An-Najah National University

Faculty of Graduate Studies

Synthesis & Characterization of N-methylpyrrole Containing Distamycin A Analogues Which Have Potential Biological Activity

By

Baraa Omar

Supervisor

Dr. Hasan Y. Alniss

Co-supervisor

Dr. Waheed J. Jondi

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This Thesis was Defended Successfully on 23 /5 /2017 and approved by:

Defense Committee Members	**Signature**
– **Dr. Hasan Y. Alniss / Supervisor**	**.....**
– **Dr. Waheed J. Jondi / Co- Supervisor**	**.....**
– **Dr. Ahmad Khasati / External Examiner**	**.....**
– **Dr. Nizar Mattar / Internal Examiner**	**.....**

Dedication

To my parents for their love, endless support, taking care, praying for me and extraordinary encouragement.

To my husband Hazem for his support, love, and encouragement.

To my son Yousef.

To my sisters Lama and Ebaa who supported me and shared my worries.

To my brothers Mohammad, Mahmoud and Rayyan for their love, sincere feelings and their moral support.

To all who prayed for me.

To all whom I loved and knew.

Acknowledgments

After a long period of hard work and writing, this note of thanks is the finishing touch to my thesis.

First of all, I'd like to thank Alimight Allah for making this thesis possible and in helping me to complete it.

I would like to express my sincere gratitude to my co-supervisor Dr. Waheed J. Jondi for his valuable guidance, continuous support and encouragement throughout my work on this thesis.

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Very great help was also provided by BERC Centre, Til Village-Nablus for the study of the biological impact of my studied compounds.

Last but not least, I wish to thank my family and all my friends who helped and supported me.

الاقرار

انا الموقعه ادناه مقدم الرسالة التي تحمل العنوان:

Synthesis & Characterization of N-methylpyrrole Containing Distamycin A Analogues Which Have Potential Biological Activity.

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

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

A	Adenine
T	Thymine
G	Guanine
C	Cytocine
MGBs	Minor Groove Binders
DNA	Deoxyribonucleic acid
DCM	Dichloromethane
DMF	N,N-Dimethylformamide
HCl	Hydrochloric acid
THF	Tetrahydrofuran
DCFC	Dry Column Flash Chromatography
m.p.	Melting point
NMR	Nuclear Magnetic Resonance
I.R.	Infrared
ppm	Part per million
mol	Mole
DPPH	1,1-Diphenyl-2-picryl-hydrazyl
OD	Optical Density

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Abstract

A new set of Distamycin A analogues have been synthesized to improve their binding with minor groove by changing molecular masses and lipophilicity with the N-terminal alkyl group. This may increase biological activity as anti-cancer, in addition to its anti-bacterial activity. The compounds are N(5((3(dimethylamino) propyl) carbamoyl)-1-methyl-1H-pyrrol-3-yl) nicotinamide (B1), 4-benzamido-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide(B2), 4-acetamido-N-(3(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide(B3), N-(5-((5-((3-(dimethylamino) propyl) carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrol-3-yl) nicotinamide(B4),4-benzamido-N-(5-((3-(dimethylamino)propyl) carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide (B5), 4-acetamido-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide(B6),4-benzamido-N-(5((5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide(B7), 4-benzamido-N-(3-((3-(dimethylamino) propyl) carbamoyl) phenyl)- 1-methyl-1H-pyrrole-2-carboxamide(B8), 4-benzamido-N-(5-((3-((3-(dimethylamino)

propyl)carbamoyl)phenyl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide(B9), N-(5-((5-((3-((3-(dimethylamino)propyl) carbamoyl)phenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)picolinamide(B10), 4-benzamido-1-methyl-N-(1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)-1H-pyrrole-2-carboxamide(B11), 4-benzamido-1- methyl-N- (1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)-1H- pyrrole-2- carboxamide (B12), N-(1-methyl-5- ((1-methyl-5- ((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)nicotinamide(B13), using acceptable methods shown in the experimental part. The structures of those compounds were confirmed by Fourier Transform Infrared (FT-IR), and Proton Nuclear Magnetic Resonance (^{1}H-NMR). Although the compounds didn't show significant activity as antioxidants, but (B3 and B13) have reductive potential activity (601.8 and 277.2 respectively) compared with (Gallic acid=470.7). On the other hand, they showed antifungal activity against the tested dematophytes. B6 and B8 revealed 100% inhibition against *T. rubrurm* CBS 392.58 and more than 80% against the other type's fungi at the concentration 240µg/ml .

Chapter One

Introduction

1.1 Medicinal Chemistry

Medicinal chemistry involves isolation of compounds from nature or synthesis of new molecules, investigations of the relationships among the structure of natural and/or synthetic compounds and their biological activities, elucidations of their interactions with receptors of various kinds, including enzymes and DNA, the determination of their absorption, transport, and distribution properties, and studies of the metabolic transformations of these chemicals into other chemicals and their excretion and toxicity [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 [1].

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 [2].

1.2 DNA Structure

Deoxyribonucleic acid (DNA) is a molecule of great biological significance. The whole DNA content of a cell is termed Genome. The Genome is unique to an organism, and is the information bank controlling all life processes of the organism, DNA being the form in which this information is kept. Stretches of DNA are called genes. They have extremely important function of coding for proteins. The function of the remnant of the Genome, loosely termed as non-gene regions, is not very clearly known [3,4].

DNA is a polymer of repeated units called nucleotides. Each nucleotide consists of 5-carbon sugar (deoxyribose), nitrogen containing base attached to the sugar, and phosphate group (Figure1.1) [5,6,7].

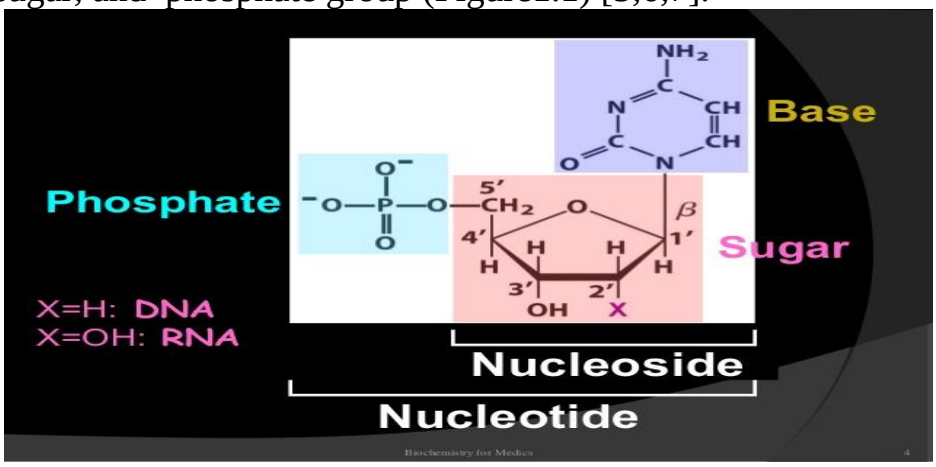


Figure (1.1) : DNA Structure[7].

There are four different types of nucleotides found in DNA, differing only in nitrogenous base. The DNA bases are adenine (A), guanine (G), cytocine (C) and thymine (T).

Adenine and guanine are purine derivatives and composed of two fused heteroaromatic planar rings. Cytocine and thymine are pyrimidine derivatives and are composed of one heteroaromatic planar ring (Figure 1.2) [5,6,8].

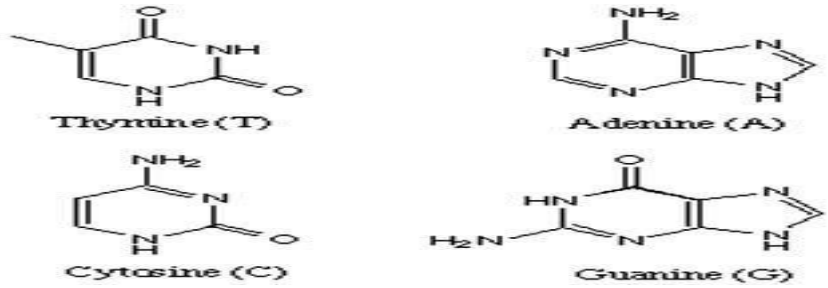


Figure (1.2): Structure of DNA Bases[8].

Most DNA molecules are double-stranded helices. These two strands run in opposite directions to each other and are therefore anti-parallel, one backbone being 3' (three prime) and the other 5' (five prime). This refers to the direction of 3rd and 5th carbon on the sugar molecule is facing. Attached to each sugar is one of four types of molecules called nucleobases [9].

The backbone of DNA strand is formed from alternating phosphate and sugar residues. The sugar in DNA is 2-deoxyribose, which is a pentose (five-carbon) sugar [10].

The sugars are connected together by phosphate groups that form phosphodiester bonds between the third and fifth carbon atoms of adjacent sugar rings. These asymmetric bonds denote a strand of DNA has a direction. In a double helix the direction of nucleotides in one strand is opposite to their direction in the other strand, the strands are antiparallel [10].

The asymmetric ends of DNA strands are called 5′ (five prime) and 3′ (three prime) ends, with 5' end having a terminal phosphate group and 3' end a terminal hydroxyl group (Figure 1.3) [11].

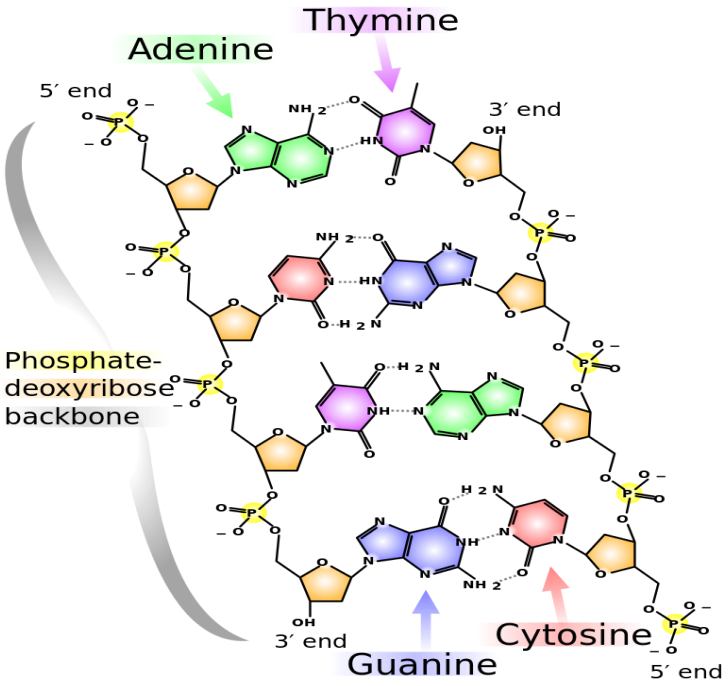


Figure (1.3) : Phosphodiester Bonds Between the Third and Fifth Carbon Atoms of Adjacent Sugar Rings[11].

In a DNA double helix, each type of nucleobase on one strand connects with just one type of nucleobase on the other strand. This is called complementary base pairing. Here, purines form hydrogen bonds to pyrimidines, cytosine(C) bonding only to guanine(G) in three hydrogen bonds, and adenine(A) bonding only to thymine(T) in two hydrogen bonds. This arrangement of two nucleotides binding together across the double helix is called a base pair. As hydrogen bonds are not covalent, they can be broken and rejoined relatively easily. The two strands of DNA in a double helix can therefore be pulled apart like a zipper, either by a mechanical force or high temperature [12].

The two types of base pairs form different numbers of hydrogen bonds, AT forming two hydrogen bonds, and GC forming three hydrogen bonds (Figure 1.4). DNA with low GC-content is less stable than DNA with high GC-content [13].

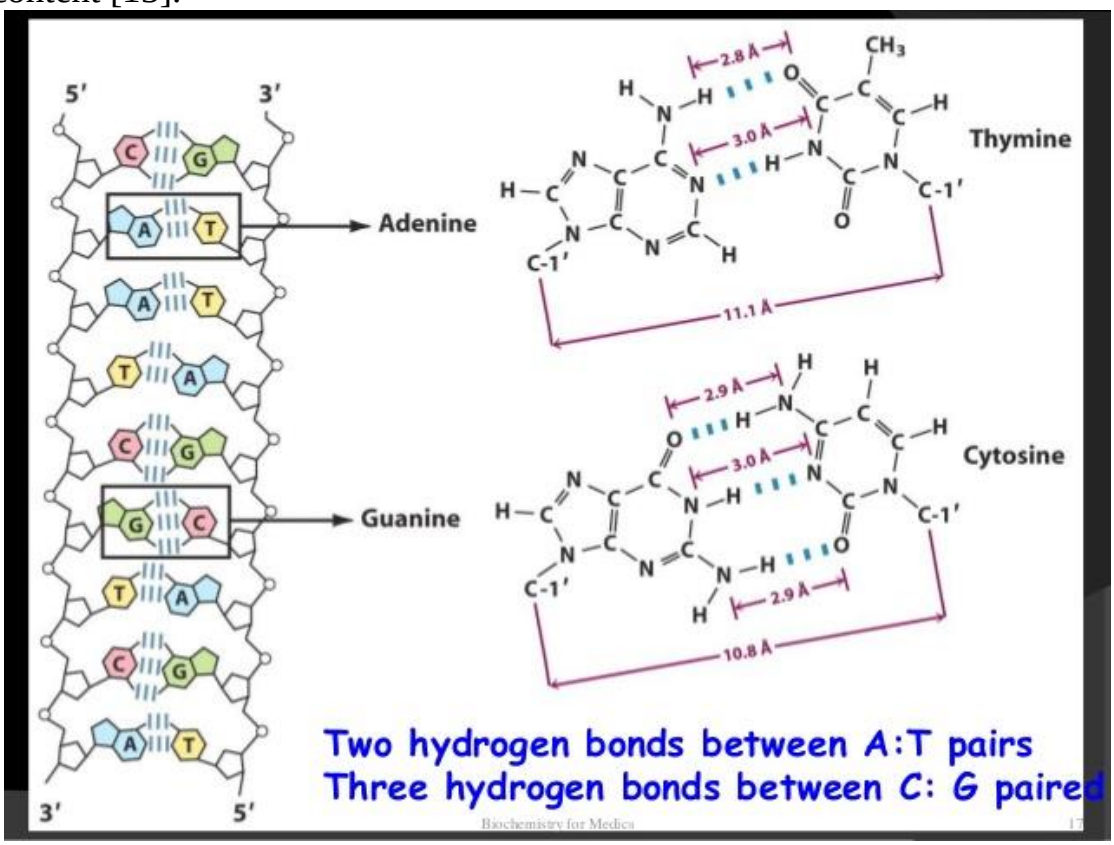


Figure (1.4): Hydrogen Bonds Between A:T Pairs and C:G Pairs[13].

DNA exists in many possible conformations that include A-DNA, B-DNA, and Z-DNA forms, although, only B-DNA and Z-DNA have been directly observed in functional organisms. The conformation that DNA adopts depends on the hydration level, DNA sequence, the amount and direction of supercoiling, chemical modifications of the bases, the type and concentration of metal ions, as well as the presence of polyamines in solution. But the dominant form of DNA in solution (B-DNA) exists as a right-hand helix (Figure 1.5) [14,15].

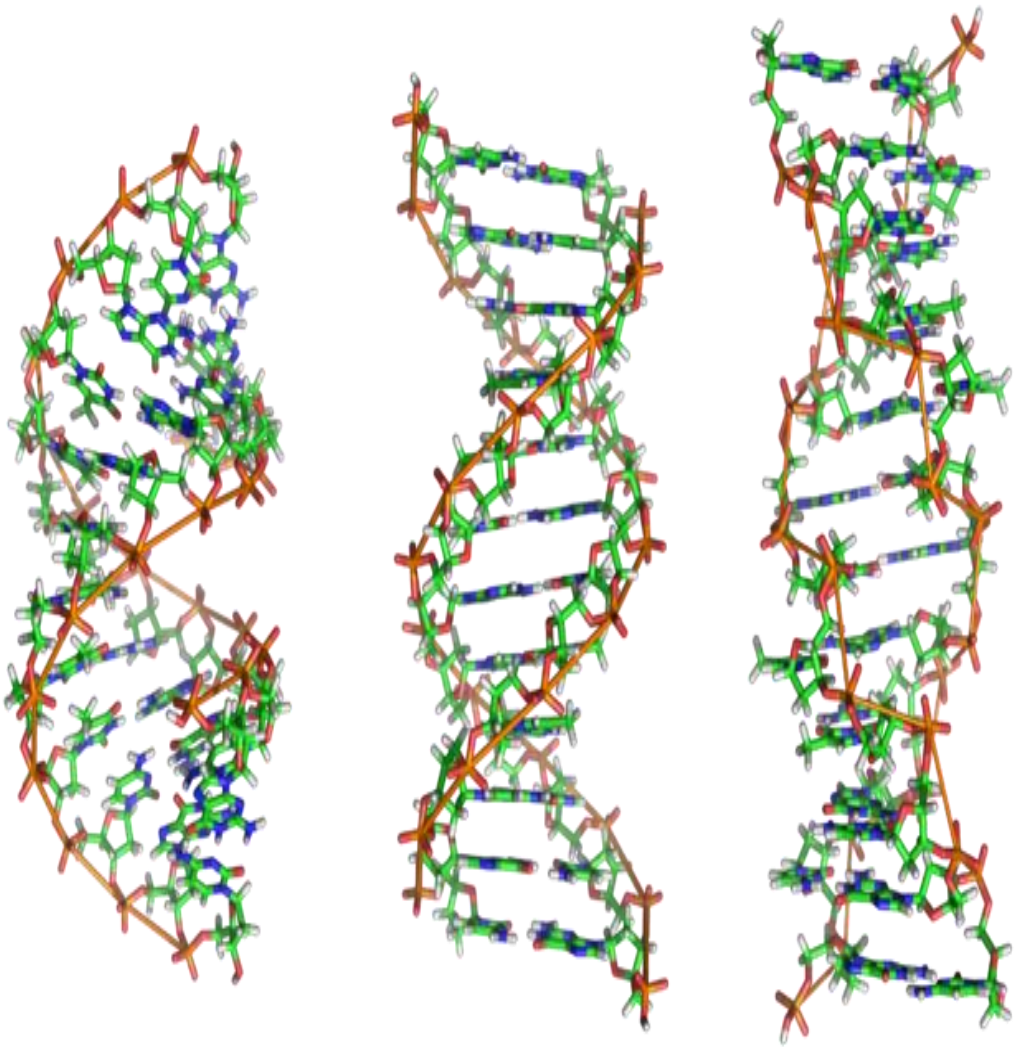


Figure (1.5) : From Left to Right, the Structures of A, B and Z DNA[14].

1.3 Properties of DNA

DNA was first isolated and described by Friedrich Miescher and the double helix structure of DNA was first discovered by James D. Watson and Francis Crick [16]. The structure of DNA of all species comprises two helical chains each coiled round the same axis, and each with a pitch of 34 ångströms (3.4 nanometres) and a radius of 10 ångströms (1.0 nanometres) [17].

The major function of DNA is to store and transmit genetic information. To accomplish this function DNA must have two properties. It must be chemically stable so as to minimize the possibility of damage. DNA must

also be capable of copying the information it contains. The two-stranded structure of DNA gives it both of these properties. The nucleotide sequence contains the information found in DNA. The nucleotides join the two strands through hydrogen bonds. Because each nucleotide has a unique complimentary nucleotide, each strand contains all the information required to synthesize a new DNA molecule. The double stranded structure also makes the molecule more stable [18].

DNA polymers can be very large molecules containing millions of nucleotides. For example, the largest human chromosome, chromosome number 1, is approximately 220 million base pairs long [19].

In living organisms DNA does not usually present as a single molecule, but instead as a pair of molecules that are held tightly together [11,17]. These two long strands entwine as vines, in the form of a double helix. The nucleotide repeats contain both the segment of the backbone of the molecule, which holds the chain together, and a nucleobase, which interacts with the other DNA strand in the helix. A nucleobase connected to a sugar is called a nucleoside and a base connected to a sugar and one or more phosphate groups is called a nucleotide. A polymer comprising multiple linked nucleotides (as in DNA) is called a polynucleotide [20].

Small molecules may interact with DNA. The main classes of DNA binding molecules are: groove binders that sit in the minor groove, intercalators that sandwich between base pairs, alkylators that can chemically react with DNA resulting in DNA alkylation and DNA cleavage agents that have the ability

to break DNA chains (Figure 1.6) [21]. Each of these classes of molecules has a different structure and interacts with DNA in a different way [22].

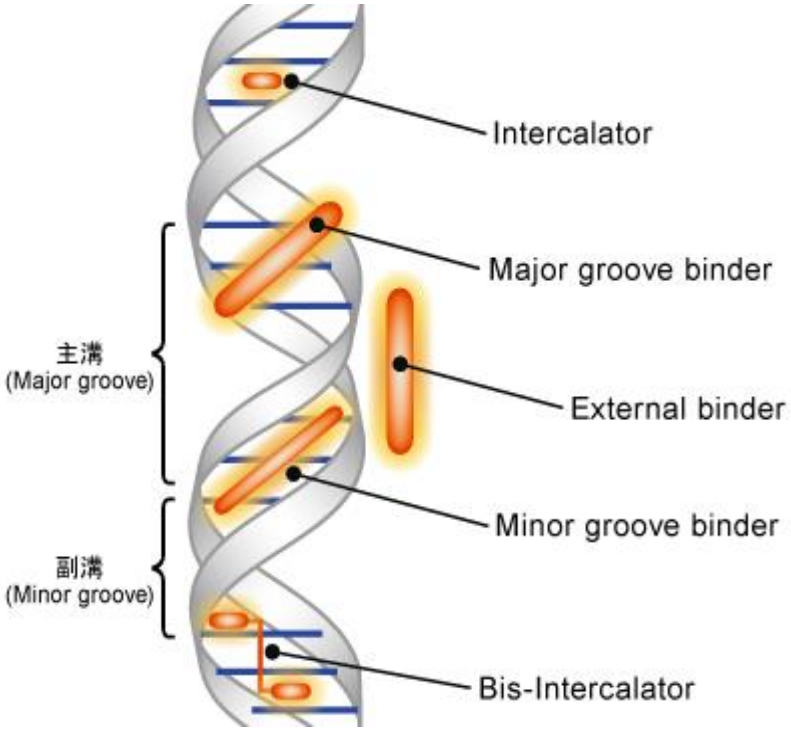


Figure (1.6): The Main Classes of DNA Binding Molecules[21].

1.4 Natural Compounds that Bind to the Minor Groove

Minor groove binding drugs are generally crescent shaped, which complement the shape of the groove and facilitates binding by promoting van der Waals interactions. In addition, these drugs can form hydrogen bonds with DNA bases, typically to the two oxygen of thymine and to the three nitrogen of adenine [23]. Most minor groove binding drugs connect to A/T rich sequences. This preference in addition to the designed propensity for the electronegative pockets of AT sequences is probably due to better van der Waals contacts between the ligand and groove walls in this region, since A/T regions are narrower than G/C groove regions and also because of steric hindrance in the G/C groove regions, presented by the C2 amino group of

the guanine base. Distamycin and its analogues are examples of minor groove binder [24].

During the past years, studies have shown that antitumour activity of DNA-binding drugs is, at least in part, the result of the inhibition of enzymes that organize DNA topology: the topoisomerases [25].

1.4.1 Distamycin A

Distamycin A is natural product possessing amido groups and three *N*-methylpyrrole rings (Figure 1.7). It was obtained by submerged fermentation and butanol extraction of the mycelial mass of a *Streptomyces* sp. It shows antibacterial, antiviral and antitumor properties in some systems as well as inhibition of DNA synthesis in vitro [26].

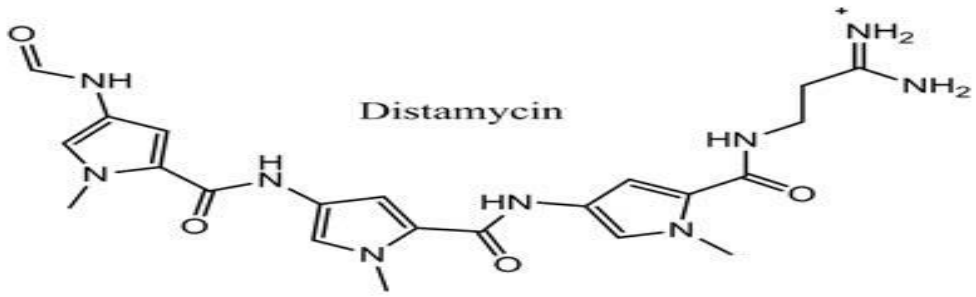


Figure (1.7): Structure of Distamycin[26].

Distamycin A interacts with AT-rich regions in double stranded DNA [27]. The preference of distamycin A for binding to AT residues seems to be determined primarily by van der Waals contacts between the pyrrole rings of the antibiotic and various surfaces on the DNA, and its ability to form hydrogen bonds with the DNA bases, and electrostatic interactions between its positively charged tail and negatively charged backbone of DNA [28,29].

Distamycin A can bind with the minor groove of DNA as anti-parallel dimer (Figure 1.8) or as a monomer (Figure 1.9). This binding distorts the DNA structure by widening the minor groove. The 2-amino group of guanine prevents distamycin A from binding to the minor groove of GC base pairs by steric hindrance, thus conferring AT-selectivity on the drug molecule [28].

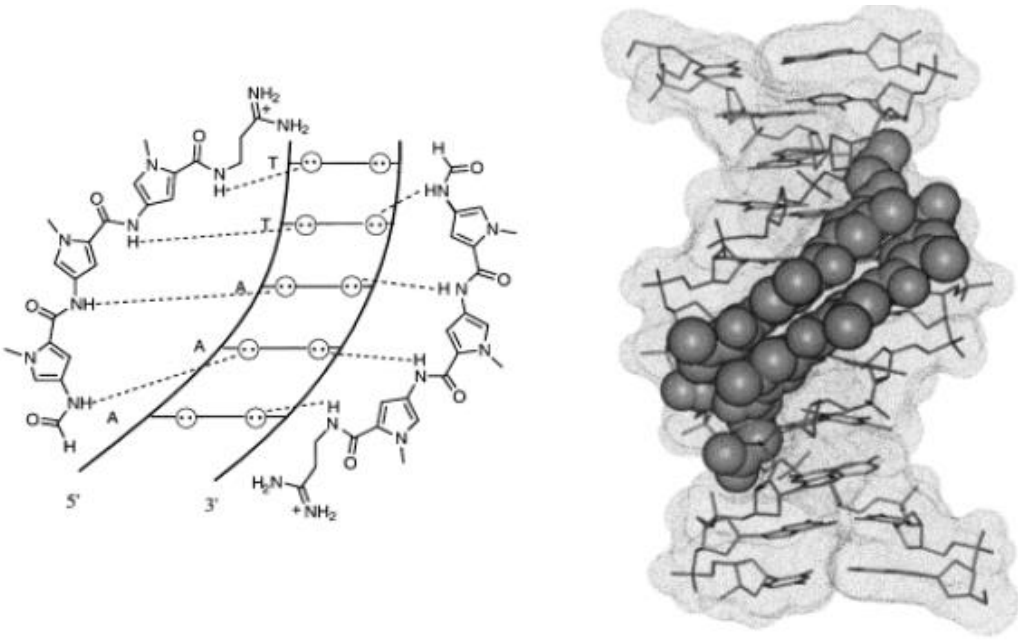
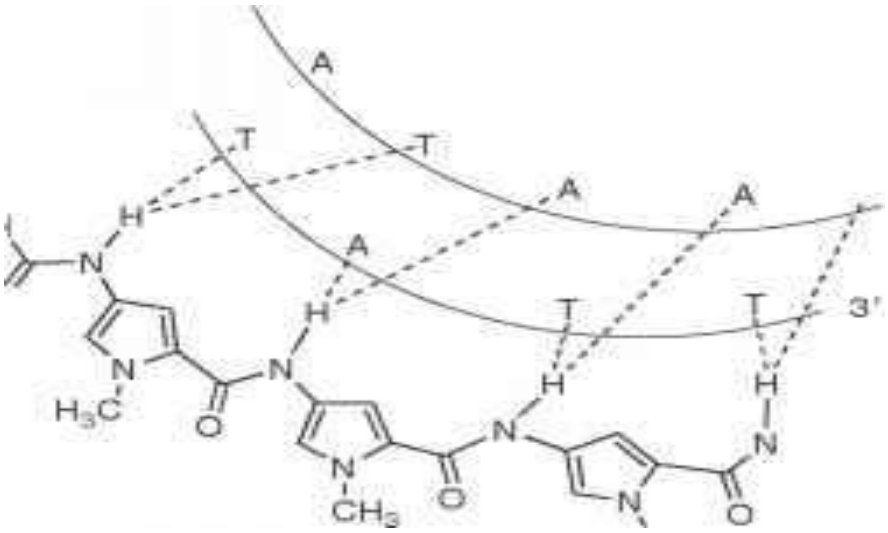


Figure (1.8): Distamycin A Bound in the Minor Groove as Anti-parallel Dimer (2:1) [28] .



Figure(1. 9): Distamycin A Bound in The Minor Groove of DNA as a Monomer (1:1) [28].

Binding of distamycin A occurs either in the form of a 1:1 complex or as a 2:1(drug: DNA ratios complex) the minor groove can accommodate not only a single distamycin A molecule, but also side-by-side antiparallel binding of two distamycin A molecules [30,31]. The measurements, showing a 2 Å decrease in minor groove width upon 1:1 binding and a subsequent 6 Å increase upon formation of the 2:1 complex [32].

Distamycin A is too toxic to find application in cancer therapy [33]. For this reason a lot of various distamycin A analogues have been synthesized, with modification of the side chain, the binding increases as a function of number of repeated pyrrole, as a result of availability of hydrogen bonds and Van der Waals surface. Some of these analogues show an enhanced biological activity with increasing chain length due to increasing hydrogen bonds [33]. Examples of distamycin A analogue: Pentamidine and Berenil (Figure 1.10) [23].

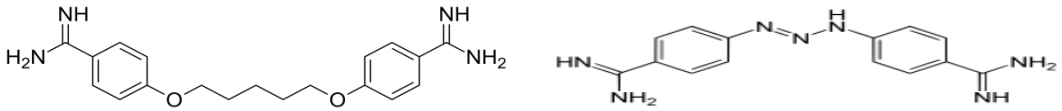


Figure (1.10): Examples of Distamycin A Analogue Pentamidine and Berenil [23].

1.4.2 Netropsin

Netropsin is a polyamide with antibiotic and antiviral activity, netropsin was discovered by Finlay et al. and first isolated from the actinobacterium Streptomyces netropsis. It belongs to the class of pyrrole amidine antibiotics (Figure 1.11) [34].

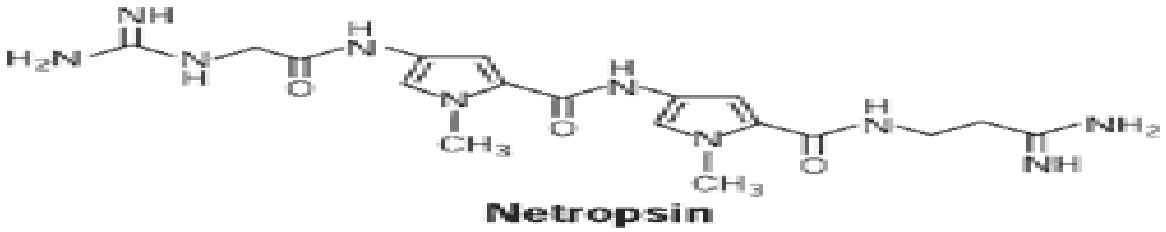


Figure (1.11): Structure of Netropsin[34].

Other names for the compound are congocidine and sinanomycin [35].

Netropsin binds to the minor groove of AT-rich sequences of double stranded DNA. In contrast, netropsin does not bind single stranded DNA or double stranded RNA[35].

Netropsin is active both against Gram-positive bacteria and Gram-negative bacteria[35]. Unlike distamycin A, netropsin binds exclusively into a narrow B-DNA minor groove to form 1:1 complexes and not 2:1 complexes and this due to the repulsive force which would occur having two positively charged groups side by side [36].

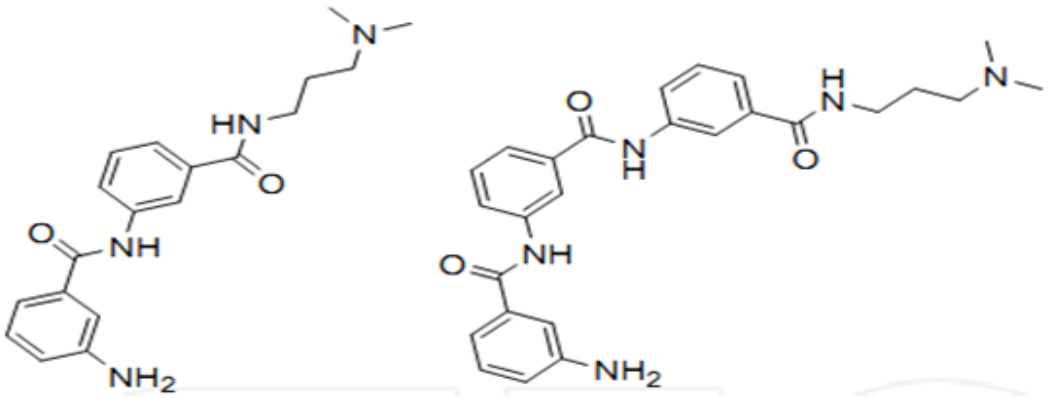
The two differences between netropsin and distamycin A are: 1) distamycin A has three pyrrole rings and netropsin has two pyrrole rings, and 2) netropsin has two positively charged terminal groups and distamycin A has one cationic amidine terminus and a formamido (f) group at the other terminus [37].

1.5 Synthesis of Distamycin A analogues

Since their discovery, distamycin A and netropsin have served as templates for the design of new compounds with similar interaction to DNA. The class of synthetic polyamides developed after the models of the distamycin A and netropsin, are called lexitropsins. Lexitropsins linked with molecules of

different known drugs, e.g. alkylating agents received the name combilexins [38].

Lexitropsins (carbocyclic distamycin A and netropsin analogues), which contain benzene in place of N-methylpyrrole rings, with a minor modification of cationic heads, connect to AT sequences less strongly than the extensively studied MGB, while these compounds show sequence selectivity (Figure 1.12) [39,40].



Figure(1.12): Structures of Carbocyclic Netropsin and Distamycin A Analogue Respectively[40].

In the mid-late 1980's, Lown and Dickerson et al. developed synthetic polyamides, lexitropsins, which could target G-C and C-G sequences. This was achieved by replacing an N-methyl pyrrole (Py) with an N-methyl imidazole (Im). The additional nitrogen in the imidazole ring performs as a hydrogen bond acceptor and can hydrogen bond with the exocyclic amino group of guanine (Figure1.13) [36,41].

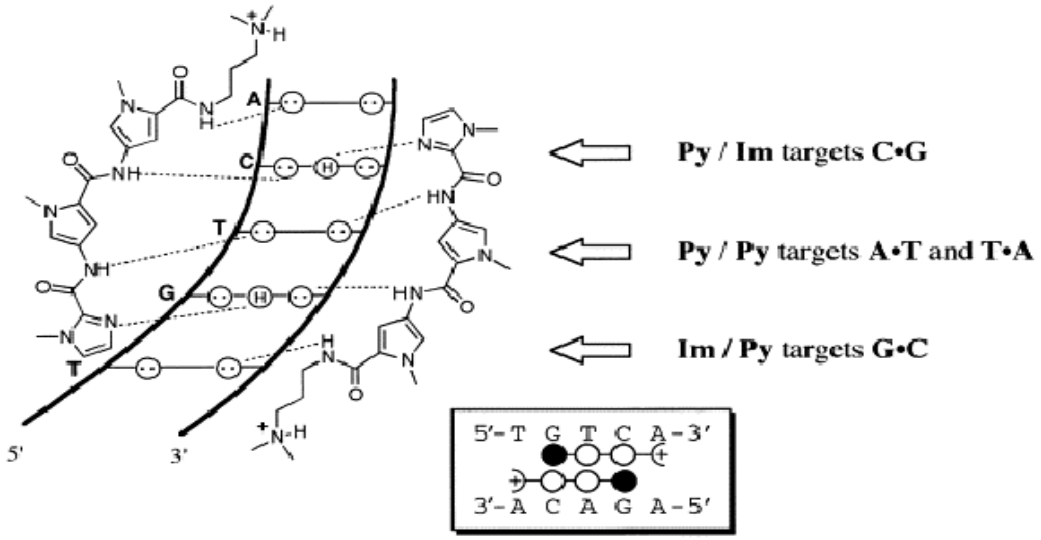


Figure (1.13): Schematic Representation of G-C Recognition of Im-Py and the Binding of Im-Py-Py to 5'-TGTCA-3'. The Black Circles of the Inset Signify Im Rings, the Open Circles Py Rings [36,41].

Baird and Dervan synthesized poly amides containing imidazole and pyrrole amino acids using tert-butyloxycarbonyl-protection strategy which is a solid phase synthesis [42].

Boger and co-workers also used this strategy to synthesize a series of 2,640 compounds inspired by distamycin A structure. They used solution-phase synthesis and acid/ base liquid-liquid extraction techniques for isolation of these compounds [43].

Brucoli and others employed SynPhase Lanterns to make a series 72 novel distamycin A analogues, where one of pyrrole rings was substituted by biaryl motifs (Figure 1.14) [44,45].

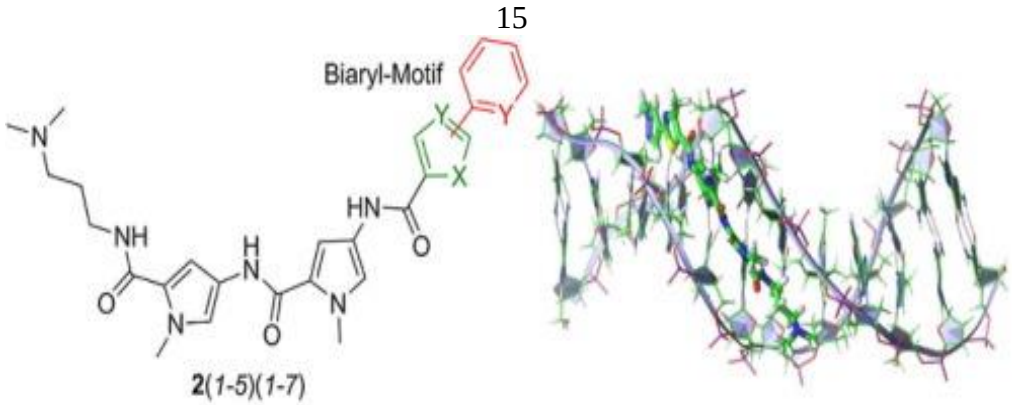


Figure (1.14): Biaryl-motifs Containing Polyamides[45].

Baraldi's group synthesized and evaluated series of α-methylene-γ-butyrolactone-lexitropsin hybrids [46], while Bhattacharya and Thomas reported the first example of cholesterol-conjugated distamycin A analogues, which retain their strong binding capacity to double-stranded (ds)-DNA [47].

1.6 Antimicrobial Activity of Distamycin A Analogues

Since their discovery and isolation, distamycin A and netropsin have been known to have biological activity as antiviral, antibiotic and antitumor therapeutics, leading to the hypothesis that these two molecules are synthesized naturally by streptomycete strains as a potential defense mechanism [28]. Though too toxic for clinical use, these compounds have been used to elucidate the origins of sequence specificity and biological activity. These molecules are thought to interfere with topoisomerase in some fashion, although their true mechanism is not completely understood. Excitingly, synthetic Py- and Im-containing polyamides have not shown the same levels of toxicity as netropsin and distamycin A [48].

1.7 Proposed Synthetic Pathway for Distamycin A Analogues

The proposed synthetic pathway for one of these analogues is described in Figure (1.15). The amide bond between the aromatic hetero-aromatic rings is formed by reacting 1 with an acid amine in the right-hand side of the compound and then reducing the nitro group and reacting the resulting compound with an acid chloride in the left-hand side of the compound.

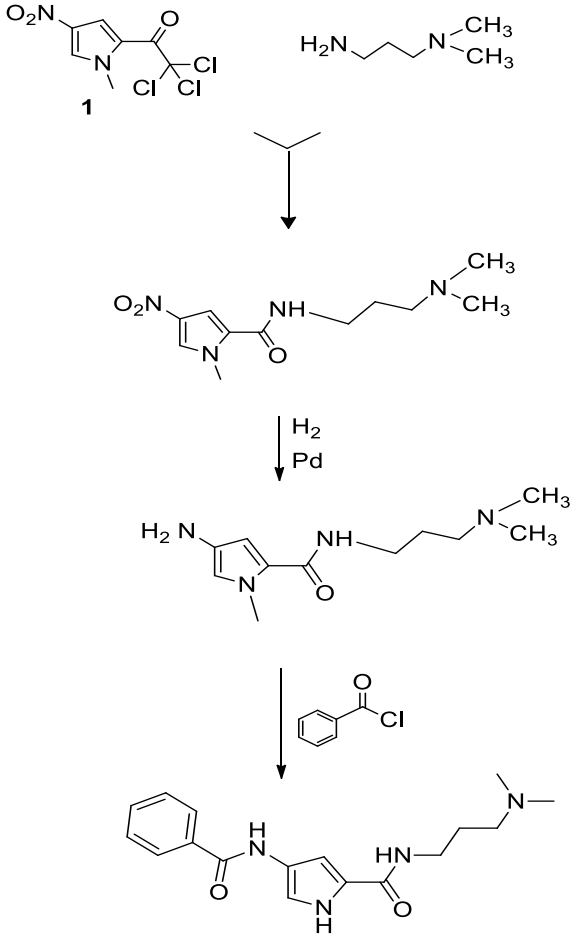


Figure (1.15): The Proposed Synthetic Pathway for One of These Analogues.

The proposed synthetic pathway for another analogue is described in Figure (1.16).

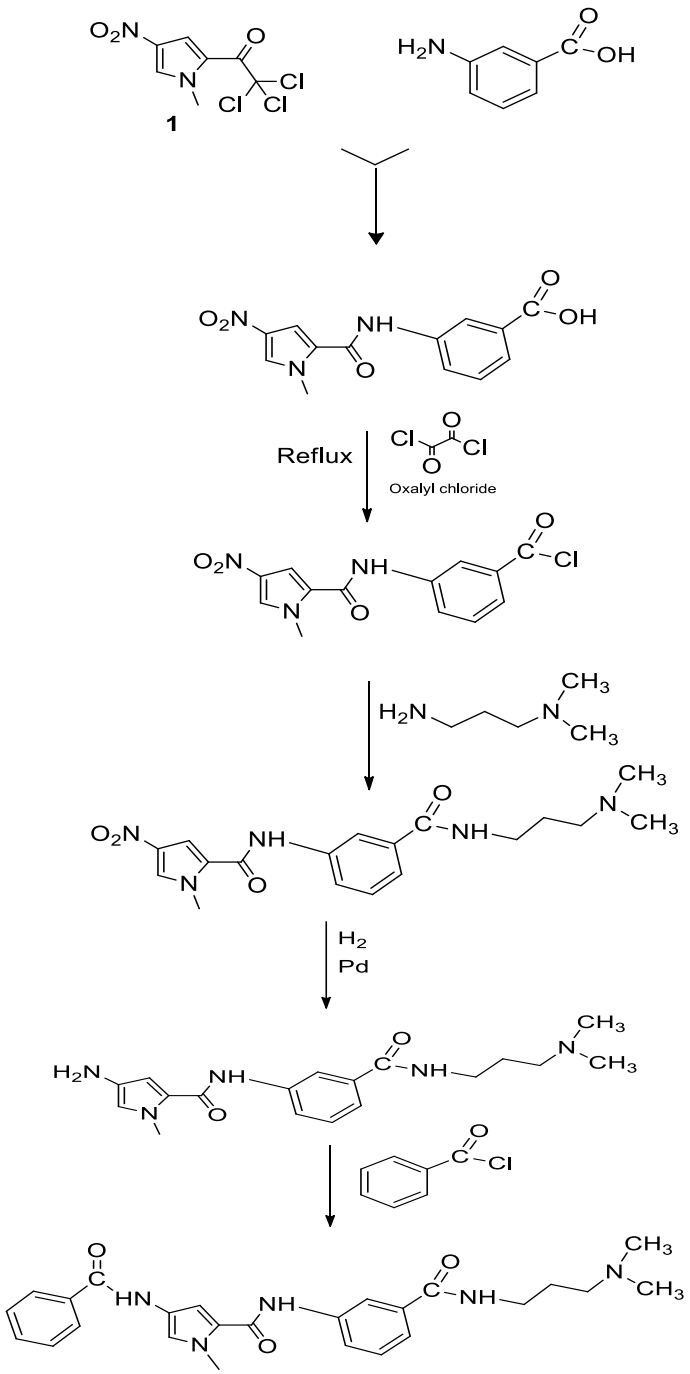


Figure (1.16): The Proposed Synthetic Pathway for Another Analogue**.**

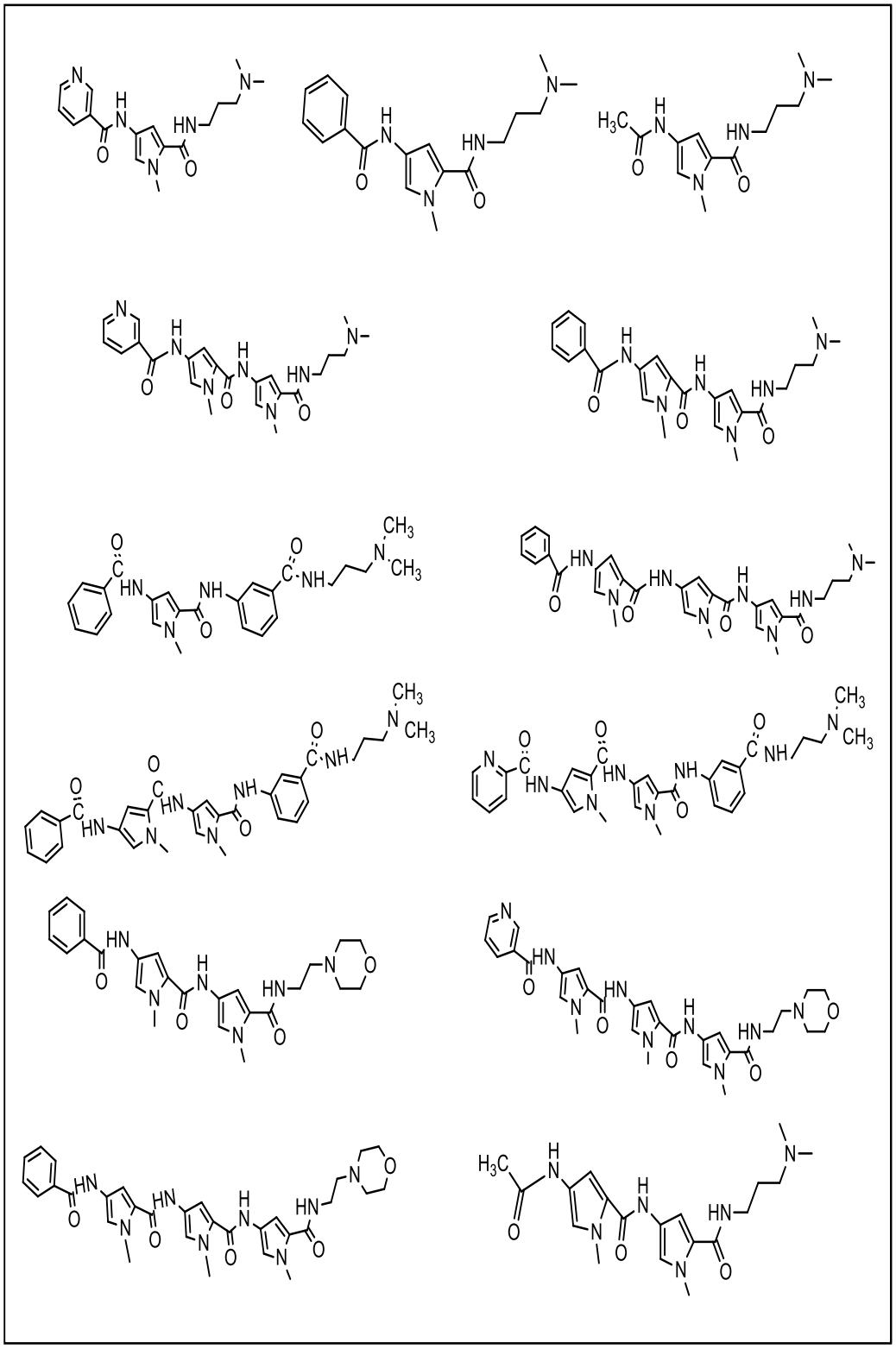


Figure (1.17):Examples of Proposed MGBs With Enhanced Lipophilicity and Small Molecular Weight.

1.8 Aim of the study

The main aim of this project is to synthesize new distamycin A analogues starting from the N-methylpyrrole as a core structure for these compounds. N-methylpyrrole will impose the curvature and the angle of these crescent-shaped molecules which help them to fit snugly into the minor groove of DNA. The proposed analogues of distamycin A will have small molecular mass and enhanced lipophilicity in order to improve their binding with minor groove of DNA and increase the absorption and cell permeability of these compounds.

The second aim is to examine the biological activity of these compounds.

Chapter Two

Materials and Methods

2.1 Chemicals

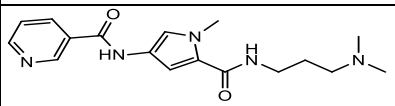
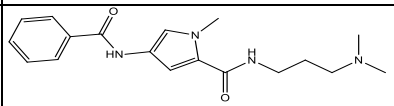
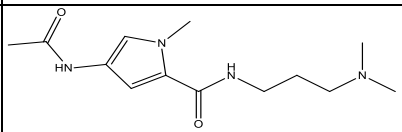
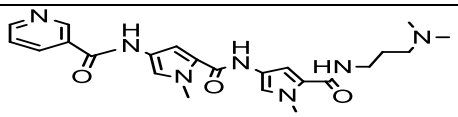
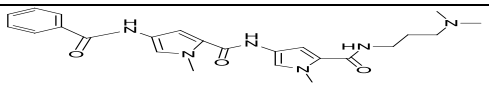
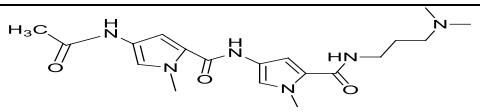
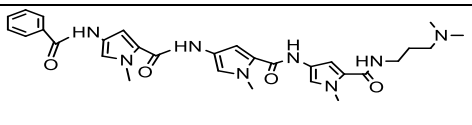
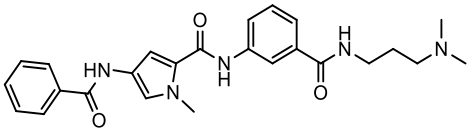
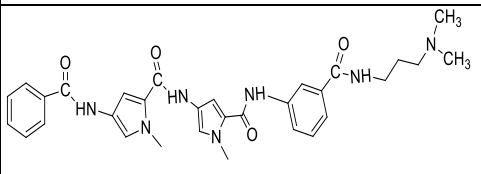
All chemicals used in this study were purchased from Sigma Aldrich Chemical Company. The following chemicals were utilized: N-methylpyrrole, trichloroacetyl chloride, acetic anhydride, nitric acid, 3-dimethylamino-1-propylamine, 2-morpholinoethanamine, benzoyl chloride, nicotinyl chloride, 3-aminobenzoic acid, DCM, triethylamine, sodium carbonate, ethyl acetate, DMF, oxalyl chloride, THF, ethanol, methanol, n-hexane. All chemicals and reagents were of analytical grade and were used without further purification.

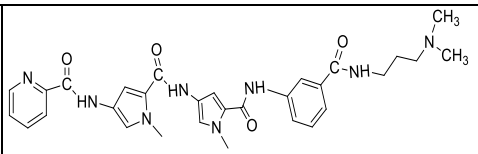
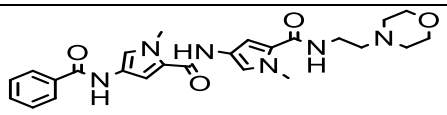
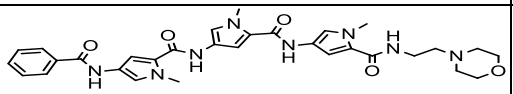
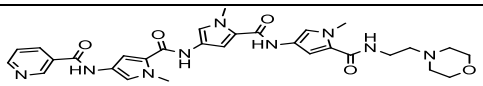
As for biological activities, all tested microorganisms in this work were obtained from Biodiversity & Environmental Research Center (BERC) Til Village-Nablus.

2.2 Physical Measurements

Melting point of each product was determined by Stuart meting point apparatus, SMP3. IR was performed through Fourier transform infrared spectrophotometer (Necolet Is5 - ID3).^{1}H –NMR was measured in Jordan University of Science and Technology / Jordan (Bruker 400 MHz).

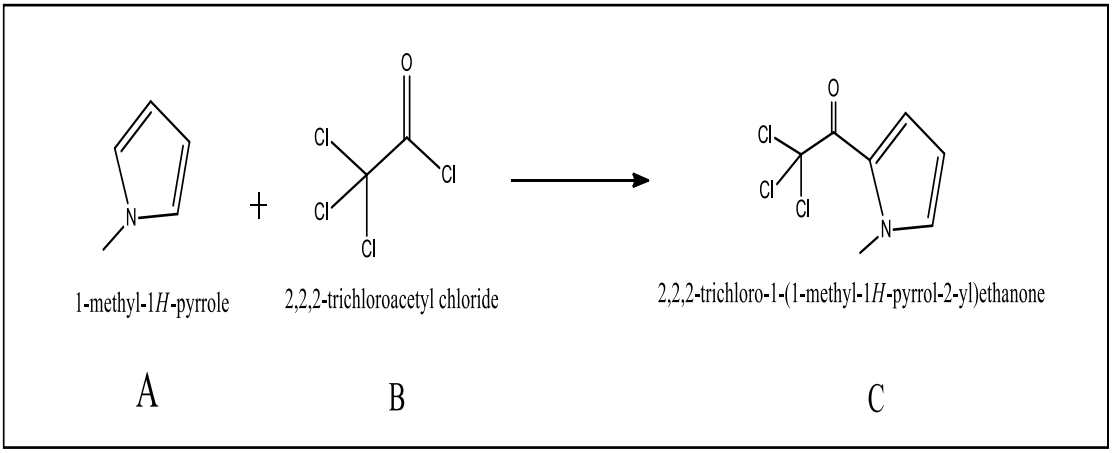
Table (2.1):Chemical Formulas of Prepared Compounds

No.	Compound	Formula	page
B1	N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)nicotinamide		29
B2	4-benzamido-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide		30
B3	4-acetamido-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide		30
B4	N-(5-((5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)nicotinamide		36
B5	4-benzamido-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide		37
B6	4-acetamido-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide		38
B7	4-benzamido-N-(5-((5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide		39
B8	4-benzamido-N-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)-1-methyl-1H-pyrrole-2-carboxamide		47
B9	4-benzamido-N-(5-((3-((3-(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide		60

B10	N-(5-((5-((3-((3-(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)picolinamide		61
B11	4-benzamido-1-methyl-N-(1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)-1H-pyrrole-2-carboxamide		62
B12	4-benzamido-1-methyl-N-(1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)-1H-pyrrole-2-carboxamide		64
B13	N-(1-methyl-5-((1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)nicotinamide		65

2.3.1 Preparation of Starting Materials.

A: Preparationof2,2,2-trichloro-1-(1-methyl-1H-pyrrol-2-yl) ethanoneC.

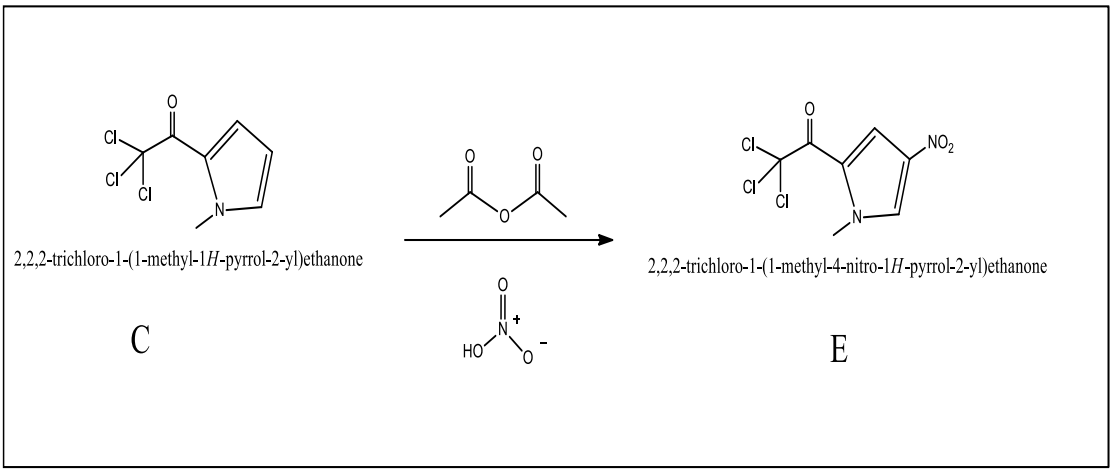


N-methylpyrrole (16.2g, 0.2mol) was dissolved in (40ml) DCM and then added dropwise during 2.5 hour to a solution of (36.2g, 0.1993mol) of trichloroacetyl chloride in (85ml) DCM, which was placed in 250 ml round-

bottomed flask. The mixture was then left to stir overnight. After that, the solvent was removed under reduced pressure to yield the crude product. Finally, the crude product was purified through Dry Column Flash Chromatography (DCFC)to yield a white-yellow crystals as a product.

Yield; 35g, 77%, m.p. = 62-64°C.

B: Preparation of 2,2,2-trichloro-1-(1-methyl-4-nitro-1H-pyrrol-2-yl) ethanone E.

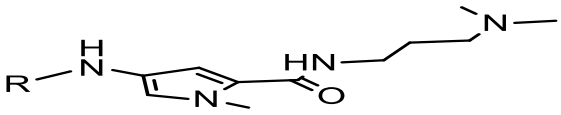


Acetic anhydride (60ml) was placed in a round-bottomed flask, nitric acid(70%, v/v,8ml)was then added drop-wise at -30°C,and the solution was left with stirring for 20 min. After that, this solution was added dropwise to solution of 2,2,2-trichloro-1-(1-methyl-1H-pyrrol-2-yl) ethanone (10g, 0.04421mol) in (40ml) acetic anhydride in 250 ml round-bottomed flask at -30°C and then allowed to warm up to 0°C. After that, this solution was cooled to -40°C. Finally, water was added drop wise to the product until off-white-yellow solid was precipitated. This product was collected and washed with hexane, before being dried under reduced pressure.

Yield; 10g, 83%, m.p. =133-135°C .

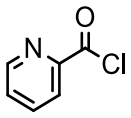
2.3.2 Preparation of B1,B2,B3 Compounds .

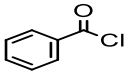
The general structure of those compounds is,

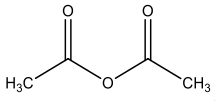


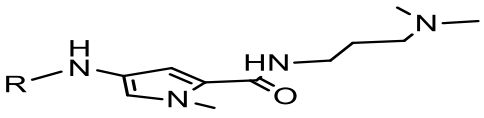
But the difference between them is in the last amide link.

Where R functional chemical group can be any of the following:

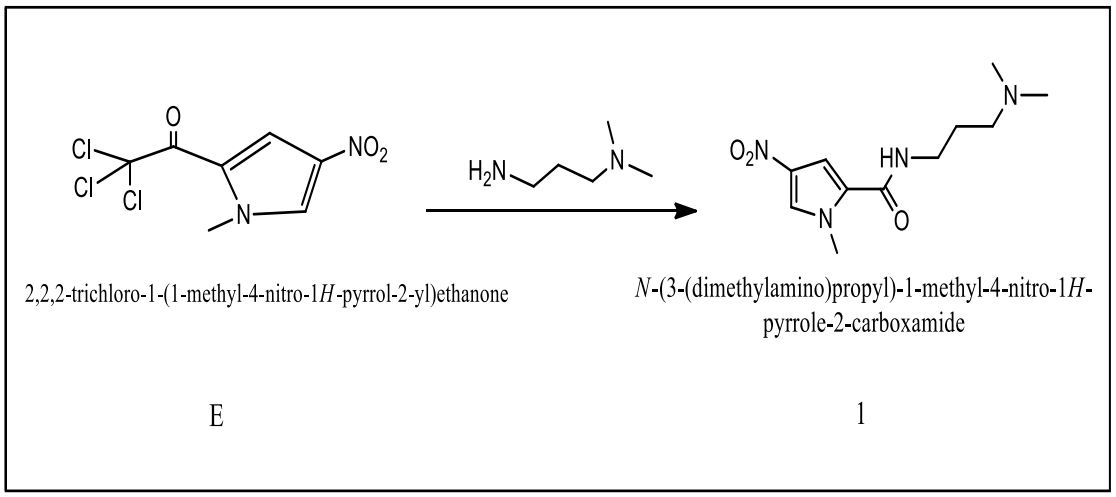
I (R =  , nicotinyl chloride).

II (R =  , benzoyl chloride).

II (R =  , acetic anhydride).

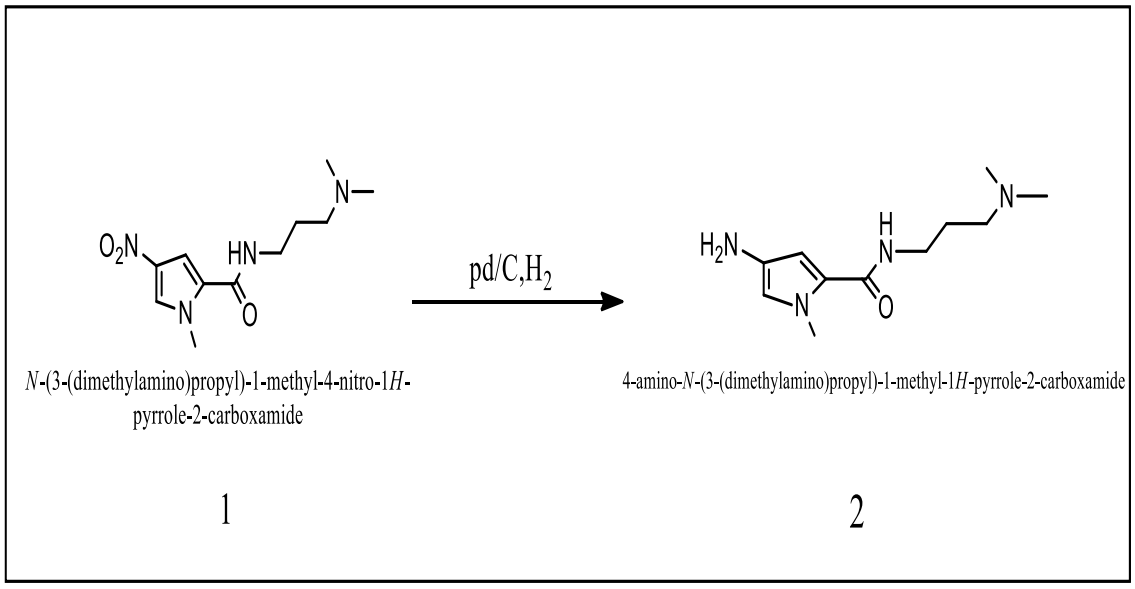
Preparation of  **is shown in the next steps:**

1: Preparation of N-(3-(dimethylamino)propyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide1.



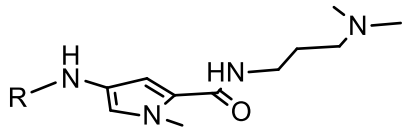
2,2,2-trichloro-1-(1-methyl-4-nitro-1H-pyrrol-2-yl) ethanone (2g, 0.00737mol) was dissolved in THF (40ml), 3-dimethylamino-1-propylamine (1g, 0.0098mol) was added to the solution and left to stir over night. The product was dried under reduced pressure, and water (60ml) was added. The product was extracted with ethyl acetate (80ml) and then the solvent was removed under reduced pressure to get a bright yellow powder as a product.

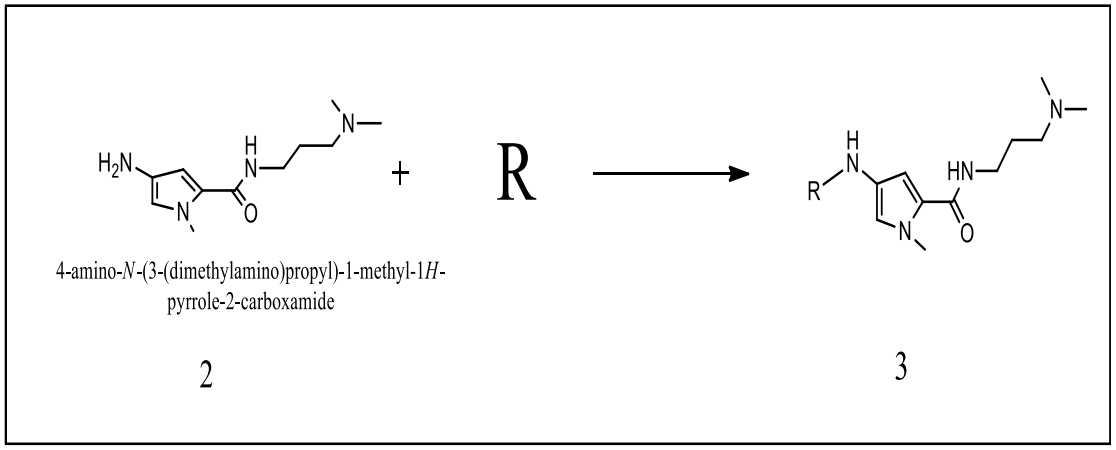
2: Preparation of 4-amino-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide 2 .



N-(3-(dimethylamino)propyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide (1.5g, 0.0062mol) was dissolved in (50ml) methanol and (5ml) THF in 250ml round-bottomed flask,(0.5g) Pd/C was also added slowly at 0°C. After that, the suspension was placed under hydrogen, and left with stirring for 4 hr. The suspension was then filtered through silica gel (6g), and the solvent was removed under reduced pressure to get the product which was

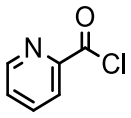
used immediately in the next step for synthesis because of the lack of stability of this product.

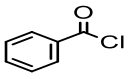
3 : Preparation of  **3.**

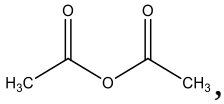


The proper amount of R was dissolved in DCM (10ml). This solution was then added dropwise to proper amount of 4-amino-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide which was dissolved in THF (20ml) and trimethylamine (3ml). After that, the solution was left to stir over night. A brown precipitate was formed, filtered and dried under reduced pressure. In the next step, the product was extracted with aqueous solution saturated with sodium carbonate and ethyl acetate, then the solvent was removed under reduced pressure to get the product, which was purified by Dry Column Flash Chromatography (DCFC) and by recrystallization.

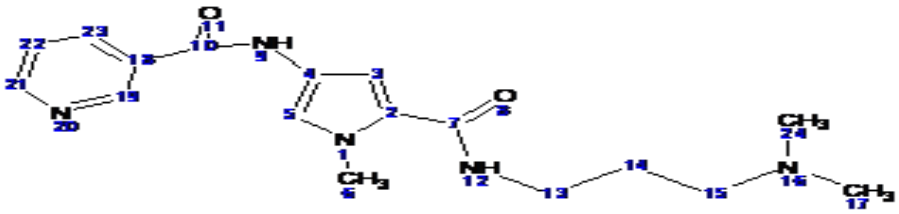
Where R functional chemical group can be any of the following:

I (R =  , nicotinyl chloride).

II (R =  , Benzoyl chloride).

II (R =  , acetic anhydride).

Preparation of N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)nicotinamide I (B1).

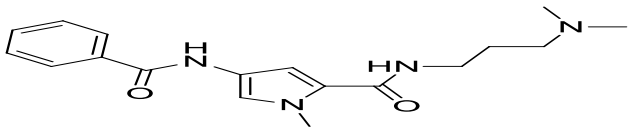


The reaction of nicotinyl chloride (0.64g, 0.00359mol) with 4-amino-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide(0.8g, 0.00357mol) produced (I). (1g, 85 %) (m.p.= 263-265 ºC, lit. not found).

IR:3272;2955;2868;2827;2778;1719;1635;1535;1437;1402;1286;1260; 1098; 1025; 794; 701; 512 cm^{-1}.

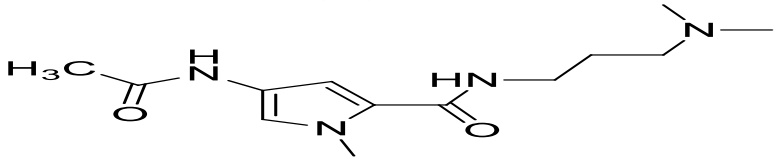
1HNMR:δ=H_3(1H):7.4;H_5(1H):6.7;H_6(3H):3.5;H_{12},H_9(2H):10.8;H_{13}(2H):3.7;H_{14}(2H):1.4;H_{15}(2H):2.4;H_{17},H_{24}(6H):2.3;H_{19}(1H):9.4;H_{21}(1H):8.9;H_{22}(1H):7.7;H_{23}(1H):8.5 ppm.

Preparation of 4-benzamido-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide II (B2).



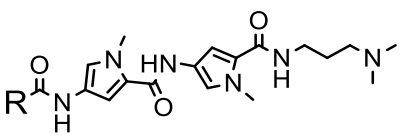
The reaction of benzoyl chloride (1g, 0.00711mol) with 4-amino-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide(1.5g, 0.00669mol) produced (II). (1.22 g, 55%) (m.p.= 261-263 ºC, lit. not found). **IR**:2976;2943;2737;2591;2529;2488;2357;1699;1541;1475;1442;1396;1382;1330;1262;1170;1074;1033;849;805;719;634;597;543;531cm^{-1}.

Preparation of 4-acetamido-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide III (B3).



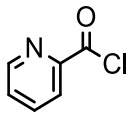
The reaction of acetic anhydride (0.5g, 0.00458mol) with 4-amino-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide (1g, 0.00446mol) produced (III). (1g, 85%) (m.p.= 149-151 ºC, lit. not found). **IR**:3261;2939;2864;2301;1673;1633;1574;1535;1436;1370;1336;1254;1196;1154;1016;973;920;871;806;755;684;650;604;566;538 cm^{-1}.

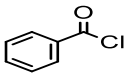
2.3.3Preparation of B4,B5,B6 compounds .
The general structure of those compounds is,

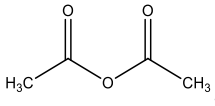


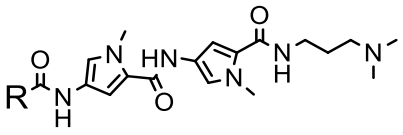
But the difference between them is in the last amide link.

Where R functional chemical group can be any of the following:

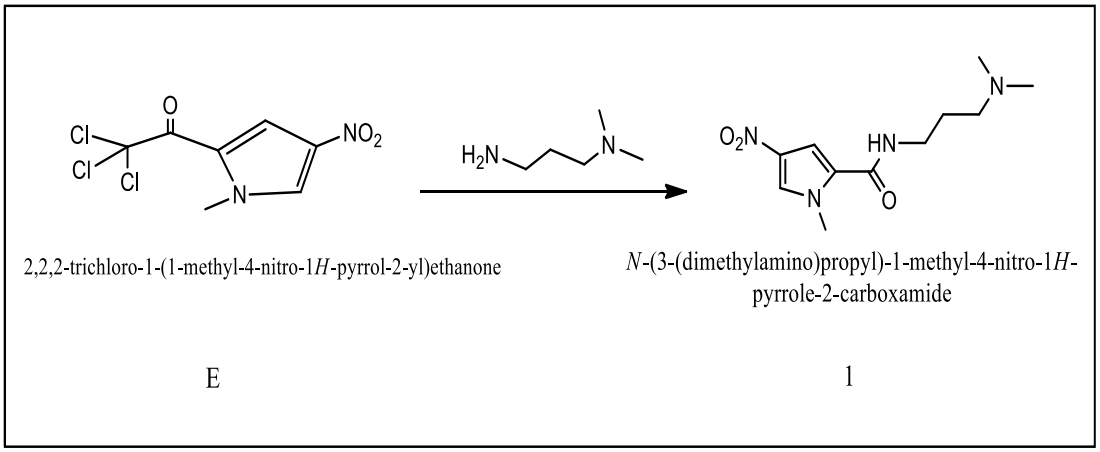
I (R =  , nicotinyl chloride).

II (R =  , benzoyl chloride).

II (R =  , acetic anhydride).

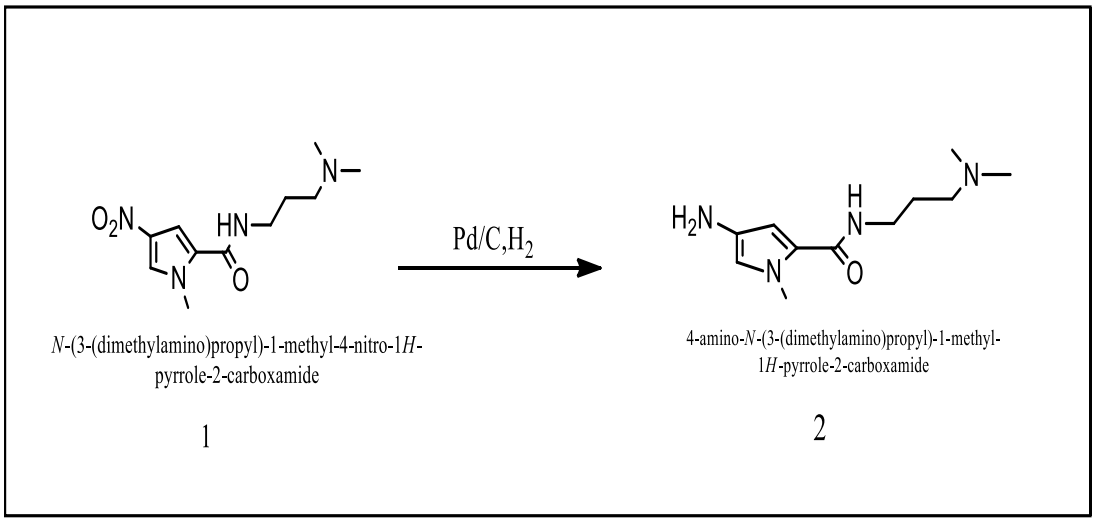
Preparation of  **is shown in the next steps:**

1: Preparation of N-(3-(dimethylamino)propyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide <u>1.</u>



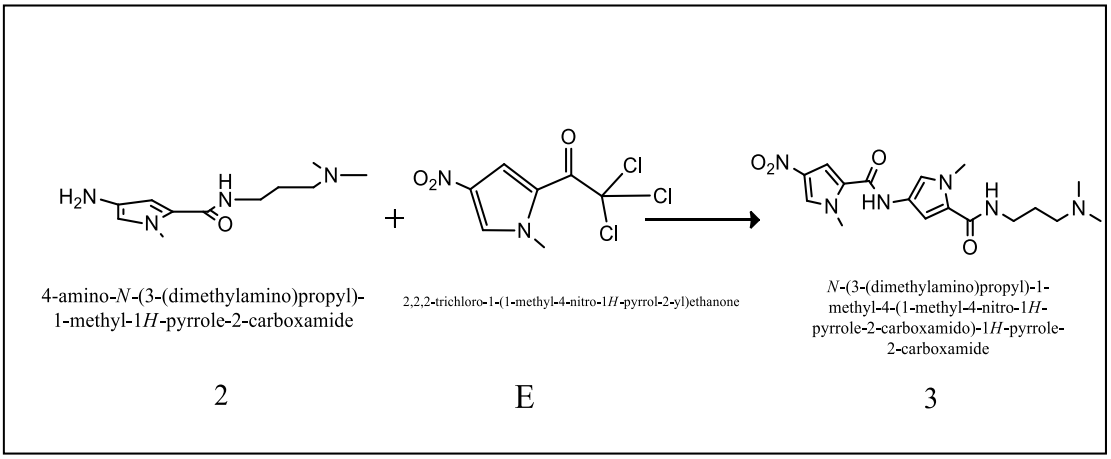
2,2,2-trichloro-1-(1-methyl-4-nitro-1H-pyrrol-2-yl) ethanone (2g, 0.00737mol) was dissolved in THF(40ml), 3-dimethylamino-1-propylamine (1g, 0.0098mol) was added to the solution and left to stir over night. The product was dried under reduced pressure, and water (60ml) was added. The product was extracted with ethyl acetate(80ml) and then the solvent was removed under reduced pressure to get a bright yellow powder as a product.

2: Preparation of 4-amino-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide 2 .



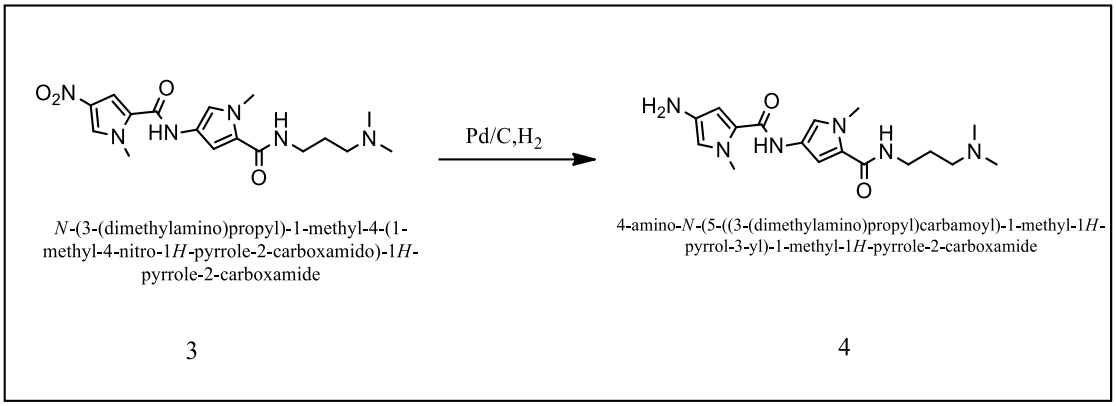
N-(3-(dimethylamino)propyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide (1g, 0.00394mol) was dissolved in (50ml) methanol and (5ml) THF in 250 ml round-bottomed flask,(0.33g) Pd/C was also added slowly at 0°C. After that, the suspension was placed under hydrogen, and left with stirring for 4 hr. The suspension was then filtered through silica gel (6g), and the solvent was removed under reduced pressure to get the product which was used immediately in the next step for synthesis because of the lack of stability of this product.

3: Preparation of N-(3-(dimethylamino)propyl)-1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamide3.

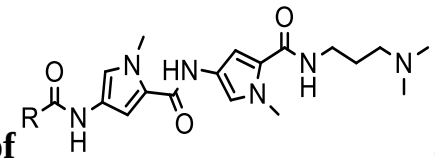


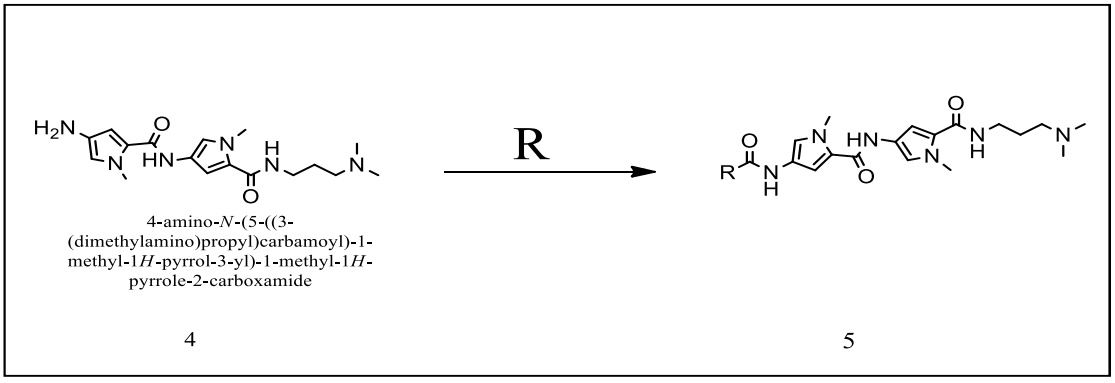
2,2,2-trichloro-1-(1-methyl-4-nitro-1H-pyrrol-2-yl) ethanone (1.2g, 0.00449 mol) was dissolved in DCM (10ml). This solution was then added dropwise to 4-amino-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-Carboxamide (1g, 0.00446mol) which was dissolved in THF(20ml) and triethylamine (2ml). After that, the solution was left to stir over night. A brown precipitate was formed, filtered and dried under reduced pressure. In the next step, the product was extracted with aqueous solution saturated with sodium carbonate and ethyl acetate, then the solvent was removed under reduced pressure to get the product.

4: Preparation of 4-amino-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide 4.



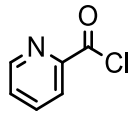
N-(3-(dimethylamino)propyl)-1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamide (1.5g,0.00399mol) was dissolved in (70ml) methanol and (5ml) THF in 250 ml round-bottomed flask, (0.5g) Pd/C was also added slowly at 0°C. After that, the suspension was placed under hydrogen, and left with stirring for 4 hr. The suspension was then filtered through silica gel (6g), and the solvent was removed under reduced pressure to get the product which was used immediately in the next step of synthesis because of the lack of stability of this product.

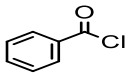
5: Preparation of  **5**.

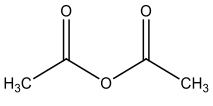


The proper amount of R was dissolved in DCM (10ml). This solution was then added dropwise to proper amount of 4-amino-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide which was dissolved in THF (20ml) and triethylamine (2ml). After that, the solution was left to stir over night. A brown precipitate was formed, filtered and dried under reduced pressure. In the next step, the product was extracted with aqueous solution saturated with sodium carbonate and ethyl acetate, then the solvent was removed under reduced pressure to get the product, which was purified by Dry Column Flash Chromatography (DCFC) and by recrystallization.

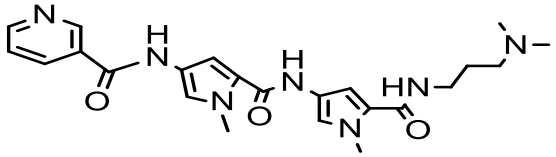
Where R functional chemical group can be any of the following:

I (R =  , nicotinyl chloride)

II (R =  , benzoyl chloride)

III (R =  , acetic anhydride)

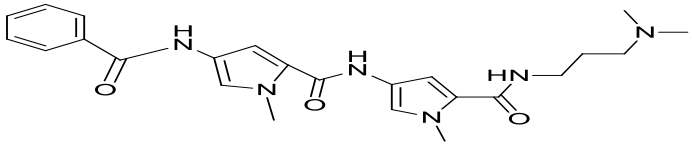
Preparation of N-(5-((5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)nicotinamide I(B4).



The reaction of nicotinyl chloride (0.6g, 0.00337mol) with 4-amino-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide (1g, 0.00289mol) produced (I). (0.3g, 23 %) (m.p.= 249-250 ºC, lit. not found).

IR:2943;2597;2492;2359;1702;1475;1442;1396;1320;1297;1171;1074;1033;849;806;745;691;639;554;520 cm^{-1}.

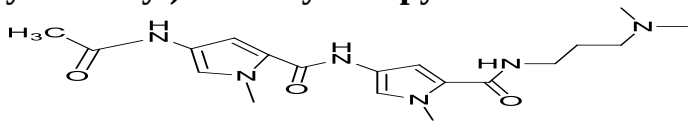
Preparation of4-benzamido-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide II(B5).



The reaction of benzoyl chloride (0.62g, 0.0044mol) with (4-amino-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide (1.5g, 0.00434mol) produced (II). (0.33g, 17%) (m.p.= 248-250 ºC, lit. not found).

IR:2977;2944;2737;2597;2491;1573;1473;1442;1395;1314;1170;1071;1033;848;805;706;581 cm^{-1}.

Preparation of 4-acetamido-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole -2-carboxamide III(B6).

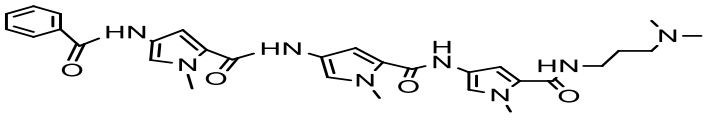


The reaction of acetic anhydride (0.5g, 0.00489mol) with 4-amino-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide (1.5g, 0.00434mol) produced (III). (0.3g, 18%) (m.p.= 149-151 ºC, lit. not found).

IR:3274;3138;2921;2851;2604;2497;1700;1646;1577;1537;1445;1391;1306;1248;1187;1096;1058;1030;958;808;750;698;598;565;526 cm^{-1}.

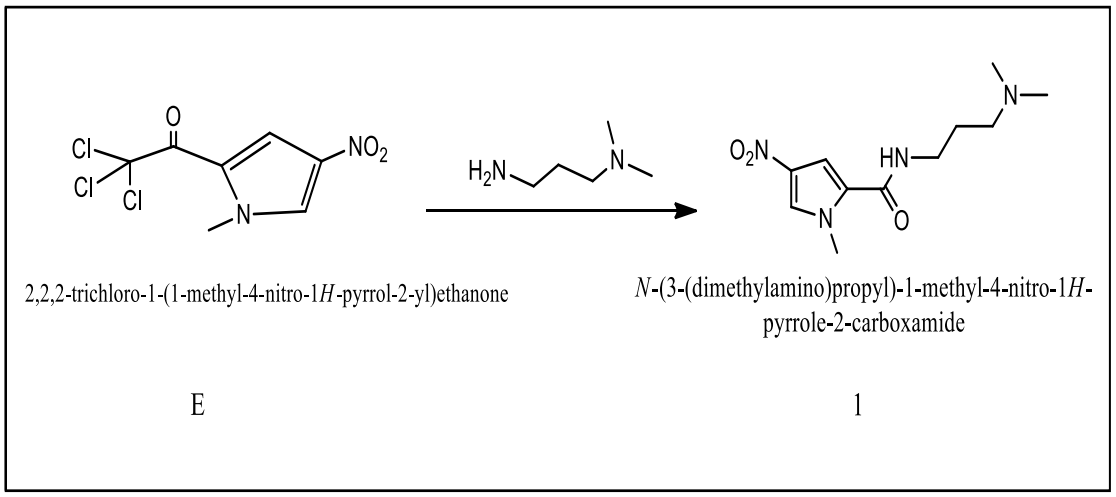
2.3.4 Preparation of B7 Compound

B7= 4-benzamido-N-(5-((5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide.



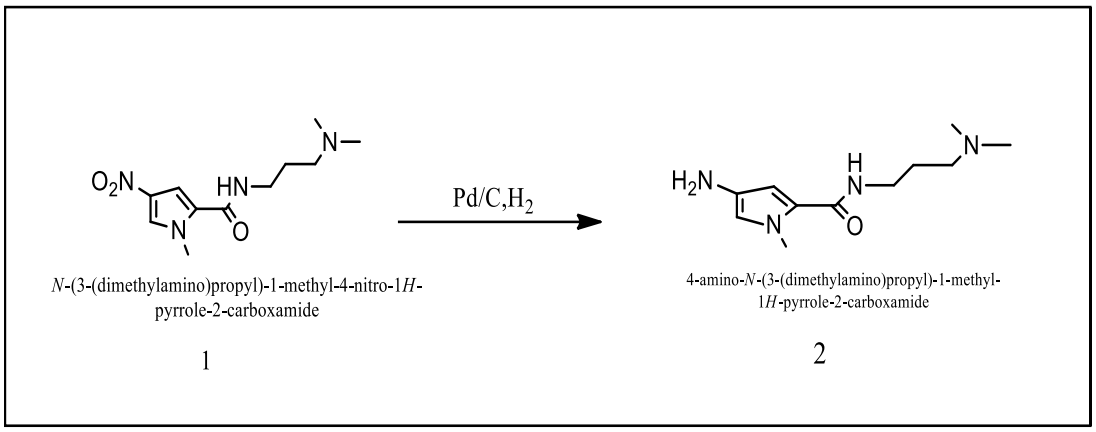
Shown in the next steps:

1: Preparation of N-(3-(dimethylamino)propyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide <u>1.</u>



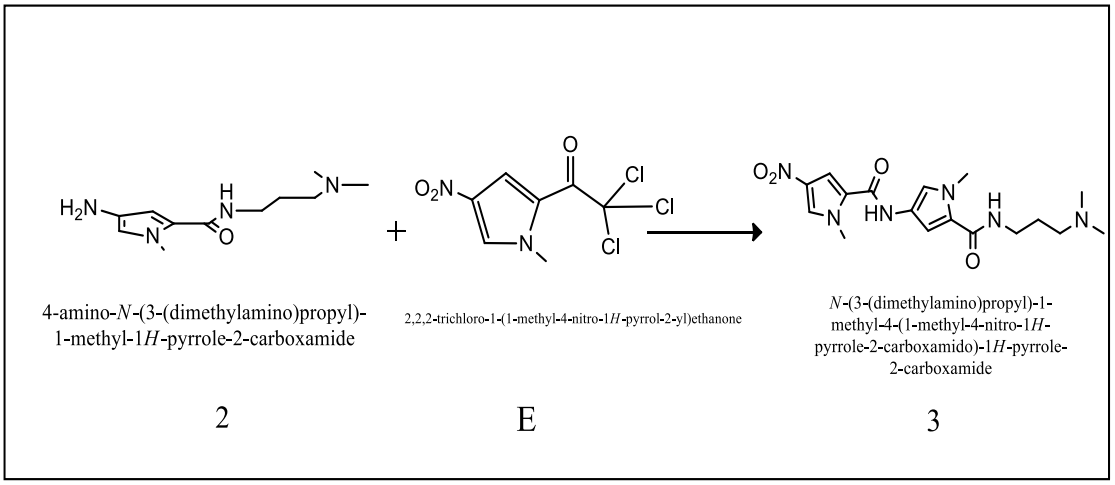
2,2,2-trichloro-1-(1-methyl-4-nitro-1H-pyrrol-2-yl) ethanone (2g, 0.00737mol) was dissolved in THF(40ml), 3-dimethylamino-1-propylamine (1g, 0.0098mol) was added to the solution and left to stir over night. The product was dried under reduced pressure, water (60ml) was added and the product was extracted with ethyl acetate(80ml) and then the solvent was removed under reduced pressure to get a bright yellow powder as a product.

2: Preparation of 4-amino-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide 2 .



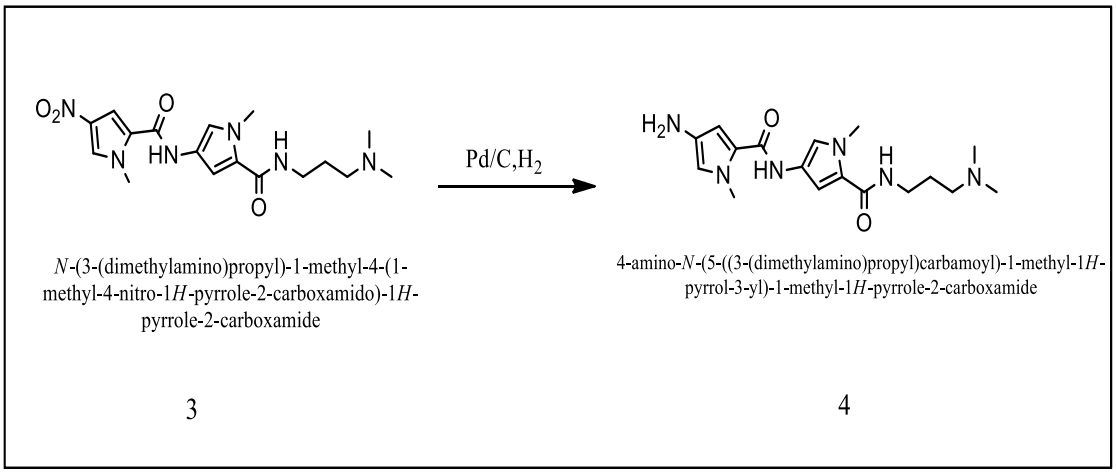
N-(3-(dimethylamino)propyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide (1.5g, 0.00625mol) was dissolved in (50ml) methanol and (5ml) THF in 250 ml round-bottomed flask,(0.5g) Pd/C was also added slowly at 0°C. After that, the suspension was placed under hydrogen, and left with stirring for 4 hr. The suspension was then filtered through silica gel (6g), and the solvent was removed under reduced pressure to get the product which was used immediately in the next step for synthesis because of the lack of stability of this product.

3: Preparation of N-(3-(dimethylamino)propyl)-1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamide 3.



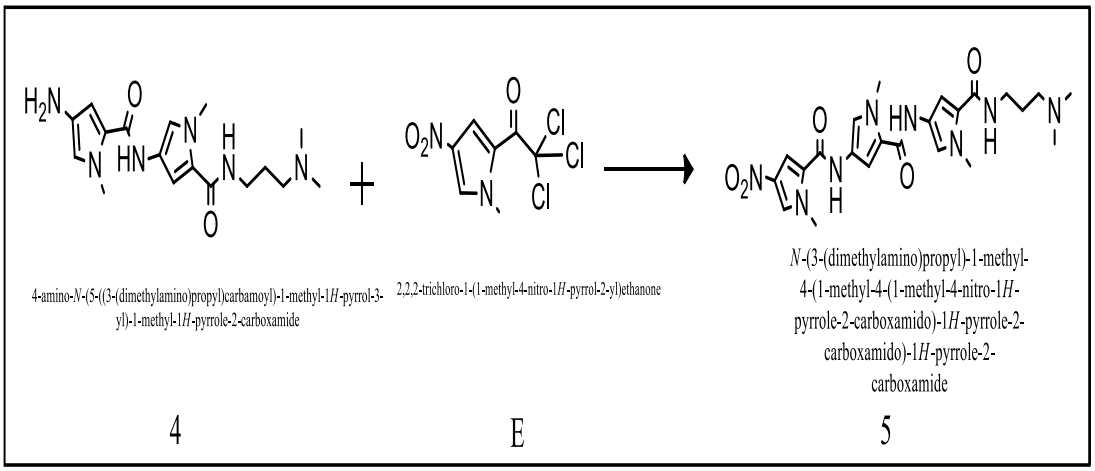
2,2,2-trichloro-1-(1-methyl-4-nitro-1H-pyrrol-2-yl) ethanone (1.8g, 0.00671mol) was dissolved in DCM (10ml). This solution was then added dropwise to 4-amino-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide(1.5g, 0.00669mol) which was dissolved in THF(20ml) and triethylamine (3ml). After that, the solution was left to stir over night. A brown precipitate was formed, filtered and dried under reduced pressure. In the next step, the product was extracted with aqueous solution saturated with sodium carbonate and ethyl acetate, then the solvent was removed under reduced pressure to get the product.

4: Preparation of 4-amino-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide.



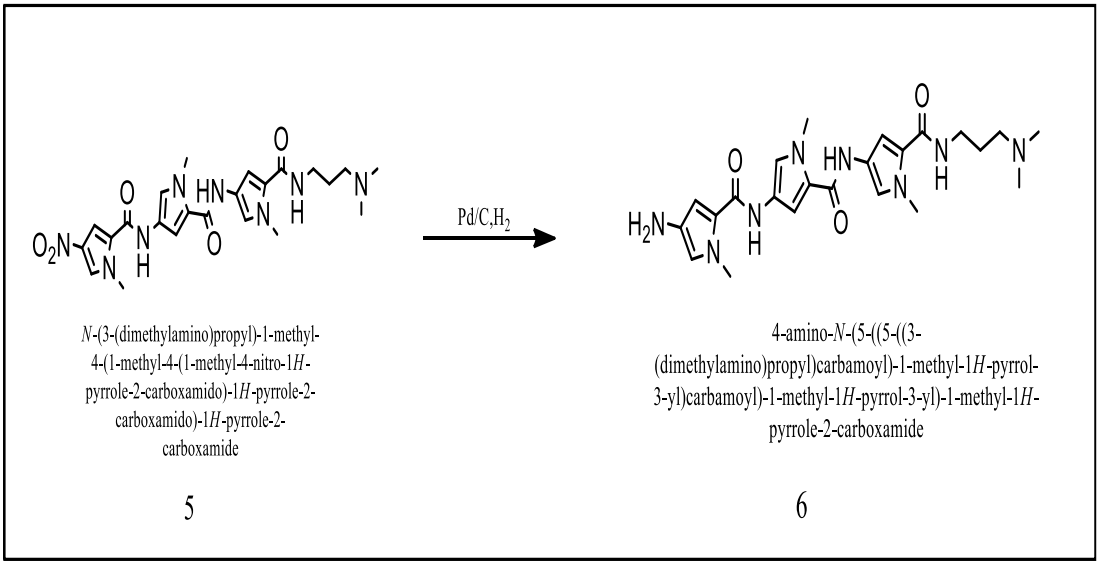
N-(3-(dimethylamino)propyl)-1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamide (2.3g,0.00637mol) was dissolved in (50ml) methanol and (5ml) THF in 250 ml round-bottomed flask, (0.9g) Pd/C was also added slowly at 0°C. After that, the suspension was placed under hydrogen, and left with stirring for 4 hr. The suspension was then filtered through silica gel (6g), and the solvent was removed under reduced pressure to get the product which was used immediately in the next step for synthesis because of the lack of stability of this product.

5: Preparation of N-(3-(dimethylamino)propyl)-1-methyl-4-(1-methyl-4-(1-methyl-4-nitro-1H-pyrrol-2-carboxamido)-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamide 5.



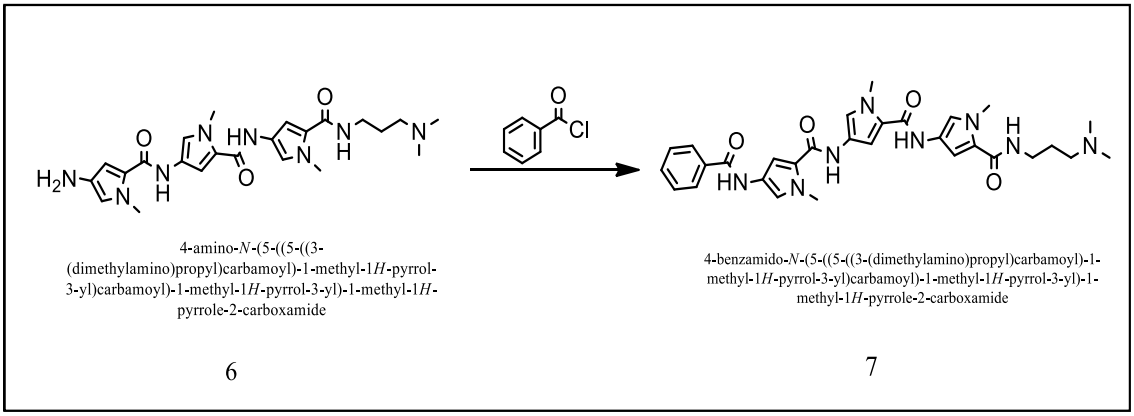
2,2,2-trichloro-1-(1-methyl-4-nitro-1H-pyrrol-2-yl) ethanone (1.56g, 0.0059mol) was dissolved in DCM (10ml). This solution was then added dropwise to 4-amino-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide (2g, 0.00578mol) which was dissolved in THF(20ml) and triethylamine (3ml). After that, the solution was left to stir over night. A brown precipitate was formed, filtered and dried under reduced pressure. In the next step, the product was extracted with aqueous solution saturated with sodium carbonate and ethyl acetate, then the solvent was removed under reduced pressure to get the product.

6: Preparation of 4-amino-N-(5-((5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide 6.



N-(3-(dimethylamino)propyl)-1-methyl-4-(1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamide (2.6g, 0.00522mol) was dissolved in (50ml) methanol and (5ml) THF in 250 ml round-bottomed flask,(0.9g) Pd/C was also added slowly at 0°C. After that, the suspension was placed under hydrogen, and left with stirring for 4 hr. The suspension was then filtered through silica gel (6g), and the solvent was removed under reduced pressure to get the product which was used immediately in the next step for synthesis because of the lack of stability of this product.

7: Preparation of 4-benzamido-N-(5-((5-((3-(dimethylamino) propyl) carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide .



Benzoyl chloride (0.3g, 0.00284mol) was dissolved in DCM (10ml). This solution was then added dropwise to 4-amino-N-(5-((5-((3-(dimethylamino) propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide(1g, 0.00214mol)which was dissolved in THF(20ml) and trimethylamine (2ml). After that, the solution was left to stir over night. A brown precipitate was formed, filtered and dried under reduced pressure. In the next step, the product was extracted with aqueous solution saturated with sodium carbonate and ethyl acetate, then the solvent was removed under reduced pressure to get the product, which was purified by Dry Column Flash Chromatography (DCFC) and by recrystallization.

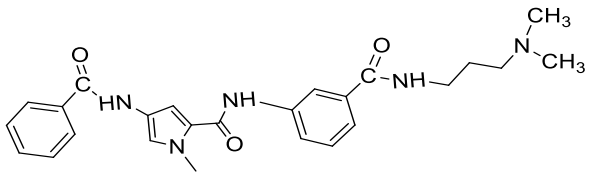
Yield; 0.15g, 13%, mp; 262-263.

IR:2977;2945;2738;2600;2494;1474;1442;1396;1170;1072;1033;850;805; 576;552 cm^{-1}.

^{1}H NMR: δ=H_3(1H):6.9; H_5(1H):7.3; H_6(3H):3.5; H_{14}(1H):6.6; H_{16}(1H):7.4; H_{17},H_{24},H_{36},H_7(4H):9.9;H_{19}(1H):6.7;H_{22}(1H):7.4; H_{23}(3H):3.5; H_{27}(3H):3.5; H_{31}(1H):7.9;H_{32}(1H):7.6;H_{33}(1H):7.6;H_{34}(1H):7.6;H_{35}(1H):7.9;H_{37}(2H):3.5; H_{38}(2H):1.4; H_{39}(2H):2.4; H_{41},H_{42}(6H):2.3 ppm.

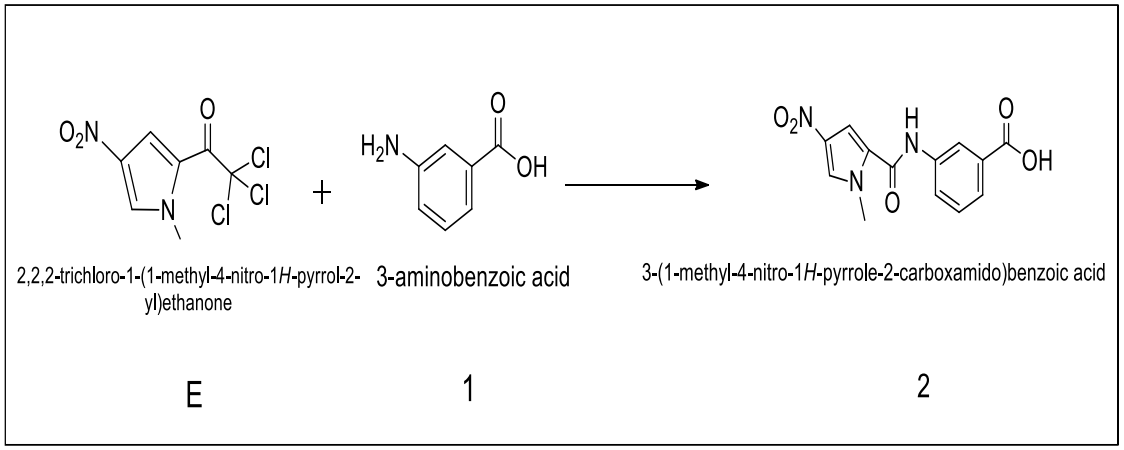
2.3.5 Preparation of B8 Compound.

B8 =4-benzamido-N-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)-1-methyl-1H-pyrrrole-2-carboxamide.



is shown in the next steps:

1: Preparation of3-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)benzoic acid2.

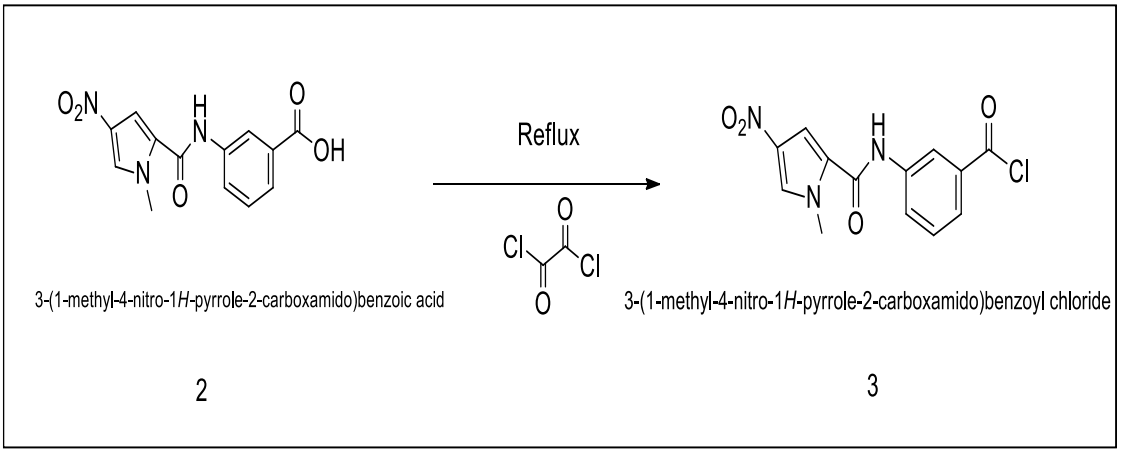


2,2,2-trichloro-1-(1-methyl-4-nitro-1H-pyrrol-2-yl) ethanone (2g, 0.007375mol) was dissolved in DCM(20ml). After that, 3-aminobenzoic acid (1.1g, 0.008mol) in THF(10ml) was added to the solution and allowed

to stir over night, during this time the product precipitated as a white solid, and then this product was dried under reduced pressure. Finally, water (60ml) was acidified and then added, and the product was extracted with ethyl acetate(80ml) and then the solvent was removed under reduced pressure to get the product as a white solid powder.

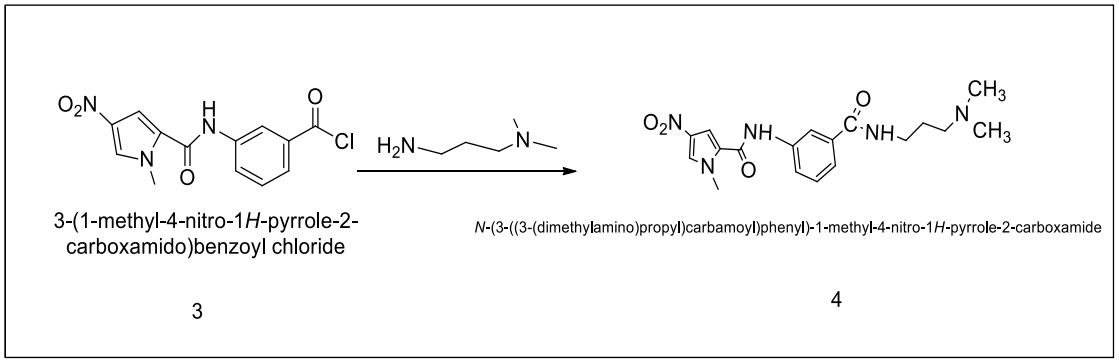
Yield; 2 g,94%, m.p. = 145-148°C.

2: Preparation of 3-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)benzoyl chloride 3.



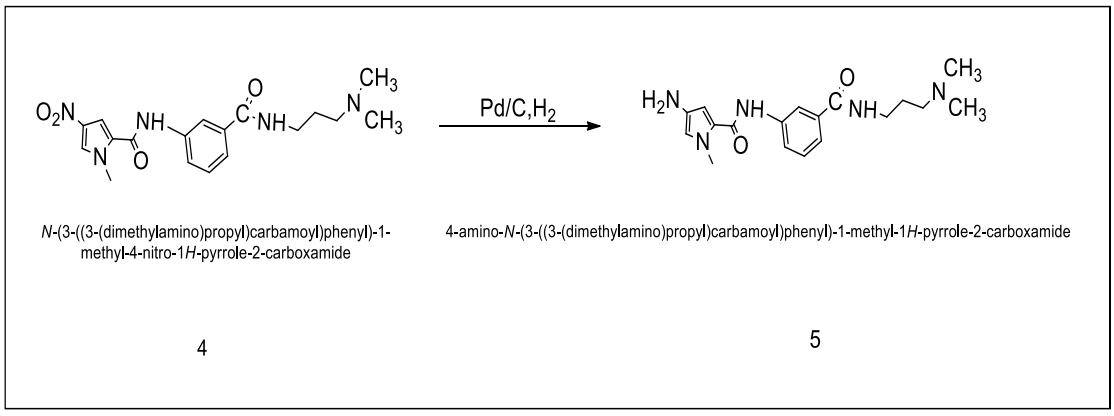
3-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)benzoic acid(2g, 0.00692mol)was dissolved in DCM (80ml) in a round-bottomed flask (250ml). DMF (1ml) was then added as a catalyst. After that, oxalyl chloride (10ml) was added and the solution was refluxed for 2 hr. Finally, the solvent was removed under reduced pressure to get the product which was used directly in the next step of synthesis because of the lack of stability of the acid chloride.

3: Preparation of N-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide 4.



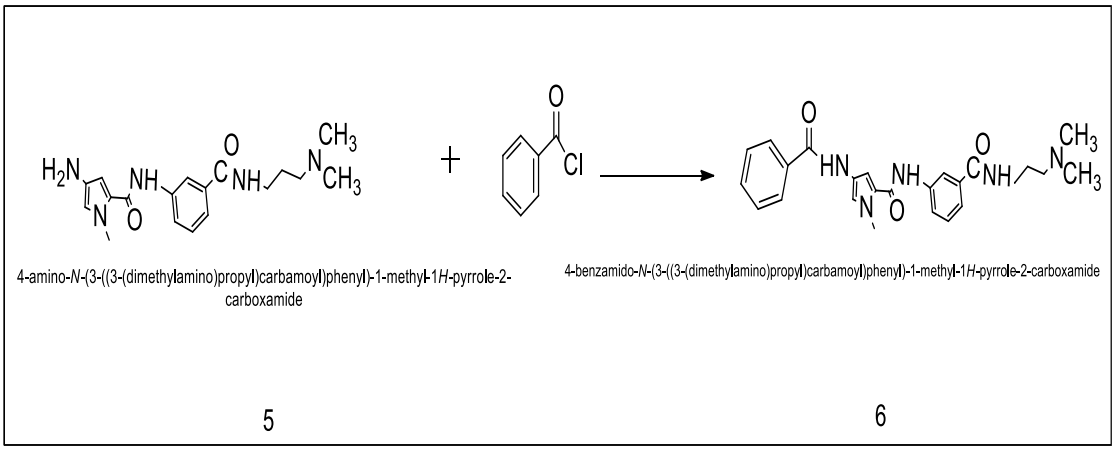
3-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)benzoyl chloride (3.5g, 0.01139mol) was dissolved in DCM (10ml). This solution was then added dropwise to 3-dimethylamino-1-propylamine(1.2g,0.01174mol) which was dissolved in THF(20ml) and triethylamine (3ml). After that, the solution was left to stir over night. A brown precipitate was formed, filtered and dried under reduced pressure. In the next step, the product was extracted with aqueous solution saturated with sodium carbonate and ethyl acetate, then the solvent was removed under reduced pressure to get the product.

4: Preparation of 4-amino-N-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)-1-methyl-1H-pyrrole-2-carboxamide 5 .



N-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide(1.7g, 0.00456) was dissolved in (70ml) methanol and (5ml) THF in 250 ml round - bottomed flask ,(0.5g) Pd/C was also added slowly at 0°C. After that, the suspension was placed under hydrogen, and left with stirring for 4 hr. The suspension was then filtered through silica gel (6 g), and the solvent was removed under reduced pressure to get the product which was used immediately in the next step of synthesis because of the lack of stability of this product.

5: Preparation of 4-benzamido-N-(3-((3-(dimethylamino) propyl) carbamoyl)phenyl)-1-methyl-1H-pyrrole-2-carboxamide6.



Benzoyl chloride (0.5g, 0.00356mol) was dissolved in DCM (10ml). This solution was then added dropwise to 4-amino-N-(3-((3-(dimethylamino) propyl) carbamoyl) phenyl)-1-methyl-1H-pyrrole-2-carboxamide (1.2g, 0.0035mol) which was dissolved in THF (20ml) and trimethylamine (2ml). After that, the solution was left to stir over night. A brown precipitate was formed, filtered and dried under reduced pressure. In the next step, the product was extracted with aqueous solution saturated with sodium

carbonate and ethyl acetate, then the solvent was removed under reduced pressure to get the product, which was purified by Dry Column Flash Chromatography (DCFC) and by recrystallization.

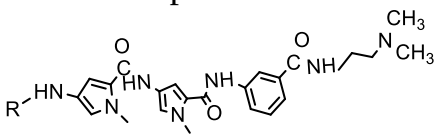
Yield;0.4g,26%, m.p= 260-261°C

IR:3370;2977;2944;2737;2597;2529;2493;2358;1622;1539;1473;1441;1395;1170;1071;1032;849;804;559;536;519 cm^{-1}.

^{1}H NMR: δ =H_3(1H):7.3; H_5(1H):7.2;H_6(3H):3.5; H_{13}(1H):7.9; H_{14}(1H):7.7; H_{15}(1H):7.7; H_{16}(1H)7.7; H_{17}(1H):7.9; H_{18},H_{28},H7(3H):7.8; H_{21}(1H):7.4; H_{22}(1H):7.6;H_{23}(1H):7.7;H_{25}(1H):7.5;H_{29}(2H):3.4;H_{30}(2H):1.4;H_{31}(2H):2.4; ,H_{33},H_{34}(6H):2.3 ppm.

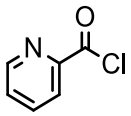
2.3.6: Preparation of B9, B10 Compounds

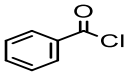
The general structure of these compounds is

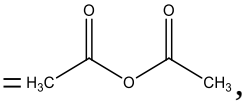


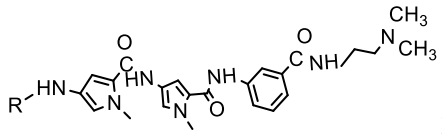
The difference between them is in the last amide link.

Where R functional chemical group can be any of the following:

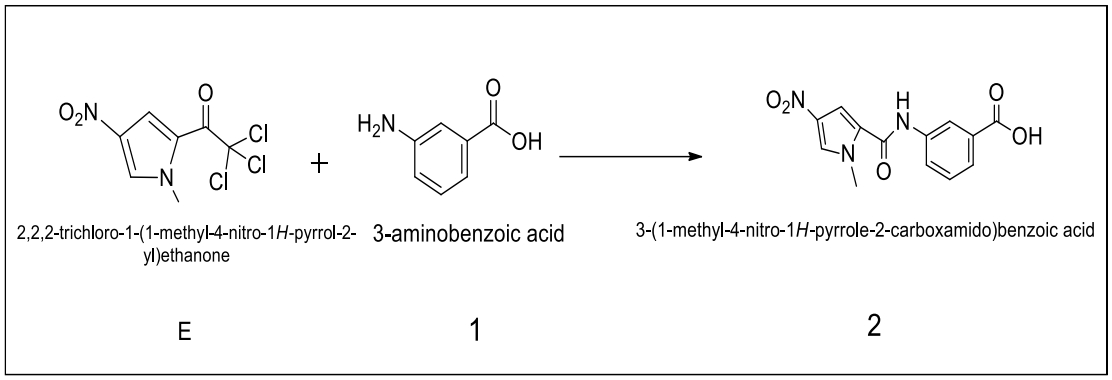
I (R =  , nicotinyl chloride).

II (R =  , benzoyl chloride).

II (R =  , acetic anhydride).

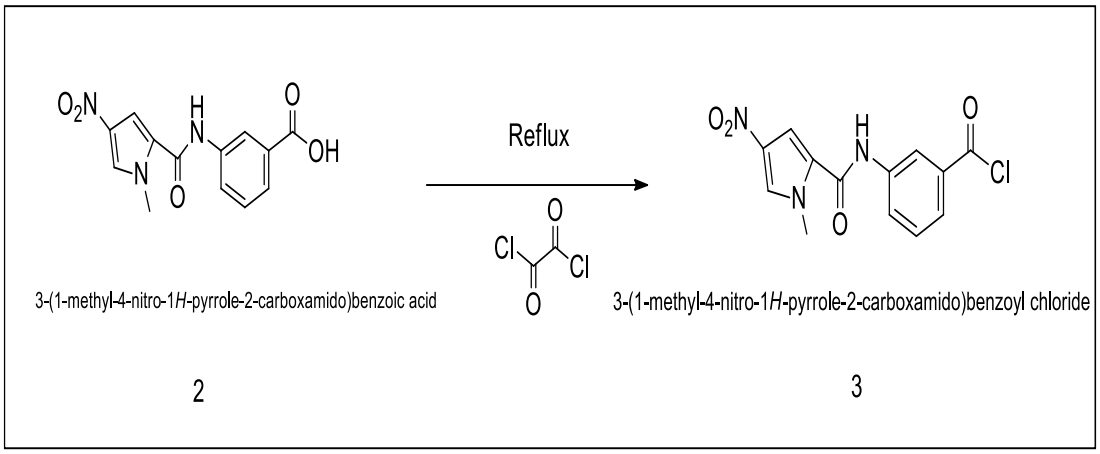
Preparation of  **is shown in the next steps:**

1:**Preparation of 3-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)benzoic acid 2.**



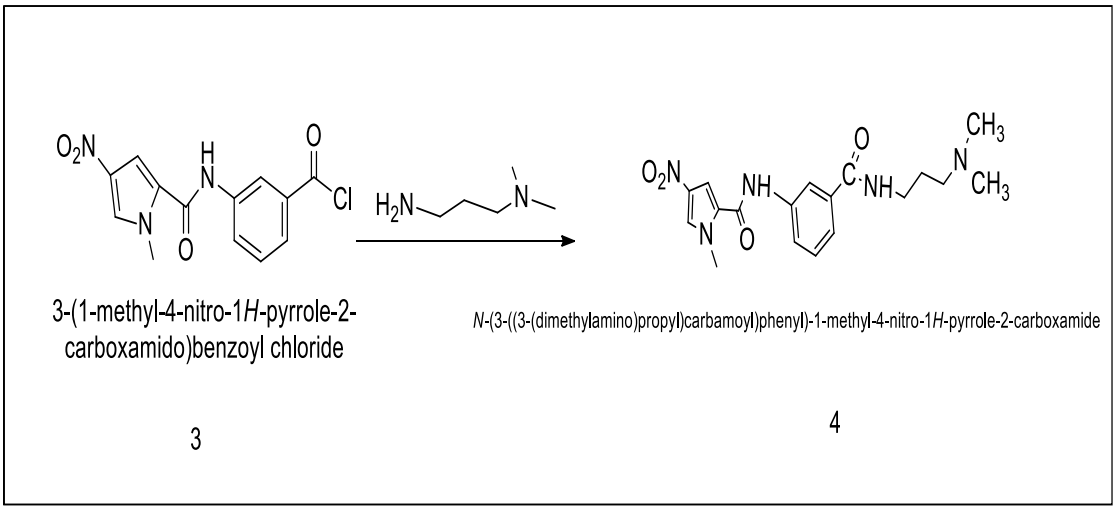
2,2,2-trichloro-1-(1-methyl-4-nitro-1H-pyrrol-2-yl) ethanone (2g, 0.00737mol) was dissolved in DCM (20ml). After that, 3-aminobenzoic acid (1.1g, 0.008mol) in THF(10ml) was added to the solution and left to stir over night, during this time the product precipitated as a white solid, and then this product was dried under reduced pressure. Finally, water (60ml) was acidified and then added, and the product was extracted with ethyl acetate (80ml) and then the solvent was removed under reduced pressure to get the product as a white solid powder.

2: Preparation of 3-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)benzoyl chloride 3.



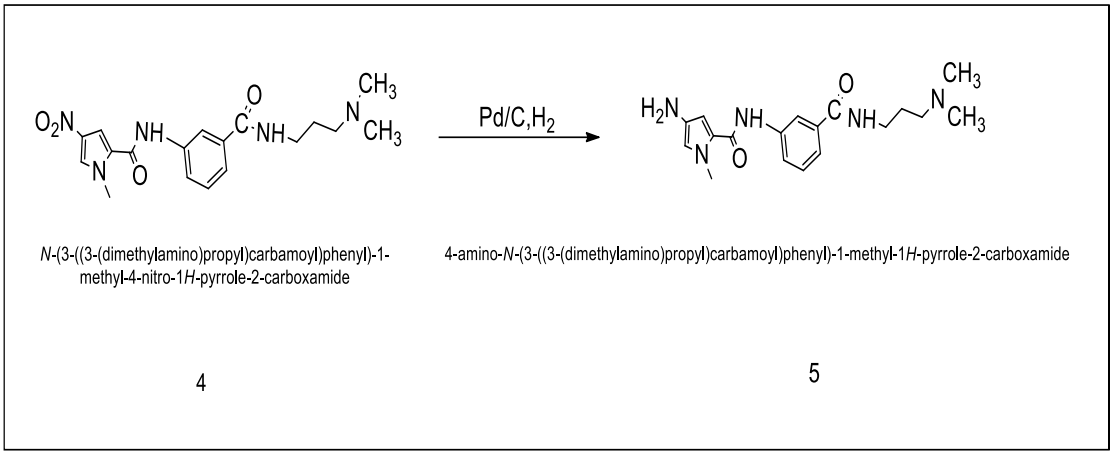
3-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)benzoic acid (2g, 0.00691mol) was dissolved in DCM (80 ml) in a round-bottomed flask (250ml). DMF (1 ml) was then added as a catalyst. After that, oxalyl chloride (10ml) was added and the solution was refluxed for 2 hr. Finally, the solvent was removed under reduced pressure to get the product which was used directly in the next step of synthesis because of the lack of stability of the acid chloride.

3: Preparation of N-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide 4.



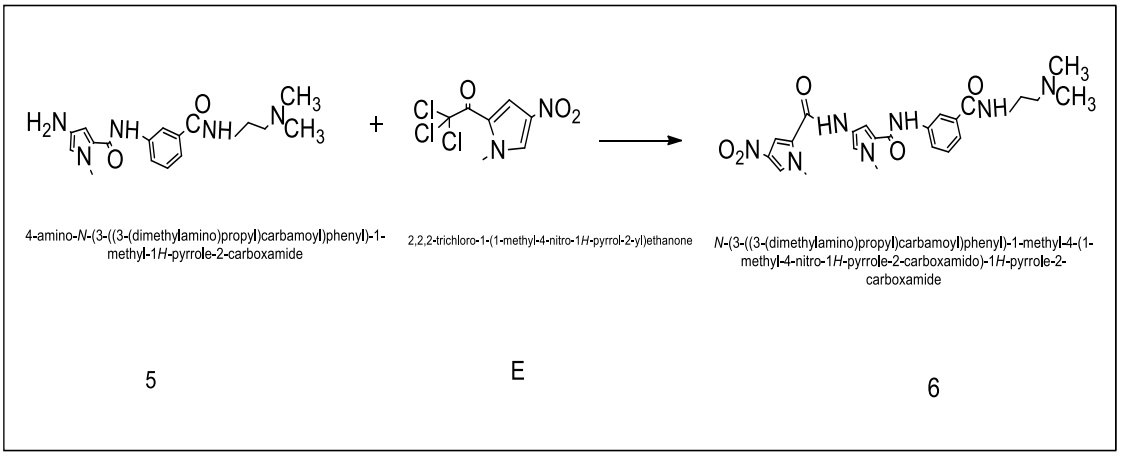
3-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)benzoyl chloride (3.5g, 0.01137mol) was dissolved in DCM (10ml). This solution was then added dropwise to 3-dimethylamino-1-propylamine (1.2g, 0.01174mol) which was dissolved in THF(20ml) and triethylamine (3ml). After that, the solution was left to stir over night. A brown precipitate was formed, filtered and dried under reduced pressure. In the next step, the product was extracted with aqueous solution saturated with sodium carbonate and ethyl acetate, then the solvent was removed under reduced pressure to get the product.

4: Preparation of 4-amino-N-(3-((3-(dimethylamino) propyl) carbamoyl) phenyl)-1-methyl-1H-pyrrole-2-carboxamide 5 .



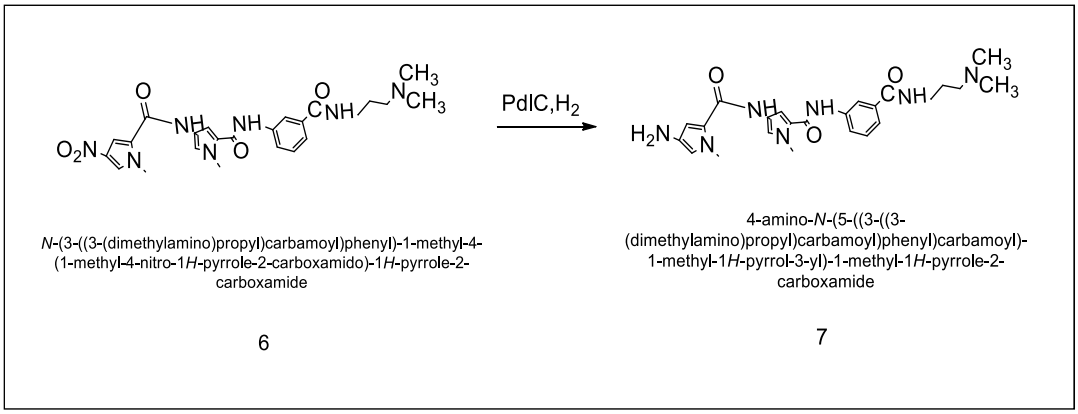
N-(3-((3-(dimethylamino) propyl)carbamoyl)phenyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide(4g, 0.0107) was dissolved in (70ml) methanol and (5ml) THF in 250 ml in round-bottomed flask,(1.3g) Pd/C was also added slowly at 0°C. After that, the suspension was placed under hydrogen, and left with stirring for 4 hr. The suspension was then filtered through silica gel (6g), and the solvent was removed under reduced pressure to get the product which was used immediately in the next step of synthesis because of the lack of stability of this product.

5: Preparation of N-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)-1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamide.

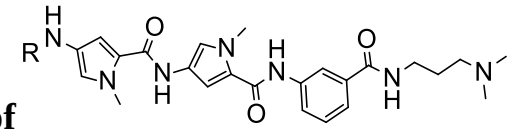


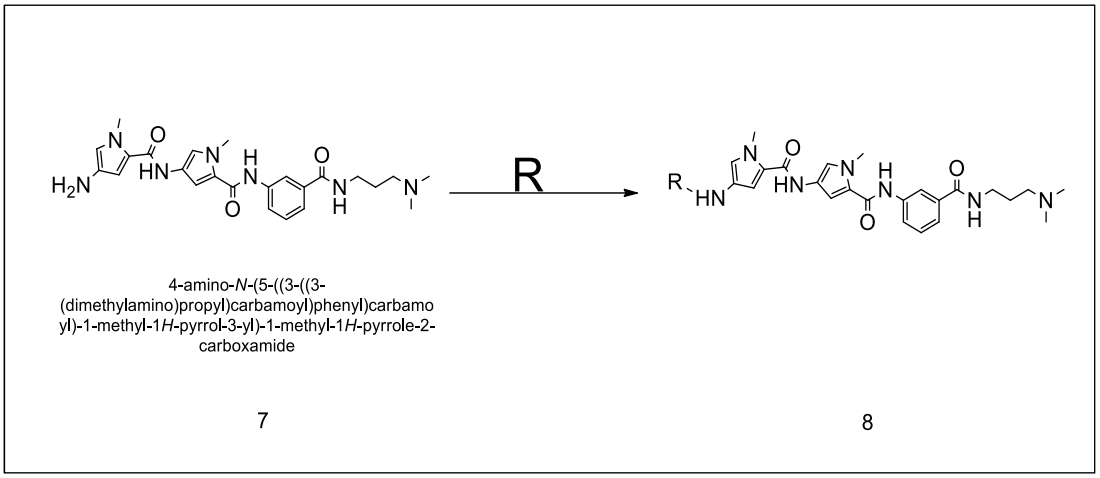
2,2,2-trichloro-1-(1-methyl-4-nitro-1H-pyrrol-2-yl) ethanone (2.4g,0 .00884mol) was dissolved in DCM (20ml). This solution was then added dropwise to 4-amino-N-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)-1-methyl-1H-pyrrole-2-carboxamide (3g,0.00873mol) which was dissolved in THF(30ml) and trimethylamine (5ml). After that, the solution was left to stir over night. A brown precipitate was formed, filtered and dried under reduced pressure. In the next step, the product was extracted with aqueous solution saturated with sodium carbonate and ethyl acetate, then the solvent was removed under reduced pressure to get the product.

6: Preparation of 4-amino-N-(5-((3-((3-(dimethylamino) propyl) carbamoyl) phenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide.



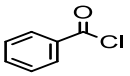
N-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)-1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamide (2.5g, 0.00505) was dissolved in (80ml) methanol and (5ml) THF in 250 ml round-bottomed flask,(0.8g) Pd/C was also added slowly at 0°C. After that, the suspension was placed under hydrogen, and left with stirring for 4 hr. The suspension was then filtered through silica gel (6 g), and the solvent was removed under reduced pressure to get the product which was used immediately in the next step of synthesis because of the lack of stability of this product.

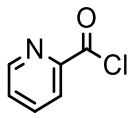
7: Preparation of  .



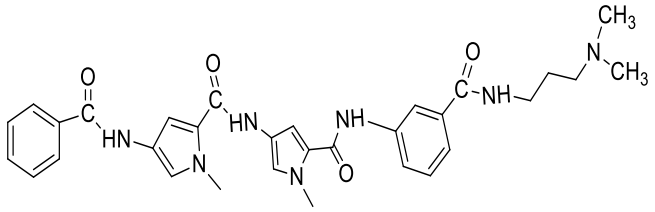
The proper amount of R was dissolved in DCM (10ml). This solution was then added dropwise to 4-amino-N-(5-((3-((3-(dimethylamino) propyl) carbamoyl)phenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide(1g, 0.00215mol) which was dissolved in THF(20ml) and trimethylamine (2ml). After that, the solution was left to stir over night. A brown precipitate was formed, filtered and dried under reduced pressure. In the next step, the product was extracted with aqueous solution saturated with sodium carbonate and ethyl acetate, then the solvent was removed under reduced pressure to get the product, which was purified by Dry Column Flash Chromatography (DCFC) and by recrystallization.

Where R functional chemical group can be any of the following:

I (R =  , benzoyl chloride)

II (R =  , nicotinyl chloride)

Preparation of4-benzamido-N-(5-((3-((3-(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide I(B9).

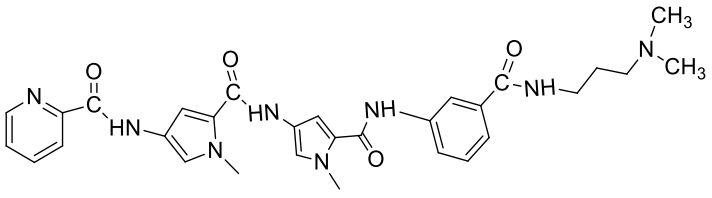


The reaction of benzoyl chloride (0.3g, 0.00221mol) with 4-amino-N-(5-((3-((3-(dimethylamino) propyl) carbamoyl) phenyl) carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide(1g, 0.00215mol) produced (I).(0.3g,25%) (m.p.= 260-262 ºC, lit. not found).

IR:3856;3752;3378;2977;2944;2738;2599;2529;2494;1542;1474;1442;1395;1170;1072;1033;849;805;709;516 cm^{-1}.

^{1}H NMR:δ=H_3(1H):6.7; H_5(1H):7.1; H_6(3H):3.5; H_{14}(1H):6.6; H_{16}(1H):7.4; H_{17},H_{26},H_{37},H_7(4H):8.7;H_{20}(1H):7.4;H_{21}(1H):7.6;H_{22}(1H):7.7; H_{24}(1H):7.5; H_{25}(3H):3.5;H_{32}(1H):7.9;H_{33}(1H):7.6;H_{34}(1H):7.6;H_{35}(1H):7.6;H_{36}(1H):7.9; H_{38}(2H)3.4; H_{39}(2H):1.4; H_{40}(2H):2.4; H_{42},H_{43}(6H):2.3 ppm.

Preparation ofN-(5-((5-((3-((3-(dimethylamino)propyl)carbamoyl) phenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)picolinamide II(B10).



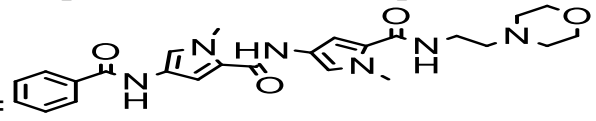
The reaction of nicotinyl chloride (0.4g, 0.0022mol) with4-amino-N-(5-((3-((3-(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)-1-methyl-1H-

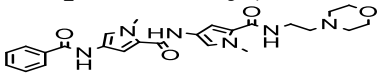
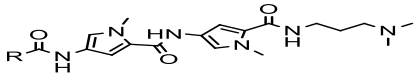
pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide(1g, 0.00215mol produced (II). (0.4g, 33%) (m.p.= 260-262 ºC, lit. not found).

IR:2978;2946;2739;2604;2531;2498;2360;1707;1546;1477; 1444;1397;1172; 1073; 1036; 851; 807; 669; 576; 563; 544; 526;512 cm^{-1}.

^{1}H NMR: δ=H_3(1H):6.7; H_5(1H):7.1; H_6(3H):3.5; H_{14}(1H):6;H_{16}(1H):6.8; H_7,H_{17},H_{26},H_{37}(4H):8.6;H_{20}(1H):7.4;H_{21}(1H):7.6; H_{22}(1H):7.7; H_{24}(1H):7.5; H_{25}(3H):3.5;H_{33}(1H):8.9;H_{34}(1H):7.8;H_{35}(1H):8.2;H_{36}(1H):8.3;H_{38}(2H):3.4; H_{39}(2H):1.4; H_{40}(2H):2.4; H_{42},H_{43}(6H):2.3 ppm.

2.3.7 Preparation of B11 Compound

B11=  .

Preparation of 4-benzamido-1-methyl-N-(1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)-1H-pyrrole-2-carboxamide  **in the same procedure of the preparation of**  **(2.3.3).**

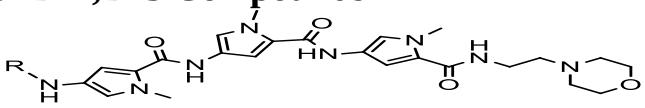
The reaction of benzoyl chloride (0.75g, 0.00569mol) with (4-amino-1-methyl-N-(1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)-1H-pyrrole-2-carboxamide(2g, 0.00535mol) produced(2.3.7). (0.15g, 5%) (m.p.= 261-262 ºC, lit. not found).

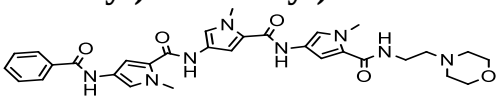
IR:3384;2977;2944;2737;2601;2530;2495;1542;1473;1441;1395;1363;1170;1071;1032;849;804;605;566;549;527;504 cm^{-1}.

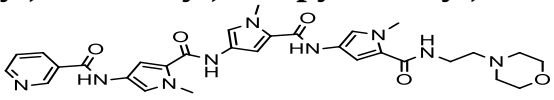
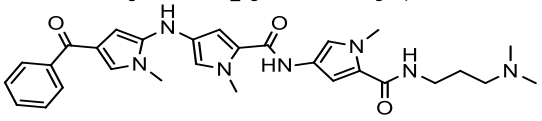
^{1}H NMR: δ =H_2(2H):3.5; H_3(2H):2.4; H_5(2H):2.4; H_6(2H):3.5; H_7(2H)2.9; H_8(2H):3.7;H_{14}(1H):6.7;H_{16}(1H):7.5;H_{17}(3H):3.5;H_{18},H_{27},H_9(3H):9.9;

H_{22}(1H):7.3;H_{25}(1H):7.5;H_{26}(3H):3.5;H_{31}(1H):7.9;H_{32}(1H):7.7;H_{33}(1H):7.7; H_{34}(1H):7.7; H_{35}(1H):7.9 ppm.

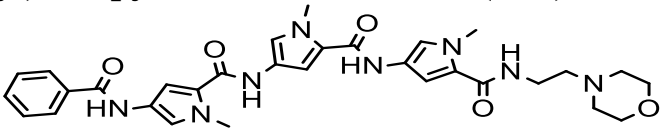
2.3.8Preparation of B12,B13 Compounds

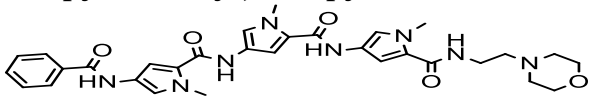
General structure 

Preparation of of 4-benzamido-1-methyl-N-(1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)-1H-pyrrole-2-carboxamide 

, and preparation of N-(1-methyl-5-((1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)nicotinamide  **,in the same procedure of the preparation of4-((4-benzoyl-1-methyl-1H-pyrrol-2-yl)amino)-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide**  **(2.3.4)(B7).**

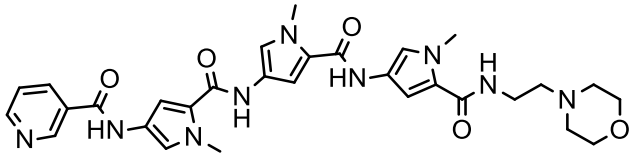
Preparation of 4-benzamido-1-methyl-N-(1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)-1H-pyrrole-2-carboxamide(B12).

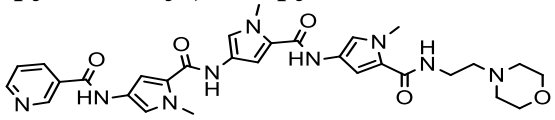


The reaction of benzoyl chloride (0.71g, 0.00505mol) with 4-amino-1-methyl-N-(1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrole-3-yl)-1H-pyrrole-2-carboxamide(2.5g, 0.00504mol) produced  . (0.1g, 3%) (m.p.= 249-251 ºC, lit. not found).

IR:3855;3822;3752;3736;3676;3650;3630;3139;2976;2946;2738;2599;2530;2494;1705;1538;1474;1442;1420;1396;1365;1313;1249;1190;1171;1112;1072;1033;984;943;851;827;805;749;660;585;550;537;514 cm^{-1}.

Preparation of N-(1-methyl-5-((1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)nicotinamide(B13)



The reaction of nicotinyl chloride (0.9g, 0.00506mol) with 4-amino-1-methyl-N-(1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrole-3-yl)-1H-pyrrole-2-carboxamide(2.5g, 0.00504mol) to produced  (0.1g, 3%) (m.p.= 261-262 ºC, lit. not found).

IR:3752;2976;2943;2738;2598;2530;2494;1541;1474;1442;1396;1364;1330;1170;1072;1033;849;805;631;590;577;552;537;520;510 cm^{-1}.

2.4 Results and Discussion

2.4.1Obstacle Faced while Carrying out this Project

The products' amounts were very small, since the starting materials were very expensive, and we couldn't afford the expenses. For that, we used small amounts of starting materials.

These synthetic reactions are multi-steps reactions which also affected the yield and the process consumed extra time.

The reduction step was the most difficult and time consuming.

2.4.2 NMR

NMR Spectra for B7Compound

(7.3-9.1 ppm) many complex signals for the aromatic protons and amide protons.

A signal at (2.3ppm) for two CH_3 on nitrogen.

Signals at (3.5-4.8 ppm) which is expected to the pyrrole CH_3.

Signals between (2.3-3.5) for two CH_2 on nitrogen.

A signal at (1.4ppm) for aliphatic CH_2.

NMR Spectra for B10Compound

(7.3-9.1 ppm) many complex signals for the aromatic protons and amide protons.

A signal at (2.3ppm) for two CH_3 on nitrogen.

Signals at (3.5-4.8 ppm) which is expected to the pyrrole CH_3.

Signals between (2.3-3.5) fortwoCH_2 on nitrogen.

A signal at (1.4ppm) for aliphatic CH_2.

2.4.3 Melting point

In general, the majority of products have high melting points more than 240 C °. For examples, melting point for B1(263-265), B9(260-262),B11(261-262).

2.4.4 IR

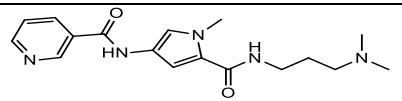
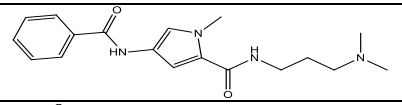
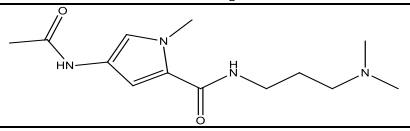
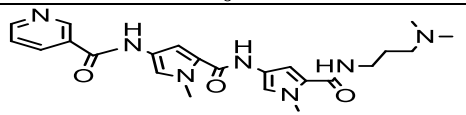
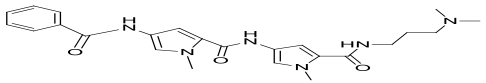
IR spectra for our products are shown in the appendix.

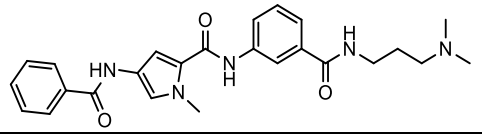
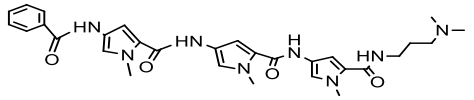
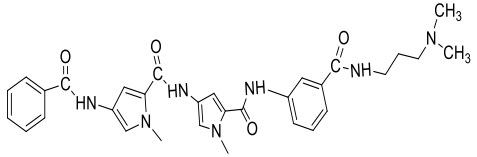
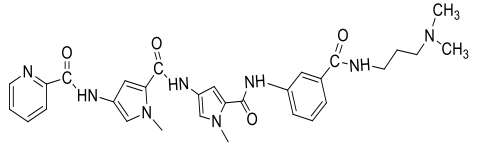
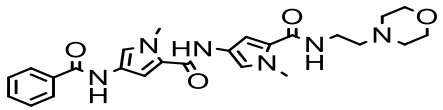
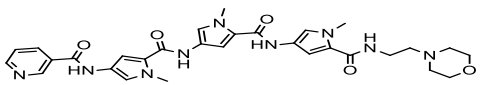
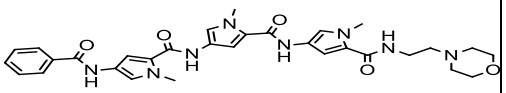
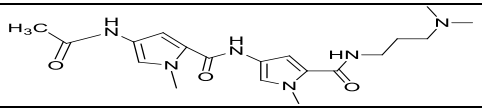
For instance, compoud B7showed absorption bands around 2945cm^{-1} (CH, str), 1474 cm^{-1} the highly conjugated(C=O, str), 1072 cm^{-1}(CN, str), 850cm^{-1}(Aromatic CH, str).

Chapter Three

Biological Activities

Table (3.1):Synthesized Compounds Used for Biological Activity

Abbreviations for Compounds in Chapter 3 Only	Compound	Formula	Abbreviations for Compounds in Chapter 2
B1	N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)nicotinamide		B1
B2	4-benzamido-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide		B2
B3	4-acetamido-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide		B3
B4	N-(5-((5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)nicotinamide		B4
B5	4-benzamido-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide		B5

B6	4-benzamido-N-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)-1-methyl-1H-pyrrole-2-carboxamide		B8
B7	4-benzamido-N-(5-((5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide		B7
B8	4-benzamido-N-(5-((3-((3-(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide		B9
B9	N-(5-((5-((3-((3-(dimethylamino)propyl)carbamoyl)phenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)picolinamide		B10
B10	4-benzamido-1-methyl-N-(1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)-1H-pyrrole-2-carboxamide		B11
B11	N-(1-methyl-5-((1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)nicotinamide		B13
B12	4-benzamido-1-methyl-N-(1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)-1H-pyrrole-2-carboxamide		B12
B13	4-acetamido-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole -2-carboxamide		B6

3.1 Introduction

This research is focused on the synthesis of molecules that possess unique biological activity, useful for biological and chemical research. Also, it is intended for controlling post-translational protein modifications and elucidating the relationship between biological activity and the structures of compounds. Also, this research study the behavior of molecules in 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 [49]. 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 [50]. Different Biological assays were done to test the activities of the synthesized compounds such as antioxidant activity, reductive potential, antibacterial and antifungal activities.

3.2 Materials and Methods

1-Chemicals

Chloramphenicol, peptone, agar, dextrose, ethanol, Muller–Hinton agar, gentamicin, ampicilline, chloramphenicol, econazole, and1,1- diphenly-2-picrylhydrazyl (DPPH). All chemicals and reagents were of analytical grade.

2- 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 radicals by DPPH in percent (I%) was calculated in following way:

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

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

3-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, which was then 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 $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm in a Shimadzu 160-UV spectrophotometer [51].

4- Antibacterial Activity Testing

The antibacterial activity of the synthesized compounds was determined against the following microorganisms: Staphylococcus aureus (ATCC 25923), Salmonella, (ATCC14028), Klebsiella pneumonia (ATCC 13883), Proteus vulgaris (ATCC 13315), and Pseudomonas aeruginosa (ATCC 27853), All 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 the 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) and placed on the inoculated agar. (4 discs per agar plate).. After 24 hr 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).

5- Antifungal Activity Testing

The antifungal activity test was done against the following dermatophytes: Trichophyton rubrum (CBS 392.58), Trichophyton mentagophytes (CBS 106.67and Microsporum canis (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[51]. 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 control, 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.

3.3 Results and Discussion

Antioxidant activity

Table 2 and figure 1 showed the percent inhibition of the tested compounds in DPPH assay. None of the compounds showed significant antioxidant activity compared with Gallic acid(percent inhibition =91.34 at the concentration 150 µg/ml and IC50=62 µg/ml) Figure 3. Values of percent

inhibition for the compounds were (B7=28.95 and B9=23.51 at the concentration 125 µg/ml, respectively.

Table (3.2): DPPH Assay for the Compounds

	Compounds												
Conc.	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	G.A
25	-2.56	-1.11	-3.39	-3.66	-3.96	-1.7	-1.63	-1.65	-0.43	-3.39	-1.8	-2.42	20
50	-1.38	-0.94	-3.18	-2.7	-2.32	-0.4	0.43	-0.27	0.11	-0.71	0.74	-2.07	44
75	-0.42	0.77	-1.75	-2.19	-1.26	-0.8	2.16	1.95	0.53	2.77	1.85	-1.95	61
100	0.06	2.5	-1.53	-0.45	-1.13	3.92	4.04	4.56	0.8	6.49	14.3	-1.37	72
125	2.72	8.21	-0.38	1.15	-0.14	5.79	29	7.28	23.5	9.71	18.5	-1.01	81
													92

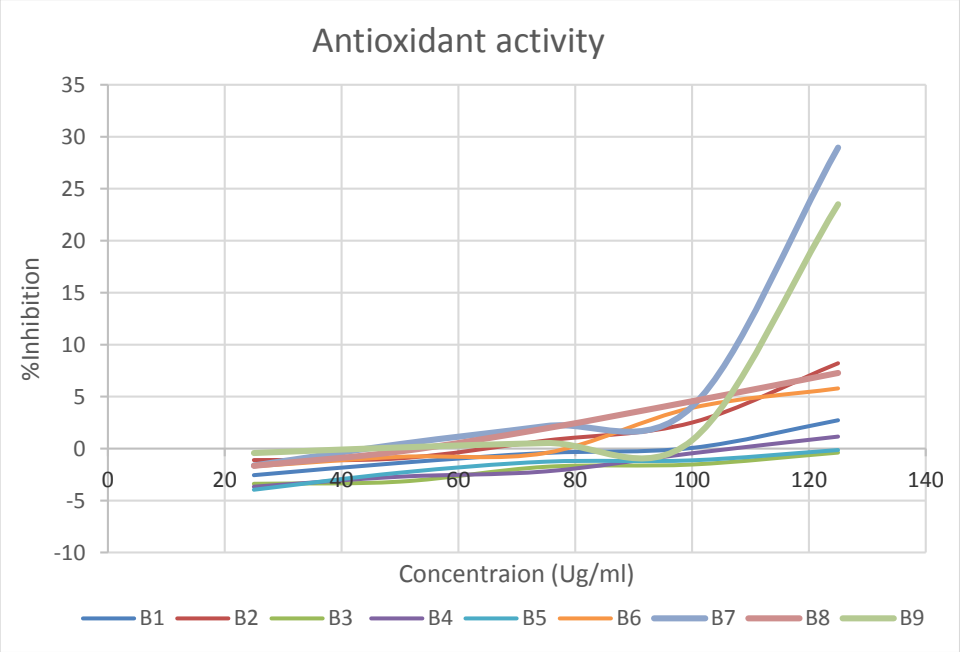


Figure (3.1): Antioxidant Activity of the Compounds

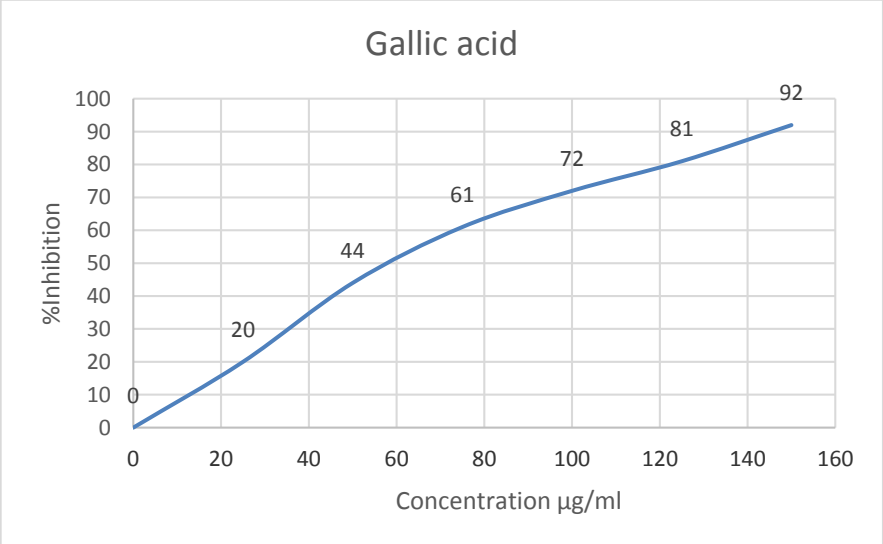


Figure (3.2): Antioxidant Activity of Gallic Acid

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 [51].The following Table and Figure showed the reductive potential activity of the compounds compared with Gallic acid as a standard. Compounds (B3=601.8 and B13=277.2) revealed the highest values compared with (Gallic acid=470.7). (B1=133.4, B2=123.6, B4=118)were the second set in the activity. The other compounds are relatively very low compared to Gallic acid.

Table (3.3): Reductive Potential for Compounds and Gallic Acid

Comp.	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	Gallic
Red. Pot	133.4	123.6	601.8	118.7	34.1	40.4	62.6	30.1	14	7.4	12.1	7	277.2	470.7

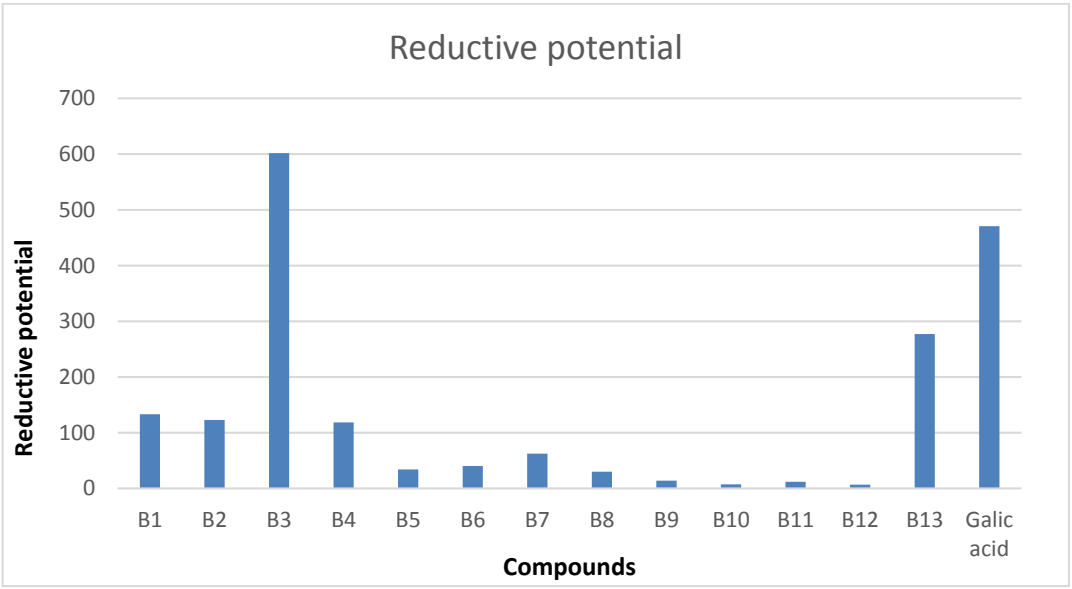


Figure (3.3): Reductive Potential for the Compounds and Gallic Acid

Antibacterial Activity

Figure 4 showed the activity of the tested compounds against six types of bacteria, using disk diffusion method. None 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).

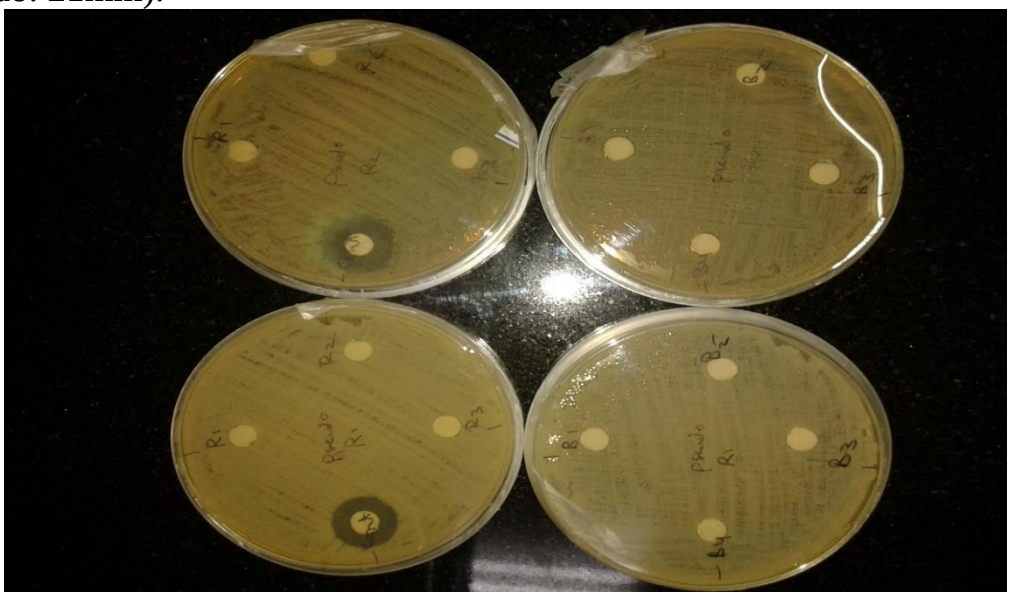


Figure (3.4): Disk Diffusion Test Against Bacteria Strains

Antifungal Activity

Table 4 and Figure5 showed the activity of the compounds at different concentrations against *M. canis* . Compound B8 revealed 81.1% at the concentration 240 µg/ml and B5 revealed 79.4% at the same concentration

Table (3.4): Inhibition Percent of M. canis CBS 132.88

Compd.	Conc.ug/ml	% Inhibition
B1c1	50	17.7
B1C2	200	22.8
B2C1	25	28.5
B2C2	100	45.6
B3C	25	12
B3C2	100	31.6
B4C1	25	7
B4C2	100	21.5
B4C3	200	58.2
B5C1	120	31.1
B5C2	240	79.4
B6C1	120	28.3
B6C2	240	62.2
B7C1	120	18.3
B7C2	240	24.4
B8C1	120	18.3
B8C2	240	81.1

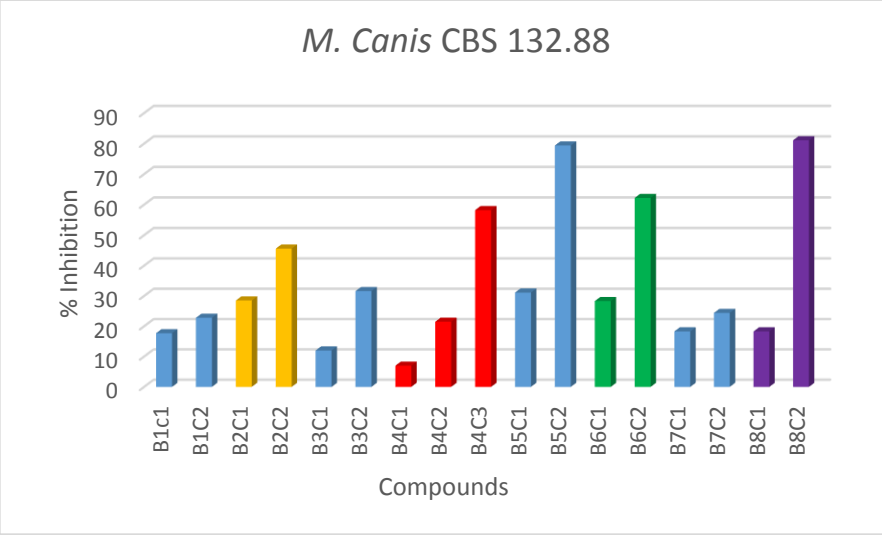


Figure (3.5): Antifungal Activity Against M. canis

Table5, Figure 6 showed the activity of the compounds against *T. mentagrophytes*.

Compound B8 and B6 again revealed the highest activity against *T. ment*. They showed 55.1, 56.5 % inhibition, respectively at the concentration 240 µg/ml. On the other hand, B2 showed 53.6% inhibition at 100 µg/ml.

Table (3.5): Antifungal Activity against T. ment. CBS 106.67

Compd.	Conc. ug/ml	% Inhibition
B1c1	50	15
B1C2	100	27.9
B2C1	25	18.6
B2C2	100	53.6
B3C	25	20
B3C2	100	30
B4C1	25	20
B4C2	100	27.1
B5C1	120	36.2
B5C2	240	42.8
B6C1	120	35.5
B6C2	240	56.5
B7C1	120	16.7
B7C2	240	28.3
B8C1	120	33.3
B8C2	240	55.1

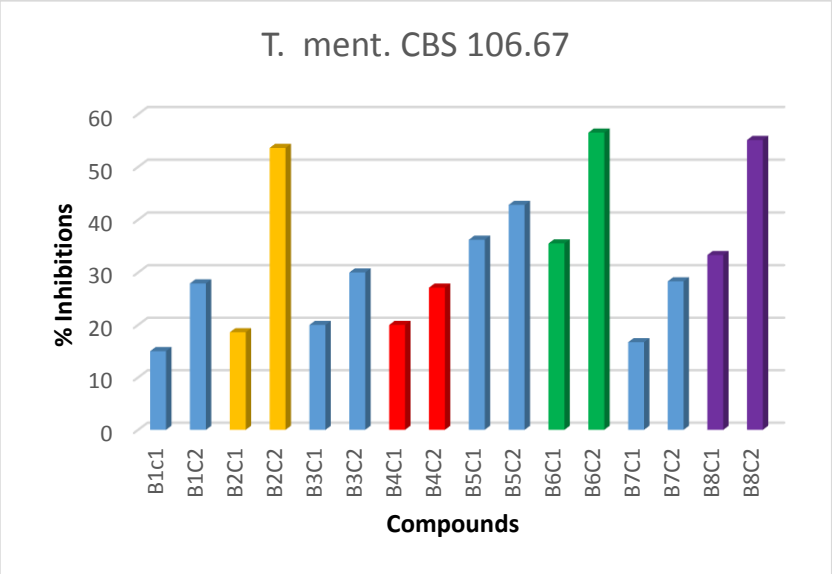


Figure (3.6): Antifungal Activity of Compounds Against T. Mentagrophytes

Table (3.6): T.rubrum CBS 392.58

Compd.	Conc. ug/ml	% Inhibtion
B1c1	50	6.1
B1C2	200	8.5
B2C1	25	4.9
B2C2	100	8.5
B3C	25	15.9
B3C2	100	22
B4C1	100	18.3
B4C2	200	28
B4C3	25	8.5
B5C1	120	55
B5C2	240	79.1
B6C1	120	51.2
B6C2	240	100
B7C1	120	15.5
B7C2	240	30.2
B8C1	120	38
B8C2	240	100

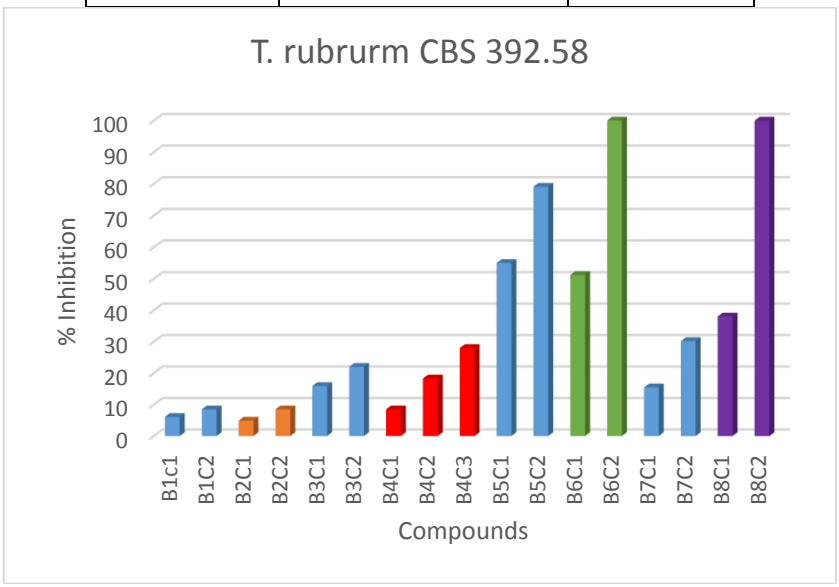


Figure (3.7): Antifungal Activity of Compounds Against T. rubrum

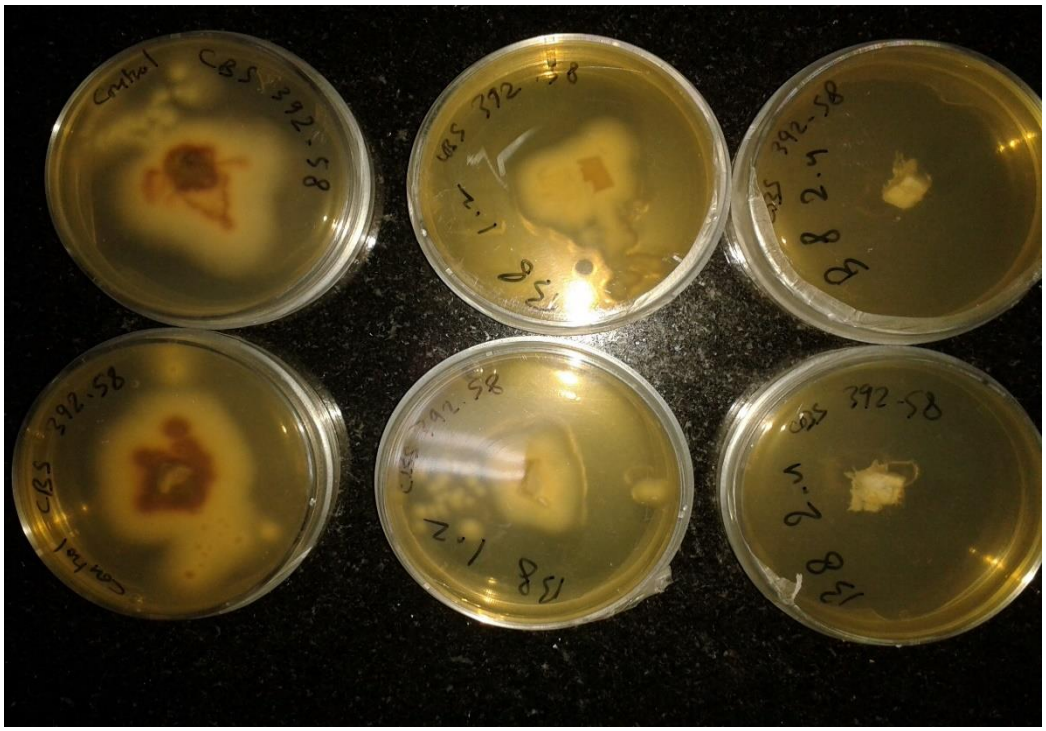


Figure (3.8): Antifungal Activity of Compound B8 Against T.rubrum

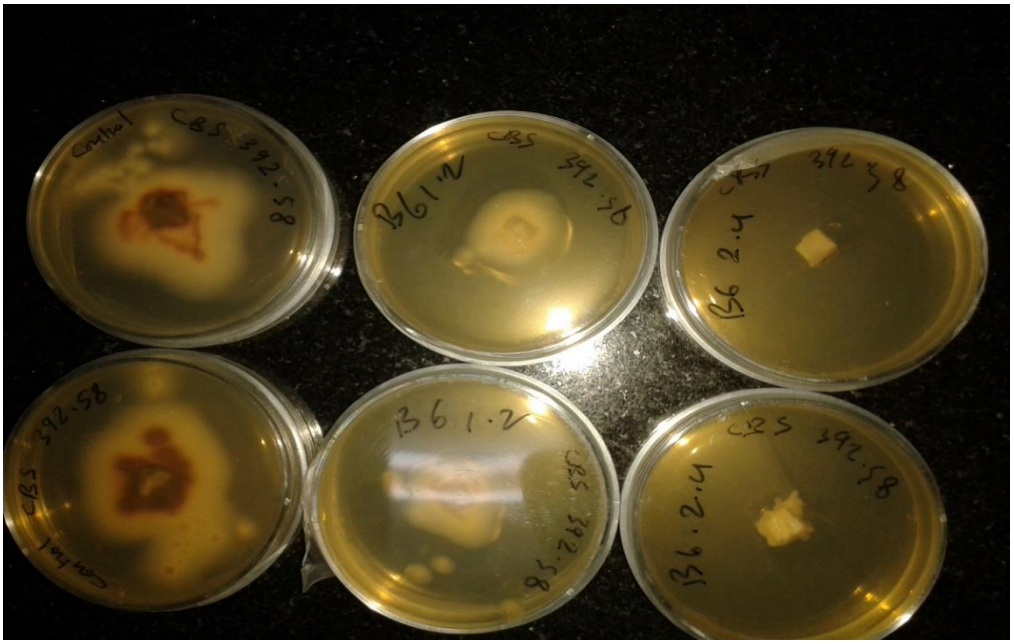


Figure (3.9): Antifungal Activity of Compound B6 Against T. rubrum

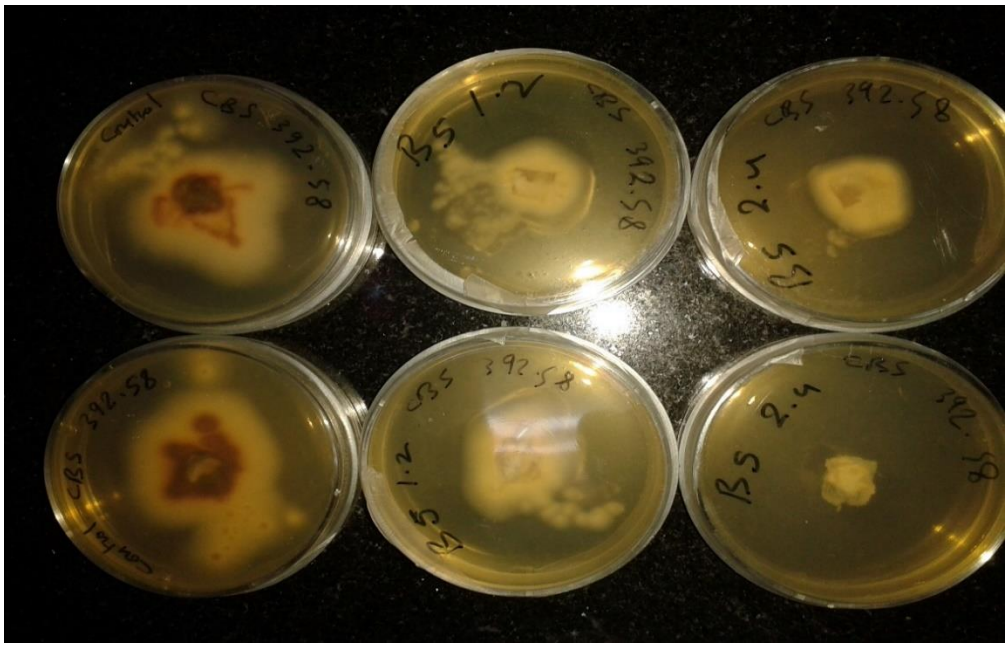


Figure (3.10): Antifumgal Activity of Compound B5 Against T. rubrum

Table (3.7): The Antifungal Activity of the B5,B6 and B8 Compounds Against the Test Pathogens at the Concentration 240 µg/ml.

	M. canis	T. Mentagrophytes	*T. rubrum*
B5	79.4%	42.8%	79.1%
B6	62.2%	56.5%	100%
B8	81.1%	55.1%	100%

B8 showed 100% inhibition against *T. rubrum* , 81.1% inhibition against *M. canis*and 55.1% inhibition against T. Mentagrophytes .

B6 revealed 100% inhibition against *T. rubrum* , 62.2% inhibition against *M. canis* and 56.5% inhibition against T. Mentagrophytes .

Also, B5 showed79.1% inhibition against *T. rubrum* ,79.4% inhibition against *M. canis* and 42.8% inhibition against T. Mentagrophytes .

Suggestion for Further Work

1- To prepare another distamycin analogue by replacing N-mehylpyrrole with another ring.

2-To do more tests on these compounds, for example, against cancer cell.

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إعداد
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إشراف
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قدمت هذه الرسالة استكمالاً لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.
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الملخص

لقد قمت بتحضير ثلاثة عشر مركبا من أشباه الDistamycin وهي مركبات أميديه ترتبط بالحمض النووي (DNA)والأشباه المقترحة لها كتل مولية منخفضة تعمل على تعزيز خاصية الذوبان في الدهون لتحسين ارتباطها بالحمض النووي (DNA) وزيادة امتصاص ونفاذية الخلية لهذه المركبات وقد يؤدي ذلك لزيادة فعالية هذه الأشباه كمضادات حيوية ضد البكتيريا والسرطانات وهذه الاشباه هي :

N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl) nicotinamide (B1),4-benzamido-N-(3-(dimethylamino)propyl)-1-methyl-1H-pyrrole-2-carboxamide(B2), 4-acetamido-N-(3-(dimethylamino) propyl)-1-methyl-1H-pyrrole-2-carboxamide(B3),N-(5-((5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)nicotinamide(B4),4-benzamido-N-(5-((3-(dimethylamino)propyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide(B5),4-acetamido-N-(5-((3-(dimethylamino) propyl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide(B6),4-benzamido-N-(5-((5-((3-(dimethylamino) propyl) carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrole-3-

yl)-1-methyl-1H-pyrrole-2-carboxamide (B7),4-benzamido-N-(3-((3-(dimethylamino)propyl)carbamoyl)phenyl)-1-methyl-1H-pyrrole-2-carboxamide(B8),4-benzamido-N-(5-((3-((3-(dimethylamino) propyl) carbamoyl)phenyl)carbamoyl)-1-methyl-1H-pyrrole-3-yl)-1-methyl-1H-pyrrole-2-carboxamide(B9),N-(5-((5-((3-((3-(dimethylamino) propyl) carbamoyl)phenyl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)carbamoyl)-1-methyl-1H-pyrrol-3-yl)picolinamide(B10), 4-benzamido-1-methyl-N-(1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)-1H-pyrrole-2-carboxamide(B11),4-benzamido-1-methyl-N-(1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)-1H-pyrrole-2-carboxamide(B12), N-(1-methyl-5-((1-methyl-5-((1-methyl-5-((2-morpholinoethyl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)carbamoyl)-1H-pyrrol-3-yl)nicotinamide(B13),

جميع هذه المركبات تمت دراسة خصائصها باستخدام القياسات الفيزيائية التالية :***^{1}H-NMR,IR***

ومن ثم قمنا بدراسة الآثار البيولوجية متمثلة بالفحوصات التالية:

Anti-fungal, Anti-oxidant, Anti-bacterial, Reductive potential.

وقد أظهرت هذه المركبات نتائج مهمة ضد ثلاثة أنواع من الفطريات فعندما استخدمنا الفطر *T. rubrurm CBS 392.58* لاحظنا أن بعض المركبات ثبطت من نمو هذا الفطر بنسبة (100%) ومن الأمثلة على هذه المركبات(*B6,B8*) على تركيز(*240µg/ml*) وثبط هذا الفطر أيضا بنسبة(79%) بواسطة المركب(*B5*) على نفس التركيز. وعندما استخدمنا الفطر *M.Canis CBS 132.88* فان المركبان(*B5*) و(*B8*) قاما بتثبيط نمو هذا الفطر بنسبة(79.4%) (81.1%)على الترتيب على تركيز (*240µg/ml*). والفطر *T. ment. CBS 106.67* ثبط بنسبة(56%)بواسطة المركبين(*B6,B8*) على تركيز *(240µg/ml)* وثبط بنسبة(53%) على تركيز *(100 µg/ml)* بواسطة المركب (*B2*).

وعندما قمنا بفحص قوة العامل المختزل وجدنا أن المركب B3 له أعلى قيمة (B3=601.8) ويليه المركبB13 (B13=277.2) مقارنة.Gallic acid(Gallic acid=470.7)

لم تظهر مركباتنا أي أثر ضد البكتيرياعلى تركيز(50 µg/disk) بالمقارنة مع gentamycin ولم تظهر أي أثر قوي ضد الأكسدة بالمقارنة مع Gallic acid.