An-Najah National University

Faculty of Graduate Studies

Synthesis of Amide-Linked Minor Groove Binders (MGBs) to Target the Androgen Response Element (ARE) Sequence

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Dedication

I humbly dedicate this thesis to my parents for their love, endless supports and extraordinary encouragement. I would like also to dedicate it to my brother Islam.

Acknowledgments

Praise and thanks to Almighty Allah, for helping me to complete this thesis, without his help my effort would have gone astray. It would be impossible to accomplish this thesis without the help and support of several persons: First of all I would like to express my sincere thanks to my supervisor Dr.Hasan Alniss for his excellent guidance throughout this work. I appreciate his positive attitude and words of encouragement and support that motivated me during this research.

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Abu Jabal Saber

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل عنوان

Synthesis of Amide-Linked Minor Groove Binders (MGBs) to Target the Androgen Response Element (ARE) Sequence

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

Declaration

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

Student's name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

A	Adenine
C	Cytosine
$CDCl_3$	Deuterated chloroform
ESI-MS	Electro spray Ionization Mass Spectrometry
DCM	Dichloromethane
DMF	N,N-Dimethylformamide
DMAP	Dimethylaminopropylamine
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
Dp	Dimethylaminopropylamine
G	Guanine
HCl	Hydrochloric acid
Hp	Hydroxy pyrrole
Hz	Hertz
Im	Imidazole
I.R.	Infrared
M	Molar concentration
Me	Methyl
mg	Milligram
MHz	Megahertz
MGB	Minor Groove Binder
ml	Millilitre
mol	Mole
m.p.	Melting point
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
ppm	Part per million
Pyr	N-Methylpyrrole
Spec.	Spectroscopy
T	Thymine
TFA	Trifluoroacetic Acid

THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
	NMR data
s	SINGLET
d	DOUBLET
t	TRIPLET
q	QUARTET
Q	QUINTET
S	SEPTET
m	MULTIPLET

Synthesis of Amide-Linked Minor Groove Binders(MGBs) to Target the Androgen Response Element(ARE) Sequence

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Abstract

Minor groove binders (MGBs) are molecules which bind selectively to the minor groove of DNA. Distamycin **1** and netropsin **2** are naturally occurring MGBs, and are members of the polypyrrole class of compounds. These compounds consist of two and three methylpyrrole monomers linked by amides, with a head and tail group also linked by amides. They have potential antiviral, antibiotic, and antioncolytic properties, however they are also toxic. These biological effects arise from the molecule binding to DNA in regions where there are short runs of A:T base pairs. Much work has been carried out to develop analogues of distamycin and netropsin which have improved biological activity and a reduced toxicity profile.

The aim of this project is to synthesize amide-linked minor groove binders as potential antibacterial and anticancer agents. The proposed analogues of distamycin have low molecular weight and enhanced lipophilicity to improve their binding with the minor groove of DNA and increase the absorption and the cell permeability of these compounds to offer them better chances of becoming commercial drugs. In this thesis, we describe a successful development of novel routes for the synthesis of distamycin analogue compound (Thiazotropsin C).

CHAPTER 1

Introduction

1.1 DNA Structure

Deoxyribonucleic acids (DNA) are informational molecules encoding the genetic instructions used in the development, and function of all known living organisms and many viruses, along with RNA and proteins [1].

DNA is one of the three major macromolecules that are essential for all known forms of life. Genetic information is encoded as a sequence of nucleotides (Guanine, adenine, thymine and cytosine) recorded using the letters G, A, T and C [2-4].

Accordingly, Watson and Crick concluded that adenine must be matched with thymine and guanine with cytosine (**Figure** 1.1). [5-7]. Most DNA molecules are double –stranded helix, consisting of two long polymers of simple units called nucleotides, with backbone made of alternating sugar (deoxyribose) and phosphate groups with the nucleo bases (G, A, T, C) attached to the sugars.

DNA consists of two anti-parallel polynucleotide chains, stabilised by hydrogen bonds which form a helical structure [8]. The two strands are held together by interstrand hydrogen bonding; A pairs with T (2 H-bonds), and G pairs with C (3 H-bonds) (**Figure** 1.2). The dominant form of DNA

in solution (B-DNA) exists as a right-handed helix and is characterized by a shallow, wide major groove and a deep, narrow minor groove [11].

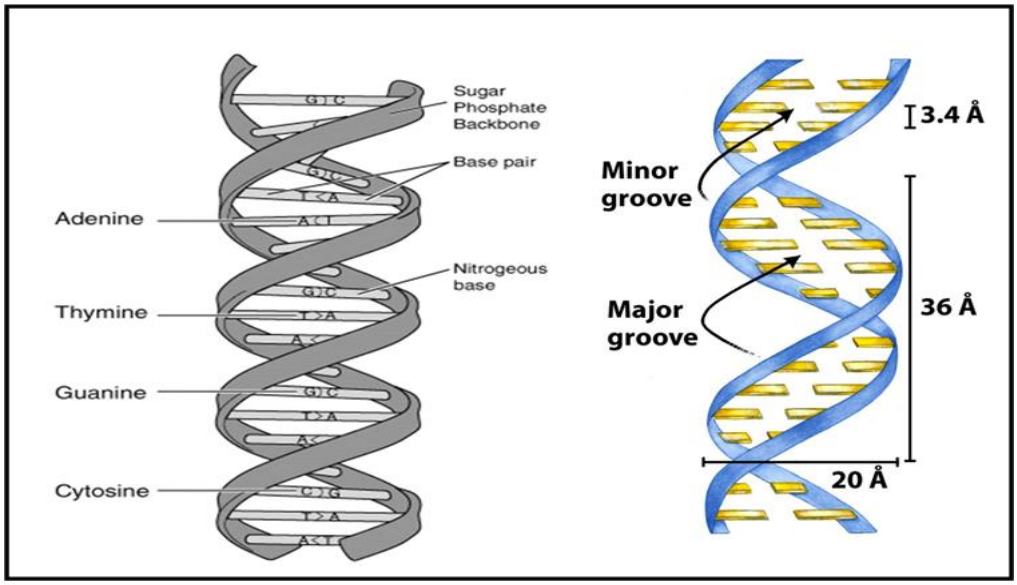


Figure (1.1): DNA Structure. [9].

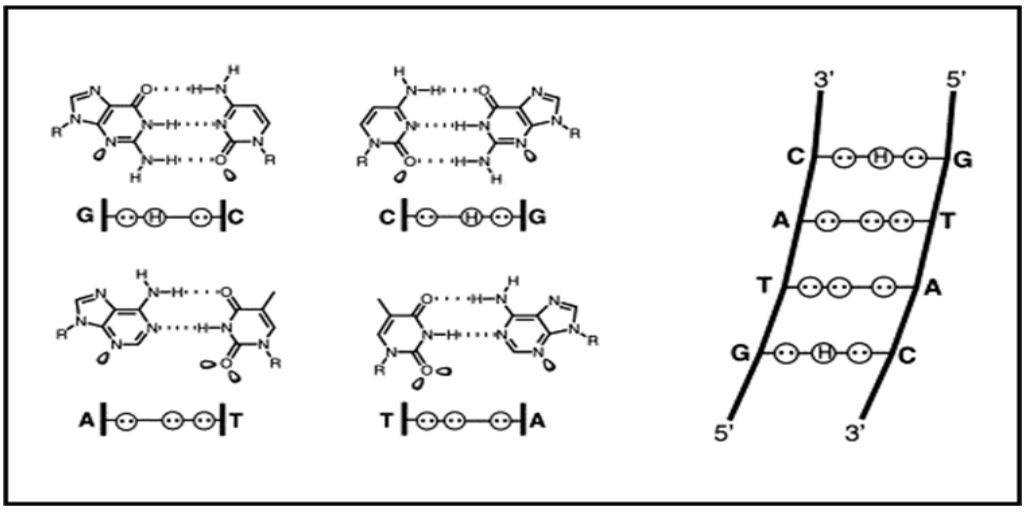


Figure (1.2): Hydrogen Bonds Formed in Base Pairing [10].

The chemical and structural properties of both the minor and major grooves are characteristic of any DNA sequence, which forms the basis of DNA molecular recognition by small molecules and proteins [11].

DNA is suited for biological information storage since the DNA backbone resistance to cleavage and the double stranded structure provides the molecule with a built- in duplicate of the encoded information. The two strands are complementary – each is, in a sense, the mirror image, or a mold, of the other. All strands of DNA are made of four kinds of subunits, called nucleotides [12].

The chemistry of nucleotides allows a single strand to contain any sequence, but the opposite strand must always be of complementary sequence. In other words, if there is an A at a given position in one strand, only a T can go into a position opposite to it on the complementary strand. In the same way, C and G [13].

1.2 Properties of DNA

DNA was first identified and isolated by Fridrich Miescher and the double helix structure of DNA was first discovered by James D. Watson and Francis Crick [14]. The structure of DNA of all species comprises two helical chains each coiled round the same axis, and each with a pitch 34 angstroms (3.4 nanometers) and a radius of 10 angstroms (1.00 nanometers) [15].

The four bases found in DNA are adenine, cytosine, thymine and guanine. These bases are attached to the sugar/ phosphate to form the complete nucleotide. DNA contains 1. a nitrogenous base (either a purine or pyrimidine) 2. a pentose, and 3. Phosphate group . In DNA the pentose

is 2-deoxyribose, that means DNA contain the purine nitrogenous bases adenine (A) and guanine (G) and pyrimidine cytosine (C) and thymine (T), These information are summarized in **Table** 1-1,and the corresponding chemical formulas are shown in (**Figure** 1.3) [16].

Table (1.1): Chemical Constituents of DNA.

	DNA
purines	Adenine Guanine
pyrimidines	Cytosine thymine
Pentose	2-Deoxyribose Phosphoric acid

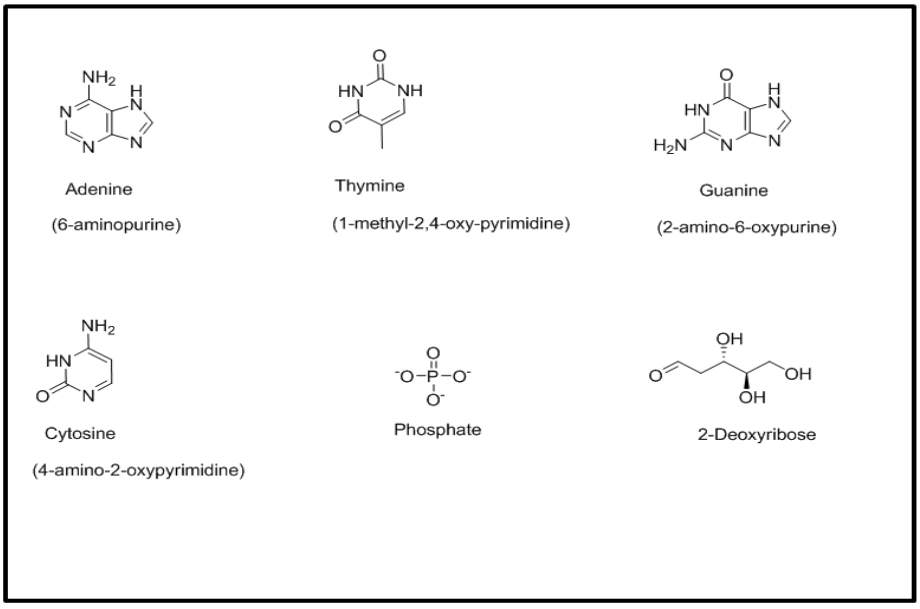


Figure (1.3): Structural Formulas of the Constituents of DNA.

DNA polymers can be very large molecules containing millions of nucleotides, for instance, the largest human chromosome, chromosome number 1 is approximately 220 million base pairs long [17].

An important property of DNA is that it can replicate, or make copies of itself. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell [18].

1.3 Natural Compounds that Bind to the Minor Groove

1.3.1 Netropsin

Many of organic and inorganic compounds [19] have been reported to interact with minor grooves of DNA. An important example of these compounds is Netropsin (**Figure** 1.4) [20].

It is one of the most widely studied DNA minor groove binding drugs, naturally occurring antibiotic [21], which interacts with the deep and narrow minor groove of B-DNA [22]. It is isolated from streptomyces distallicus [23]. Netropsin has a crescent shape that complements the convex floor of the minor groove, as in the (**Figure** 1.5) [20-24].

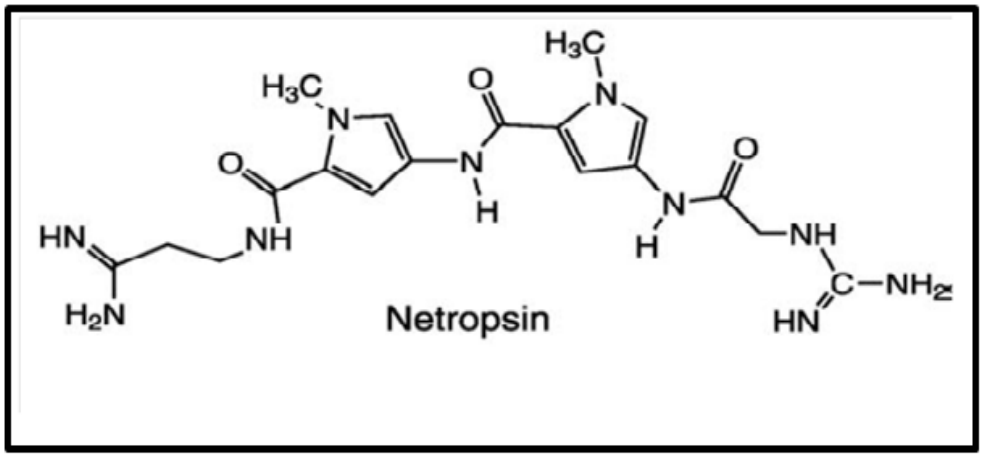


Figure (1.4): Netropsin Structure

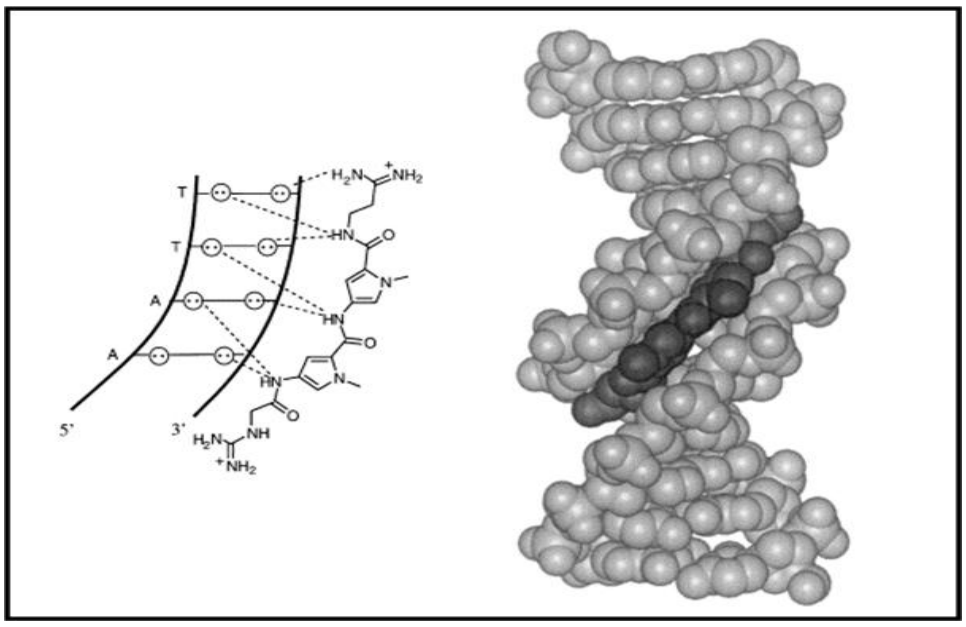


Figure (1.5): Netropsin Bound in the Minor Groove 1:1. [10]

Netropsin exhibits antibacterial, antifungal, and antiviral activities and inhibits DNA and RNA tumor viruses in mammalian cells [25] . It has been used as lead compound in cancer research because of its binding ability to specific sequences within minor groove of double –helical DNA [26].

Netropsin complexes with douplex DNA but does not complex which single – stranded DNA , double- stranded RNA , or DNA –RNA hybrids ,with a special preference for AT sites [27-29].

Antibiotic netropsin is known to bind specifically to A and T regions in DNA,the mode of binding being non-intercalative. Obviously, H-bonding between the proton donors of netropsin and acceptors N-3 of A and O-2 of T comes as a strong possibility which might render this specificity [30].

Other names for netropsin are Congocidin and Sinanomysin . [31]. In contrast, netropsin does not bind single stranded DNA or double stranded RNA. Crystallographic structure of DNA – bound Netropsin has been obtained and elucidate details of how the drug binds in the minor groove [32].

Netropsin is active both against Gram-positive bacteria and Gram-negative bacteria [33].

1.3.2 Distamycin

Distamycin (**Figure** 1.6) is a naturally occurring minor groove binder and has similar properties to that observed with netropsin.

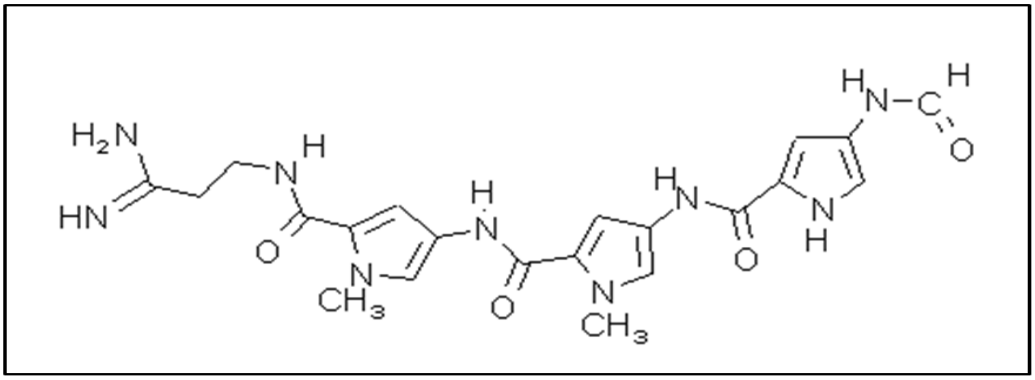


Figure (1.6): Structure of Distamycin

Both molecules, distamycin and netropsin have shown antibacterial, antiviral and anticancer activity. Netropsin as shown in (**Figure** 1.5) binds exclusively in a 1:1 complex with DNA [10, 34] almost certainly due to the repulsive force which would occur having two positively charged groups side by side. Distamycin however, whilst also binding in a 1:1 complex in a similar way to netropsin, also has the ability to bind in a 2:1 complex with DNA as an anti-parallel dimer which is only possible as the positively charged tails are well separated (**Figure** 1.7), each monomer reading a single strand of DNA. This has been shown not to significantly distort the overall DNA structure but widens the groove width by 2 Å [34].

The series of hydrogen bonds is possible in A:T regions as the topology of the groove can be considered a smooth curve, however introducing a G:C base pair with the exocyclic N-2 of guanine which points up into the groove leads to a steric hindrance (clash) with the proton on C-3 of the pyrrole rings, thus preventing the necessary hydrogen bonds from being formed effectively.

The combination of the previous factors leads to the sequence specificity seen with these molecules.

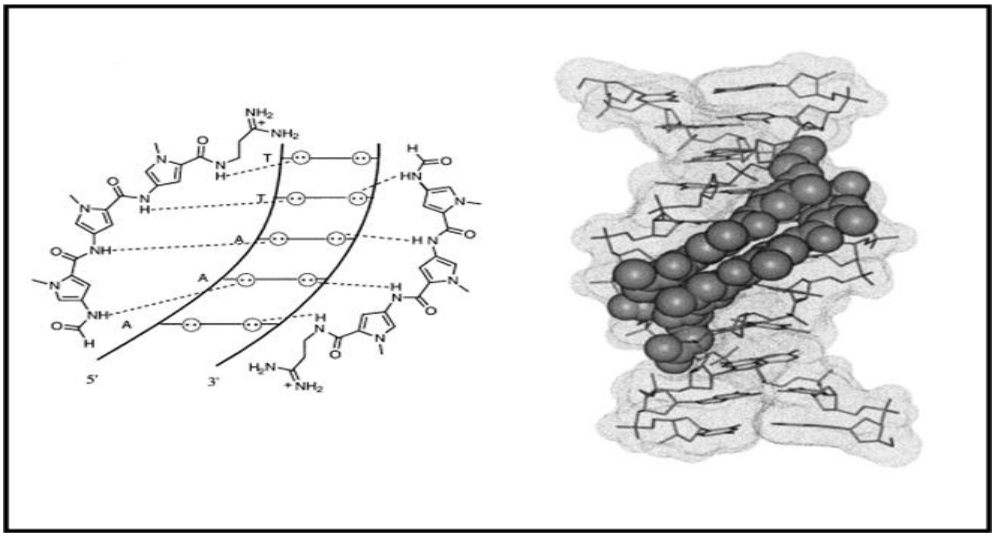


Figure (1.7): Distamycin Bound in the Minor Groove 2:1. [10]

1.4 Design of a Distamycin Analogue as an Anticancer Agent (Thiazotropsin C)

The cationic lexitropsins (analouges of distamycin), which bind non-covalently to the minor groove of DNA have shown therapeutic potential in the treatment of cancer, viral, fungal and bacterial diseases. The ring slippage of the side-by-side minor groove binders (such as thiazotropsin A[35], (**Figure** 1.8), has enabled the extension of DNA sequences with six pairs to these ligands. On the other hand and in contrast to expectations based on a four-pairs based on the size of the ligand.

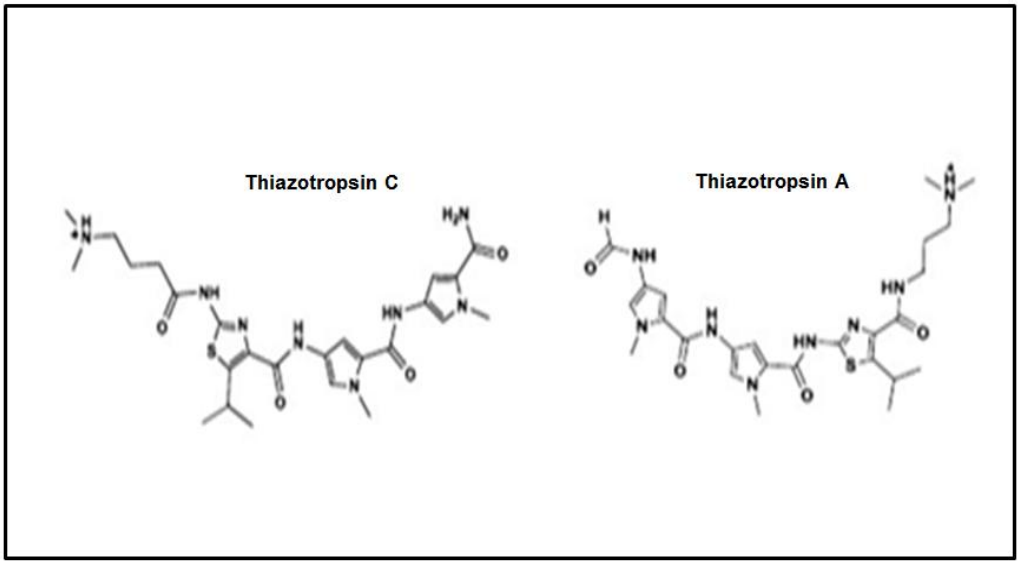


Figure (1.8) Structure of Thiazotropsin A [35], and its Proposed Analogue, Thiazotropsin C.

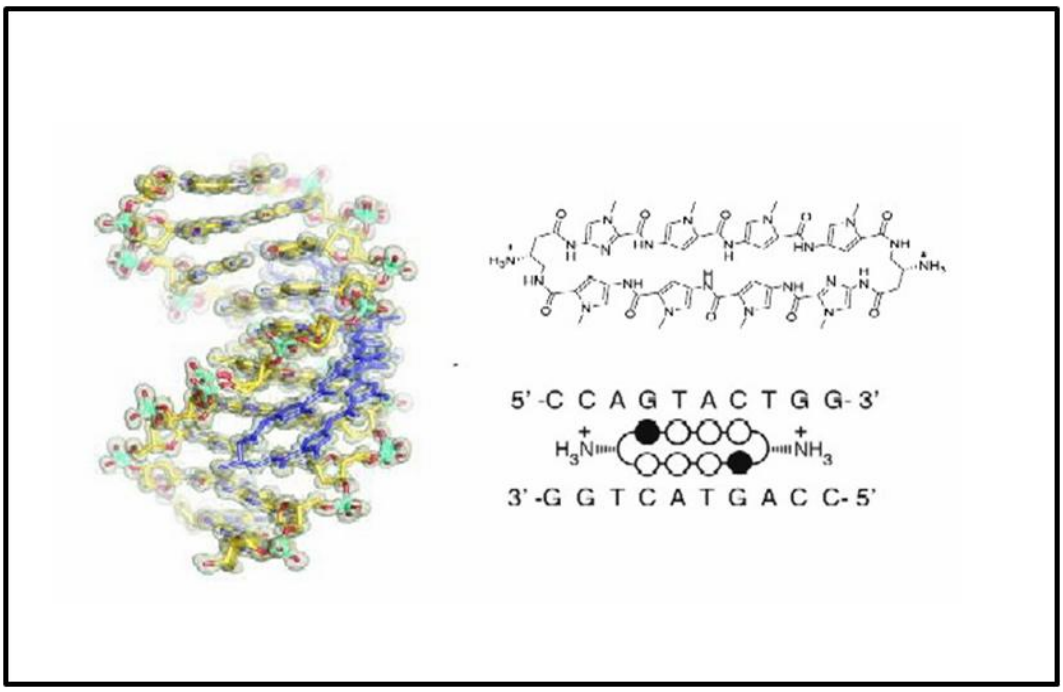
The importance of this in terms of gene targeting is that such ligands with small molecular weight and enhanced lipophilicity can be used to disrupt the binding of transcription factors to the response element of the target gene that is composed of six base pair sequences.

There are many examples of transcription factors that regulate hormone response element, which consists of a sequence of six DNA base pairs, such as the androgen receptor (AR), glucocorticoid receptors (GR) mineralocorticoid receptor (MR) and progesterone receptor (PR). All these receptors recognise the same DNA response element [36], which is organized as repeats of the consensus DNA sequence 5′-WGWWCW-3′ (W is A or T), with a three nucleotide spacer.

Any defect in transcription factors leads to a problem in gene expression, which was observed in many human cancers such as prostate cancer. The prostate specific antigen (PSA) gene which is usually used as an indicator for the diagnosis of prostate cancer is regulated through the

interaction of AR with the androgen response element(ARE) of the PSA gene. The AR-ARE interaction can be disrupted via small molecules (e.g. MGBs) targeting the ARE. Blocking the AR from binding to the ARE is expected to reduce the transcription of PSA gene. Recent data from Dervan′s laboratory have shown that a cyclic polyamide minor groove binder, consists of 8 imidazole / pyrrole rings(**Figure** 1.9), which binds with ARE(5′-WGWWCW-3′) is able to regulate the expression of AR target genes in the cell culture studies [37]. Although it was concluded from this study that the relatively large cyclic polyamide is cell permeable, absorption studies carried out on this compound showed low Caco-2 permeability, suggesting that it may not be orally available.

The ability of lexitropsins to recognize a DNA sequence very similar to that of the ARE (for instance, thiazotropsin A can recognize the 5′-WCWWGW-3′. sequence, where W is A or T) [35], is encouraging and development of these ligands is therefore of value in order to target the ARE sequence.



Figure(1.9): Crystal Structure of the 8 Ring Cyclic Polyamide that Targets the 5′-AGTACT-3′Sequence [38].

1.5 The Rationale Behind the Design of Thiazotropsin C.

The small molecular weight of thiazotropsin C which is composed of three aromatic rings and its enhanced lipophilicity via the isopropyl thiazole ring could offer it additional beneficial physical properties compared with the large molecular weight cyclic polyamide ligands (**Figure** 1.9). Furthermore, additional lipophilic functional groups could be introduced without sacrificing binding affinity as these substituents point out from the minor grooves.

Different ligands can be designed in order to inhibit AR-ARE interactions based on the structure of thiazotropsin A. One ligand structure is described here by way of example to target the 5′-WGWWCW-3′ sequence. The proposed structure is an analogue of thiazotropsin A (thiazotropsin C) (see **Figure** 1.8).

In the proposed ligand structure, the position of both the DMAP tail (dimethylaminopropyl) and the isopropyl thiazole in thiazotropsin A are changed : the DMAP group is attached to the formyl head and the isopropyl thiazole replaces the pyrrole ring located towards the N-terminus. Studying the DNA binding behavior of this compound may reveal further factors that dictate the preferred orientation of 2:1 anti-parallel dimer. In all of the studies that have been conducted, the ligands bind to the minor groove as dimers in anti-parallel side-by-side fashion and only a single orientation of the ligand has been observed : the positively charged DMAP groups are oriented toward the 3′-ends of the DNA strands . In theory, if thiazotropsin C can bind to the minor groove as a dimer, there are two possible orientations that can be adopted by the ligand with respect to the DNA helix (**Figure** 1.10). In the first orientation (**Figure** 1.10.A), the ligand recognize the 5′-WGWWCW-3′ sequence as a dimer, with its positively charged DMAP group lying toward the 5′-end and the carbonyl group of amide (coloured red) linking the aromatic rings is closer to the 5′-end than its partnering NH. If the biophysical experiments confirm the binding of thiazotropsin C to the 5′-WGWWCW-3′ sequence, such results would suggest that the factor that dictates the orientation of lexitropsin ligands in the minor groove is the orientation of the amide links with respects to the 5′-3′-ends. In this case, the amide link always prefers an orientation where its carbonyl group is closer to the 5′-end than the neighboring NH regardless of the DMAP group position in the ligand structure whether it is attached to the head or the tail position.

In the second orientation (**Figure** 1.10.B), the ligand recognises the 5′-WCWWGW-3′ sequence as a dimer, with its positively charged DMAP group lying toward the 3′-end and the carbonyl group (coloured blue) linking the aromatic rings is closer to the 3′-end than the neighboring NH. If the biophysical experiments confirm the binding of thiazotropsin C to the 5′-WCWWGW-3′ sequence, such results would suggest that the orientation of lextropsins in the minor groove is dictated by the positively charged DMAP tail of the ligand. In this case, the DMAP group always prefers to point toward the 3′-end regardless of its position in the ligand structure either at the head or the tail position, and regardless of the two possible orientations of the amide links (red/blue coloured amides) with respect to the 5′-3′ –ends.

Its expected that the ligand would not exhibit both orientations, because lexitropsins usually prefer to adopt a single orientation . If no binding or very weak binding is observed with this ligand, this presumably indicates a significant steric hindrance (clash) between the DMAP group and the backbone of DNA.

The possibility that the ligand dimer slides between six and five base pairs sequence cannot be ruled out.

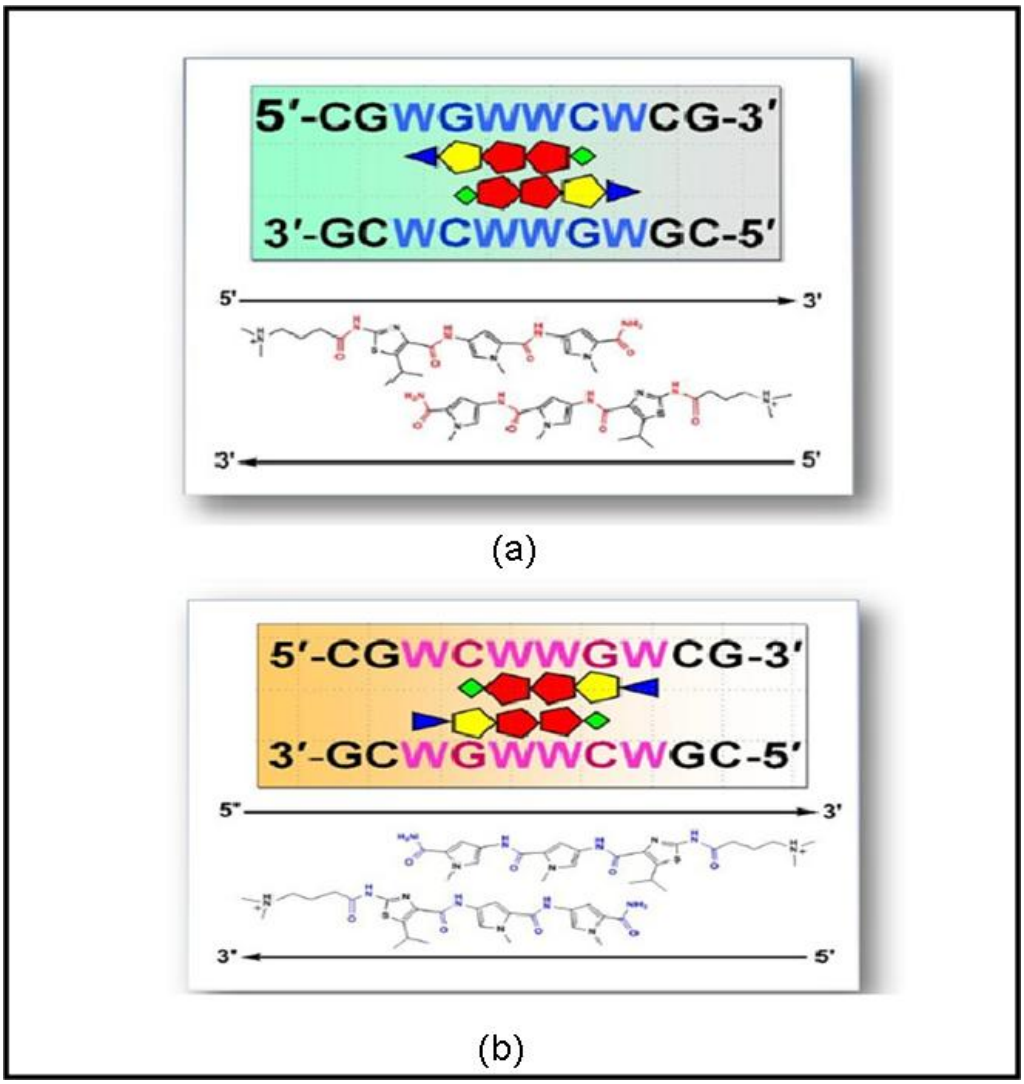


Figure (1.10): Representation of the possible binding orientations that may be Adopted by thiazotropsin C with the DNA sequence 5′-WGWWCW-3′.A)The ligand aligned in a 3′-5′direction B)The ligand aligned in a 5′-3′direction.

However, footprinting studies performed on thiazotropsin A [39], and thiazotropsin B [40], have shown that these ligand are selective for the six base pair sequences 5′-ACTAGT-3′ and 5′-ACGCGT-3′,respectively. The outcome of such study would be of great importance in terms of both the development of lexitropsins to target specific genes and to gain more insight into the molecular basis of minor groove recognition by lexitropsins.

1.6 Proposed Pathway for Synthesis of Thiazotropsin C

From the retrosynthetic analysis shown below (**Figure** 1.11), the final product, thiazotropsin C **7**, can be prepared using a convergent synthesis through coupling the thiazole tail **5** with mononitro-substituted diamide-substituted pyrrole dimer **6** after the reduction of the nitro group. **5** can be prepared from the coupling of **1** with **2** and **6** can be prepared by the coupling of **3** with **4**.

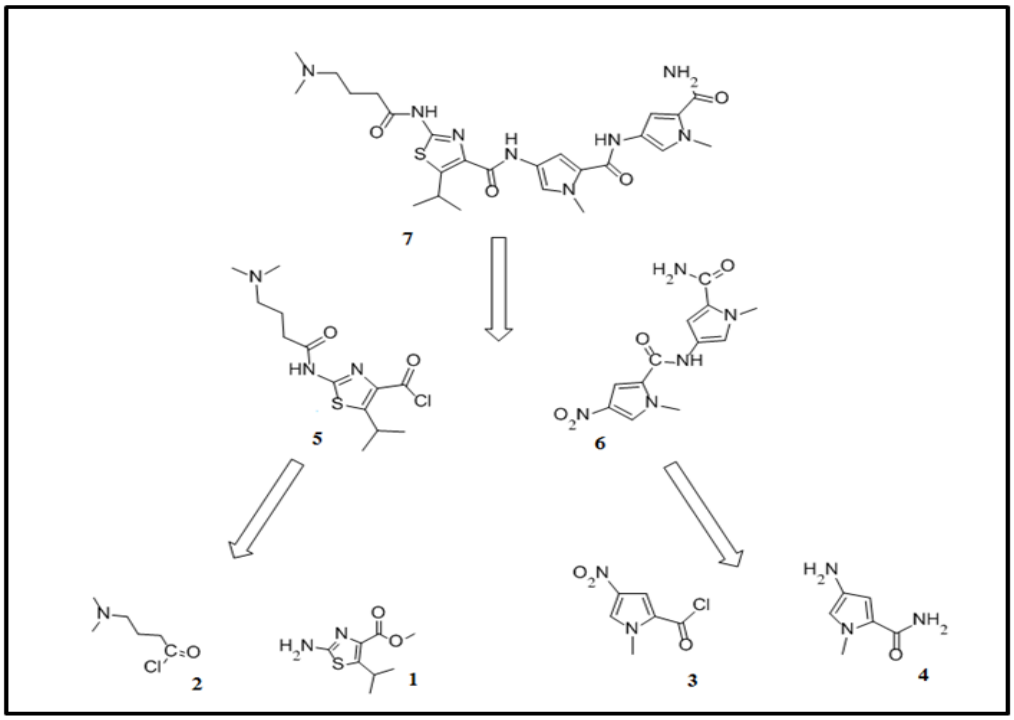


Figure (1.11): Retrosynthetic Analysis of Thiazotropsin C

1.7 Aim of The Project

The aim of this project is to design and synthesise an amide-linked minor groove binder to target the androgen response element sequence (ARE) associated with prostate cancer. The outcome of such a study would

be of great importance in terms of both the development of new analogues of distamycin as potential anticancer /antibacterial agents and to gain more insight into the molecular basis of minor groove recognition by small molecules.

CHAPTER 2

Materials and Methods

2.1 General Experimental

2.1.1 Materials

All chemicals used in this study were purchased from Sigma Aldrich Chemical Company

2.1.2 Melting Point Determination

A Stuart Scientific Melting Point SMP1 apparatus was used for the melting point determinations with degrees Celsius (ºC) as the units.

2.1.3 Infrared Spectroscopy

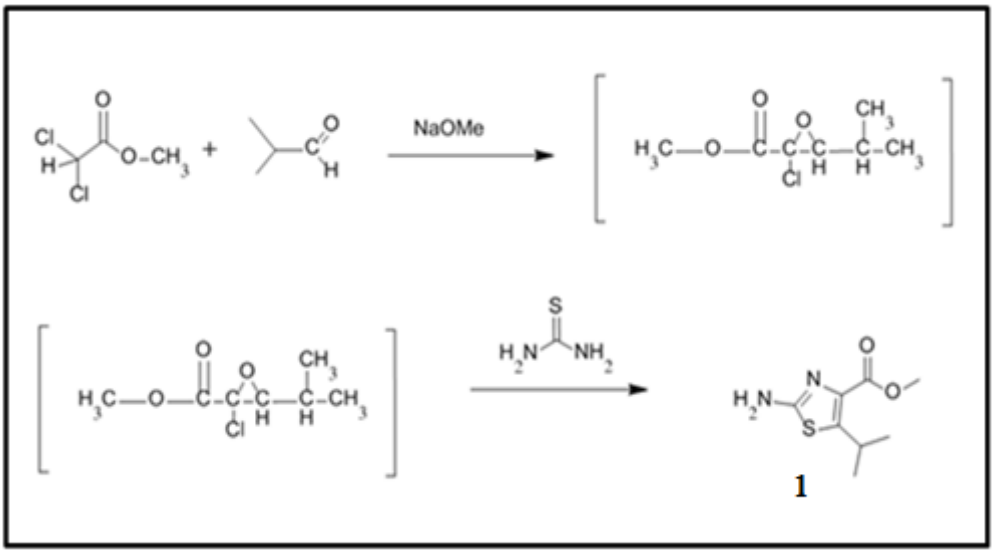
Infrared Spectra were run on Mattson Genesis Series FTIR spectrometers with samples prepared in KBr discs. The spectra were shown as transmittance output in frequencies expressed as υ_{max} in cm^{-1}.

2.1.4 Mass Spectrometry

High resolution mass spectra (MS) were obtained on a Thermo Scientific spectrometer using electrospray ionization (ESI) in a Fourier Transform analyser. Mass to charge ratio (m/z) are quoted for the molecular mass.

2.2 Chemical Synthesis

2.2.1 Preparation of methyl 2-amino-5-isopropyl-1,3-thiazole-4-carboxylate 1.



Sodium (3.06 g, 177.29mmol) was dissolved in methanol (50 ml) (could be used for HPLC) at 0 °C and allowed to stir for 30 min. This solution was added dropwise to a solution of isobutyraldehyde (14 ml, 154 mmol) and methyl-dichloroacetate (20 ml, 193 mmol) in ether (50 ml) over 45 min at 0 °C. The solution was allowed to stir for 30 min. Diethylether (50 ml) and brine (50 ml) (saturated NaCl solution) were then added. Ether layer was separated and dried over $MgSO_4$(anhydrous), the solvent was removed under reduced pressure to yield a pale yellow oil.

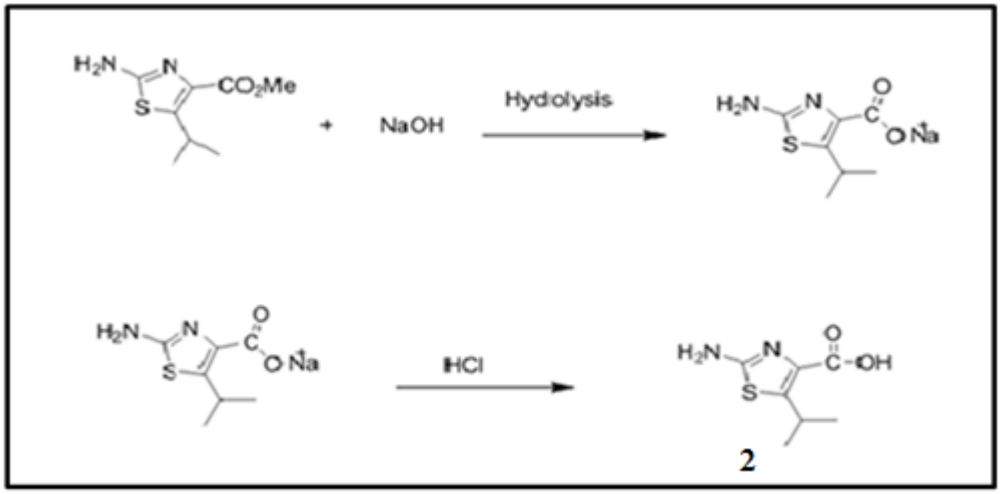
The oil was then dissolved in a solution of thiourea (8.52 g, 112mmol) in methanol (HPLC) (60 ml) and the solution was refluxed for 3 hr. The solvent was then removed under reduced pressure using rotary

evaporator and the crude product was taken up in 100 ml distilled water neutralized with ammonium - hydroxide solution, and extracted with (100 ml) DCM, 100 ml ethyl acetate and dried over $MgSO_4$ (anhydrous) to yield the crude thiazole. Recrystallization from methanol/water gave the desired product.

Yield; 14 g, 65%, m.p. =151-153°C . (Lit =150-151°C).

IR (KBr): 3432, 3121, 2949, 1685, 1433, 1327, 1048 cm^{-1}.

2.2.2 Preparation of 2-amino-5-isopropylthiazole-4-carboxylic acid 2.

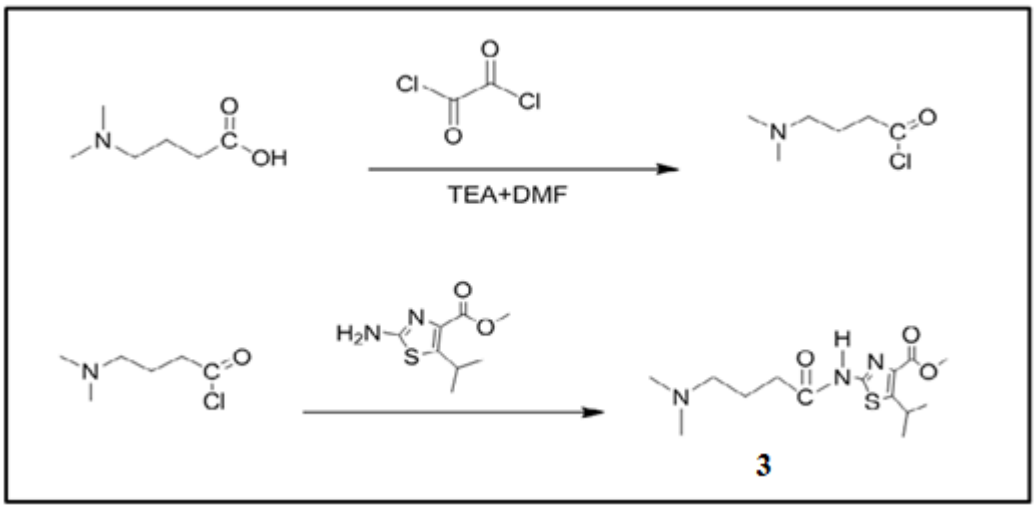


Thiazole (1.00 g, 4.99mmol) and sodium hydroxide (1.00g, 25mmol) were placed in a round-bottomed flask (250 ml) , followed by addition of water (20 ml) and THF (5 ml) then refluxed for 2hr. The THF solvent was then removed under vacuum. The round bottomed flask was then placed in an ice, and water (40 ml) was added to the reaction mixture then acidified by addition of HCl to make pH (4-5) , to obtain a precipitate which was filtered to obtain a yellow solid product.

Yield; 0.5g, 53 %, m.p. =255-257°C .

IR (KBr): 3738, 3644, 3554, 2974, 2749, 2349, 1625 cm^{-1}.

2.2.3 Preparation of methyl 2-(4-(dimethylamino)butanamido) -5- isopropylthiazole-4-carboxylate 3.



4-(N,N-Dimethylamino) butanoic acid (1.00 g, 7.62mmol) was placed in a round-bottomed flask (100 ml) and a solution of DCM (20 ml), DMF (0.6 ml) and oxalyl chloride (2 ml) were added to the flask. The reaction mixture was then refluxed for 4 hr.

DCM solvent was then removed under vacuum, then DCM (10 ml) was added and removed for another time .

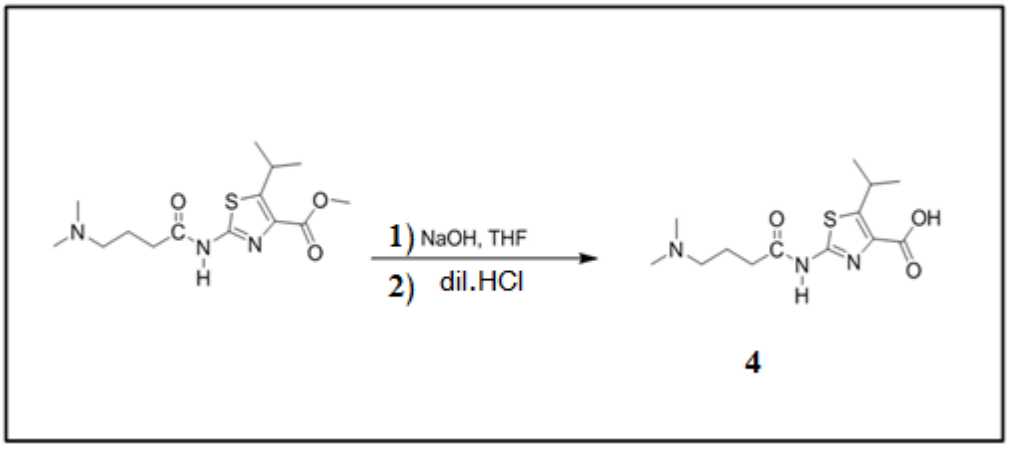
The reaction flask was placed in an ice bath and a solution of triethylamine TEA (1.6 m l), DCM (20 ml), and thiazole (1.2 g,5.99mmol) were added to it, stirring overnight, and finally DCM solvent was removed under vacuum using rotary evaporator at 40 °C .

The reaction mixture was made alkaline by adding a solution of $NaHCO_3$,then extracted with ethyl acetate (40 ml) twice, and the solution was dried by using $MgSO_4$(anhydrous), then ethyl acetate was removed under vacuum using rotary evaporator and the product was obtained as a pale yellow oil.

Yield ; 1.5g, 65% .

IR (KBr): 2963, 2647, 2340, 1714, 1537, 1193, 637 cm^{-1} .

2.2.4 Preparation of 2-(4-(dimethylamino)butanamido)-5-isopropylthiazole-4-carboxylic acid 4.



4

A solution of water (20 ml), sodium hydroxide (0.0634 g, 1.58mmol) and tetrahydrofuran (5 ml) were added to the methyl 2-(4(dimethylamino) butanamido)-5-isopropylthiazole-4-carboxylate(0.5g) prepared in section 2.2.3, then the reaction mixture was stirred overnight at room temperature.

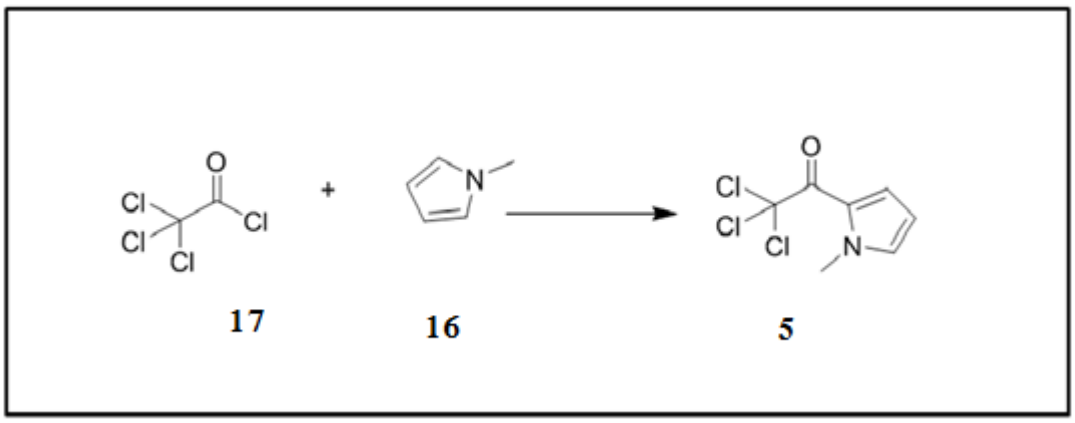
The organic solvent (tetrahydrofurane) was removed under reduced pressure using rotary evaporator. The aqueous reaction mixture was acidified by adding a dilute solution of HCl to make pH (3-4.5).

Finally, when a precipitate appeared, the solvent was centrifuged, when the precipitate did not appear, freeze drying was performed in Biology labs.

Yield; 0.4g, 83%, m.p. = 260-263°C .

IR (KBr): 3481, 3038, 2956, 2684, 1683, 1625, 1544, 792 cm^{-1}.

2.2.5 Preparation of 2-trichloroacetyl-N-methylpyrrole 5.

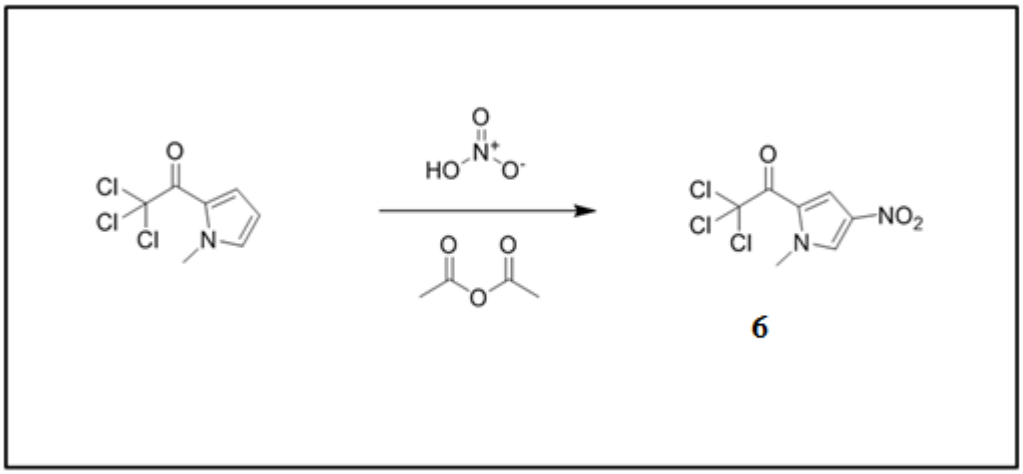


A solution of trichloro acetylchloride (10 g, 54.99mmol), in DCM (36 ml) was placed in a 250 ml round bottomed flask at room temperature under nitrogen atmosphere, then a solution of N-methylpyrrole (5 g, 61.63mmol) in DCM (22 ml), was added drop- wise over 2.5 hr. Then the solution was allowed to stir overnight. The solvent was then removed under vacuum to yield the crude product. Finally, the crude product was then filtered through a silica column to yield the product as a white- yellow solid crystals.

Yield; 9g, 75%, m.p. = 62-64°C . (Lit =64-65°C)

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

2.2.6 Preparation of 2,2,2-trichloro-1-(1-methyl-4-nitro-1*H*-pyrrol-2-yl) ethanone 6.



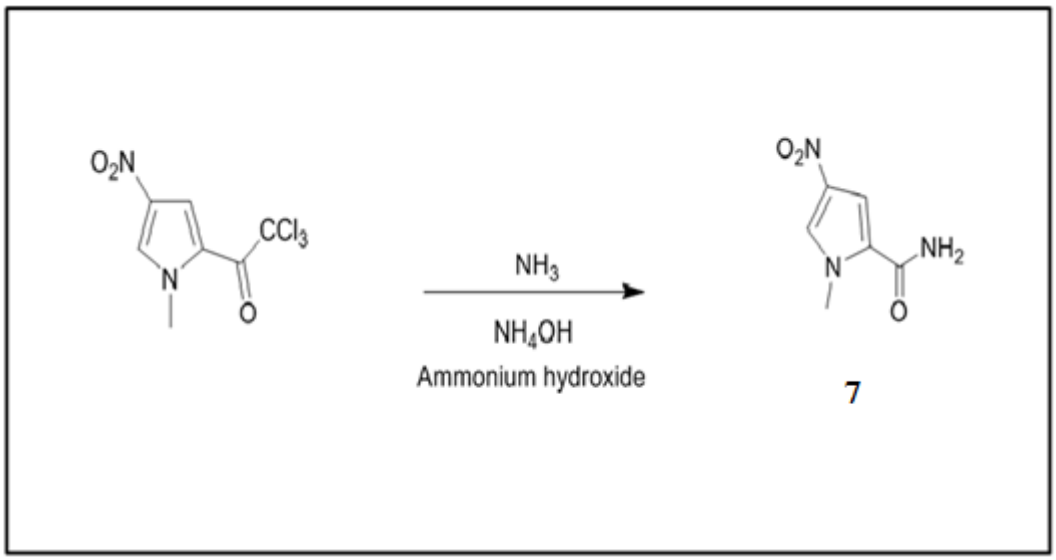
6

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

Yield; 9g, 75%, m.p. =133-135°C . (Lit = 137-139°C).

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

2.2.7 Preparation of 1-methyl-4-nitro-1H-pyrrole-2-carboxamide 7.

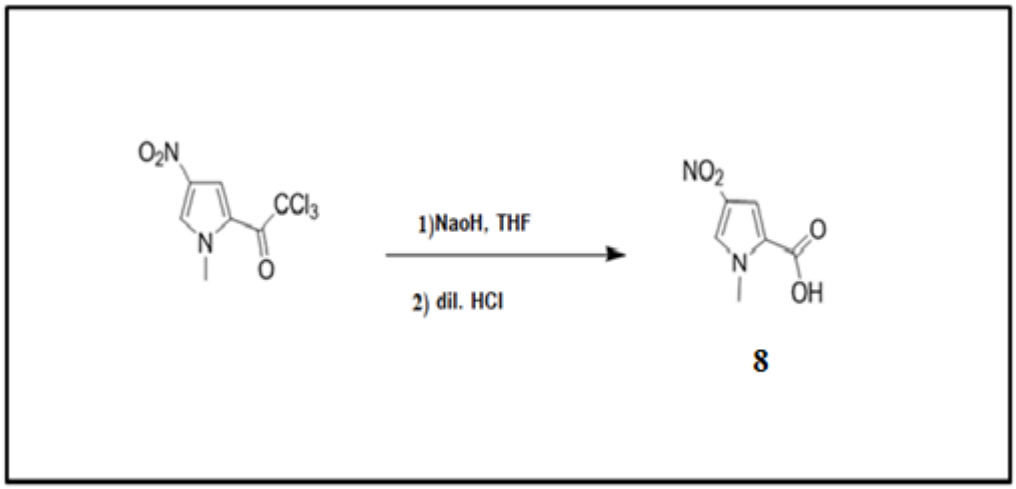


2,2,2-Trichloro-1-(1-methyl-4-nitro-1H-pyrrole-2-yl)ethanone (0.5g, 184mmol) was dissolved in THF (10 ml), ammonium hydroxide (10 ml) was then added to the solution . The solution was allowed to stir overnight, during this time the product precipitated as a white solid, which was dried under reduced pressure, after that water (15 ml) was added, and the product was extracted with ethyl acetate (20 ml) and then the solvent was removed under reduced pressure to obtain the product as a white solid powder.

Yield; 0.25g, 80%, m.p. = 211-213°C .

IR (KBr): 3431, 3349, 3195, 3132, 2922, 1671, 1100 cm^{-1}.

2.2.8 Preparation of 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid 8.



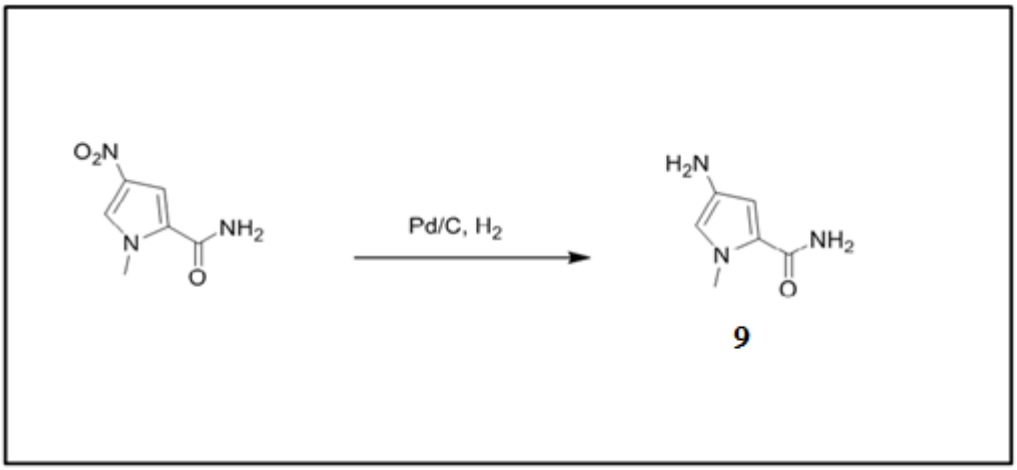
8

2,2,2,-Trichloro-1-(1-methyl-4-nitro-1H-pyrrole-2-yl)ethanone (16.00 g, 58.93mmol) was dissolved in THF (35 ml). A solution of sodium hydroxide (7g, 175mmol) in water (70 ml) was added and the solution was allowed to reflux for 2hr. The reaction mixture was evaporated to get rid of THF and the solution was cooled to 0°C and acidified with diluted hydrochloric acid, during which the product precipitated as a white /off-white solid.

Yield; 8g, 80%, m.p. = 198-200°C . (Lit = 198-200°C)

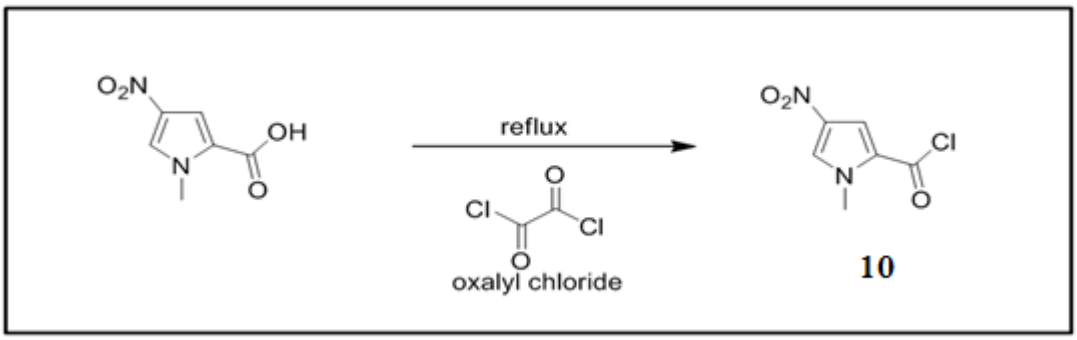
IR (KBr): 3852, 3748, 3534, 3137, 2345, 1684, 1613, 1416, 1314,751 cm^{-1}.

2.2.9 Preparation of 4-amino-1-methyl-1H-pyrrole-2-carboxamide 9.



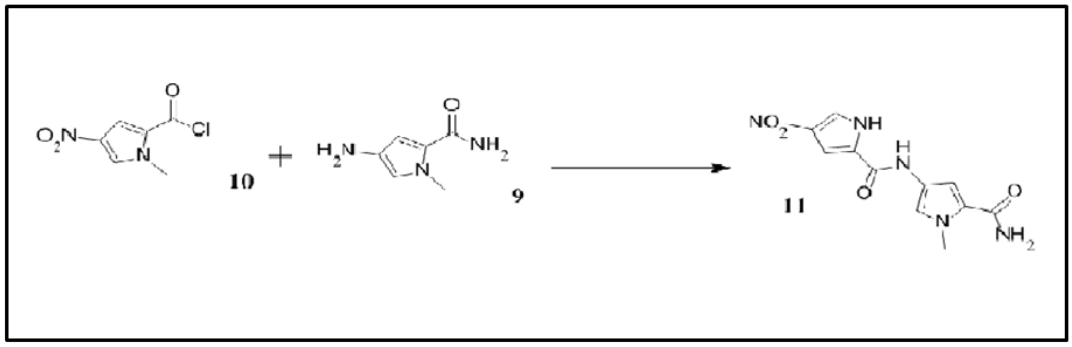
1-methyl-4-nitro-1H-pyrrole-2-carboxamide (1 g, 5.91mmol) was dissolved in methanol (50 ml) and a few amount of THF in 250 ml in round-bottomed flask, then (0.5 g) Pd/C was added slowly at 0 °C . The suspension was then placed under hydrogen, and allowed to stir for 4 hr. The suspension was then filtered through celite (6 g), and the solvent was removed under reduced pressure to yield the product which was used directly in the next step of synthesis due to the lack of stability for this aminopyrrole product.

2.2.10 Preparation of 1-methyl-4-nitro-1H-pyrrole-2-carbonyl chloride 10.



1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid (1 g, 5.87mmol) was placed in a round-bottomed flask (250 ml) and dissolved in DCM (30 ml). DMF (0.5 ml) was added as a catalyst. Oxalyl chloride (2 ml) was then added and the solution was refluxed for 2 hr. Finally, the solvent was removed under reduced pressure to obtain the product which was used directly in the next step of synthesis due to the lack of stability of the acid chloride.

2.2.11 Preparation of N-(5-carbonyl-1-methyl-1H-pyrrol-3-yl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide 11.



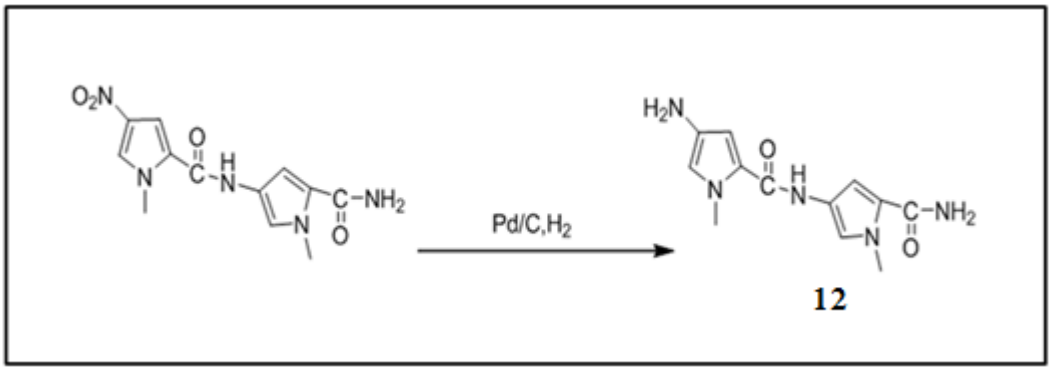
4-amino-1-methyl-1H-pyrrole-2-carboxamide **9** was dissolved in THF (20 ml) , and triethylamine (1 ml), followed by drop-wise addition of 1-methyl-4-nitro-1H-pyrrole-2-carbonyl chloride **10** (0.5 g, 2.65mmol) in DCM (10 ml). This solution was then allowed to stir overnight, during which a yellow precipitate formed, which was filtered and dried under reduced pressure. The product was extracted several times firstly with ethyl acetate (100 ml)and water (40 ml), secondly with distilled water (40 ml) only , after that with distilled water (38 ml) together with concentrated HCl (2 ml). Finally, washing with distilled

water (30 ml), then organic layer was taken and the solvent was removed under reduced pressure to obtain the product.

Yield; 0.8g, 50%, m.p= 153-156°C .

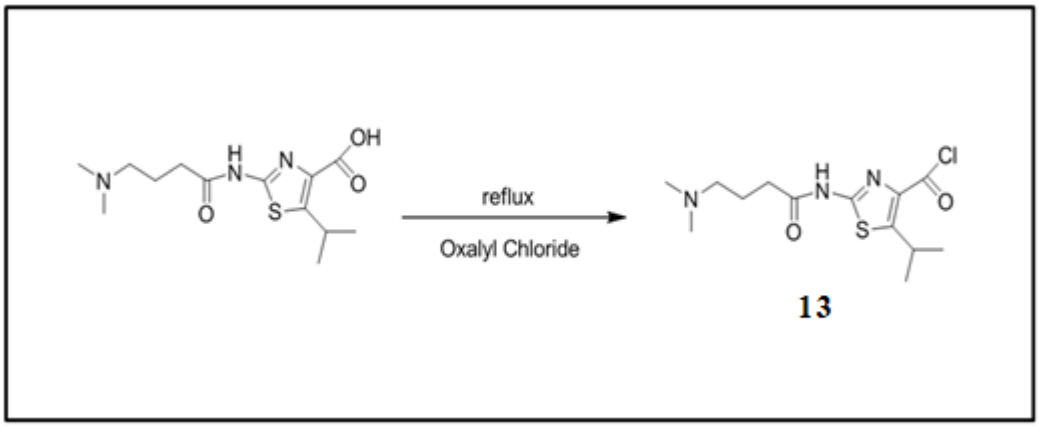
IR (KBr): 3444, 3137, 2958, 2921, 2835, 1719, 1623, 1297, 1101, 657cm^{-1}.

2.2.12 Preparation of 4-amino-N-(5-carbonyl-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide 12.



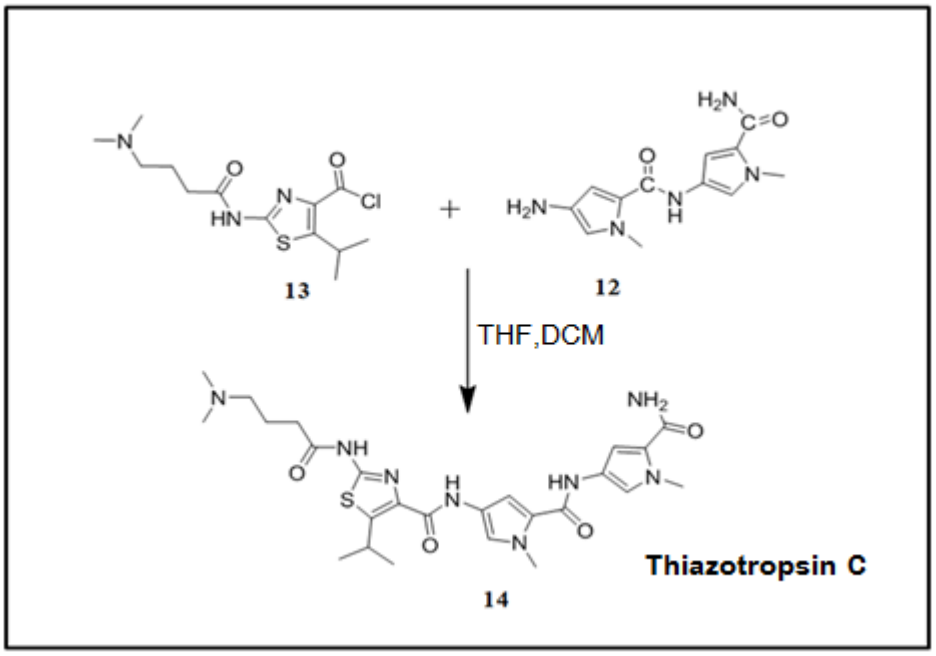
N-(5-carbonyl-1-methyl-1H-pyrrol-3-yl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide (0.35g, 1.2017mmoles) was dissolved in methanol (30 ml) and a little amount of THF in 250 ml round bottomed flask, then (0.2 g) Pd/C was added slowly at 0 °C. The suspension was then placed under hydrogen, and allowed to stir for 4 hr. The suspension was then filtered through celite (6 g), and the solvent was removed under reduced pressure to yield the product which was used directly in the next step of synthesis due to the lack of stability of this product.

2.2.13 Preparation of 2-(4-(dimethylamino)butanamido)-5-isopropylthiazole-4-carbonylchloride 13.



2-(4-(Dimethylamino)butanamido)-5-isopropylthiazole-4-carboxylic acid (0.359g, 1.2017mmoles) was placed in a round-bottomed flask (250 ml) , DCM (30 ml) was added. DMF (0.5 ml) was added as a catalyst. The oxalyl chloride (1.5ml) was then added and the solution was refluxed for 2 hr. The solvent DCM was removed under vacuum to obtain the product which was used directly in the next step of synthesis due to the lack of stability of this product.

2.2.14 Preparation of N-(5-(5-carbonyl-1-methyl-1H-pyrrol-3-ylcarbomyl)-1-methyl-1H-pyrrol-3-yl)-2-(4-(dimethylamino)butanamid)-5-isopropylthiazole-4-carboxamide 14.



4-amino-N-(5-carbonyl-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide **12** was dissolved in THF (20 ml), and triethylamine (1ml), followed by the dropwise addition of 2-(4-(dimethylamino)butanamido)-5-isopropylthiazole-4-carbonylchloride **13** (0.2 g, 0.629mmol) in DCM (10 ml). This solution was allowed to stir overnight, during which time a yellow precipitate formed, which was filtered and dried under vacuum. The product was then purified using preparative TLC.

Yield; 0.4g, 63%, decomposition at high temperature.

IR (KBr): 3384, 2962, 2667, 2484,1719, 1622, 1399 cm^{-1} .

CHAPTER 3

Result and Discussion

3.1 Synthesis of Distamycin Analogue

The aim of this project, as previously mentioned, is to synthesise a distamycin analogue to target the androgen response element sequence (WGWWCW where W is A or T). Thiazotropsin A is a distamycin analogue that was proven to be able to bind to the sequence WCWWGW. In this project, we propose to synthesise an analogue of thiazotropsin A (thiazotropsin C , see **Figure** 3.1) by reversing the position of both the DMAP tail and the isopropylthiazole ring from the right hand side (in thiazotropsin A) to the left hand side (in thiazotropsin C) in order to target androgen response element sequence (WGWWCW). These compounds (MGBs), which bind non–covalently to the minor groove of DNA have shown therapeutic potential in the treatment of cancer, viral, fungal and bacterial diseases.

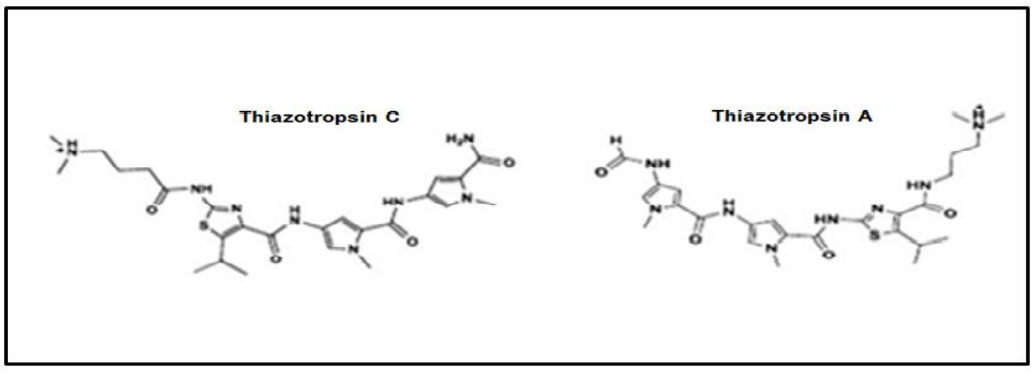


Figure (3.1): Structure of Thiazotropsin A , and its Proposed Analogue, Thiazotropsin C.

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

3.2 Synthesis of the MGB Monomers

2,2,2-trichloro-1-(1-methyl-4-nitro-1H-pyrrole-2-yl) ethanone **6** (**Figure** 3.2 & 3.4) was prepared by the nitration of 2-trichloroacetyl-N-methylpyrrole with nitric acid in acetic anhydride , in a good yield (75%). 2-trichloro-acetyl-N-methylpyrrole **5** was also prepared in a good yield (73%) by the reaction of trichloroacetylchloride **17** and N-methylpyrrole **16.**

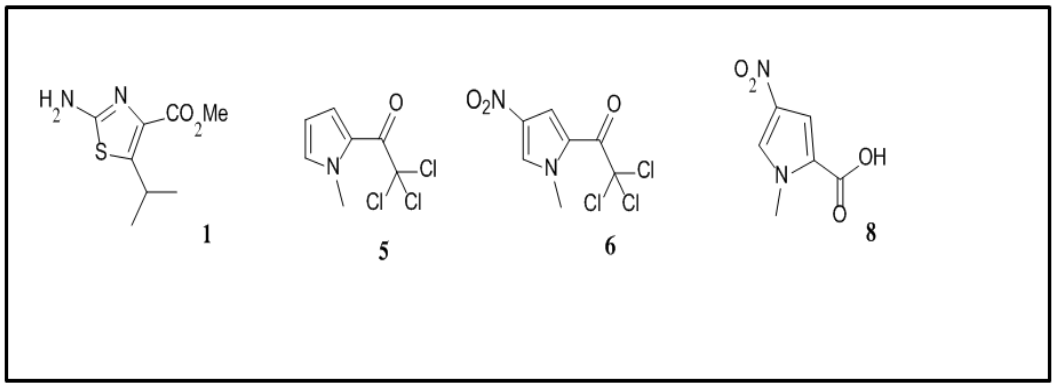


Figure (3.2): Structure of Some Monomers Used in the Synthesis of MGBs.

The pyrrole carboxylic acid **8** is a fairly commercially expensive monomer, so the low yield seen for the nitration is a problem. To resolve this issue, an alternative preparation was carried out as described below (**Figure** 3.3). This allowed the desired carboxylic acid to be prepared in an improved yield, and from less expensive starting materials. The nitration of **5** yields **6**, the desired nitration takes place in good yield (75%), and **6** can in turn be hydrolysed with sodium hydroxide to give **8** in a very good yield (80%).

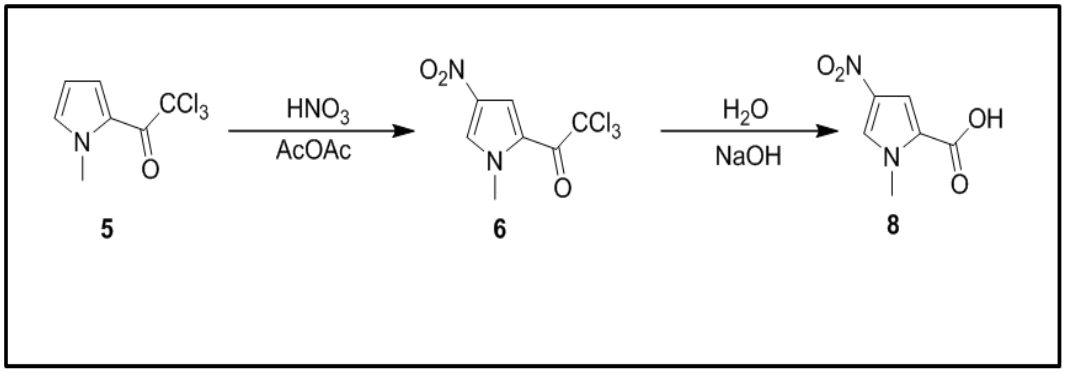


Figure (3.3): Preparation of Nitropyrrole Carboxylic Acid .

The amide product **7** obtained in two steps: 2-trichloro-acetyl-N-methylpyrrole **5** was reacted with nitric acid in acetic anhydride to give **6** , which was then reacted with ammonium hydroxide to give **7** in an (80%) yield (**Figure** 3.4).

Methyl-2-amino-5-isopropyl-1,3-thiazole-4-carboxylate **1** was also prepared in a reasonable yield (64%) by reacting methyl dichloroacetate with isobutyraldehyde at 0ºC in diethyl ether in the presence of sodium methoxide as a strong base to give the α-chloro glycidic ester intermediate **18 (**Darzens reaction**)**.

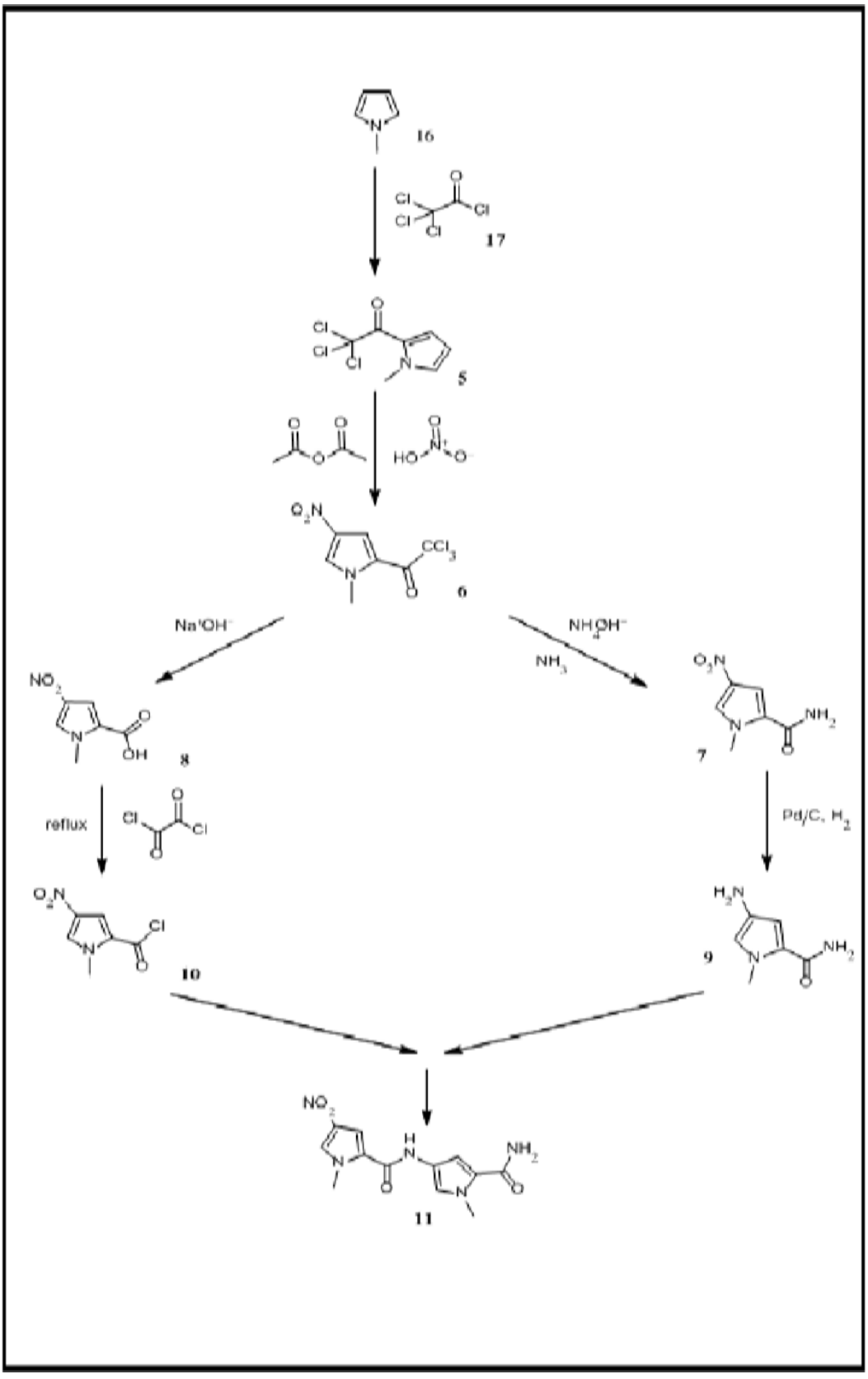


Figure (3.4): Synthesis of the Amide Linked Minor Groove Binder.

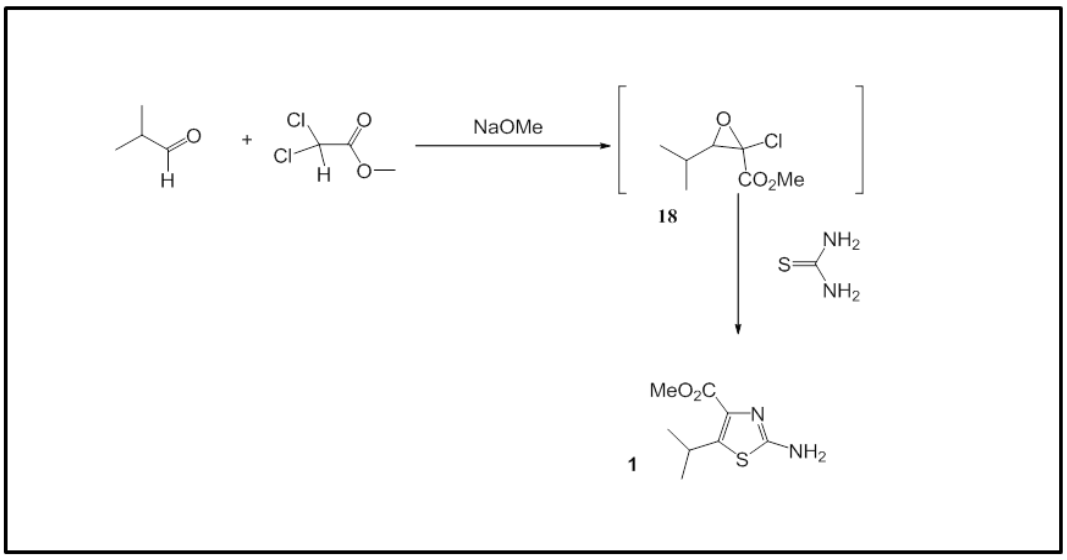


Figure (3.5): Preparation of Isopropyl Thiazole.

1 is outlined above in (**Figure** 3.5) and the mechanism of Darzens reaction is described in (**Figure** 3.6).

Since the α-chloro glycidic ester is highly reactive , it was directly used for the second step without further purification. The second step involves the coupling between the the α-chloro glycidic intermediate and thiourea in dry methanol to give the methyl 2-amino-5-isopropyl-1,3-thiazole-4-carboxylate **1.** The reaction proceeds via nucleophilic attack of the thiourea sulfur atom on the epoxide carbon which leads to epoxide ring opening and the formation of new α-carbonyl moiety. Formation of the thiazole ring was achieved by subsequent nucleophilic attack of the thiourea amino group on the α-carbonyl carbon in a common Schiff's base

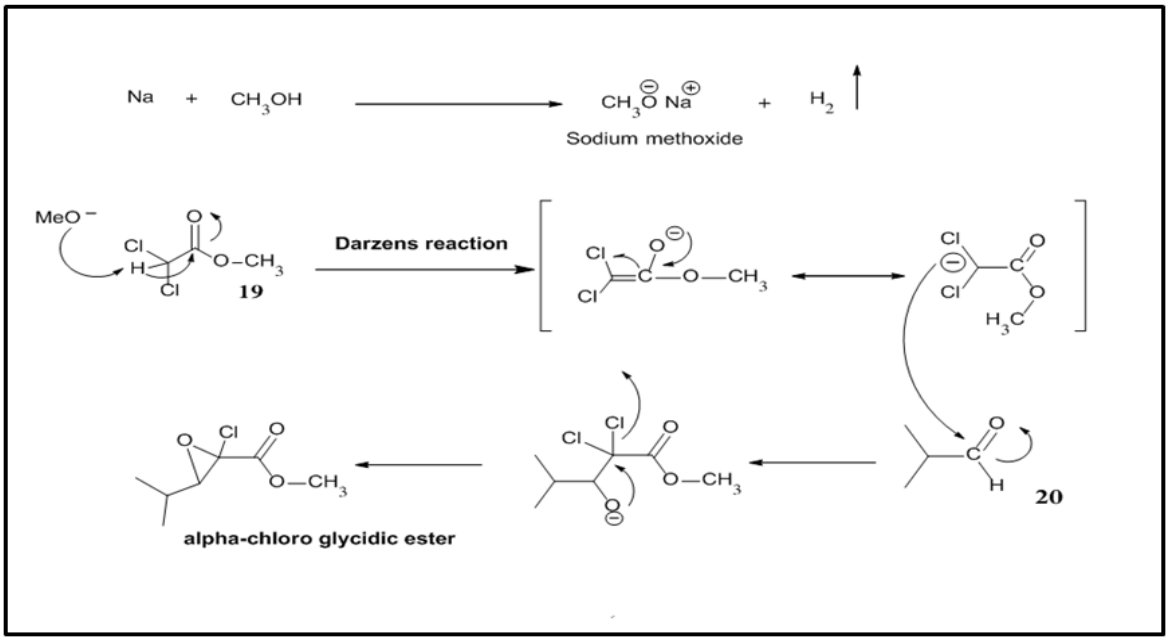


Figure (3.6): The Mechanism of Darzens Reaction.

reaction to form the thiazolidine ring. Two dehydration steps of the thiazolidine ring then restore the aromaticity of the thiazole ring. (See **Figure** 3.7).

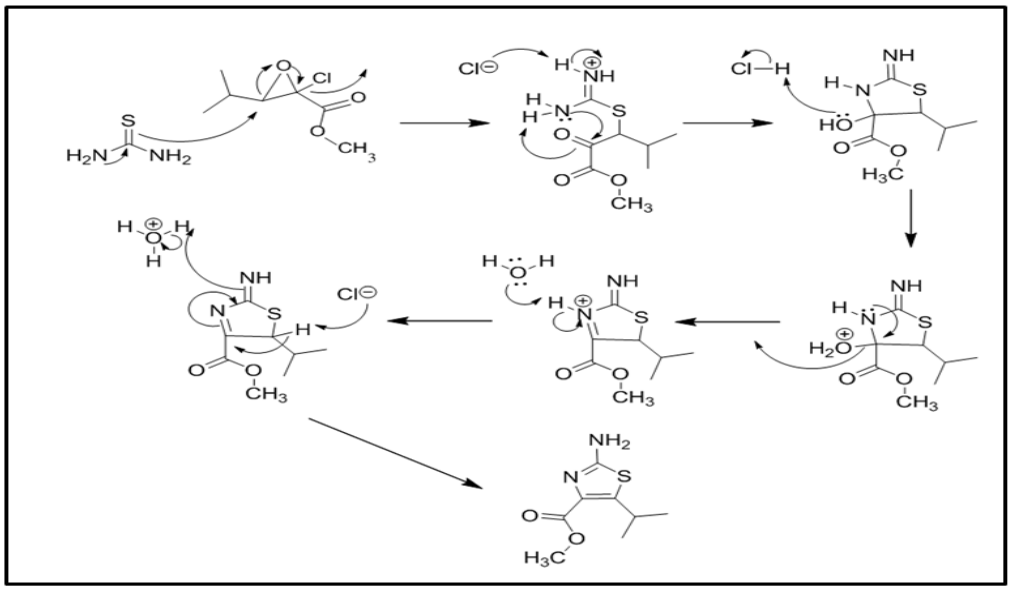


Figure (3.7): Mechanism of Thiazole Ring Formation by the Coupling between α-chloro glycidic Ester and Thiourea.

3.3 Synthesis of the Amide-Linked Pyrrole Dimer

4-amino-N-(5-carbonyl-1-methyl-1H-pyrrol-3-yl)-1-methyl-1H-pyrrole-2-carboxamide **12** (**Figure** 3.8) was prepared by the coupling between **9** and**10** this followed by catalytic hydrogenation using Pd-C/H_2. **10** was prepared by refluxing the carboxylic acid **8** with oxalyl chloride to obtain the acid chloride.

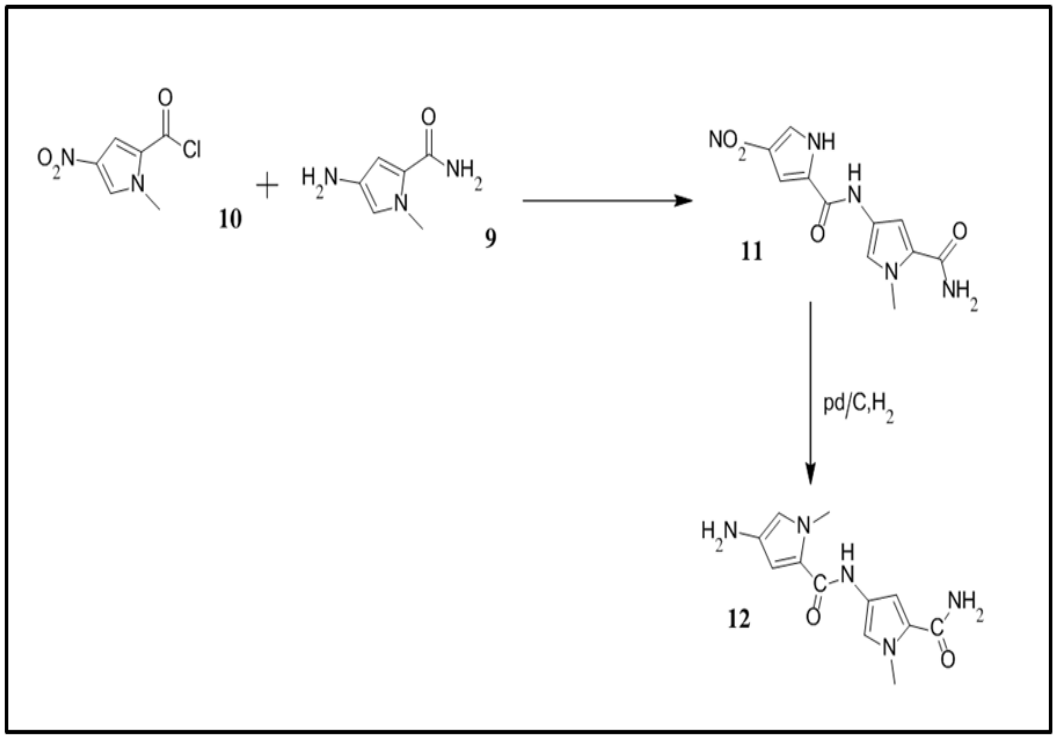


Figure (3.8): Synthesis of the Amide Linked Pyrrole Dimer

Compound **9** was prepared by reducing the nitro compound **7** by calalytic hydrogenation (see **Figure** 3.4). The amino compound **12** was used directly in the next step of synthesis due to the lack of stability of the amino group attached to the pyrrole ring.

3.4 Synthesis of 2-(4-(dimethylamino)butanamido)-5-isopropylthiazole-4-carbonylchloride 13.

4-(Dimethylamino) butanoyl chloride **21** (**Figure** 3.10) was prepared by refluxing **15** in oxalyl chloride to produce the acid chloride, which was directly coupled with the amino group of **1** to obtain **3** in (65%) yield. Compound **4** was obtained by the basic hydrolysis of the methyl ester in compound **3** to corresponding carboxylic acid in very good yield (83%). This was followed by refluxing in oxalyl chloride to give the acid chloride **13** which was used directly in the next step of synthesis due to the lack of stability of the acid chloride**.**

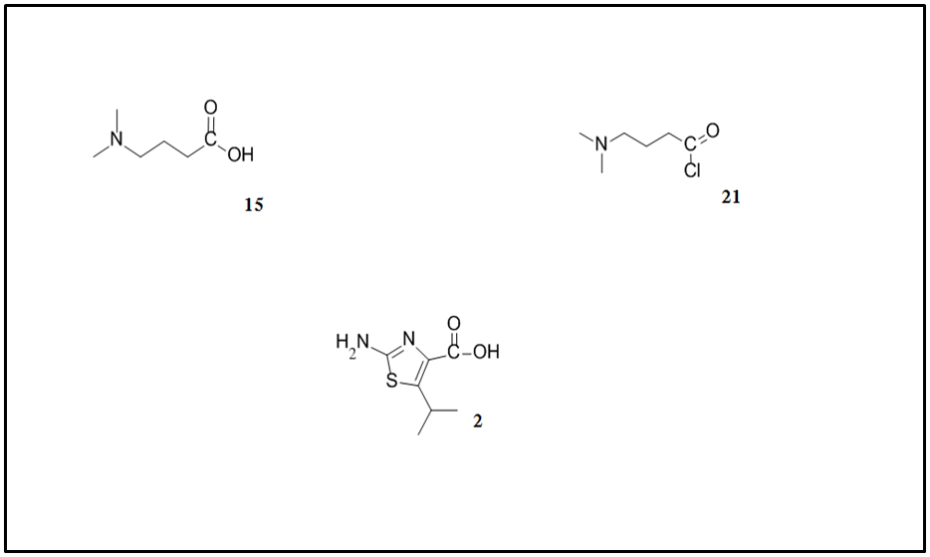


Figure (3.9): Structure of Some Monomers

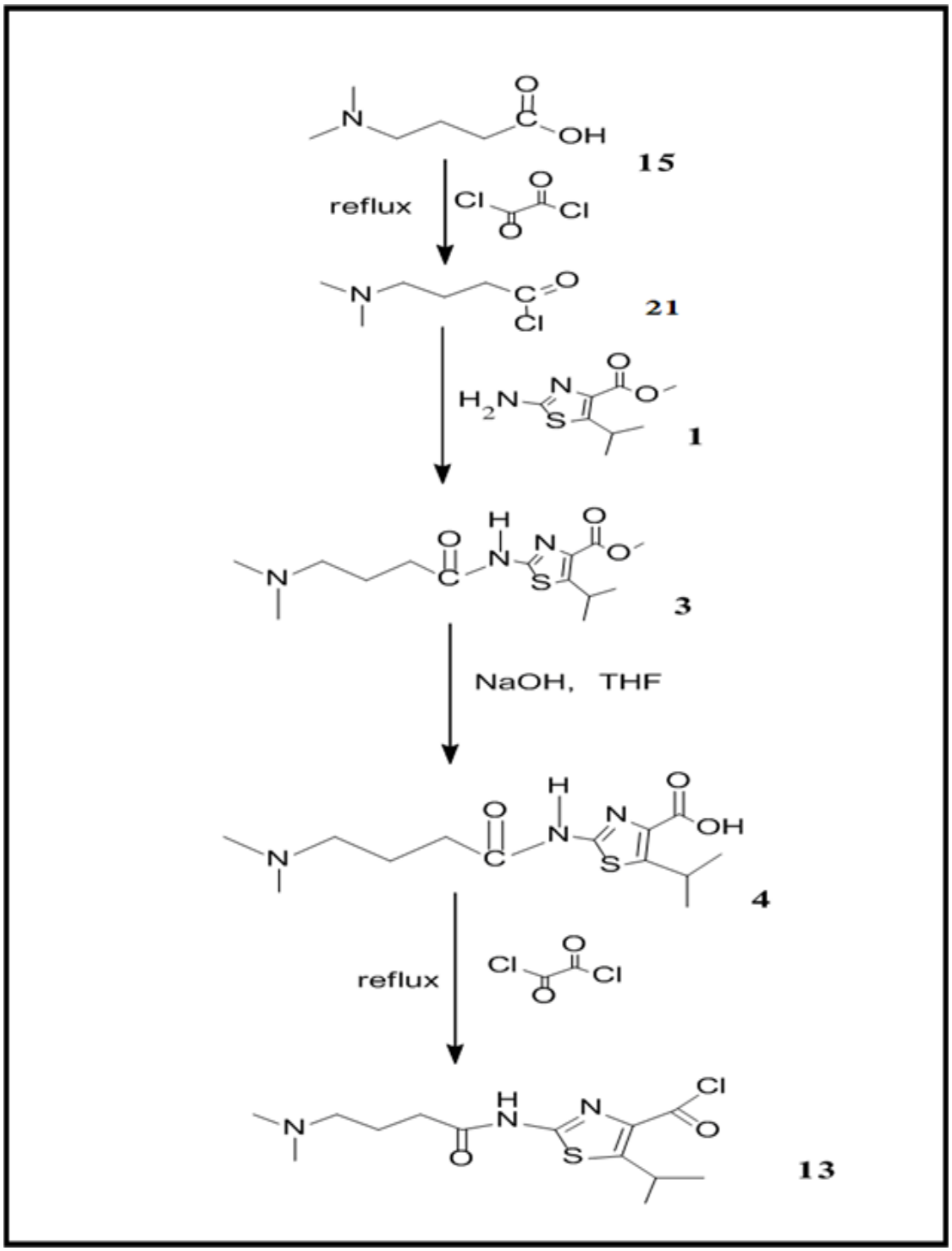


Figure (3.10): Synthesis of 2-(4-(dimethylamino)butanamido)-5-isopropylthizole-4-Carbonylchloride **13**.

3.5 Synthesis of Thiazotropsin C

N-(5-(5-carbonyl-1-methyl-1H-pyrrol-3-ylcarbomyl)-1-methyl-1H-pyrrol-3-yl)-2-(4-(dimethylamino)butanamid)-5-isopropylthiazole-4-carboxamide **14** (**Figure** 3.11) was prepared by coupling of the pyrrole dimer amine **12** with the acid chloride **13** in a good yield.

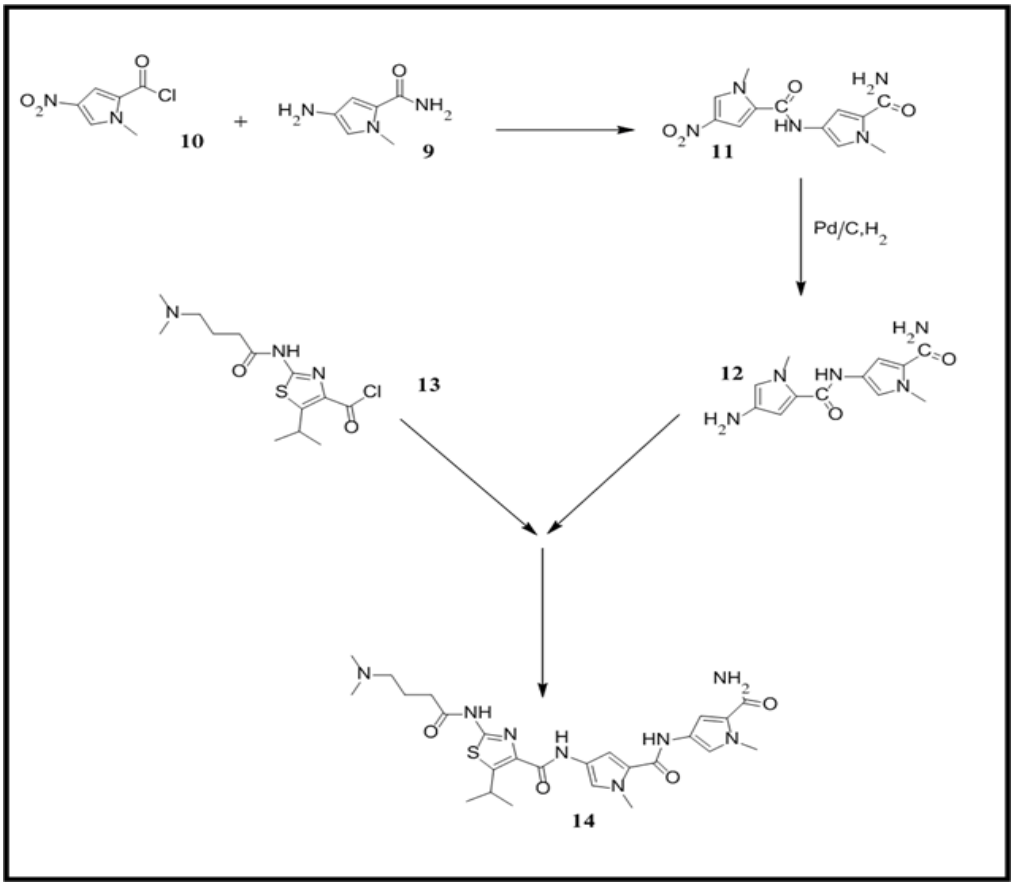


Figure (3.11): Synthesis of Thiazotropsin C.

The Researcher believe that compound **14** (Thiazotropsin C) was synthesized based on the FT-IR data and ESI-FTMS data.

Based on the FT-IR spectra , the following functional groups are tentatively assigned as shown in the Table (3.1) below.

Table (3.1): Table of IR Absorptions.

Functional Group	**Experimental υ in cm^{-1}**	**Theoretical υ in cm^{-1}**
Amine N—H Stretch	3384	3300-3500
Amide N—H Stretch	3644	3500-3700
O—H Stretch	2684-2956	2500-3000(broad)
Aromatic C—H stretch	792	680-860
C═C Stretch	3038	3300
C—H Stretch	2949	2850-2950
C═O Stretch	1719	1710-1780
C—N Stretch	1203	1020-1220

The IR spectrum of our compound Thiazotropsin C **14** showed absorption bands around 3384 cm^{-1} (NH, str), 2962 cm^{-1} (CH, str), 1622 cm^{-1} (C═O, str), 1014 cm^{-1} (C—N, str). Further spectra are shown in the appendix.

Based on the ESI-FTMS some of masses are tentatively assigned in the Table (3.2) below.

Note: Our product has molecular weight of 542.

Table (3.2) Mass spectra.

Masses	Compound No.
291	No.11
549	No.14 + lithium attached.
560	No.14 + water attached.

The molecular ion peak appearing at m/z 291 is believed to be compound no.11, The ion peak at m/z is related to thiazotropsin C (14) with lithium attached, and ion peak appearing at m/z 560 is believed to be thiazotropsin C with water attached.

3.6 Conclusion and Future Work

In conclusion, we provided a successful and convenient synthetic route for preparing a new distamycin analogue (thiazotropsin C), using a wet lab synthesis. The next step in this process will be to study the biological activity of this compound. If the biological results are encouraging, this molecule will be taken as a lead compound and several modifications can be done at the head and tail positions of the molecule in order to improve its biological activity.

Further work will be concerned with developing the possible analytical uses for these minor groove binders. For instance, these compounds can be sent abroad for foot-printing and isothermal titration calorimetry (ITC) studies to determine their exact binding site, and to

determine whether such modifications in the structure of the distamycin analogues has any effect on the preferred sequence for binding.

Finally, the main obstacle we faced while carrying out this project is the absence of some important analytical instruments at An-Najah University, which are required for both purification and identification of organic compounds, such as preparative HPLC, mass spectrometer (MS), and NMR spectrometer. The lack of these instruments has slowed our progress and forced us to rely on old fashion methods for purification and identification of organic compounds.

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Appendix

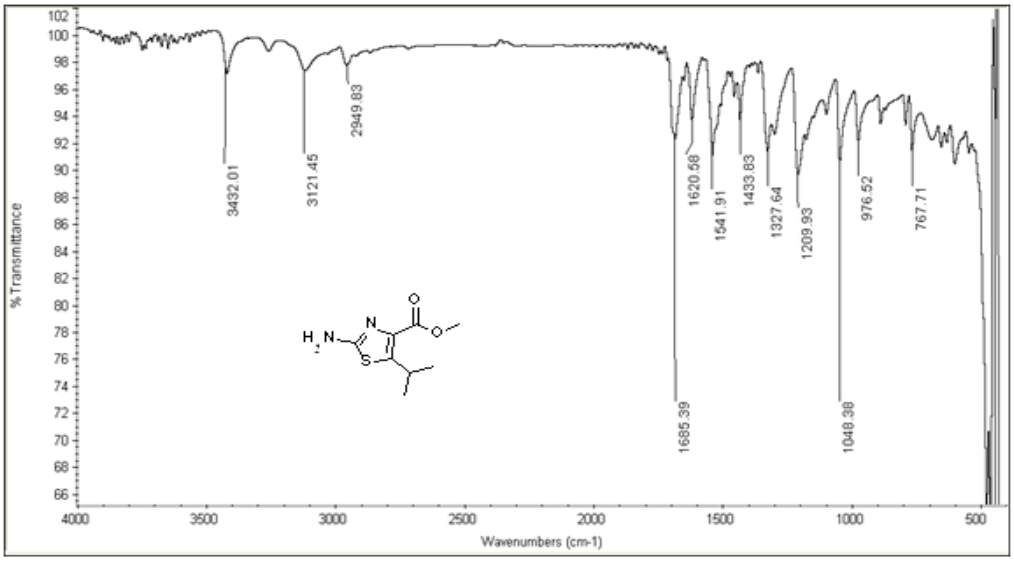


Figure 1: FT-IR spectrum of methyl 2-amino-5-isopropyl-1,3-thiazole-4-carboxylate **1**.

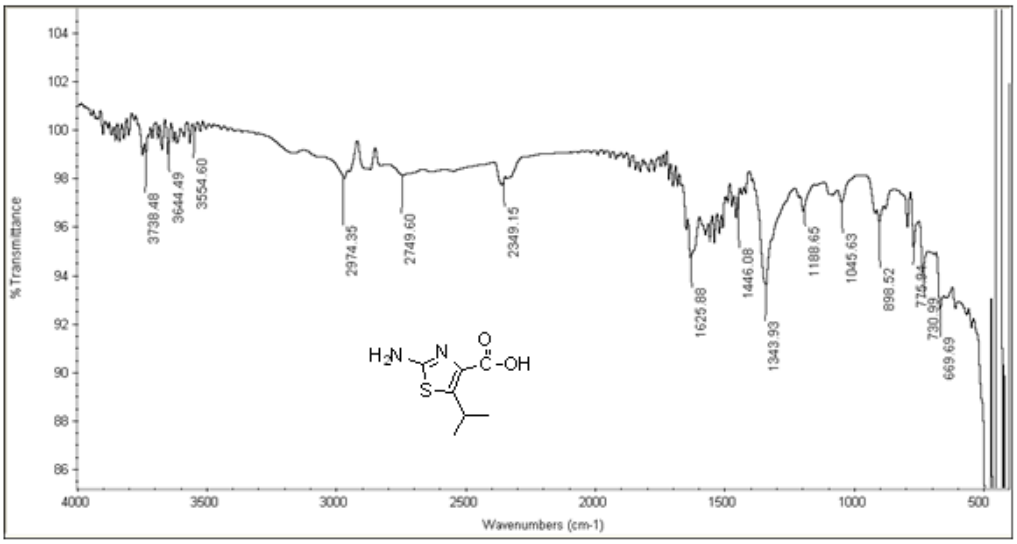


Figure 2: FT-IR spectrum of 2-amino-5-isopropylthiazole-4-carboxylic acid **2**.

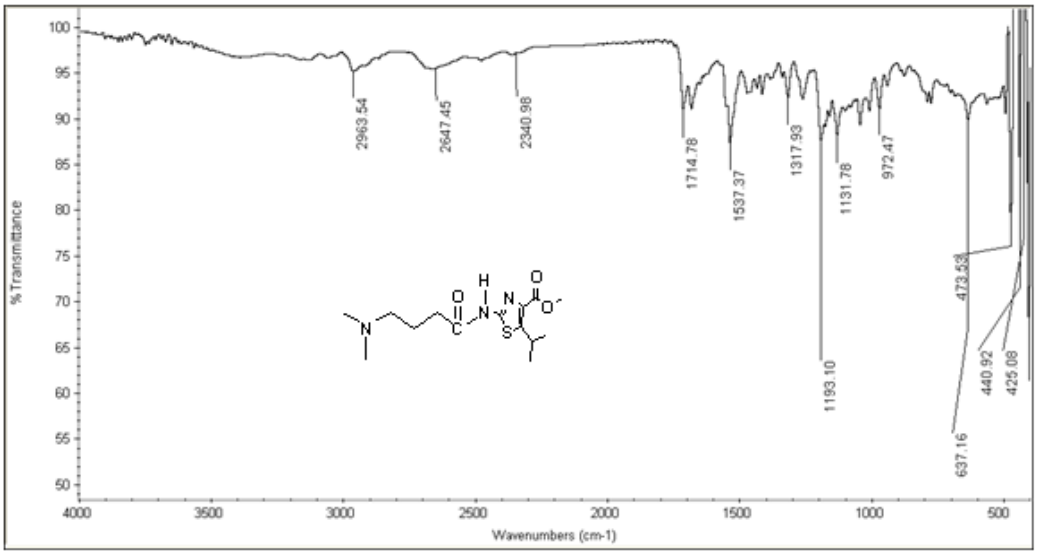


Figure 3 : FT-IR spectrum of methyl 2-(4-(dimethylamino)butanamido)-5-isopropylthiazole-4-carboxylate **3**.

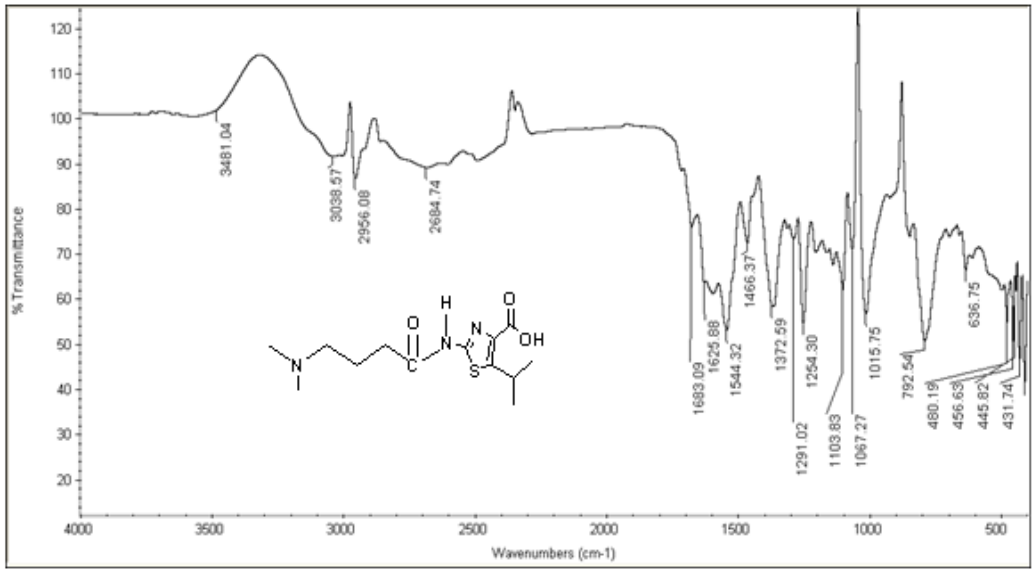


Figure 4 : FT-IR spectrum of 2-(4-(dimethylamino)butanamido)-5-isopropylthiazole-4- carboxyli acid **4**.

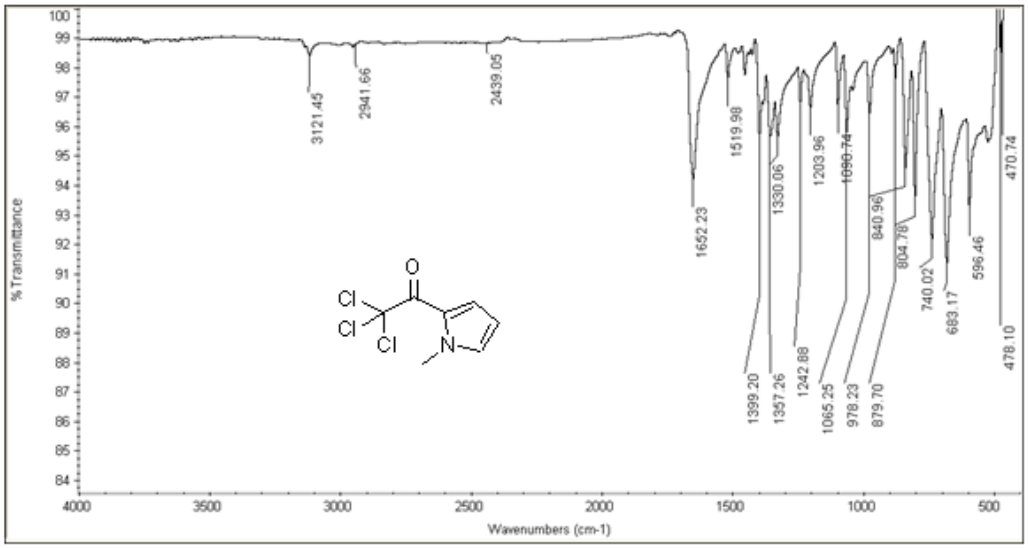


Figure 5 : FT-IR spectrum of 2-trichloroacetyl-N-methylpyrrole **5**.

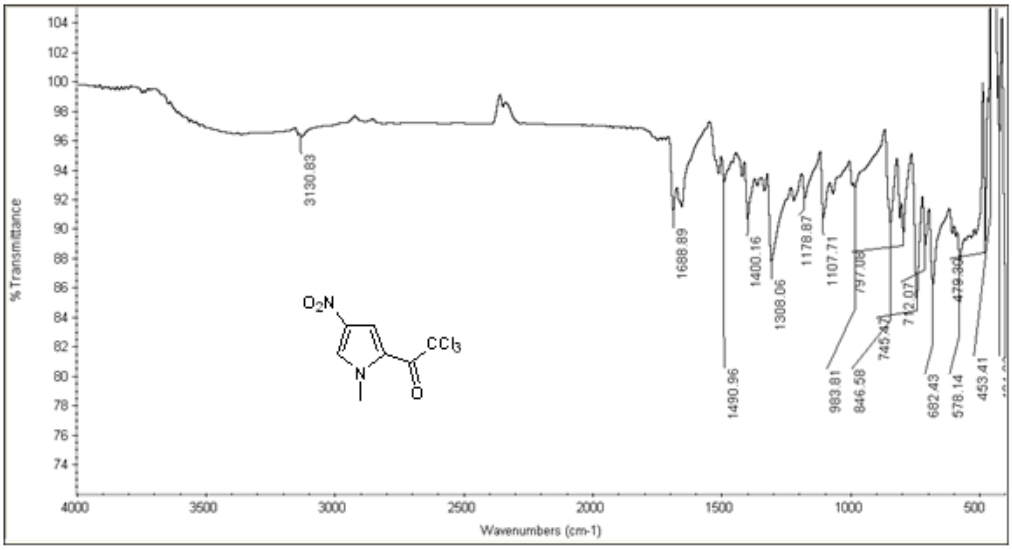


Figure 6 : FT-IR spectrum of 2,2,2-trichloro-1-(1-methyl-4-nitro-1*H*-pyrrol-2-yl) ethanone **6**.

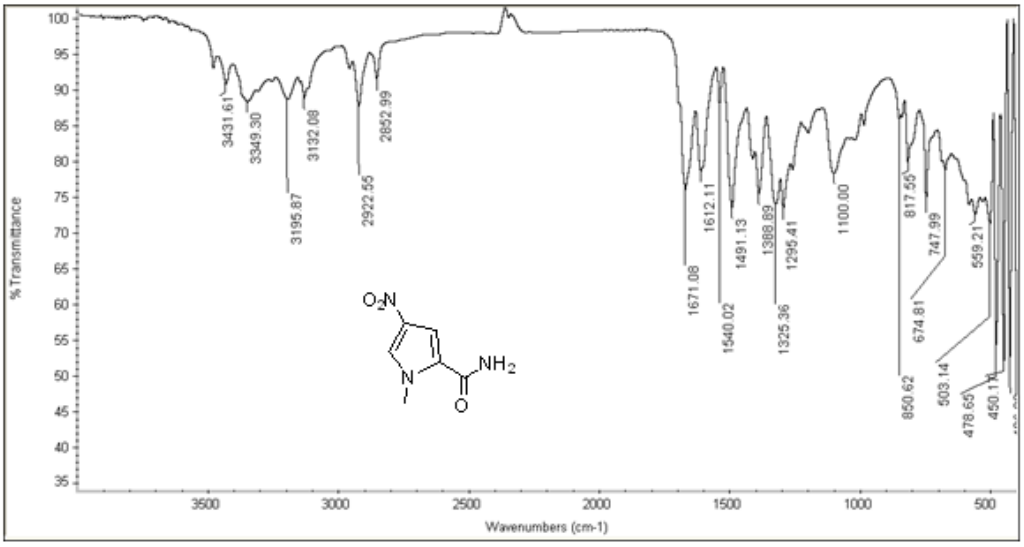


Figure 7 : FT-IR spectrum of 1-methyl-4-nitro-1H-pyrrole-2-carboxamide **7**.

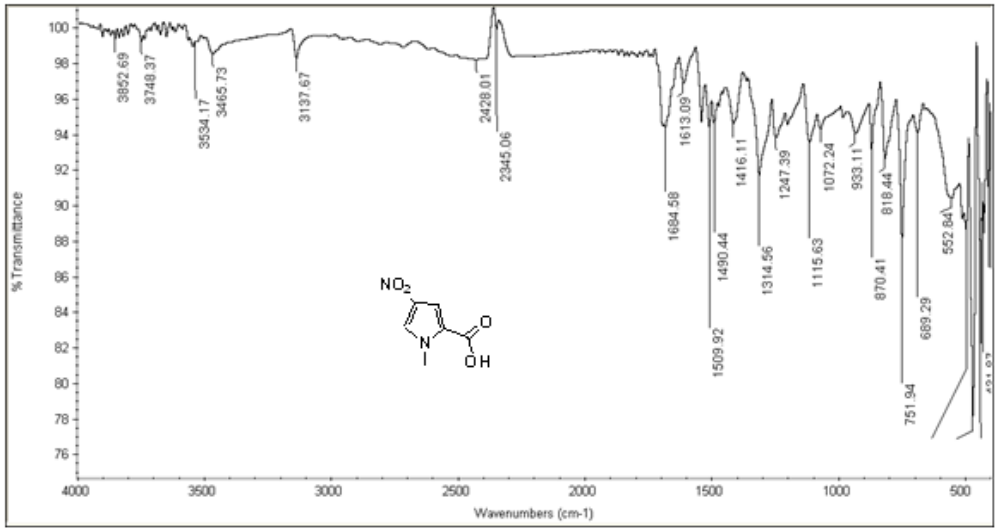


Figure 8 : FT-IR spectrum of 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid **8**.

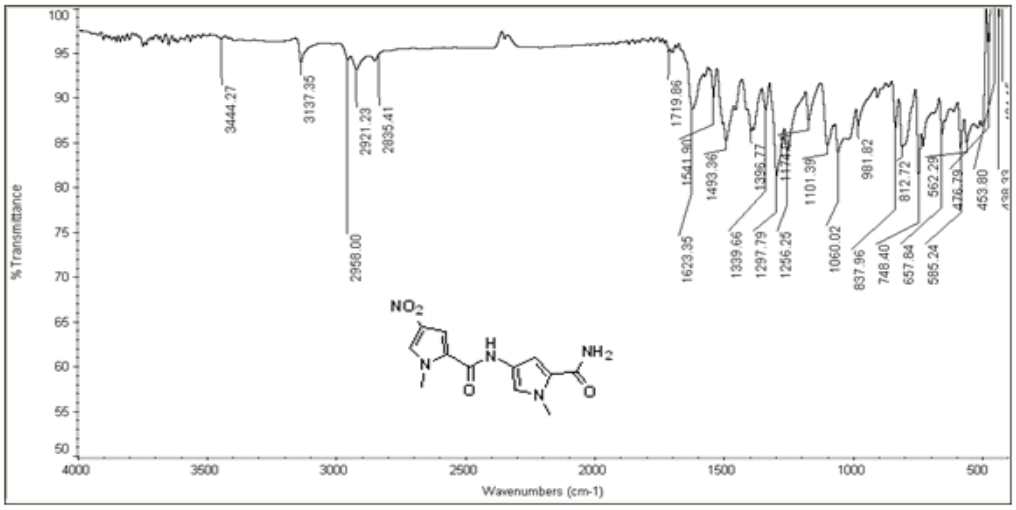


Figure 9 : FT-IR spectrum of N-(5-carbonyl-1-methyl-1H-pyrrol-3-yl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide **11**.

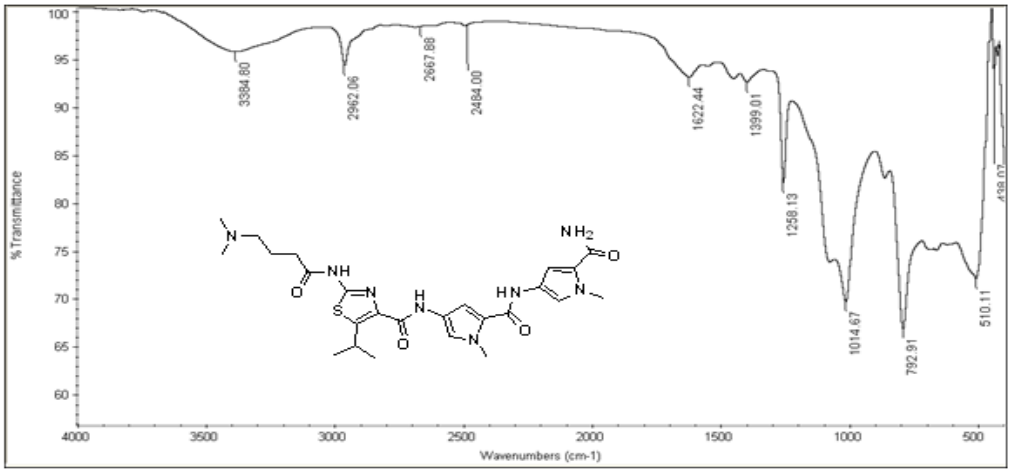


Figure 10 : FT-IR spectrum of N-(5-(5-carbonyl-1-methyl-1H-pyrrol-3-ylcarbomyl)-1-methyl-1H-pyrrol-3-yl)-2-(4-(dimethylamino)butanamid)-5-isopropylthiazole-4-carboxamide (Thiazotropsin C) **14**.

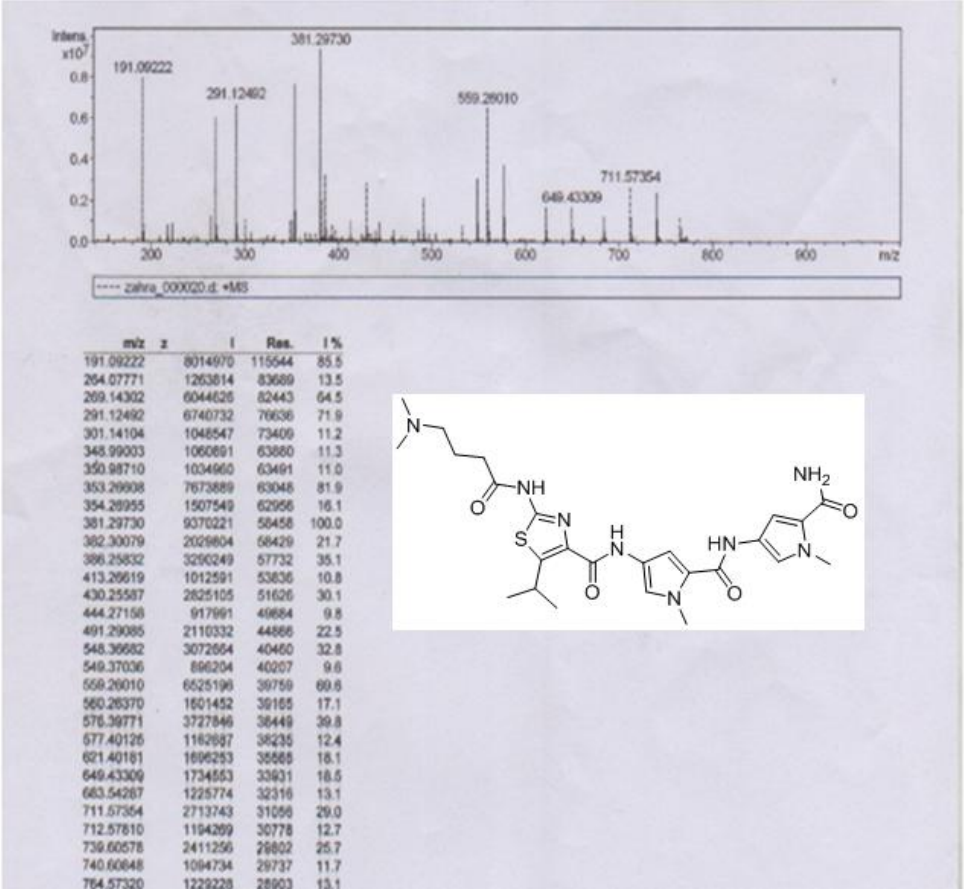
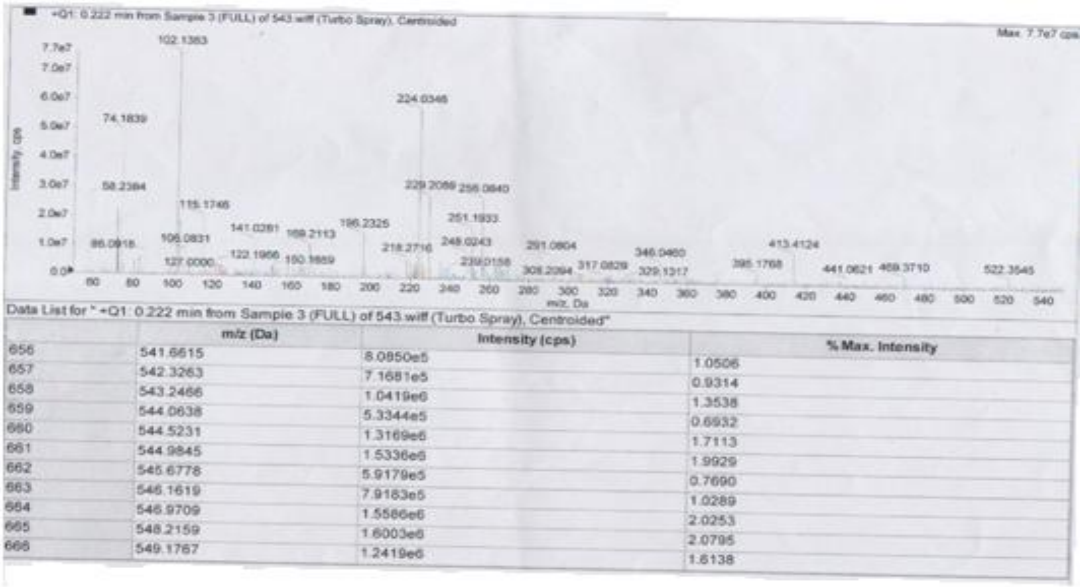
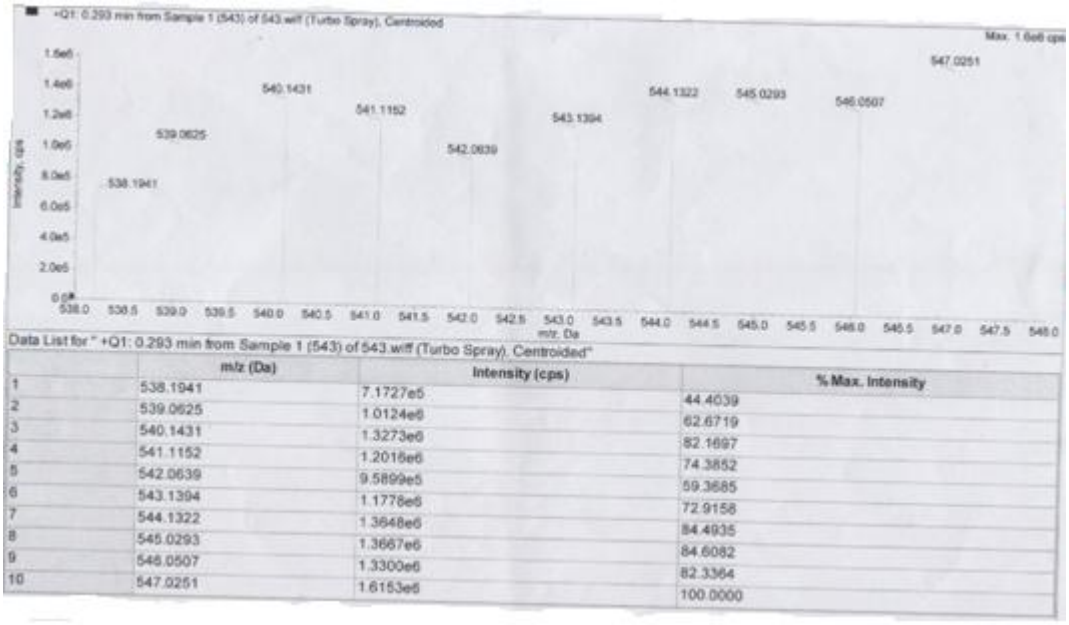


Figure (11-1): ESI-MS spectrum of N-(5-(5-carbonyl-1-methyl-1H-pyrrol-3-ylcarbomyl)-1-methyl-1H-pyrrol-3-yl)-2-(4-(dimethylamino)butanamid)-5-isopropylthiazole-4-carboxamide (Thiazotrpsin C) **14**.

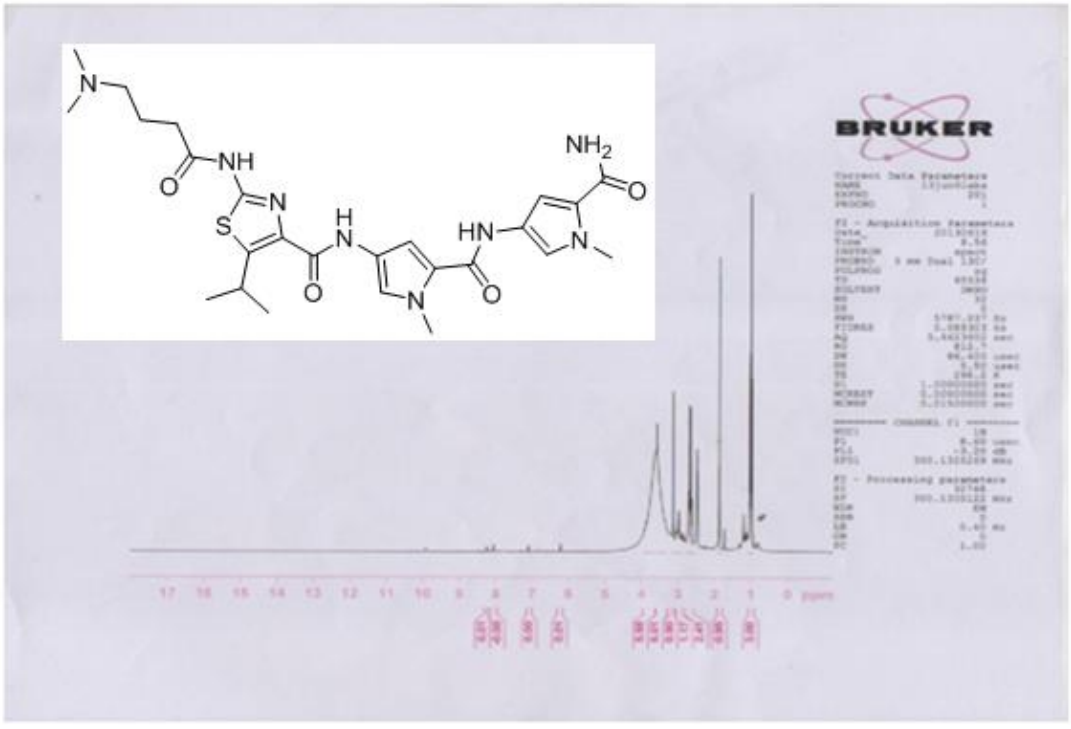




Data List for " +Q1: 0.293 min from Sample 1 (543) of 543.wiff (Turbo Spray), Centroided"

	m/z (Da)	Intensity (cps)	% Max. Intensity
1	538.1941	7.1727e5	44.4039
2	539.0625	1.0124e6	62.6719
3	540.1431	1.3273e6	82.1697
4	541.1152	1.2018e6	74.3852
5	542.0639	9.5899e5	59.3685
6	543.1394	1.1778e6	72.9158
7	544.1322	1.3648e6	84.4935
8	545.0293	1.3667e6	84.6082
9	546.0507	1.3300e6	82.3364
10	547.0251	1.6153e6	100.0000

Figure (11-2): MS spectra of N-(5-(5-carbonyl-1-methyl-1H-pyrrol-3-ylcarbomyl)-1-methyl-1H-pyrrol-3-yl)-2-(4-(dimethylamino)butanamid)-5-isopropylthiazole-4-carboxamide (Thiazotropsin C) **14**.



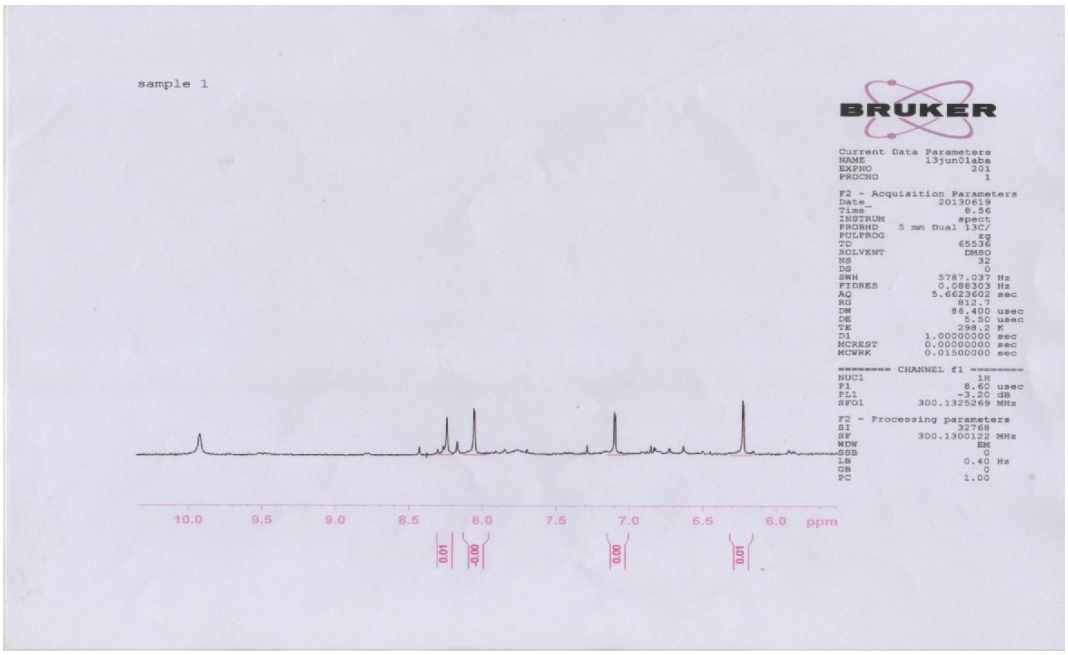


Figure (12): [1]HNMR spectra of N-(5-(5-carbonyl-1-methyl-1H-pyrrol-3-ylcarbomyl)-1-methyl-1H-pyrrol-3-yl)-2-(4-(dimethylamino)butanamid)-5-isopropylthiazole-4-carboxamide (Thiazotropsin C) **14**.

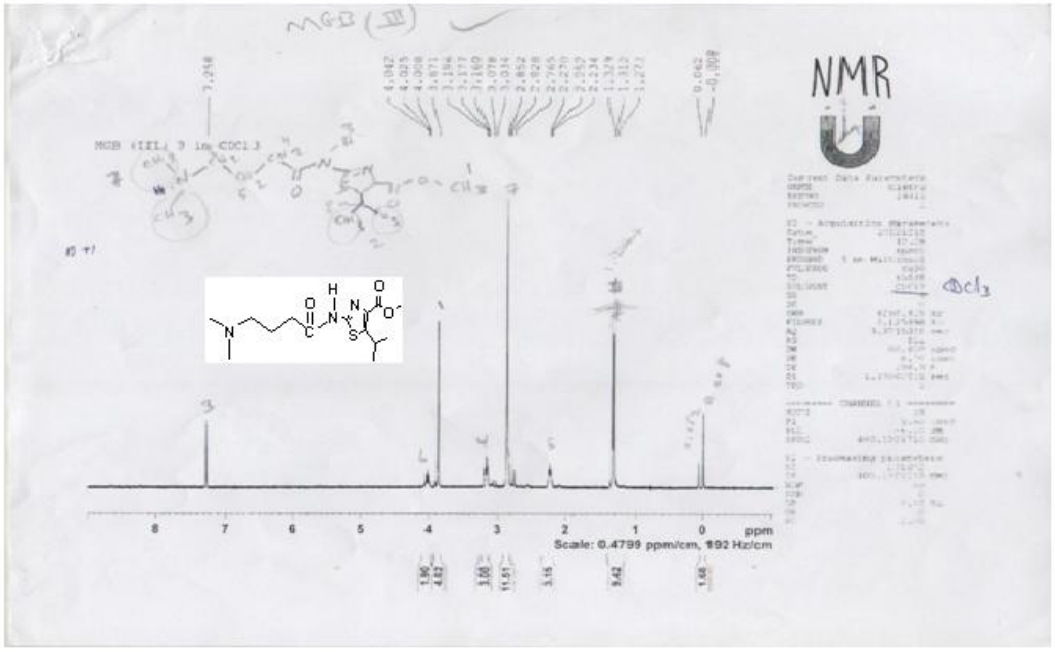


Figure (13): [1]HNMR spectrum of methyl 2-(4-(dimethylamino)butanamido)-5-isopropylthizole-4-carboxylate **3**.

جامعة النجاح الوطنية

كلية الدراسات العليا

تصنيع مركبات أميدية ترتبط بال (DNA) لاستخدامها في استهداف سلسلة ال (ARE)

إعداد

صابر محمود ابو جبل

إشراف

د. حسن النيص

أ.د. محمد النوري

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

2013

تصنيع مركبات أميدية ترتبط بالـــ (DNA) لاستخدامها في استهداف سلسلة الـــ (ARE)

إعداد

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أ.د. محمد النوري

الملخص

(MGBs) هي عبارة عن جزيئات ترتبط بالحمض النووي **(DNA)** . ال **Distamycin** وال **Netropsin** مركبات تتكون من مونومرات, هذه المونومرات تحتوي على حلقتين و ثلاث من الميثل بيرول ترتبط بروابط اميدية , هذه المركبات تمتلك فعالية علاجية قوية ضد الفيروسات والبكتيريا والسرطانات, وهي تلعب دور المضادات الحيوية , وبالتالي فهي مركبات سامة .

هذه الآثار البيولوجية تنشأ من ارتباط هذه الجزيئات بالحمض النووي **(DNA)** خاصة في الأجزاء التي يتواجد فيها سلاسل قصيرة من القواعد النيتروجينية من نوع A,T .

لقد كان هناك الكثير من العمل من اجل تطوير الأشباه لل **Distamycin** وال **Netropsin**, التي أثبتت فعاليتها العلاجية المحسنة وقدرتها على تخفيض درجة السمية .

أن الهدف من هذا المشروع هو تصنيع مركبات أميدية ترتبط بالحمض النووي **(DNA)** لاستهداف سلسلة عنصر استجابة الأندروجين **(ARE)** كمضادات حيوية ضد البكتيريا وضد السرطان .

الشبيه (المركب) المقترح لل **Distamycin** في هذه الأطروحة له كتلة مولية منخفضة تعمل على تعزيز خاصية الذوبان في الدهون لتحسين ارتباطها بالحمض النووي **(DNA)** , وزيادة الامتصاص ونفاذية الخلية لهذه الأشباه (المركبات) مما يزيد من فرصتها لتحل محل الأدوية التي يمكن أن يستخدمها الإنسان عن طريق الفم.

وفي هذه الاطروحة وصف ناجح لتطوير وتصنيع الشبيه للـ **Distamycin** وهو **(Thiazotropsin C)** .