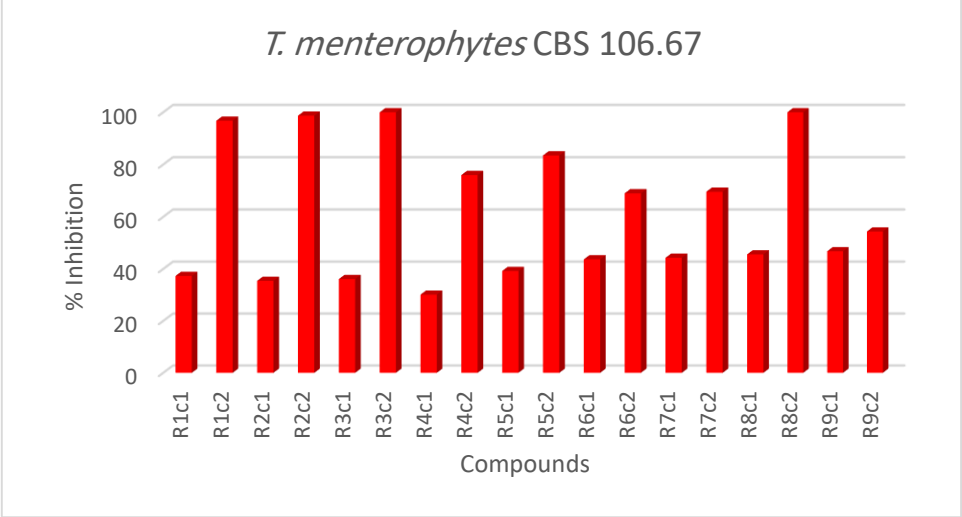


Figure4.5: Antifungal Activity of the Compounds Against T. mentagrophytes

Table 4.5: Antifungal of the Compounds Against T.rubrum

Control	Conc.	28	26	27	26	26.8				
	µg/ml									
R1c1	50	21	20	22	20	20.8	0.47	47.5	R1c2	47.5
R1c2	200	1	1	0	0	0.5	0.99	98.7	R1c1	98.7
R2c1	50	20	21	20	19	20	0.49	49.3	R2c2	49.3
R2c2	200	0	0	0	0	0	1	100	R2c1	100
R3c1	50	19	20	22	18	19.8	0.5	50	R3c2	50
R3c2	200	0	0	0	0	0	1	100	R3c1	100
R4c1	120	25	24	24	23	24	0.39	39.2	R4c1	39.2
R4c2	240	12	11	10	9	10.5	0.73	73.4	R4c2	73.4
R5c1	120	25	24	25	23	24.3	0.39	38.6	R5c1	38.6
R5c2	240	7	6	7	6	6.5	0.84	83.5	R5c2	83.5
R6c1	120	21	22	21	23	21.8	0.45	44.9	R6c1	44.9
R6c2	240	7	8	6	6	6.75	0.83	82.9	R6c2	82.9
R7c1	120	21	19	18	17	18.8	0.53	52.5	R7c1	52.5
R7c2	240	0	0	0	0	0	1	100	R7c2	100
R8c1	120	13	11	11	14	12.3	0.69	69	R8c1	69
R8c2	240	6	7	6	8	6.75	0.83	82.9	R8c2	82.9
R9c1	120	22	23	24	35	26	0.34	34.2	R9c1	34.2
R9c2	240	10	11	13	10	11	0.72	72.2	R9c2	72.2

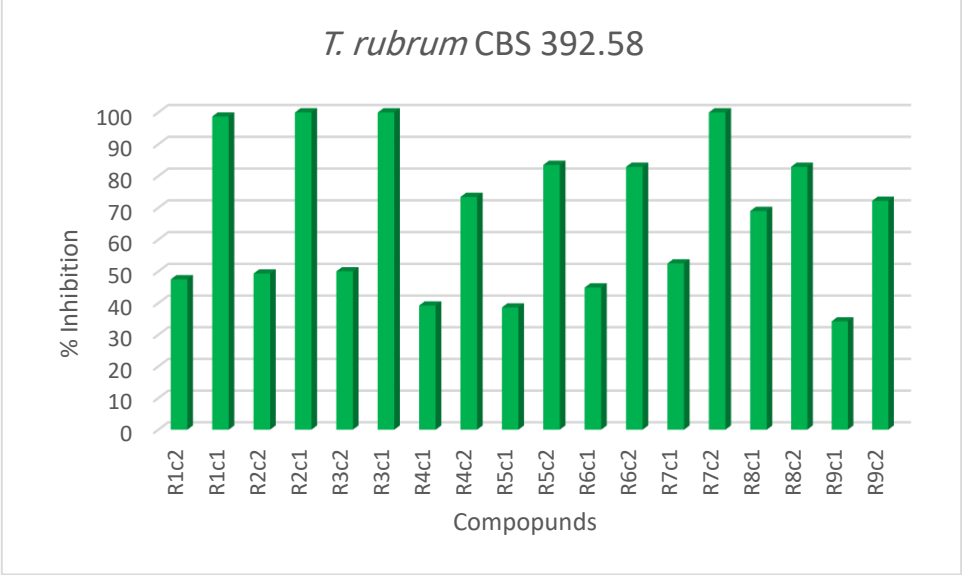


Figure 4.6: Antifungal Activity of the Compounds Against T. rubrum.

Table 4.5 and Figure 4.6 showed the activity of the compounds against T. rubrum. The selected fungi.R1c2, R2c2 and R3c2 revealed 100% inhibition at the concentration 200µg/ml. Generally speaking, all tested fungi are sensitive to compounds 1, 2 and 3 with 100% inhibition at (200-250µg/ml). Those compounds are promising to be new matrices in manufacturing new drugs.

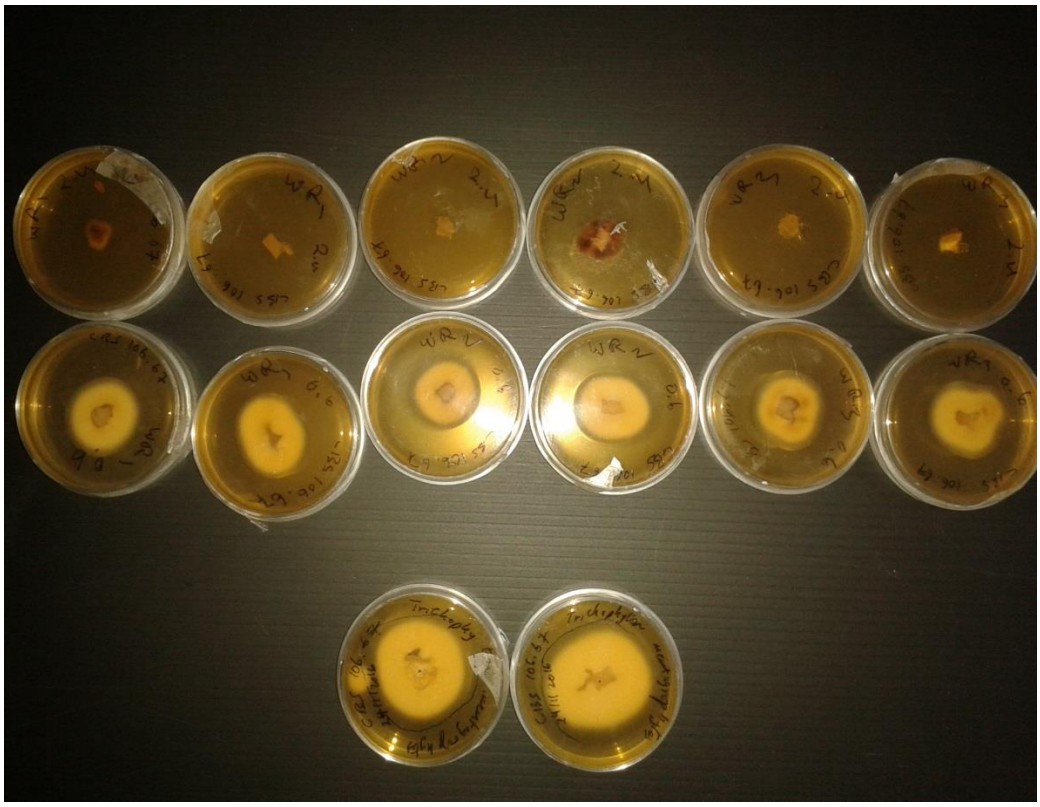


Figure 4.7: Disk Diffusion Test Against M. canis

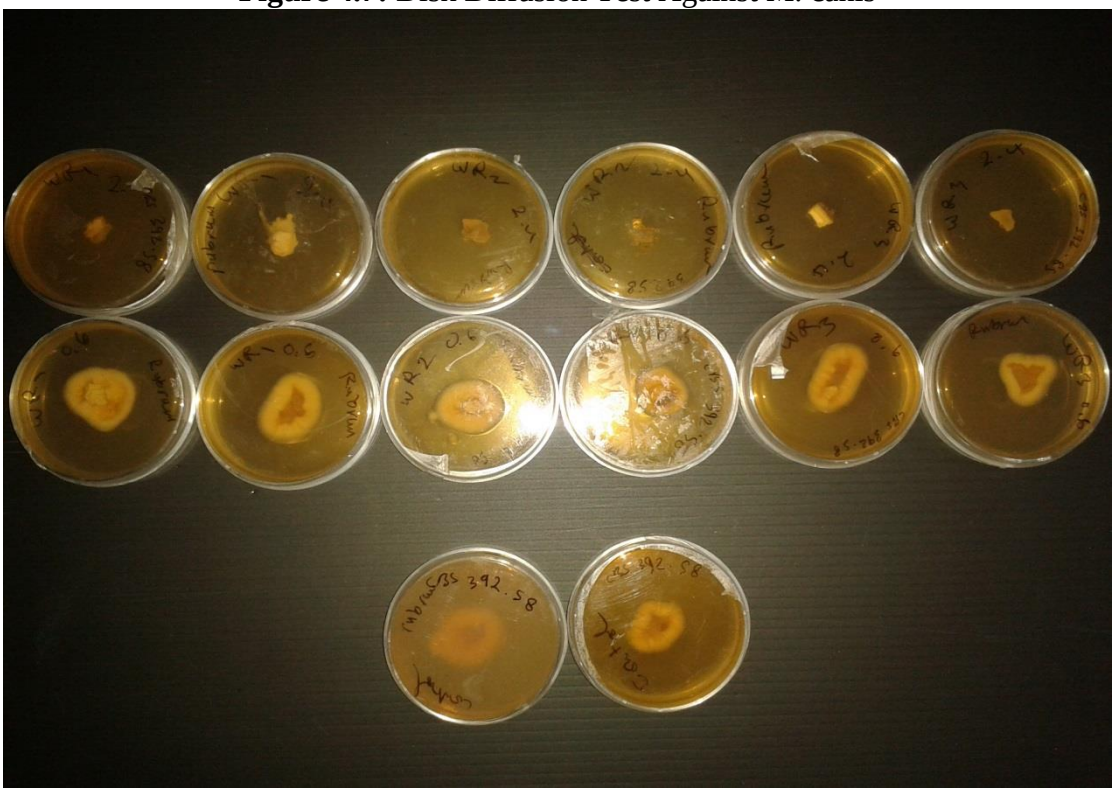


Figure4.8: Disk Diffusion Test Against T. mentagrophytes

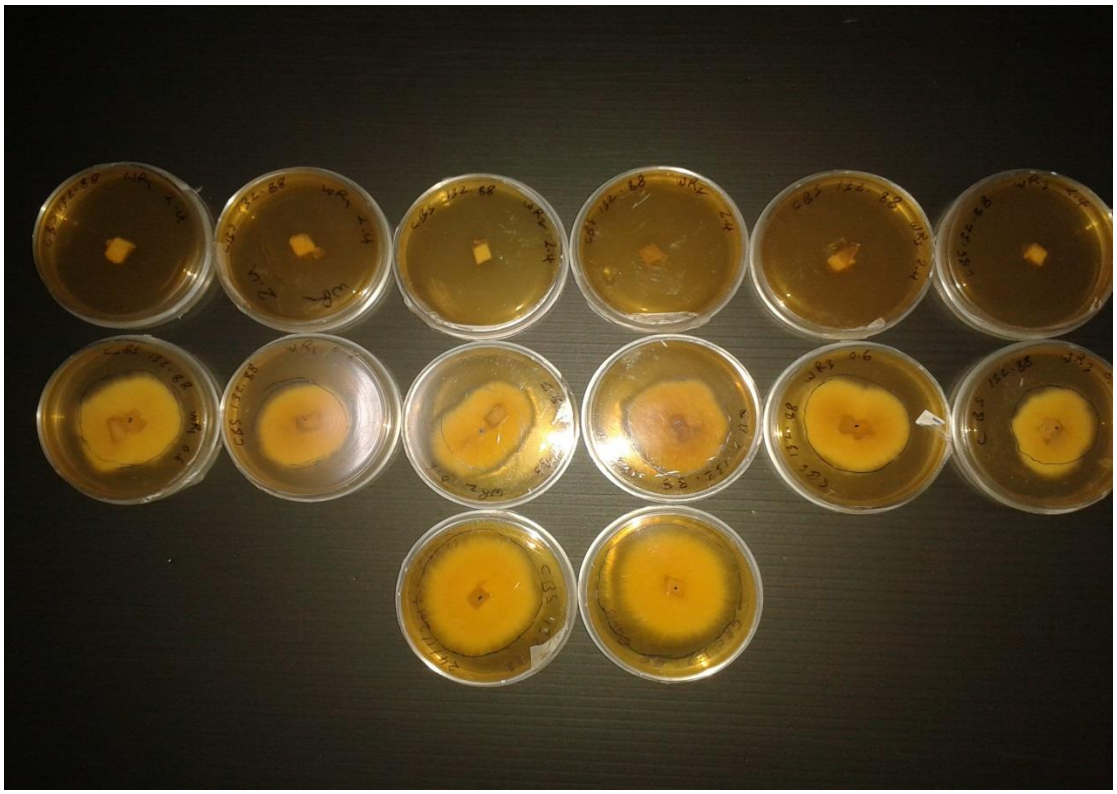


Figure4.9: Disk diffusion test against T. rubrum

4.8 Suggestion for Further work:

To prepare other analogues of distamycin by changing the head and tail of the compound which has been prepared, and study their biological activities.

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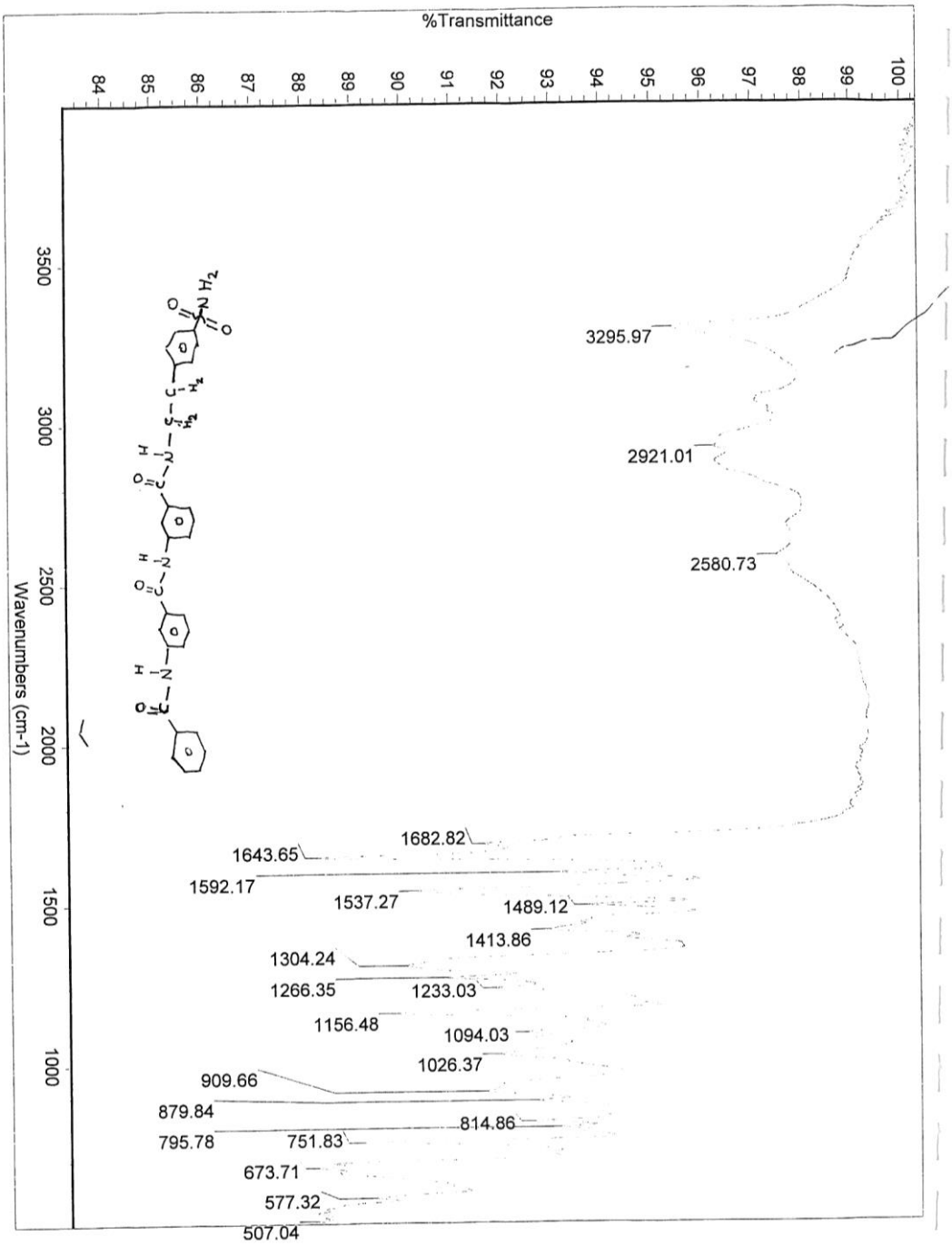
Appendix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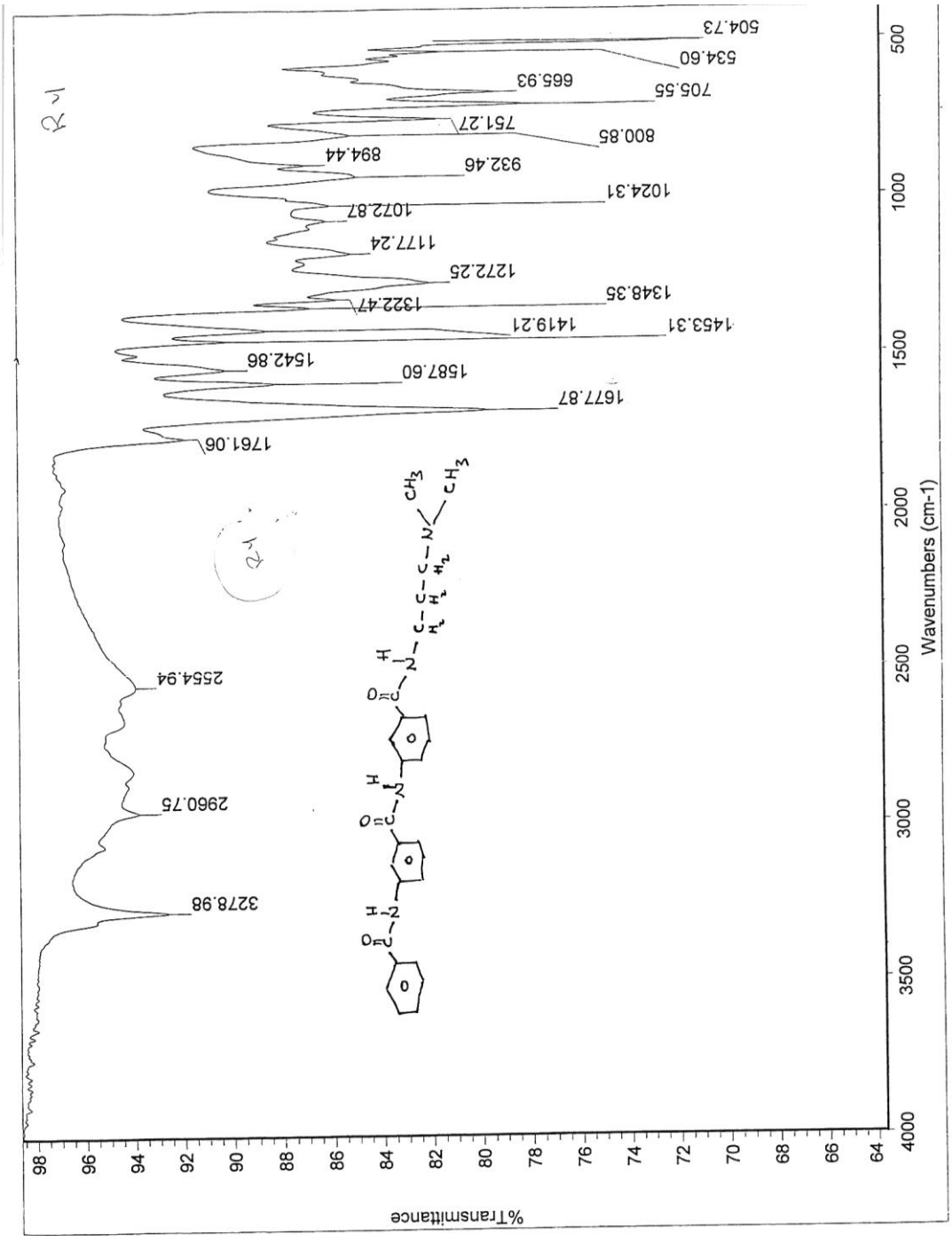
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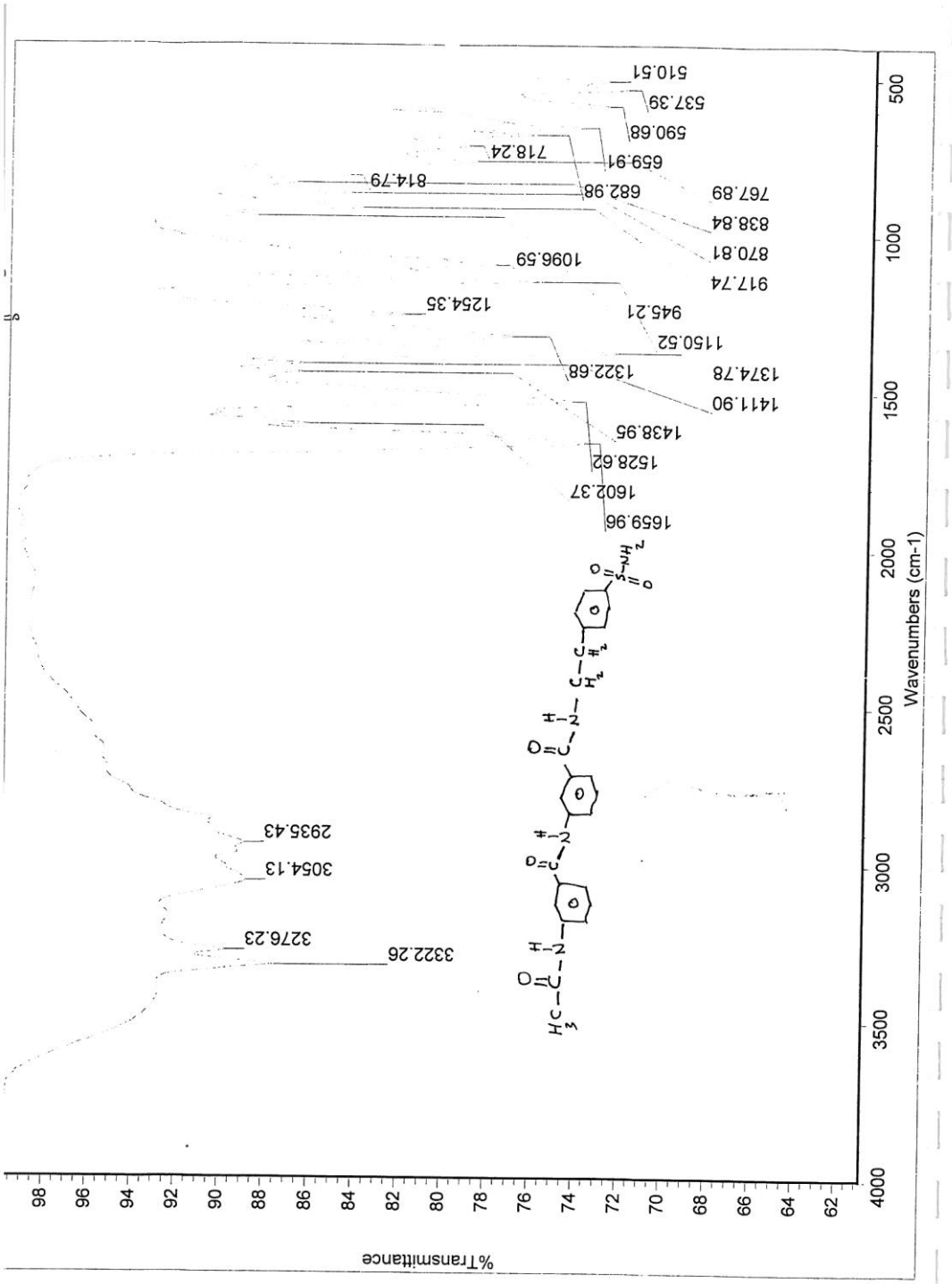
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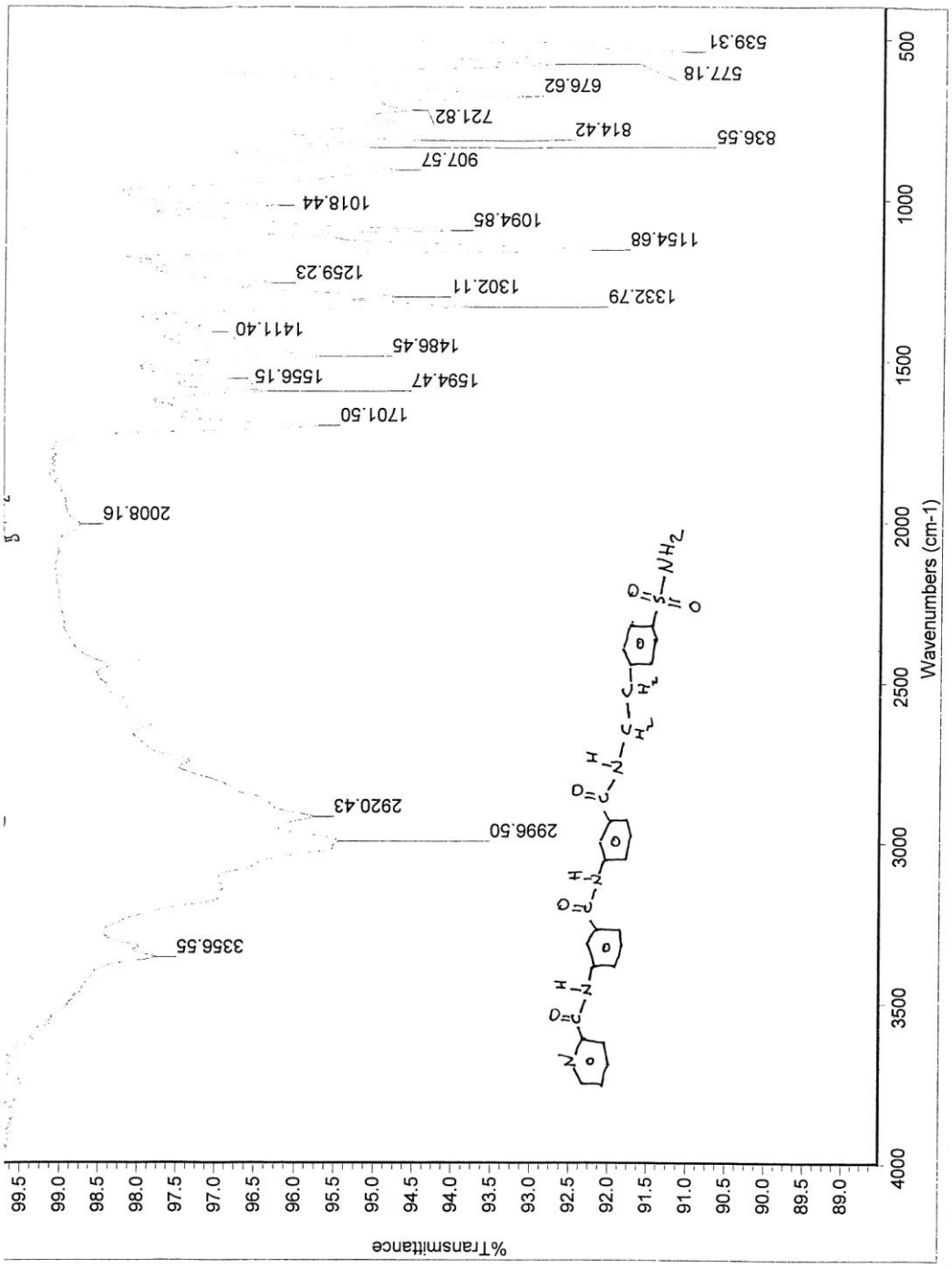
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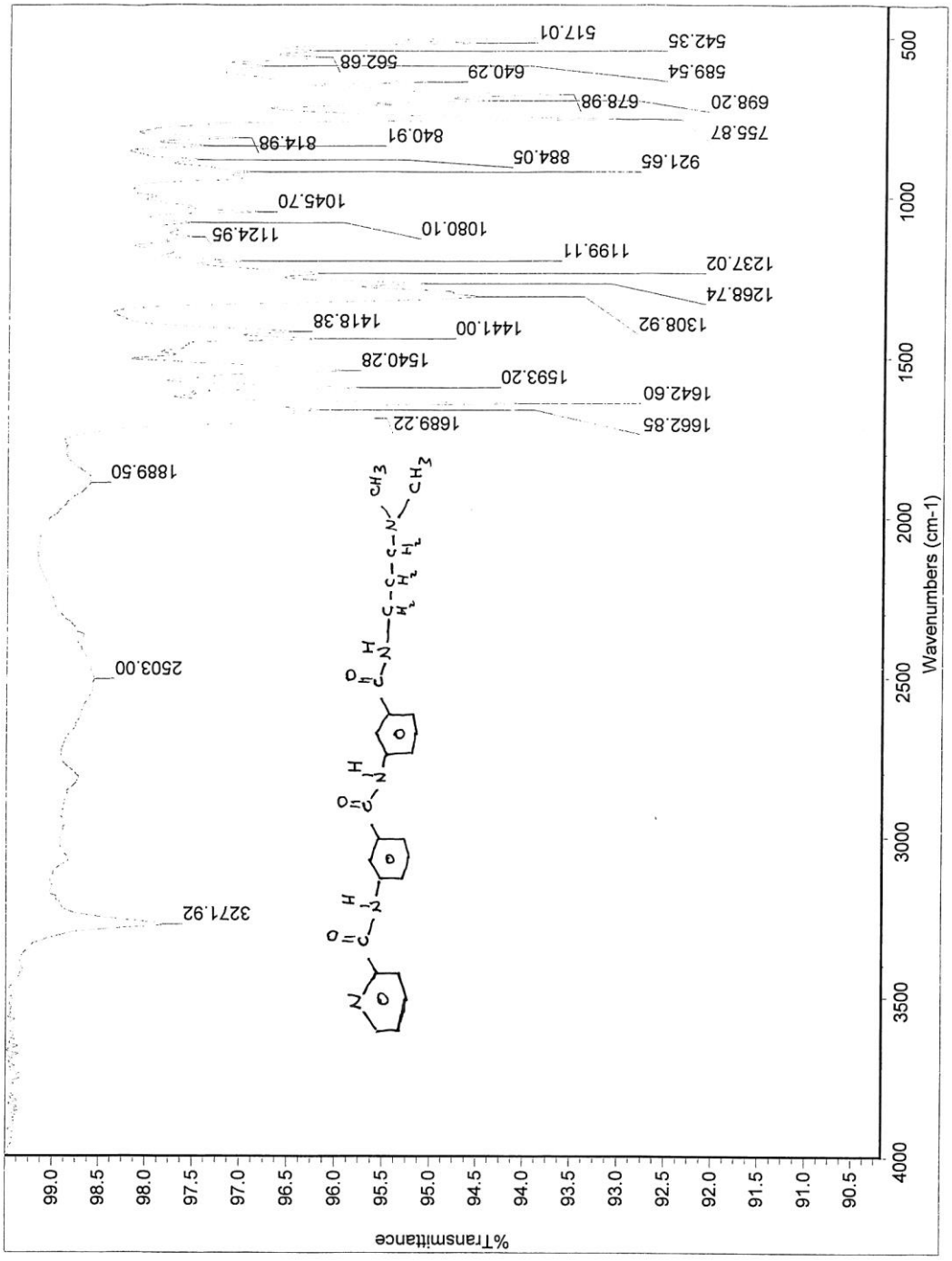
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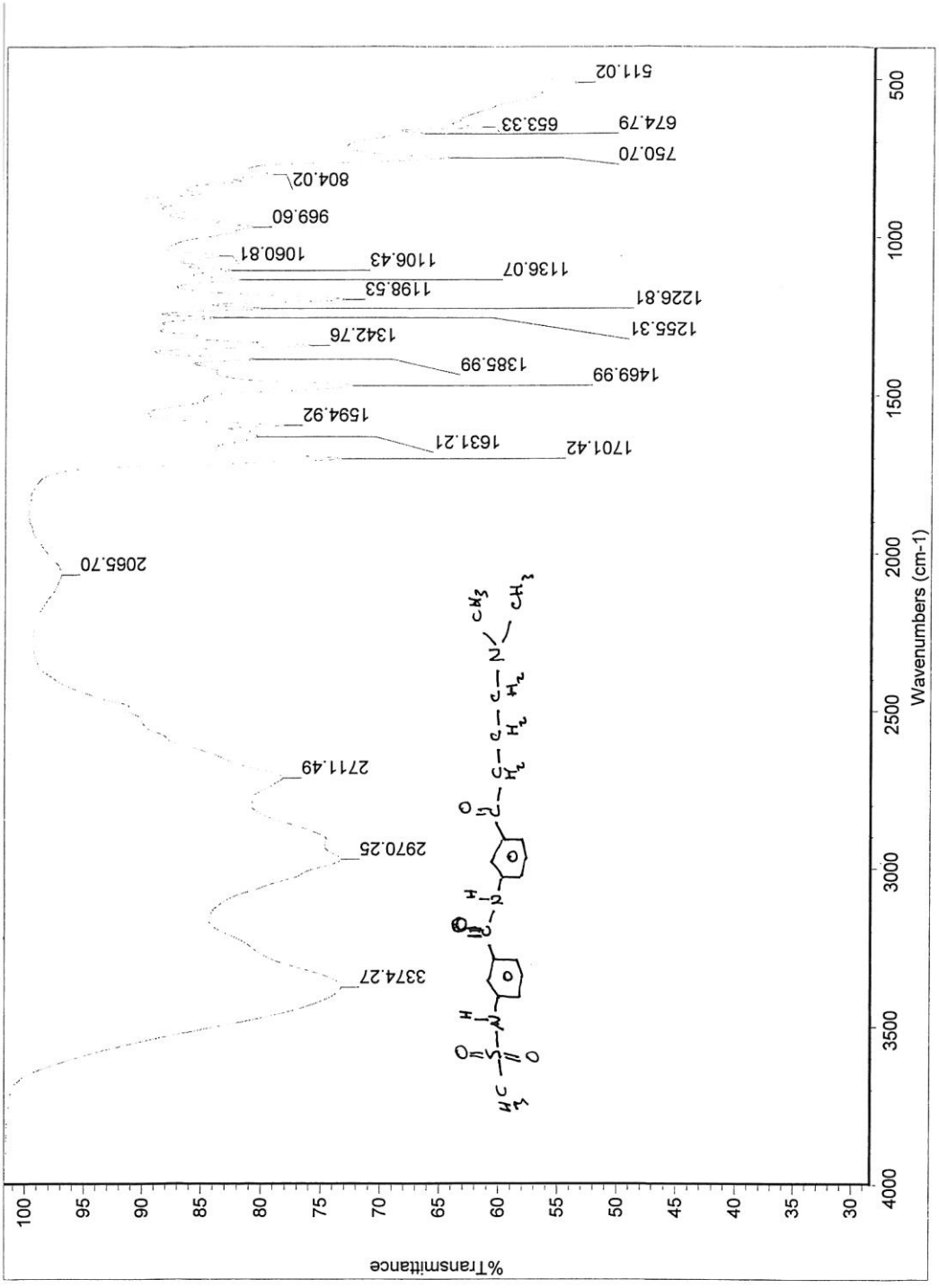
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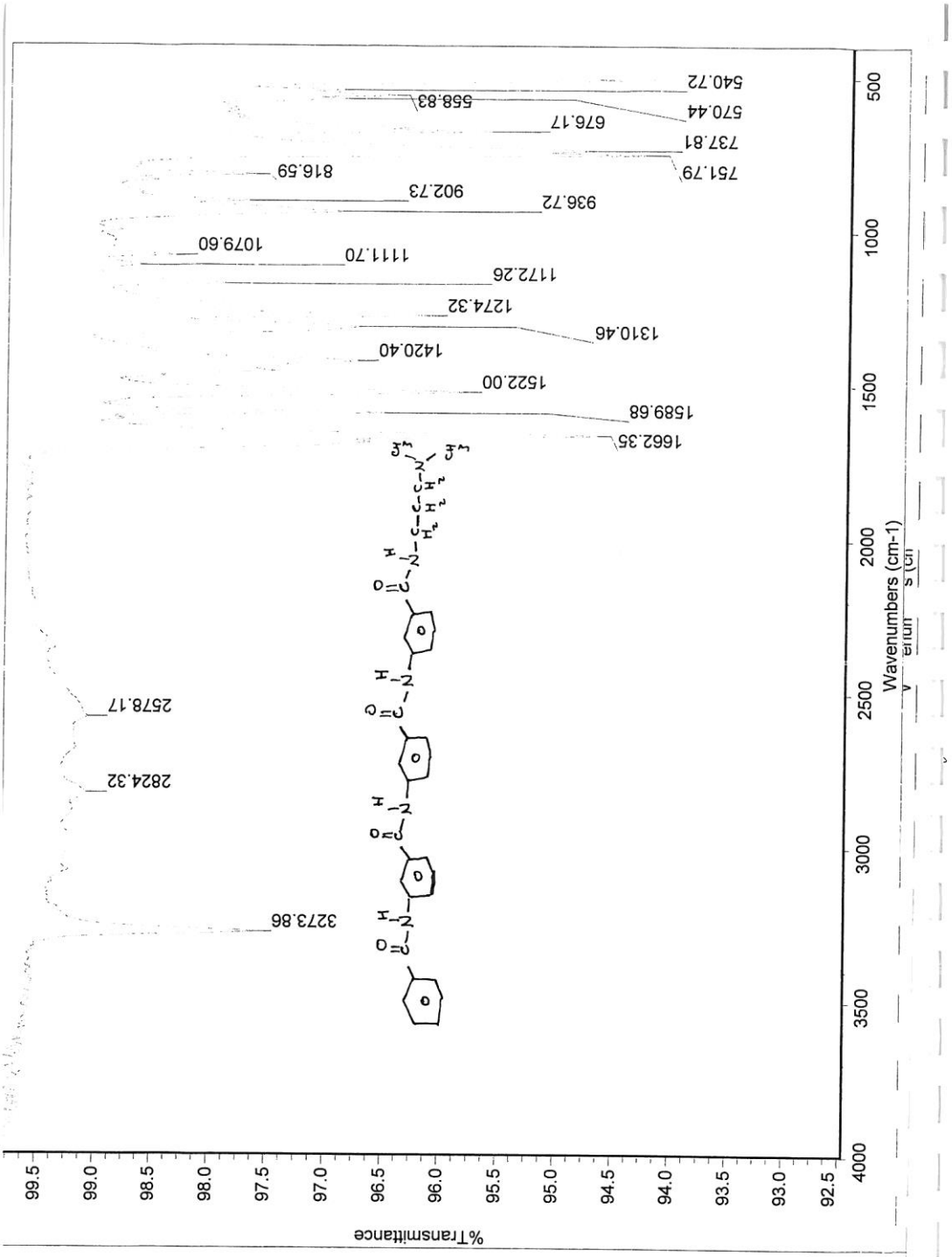
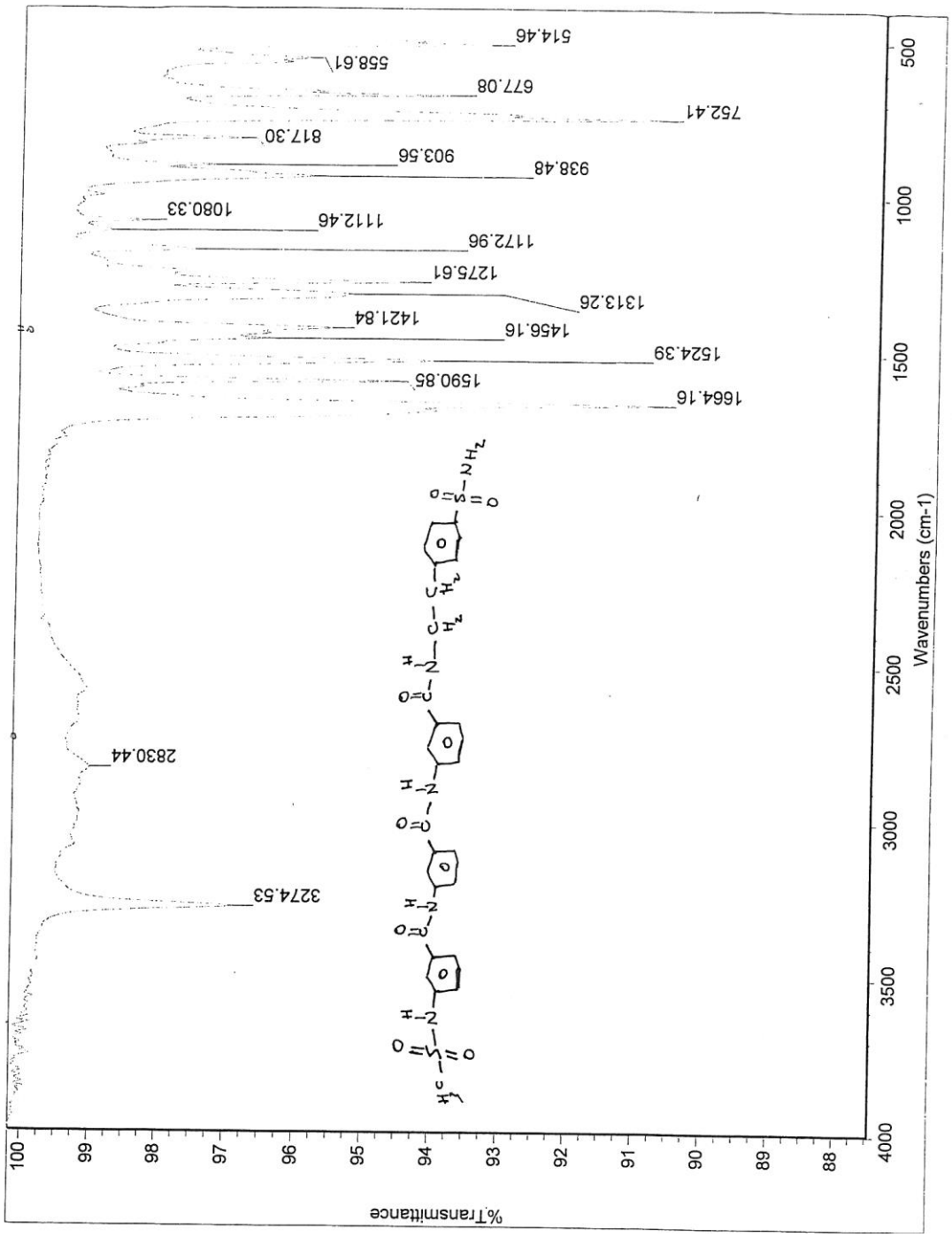
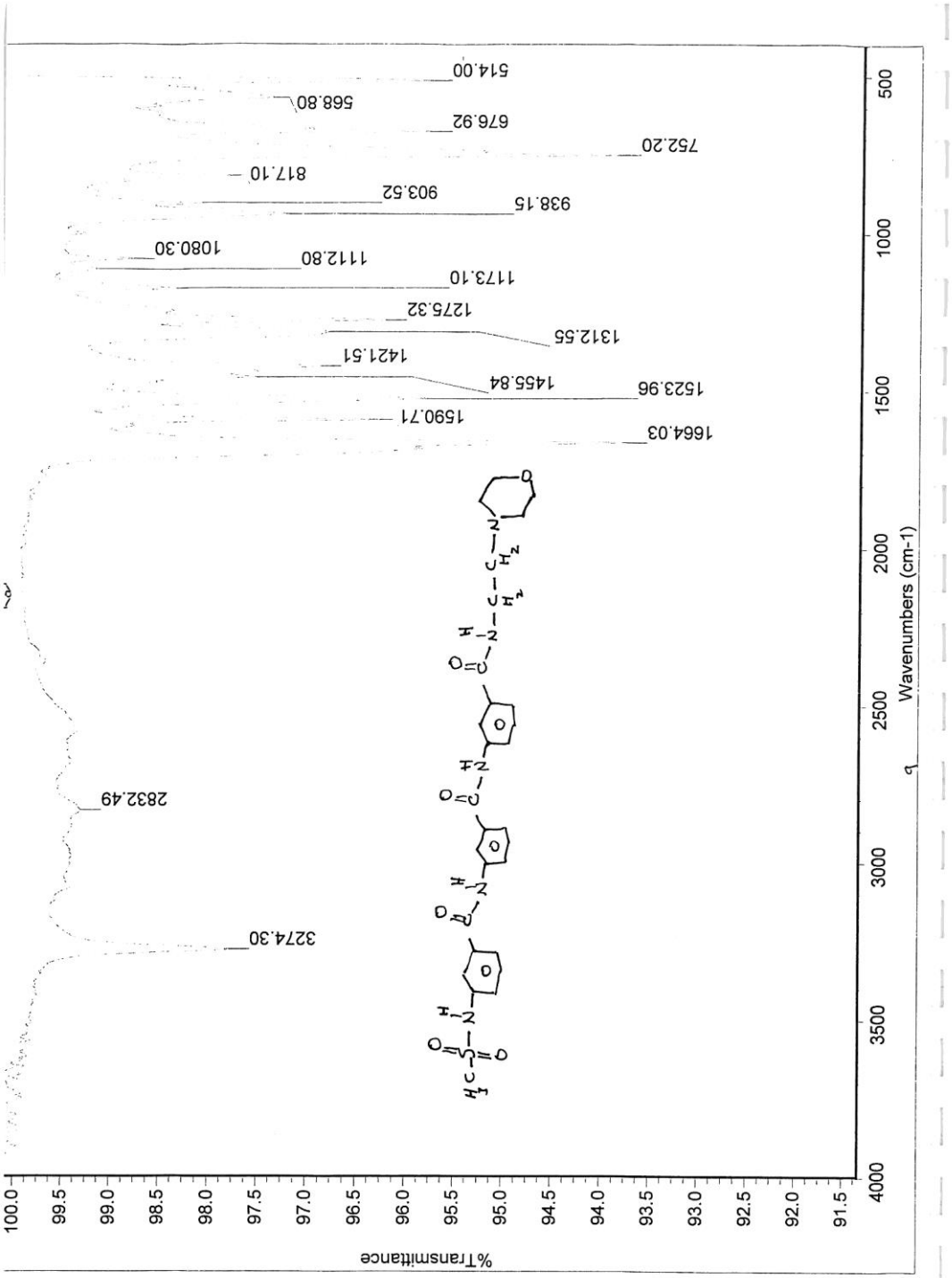
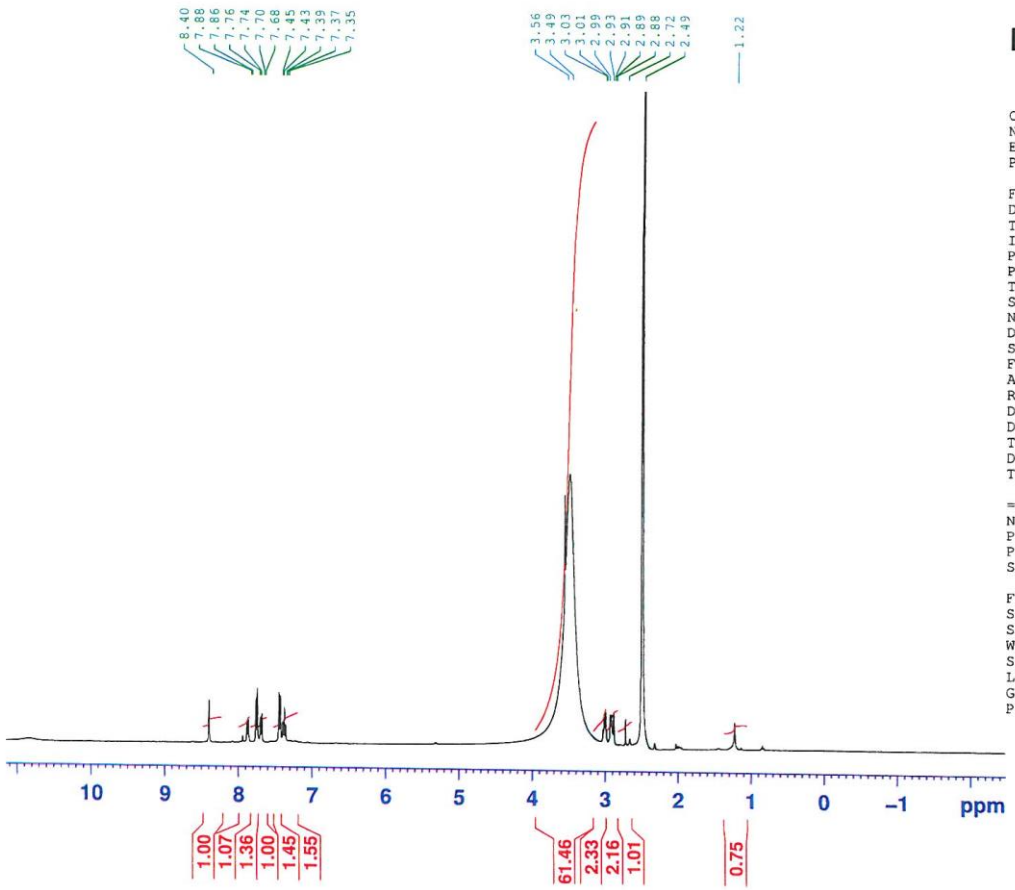
30
4000
3500
3000
2500
2000
1500
1000
500
3374.27
2970.25
2711.49
2065.70
1701.42
1631.21
1594.92
1469.99
1385.99
1342.76
1255.31
1226.81
1198.53
1136.07
1106.43
1060.81
969.60
804.02
750.70
674.79
653.33
511.02


%Transmittance
Wavenumbers (cm-1)
3273.86
2824.32
2578.17
1662.35
1589.68
1522.00
1420.40
1310.46
1274.32
1172.26
1111.70
1079.60
936.72
902.73
816.59
751.79
737.81
676.17
570.44
558.83
540.72


%Transmittance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
500
100
99
98
97
96
95
94
93
92
91
90
89
88
3274.53
2830.44
1664.16
1590.85
1524.39
1456.16
1421.84
1313.26
1275.61
1172.96
1112.46
1080.33
938.48
903.56
817.30
752.41
677.08
558.61
514.46


%Transmittance
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
500
100.0
99.5
99.0
98.5
98.0
97.5
97.0
96.5
96.0
95.5
95.0
94.5
94.0
93.5
93.0
92.5
92.0
91.5
3274.30
2832.49
1664.03
1590.71
1523.96
1455.84
1421.51
1312.55
1275.32
1173.10
1112.80
1080.30
938.15
903.52
817.10
752.20
676.92
568.80
514.00

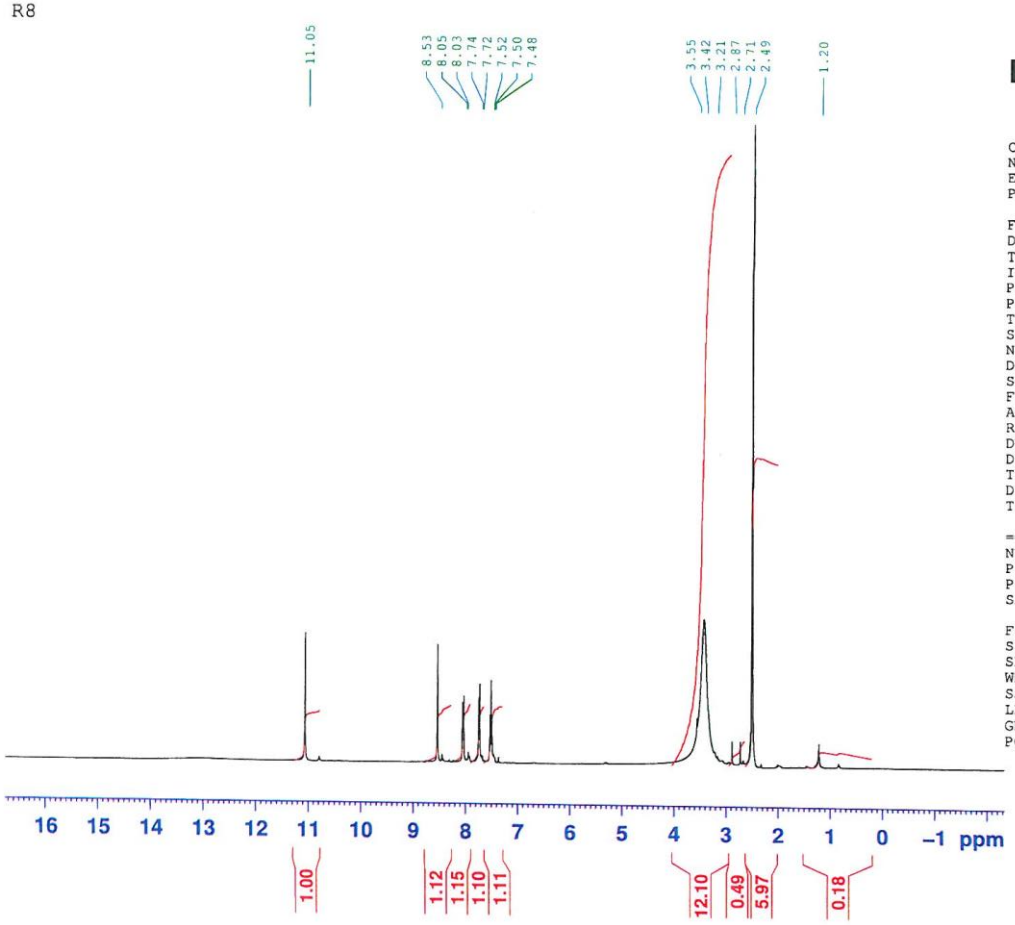
R 2



BRUKER

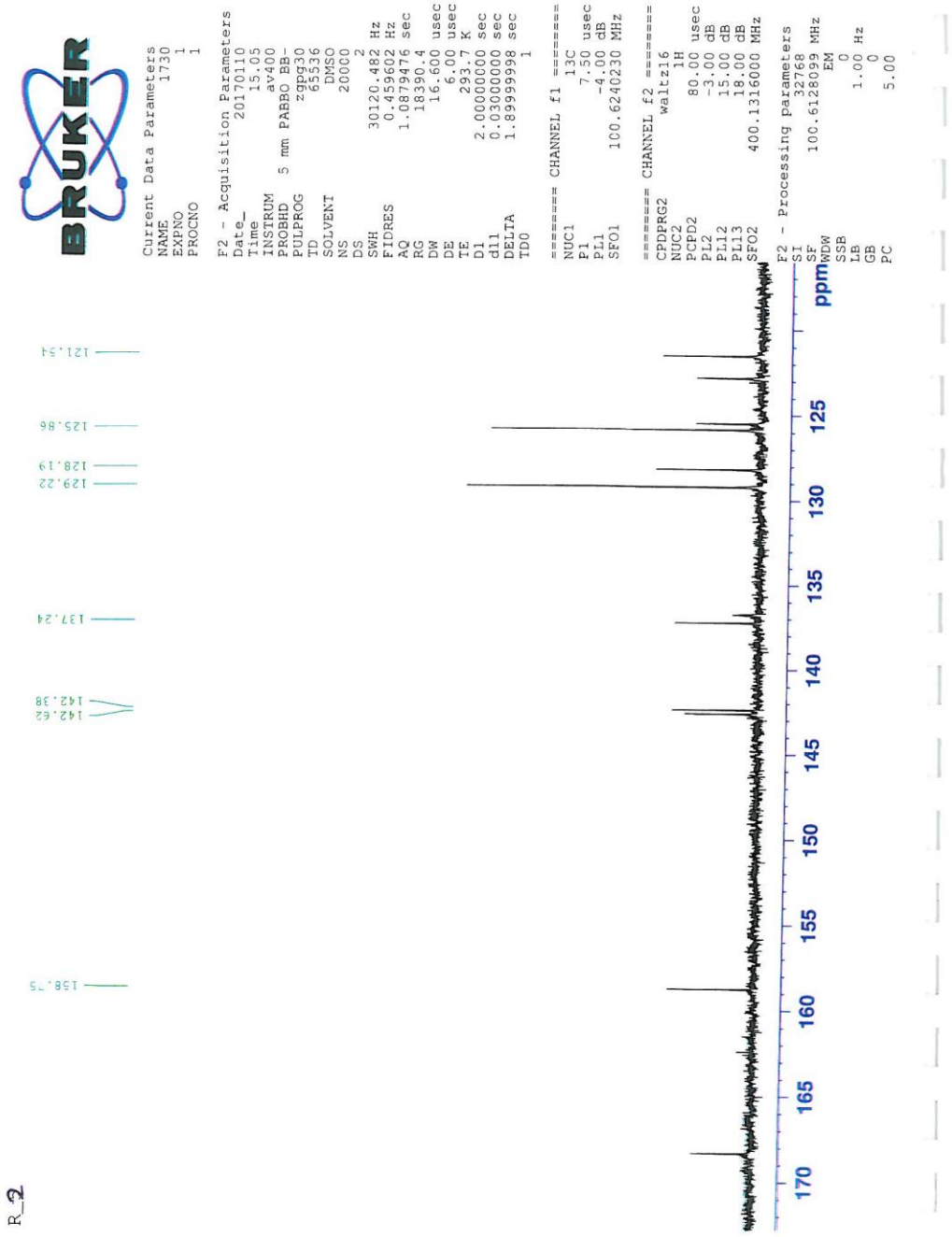
```
Current Data Parameters
NAME                  Master
EXPNO                    944
PROCNO                     1

F2 - Acquisition Parameters
Date_              20170110
Time                  13.55
INSTRUM                av400
PROBHD    5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                        14
DS                         2
SWH                9803.922 Hz
FIDRES             0.149596 Hz
AQ                 3.3423860 sec
RG                       128
DW                  51.000 usec
DE                   6.00 usec
TE                  293.6 K
D1                 1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                      1H
P1                   10.00 usec
PL1                  -3.00 dB
SFO1           400.1340013 MHz

F2 - Processing parameters
SI                     32768
SF             400.1319009 MHz
WDW                       EM
SSB                        0
LB                    0.30 Hz
GB                         0
PC                    2.00
```

R8

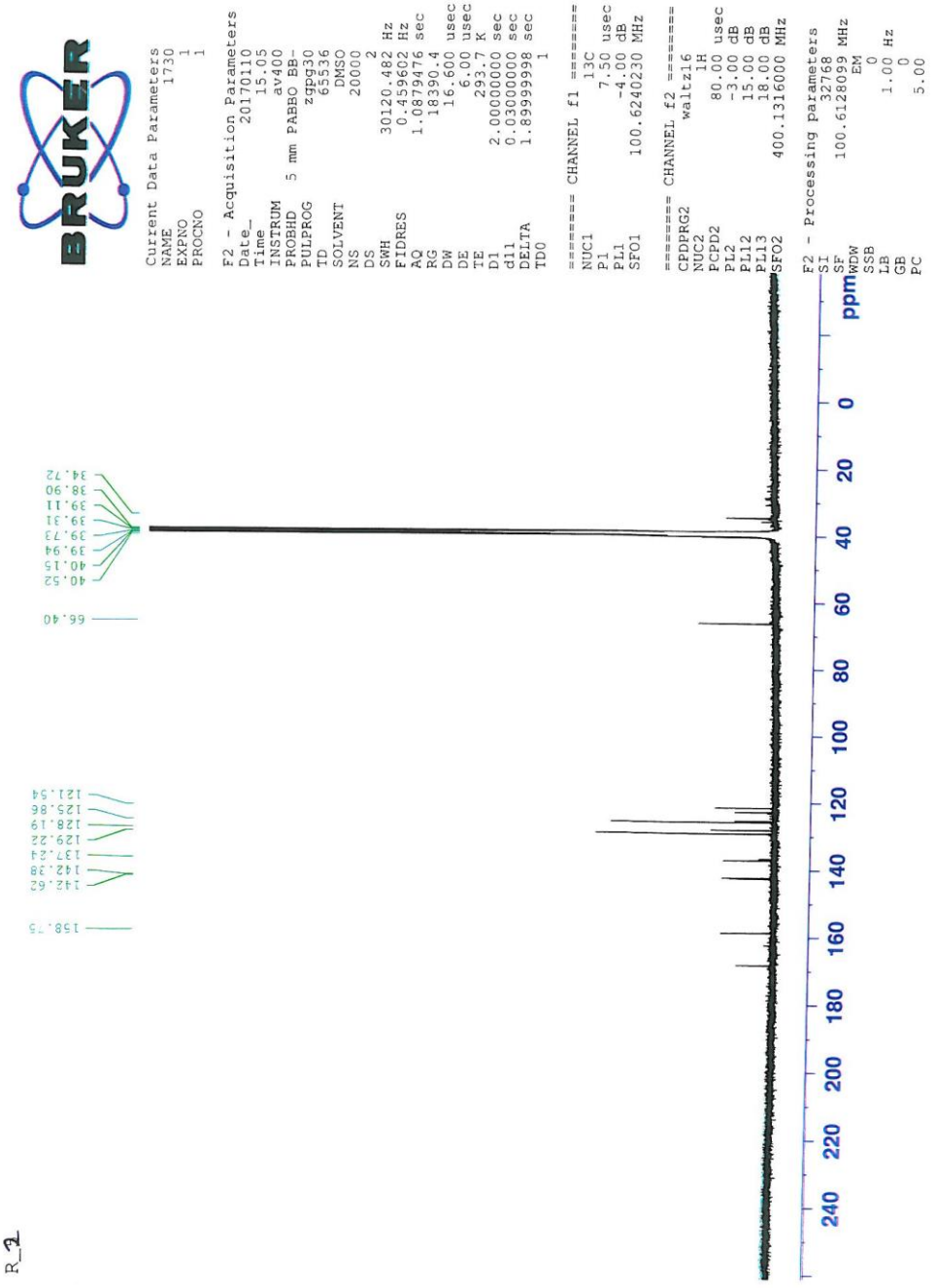


BRUKER

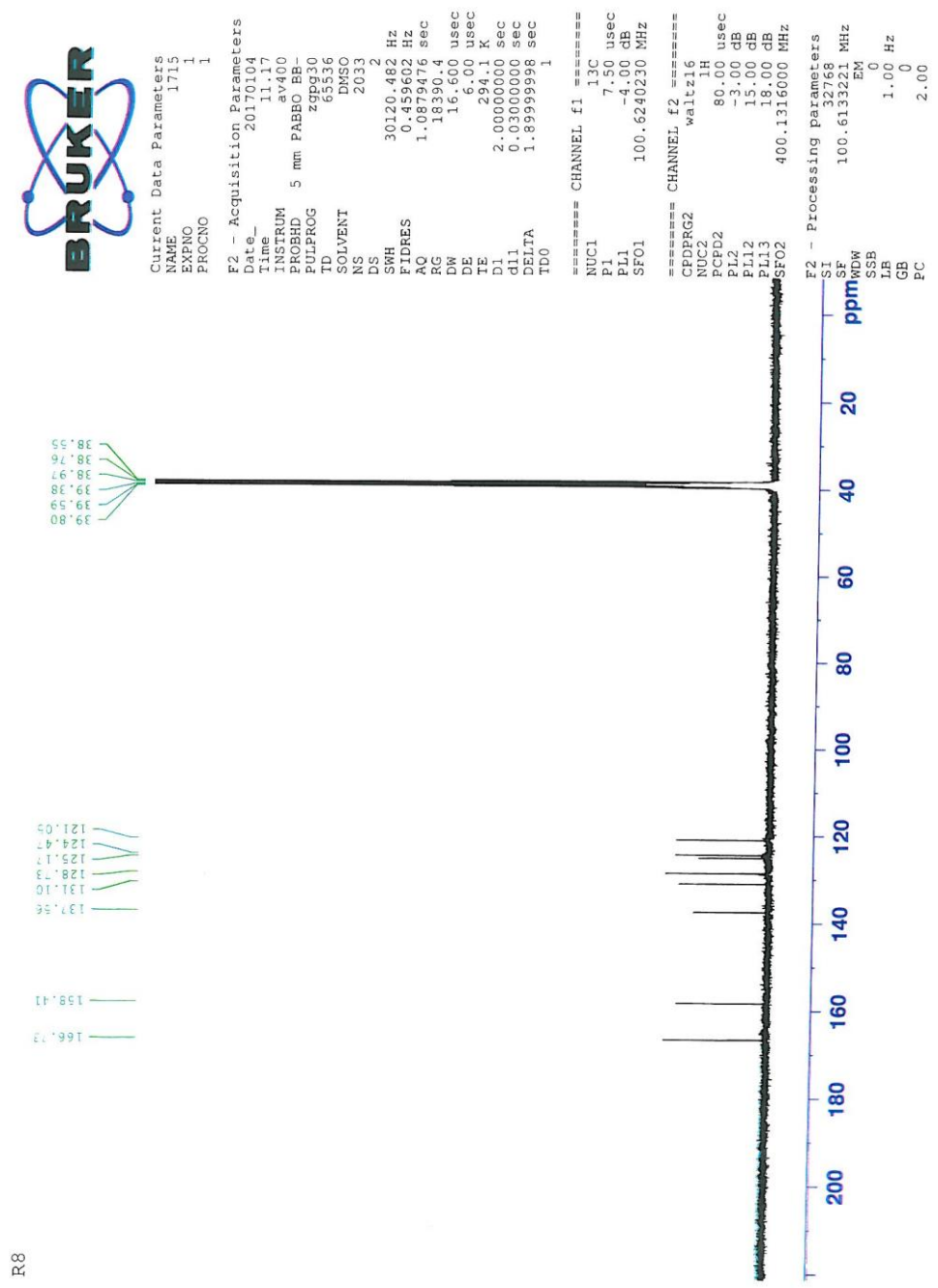
```
Current Data Parameters
NAME                  Master
EXPNO                    934
PROCNO                     1

F2 - Acquisition Parameters
Date_              20170104
Time                   9.29
INSTRUM               av400
PROBHD    5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       10
DS                        2
SWH                9803.922 Hz
FIDRES            0.149596 Hz
AQ                3.3423860 sec
RG                    143.7
DW                   51.000 usec
DE                    6.00 usec
TE                   292.3 K
D1                1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                      1H
P1                    10.00 usec
PL1                   -3.00 dB
SFO1          400.1340013 MHz

F2 - Processing parameters
SI                        32768
SF            400.1319009 MHz
WDW                        EM
SSB                         0
LB                     0.30 Hz
GB                          0
PC                       2.00
```


BRUKER
Current Data Parameters
NAME 1730
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170110
Time 15.05
INSTRUM av400
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 20000
DS 2
SWH 30120.482 Hz
FIDRES 0.459602 Hz
AQ 1.08794760 sec
RG 18390.4
DW 16.600 usec
DE 6.00 usec
TE 293.7 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 7.50 usec
PL1 -4.00 dB
SFO1 100.6240230 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 15.00 dB
PL13 18.00 dB
SFO2 400.1316000 MHz
F2 - Processing parameters
SI 32768
SF 100.6128099 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 5.00
158.75
142.62
142.38
137.24
129.22
128.19
125.86
121.54
ppm
170
165
160
155
150
145
140
135
130
125

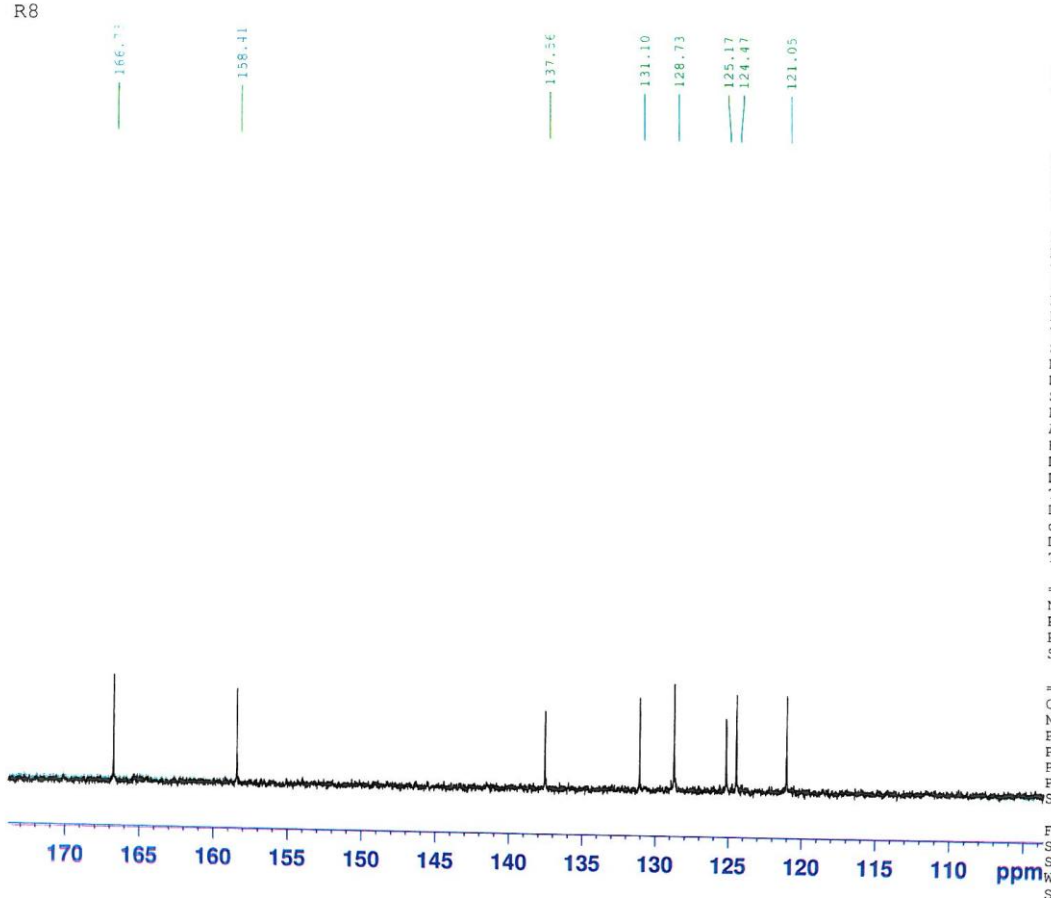
R_2


BRUKER
Current Data Parameters
NAME 1730
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170110
Time 15.05
INSTRUM av400
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 20000
DS 2
SWH 30120.482 Hz
FIDRES 0.459602 Hz
AQ 1.0879476 sec
RG 18390.4
DW 16.600 usec
DE 6.00 usec
TE 293.7 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 7.50 usec
PL1 -4.00 dB
SFO1 100.6240230 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 15.00 dB
PL13 18.00 dB
SFO2 400.1316000 MHz
F2 - Processing parameters
SI 32768
SF 100.6128099 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 5.00
34.72
38.90
39.11
39.31
39.73
39.94
40.15
40.52
66.40
121.54
125.86
128.19
129.22
137.24
142.38
142.62
158.75
ppm
240
220
200
180
160
140
120
100
80
60
40
20
0

R_2


BRUKER
Current Data Parameters
NAME 1715
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20170104
Time 11.17
INSTRUM av400
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 2033
DS 2
SWH 30120.482 Hz
FIDRES 0.4594602 Hz
AQ 1.0879476 sec
RG 18390.4
DW 16.600 usec
DE 6.00 usec
TE 294.1 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 7.50 usec
PL1 -4.00 dB
SFO1 100.6240230 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 15.00 dB
PL13 18.00 dB
SFO2 400.1316000 MHz
F2 - Processing parameters
SI 32768
SF 100.6133221 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 2.00
ppm
200
180
160
140
120
100
80
60
40
20
166.73
158.41
137.56
131.10
128.73
125.17
124.47
121.05
39.80
39.59
39.38
38.97
38.76
38.55
R8

R8



Current Data Parameters

NAME	1715
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20170104
Time	11.17
INSTRUM	av400
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	2033
DS	2
SWH	30120.482 Hz
FIDRES	0.459602 Hz
AQ	1.0879476 sec
RG	18390.4
DW	16.600 usec
DE	6.00 usec
TE	294.1 K
D1	2.00000000 sec
d11	0.03000000 sec
DELTA	1.89999998 sec
TD0	1

======== CHANNEL f1 ========

NUC1	13C
P1	7.50 usec
PL1	-4.00 dB
SFO1	100.6240230 MHz

======== CHANNEL f2 ========

CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-3.00 dB
PL12	15.00 dB
PL13	18.00 dB
SFO2	400.1316000 MHz

F2 - Processing parameters

SI	32768
SF	100.6133221 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	2.00

جامعة النجاح الوطنية
كلية الدراسات العليا

انتاج وتحديد هوية مشتقات الدستمايسن التي تحتوي على حمض الأمينوبزويك والتي لها نشاط بيولوجي محتمل

إعداد
رماح سعيد محمد عبد الرازق

إشراف
د. وحيد الجندي
د. حسن النيص

قدمت هذه الاطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.
2017

إنتاج وتحديد هوية مشتقات الدستمايسن التي تحتوي على حمض الأمينوبنزويك والتي لها نشاط بيولوجي محتمل

إعداد

رماح سعيد محمد عبد الرازق

إشراف

د. وحيد الجندي

د. حسن النيص

الملخص

لقد تم استحضار مركبات شبيهة الدستمايسن. من خلال إنتاج لهذه المركبات، بعض المركبات الوسيطة تم استحضارها: 3-benzamido-N-(3-((4- sulfamonylphenethyl) carbamoyl)phenyl)benzamide (R_1), المركبات الوسيطة لهذا المشتق هي:-3 Benzoylamino-benzoic acide (1), 3-benzoylamino-benzoyl chloride(2), 3-(3-Benzoylamino-benzoylamino)-benzoic acid(3), 3-(3-Benzoylamino-benzoylamino)-benzoyl chloride(4). المركبات الوسيطة (R_2) هي: 3-acetamido-*N*-(3-((4-sulfamoylphenethyl) carbamoyl)phenyl)benzamide (R_2) : 3-Acetylamino-benzoic acid(5),3-ل Acetylamino-benzoyl chloride(6), 3-(3-Acetylamino-benzoylamino)-benzoic acid(7), 3-(3-Acetylamino-benzoylamino)-benzoyl chloride(8). المركبات الوسيطة ل(R3)هي:*N*-(3-((3-((4-sulfamoylphenethyl) carbamoyl)phenyl) carbamoyl)phenyl) nicotinamide(R_3) : 3-[(Pyridine-3-carbonyl)-amino]-benzoic acid(9), 3-[(Pyridine-3-carbonyl)-amino]-benzoyl chloride(10), 3-{3-[(Pyridine-3-carbonyl)-amino]-benzoylamino}-benzoic acid(11), 3-{3-[(Pyridine-3-carbonyl)-amino]-benzoylamino}-benzoyl chloride(12).

المركبات الوسيطة ل R_4 هي: 3-benzamido-N-(3-((3-(dimethylamino)propyl) carbamoyl)phenyl)benzamide(R_4) were: 3-Benzoylamino-benzoic acid(1), 3-Benzoylamino-benzoyl chloride(2), 3-(3-Benzoylamino-benzoylamino)-benzoic acid(3), 3-(3-Benzoylamino-benzoylamino)-benzoyl chloride(4).

المركبات الوسيطة ل R_5هي N-(3-((3-((3-(dimethylamino)propyl)carbamoyl) : phenyl)nicotinamide(R_5) were: 3-[(Pyridine-3-carbonyl)-amino]-benzoic acid(9), 3-[(Pyridine-3-carbonyl)-amino]-benzoyl chloride(10), 3-{3-[(Pyridine-3-carbonyl)-amino]-benzoylamino}-benzoic acid(11), 3-{3-[(Pyridine-3-carbonyl)-amino]-benzoylamino}-benzoyl chloride(12).

المركبات الوسيطة ل R_6هي N-(3-(dimethylamino)propyl)-3-(3- : (methylsulfonamido)benzamido)benzamide(R_6) were: 3-Methanesulfonylamino-benzoic acid(13), 3-Methanesulfonylamino-benzoyl chloride(14), 3-(3-Methanesulfonylamino-benzoylamino)-benzoic acid(15),3-(3-Methanesulfonylamino-benzoylamino)-benzoyl chloride(16). 3-benzamido-N-(3-((3-((3-(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)phenyl)Benzamide(R_7) were: 3-Benzoylamino-benzoic acid(1), 3-Benzoylamino-benzoyl chloride(2), 3-(3-Benzoylamino-benzoylamino)-benzoic acid(3), 3-(3-Benzoylamino-benzoylamino)-benzoyl chloride(4), 3-[3-(3-Benzoylamino-benzoylamino)benzoylamino]-benzoic acid(17),3-[3-(3-Benzoylamino- benzoylamino)benzoylamino]-benzoyl chloride(18).

المركبات الوسيطة ل R_8:هي3-(methylsulfonamido)-*N*-(3-((3-((4-sulfamoylphenethyl)carbamoyl)phenyl)carbamoyl)phenyl)benzamide(R_8)we

re: 3-methanesulfonylamino-benzoic acid(13),3-methanesulfonylamino-benzoyl chloride(14),3-(3-Methanesulfonylamino-benzoylamino)-benzoic acid(15),3-(3-methanesulfonylamino-benzoylamino)-benzoyl chloride(16), 3-[3-(3-methanesulfonylamino-benzoylamino)-benzoylamino]-benzoic acid(19), 3-[3-(3-methanesulfonylamino-benzoylamino)-benzoylamino]-benzoyl chloride(20).

المركبات الوسيطة R9 هي: 3-(methylsulfonamido)-*N*-(3-((3-((2-morpholinoethyl) carbamoyl)phenyl) carbamoyl)phenyl) benzamide(R_9) were: 3-Methanesulfonylamino-benzoic acid(13),3-methanesulfonylamino-benzoyl chloride(14),3-(3-Methanesulfonylamino-benzoylamino)-benzoic acid(15),3-(3-Methanesulfonylamino-benzoylamino)-benzoyl chloride(16), 3-[3-(3-Methanesulfonylamino-benzoylamino)-benzoylamino]-benzoic acid(19), 3-[3-(3-methanesulfonylamino-benzoylamino)-benzoylamino]-benzoyl chloride(20).

تم إنشاء بنية جميع مشتقات الديستاميسين أعلاه من قبل البيانات المطيافية

(IR, ^{1}HNMR, C^{13}NMR)

وقد تم اختبار كل من مشتقات الديستاميسين ضد الأكسدة، إمكانات اختزال والبكتيريا والفطريات. أظهر R7 و R4 فعالية مضادات اكسدة القوية،R1 .، R2، R3 أظهرت إمكانات اختزال قوية. عدم وجود مركبات أظهرت مضاد للجراثيم. أظهرت مركباتR1 ، R2و R3 فعالية مضاد للفطريات قوية ضد .M كانيس. أظهرت المركباتR_4 وR_8 84%ضد R3,R8 M.canis. كشفت 100٪ ضد T.mentagrophytes. في حين كشف R1 و R2 أكثر من 95٪ R1 .، R2و R3 قوية ضد T.rubrum