An-Najah National University

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Synthesis, characterization & biological activity of Isopropyl Thiazole derivatives of the natural products Distamycin & Proximicin

By

Amjad Abdulrahman Mosa Abdulrahman

Supervisor

Dr. Hasan Y. Alniss

Co-Supervisor

Dr. Nidal Jaradat

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This thesis was defended successfully on 4/5/2017 and approved by:

Defense Committee Members	Signature
Dr. Hasan Alniss/ Supervisor	……
Dr. Nidal Jaradat / Supervisor	....……
Dr. Amjad Abu Salha / External Examiner	……..….
Dr. Murad Abu Al Hasan / Internal Examiner	…….....

Dedication

To My Father & Mother, the Spirit of life...

To my Brothers & Sisters, the Life Companions...

To my Wife, the Soul Mate...

To my flowers, Zaina & Abdulrahman, and to the coming next :)

I dedicate this work.

Acknowledgement

First of all, praise and thanks to God, for giving me patience and strength to complete this work.

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Amjad Abdulrahman

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Synthesis, characterization & biological activity of Isopropyl Thiazole derivatives of the natural products Distamycin & Proximicin

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم لنيل أية درجة أو لقب 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 Name: **إسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
A	Adenine
C	Cytosine
G	Guanine
T	Thymine
A°	Angstrom
MGB	Minor Groove Binder
MRSA	Methicillin-Resistant *Staphylococcus aureus*
VRSA	Vancomycin Resistant *Staphylococcus aureus*
P53	A tumor suppressor gene located on the short arm of chromosome 17 that encodes a nucleo- phosphoprotein that binds DNA and negatively regulates cell division; frequently measured as a marker of malignant diseases
S180	A murine cancer cell line
Ehrlich	Ehrlich ascites carcinoma cells
LD50	Lethal dose for 50 % of the study subjects
IV	Intravenous
P21	A gene on chromosome 6p21.2 that encodes a cyclin-dependent kinase inhibitor, which binds to and inhibits the activity CDK2or-CDK4 complexes, thus, acting as a regulator of cell cycle progression at G1
G0 phase	The resting cell phase
G1 phase	The Gap 1 phase, where the cell grows and synthesizes mRNA and proteins in preparation for subsequent steps leading to mitosis
C.*difficale*	*Clostridium difficale*
Da	Dalton
HPLC	High-Performance Liquid Chromatography
C°	Celsius degree
IR	Infra Red
FTIR	Fourier Transformed IR
%T	Transmittance
υ	Frequency
ESI-MS	Electro Spray Ionization Mass Spectrometry
NMR	Nuclear Magnetic Resonance
H^1NMR	Proton NMR
C^{13}NMR	Carbon NMR
M_+1	The Molecular ion in ESI-MS

MIC	Minimum Inhibitory Concentration
DMSO	Dimethylsulphoxide
CLSI	Clinical and Laboratory Standards Institute
H_2O	Water
CFU	Colony Forming Unit
mol	Mole
mmol	Millimole
g	Gram
mg	Milligram
min.	Minute
ml	Milliliter
µl	Microliter
DCM	Dichloromethane
MeOH	Methanol
m.p	Melting Point
****	Chemical shift
ppm	Part Per Million
Hz	Hertz
J	Coupling constant
s	Singlet
d	Doublet
m	Multiplet
t	Triplet
ddd	Doublet of doublet of doublet
quin	Quintet
dt	Doublet of triplet
TEA	Triethyl Amine
eq.	Equivalent
THF	Tetrahydrofuran
Hex.	Hexane
EtOAc	Ethyl Acetate
UV	Ultra Violet
TLC	Thin Layer Chromatography
r.b.f	Round Bottom Flask
HBD	Hydrogen Bond Donor
HBA	Hydrogen Bond Acceptor
S.*aureus*	*Staphylococcus aureus*
P.*auregenosa*	*Pseudomonas auregenosa*
E.*coli*	*Escherichia coli*

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Abstract

Minor groove binders (MGBs) are molecules which bind selectively to the minor groove of DNA. Distamycin and netropsin are naturally occurring MGBs, and are members of the polypyrrole class of compounds. They have potential antiviral, antibacterial, and anticancer properties. However, they also have toxic properties. 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 in developing analogues of distamycin and netropsin which have improved their biological activities and reduced their toxicity profile.

This project was concerned with developing a novel synthetic pathway of MGBs which allows for more varied substituents at the tail and head position of these molecules and replacing the *N*-methyl pyrrole with more lipophilic aromatic rings which, to date, has not been fully investigated. Aromatic rings such as benzene, pyridine, morpholine and indole were added to the structure of these

compounds, in order to enhance the lipophilicity and membrane permeability to generate biologically active compounds. The tail group is significant as it plays a key role in both DNA binding, and transportation of these compounds to within cells. These structural variations will allow libraries of compounds with small molecular weight to be prepared. In this project, we developed simple and novel routes for the synthesis of potential minor groove binders. The outcome of such a study would be of great importance regarding the development of new analogues of distamycin and netropsin as potential antibacterial and anticancer agents.

The synthetic pathway of the compounds started from the preparation of the isopropyl thiazole ring using the Darzen reaction and coupling the epoxide intermediate with thiourea. The second step was reacting the amine terminal of the isopropyl thiazole ring with various acid chlorides to give the second intermediate compounds in the synthetic pathway. After that the intermediate compounds where ether refluxed with an amine directly, or, where hydrolyzed and converted to acyl chlorides to be then reacted with various amines to give the final products. NMR spectroscopy was used to confirm the chemical structures of the investigated compounds, which were tested against different bacterial strains, and showed variable activities among the different compounds with the compound MGB 10 being the most active of the synthesized compounds.

Chapter 1

Introduction

1.1. Antibiotic Resistance & Cancer Overview

Antibiotic resistance is an increasing challenge in the clinical field as the rate of development of new antibiotics does not cope with the expanding range of antimicrobial resistance in the clinical settings [1]. This challenge provoked the emergence of a continually developing Databases for tracking the genetics behind developing antimicrobial resistance, with the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa and Enterobacter species*) being in the core of this attention [2-4].

With antimicrobial resistance being a global concern, the World Health Organization (WHO) had announced a set of policies that if implemented would mitigate both the emergence and the dissemination of antibiotic resisting organisms [5]. With those policies focusing on the stewardship of antibiotic use in the hospitals and community settings and reducing antibiotic use in livestock production, other researchers suggested a broader scope of management including pharmaceutical manufacturing waste along with domestic and agricultural waste released to the environment's normal flora of micro- organisms [6]. Until those policies are sufficiently applied effectively in the future, there is a great necessity

to face the current challenges of antimicrobial resistance through the development of new and novel antibiotic agents [3].

Other critical challenges in the clinical field are Cancer disease and cancer treatment, as the WHO declared in its 2015 fact sheet no. 297, cancer figures among the leading causes of morbidity and mortality worldwide with approximately 14 million new cases and 8.2 million cancer-related deaths in 2012 [7].

Cancer is a complex disease that simply starts with a cancer cell that initiates a tumor by carrying mutations in oncogenes and tumor suppressor genes. Common features of cancer include cell overgrowth, reduction of apoptosis and loss of cell cycle regulation[8]. The following are the hallmarks of cancer and cancer cells:

1. Sustained proliferation, where proliferations takes place independently from environmental signal to the cancer cell;

2. Evasion of growth suppressors, where cancer cells evades negative regulators of proliferation (such as P53);

3. Activation of metastasis and invasion through the inhibition of cell-cell contact and cell-matrix contact, also, pathways regulating cell motility are altered;

4. Induction of angiogenesis, where new blood vessels are formed to support the higher tumor need for nutrients and waste elimination;

5. Uncontrolled replicative potential; and finally

6. Immortality whereby cancer cells resist cell death [9].

Cancer treatment options include Chemotherapy, Surgery, Radiation Therapy, Hormone Therapy and Biological Therapy. While surgery and radiation therapy are considered local therapies; limiting their use for localized tumors; chemotherapy and biological therapy (including immunotherapy and gene therapy) are considered systemic. Also, hormone therapy is systemic, but it is targeted to specific hormone receptors in hormone-sensitive tumors. In fact, chemotherapy remains a mainstay in the treatment of most types of cancer where multiple (combination chemotherapy) or single drug could be used either alone or along with surgery or radiotherapy (combined modality treatment)[10].

Chemotherapeutic agents could be either cytotoxic or cytostatic regarding their effect on cancer cells, with relatively few classes of chemotherapy drugs, chemotherapeutic agents could generally be assigned into one of the groups mentioned in **Table 1.1** according to their general mechanisms of action [11].

Not surprisingly, the central role played by DNA in the biological systems has made it a long-standing investigational target for treating and diagnosing human illnesses [12].

Table 1.1: A General classification of anticancer drugs.[11]

Drug Group	**Examples**	**Effect on Cells**
Alkylating agents	Cisplatin, Carboplatin	Cytotoxic
Anthracyclines	Doxorubicin	Cytotoxic
Vinka Alkaloids	Vincristine, Vinblastine	Cytotoxic
Antimetabolites	6-mercaptopurine	Cytotoxic
Topoisomerase inhibitors	Irinotecan	Cytotoxic
Hormonal agents	Tamoxifen	Cytostatic
Targeted agents	Gefitinib, Imatinib	Cytostatic

1.2 DNA and the Minor Groove:

The primary basis of molecular biology was articulated by Crick [13] in 1970, which dictated the one-way flow of genetic information from DNA to RNA to Proteins [14].

This flow requires that the DNA sequence to be decoded into the information carrying RNA intermediary molecules that are copied from the DNA in a process called Transcription, which is a major determinant of the gene expression that allows cells to proliferate, differentiate and maintain proper homeostasis. This clear role of DNA in such processes explains the importance of such molecule to be the target of continuing scientific research and exploration [14-16].

The double helical DNA consists of two complementary antiparallel, sugar phosphate poly-deoxyribonucleotide strands which are associated with specific hydrogen bonding between the nucleotide bases [17].

The nucleotide bases found in DNA are chemically classified into Purines (Adenine, Guanine) and Pyrimidines (Thymine, Cytosine). Their chemical structures are shown in **Figure 1.1**.

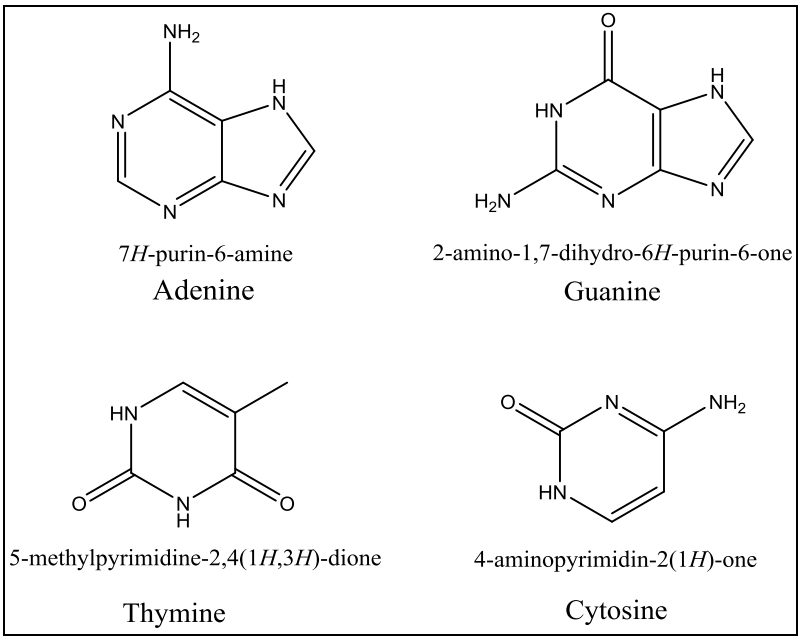


Figure 1.1: Chemical structures of nucleotide bases.

Those nucleotide bases are attached to deoxy sugar-phosphate to form the polydeoxy-nucleotide strands [18]. In the double helical DNA, the hydrogen bonding between the nucleotides held the strands together where Adenine pairs with Thymine (2 H-bonds) and Guanine pairs with Cytosine (3 H-bonds) [19,20], as illustrated in **Figure 1.2**.

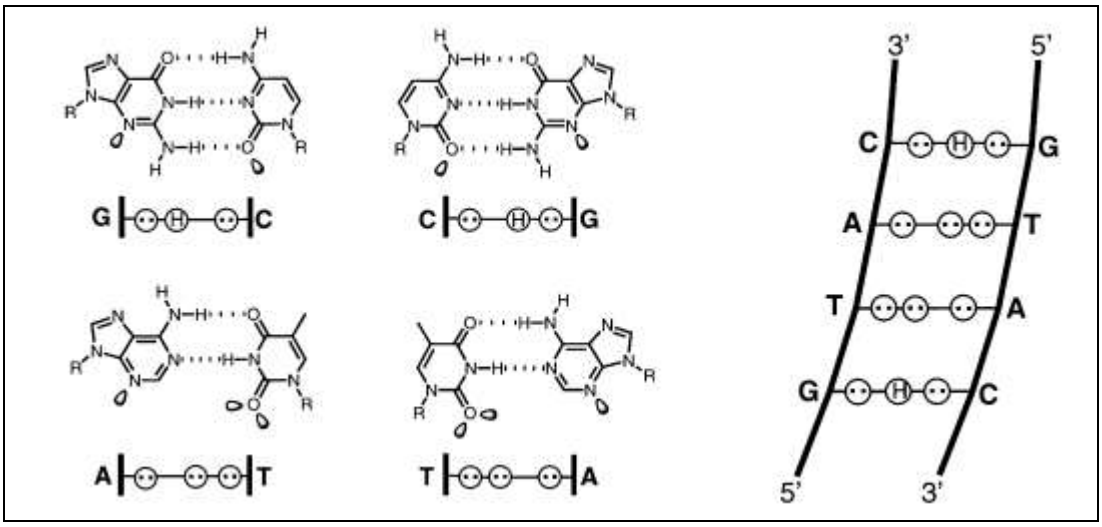


Figure 1.2: Minor groove hydrogen bonding patterns of Watson-Crick base pairs. Circles with dots represent lone pairs of N (3) of purines and O (2) of pyrimidines, and circles containing an H represent the amino group of guanine [19].

The B-DNA **(Figure 1.3)** is the biologically relevant form of DNA which is also the dominant form of DNA in solution; it exists as a right-handed helix characterized by a shallow wide major groove and a deep, narrow minor groove. The structural features of the DNA double helix imply that for a given sequence, there will be distinct chemical features of the molecular surfaces of both the major groove and the minor groove, and this

forms the basis for molecular recognition of duplex DNA by small molecules and proteins [17].

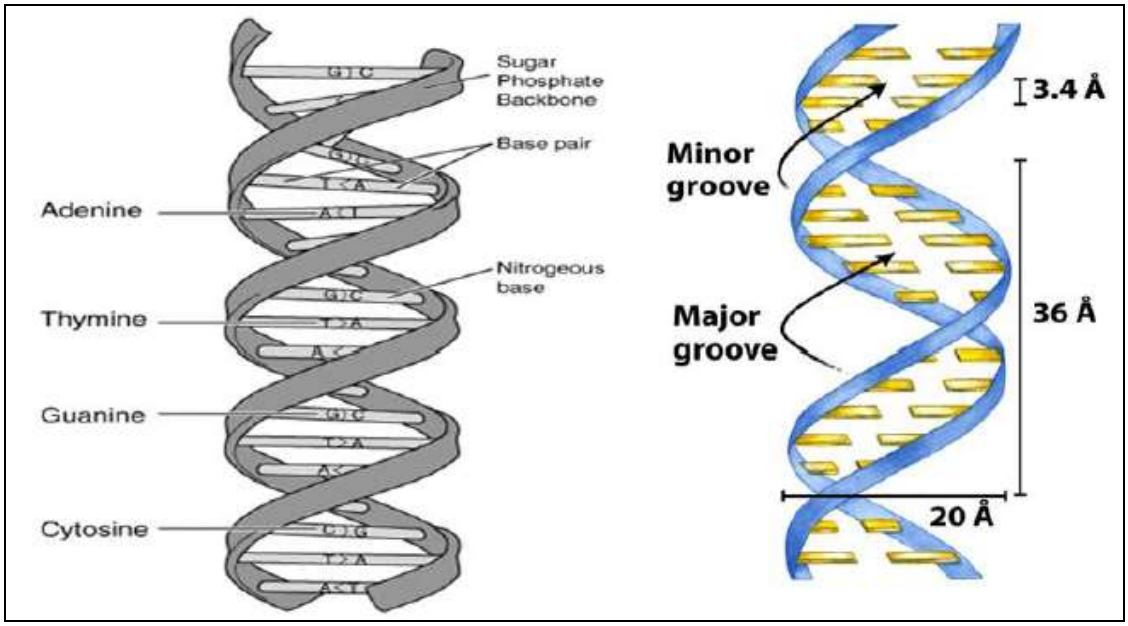


Figure 1.3: The DNA double Helix [20].

The DNA binding molecules have different interaction mechanisms; those interactions include [14]:

a) Covalent interactions with the duplex DNA; as in the Alkylating agents.

b) Duplex DNA intercalation; where intercalation between the DNA strands or intercalation in one strand takes place by insertion of planar aromatic rings between the DNA base pairs. This intercalation is driven by Vander Waals, hydrophobic and electrostatic forces and could be quite strong. Generally, intercalation is independent of specific DNA base pair sequence [14].

c) Duplex DNA Groove binding molecules; this binding could take place to either the major groove or to the minor groove. The major groove, as the name implies, is wider than the minor groove of approximately twice the width, as the average groove width values for averaged sequence B form of DNA are 11.6 Å for the major groove and 6 Å for the minor groove [21]. Due to this dimensional difference the major grooves are the sites for binding of many DNA interacting proteins [22], on the other hand, small molecules will fit the smaller dimensions of the minor groove, and this speculates the evolution of antibiotic minor groove binders that targets the DNA of pathogenic organisms [23].

d) Intercalator-minor groove binding hybrid molecules; this class, also called combilexins, have the dual binding mode of intercalators and the minor groove binding molecules. In principle, those compounds should have stronger DNA interaction due to the longer residence at the DNA binding sites that may interfere with processing enzymes [14, 24].

1.3 Minor Groove Binders (MGBs)

Minor Groove Binders (MGBs), a general term that refers to many different classes of chemical structures and compounds that have the property of binding to the minor groove of duplex DNA. Some of those compounds are of natural origins while others are synthetic chemicals [25-27].

MGBs have found wide applications in the clinical and chemical research fields; those research areas include the following: [28, 29]

- Antibacterial and antifungal agents; including compounds active against MRSA and VRSA.
- Sleeping sickness treatment;
- studies of DNA displacement in bacteria;
- also, DNA MGBs have constituted an attractive source of novel antitumor molecules; this interest stems from the ability of some of those MGBs to interact in a sequence specific fashion with the DNA.

MGBs vary in their chemical structures and complexity, with the natural polyamides Distamycin A and Netropsin **(Figure 1.4)**, being among the simplest structures [25]. The two compounds have a crescent shape that matches the curvature of the DNA minor groove, a property that is common among other MGB groups such as Anthramycins and Duocoramycins **(Figure 1.4).** Although other compounds have a different architecture yet being able to interact as MGBs, Trabectedin (Yondelis®) is an example **(Figure 1.4)**. The simplicity of the structures of both Distamycin A and Netropsin over other MGBs has made them the most studied compounds in the group were many different analogues were synthesized [30].

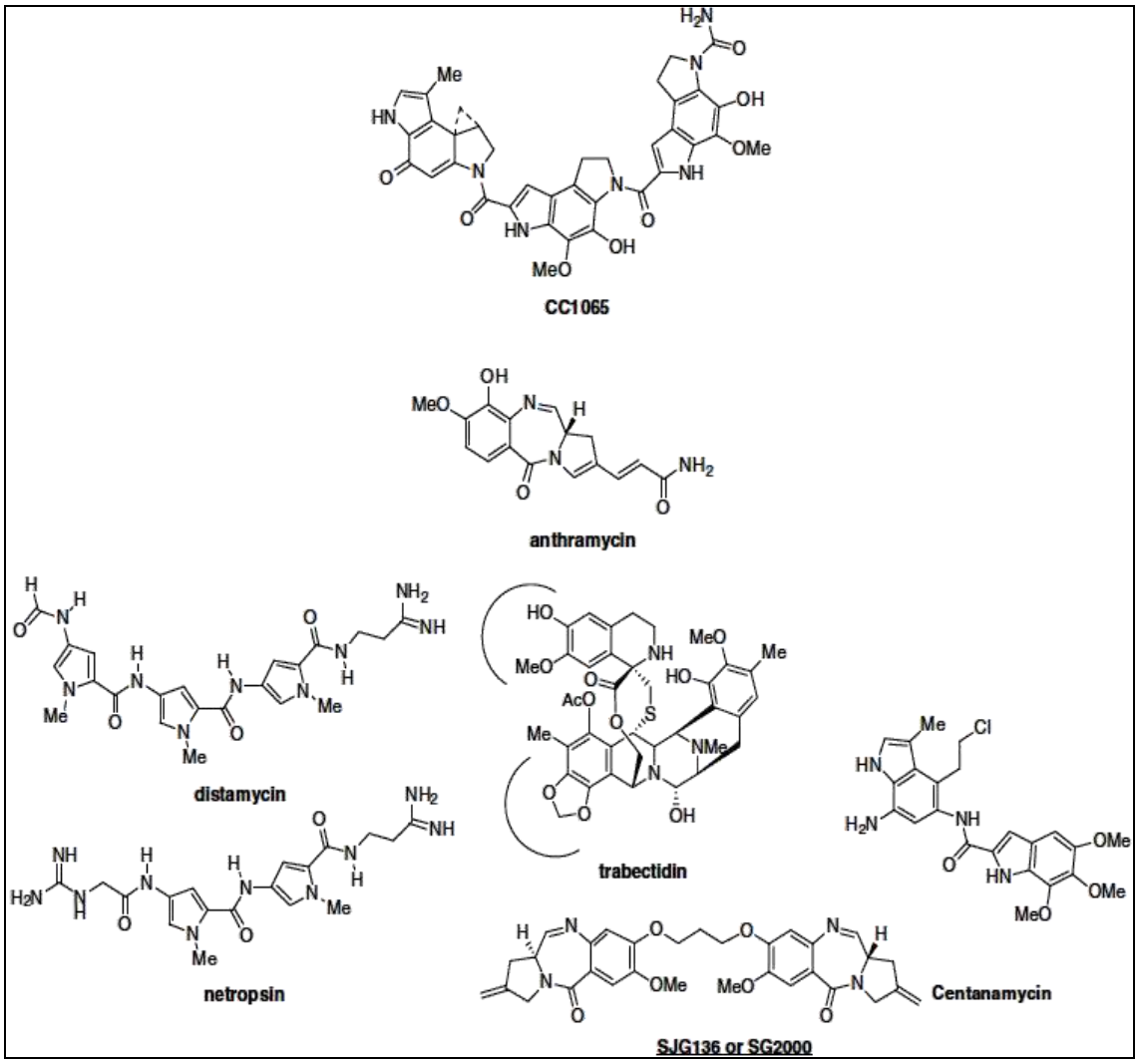


Figure 1.4: The Diverse structures of MGBs, including natural products and some synthetic derivatives [30].

In addition to the ability of an MGB to directly target a specific binding site in the minor groove of the DNA, allosteric inhibition could explain the mechanism of action of MGBs in disrupting the interaction of a transcription factor with their binding sites in the minor groove of the DNA. The allosteric inhibition can be explained by the fact that in most cases, minor groove binding alters the DNA structure by widening the

minor groove and narrowing the corresponding major groove and this makes the major groove unrecognizable by the transcription factors [30].

In this aspect, Distamycin A and Netropsin along with other MGBs have all been reported to block the binding of a transcription factor to their target site. Also, DNA may not be itself the molecular target for the action of MGBs, inhibition of DNA-Protein Complexes such as the Topoisomerases can be achieved by MGBs. For example, Eukaryotic Topoisomerase 1 was found to be inhibited by bisbenzimidazole [31], in another example Distamycin A was found to inhibit Req. Helicase; an enzyme required to maintain the integrity of DNA in both Prokaryotic and Eukaryotic cells [32].

With respect to MGBs as potential drugs it worth to mention two important points, first, there is an exceptionally wide range of building blocks such as rings and head substituents that could be connected using standard chemical reactions providing a wide variety of investigational compounds. The second point is that due to the potential selectivity of MGBs, it is possible to evolve compounds with selectivity for many different infections and malignant diseases, having in mind modern concepts in medicinal chemistry that could aid in the appropriate screening and fine tuning of chemical structures and biological targets [30]. Those two points could be implied to overcome the problem that DNA interaction is a concern regarding toxicity and mutagenicity by mining for unique targets within the pathogenic state.

1.4 Distamycin A and Netropsin as MGBs

Distamycin A (**Figure 1.5**) is a naturally occurring antibiotic agent isolated from *Streptomyces distallicus*, active against some viruses, Gram-positive bacteria and protozoa and is one of the most extensively studied members of the MGBs. Distamycin A is an oligopeptide molecule constructed with 4-amino-1-methyl pyrrole acid moieties and strong basic side chains that bind reversibly to the minor groove of duplex DNA by hydrogen bonds, Vander Waals, and electrostatic interactions, this interaction occurs with high specificity for regions containing A-T base pairs [33-36].

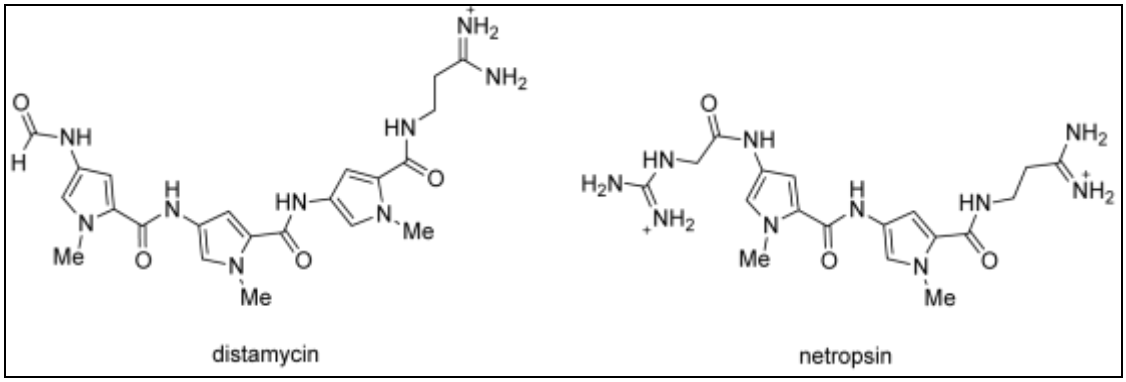


Figure 1.5: Chemical structures of Distamycin A and Netropsin [28].

In terms of geometry, Distamycin A has a curved crescent shape which enables it to match the helical part of DNA minor groove closely. This geometrical fitness allows the compound to bind with the minor groove of duplex DNA as a monomer or as an antiparallel dimer **(Figure 1.6)**, thus distorting the DNA structure by widening of the minor groove [33-36]. The antibiotic had significant effects on ascites tumors such as Ehrlich and S180 in mice; it also caused a marked decrease in the growth of solid tumors. Distamycin A had no effect on E. coli or Salmonellae when these

bacteria were cultured in media that contain nutrients that can be utilized by constitutively synthesized bacterial enzymes, rather, the bacterial growth was inhibited when those bacteria needed the biochemical utilization of nutrients from carbon sources where the bacteria must synthesize the required enzymes [37].

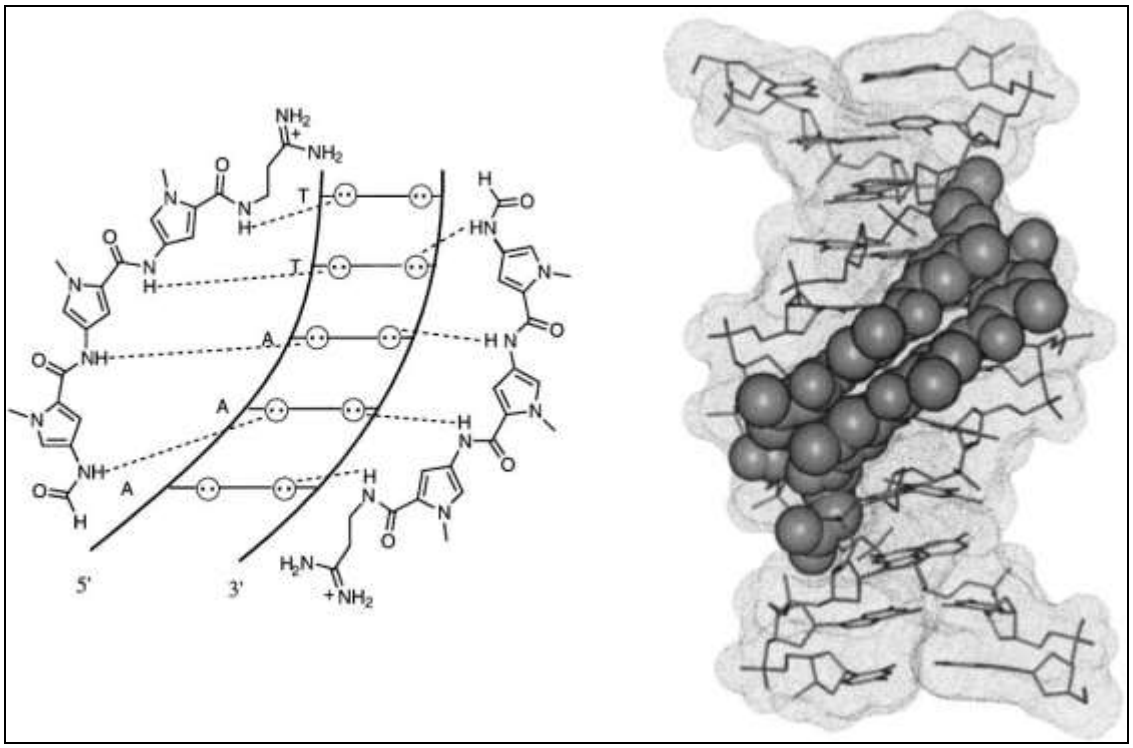


Figure 1.6: NMR structure of 2:1 complex of Distamycin-DNA complex**.** [19]

The most important antibiotic effects of Distamycin A are directed against DNA-containing viruses. Distamycin A ointment was successfully used for the topical treatment of Chickenpox, Herpes Zoster and eruptions resulting from Smallpox vaccination [37]. The toxicity of Distamycin A for animals depends on the route of administration. In mice, the LD_{50} of IV and intraperitoneal Distamycin A were 75 and 500 mg/kg respectively [37]. Distamycin A can also inhibit protein interactions with G-quadruplex (G4)

DNA, a stable four-stranded structure in which the repeating unit is a G-quartet [38].

Netropsin is a fermentation product of Streptomyces netropsis [25], as seen from its chemical structure **(Figure 1.5)**, the compound is closely related to Distamycin A. Consequently, the chemical, biochemical and biological properties of netropsin are very similar to the corresponding activity of Distamycin. The antibiotic exhibits antibacterial and antifungal properties *in vitro*. In contrast to the *in vitro* activity, Netropsin proved ineffective in chemotherapeutic studies on mice infected with both Netropsin sensitive and resistant bacteria, as for Distamycin A, the antiviral activity of Netropsin dominated [38,39].

1.5 Proximicins

Another class of compounds that possess biological activity are the marine amino furan proximicin antibiotics **(Figure 1.7)**. The characteristic structural element of Proximicins is 4-amino-furan-2-carboxylic acid, a hitherto unknown γ-amino acid. Proximicins show a structural similarity with Netropsin and Distamycin but having the furan ring in the Proximicins instead of the N-methyl-pyrrole ring of Netropsin and Distamycin [40]. The pyrrolamidone antibiotics (Netropsin and Distamycin) exhibits their biological activity by binding to AT-rich sequences in the minor groove of DNA [33], in contrast, Proximicins do not bind to DNA and show a different mode of antitumor action. Proximicins show a moderate

cytotoxic activity and induces up-regulation of cell cycle regulating proteins (p53 and p21), leading to cell cycle arrest in G0/G1-phase [40, 41].

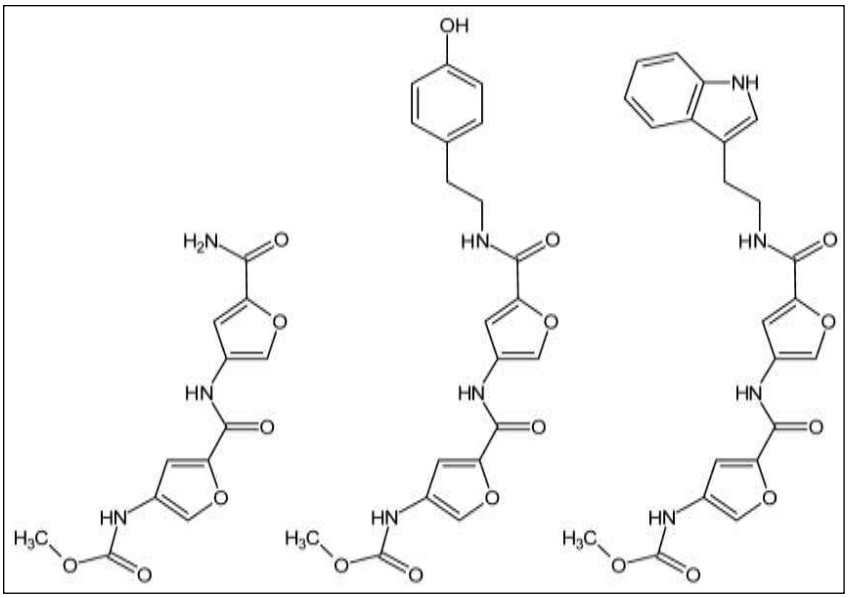


Figure 1.7: Chemical structures of Proximicin A, B, and C (from Left to Right). [40]

1.6 Distamycin, Netropsin & Proximicin Analogues, a Literature Review

There are several methods in the literature describing the attempts for the design of Distamycin analogues. Where the modifications introduced to Distamycin varied between changing the head group or the tail group of Distamycin. Other modifications were made to the rings of the molecule introducing a variety of heterocyclic rings instead of the pyrrole ring, in this aspect thiazole,imidazole, furan, oxazole and thiophene rings were investigated, where the thiazole containing analogues were of interest due to their biological activity against bacteria and/or fungi along with lower toxicity reported with respect to several mammalian cell lines [28, 42-44].

On the other hand, amide isosteres were also studied including olefinic double bonds and diazo groups [45].

Also, research about Distamycin Analogues extended to include various synthetic pathways and new synthetic strategies such as solid phase synthesis along with the common chemical reactions for the amide bond formation including acid chlorides with amines and the use of coupling agents [28, 46].

Beyond thinking about one molecule, other researchers had investigated the effect of combining active moieties of other anticancer drugs (such as alkylating groups) to Distamycin and its analogues [44,47] or having hybrid molecules of Distamycin and other active anticancer drugs [29]. Netropsin and Proximicins were also present in this aspect by investigating hybrid molecules of Netropsin-Proximicins [47].

Figure 1.8 presents the chemical structures of Distamycin, Netropsin, and Proximicins, while **Figure 1.9** presents some examples of the analogues. Meanwhile, **Table 1.2** illustrates the variation strategy for those analogues.

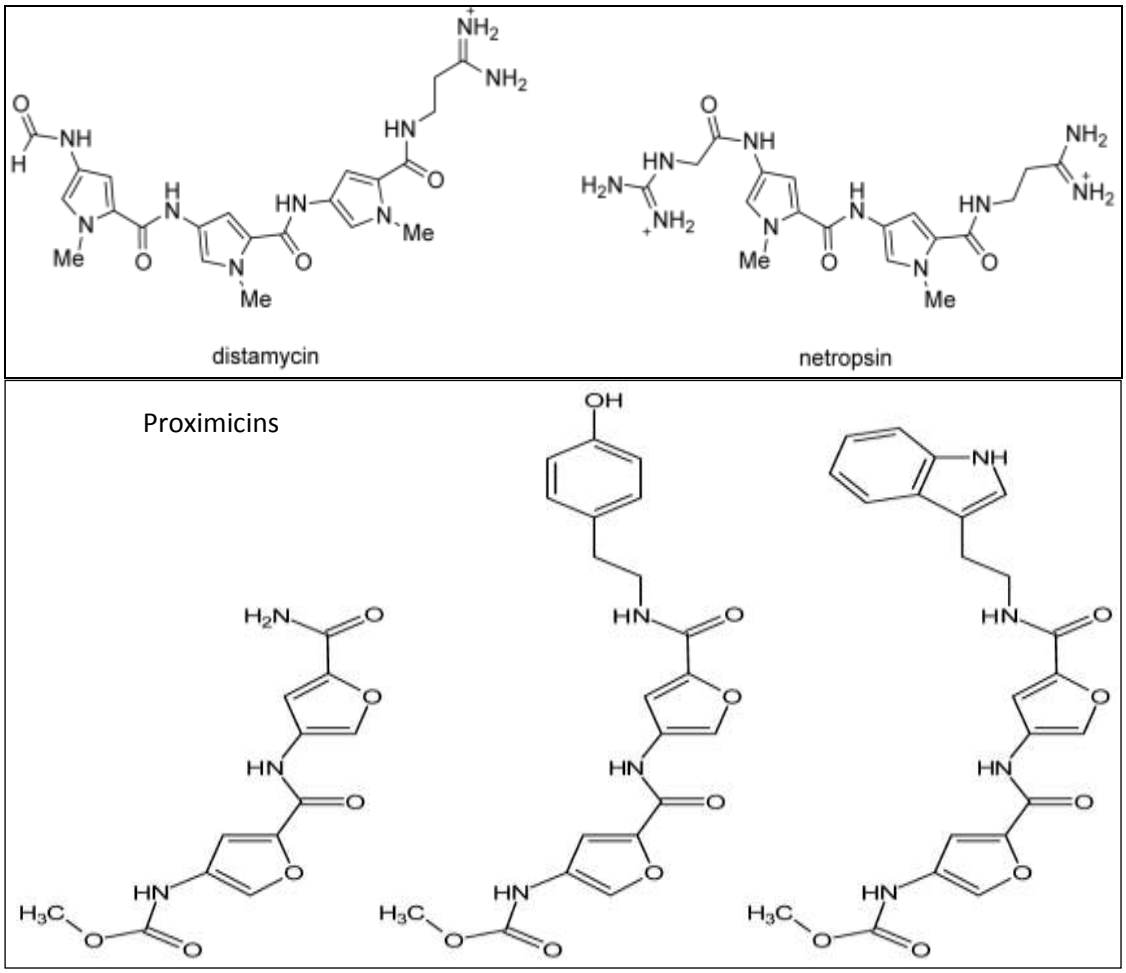


Figure 1.8: Chemical Structures of Distamycin, Netropsin, and Proximicins [28, 40].

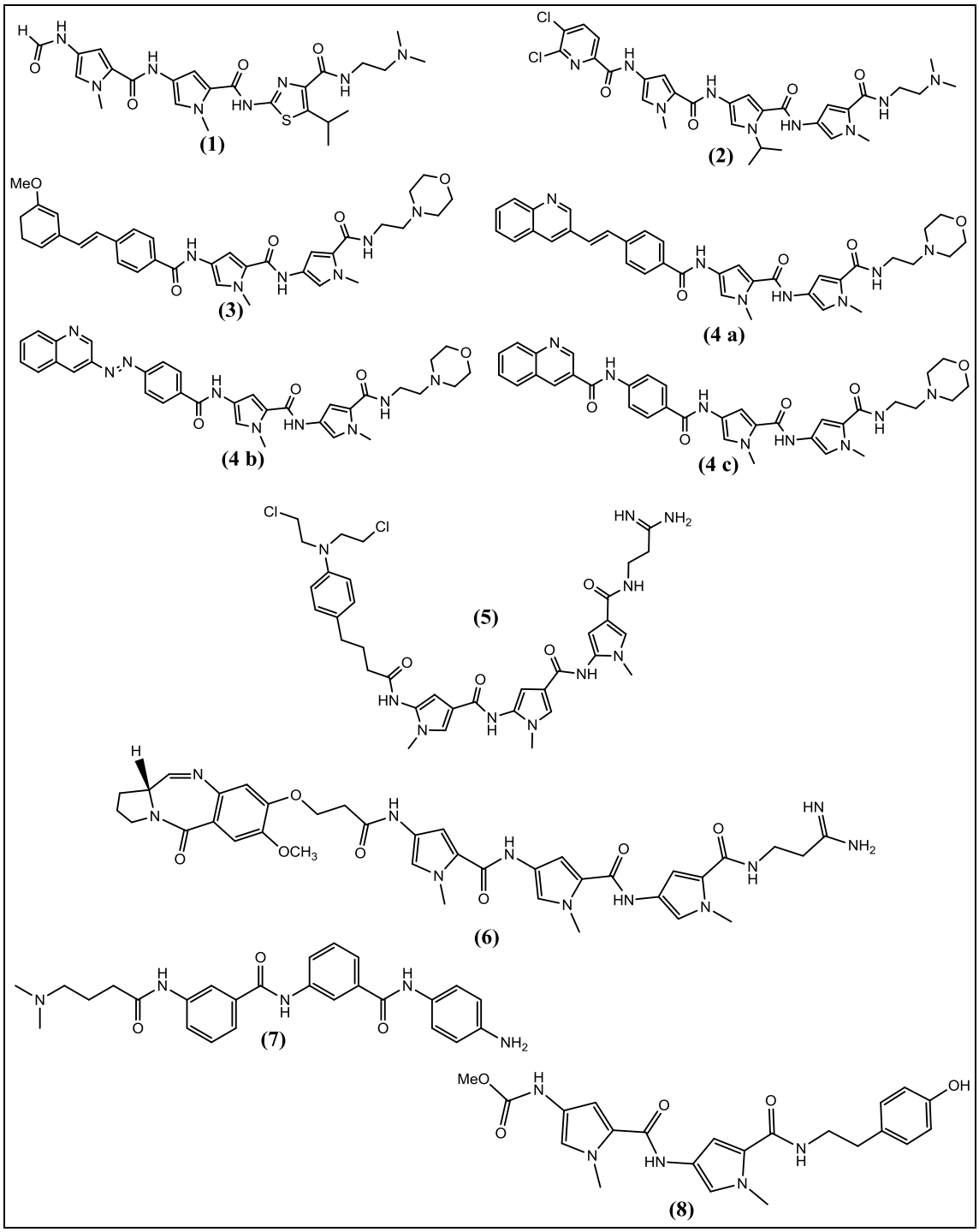


Figure 1.9: Examples of chemical structures of some analogues reported in the literature. [28, 29, 42, 44-47]

Table 1.2: Some variation strategies for Distamycin analogues in literature:

Compound no. from Figure 1.9	Modification strategies	Author(s) and reference no.
(1),(2)	Varied Head and Tail groups, varied N-alkyl chain, varied cyclic system	A. Khalaf [28]
(3),(4a-c)	Non Cationic tail group, Amide isosteres, and varied Head group	A. Khalaf et. al [42] [45]
(5)	Alkylating moiety at Head position	S. Marchini et. al [44]
(6)	Hybrid molecule of Distamycin A and other antitumor agents (anthramycin analogue DC-81)	P.Baraldi et. al [29]
(7)	Solid Phase Synthesis of MGBs	D.Danuta [46]
(8)	Hybrid molecule of Netropsin-Proximicin	F.Wolter et. al [47]

The continuing investigations on Distamycin analogues have resulted in developing many active compounds, of those, the compound MGB PB3, **Figure 1.10** developed by MGB pharma company, is heading to finish

phase 1 clinical trials for the treatment of *C. diffcale* associated diarrhea[48].

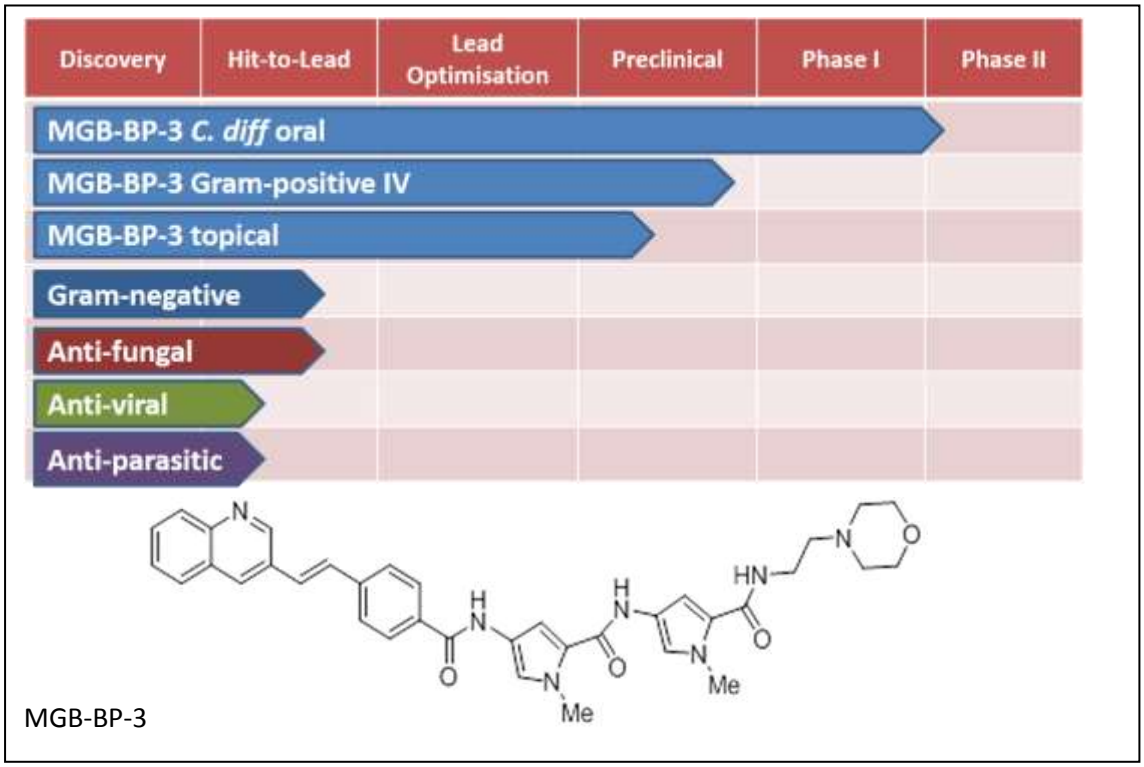


Figure1.10: MGB-BP-3 [48].

1.7 Aim and Objectives

The aim of this project is to synthesize new analogues of the natural products Distamycin A and Proximicin as potential antibacterial, antifungal, or anticancer agents. The aim of the project will be achieved by fulfilling the objectives below:

1. Introducing the isopropyl thiazole ring as the cyclic structural element in the compound instead of the pyrrole and furan rings of Distamycin A and Proximicin, respectively, thus enhancing the lipophilicity of the compound. Other lipophilic aromatic rings such

as pyridine, benzene, and quinoline can also be introduced at the terminal positions to maintain the crescent shape of these compounds which is essential for their interaction with the binding sites.

2. Simplification of the chemical structure which will be composed of two or three planar aromatic rings along with a head and tail groups, to produce compounds with favorable small molecular weight for potential biological activity and enhanced absorption.
3. The biological activities of the resulting compounds will also be examined.

1.8 Methodology and Rationale of synthesis:

This project will be concerned with developing a simple synthetic pathway of MGBs which allows for more various substituents at the tail end of these molecules and replacing the *N*-methyl pyrrole with more lipophilic aromatic rings which, to date, has not been fully investigated. The addition of lipophilic aromatic rings such as benzene, pyridine, and quinoline to the structure of these compounds, should give them enhanced lipophilicity and in turn better membrane permeability, this which we hope will generate compounds with potential biological activity.

In fact, there are several strategies which can be used in an attempt to improve the antibacterial activity of MGBs (distamycin and netropsin). Small molecule analogues to Distamycin with a molecular weight less than 500 Da will be prepared as potential antibacterial agents. Our strategy is to

enhance the antibacterial activity of these analogues by varying their lipophilicity and without increasing their molecular weight, which we suggest will improve the transportation properties of these molecules to the nuclei of cells, and therefore leading to enhanced biological activity[49-51]. The enhanced lipophilicity of these compounds may also lead to enhanced hydrophobic interactions within the groove which may again lead to enhanced activity. As mentioned before, the starting point for our work is Distamycin; the first step is to evaluate Distamycin and identify the areas in which enhanced lipophilicity could be introduced **(Figure 1.11)**.

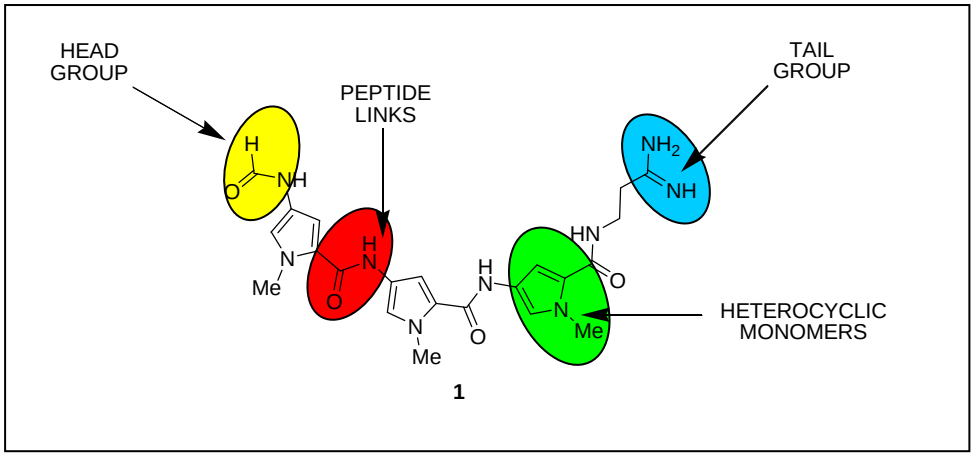


Figure 1.11: Possible positions for variations on Distamycin.

This analysis showed there are several positions which would allow for the desired variations to be carried out. If we look at each position, in turn, we can see that the head group of Distamycin is a small polar formyl group. This substituent could be altered to a range of more lipophilic head groups giving the enhanced lipophilicity required. Secondly, the peptide links between each heterocyclic monomer play a major role in the binding of these compounds to the minor groove of DNA by forming hydrogen bonds

with the DNA bases, the amide moiety, therefore, will be kept intact. Another major variation is to vary the heterocyclic monomers by either varying the ring system or its substituent. The last variation proposed would be the substitution of the amidine tail for a less polar group or tail groups with a lipophilic property. Several examples of such analogues have already been prepared which illustrated this strategy such as the compound developed by Genesoft **(Figure 1.12)** [49].

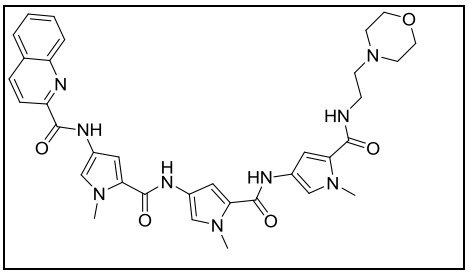


Figure 1.12: Example of MGB with enhanced lipophilicity prepared by Genesoft. [49]

This compound is shown to demonstrate the possible head group variations. The change from the formyl group to the quinoline offers the possibility of enhanced hydrophobic interaction between the larger aromatic head group and the groove walls. This compound is currently the most active polyamide published in the literature. Based on these observations, we are going to design and synthesize different analogues of Distamycin. Some of the structures of these compounds are summarized in **Figure 1.13**. Our main strategy to enhance lipophilicity is to replace the *N*-methyl pyrrole rings with more lipophilic aromatic rings such as isopropyl thiazole, benzene, pyridine, quinoline. The isopropyl thiazole was placed

in the center of all compounds because its presence in the middle of the structure is essential to keep the curvature and the crescent shape of these compounds which help them to fit snugly into the minor groove of DNA. No more than three aromatic rings will be used in all proposed analogues in order not to exceed the molecular weight of 500 Da and thus not to break the Lipinski's “rule of five” [50] for potential drugs. Another variation proposed is the substitution of the amidine tail for a more lipophilic tail groups such as tyramine. These structural variations will allow preparing libraries of compounds with small molecular weight (less than 500 D), and of preferable molecular descriptors to enhance their interaction characteristics to their targets [51]. The proposed analogues vary in their structures in one or two positions to allow comparison and evaluation of their biological activities **(Figure 1.13)**. Comparing the binding characteristics of closely related ligand structures to a specific binding site can help establish how modifications in the structure influence binding affinity.

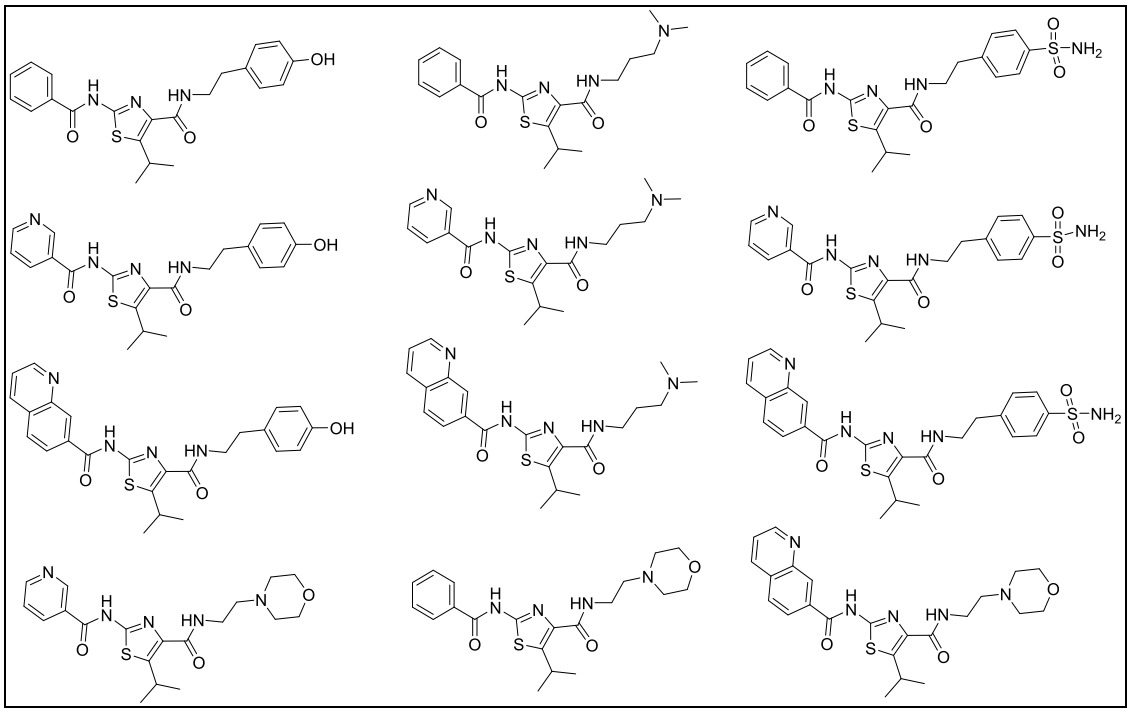


Figure 1.13: Examples of proposed MGBs with enhanced lipophilicity and small molecular weight.

The proposed synthetic pathway for one of these analogues is described in **Figure 1.14** were isopropyl thiazole was formed by reacting dichloromethyl acetate with isobutyraldehyde to form the epoxide intermediate which is reacted directly with thiourea under reflux to produce the isopropyl thiazole. The amide bond between the heteroaromatic rings is then formed by reacting an acid chloride and amine or amine with an ester under reflux conditions. **Figure 1.15** presents the full synthetic scheme for our MGBs.

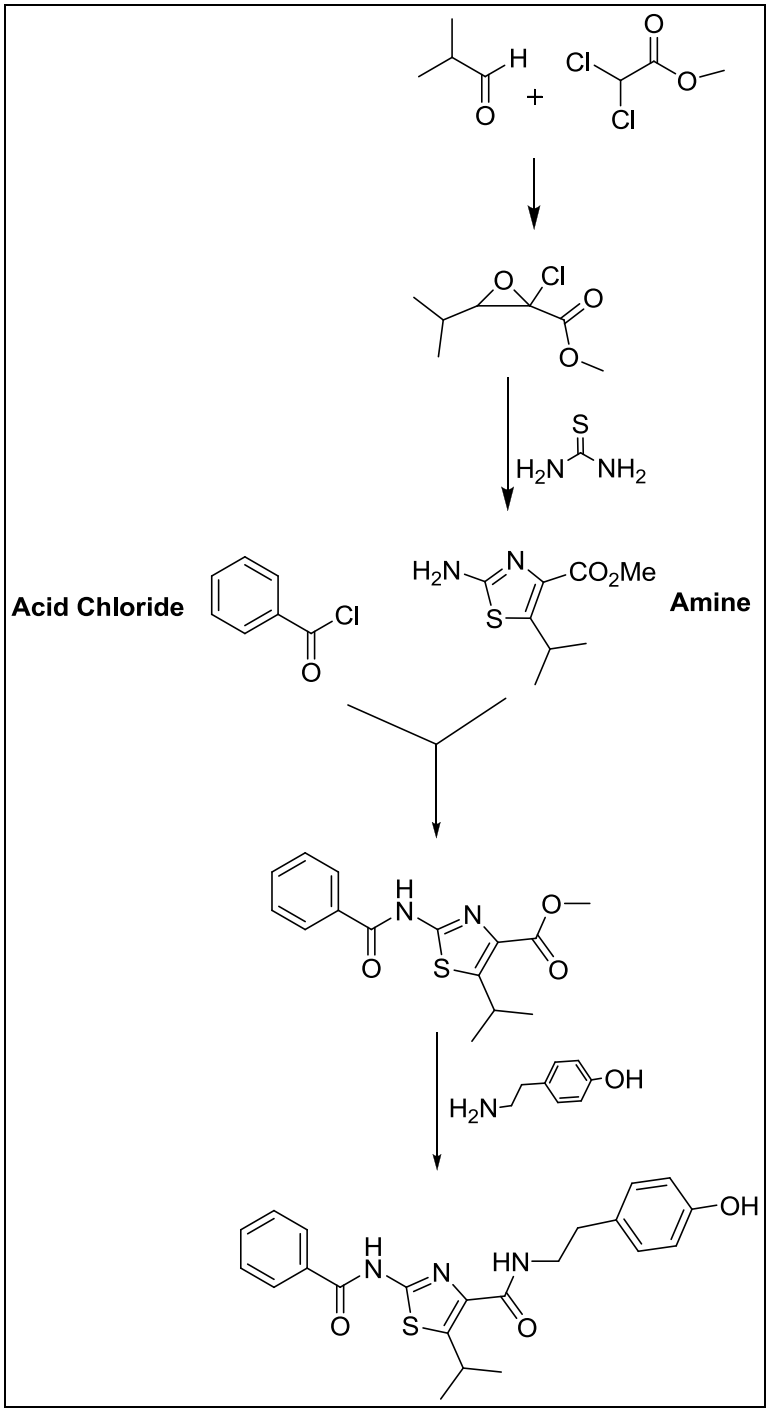


Figure 1.14: A synthetic pathway for an MGB.

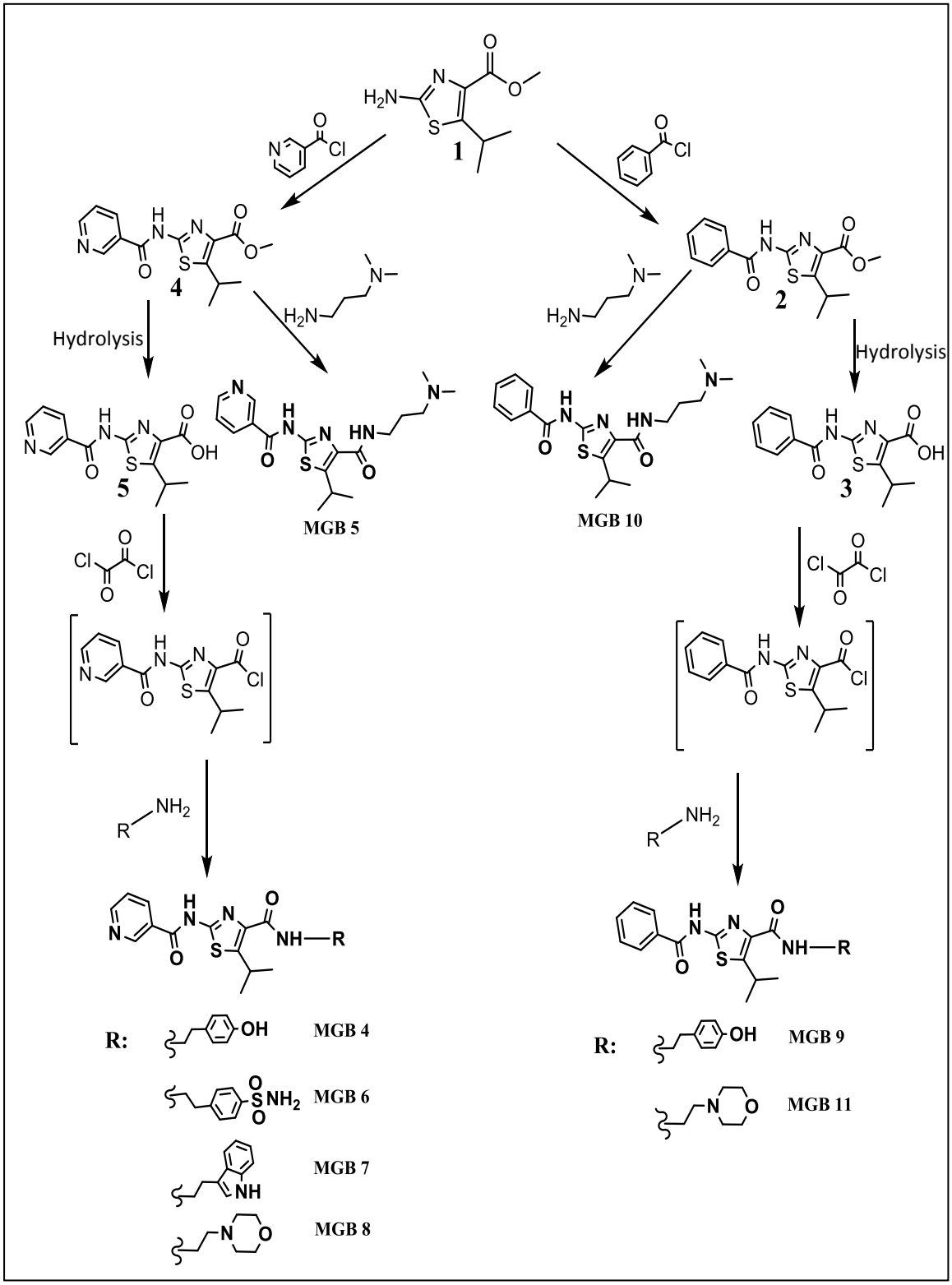


Figure 1.15: The synthetic scheme of the studied compounds.

Chapter 2

Materials and Methods

2.1. General Experimental

2.1.1 Materials

All chemicals and reagents used in synthesis were purchased from Sigma-Aldrich. HPLC grade solvents were used for synthesis. Work up solvents were purchased from Sigma-Aldrich. Other materials specific for each experiment will be mentioned later if not mentioned here.

2.1.2 Melting point determination

A Gallenkamp variable heater melting point device was used in the determination of the melting point of the compounds by visually recording the temperature in Celsius degrees (C°) at which the melting of the material in a sealed capillary tube occurs.

2.1.3 IR spectroscopy

A Thermo Scientific, NicoletiS5, iD3ATR FTIR device was used in obtaining the IR spectra of the materials and compounds directly without KBr disc formation. The spectra were shown as Transmittance (%T) output against frequency which was expressed as υ in cm^{-1}.

2.1.4 Mass Spectrometry

Mass spectra were obtained using an Agilent 6410B Triple Quad LC–MS/MS system (Agilent Technologies Inc., USA) coupled with an electrospray ionization source.

2.1.5 NMR spectroscopy

NMR data were acquired using a Bruker 500 MHz NMR spectrometer. ^{1}HNMR and ^{13}CNMR were used to confirm the structures of intermediate compounds and final products.

2.1.6 Biological Activity

2.1.6.1 The Bacterial Strains:

The antibacterial activity of the studied compounds was tested against different bacterial strains obtained from the ATCC, sub-cultured into the prepared nutrient broth and incubated at 37 °C for 24 hrs. The bacterial strains used were: *S.aureus* (ATCC 25923), *P.auregenosa* (ATCC 27853), *E.coli* (ATCC 25922) and MRSA.

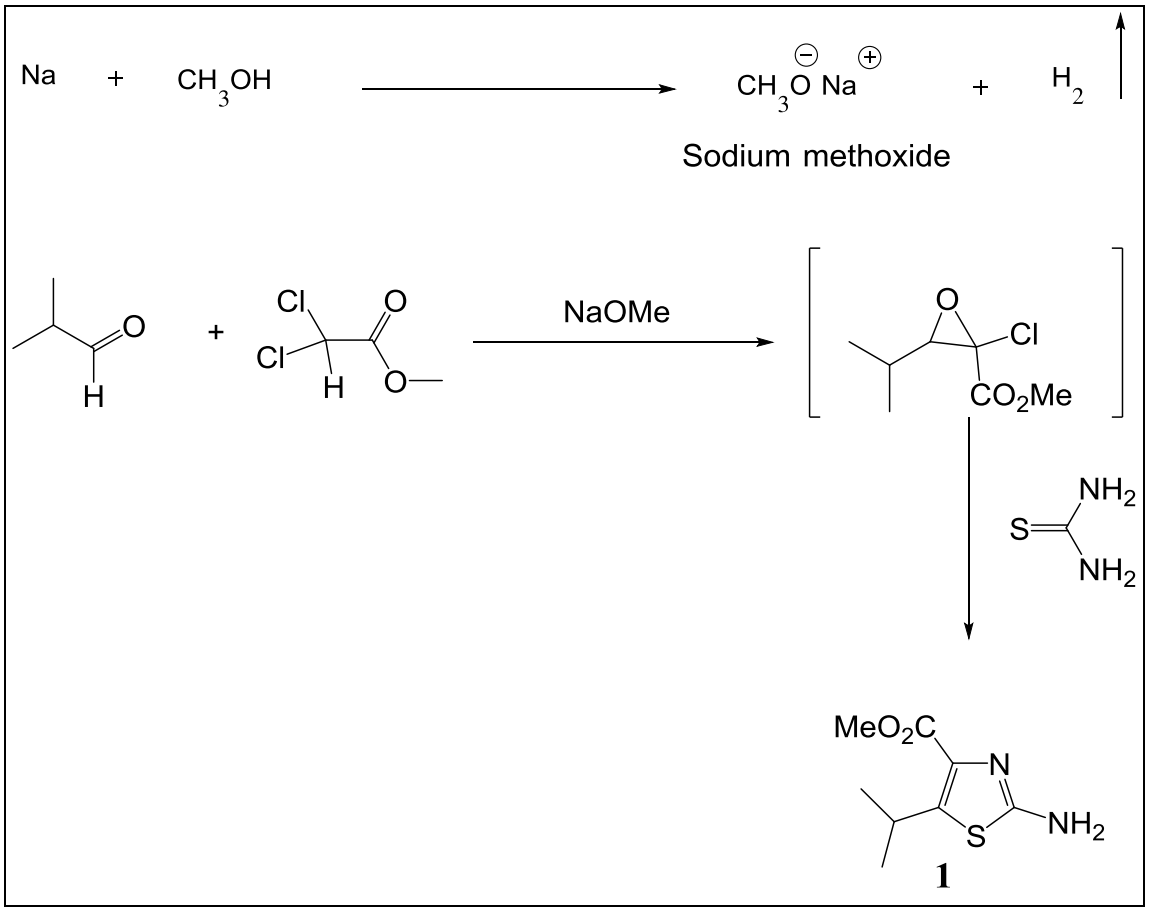
2.1.6.2 Antibacterial activity screening and MIC determination

The antibacterial activity screening and MIC determination were done using the micro-broth dilution method [52], and the procedure was done according to CLSI protocol [53]. Briefly, the compounds under investigation were dissolved in DMSO/H_2O co-solvent system. Those solutions were serially diluted (2-fold) 11 times with nutrient broth

(HIMEDIA, India). Well number 11 was considered negative control of bacterial growth, while well number 12 contained nutrient broth only and was the positive control of bacterial growth. To detect any antibacterial activity contributed to DMSO in the conditions of the broth microdilution method, a row of 10 wells with a serial 2-fold dilution of DMSO with nutrient broth was prepared with concentration from 0.098% to 50%. Overnight grown bacterial isolates were applied to all wells except negative control. The final standard bacterial concentration in each well was adjusted to 5×10^{5} CFU/ml. After inoculation of bacteria, the plates were covered and incubated at 35 °C for 18 hours. Broth microdilution method was performed in duplicate for each isolate. Minimal inhibitory concentration (MIC) was considered to be the lowest concentration that did not show any visible growth in the test media, the results were visually determined and recorded in mg/ml.

2.2 Chemical Synthesis

2.2.1 synthesis of methyl-2-amino-5-isopropyl-1,3-thiazole-4-carboxylate (1) [54]:



Sodium (12.12 g, 0.527 mol) was dissolved in 200 ml Methanol (HPLC grade) at 0 C° and allowed to be stirred for 30 min. The resulting solution was then added dropwise (over 45 min. at 0 C°) to a solution of isobutyraldehyde (56 ml, 0.616 mol.) and methyl dichloroacetate (80 ml, 0.772 mol.) in 100 ml of ether and stirred for further 30 min. Then, 100 ml

of brine (saturated NaCl solution) was added, and the reaction mixture was then extracted with diethyl ether (2 x 100 ml). The ether layer was dried over anhydrous magnesium sulfate (or sodium sulfate) and filtered, and the solvent was removed under reduced pressure to yield a pale yellow oil. The oil was dissolved in a solution of thiourea (34.08 g, 0.448 mol.) and 240 ml methanol (HPLC grade) and refluxed for 4 hrs. The solvent was then removed under reduced pressure, and the crude product was taken in 200 ml distilled water, neutralized with ammonium hydroxide solution and extracted with DCM (2 x 100 ml) to yield the crude pale yellow thiazole **(1)**. The crude product was dissolved in the smallest amount of ethanol and charcoal was added, the mixture was then hot filtrated to remove charcoal. Excess ethanol was removed under reduced pressure, and the product was recrystallized from ethanol/water (around 1:3) to yield shiny white crystals with a characteristic odor. (Methanol was replaced with ethanol in the final crystallization step for its better personal and environmental safety).

Yield: 62 g, 70 %, m.p = 149.5-152 C°. (lit. =151-152 [55])

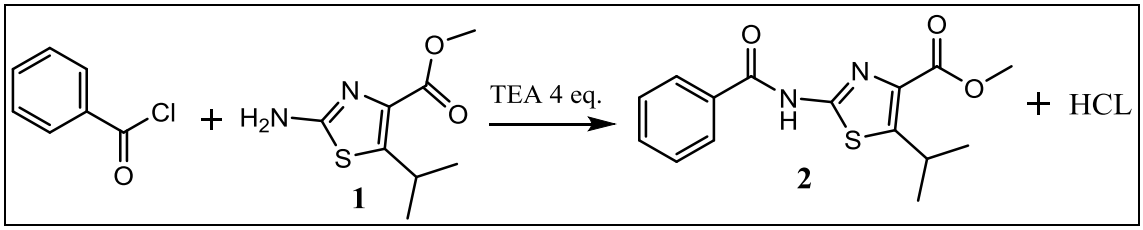
IR : 3422, 3261, 3118, 2961, 1691, 1621, 1543, 1434, 1327, 1208, 1048, 976 cm^{-1}.

ESI-MS(M+1): 200.9198 (100%), 201.9835 (9%), 202.9845 (5%).

^{1}HNMR(Methanol-d_4) : δ ppm 1.15 (d, *J*=6.60 Hz, 6 H) 3.72 (s, 3 H) 3.88 (dt, *J*=13.69, 6.85 Hz, 1 H).

^{13}C NMR (101 MHz, METHANOL-d_4) δ ppm 23.75 (s, 1 C) 27.40 (s, 1 C) 46.96 (s, 1 C) 47.95 (s, 1 C) 50.67 (s, 1 C) 133.95 (s, 1 C) 147.54 (s, 1 C) 162.67 (s, 1 C) 165.47 (s, 1 C).

2.2.2 Synthesis of methyl-2-benzamido-5-isopropylthiazole-4-carboxylate (2):



927 mg (4.62 mmol) of **(1)** previously dried overnight at 50 C° was dissolved in 20 ml of THF (HPLC grade) and was added dropwise (into an ice bath) to a solution of **(**benzoyl chloride (1.46 ml, 2 eq.), and 2.6 ml of TEA (4 eq.) in 10 ml of HPLC grade THF**)**. The reaction was stirred overnight and monitored by TLC (mobile phase: Hex/EtOAc 80:20)and visualized under UV light. After reaction completion (loss of **(1)** spot on TLC) the solvent was evaporated in a rotary evaporator, and the residue was extracted with 100 ml EA and 100 ml of saturated sodium bicarbonate solution plus 5 ml brine. The organic layer was then dried with sodium sulfate and evaporated under reduced pressure. The product was scratched as a yellowish white powder.

R_f : 0.63 (Hex/EtOAc 80:20)

Yield: 946 mg, 67 %, m.p =188-192 C°.

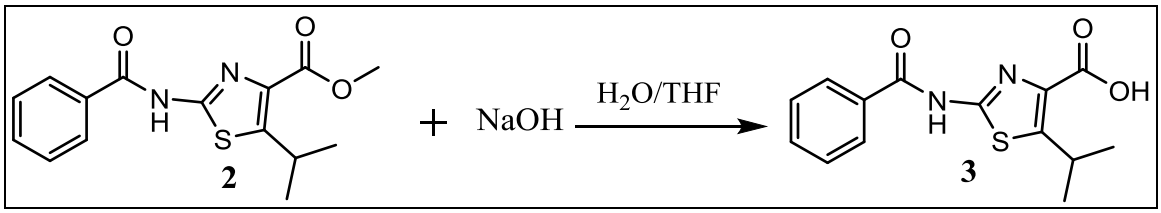
IR : 2962, 2362, 1718, 1682, 1601, 1553, 1436, 1318, 1258, 1294, 1211, 1071, 1014 cm^{-1}.

ESI-MS(M+1): 305.0315 (33%)

^{1}HNMR : (400 MHz, CHLOROFORM-d) δ ppm 1.17 - 1.35 (d, 6 H) 3.64 - 3.77 (s, 3 H) 3.79 - 3.94 (m, 1 H) 7.28 - 7.34 (m, 1 H) 7.46 - 7.49 (m, 1 H) 7.58 - 7.64 (m, 1 H) 7.69 - 7.72 (m, 1 H) 7.89 - 7.92 (m, 1 H).

^{13}C NMR (101 MHz, CHLOROFORM-*d*) δ ppm 23.73 (s, 1 C) 28.04 (s, 1 C) 52.73 (s, 1 C) 77.23 (s, 1 C) 127.93 (s, 1 C) 128.96 (s, 1 C) 129.42 (s, 1 C) 130.63 (s, 1 C) 132.03 (s, 1 C) 132.70 (s, 1 C) 134.44 (s, 1 C) 135.65 (s, 1 C) 147.63 (s, 1 C) 159.28 (s, 1 C).

2.2.3 Synthesis of methyl-2-benzamido-5-isopropylthiazole-4-carboxylic acid (3):



617 mg (2.03 mmol) of **(2)** was dispersed in 5 ml THF, and 20 ml distilled water and stirred vigorously in a suitable r.b.f. Then 1 eq. of NaOH pellets (81.2 mg, 2.03 mmol) was added to the r.b.f and stirred overnight; the reaction was monitored by TLC (Hex/EtOAc 80:20). The carboxylic acid spot remains at the baseline of the TLC plate. THF was then removed from the reaction mixture under reduced pressure, and HCl solution was used to

neutralize the mixture and to precipitate the carboxylic acid **(3)**. The product was then filtrated using a Buchner funnel and **(3)** was collected as a white powder and dried in an oven overnight at 50 C°.

Yield: 428.3 mg, 74 %, m.p =308-309.5 C°.

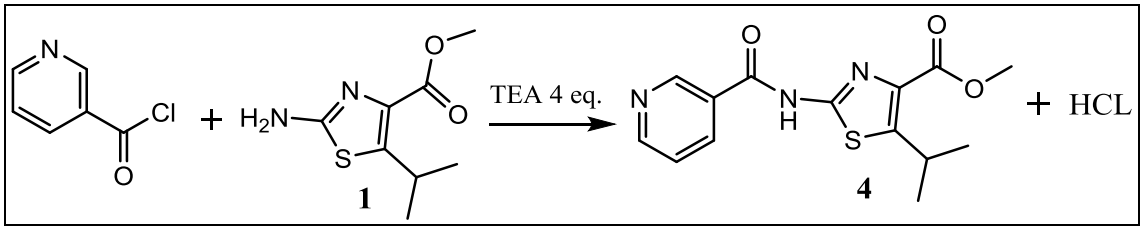
IR : 2962, 1666, 1604, 1565, 1544, 1463, 1328, 1307, 1233, 1099, 1045, 955 cm^{-1}.

ESI-MS(M+1): 291.0169 (76%)

^{1}HNMR : (400 MHz, DMSO-*d6*) δ ppm 1.31 (d, *J*=6.85 Hz, 6 H) 4.04 - 4.09 (m, 1 H) 7.51 - 7.66 (m, 3 H) 8.10 (d, *J*=7.58 Hz, 2 H).

^{13}CNMR: poor ^{13}CNMR spectrum.

2.2.4 Synthesis of methyl 5-isopropyl-2-(nicotinamido)thiazole-4-carboxylate (4):



A 900 mg (4.55 mmol) of **(1)** previously dried overnight at 50 C° was dissolved in 20 ml of THF (HPLC grade). The solution was added dropwise (in an ice bath) to a solution of **(**nicotinoyl chloride (1.6 g ml, 1.5 eq.), and 2.5 ml of TEA (4 eq.) in 10 ml of HPLC grade THF**)**. The reaction was stirred overnight and monitored by TLC (mobile phase: EtOAc 100 %) and visualized under UV light. After reaction completion

(loss of **(1)** spot on TLC) the solvent was evaporated in a rotary evaporator, and the reaction mixture was extracted with 100 ml EA and 100 ml of saturated sodium bicarbonate solution plus 5 ml brine. The organic layer was then dried with sodium sulfate and evaporated under reduced pressure. The product was obtained as a pale yellow powder.

R_f : 0.48 (EtOAc 100 %)

Yield: 0.566 mg, 40.7 %, m.p =181.2-182 C°.

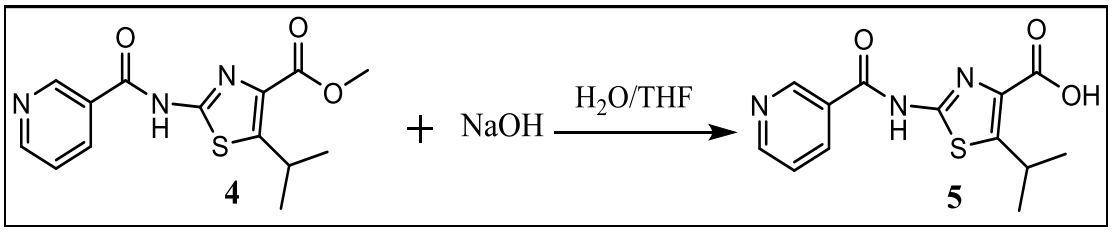
IR : 3737, 3158, 2959, 1713, 1677, 1598, 1558, 1515, 1467, 1301, 1140, 1048, 992, 910 cm^{-1}.

ESI-MS(M_+1): 305.9074 (100%), 307.0336(23%), 308.0347 (9%).

^{1}HNMR : (400 MHz, CHLOROFORM-*d*) δ ppm 1.30 - 1.35 (d, 6 H) 3.72 - 3.76 (s, 3 H) 4.02 - 4.13 (m, 1 H) 4.08 (s, 1 H) 7.40 (ddd, *J*=8.01, 4.83,0.86 Hz, 1 H) 8.15 (dt, *J*=8.13, 1.93 Hz, 1 H) 8.75 - 8.78 (m, 1 H) 9.08 - 9.11 (m, 1 H) 10.22 - 10.59 (m, 1 H).

^{13}C NMR (101 MHz, CHLOROFORM-*d*) δ ppm 24.81 (s, 1 C) 27.74 (s, 1 C) 52.01 (s, 1 C) 77.23 (s, 1 C) 123.74 (s, 1 C) 127.77 (s, 1 C) 133.62 (s, 1 C) 135.27 (s, 1 C) 148.60 (s, 1 C) 153.66 (s, 1 C) 153.85 (s, 1 C) 154.04 (s, 1 C) 162.37 (s, 1 C) 163.35 (s, 1 C).

2.2.5 Synthesis of methyl 5-isopropyl-2-(nicotinamido)thiazole-4-carboxylic acid (5):



An 856 mg (2.8 mmol) of **(4)** was dispersed in 5 ml THF and 20 distilled water and stirred vigorously in a suitable r.b.f, and 1 eq. of NaOH pellets (112 mg, 2.8 mmol) was added to the r.b.f and stirred overnight. The reaction was monitored by TLC (EtOAc 100 %). The carboxylic acid spot remains at the baseline of the TLC plate. After that, a rotary evaporator was used to remove THF from the reaction mixture, and diluted HCl solution was used to neutralize the mixture and to precipitate the carboxylic acid **(5)**. The product was then filtrated using a Buchner funnel and **(5)** was collected as a white powder and dried in an oven overnight at 50 C°.

Yield: 640.5 mg, 82 %, m.p =301.2-302 C°.

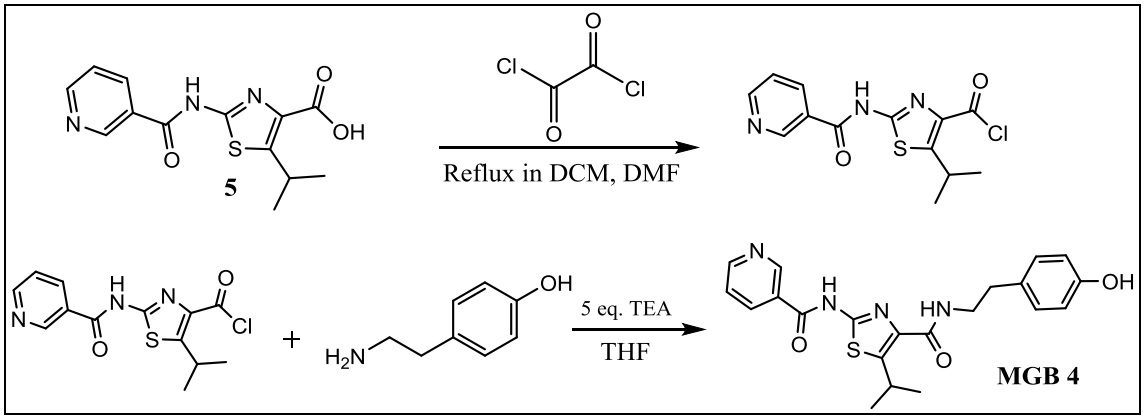
IR : 2961, 1663, 1593, 1568, 1542, 1464, 1326, 1308, 1048, 949, 920 cm^{-1}.

ESI-MS(M_+1): 291.6425 (100%), 292.9585 (63%), 294.0201 (28%).

^{1}HNMR : (400 MHz, DMSO-*d*6) δ ppm 1.26 - 1.36 (d, 6 H) 4.04 - 4.13 (m, 1 H) 7.53 - 7.63 (m, 1 H) 8.38 - 8.47 (m, 1 H) 8.75 - 8.82 (m, 1 H) 8.79 (s, 1 H) 9.20 (d, *J*=1.71 Hz, 1 H) 13.44 - 13.58 (m, 1 H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 25.19 (s, 2 C) 27.37 (s, 1 C) 124.10 (s, 1 C) 128.48 (s, 1 C) 135.49 (s, 1 C) 136.33 (s, 1 C) 150.25 (s, 1 C) 153.41 (s, 1 C) 158.44 (s, 1 C) 158.82 (s, 1 C) 164.79 (s, 1 C) 166.32 (s, 1 C).

2.2.6 Synthesis of N-(4-hydroxyphenethyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide (MGB 4):



A 102 mg (0.344 mmol) of **(5)** were refluxed for 3 hrs in 10 ml DCM (HPLC grade) with 2 eq. of oxalyl chloride (344 µl of 2 M oxalyl chloride in DCM, HPLC grade) and 25 µl DMF as a catalyst. The solvent was then removed under reduced pressure, and the intermediate product was dissolved immediately in 5-10 ml THF (HPLC grade). The r.b.f was placed in an ice bath, and a previously prepared solution of tyramine (47.19 mg, 0.344 mmol) and 240 µl of TEA (5 eq.) were added dropwise to the chlorinated derivative of **(5).** The reaction mixture was then stirred overnight and monitored by TLC (mobile phase: EtOAc 100%) for detecting new spots. The solvent was then removed under reduced

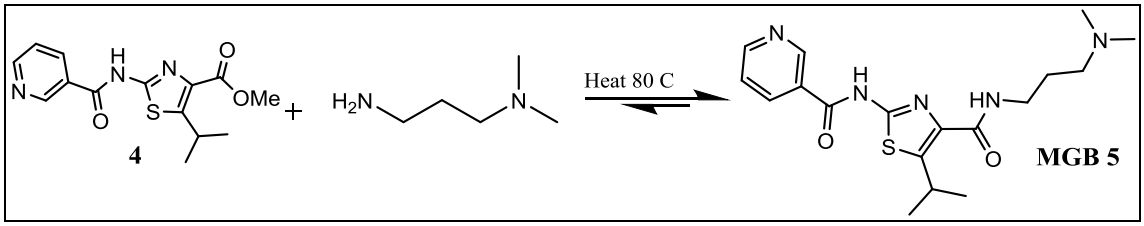
pressure, and the product was extracted with EtOAc (2 x 50 ml) from 50 ml of saturated sodium bicarbonate solution plus 5 ml brine discarding the aqueous layer. The product **MGB 4** was then purified by column chromatography (mobile phase: Hex: EtOAc 30:70).

R_f : 0.57 (EtOAc 100 %)

Yield: 57.2 mg, 40.5 %, m.p =224.2-226.5 C°.

IR : 3278, 2962, 2924, 1649, 1591, 1557, 1515, 1319, 1258, 1012, 864 cm^{-1}.

2.2.7 Synthesis of N-(3-(dimethylamino)propyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide (MGB 5) :



A 200 mg (0.65 mmol) of **(4)** were dissolved in 5 ml of *N, N*-dimethyl-amino propylamine, and the reaction was heated to 80 C° and left stirring overnight. The reaction was monitored by TLC (mobile phase: EtOAc: MeOH 9:1 + 1% TEA). The reaction was then treated with 50 ml saturated sodium bicarbonate solution + 5 ml brine and the product was taken with 2 x 50 ml of ether. The product was then purified by column chromatography (mobile phase: EtOAc: MeOH 95:5 + 1% TEA) and then converted to the HCl salt by dissolving in 1% aqueous HCl plus 5 ml methanol followed by freeze drying.

R_f: 0.31 (EtOAc: MeOH 9:1 + 1% TEA)

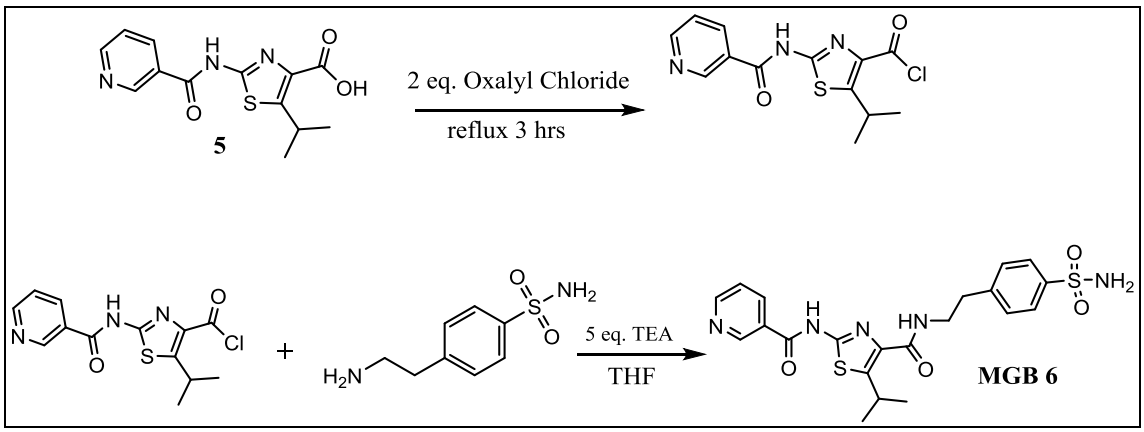
Yield & m.p: semisolid that weighed higher than 100% yield due to impurities.

IR: 3306, 2962, 1630, 1545, 1504, 1401, 1258, 1011, 864 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-*d*6) δ ppm 1.10 - 1.29 (d, 6 H) 1.78 - 2.01 (m, 2 H) 2.24 - 2.45 (m, 1 H) 2.98 - 3.20 (m, 4 H) 3.21 - 3.39 (s, 6 H) 3.58 - 3.74 (m,3 H) 4.03 - 4.20 (m, 1 H) 8.03 (br t, *J*=5.80 Hz, 1 H) 8.42 (s, 1 H) 8.57 (s, 1 H) 8.69 (s, 1 H) 10.07 (br s, 1 H).

^{13}C NMR (126 MHz, DMSO-*d*6) δ ppm 22.55 (s, 1 C) 26.55 (s, 1 C) 29.15 (s, 1 C) 36.07 (s, 1 C) 40.00 (s, 1 C) 40.17 (s, 1 C) 40.34 (s, 1 C) 40.51 (s, 1 C) 43.45 (s, 1 C) 55.01 (s, 1 C) 124.37 (s, 1 C) 127.68 (s, 1 C) 135.74 (s, 1 C) 137.11 (s, 1 C) 141.20 (s, 1 C) 141.68 (s, 1 C) 154.73 (s, 1 C) 162.35 (s, 1 C) 163.01 (s, 1 C) 164.56 (s, 1 C)

2.2.8 Synthesis of 5-isopropyl-2-(nicotinamido)-N-(4-sulfamoylphenethyl)thiazole-4-carboxamide (MGB 6):



A 102 mg (0.342 mmol) of **(5)** were refluxed for 3 hrs in 10 ml DCM

(HPLC grade) with 2 eq. of oxalyl chloride (370 µl of 2 M oxalyl chloride in DCM) and 25 µl DMF as a catalyst. The solvent was then removed under reduced pressure, and the intermediate product was dissolved immediately in 5-10 ml THF (HPLC grade). The r.b.f was placed in an ice bath, and a previously prepared solution of aminoethyl-benzene sulphonamide (70.5 mg, 0.35 mmol) and 240 µl of TEA (5 eq.) were added dropwise to the chlorinated derivative of **(5)**. The reaction was stirred overnight and monitored by TLC (mobile phase: EtOAc 100%) for detecting new spots. The solvent was then removed under reduced pressure, and the product was extracted with EtOAc (2 x 50 ml) from 50 ml of saturated sodium bicarbonate solution plus 5 ml brine discarding the aqueous layer. The product **MGB 6** was then obtained by column chromatography (mobile phase: Hex: EtOAc 30:70).

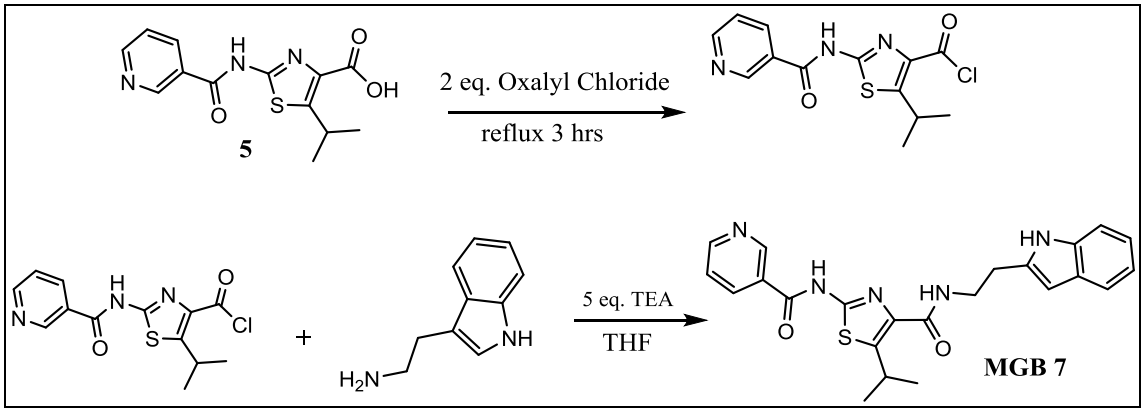
R_f : 0.49 (EtOAc 100 %)

Yield: 140 mg, 82 %, m.p =234-236 C°.

IR : 2962, 1649, 1542, 1415, 1304, 1258, 1010, 864 cm^{-1}.

ESI-MS (M_+1): 474.1378 (43%).

2.2.9 Synthesis of N-(2-(1H-indol-2-yl)ethyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide (MGB 7):



A 100 mg (0.342 mmol) of **(5)** were refluxed for 3 hrs in 10 ml DCM (HPLC grade) with 2 eq. of oxalyl chloride (344 µl of 2 M oxalyl chloride in DCM) and 25 µl DMF as a catalyst. The solvent was then removed under reduced pressure, and the intermediate product was dissolved immediately in 5-10 ml THF (HPLC grade). The r.b.f was placed in an ice bath, and a previously prepared solution of tryptamine (55.11 mg, 0.344 mmol) and 240 µl of TEA (5 eq.) were added dropwise to the chlorinated derivative of **(5)**. The reaction was stirred overnight and monitored by TLC (mobile phase: EtOAc 100%) for detecting new spots. The solvent was then removed under reduced pressure, and the product was extracted with EtOAc (2 x 50 ml) from 50 ml of saturated sodium bicarbonate solution plus 5 ml brine discarding the aqueous layer. The product **MGB 7** was then obtained by column chromatography (mobile phase: Hex: EtOAc 50:50 to EtOAc 100%).

R_f : 0.4 (EtOAc 100 %)

Yield: 131 mg, 86 %, m.p = decomposed over 300 C°.

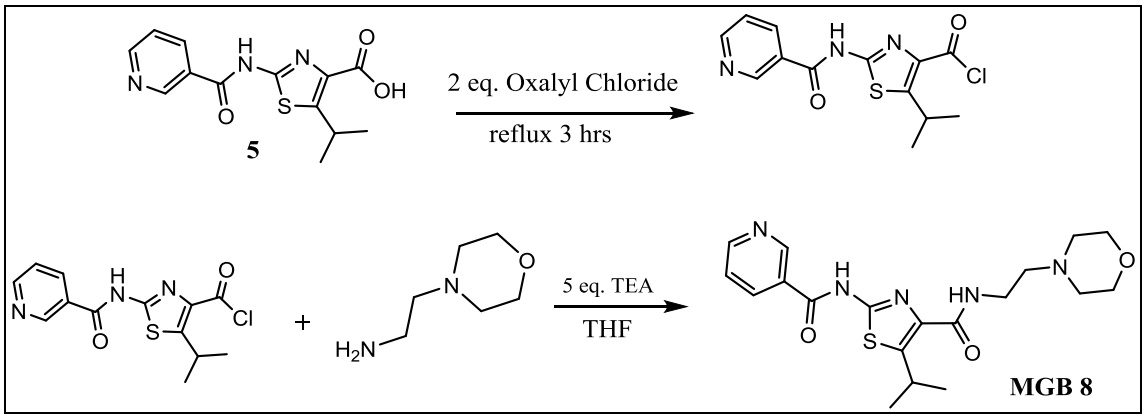
IR : 2962, 2920, 2851, 1650, 1542, 1416, 1259, 1085, 1015, 865 cm^{-1}.

ESI-MS (M_+1): 434.1616 (29%)

^{1}H NMR (500 MHz, DMSO-*d*6) δ ppm 1.29 (s, 6 H) 2.95 (s, 2 H) 3.17 (s, 1 H) 4.29 (s, 2 H) 5.75 (s, 2 H) 6.99 (s, 1 H) 7.08 (s, 1 H) 7.19 (s, 1 H) 7.34 (s, 1 H) 7.60 (s, 1 H) 8.31 (s, 1 H) 8.40 (s, 2 H) 8.81 (s, 1 H) 10.43 (s, 1 H) 10.84 (s, 2 H) 12.82 (s, 2 H).

^{13}CNMR: poor result, no peaks detected in the spectrum.

2.2.10 Synthesis of 5-isopropyl-N-(2-morpholinoethyl)-2-(nicotinamido)thiazole-4-carboxamide (MGB 8):



A 200 mg (0.68 mmol) of **(5)** were refluxed for 3 hrs in 10 ml DCM (HPLC grade) with 2 eq. of oxalyl chloride (690 µl of 2 M oxalyl chloride in DCM) and 25 µl DMF as a catalyst. The solvent was then removed under reduced pressure, and the intermediate product was dissolved immediately in 5-10 ml THF (HPLC grade). The r.b.f was placed in an ice bath, and a previously prepared solution of aminoethyl morpholine (90 µl,

0.68 mmol) and 240 µl of TEA (5 eq.) were added dropwise to the chlorinated derivative of **(5)**. The reaction was stirred overnight and monitored by TLC (mobile phase: MeOH: DCM 1:9) for detecting new spots. The solvent was then removed under reduced pressure, and the product was extracted with Ether (2x50ml) from 50 ml of saturated sodium bicarbonate solution plus 5 ml brine discarding the aqueous layer. The product **MGB 8** was then obtained by crystallization upon addition of Hexane to the Ether solution.

R_f : 0.675 (MeOH: DCM 1:9)

Yield: 94.2 mg, 26.77 %, m.p = 141-142.2 C°.

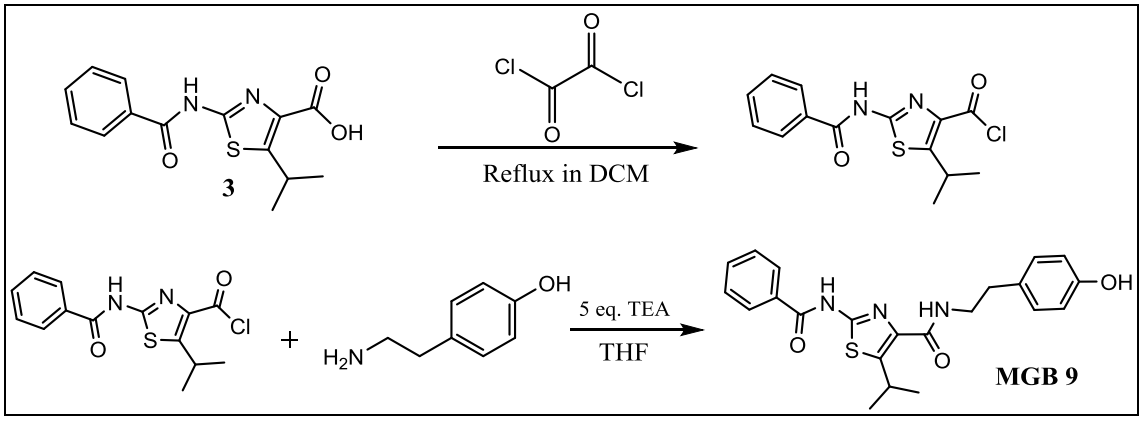
IR : 3853, 3838, 3801, 3749, 3674, 3649, 3616, 3566, 3403, 2937, 2866, 2820, 1748, 1716, 1699, 1653, 1026, 853 cm^{-1}.

ESI-MS (M_+1): around 405 (less than 5%).

^{1}H NMR (500 MHz, DMSO-*d6*) δ ppm 1.18 (d, *J*=6.71 Hz, 6 H) 2.36 - 2.45 (m, 6 H) 2.95 - 2.96 (m, 3 H) 3.10 (s, 3 H) 3.57 (t, *J*=4.58 Hz, 4 H) 4.22 (s, 1 H) 7.51 (s, 1 H) 8.22 (s, 1 H) 8.70 (s, 1 H) 8.98 (s, 1 H).

^{13}C NMR (126 MHz, DMSO-*d6*) δ ppm 25.41 (s, 1 C) 27.03 (s, 1 C) 37.03 (s, 1 C) 39.86 (s, 1 C) 40.02 (s, 1 C) 40.19 (s, 1 C) 40.35 (s, 1 C) 40.52 (s, 1 C) 53.68 (s, 1 C) 56.75 (s, 1 C) 66.69 (s, 1 C) 123.94 (s, 1 C) 130.46 (s, 1 C) 135.38 (s, 1 C) 138.31 (s, 1 C) 140.39 (s, 1 C) 145.96 (s, 1 C) 148.76 (s, 1 C) 157.28 (s, 1 C) 162.56 (s, 1 C) 165.86 (s, 1 C).

2.2.11 Synthesis of 2-benzamido-N-(4-hydroxyphenethyl)-5-isopropylthiazole-4-carboxamide (MGB 9):



A 100 mg (0.344 mmol) of **(3)** were refluxed for 3 hrs in 10 ml DCM (HPLC grade) with 2 eq. of oxalyl chloride (330 µl of 2 M oxalyl chloride in DCM) and 25 µl DMF as a catalyst. The solvent was then removed under reduced pressure and the intermediate product was dissolved immediately in 5-10 ml THF (HPLC grade), the r.b.f was placed in an ice bath and a previously prepared solution of tyramine (47.19 mg, 0.344 mmol) and 240 µl of TEA (5 eq.) were added drop wise to the chlorinated derivative of **(5)**. The reaction was stirred overnight and monitored by TLC (mobile phase: EtOAc : Hex 1:1) for detecting new spots. The solvent was then removed under reduced pressure and the product was extracted with EtOAc (2 x 50 ml) from 50 ml of saturated sodium bicarbonate solution plus 5 ml brine discarding the aqueous layer. The product **MGB 9** was then purified by column chromatography (mobile phase: EtOAc: Hex 1:1) .

R_f : 0.41 (EtOAc: Hex 1:1).

Yield: 48 mg, 33.5 %, m.p = 129.5-132 C°.

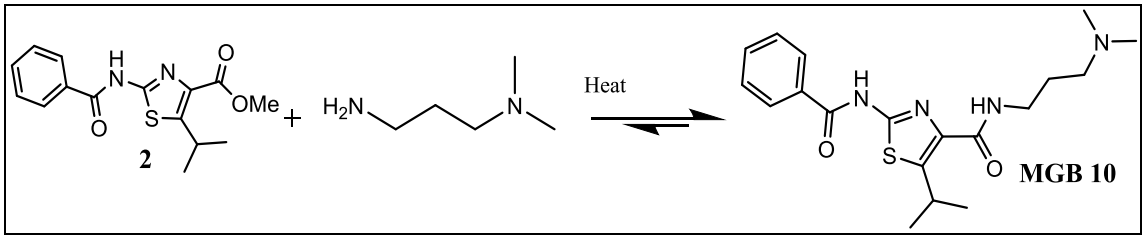
IR : 2962, 2909, 2844, 1645, 1544, 1504, 1447, 1258, 1013, 792 cm^{-1}.

ESI-MS (M_+1): 410.0128 (100%), 410.3256 (88%), 411.0764 (38%).

^{1}H NMR (500 MHz, DMSO-*d*6) δ ppm 1.34 (d, *J*=7.02 Hz, 6 H) 2.77 (t, *J*=7.48 Hz, 2 H) 3.52 - 3.58 (m, 2 H) 4.30 (sept, *J*=6.87 Hz, 1 H) 6.73 - 6.77 (m, 2 H) 7.10 (m, *J*=8.24 Hz, 2 H) 7.61 (t, *J*=7.63 Hz, 2 H) 7.69 - 7.73 (m, 1 H) 7.88 (t, *J*=5.95 Hz, 1 H) 8.11 (d, *J*=7.38 Hz, 2 H) 9.30 (s, 1 H) 12.65 (br s, 1 H).

^{13}C NMR (126 MHz, DMSO-*d*6) δ ppm 25.24 (s, 1 C) 26.70 (s, 1 C) 34.90 (s, 1 C) 40.00 (s, 1 C) 40.17 (s, 1 C) 40.33 (s, 1 C) 40.72 (s, 1 C) 115.67 (s, 1 C) 128.55 (s, 1 C) 129.13 (s, 1 C) 129.78 (s, 1 C) 129.95 (s, 1 C) 132.29 (s, 1 C) 133.22 (s, 1 C) 136.77 (s, 1 C) 146.21 (s, 1 C) 154.37 (s, 1 C) 156.12 (s, 1 C) 162.37 (s, 1 C) 165.74 (s, 1 C)

2.2.12 Synthesis of 2-benzamido-N-(3-(dimethylamino)propyl)-5-isopropylthiazole-4-carboxamide (MGB 10):



A 109 mg (0.36 mmol) of **(2)** were dissolved in 5 ml of *N, N*-dimethyl-amino propylamine and the reaction was heated to 80 C° and left stirring overnight. The reaction was monitored by TLC (mobile phase: EtOAc:

MeOH 9:1 + 1% TEA). The reaction was then treated with 50 ml saturated sodium bicarbonate solution + 5 ml brine and the product was taken with 2 x 50 ml of ether. The product was then purified by column chromatography (mobile phase: EtOAc: MeOH 9:1 + 1% TEA) and then converted to the HCl salt by dissolving in 1% aqueous HCl plus 5 ml methanol followed by freeze drying.

R_f : 0.13 after extraction, and 0.39 in reaction mixture (EtOAc: MeOH 9:1 + 1% TEA).

Yield: liquid impurity made the weight higher, m.p: semisolid

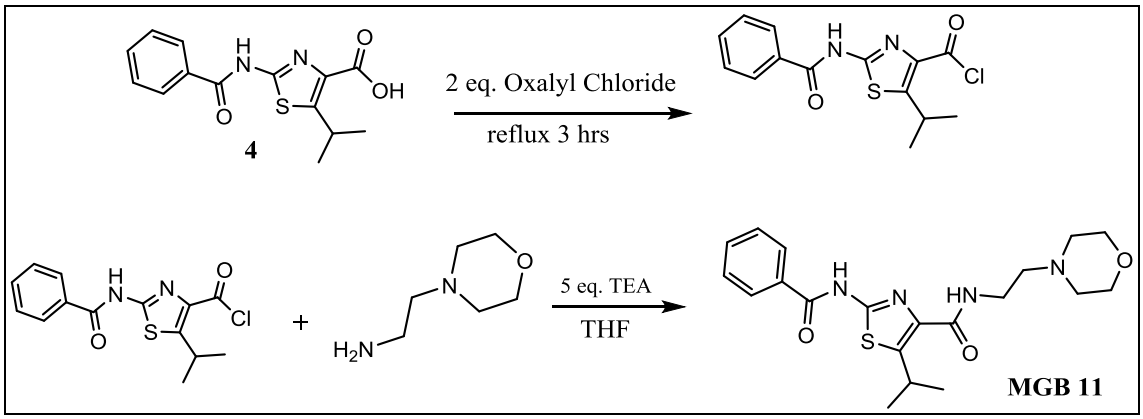
IR: 2962, 1640, 1546, 1405, 1258, 1007, 864, 759 cm^{-1}.

ESI-MS (M_+1): 374.9154 (100%), 376.0415 (37%), 377.1050 (12%).

^{1}H NMR (500 MHz, DMSO-*d*6) δ ppm 0.91 (s, 6 H) 1.50 - 1.54 (m, 6 H) 1.61 - 1.74 (m, 28 H) 2.27 (dt, *J*=3.66, 1.83 Hz, 24 H) 2.78 - 2.93 (m, 30 H) 3.10 (q, *J*=6.41 Hz, 51 H) 3.84 (s, 1 H) 3.85 (s, 1 H) 7.23 - 7.33 (m, 23 H) 7.63 - 7.66 (m, 21 H) 8.48 (br t, *J*=5.65 Hz, 10 H) 12.42 (s, 1 H).

^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 24.47 (s, 1 C) 25.61 (s, 1 C) 28.35 (s, 1 C) 35.67 (s, 1 C) 38.88 (s, 1 C) 39.05 (s, 1 C) 39.22 (s, 1 C) 39.38 (s, 1 C) 41.37 (s, 1 C) 53.87 (s, 1 C) 127.43 (s, 1 C) 127.64 (s, 1 C) 132.16 (s, 1 C) 133.55 (s, 1 C) 135.53 (s, 1 C) 139.87 (s, 1 C) 161.24 (s, 1 C) 161.68 (s, 1 C) 165.84 (s, 1 C).

2.2.13 Synthesis of 5-isopropyl-N-(2-morpholinoethyl)-2-benzamido-thiazole-4-carboxamide (MGB 11):



A 147.5 mg (0.51 mmol) of **(4)** were refluxed for 3 hrs in 10 ml DCM (HPLC grade) with an excess of oxalyl chloride (2 ml of 2 M oxalyl chloride in DCM) and 25 µl DMF as a catalyst. The solvent was then removed under reduced pressure, and the intermediate product was dissolved immediately in 5-10 ml THF (HPLC grade), the r.b.f was placed in an ice bath and a previously prepared solution of aminoethyl morpholine (90 µl, 0.68 mmol) and 240 µl of TEA (5 eq.) were added dropwise to the chlorinated derivative of **(5)**. The reaction was stirred overnight and monitored by TLC (mobile phase: EtOAc 100 %) for detecting new spots. The solvent was then removed under reduced pressure, and the product was extracted with Ether (2x50ml) from 50 ml of saturated sodium bicarbonate solution plus 5 ml brine discarding the aqueous layer. The organic layer was then evaporated, and the spots were obtained by column chromatography (mobile phase : EtOAc: MeOH 9:1). The collected spots were sent for confirming the presence of MGB 11.

Chapter 3

Results and Discussion

3.1. Design of Distamycin A analogues

The aim of this project as previously mentioned was to synthesize Distamycin A analogues with enhanced lipophilicity and obeying the Lipinski's rule of five [50]. So, our compounds will have molecular weights of less than 500 Da. Also, those analogues will have no more than 5 and 10 H-bond donors and H-bond acceptors, respectively, with log P values (estimated using ChemDraw ultra 12 software) of the suggested analogues being less than 5. **Table 3.1** provides the physical and chemical properties of synthesized compounds and for other suggested analogues.

Table 3.1: Physical and chemical properties of Distamycin A analogues.

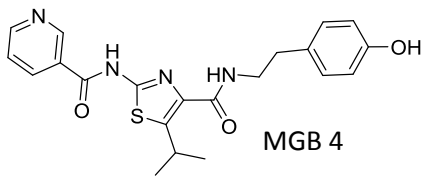
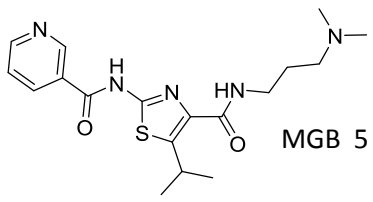
Compound	M.wt (Da)	m.p (C°)	Log P	HBD	HBA
 MGB 4	410.5	224.2-226.5	3.63	3	3
 MGB 5	375.5	Semi-solid	2.11	2	2

Table 3.1 (continued)

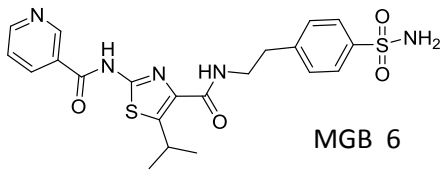
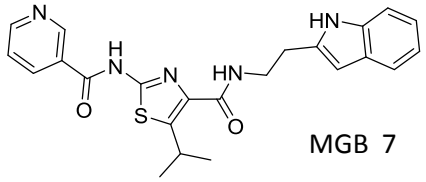
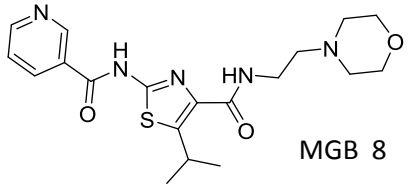
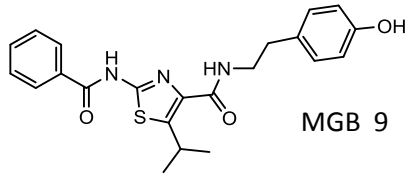
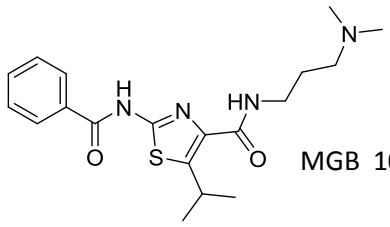
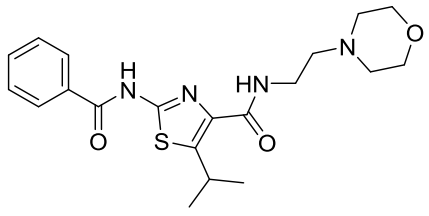
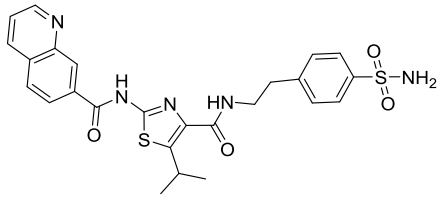
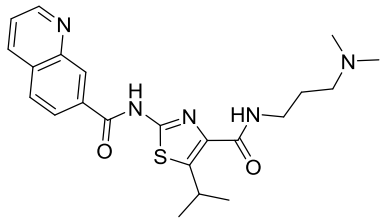
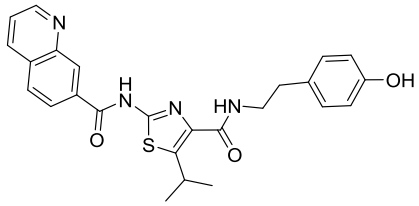
 MGB 6	473.5	234-236	2.77	4	4
 MGB 7	433.5	>300	3.44	3	2
 MGB 8	403.5	141.142.5	1.61	2	3
 MGB 9	409.5	129.5-132	4.97	3	3
 MGB 10	374.5	Semi-solid	3.45	2	2
	402.5	Semi-solid	2.95	2	3
	523	Suggested analogue	4.19	4	4

Table 3.1 (continued)

	425.5	Suggested analogue	3.54	2	2
	460.5	Suggested analogue	5.05	3	3

3.2 Synthesis of the MGBs

The starting building block of our MGBs was the methyl-2-amino-5-isopropyl-1,3-thiazole-4-carboxylate 1 **(Figure 3.1)**. It was synthesized in a good yield (70%) by reacting methyl dichloroacetate with isobutyraldehyde at 0 C° in diethyl ether in the presence of sodium methoxide as a strong base to give the α-chloroglycidic ester intermediate in accordance with the Darzens reaction **(Figure 3.2)**. Due to the high reactivity of the α-chloroglycidic ester intermediate, it was directly used in the second step to be coupled with thiourea in methanol(HPLC grade) to give compound **1**.

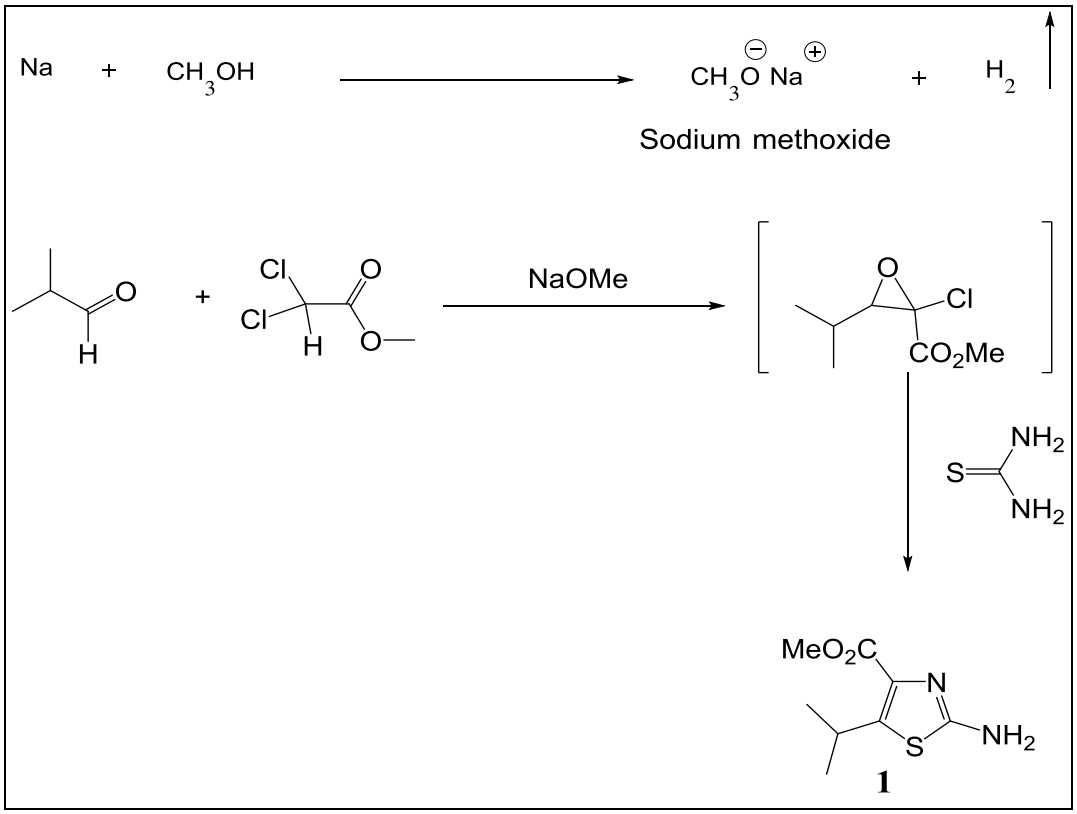


Figure 3.1 : Synthesis of methyl-2-amino-5-isopropyl-1,3-thiazole-4-carboxylate.

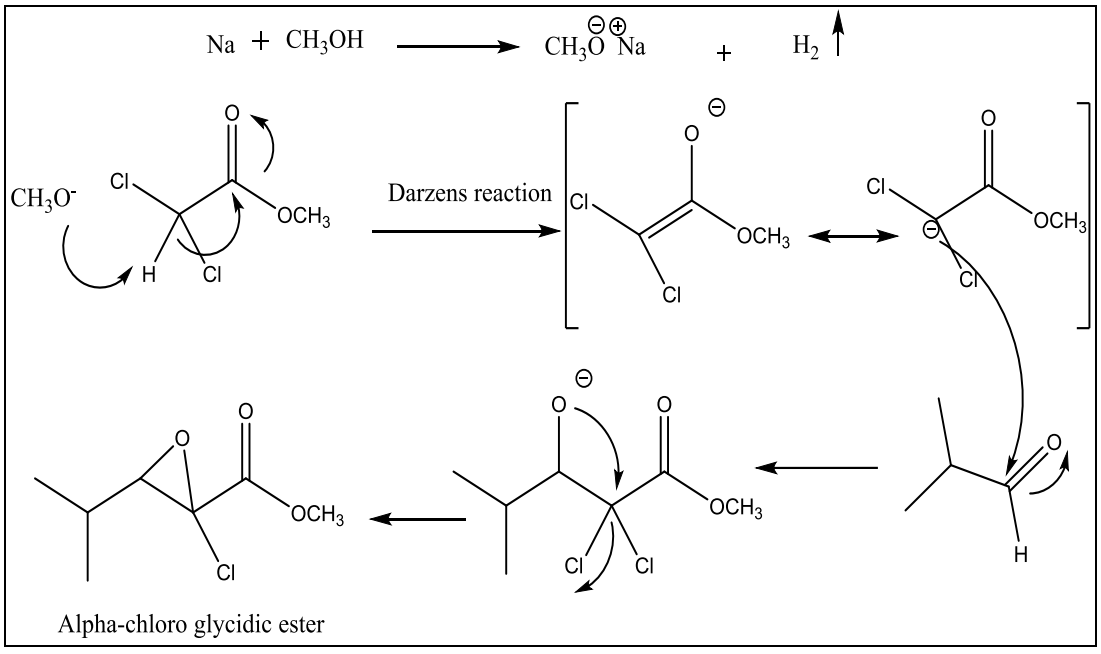


Figure 3.2: Mechanism of the Darzens reaction**.**

The reaction mechanism is outlined in **Figure 3.3**. It proceeds through nucleophilic attack of the sulphur atom of thiourea at the epoxide carbon leading to epoxide ring opening, and the formation of a new α-carbonyl moiety. The ring formation was achieved through the subsequent intramolecular nucleophilic attack of the thiourea amine on the α-carbonyl followed by two dehydration steps to form the desired compound 1.

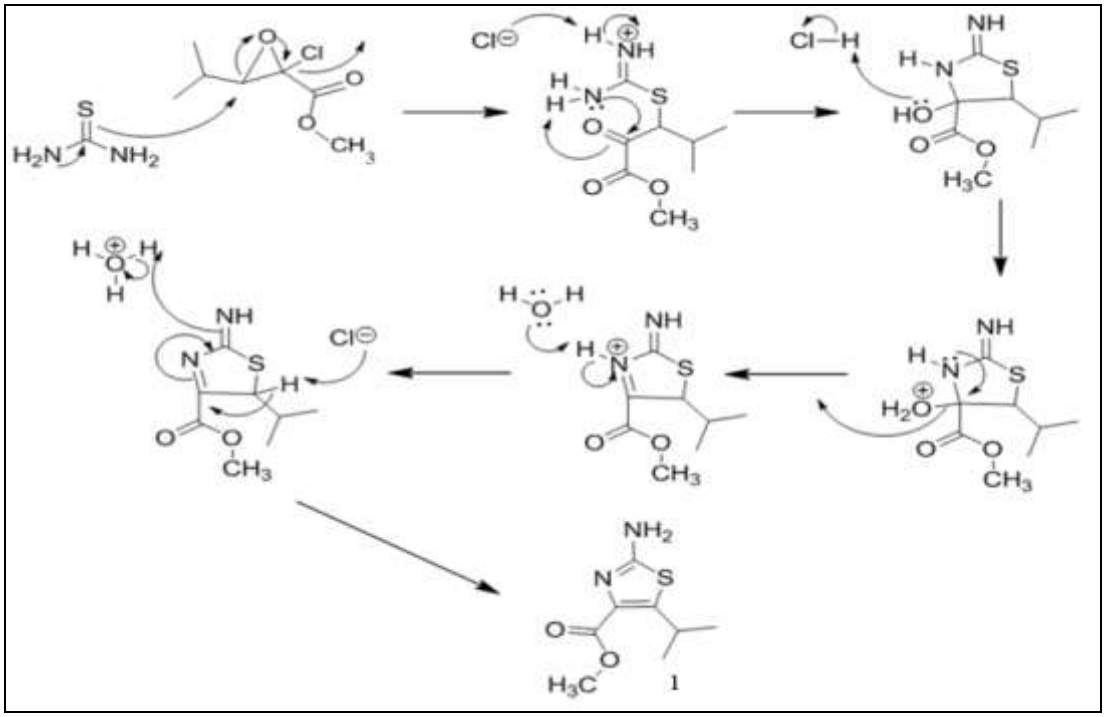


Figure 3.3: Mechanism of Thiazole ring formation through coupling of thiourea with the α-chloro glycidic ester.

The IR spectrum of compound 1 **(Figure 3.4)**, showed the stretching frequencies of the carbonyl at about 1690 cm^{-1}, and that of the alkene at 1620 cm^{-1}, and of the amine at 3421 cm^{-1}. The alkane stretching frequency also appeared around 3000 cm^{-1}. The M_+1 peak of compound 1 also

appeared in the ESI-MS spectrum **(Figure 3.5)**, at 200.92 indicating the successful synthesis of the desired compound. The ^{1}HNMR chemical shifts **(Figure 3.6)**, also confirm the chemical structure of compound 1. As the isopropyl signal appears as a doublet at 1.15 ppm for the 6 methyl hydrogens and a singlet at 3.88 ppm for the CH hydrogen, the chemical shift of the OCH_3 hydrogens appeared at 3.72 ppm as a singlet signal. The ^{1}H NMR signal for the amino group (NH_2) did not appear.

The OH and NH- protons undergo rapid exchange with the neighboring protons/or solvent protons at room temperature. NMR cannot, therefore, recognize their chemical shift position and appear as a broad signal or do not appear at all.

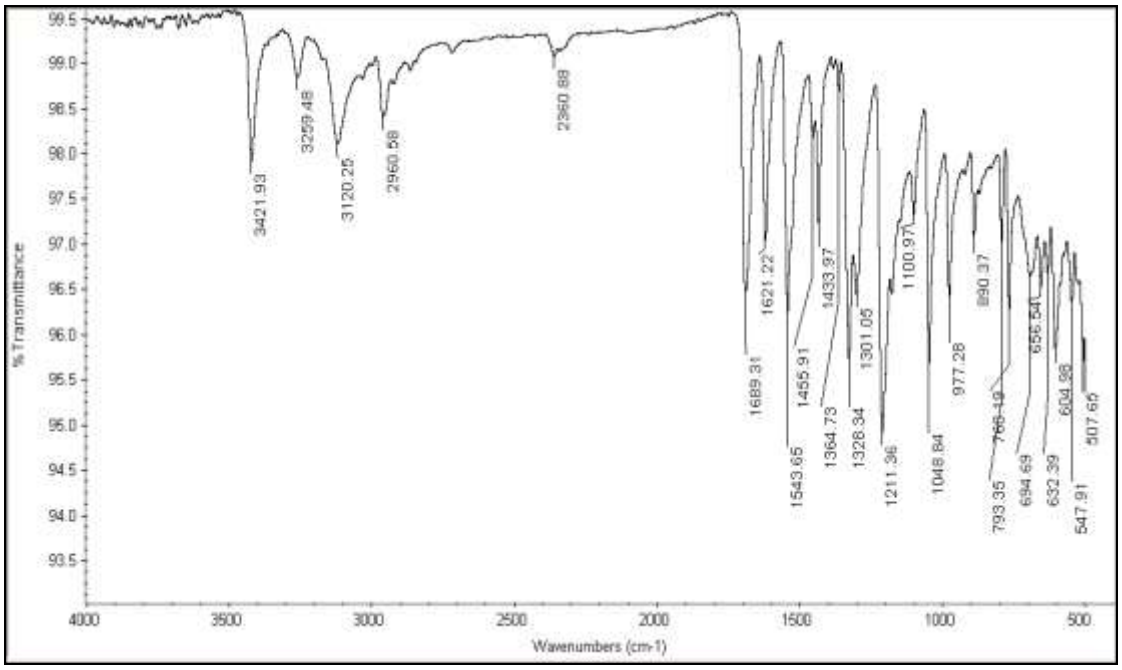


Figure 3.4: FT-IR spectrum of methyl-2-amino-5-isopropyl-1,3-thiazole-4-carboxylate.

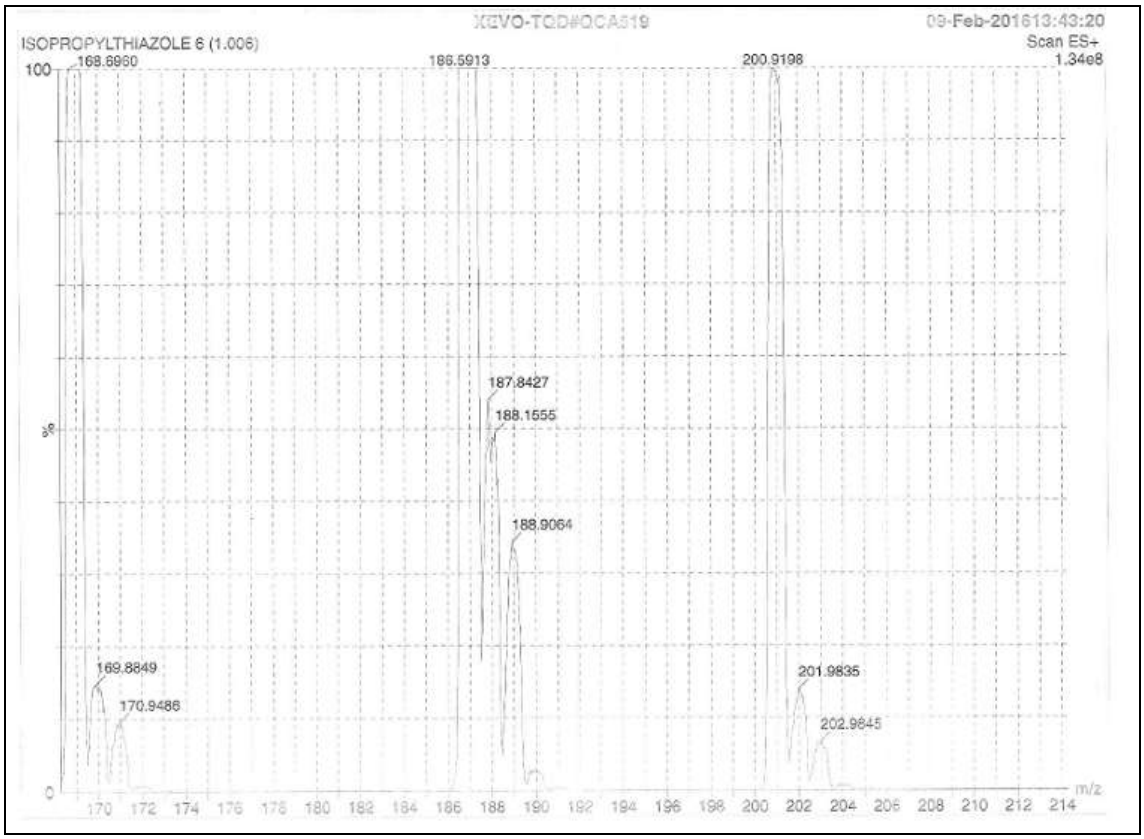


Figure 3.5: Mass spectrum of methyl-2-amino-5-isopropyl-1,3-thiazole-4-carboxylate.

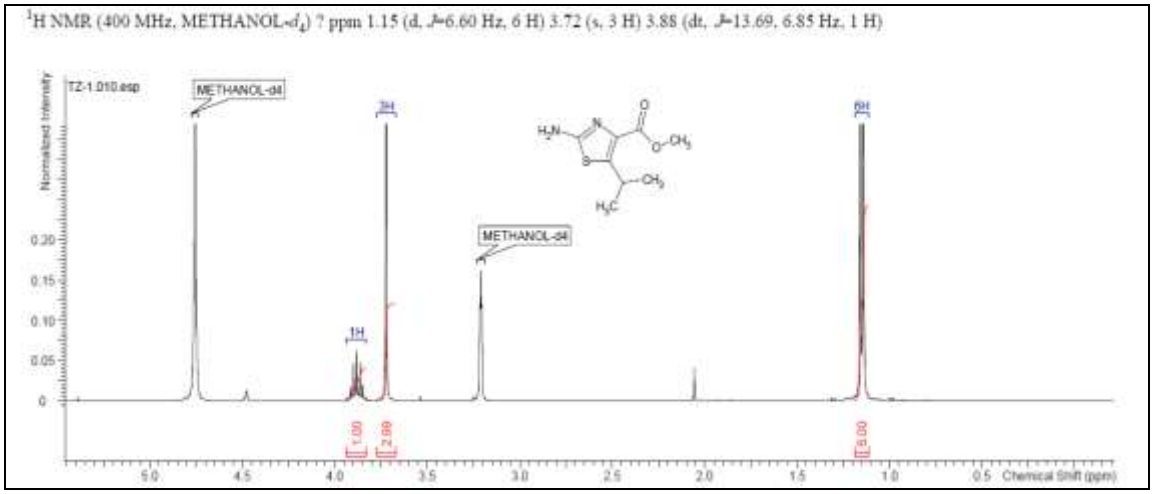


Figure 3.6: ^{1}HNMR spectrum of methyl-2-amino-5-isopropyl-1,3-thiazole-4-carboxylate (compound 1).

The second step of the MGBs synthesis was the coupling of compound 1 with various acid chlorides at the amine position to give the amide linked compounds 3 and 4 **(Figure 3.7)**. Compound 1 was reacted with nicotinoyl chloride to give compound 2. Moreover, to give compound 3, compound 1 was reacted with benzoyl chloride.

The carboxylic acid derivatives of compounds 2 and 3 were obtained by basic hydrolysis of the methyl ester with NaOH. Mass spectrometry along with ^{1}HNMR chemical shifts confirmed the chemical structures of the synthesized compounds, with new signals in the ^{1}HNMR spectra representing the chemical shifts of the new hydrogen atoms introduced to the molecules and the disappearance of some signals confirming the removal of some moieties in the generated molecules.

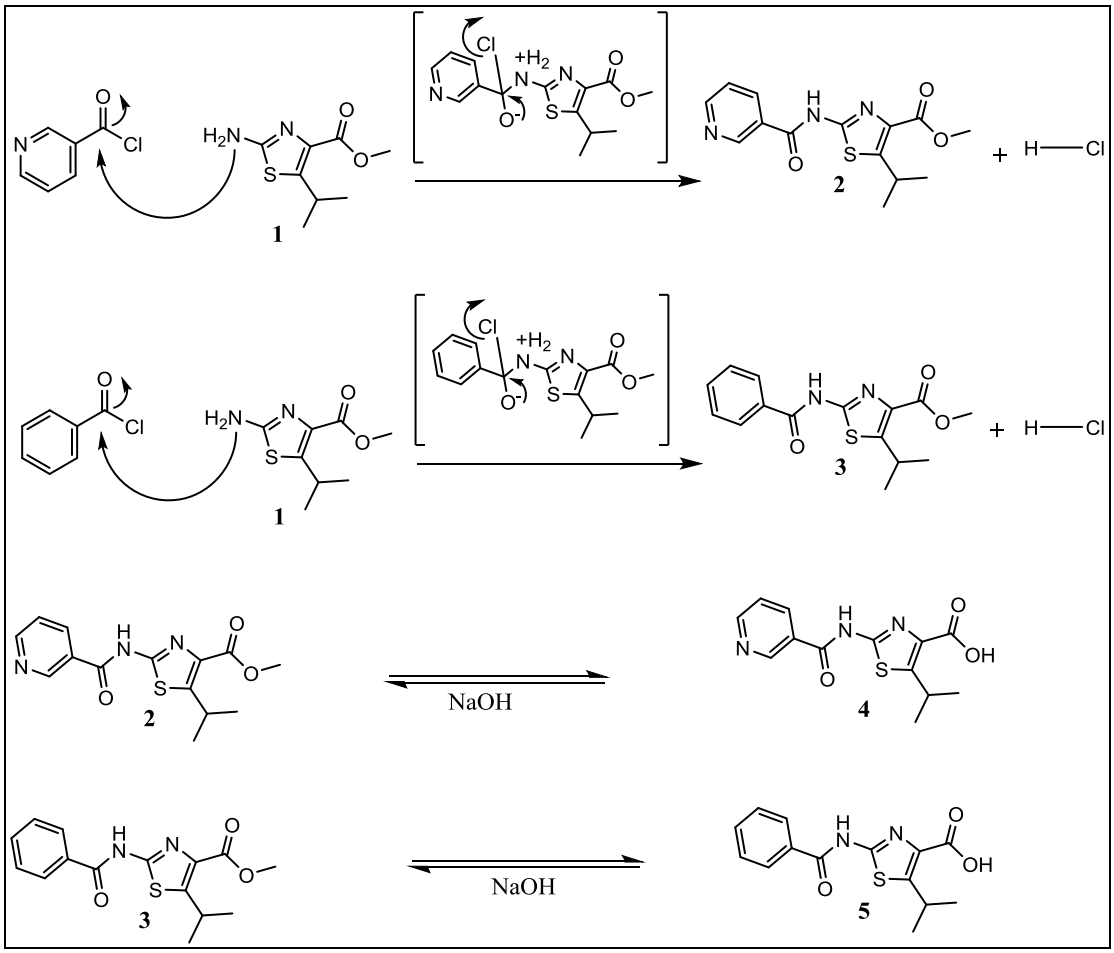


Figure 3.7: Chemical structures of compounds involved in the synthetic pathway of the investigational MGBs.

The [1]HNMR spectra of the carboxylic acid derivatives **(Figure 3.8 and Figure 3.9)**, also revealed the presence of water molecules in these compounds even after drying at 50 C° overnight indicating that the carboxylic acid might form hydrous crystals with strong hydrogen bonds with water.

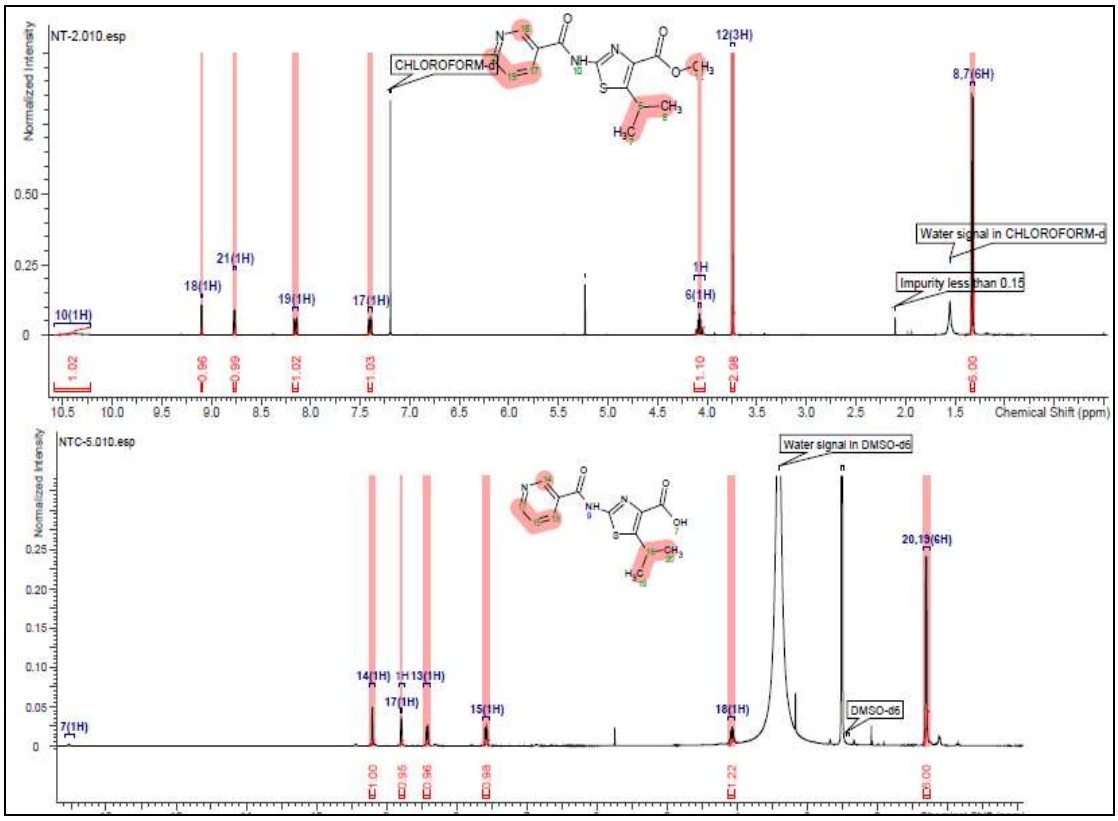


Figure 3.8: H^1NMR spectra of compound 2 and its CA derivative.

The presence of water in the carboxylic acid derivatives explains the low yields obtained in the next step of synthesis which involved the use of oxalyl chloride for the chlorination of compounds 4 and 5 to get the acyl chloride derivatives.

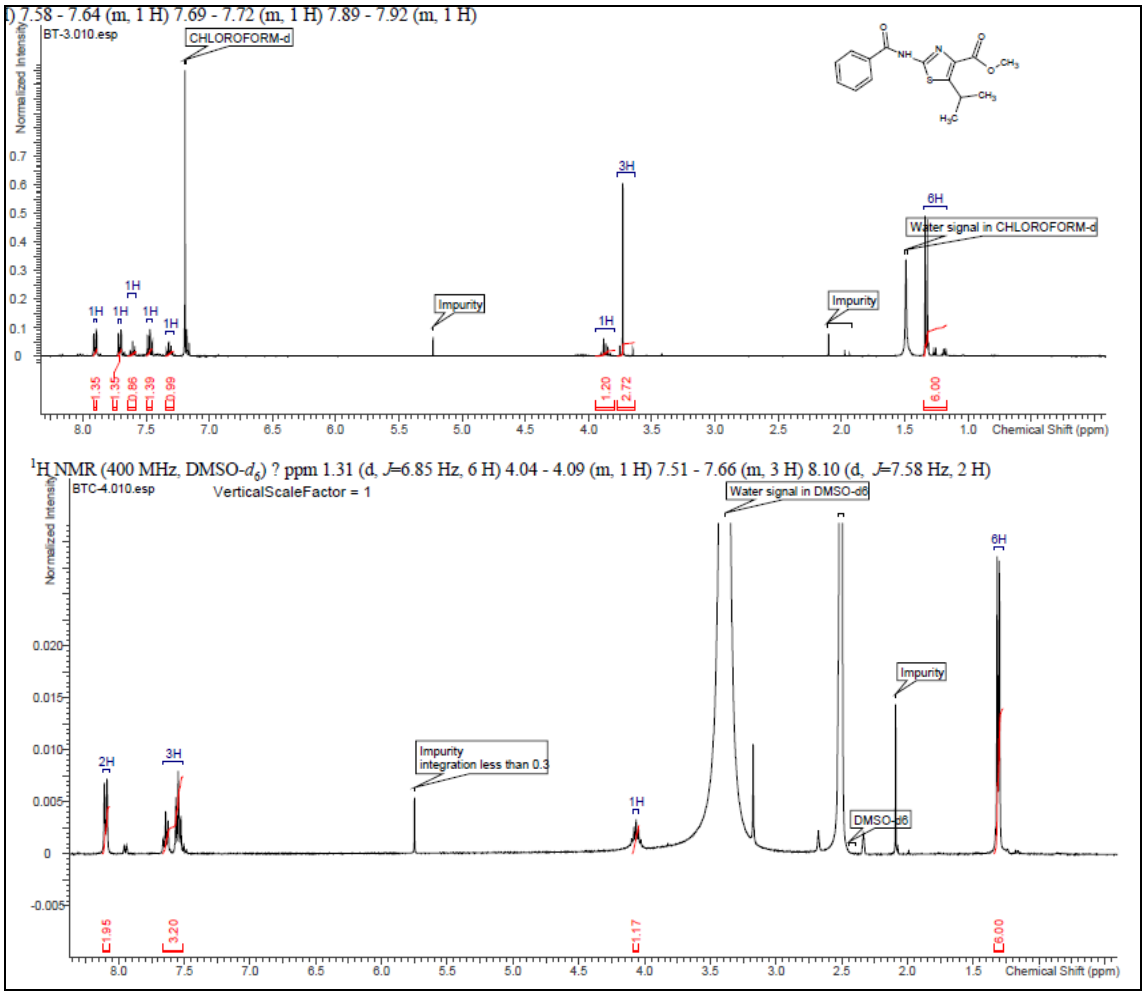


Figure.3.9: H[1]NMR spectra of compound 3 and its CA derivative.

Acyl chloride is highly reactive and unstable compound; it was therefore reacted directly with different amines to give the final compounds **(Figure 3.10)**. However, for compounds MGB 5 and MGB 10, the synthetic strategy was the direct amide formation through reacting an ester with the amine *N, N*- dimethylaminopropylamine which was used as a solvent and reagent. The reagents were activated by heating the reaction mixture to 80 C°; this temperature also served to shift the equilibrium

toward the product side by evaporating methanol from the products side of the equilibrium **(Figure 3.11)**.

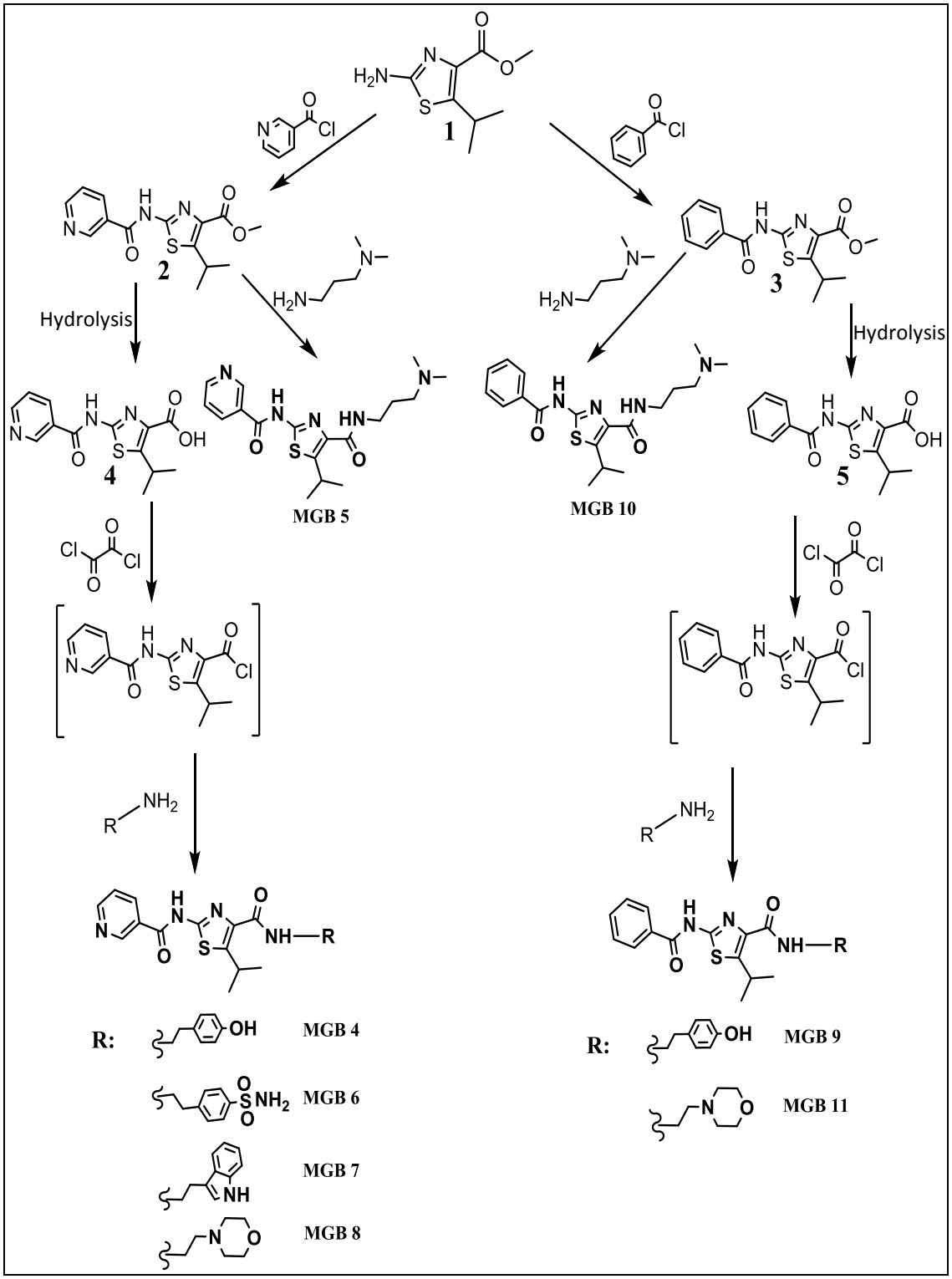


Figure 3.10: The synthetic scheme of the studied compound

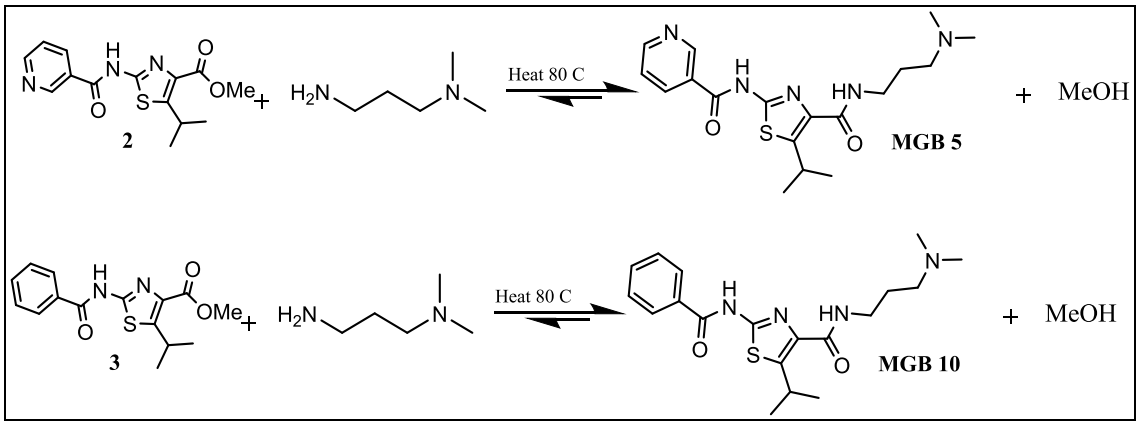


Figure 3.11: Synthesis of compound MGB 5 and MGB 10.

The chemical structures of both MGB 5 and MGB 10 were confirmed by ^{1}H, and ^{13}C NMR with the spectra for the two compounds were differing in the aromatic region, the $^{1}HNMR$ of compounds MGB 5 and MGB 10 are represented in **Figure 3,12**.

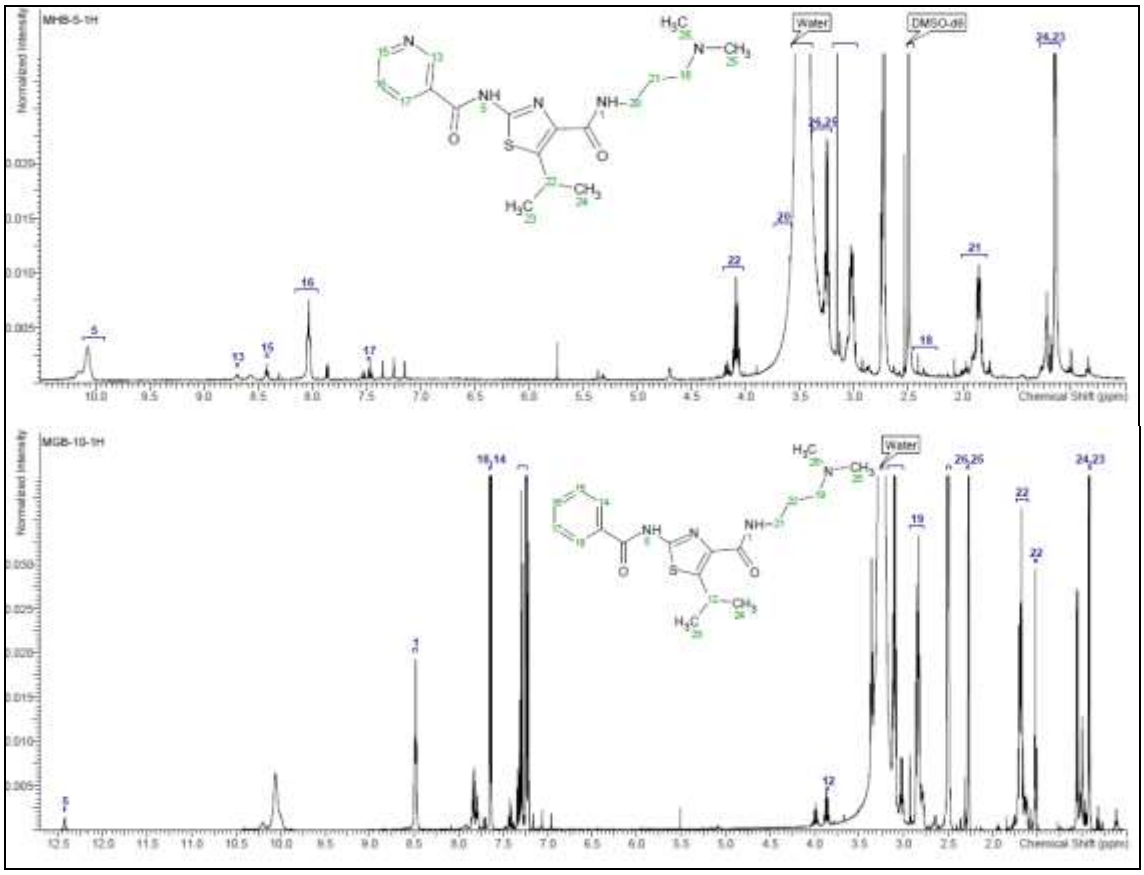


Figure 3.12: [1]HNMR of compounds MGB 5 and MGB 10.

Direct coupling using overnight reflux conditions did not succeed to couple compounds 2 and 3 with amines like Tyramine and Aminoethyl benzene sulphonamide. Compounds MGB 4 and MGB 6 were, therefore, synthesized by reacting acyl chloride with the corresponding amine **(Figure 3.13)**. The chemical structures of the compounds MGB 4 and MGB 6 were not confirmed by NMR.

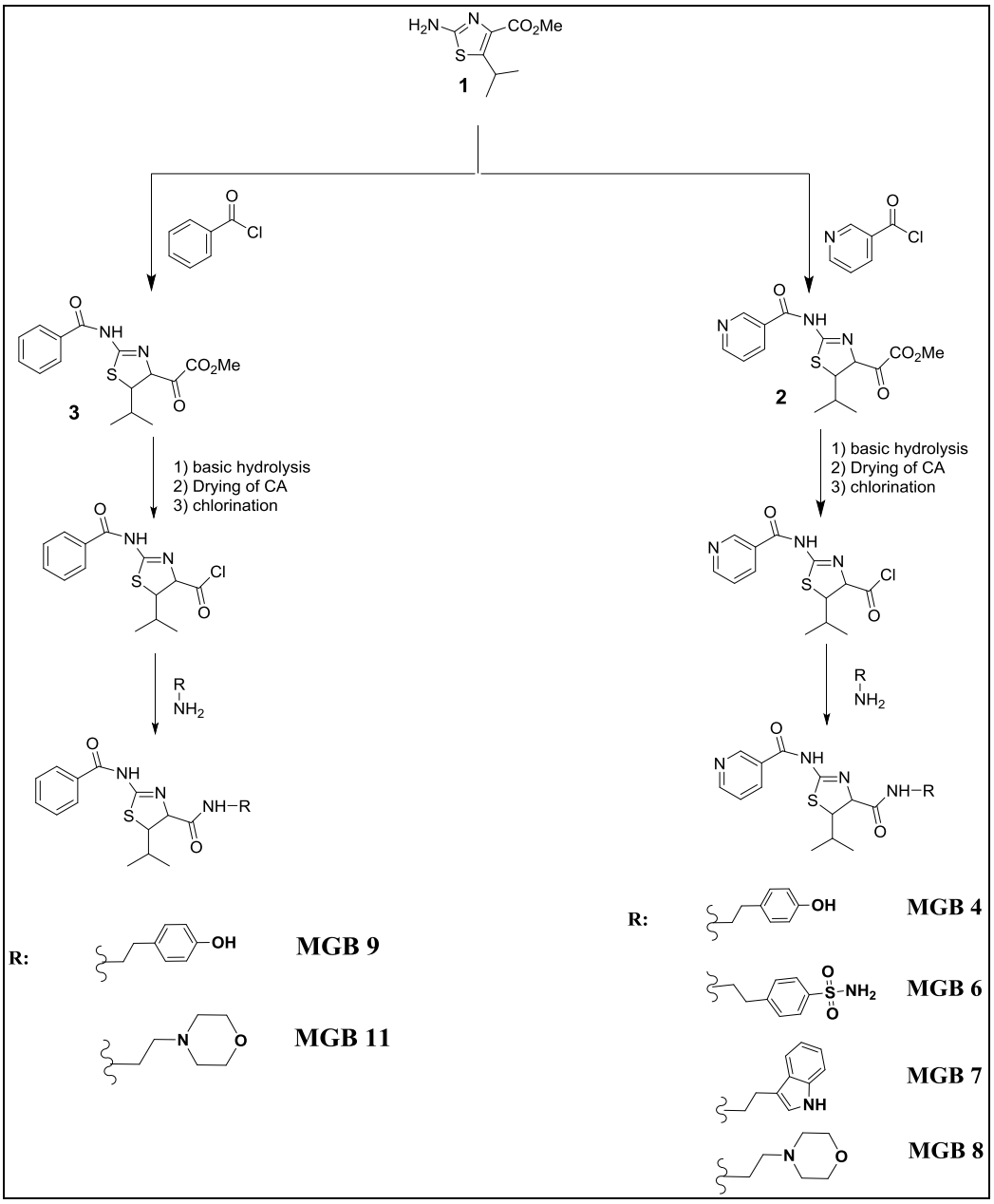


Figure 3.13: Synthetic scheme of compounds MGB 4,6,7,8,9 and 11.

Amide bond formation using acyl chloride reacted with an amine was also the synthetic strategy for compounds MGB 7, MGB 8, MGB 9 and MGB 11. To obtain MGB 7 tryptamine was reacted with the acyl chloride of compound 2 **(Figure 3.13)**, the ^{1}HNMR of MGB 7 is shown in **Figure 3.14**, and reveals crowded peaks in the aromatic region of the spectrum representing the aromatic protons in the chemical structure.

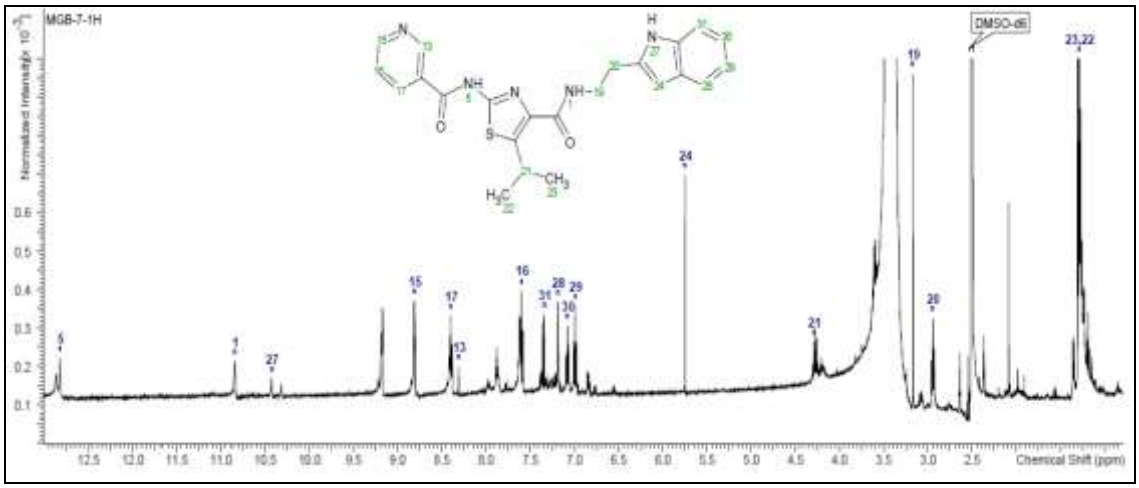


Figure 3.14: [1]HNMR of MGB 7.

MGB 8 was synthesized by reacting the chlorinated derivative of compound 2 with aminoethyl morpholine **(Figure 3.13)**, this reaction gave the best yield of the product MGB 8 obtained directly after ether/alkaline water extraction when it precipitated upon the addition of n-hexane to the ether solution of the compound. The [1]HNMR of MGB 8 is shown in **Figure 3.15**, where the peaks corresponding protons could be assigned but with low resolution.

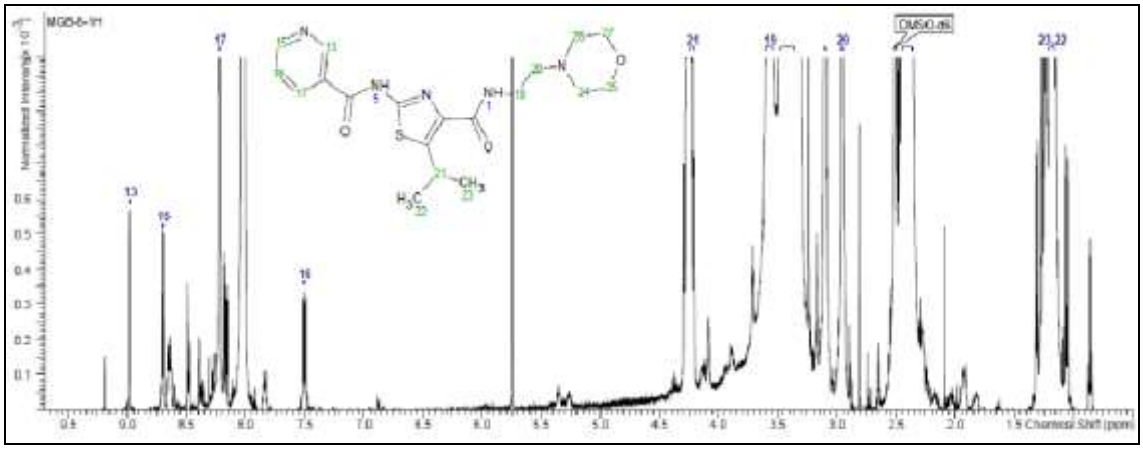


Figure 3.15: [1]HNMR of MGB 8.

The compounds MGB 9 and MGB 11 were synthesized by coupling the acyl chloride derivative of compound 3 **(Figure 3.13)**, with the amines tyramine and aminoethyl morpholine respectively. While the chemical structure of MGB 11 was not confirmed by NMR, the ^{1}HNMR of MGB 9 **(Figure 3.16)**, showed clear peaks of the protons in the compound, confirming the chemical structure of MGB 9.

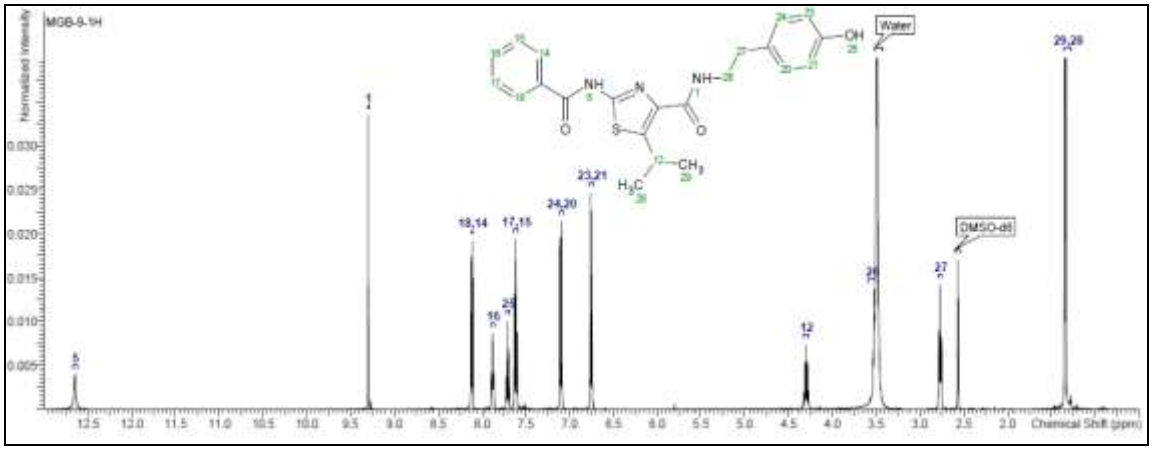


Figure 3.16: ^{1}HNMR of MGB 9.

The IR spectra of the investigated compounds were not informative and showed similarities in the stretching frequencies among the compounds.

Regarding the Mass spectrometry, compounds MGB 6, 7, 9 and 10 showed the $M_{+}1$ peak in their Mass spectra while the other compounds did not show a clear $M_{+}1$ peak of the molecules or were with very low intensity.

3.3 Biological Activity

3.3.1 Screening of Antibacterial activity

The antibacterial activity was determined using Broth Microdilution method[52,53] against the following bacterial strains: *S.aureus* (ATCC 25923) a Gram +ve bacteria, *P.auregenosa* (ATCC 27853), *E.coli* (ATCC 25928), Gram -ve bacteria and MRSA.

Compounds MGB 7 and MGB 10 showed positive antibacterial activity against both the Gram +ve and the Gram -ve strains of the studied bacteria, while the other compounds showed negative activity results for some strains and positive activity results against others. The data is presented in **Table 3.2.**

Table 3.2: Screening biological activity of the Investigational MGBs:

	S.aureus	*P.auregenosa*	*E.coli*	MRSA
MGB 4	-	-	+	+
MGB 5	-	+	-	+
MGB 6	-	-	-	-
MGB 7	+	+	+	+
MGB 8	-	-/+	-	-/+

Table 3.2 (continued)

	S.aureus	*P.auregenosa*	*E.coli*	MRSA
MGB 9	-	+	+	-
MGB 10	+	+	+	+

- : no activity +: active -/+: partial inhibition

3.3.2 MIC results

The investigational compounds showed weak antibacterial activity against the studied bacterial strains, with compound MGB 10 having the lowest observed MIC value among the studied compounds, with MIC value against *E.coli* being the lowest.

Table 3.3, shows the results of the micro broth dilution experiments carried out for the prepared samples of the synthesized compounds. The poor solubility of the crude compounds was the main obstacles in preparing the sample solutions and when incubating the samples solutions with the broth and bacteria. Later after NMR analysis of the synthesized compounds, it was also revealed that the purity of the products was not enough to account for the accuracy of the obtained MIC results. Antiviral, antifungal and anticancer activities need to be investigated.

Table 3.3: MIC of the investigational MGBs against different bacteria.

Bacterial Strain ⇨ / Compound ⇩	*S.aureus*		*P.auregenosa*		*E.coli*		MRSA	
	Conc. tested	MIC*	Conc. Tested	MIC*	Conc. tested	MIC*	Conc. tested	MIC*
MGB4 1st spot	0.918 mg/ml	------	0.918 mg/ml	------	0.918 mg/ml	0.918	1.835 mg/ml	1.835 ±
MGB4 2nd spot	0.25 mg/ml	------	0.125 mg/ml	0.125	0.125 mg/ml	------	0.25 mg/ml	0.125 ±
MGB 5 **	0.85 mg/ml	------	---------	------	----------	------	----------	------
MGB 6 **	0.125 mg/ml	------	0.0625 mg/ml	------	0.125 mg/ml	------	0.25 mg/ml	------
MGB 7 1st spot	0.049 mg/ml	0.049 ±	0.049 mg/ml	0.049	0.049 mg/ml	0.049	0.98 mg/ml	0.049
MGB 7 2nd spot	0.083 mg/ml	0.083 ±	0.083 mg/ml	0.083	0.083 mg/ml	0.083	0.167 mg/ml	0.083
MGB 8 **	1 mg/ml	------	1 mg/ml	1 ±	1 mg/ml	-----	1 mg/ml	0.5 ±
MGB 9 **	0.125 mg/ml	------	0.125 mg/ml	0.125±	0.031 mg/ml	0.063	0.5 mg/ml	-----
MGB 10	1 mg/ml	1	1 mg/ml	1	0.063 mg/ml	0.031	1 mg/ml	0.5

*** MIC in mg/ ml**

**** Turbid before culture incubation (the turbidity in the wells of the plates means bacterial growth, i.e., lack of antibacterial activity.)**

± Partial inhibition.

3.4.Conclusion and Future work

In this project, we have provided a simple synthetic pathway for new analogues of the natural compounds Distamycin A, Netropsin, and Proximicins. The antibacterial activity of the new compounds was weak. However, the spectral analysis of the final products revealed that these compounds were not pure. There is a need, therefore, to purify the final products by using advanced techniques like HPLC to confirm that the concentrations used in the biological activity studies were accurate. To build on the results of the current study, the following steps should be followed:

- Using advanced purification techniques such preparative HPLC to obtain pure compounds keeping in mind the possibility of salt formation for the compounds to enhance their water solubility.
- Expanding the study of antibacterial activities of the compounds against different strains.
- Screening the antiviral and antifungal activity of the compounds.
- Screening the anticancer activity of the compounds.
- If encouraging results are shown, investigating the mechanism of action of those compounds using different biophysical techniques, such as Isothermal Titration Calorimetry (ITC), and Circular

Dichroism (CD) should be carried out to investigate drug-DNA interaction.

3.5 Limitations of the study

The main obstacle for this study was the lack of necessary instruments at An-Najah National University, such as preparative HPLC, Mass Spectrometer, and NMR instrument. These techniques are essential for the purification and characterization of the chemical compounds, and with the absence of such instruments, primitive techniques were used for purification and identification during the synthesis of studied compounds, and this has slowed down our work and make it more difficult.

Other limitations of this study were the poor solubility of some compounds which may lead to false negative results in the MIC determination since visual inspection of turbidity was the sign of bacterial growth. Another limitation was that not all compounds structures were confirmed using NMR analysis. Moreover, for some compounds, the NMR spectra revealed that the obtained compounds were not pure due to the lack of the required instruments necessary for the purification of such compounds.

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Appendix

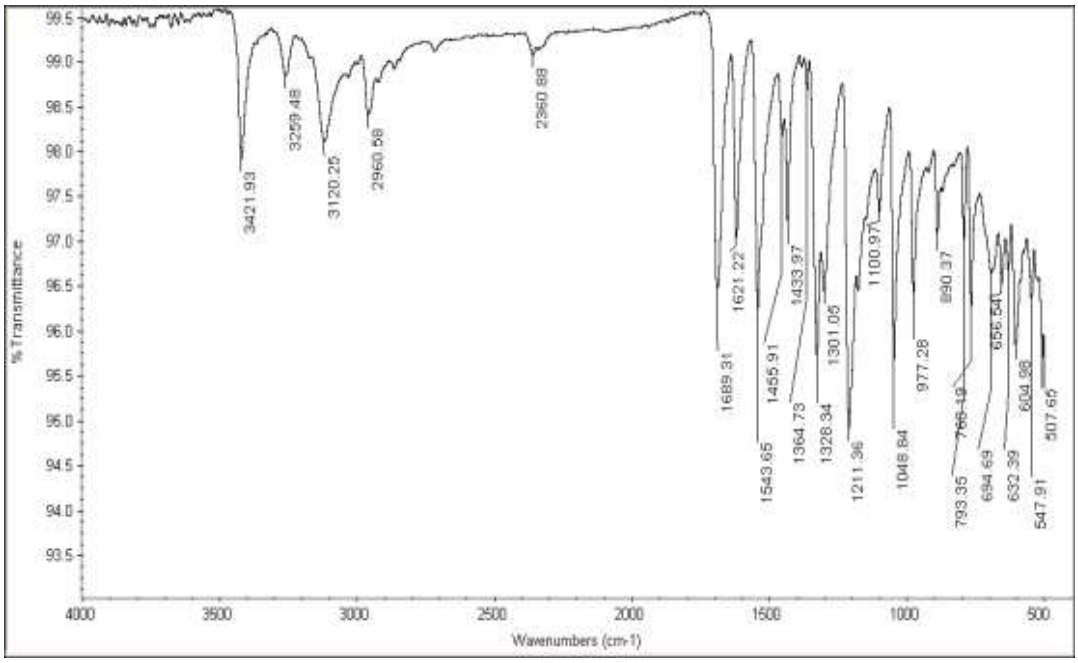

Figure 1: FT-IR of methyl-2-amino-5-isopropyl-1,3-thiazole-4-carboxylate

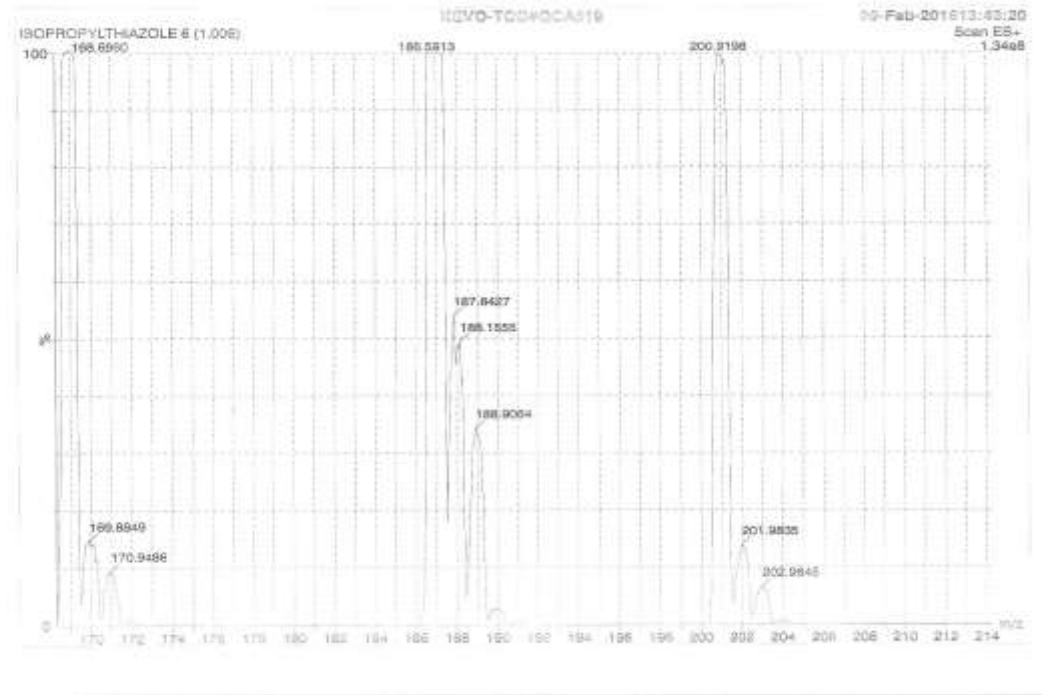

Figure 2: ESI-MS spectrum of methyl-2-amino-5-isopropyl-1,3-thiazole-4-carboxylate.

^{1}H NMR (400 MHz, METHANOL-d_4) δ ppm 1.23 - 1.30 (m, 6 H) 1.26 (s, 1 H) 3.78 - 3.89 (m, 3 H) 3.84 (s, 1 H) 3.95 - 4.06 (m, 1 H)

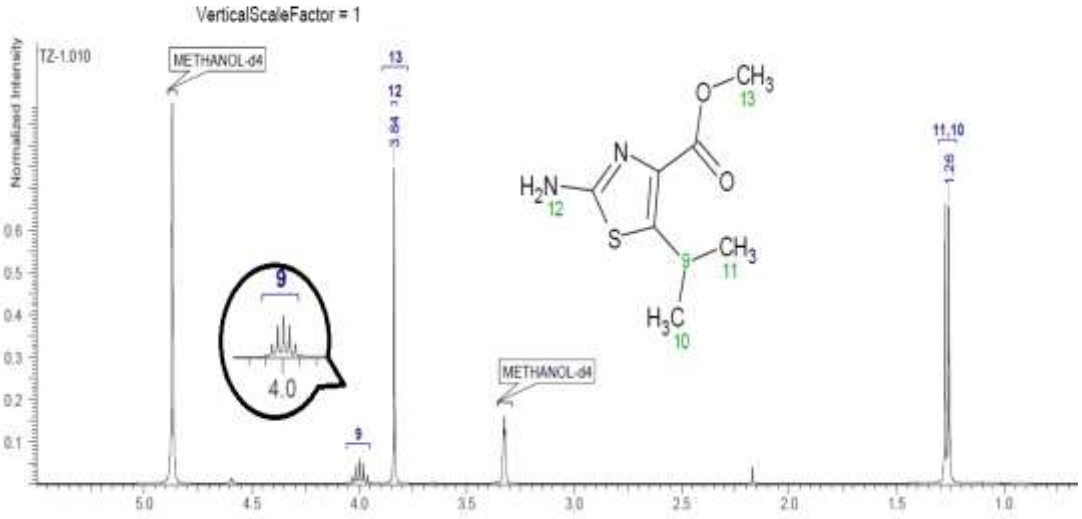

Figure 3:^{1}HNMR of methyl-2-amino-5-isopropyl-1,3- thiazole-4-carboxylate.

^{13}C NMR (101 MHz, METHANOL-d_4) δ ppm 23.75 (s, 1 C) 27.40 (s, 1 C) 46.96 (s, 1 C) 47.95 (s, 1 C) 50.67 (s, 1 C) 133.95 (s, 1 C) 147.54 (s, 1 C) 162.67 (s, 1 C) 165.47 (s, 1 C)

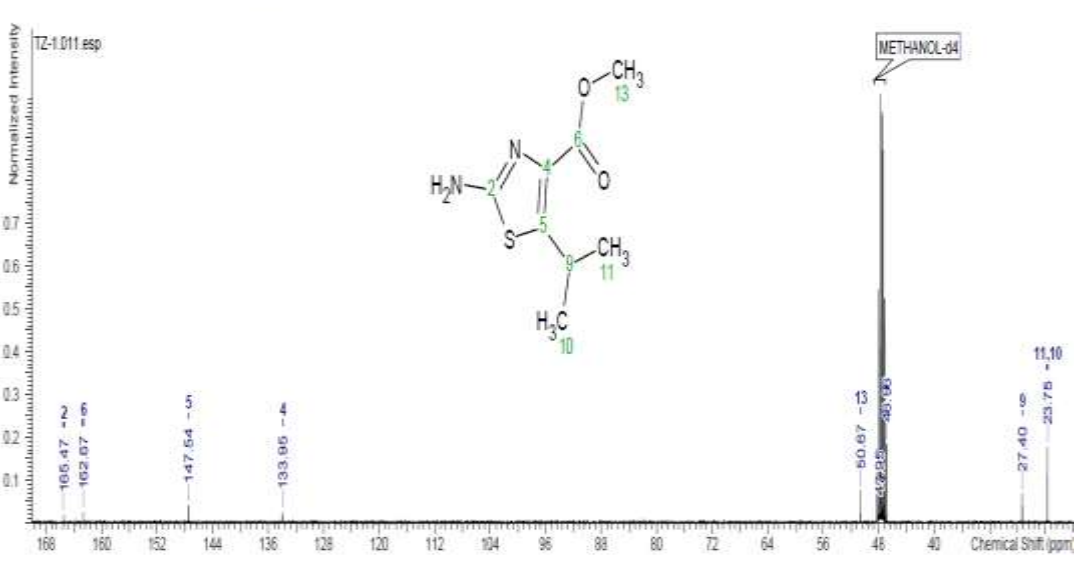

Figure 4: ^{13}CNMR of methyl-2-amino-5-isopropyl-1,3- thiazole-4-carboxylate.

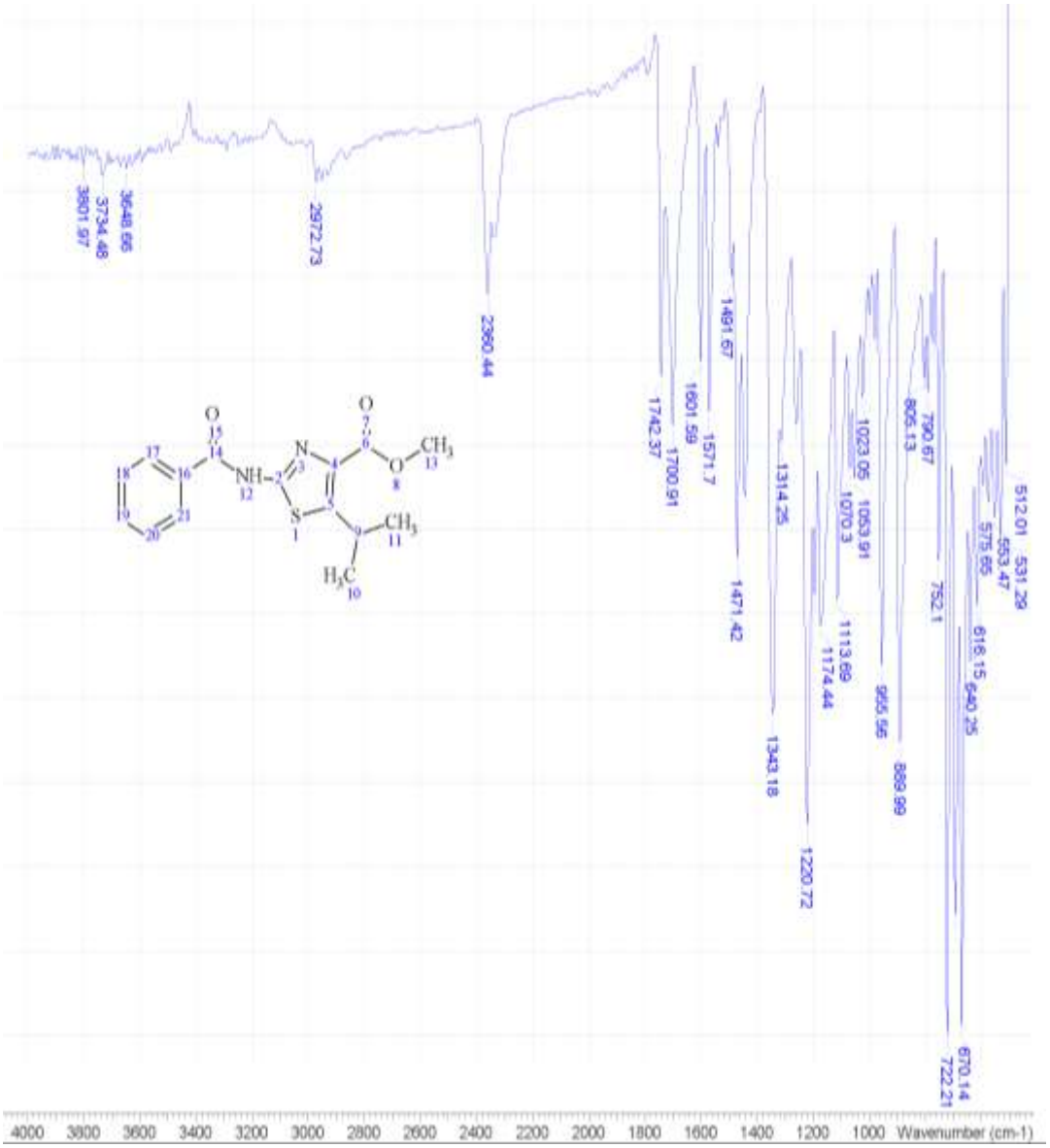

Figure 5: : FT-IR of methyl-2-benzamido-5-isopropylthiazole-4-carboxylate.

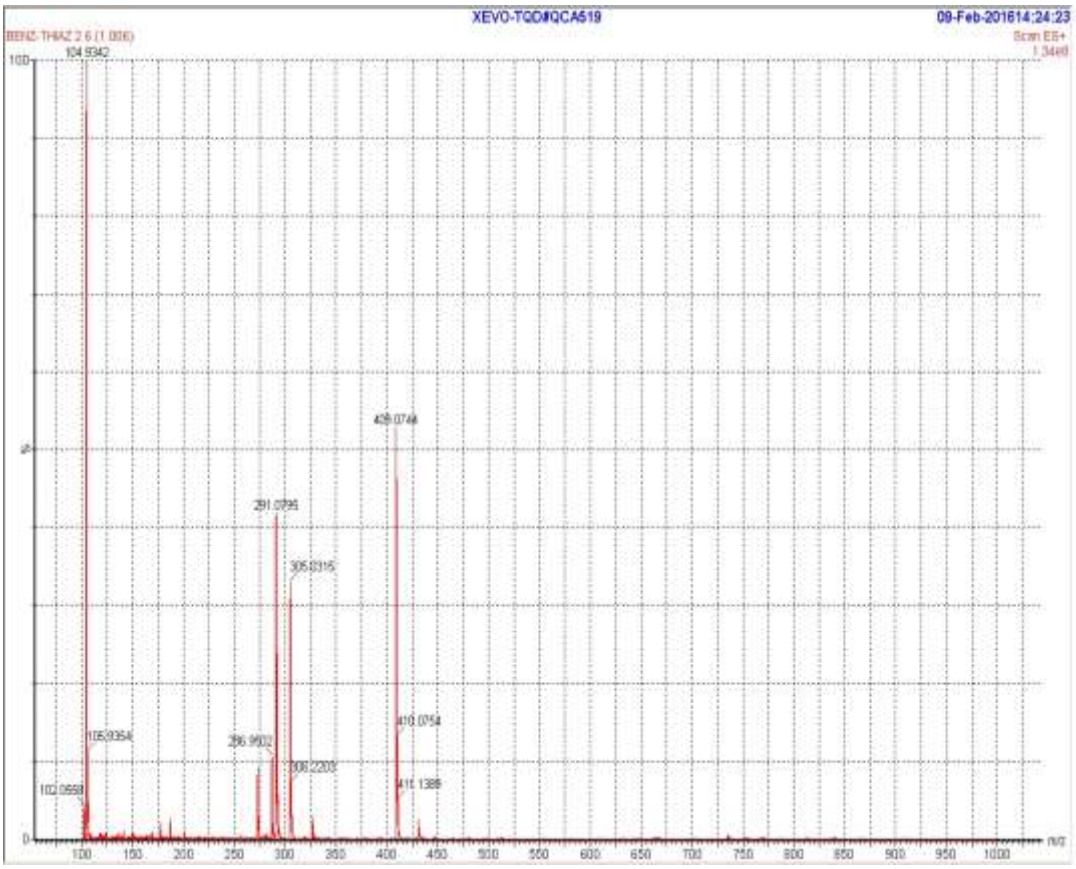

Figure 6: ESI-MS spectrum of methyl-2-benzamido-5 -isopropylthiazole-4-carboxylate.

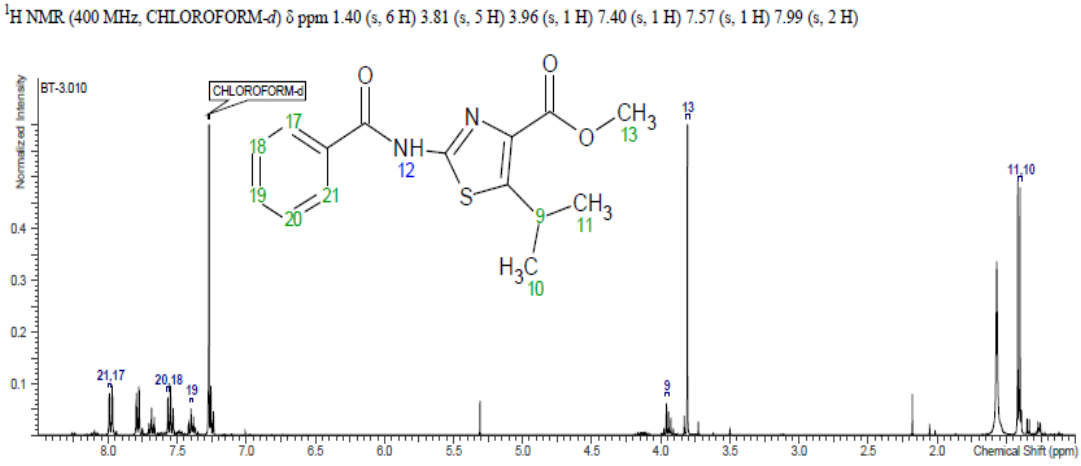

Figure 7: ^{1}HNMR of methyl-2-benzamido-5 -isopropylthiazole-4-carboxylate.

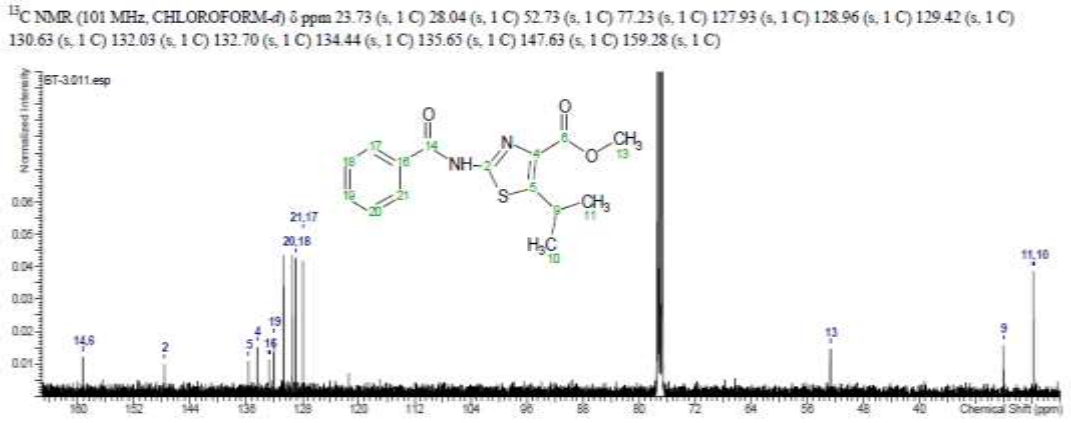

Figure 8: ^{13}CNMR of methyl-2-benzamido-5 -isopropylthiazole-4-carboxylate.

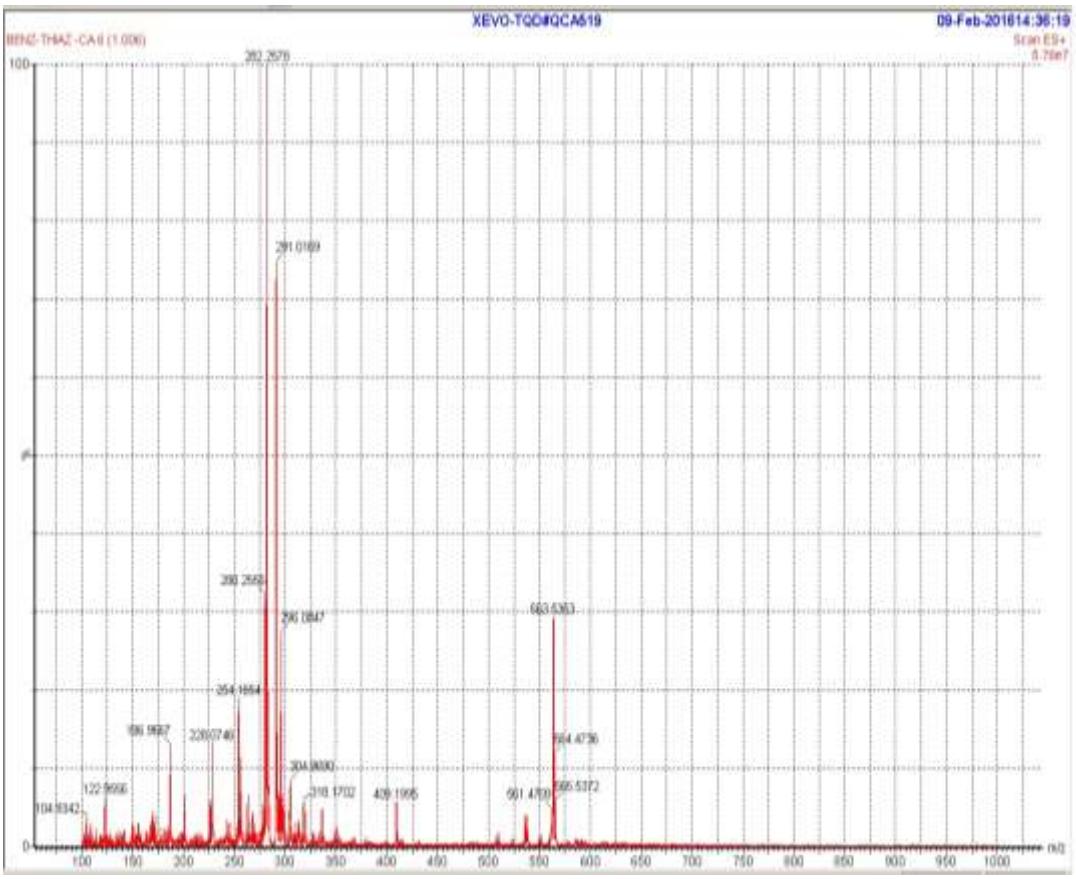

Figure 9: ESI-MS spectrum of methyl-2-benzamido-5-isopropylthiazole-4-carboxylic acid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.31 (d, J=6.85 Hz, 6 H) 2.09 (s, 1 H) 3.13 - 3.24 (m, 3 H) 3.82 (br s, 1 H) 4.06 (dt, J=13.69, 6.85 Hz, 2 H) 7.55 (t, J=7.62 Hz, 2 H) 7.62 - 7.66 (m, 1 H) 8.10 (d, J=7.58 Hz, 2 H)

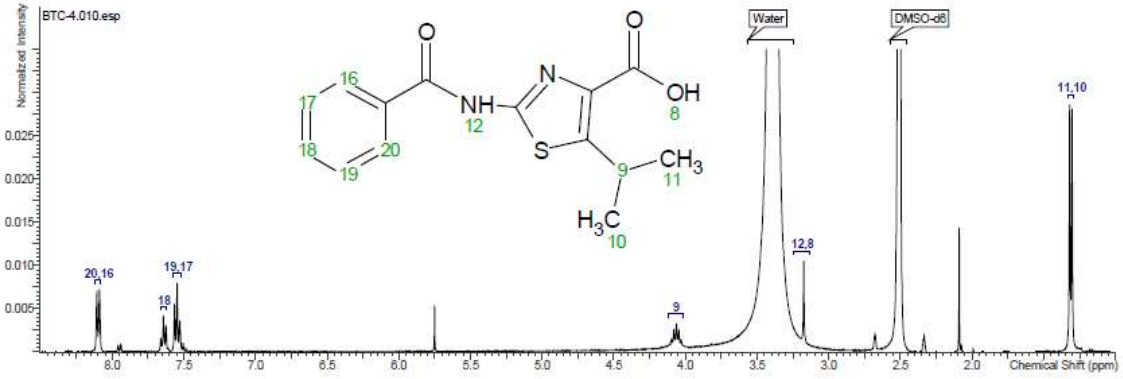

Figure 10: ^{1}HNMR spectrum of methyl-2-benzamido-5-isopropylthiazole-4-carboxylic acid.

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 25.21 (s, 1 C) 40.63 (s, 1 C) 128.62 (s, 1 C) 129.08 (s, 1 C)

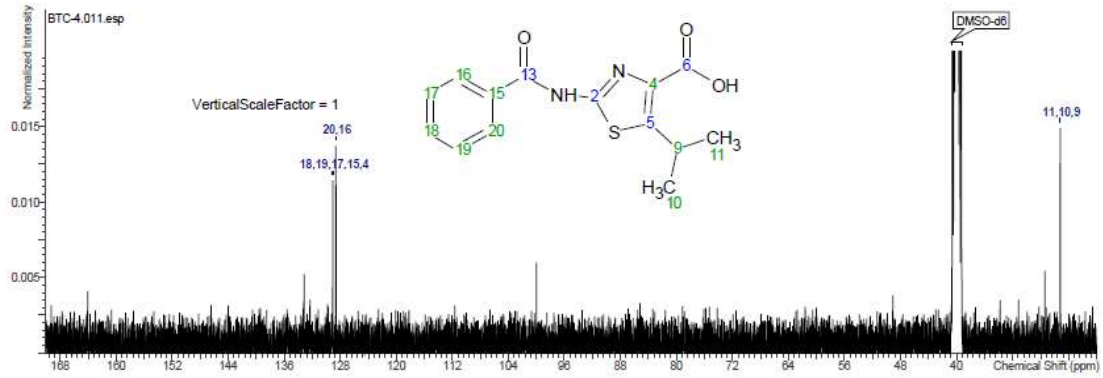

Figure 11: ^{13}CNMR spectrum of methyl-2-benzamido-5-isopropylthiazole-4-carboxylic acid.

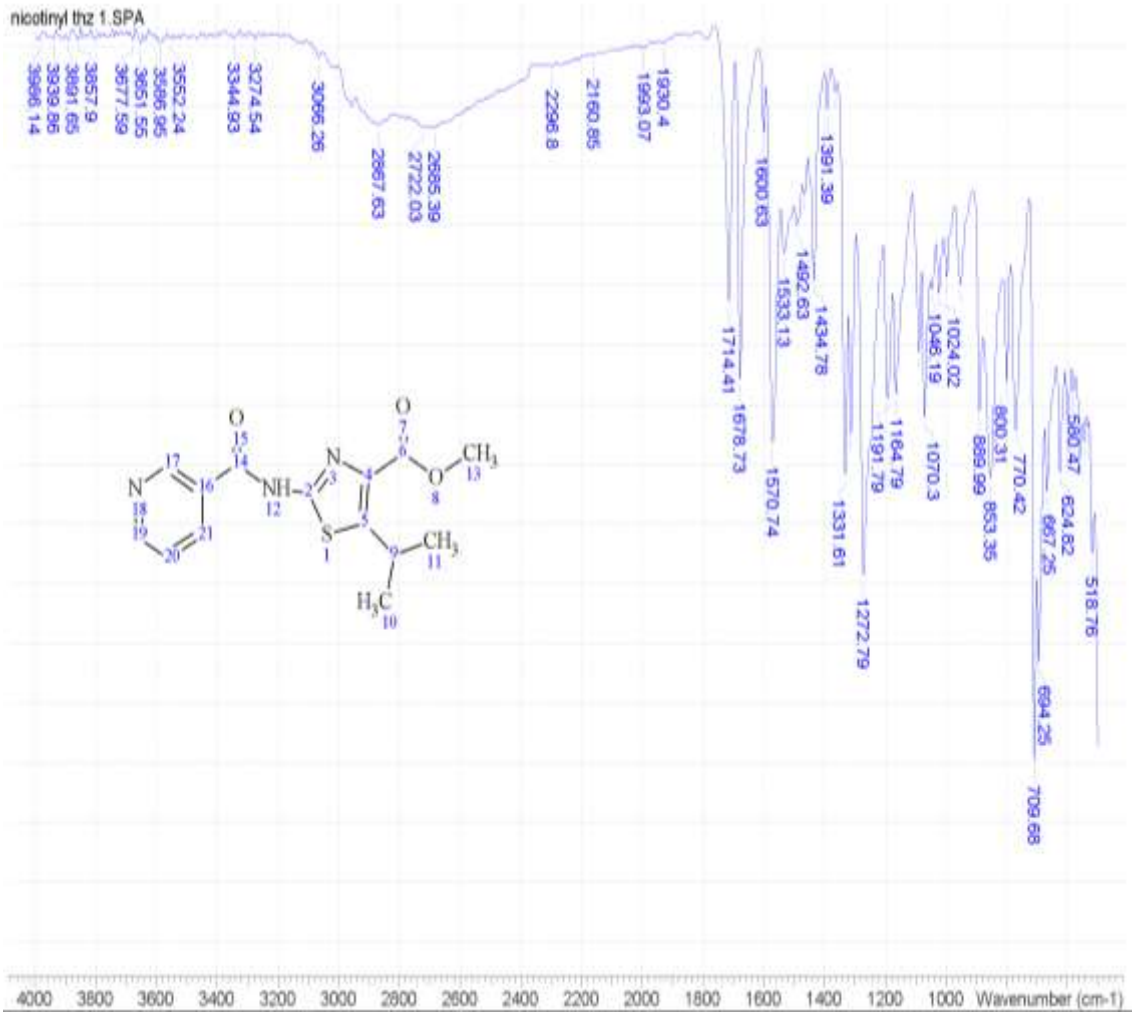

Figure 12: FT-IR spectrum of methyl 5-isopropyl-2-(nicotinamido)thiazole-4-carboxylate.

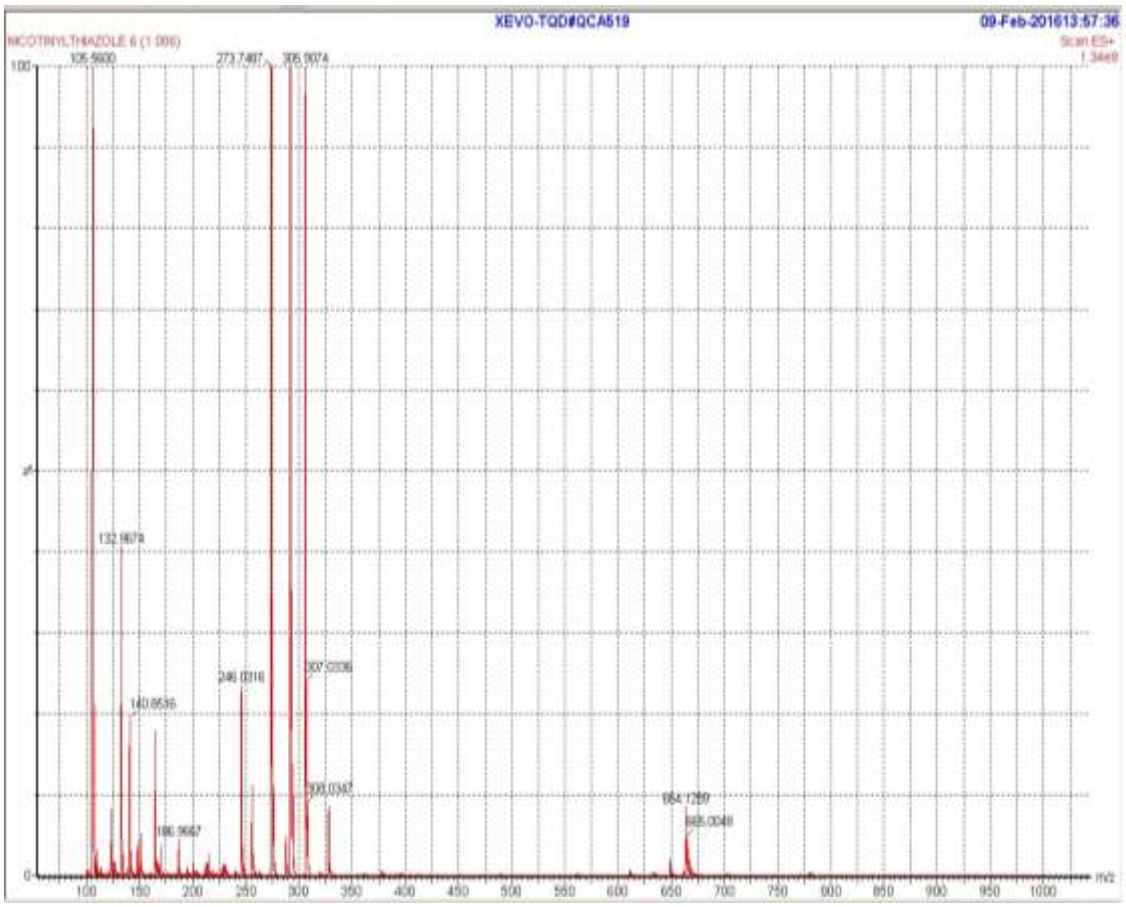

Figure 13: ESI-MS spectrum of methyl 5-isopropyl-2-(nicotinamido)thiazole-4-carboxylate.

^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.33 (d, *J*=6.85 Hz, 6 H) 3.74 (s, 3 H) 4.08 (spt, *J*=6.85 Hz, 1 H) 7.19 (s, 2 H) 7.40 (ddd, *J*=8.01, 4.83, 0.86 Hz, 1 H) 8.15 (dt, *J*=8.13, 1.93 Hz, 1 H) 8.77 (dd, *J*=4.77, 1.59 Hz, 1 H) 9.10 (s, 1 H) 10.38 (br s, 1 H)

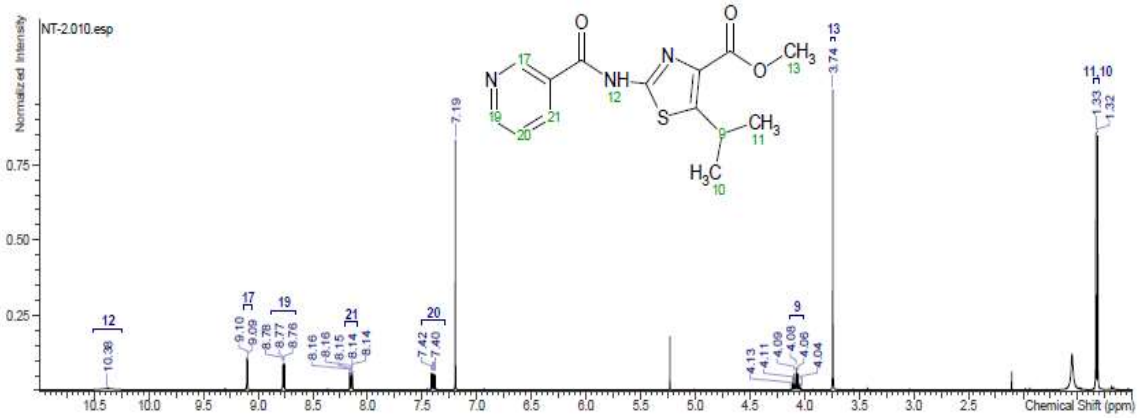

Figure 14: ^{1}HNMR spectrum of methyl 5-isopropyl-2-(nicotinamido)thiazole-4-carboxylate.

^{13}C NMR (101 MHz, CHLOROFORM-*d*) δ ppm 24.81 (s, 1 C) 27.74 (s, 1 C) 52.01 (s, 1 C) 77.23 (s, 1 C) 123.74 (s, 1 C) 127.77 (s, 1 C) 133.62 (s, 1 C) 135.27 (s, 1 C) 148.60 (s, 1 C) 153.66 (s, 1 C) 153.85 (s, 1 C) 154.04 (s, 1 C) 162.37 (s, 1 C) 163.35 (s, 1 C)

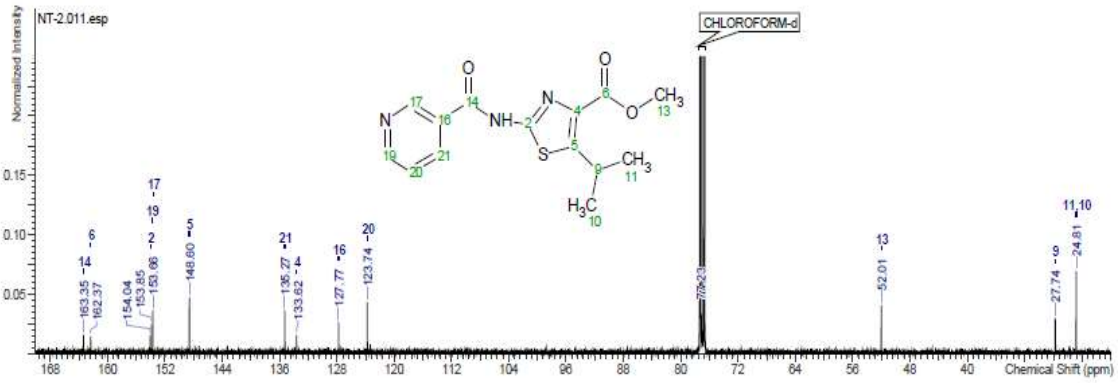

Figure 15: ^{13}CNMR spectrum of methyl 5-isopropyl-2-(nicotinamido)thiazole-4-carboxylate.

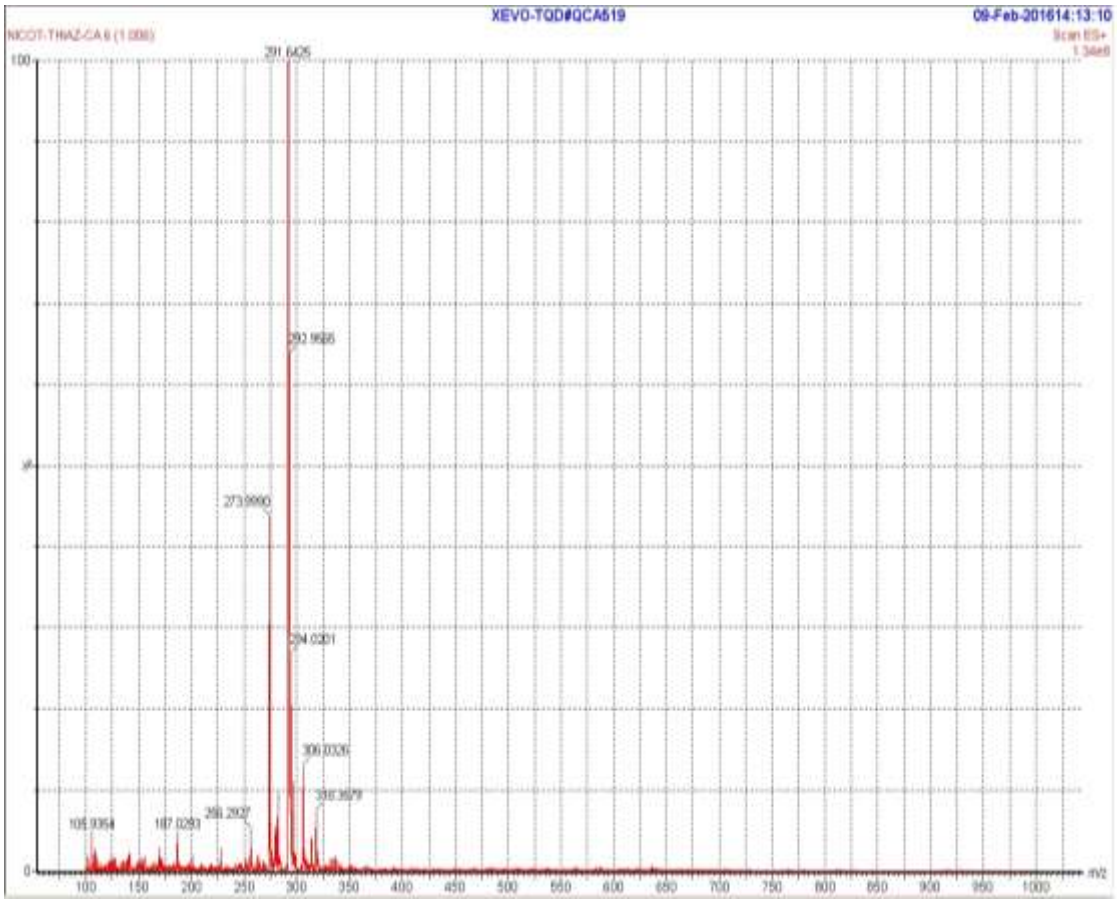

Figure 16: ESI-MS spectrum of methyl 5-isopropyl-2-(nicotinamido)thiazole-4-carboxylic acid.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.09 - 1.27 (m, 1 H) 1.31 (d, *J*=6.85 Hz, 6 H) 2.34 - 2.46 (m, 1 H) 2.53 - 2.66 (m, 1 H) 2.89 (br s, 1 H) 3.04 - 3.20 (m, 4 H) 3.95 - 4.16 (m, 2 H) 4.17 - 4.39 (m, 1 H) 7.58 (dd, *J*=7.95, 4.77 Hz, 1 H) 8.42 (br d, *J*=8.07 Hz, 1 H) 8.79 (dd, *J*=4.89, 1.47 Hz, 1 H) 9.20 (d, *J*=1.71 Hz, 1 H) 13.53 (s, 1 H)

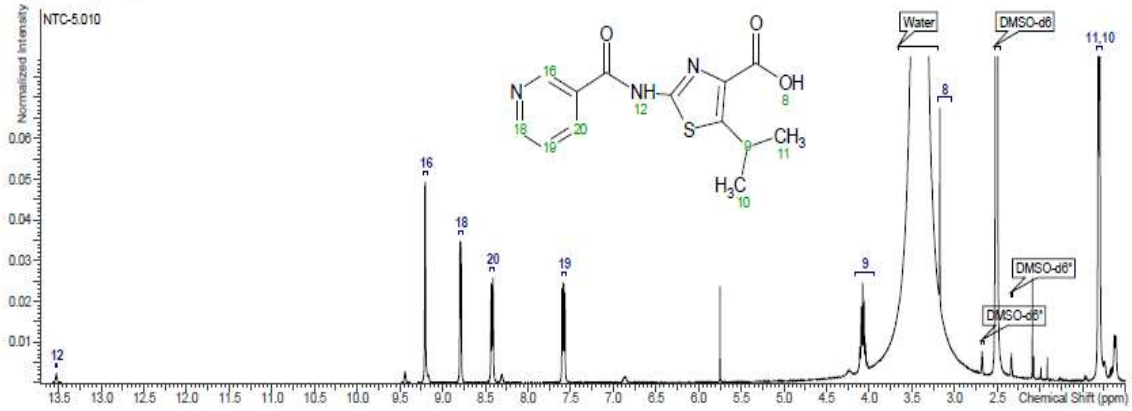

Figure 17: ^{1}HNMR spectrum of methyl 5-isopropyl-2-(nicotinamido)thiazole-4-carboxylic acid.

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm -12.08 (s, 1 C) 18.16 (s, 1 C) 19.15 (s, 1 C) 25.19 (s, 1 C) 27.37 (s, 1 C) 40.20 (s, 1 C) 40.41 (s, 1 C) 40.63 (s, 1 C) 40.77 (s, 1 C) 65.15 (s, 1 C) 67.54 (s, 1 C) 74.07 (s, 1 C) 74.25 (s, 1 C) 79.44 (s, 1 C) 83.04 (s, 1 C) 85.19 (s, 1 C) 93.23 (s, 1 C) 98.31 (s, 1 C) 111.32 (s, 1 C) 116.14 (s, 1 C) 123.32 (s, 1 C) 124.10 (s, 1 C) 128.48 (s, 1 C) 131.10 (s, 1 C) 132.69 (s, 1 C) 135.49 (s, 1 C) 136.33 (s, 1 C) 149.38 (s, 1 C) 149.63 (s, 1 C) 150.25 (s, 1 C) 153.41 (s, 1 C) 153.47 (s, 1 C) 154.53 (s, 1 C) 155.59 (s, 1 C) 158.44 (s, 1 C) 158.82 (s, 1 C) 163.94 (s, 1 C) 164.79 (s, 1 C) 166.32 (s, 1 C) 169.66 (s, 1 C) 176.18 (s, 1 C) 185.37 (s, 1 C) 205.45 (s, 1 C) 209.59 (s, 1 C)

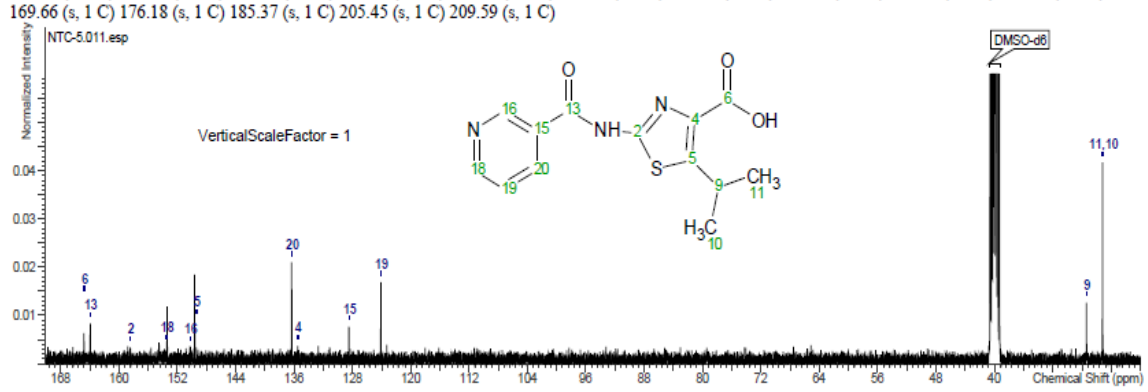

Figure 18: ^{13}CNMR spectrum of methyl 5-isopropyl-2-(nicotinamido)thiazole-4-carboxylic acid.

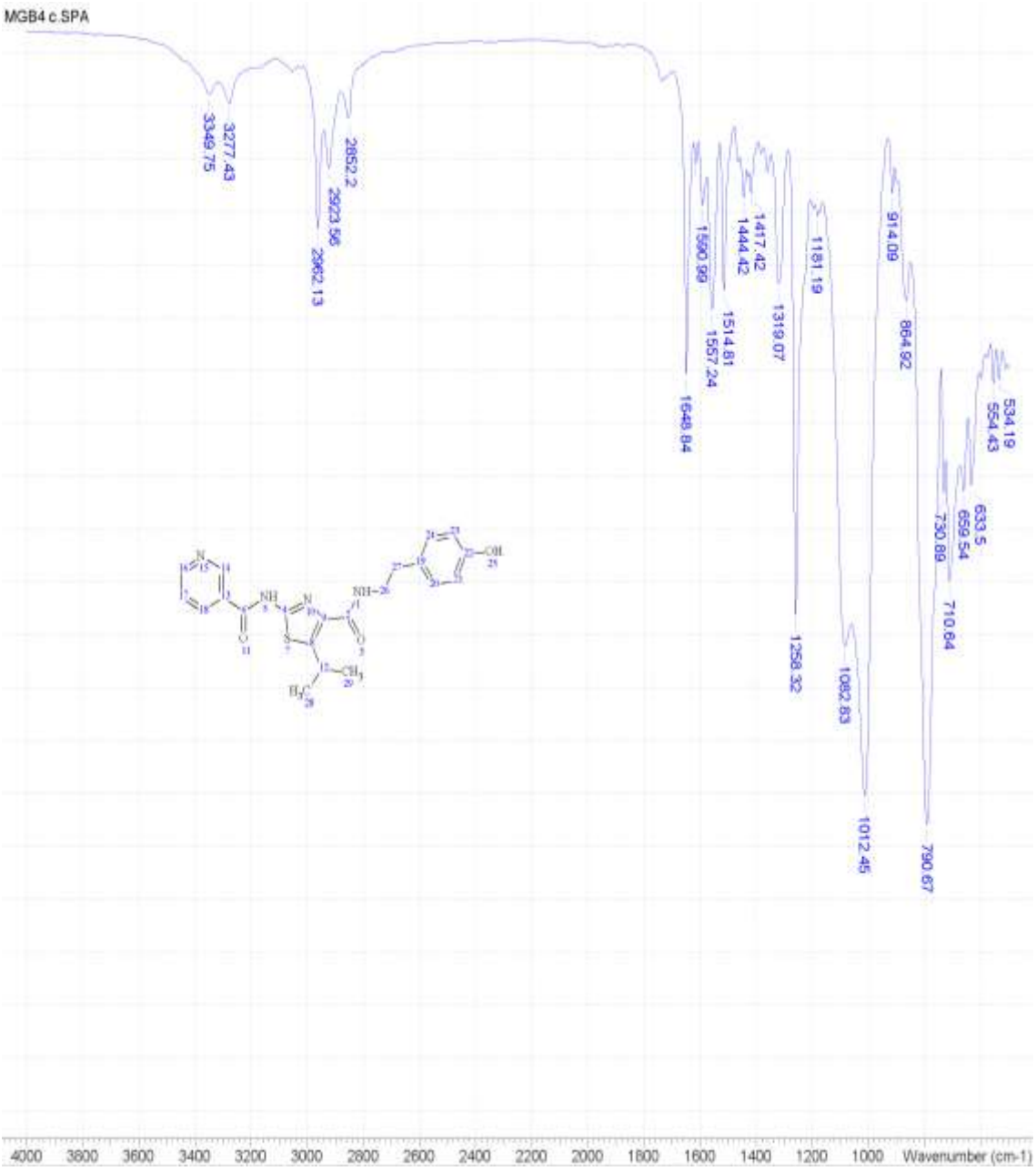

Figure 19: FT-IR spectrum of N-(4-hydroxyphenethyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide:MGB 4.

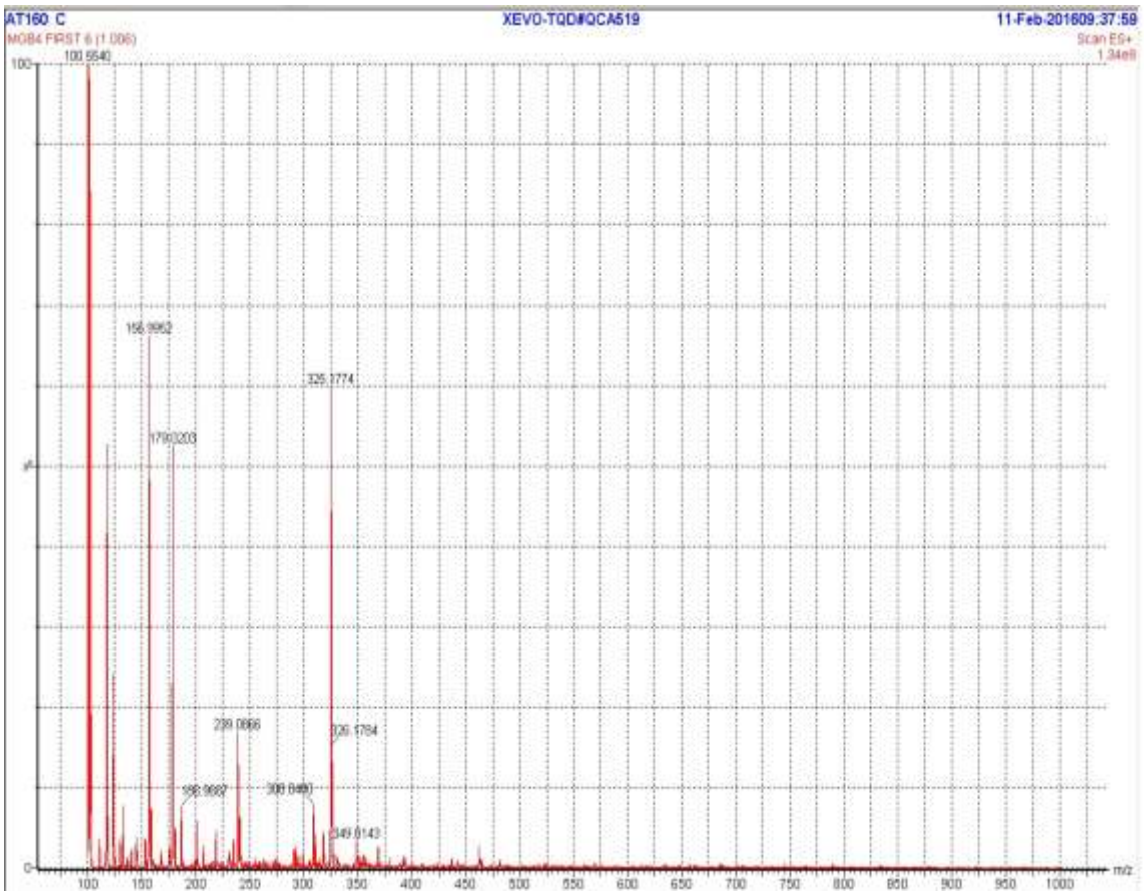

Figure 20: ESI-MS spectrum of N-(4-hydroxyphenethyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide:MGB 4.

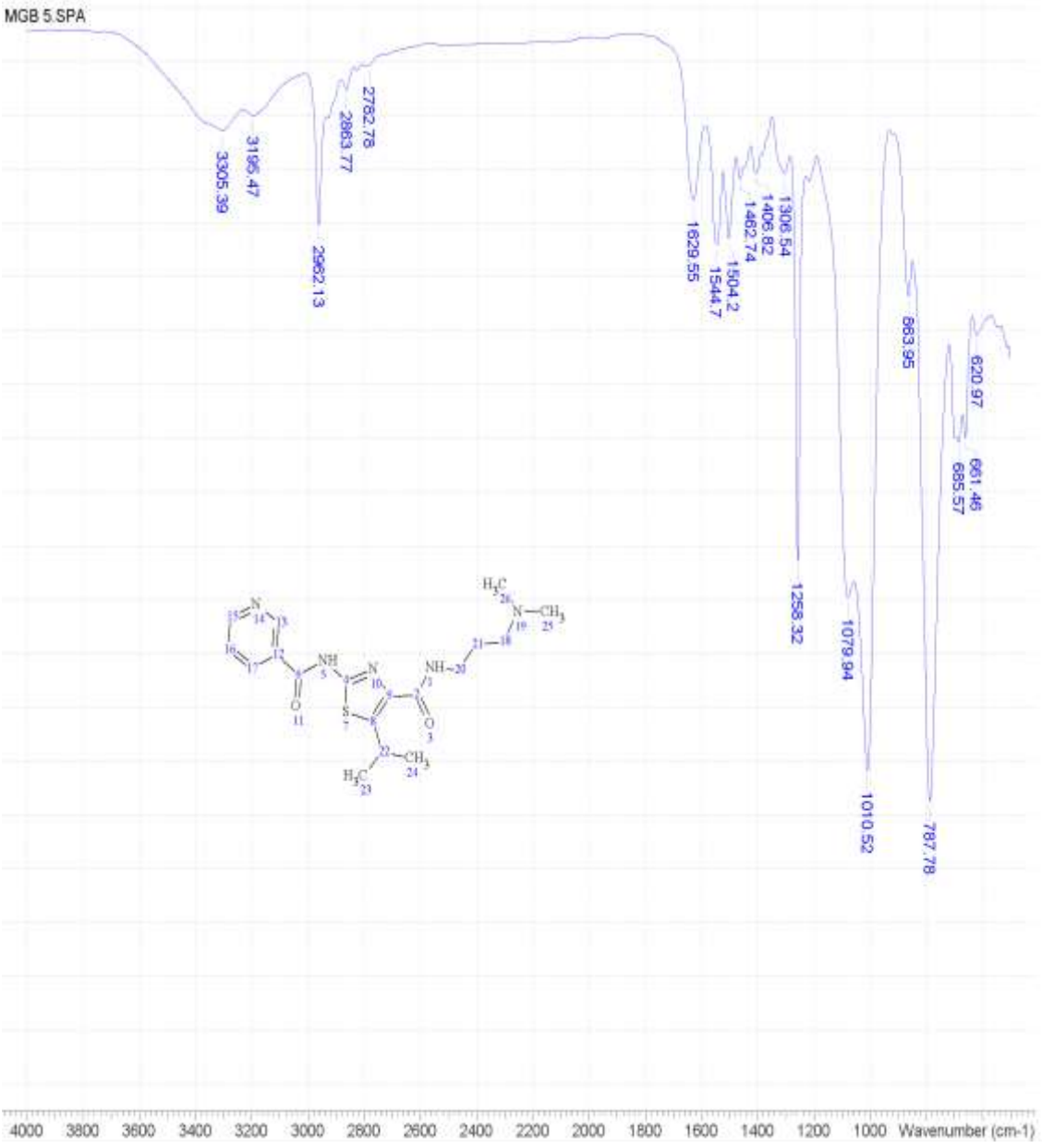

Figure 21: FT-IR spectrum of N-(3-(dimethylamino)propyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide: MGB 5.

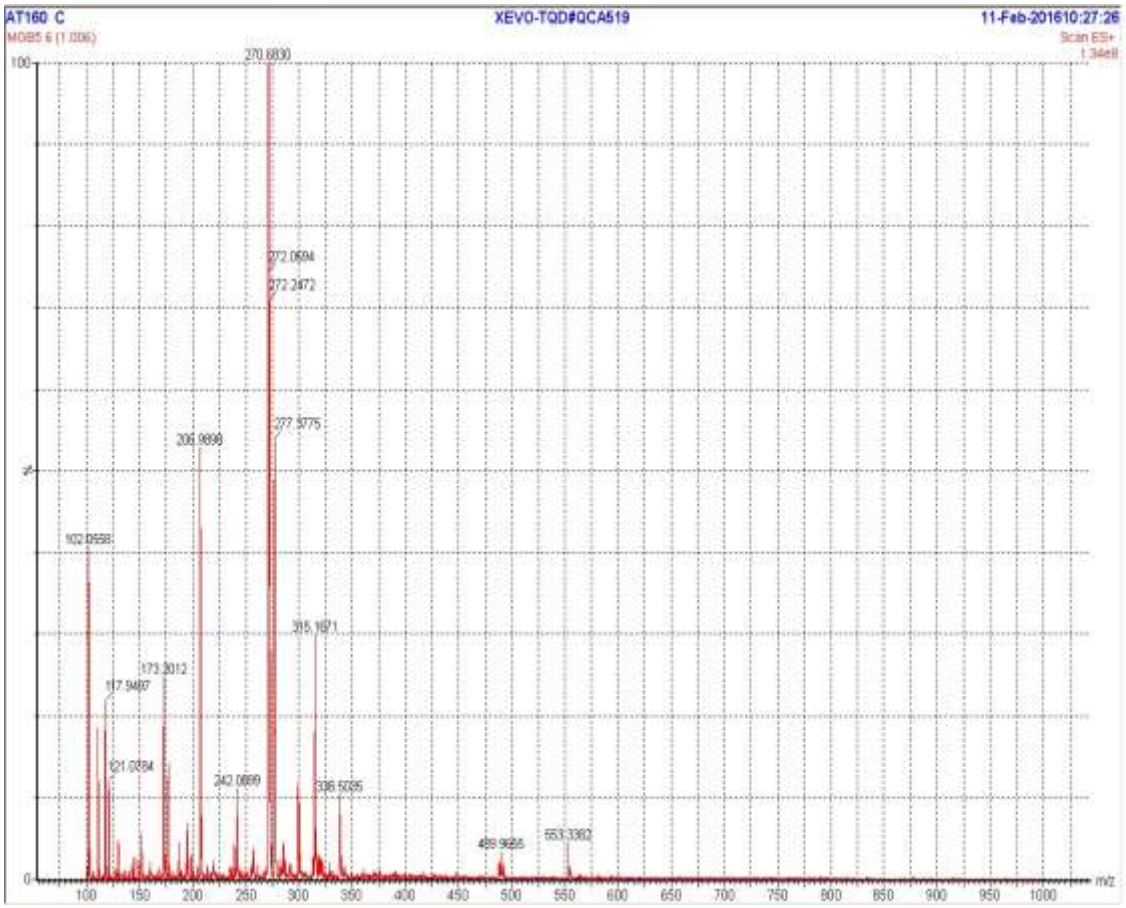

Figure 22: ESI-MS spectrum of N-(3-(dimethylamino)propyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide: MGB 5.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.10 - 1.29 (m, 6 H) 1.78 - 2.01 (m, 2 H) 2.24 - 2.45 (m, 1 H) 2.98 - 3.20 (m, 4 H) 3.21 - 3.39 (m, 6 H) 3.58 - 3.74 (m, 3 H) 4.03 - 4.20 (m, 1 H) 8.03 (br t, *J*=5.80 Hz, 1 H) 8.42 (s, 1 H) 8.57 (s, 1 H) 8.69 (s, 1 H) 10.07 (br s, 1 H)

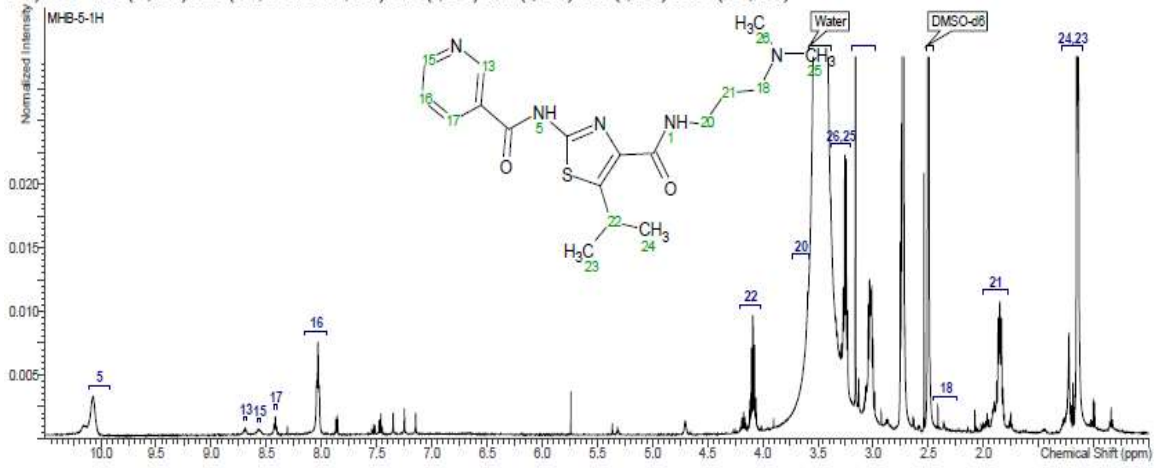

Figure 23: ^{1}HNMR spectrum of N-(3-(dimethylamino)propyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide: MGB 5.

^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 22.55 (s, 1 C) 26.55 (s, 1 C) 29.15 (s, 1 C) 36.07 (s, 1 C) 40.00 (s, 1 C) 40.17 (s, 1 C) 40.34 (s, 1 C) 40.51 (s, 1 C) 43.45 (s, 1 C) 55.01 (s, 1 C) 124.37 (s, 1 C) 127.68 (s, 1 C) 135.74 (s, 1 C) 137.11 (s, 1 C) 141.20 (s, 1 C) 141.68 (s, 1 C) 154.73 (s, 1 C) 162.35 (s, 1 C) 163.01 (s, 1 C) 164.56 (s, 1 C)

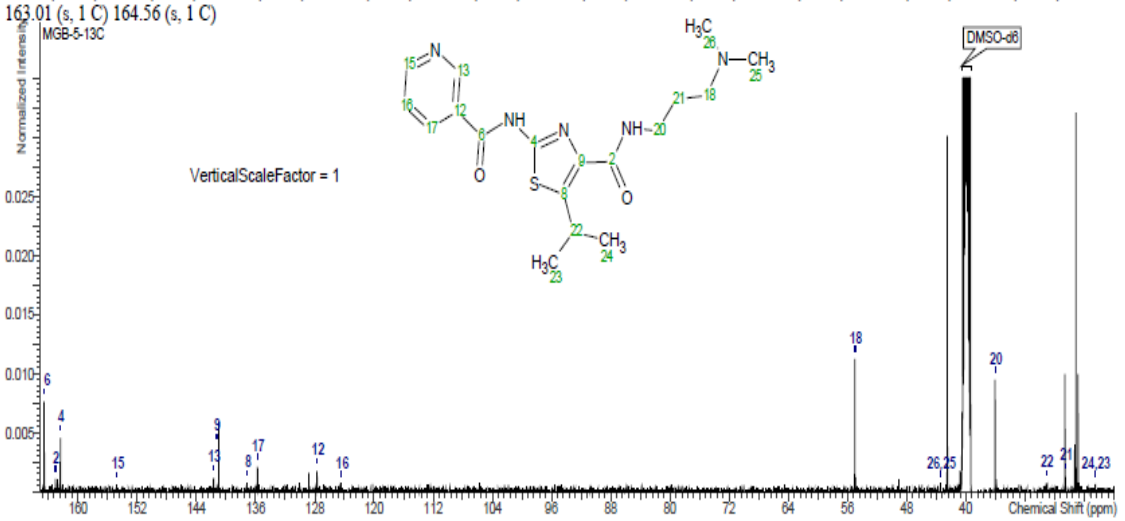

Figure 24: ^{13}CNMR spectrum of N-(3-(dimethylamino)propyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide: MGB 5.

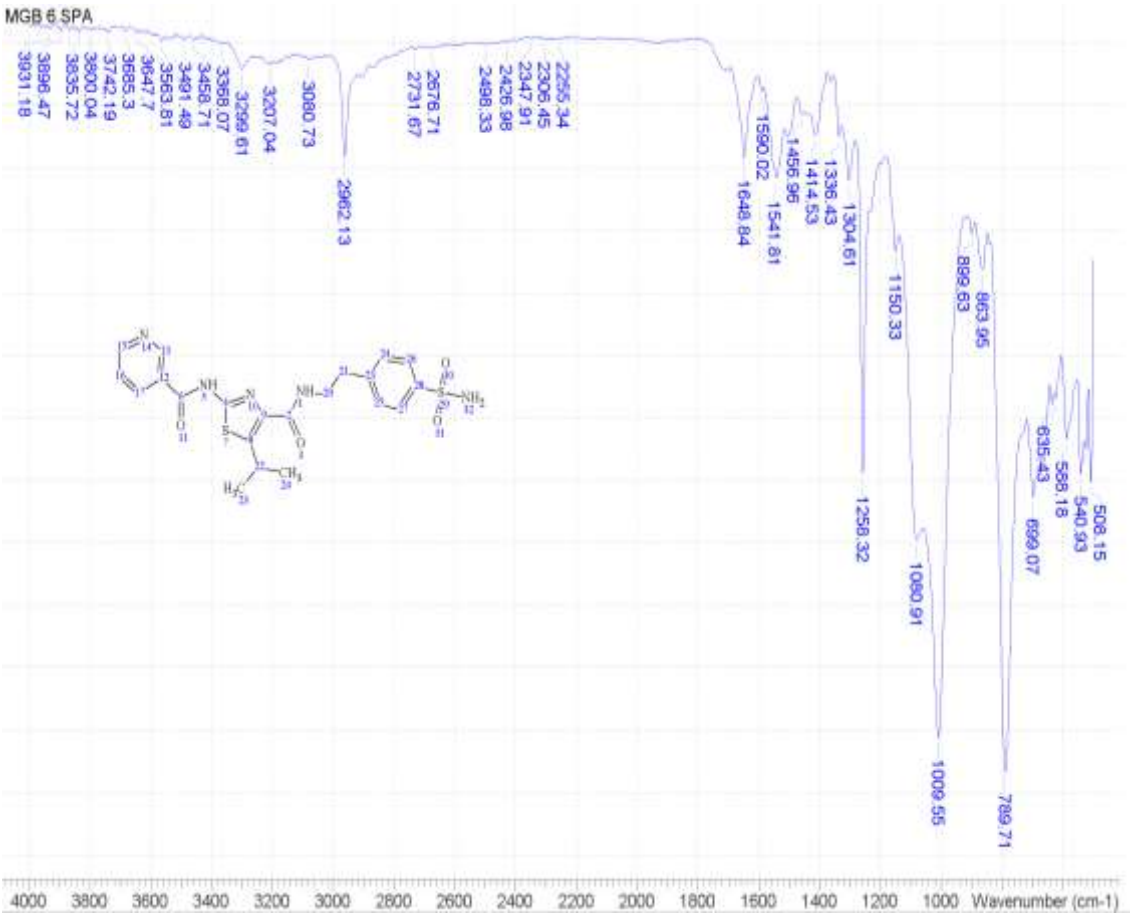

Figure 25: FT-IR spectrum of 5-isopropyl-2-(nicotinamido)-N-(4-sulfamoylphenethyl)thiazole-4-carboxamide:MGB 6.

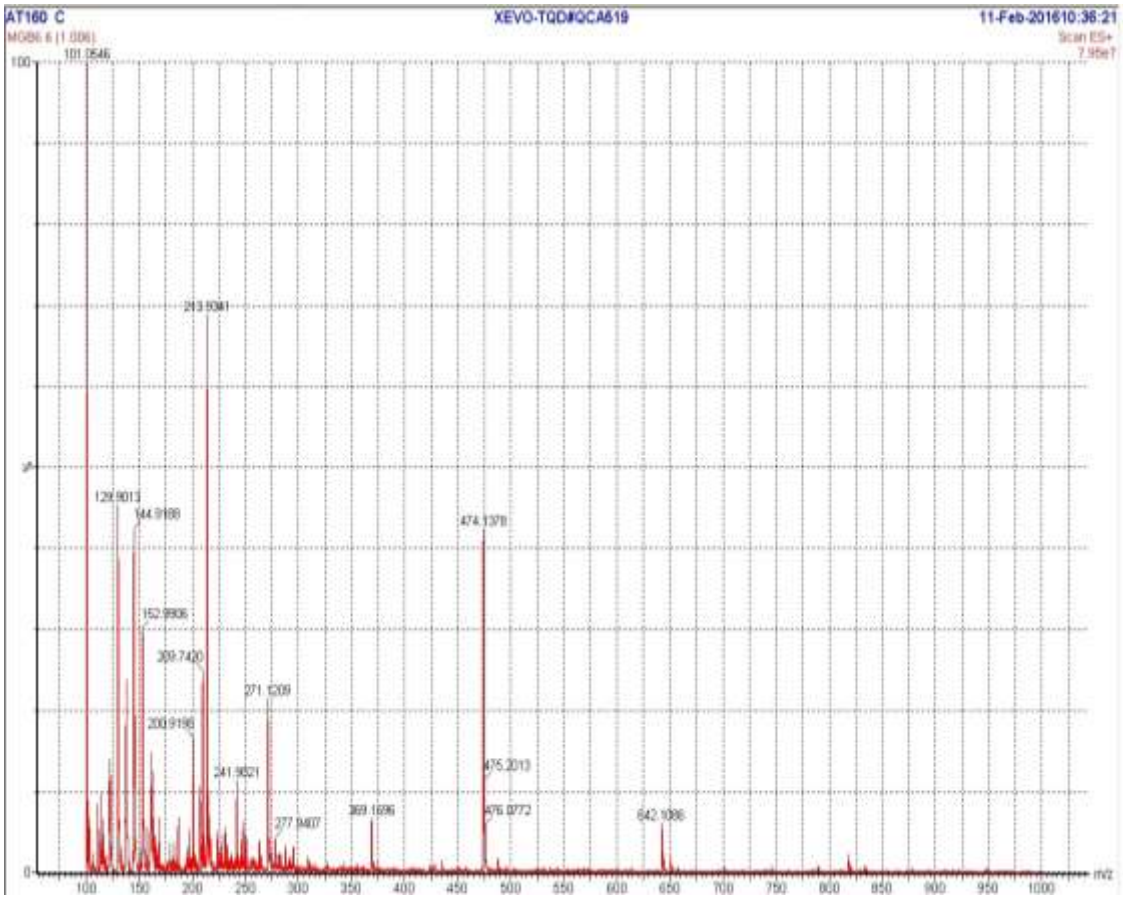

Figure 26: ESI-MS spectrum of 5-isopropyl-2-(nicotinamido)-N-(4-sulfamoylphenethyl)thiazole-4-carboxamide:MGB 6.

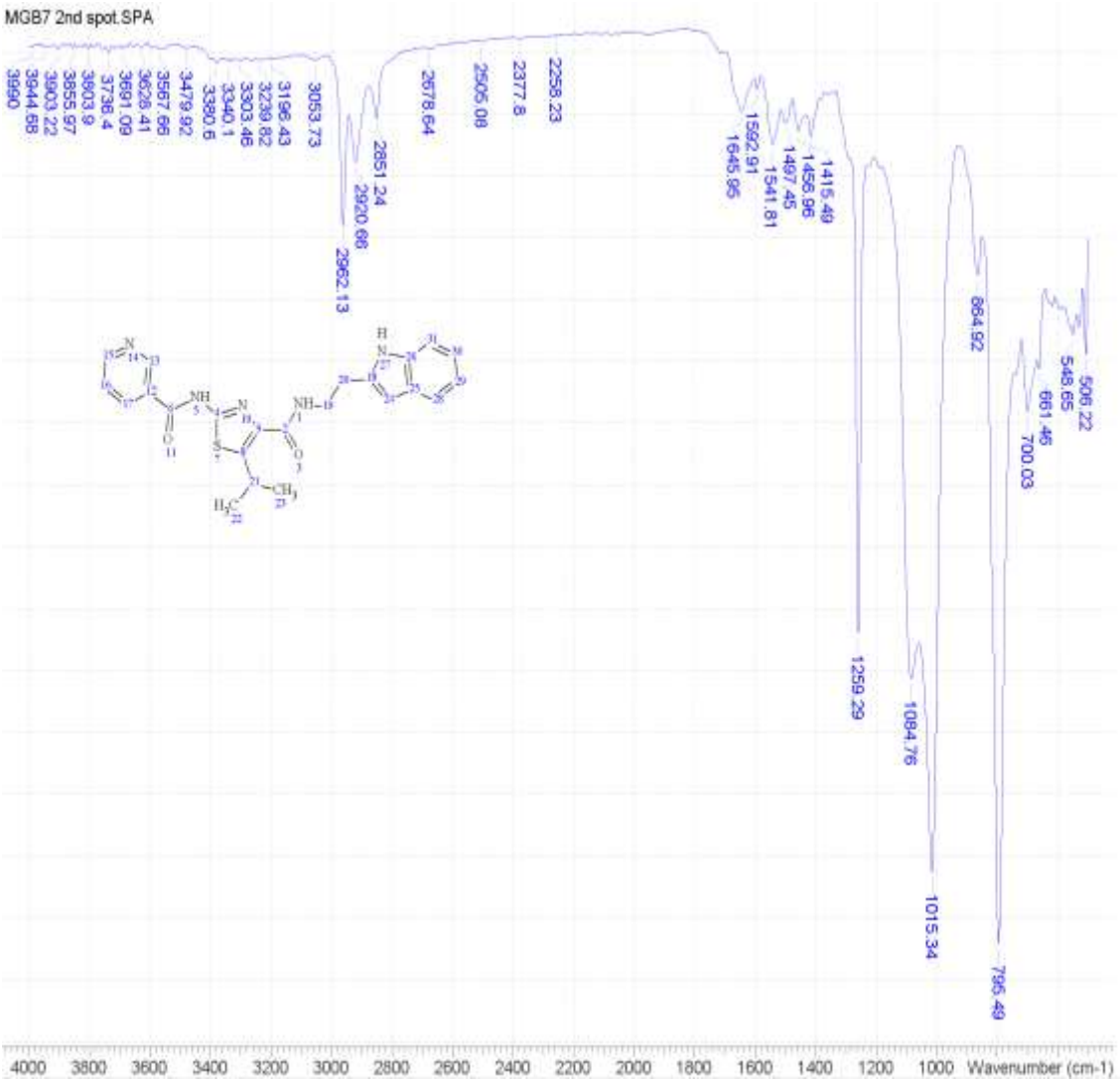

Figure 27: FT-IR spectrum of N-(2-(1H-indol-2-yl)ethyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide: MGB 7.

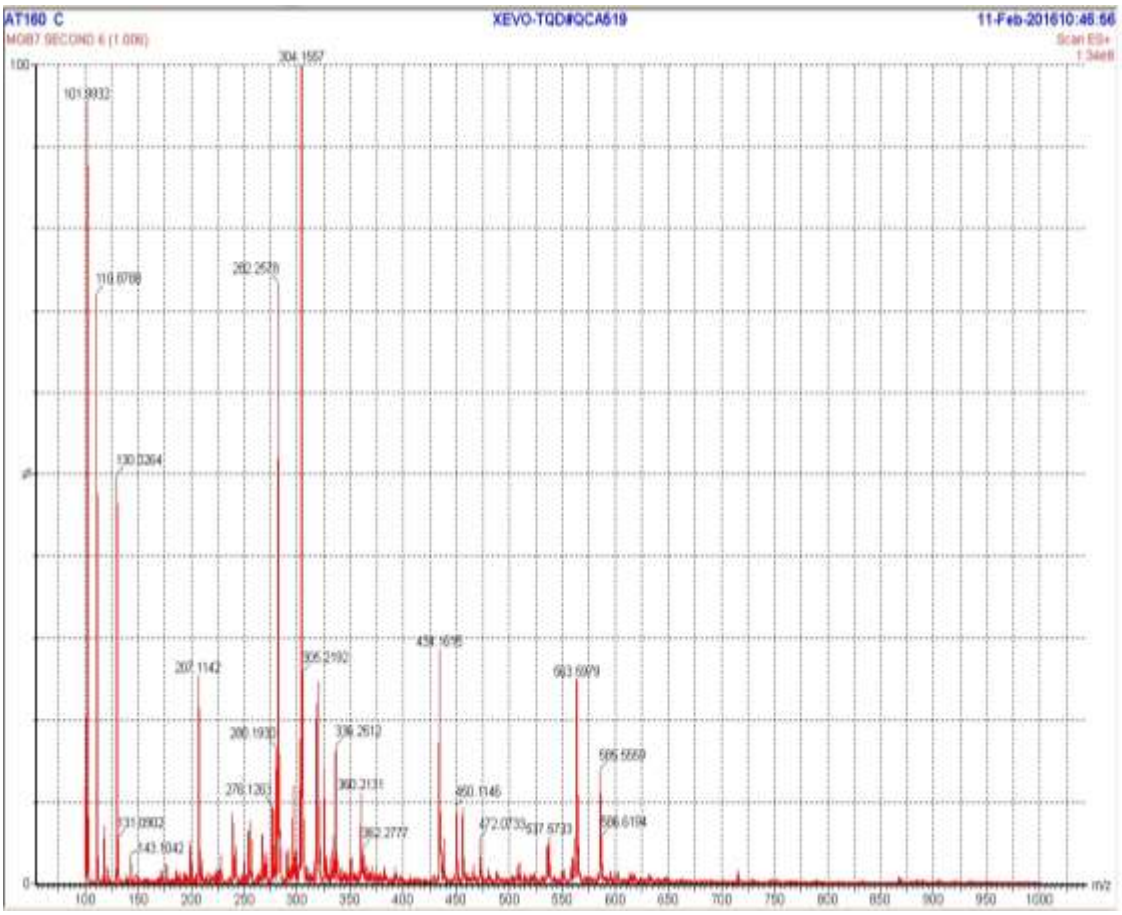

Figure 28: ESI-MS spectrum of N-(2-(1H-indol-2-yl)ethyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide: MGB 7.

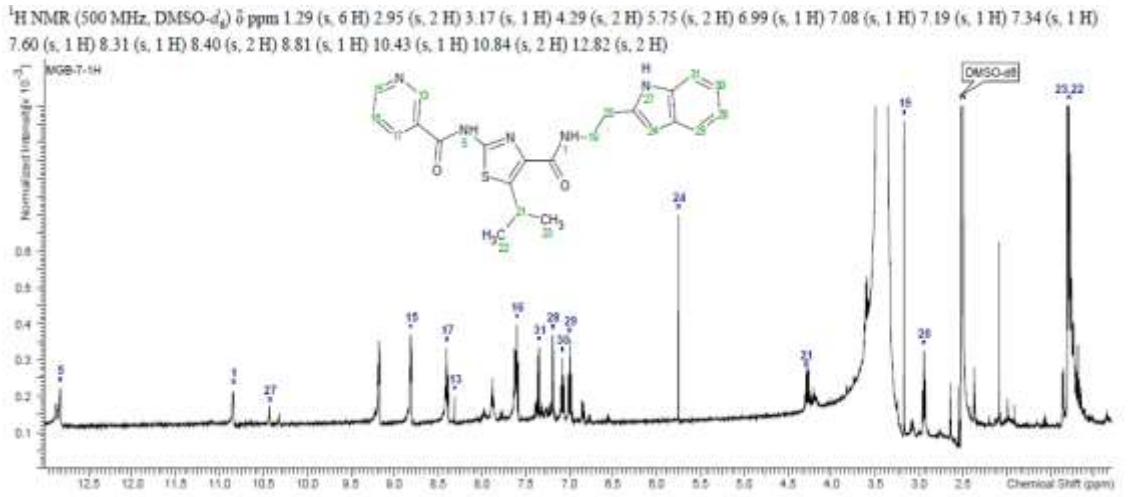

Figure 29: ^{1}HNMR spectrum of N-(2-(1H-indol-2-yl)ethyl)-5-isopropyl-2-(nicotinamido)thiazole-4-carboxamide: MGB 7.

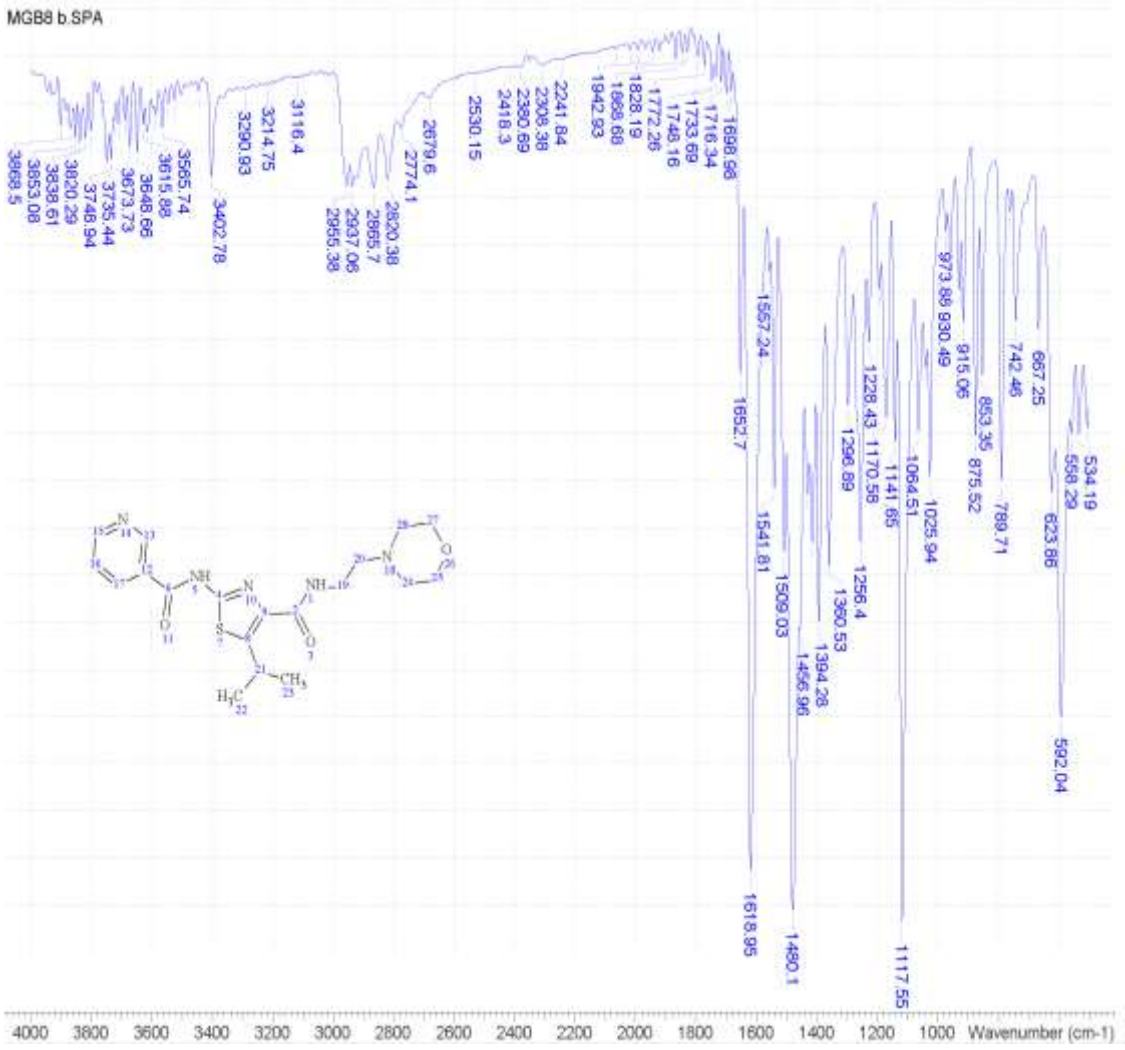

Figure 30: FT-IR spectrum of 5-isopropyl-N-(2-morpholinoethyl)-2-(nicotinamido)thiazole-4-carboxamide: MGB 8.

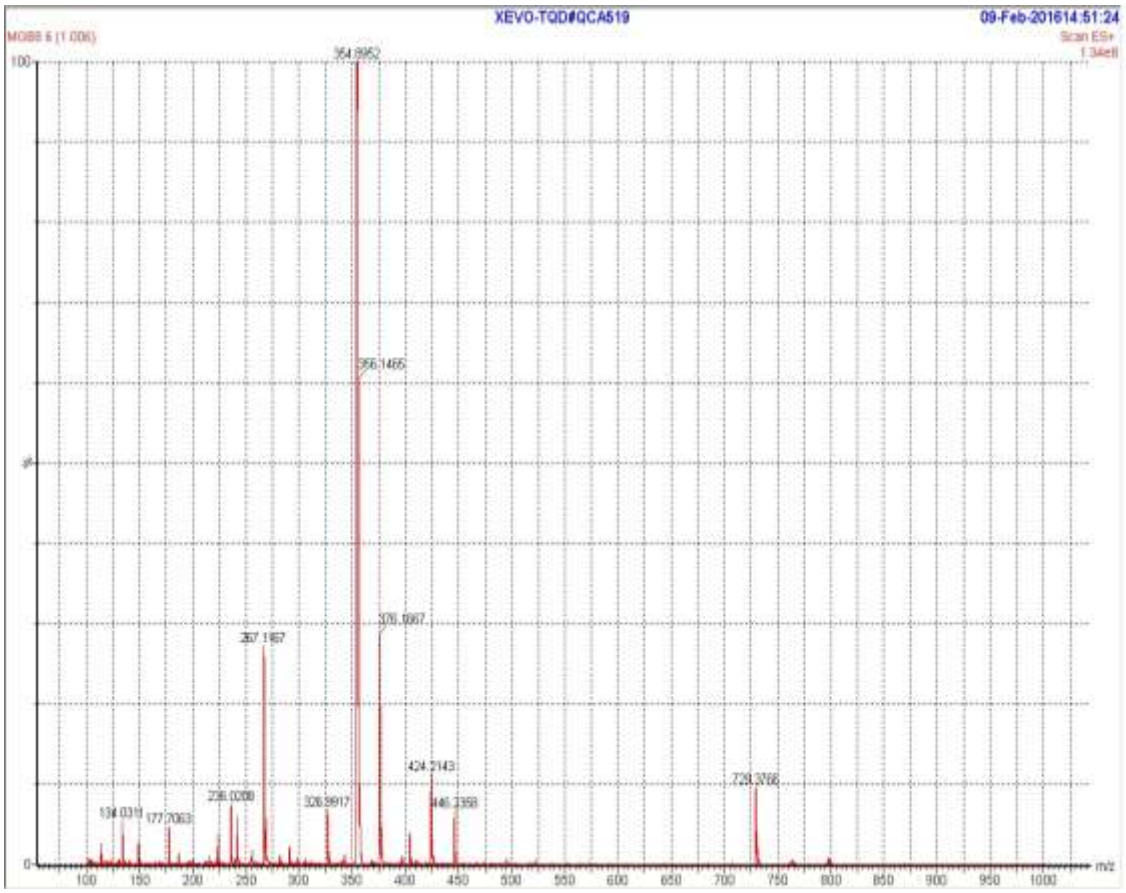

Figure 31: ESI-MS spectrum of 5-isopropyl-N-(2-morpholinoethyl)-2-(nicotinamido)thiazole-4-carboxamide: MGB 8.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.18 (d, J=6.71 Hz, 6 H) 2.36 - 2.45 (m, 6 H) 2.95 - 2.96 (m, 3 H) 3.10 (s, 3 H) 3.57 (t, J=4.58 Hz, 4 H) 4.22 (s, 1 H) 7.51 (s, 1 H) 8.22 (s, 1 H) 8.70 (s, 1 H) 8.98 (s, 1 H)

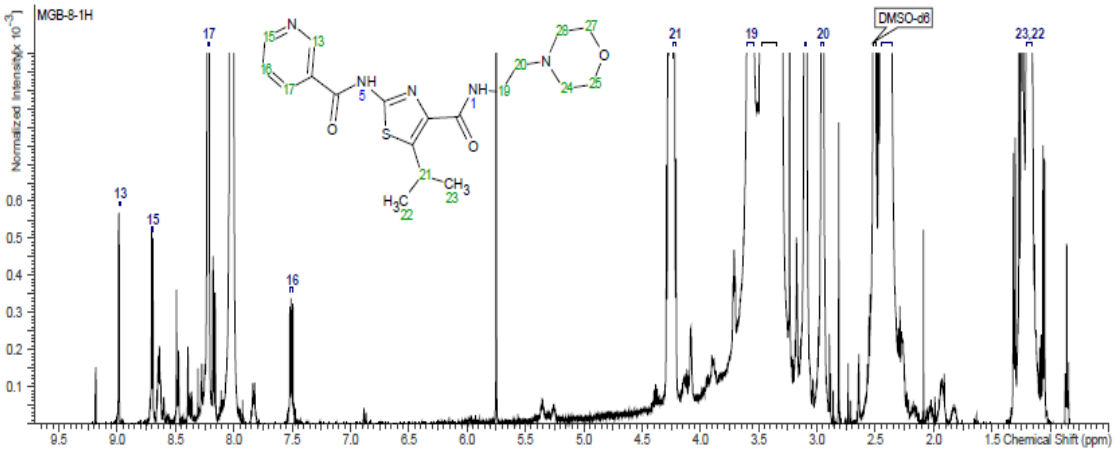

Figure 32: ^{1}HNMR spectrum of 5-isopropyl-N-(2-morpholinoethyl)-2-(nicotinamido)thiazole-4-carboxamide: MGB 8.

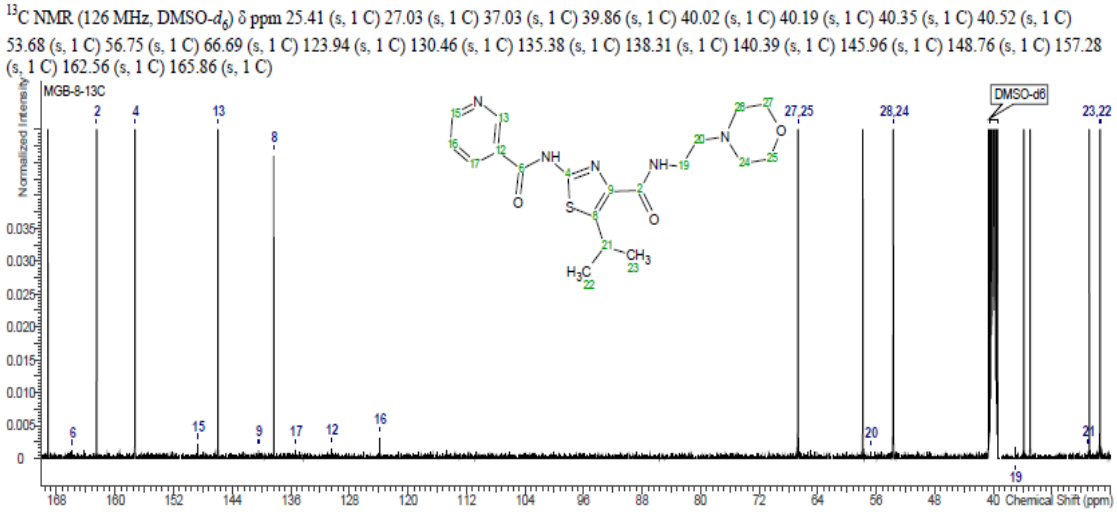

Figure 32: ^{1}HNMR spectrum of 5-isopropyl-N-(2-morpholinoethyl)-2-(nicotinamido)thiazole-4-carboxamide: MGB 8.

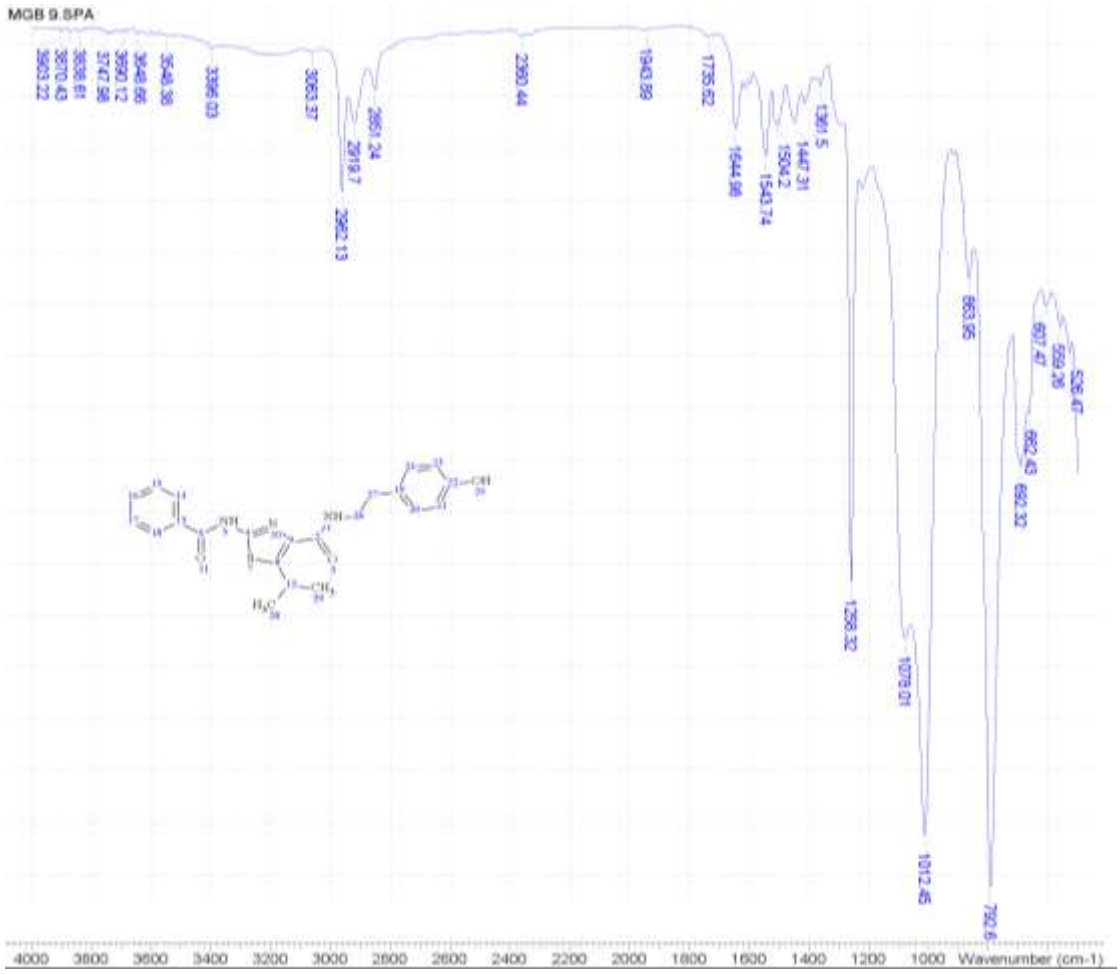

Figure 33: FT-IR spectrum of 2-benzamido-N-(4-hydroxyphenethyl)-5-isopropylthiazole-4-carboxamide: MGB 9.

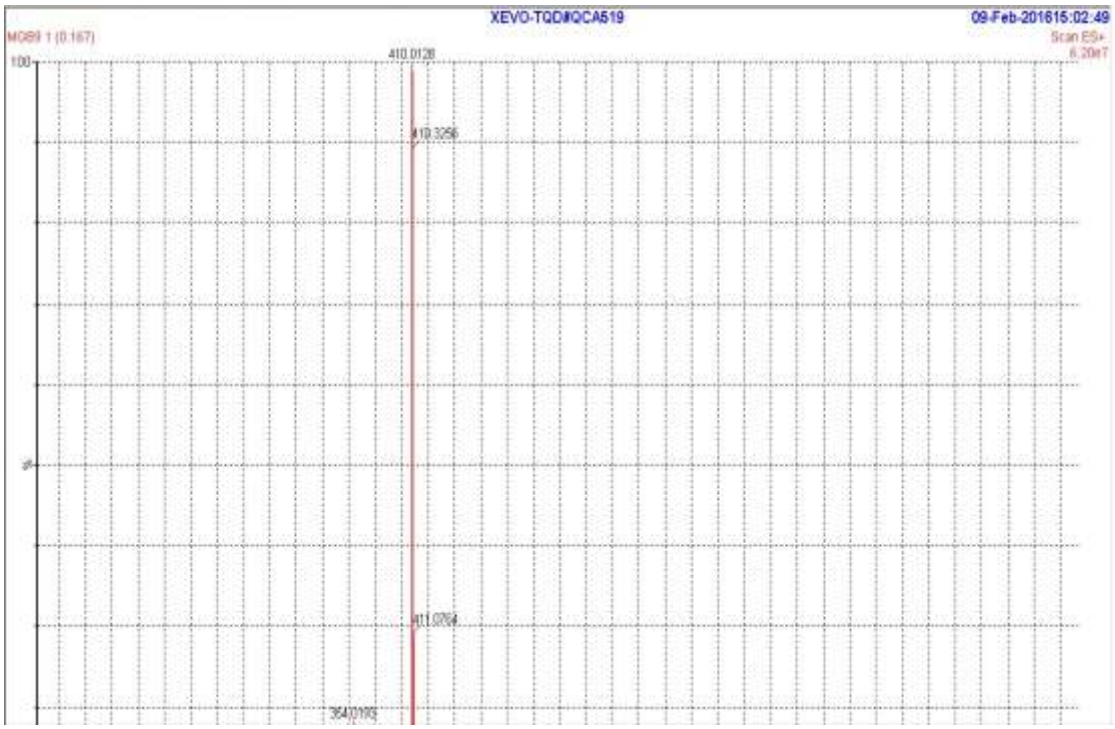

Figure 34: ESI-MS of 2-benzamido-N-(4-hydroxyphenethyl)-5-isopropylthiazole-4-carboxamide: MGB 9.

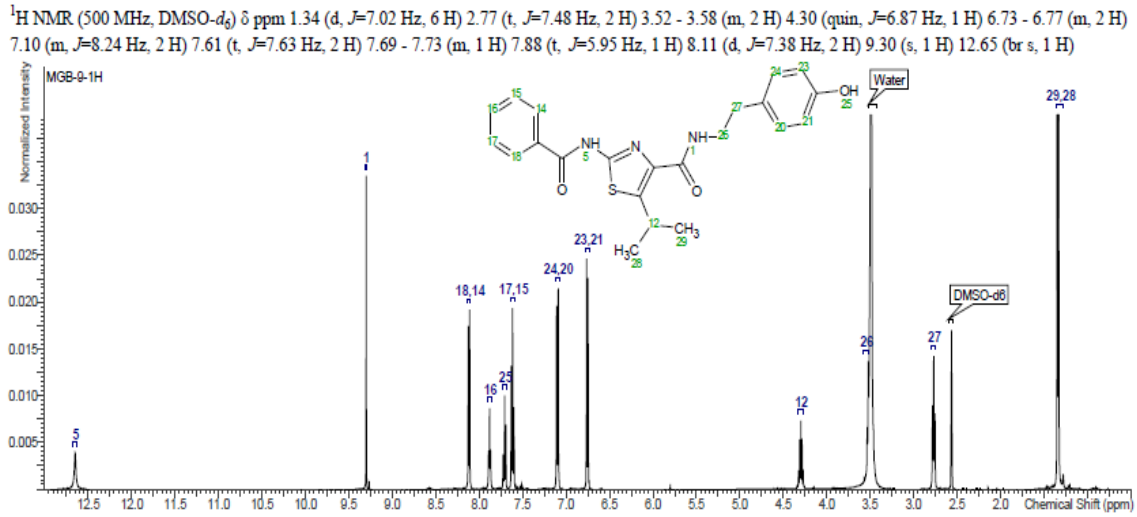

Figure 35: ^{1}HNMR spectrum of 2-benzamido-N-(4-hydroxyphenethyl)-5-isopropylthiazole-4-carboxamide: MGB 9.

^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 25.24 (s, 1 C) 26.70 (s, 1 C) 34.90 (s, 1 C) 40.00 (s, 1 C) 40.17 (s, 1 C) 40.33 (s, 1 C) 40.72 (s, 1 C) 115.67 (s, 1 C) 128.55 (s, 1 C) 129.13 (s, 1 C) 129.78 (s, 1 C) 129.95 (s, 1 C) 132.29 (s, 1 C) 133.22 (s, 1 C) 136.77 (s, 1 C) 146.21 (s, 1 C) 154.37 (s, 1 C) 156.12 (s, 1 C) 162.37 (s, 1 C) 165.74 (s, 1 C)

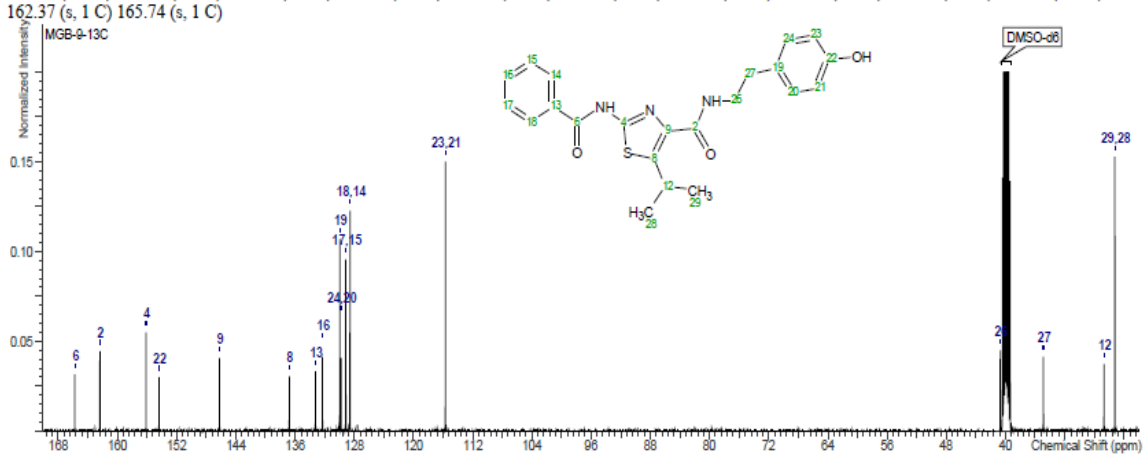

Figure 36: ^{13}CNMR spectrum of 2-benzamido-N-(4-hydroxyphenethyl)-5-isopropylthiazole-4-carboxamide: MGB 9.

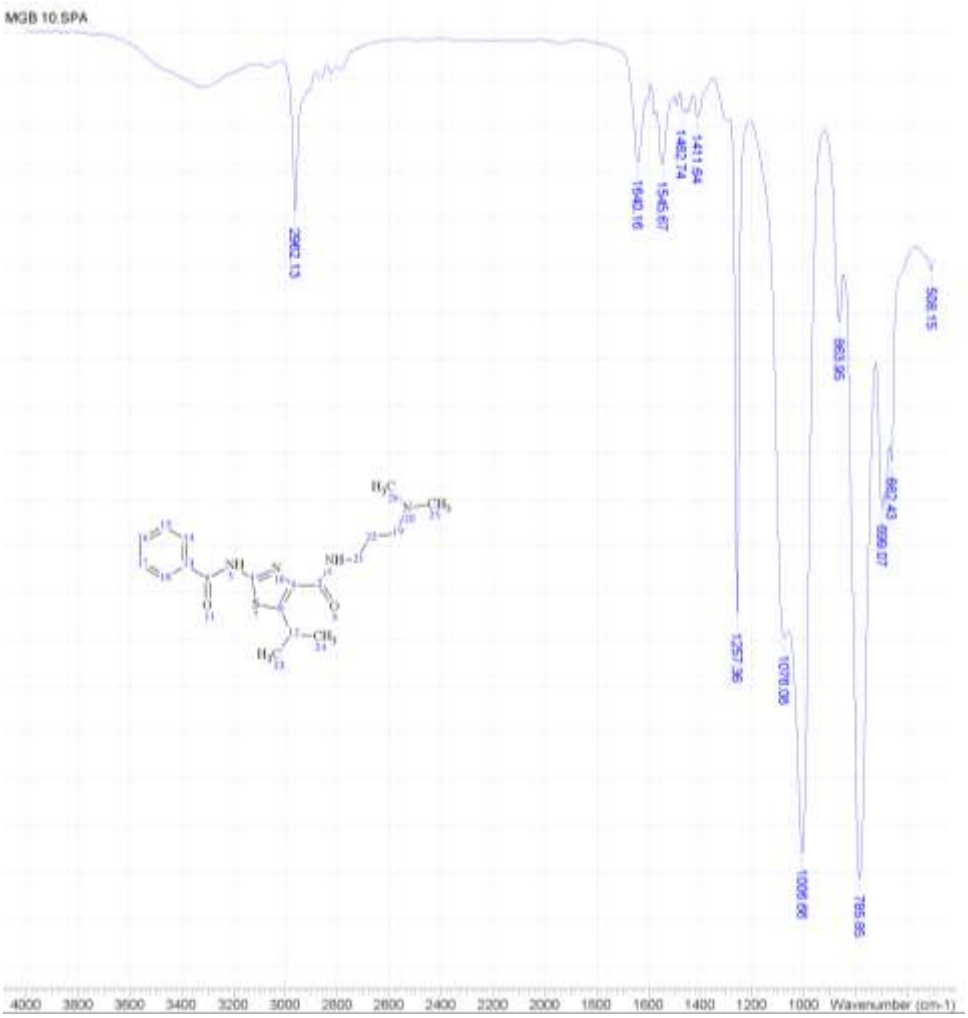

Figure 37: FT-IR spectrum of 2-benzamido-N-(3-(dimethylamino)propyl)-5-isopropylthiazole-4-carboxamide: MGB 10.

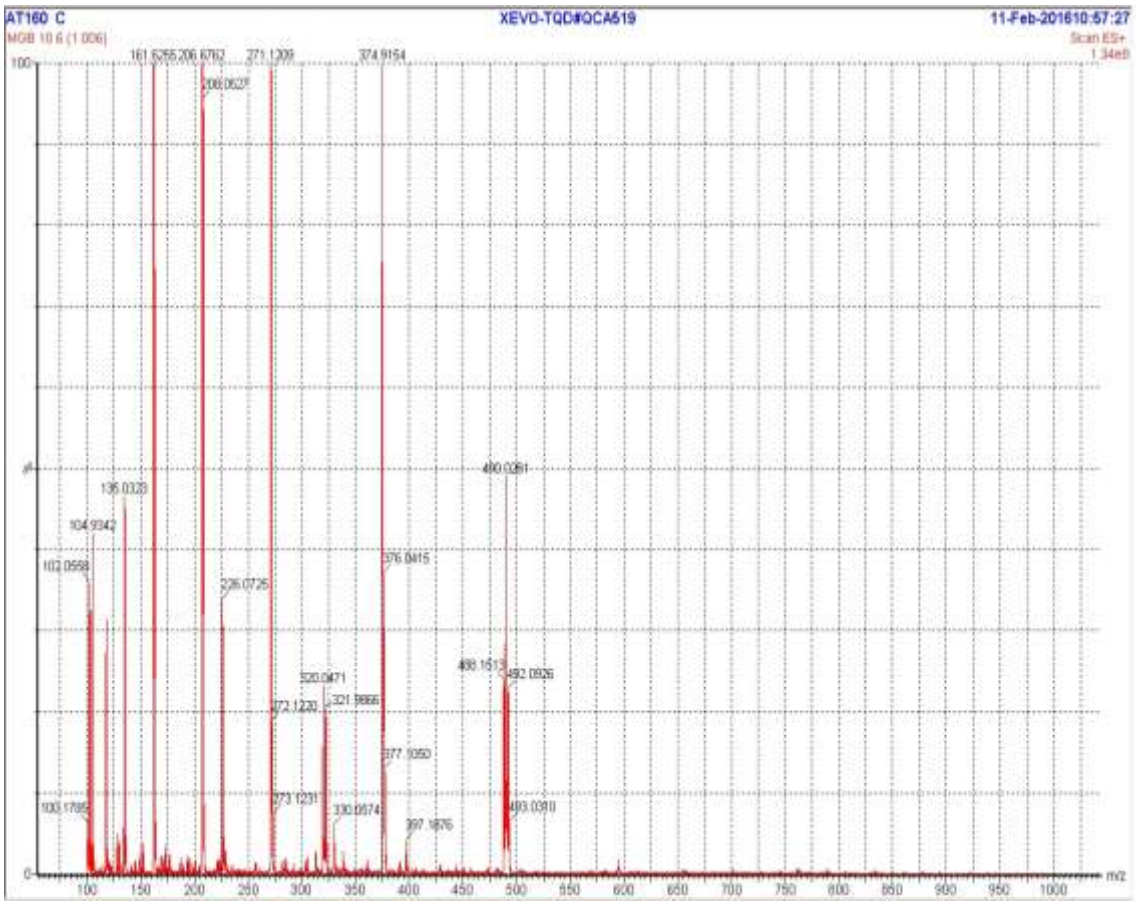

Figure 38: ESI-MS spectrum of 2-benzamido-N-(3-(dimethylamino)propyl)-5-isopropylthiazole-4-carboxamide: MGB 10.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 0.91 (s, 6 H) 1.50 - 1.54 (m, 6 H) 1.61 - 1.74 (m, 28 H) 2.27 (dt, *J*=3.66, 1.83 Hz, 24 H) 2.78 - 2.93 (m, 30 H) 3.10 (q, *J*=6.41 Hz, 51 H) 3.84 (s, 1 H) 3.85 (s, 1 H) 7.23 - 7.33 (m, 23 H) 7.63 - 7.66 (m, 21 H) 8.48 (br t, *J*=5.65 Hz, 10 H) 12.42 (s, 1 H)

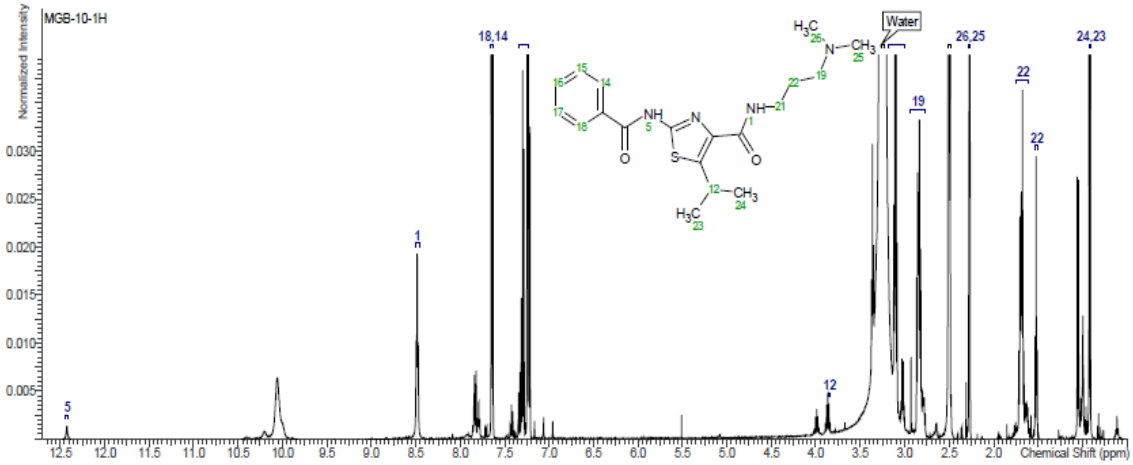

Figure 39: ^{1}HNMR spectrum of 2-benzamido-N-(3-(dimethylamino)propyl)-5-isopropylthiazole-4-carboxamide: MGB 10.

^{13}C NMR (126 MHz, DMSO-d_6) δ ppm -0.50 (s, 1 C) 13.30 (s, 1 C) 20.43 (s, 1 C) 21.44 (s, 1 C) 23.60 (s, 1 C) 23.72 (s, 1 C) 23.83 (s, 1 C) 24.05 (s, 1 C) 24.12 (s, 1 C) 24.47 (s, 1 C) 25.51 (s, 1 C) 25.61 (s, 1 C) 28.31 (s, 1 C) 28.35 (s, 1 C) 34.97 (s, 1 C) 35.08 (s, 1 C) 35.45 (s, 1 C) 35.61 (s, 1 C) 35.67 (s, 1 C) 38.88 (s, 1 C) 39.05 (s, 1 C) 39.22 (s, 1 C) 39.38 (s, 1 C) 41.37 (s, 1 C) 53.82 (s, 1 C) 53.85 (s, 1 C) 53.87 (s, 1 C) 66.37 (s, 1 C) 126.57 (s, 1 C) 126.67 (s, 1 C) 126.73 (s, 1 C) 127.43 (s, 1 C) 127.64 (s, 1 C) 127.94 (s, 1 C) 128.07 (s, 1 C) 128.61 (s, 1 C) 130.62 (s, 1 C) 131.14 (s, 1 C) 132.16 (s, 1 C) 133.55 (s, 1 C) 134.69 (s, 1 C) 135.53 (s, 1 C) 139.87 (s, 1 C) 145.36 (s, 1 C) 153.25 (s, 1 C) 161.24 (s, 1 C) 161.68 (s, 1 C) 163.42 (s, 1 C) 164.67 (s, 1 C) 165.84 (s, 1 C) 174.11 (s, 1 C)

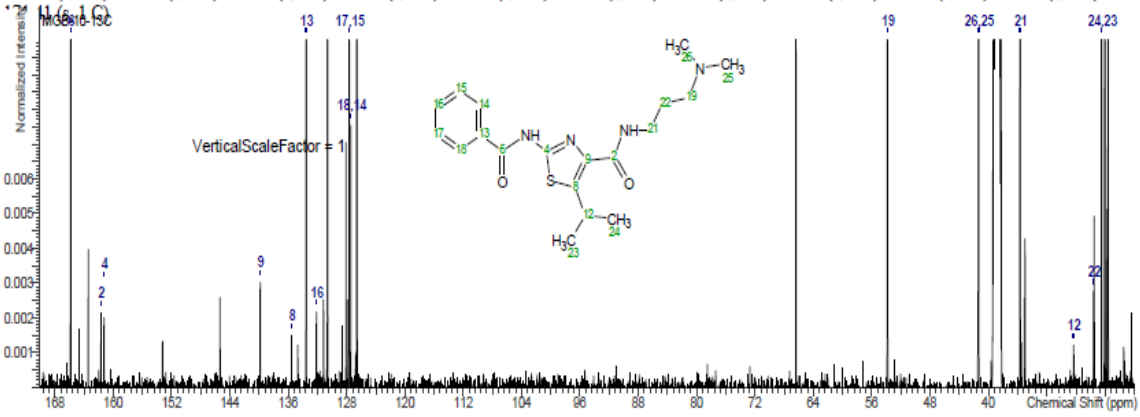

Figure 40: ^{13}CNMR spectrum of 2-benzamido-N-(3-(dimethylamino)propyl)-5-isopropylthiazole-4-carboxamide: MGB 10.

جامعة النجاح الوطنية

كلية الدراسات العليا

تصنيع وتحديد االخصائص الكيميائية و البيولوجية لمركبات ايزوبروبيل ثيازول مشتقة من المركبات الطبيعية ديستامايسين_أ و بروكسيمايسن

إعداد

امجد عبد الرحمن موسى عبد الرحمن

إشراف

د. حسن النيص

د.نضال جرادات

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

2017

تصنيع وتحديد االخصائص الكيميائية و البيولوجية لمركبات ايزوبروبيل ثيازول مشتقة من المركبات الطبيعية ديستامايسين_أ و بروكسيمايسن

إعداد

امجد عبد الرحمن موسى عبد الرحمن

إشراف

د.حسن النيص

د.نضال جرادات

الملخص

المركبات الطبيعية ديستاميسين ا و نيتروبسين هي مركبات طبيعية تنتمي الى فصياة المركبات التي لها المقدرة على الارتباط بمركب (DNA) في مناطق معينة و بشكل انتقائي. هذه المركبات تصنف كيميائيا على انها مركبات عديد البايرول و لها خصائص بيولوجية مضادة للفيروسات و البكتيريا كما انها تمتلك خصائص مضادة للسرطان و لكن هذه المركبات ايضا سامة. و قد تم بذل العديد من الجهود و المحاولات لتصنيع مركبات شبيهة لهذه المركبات في محاولة للحصول على نتائج افضل او لتقليل سمية هذه المركبات.

تم اعداد هذا البحث بهدف تصنيع و دراسة بعض الخصائص الكيميائية و الفيزيائية و البيولوجية لمركبات شبيهة بالديستاميسين ا و النيتروبسين والبروكسيميسين . هذه المركبات المقترحة تم تصميمها بما يسمح بتعدد الخيارات المتاحة لادخال التعديلات على المركبات للحصول على تنوع في الخصائص الكيميائية والبيولوجية لهذه المركبات و للمقارنة و دراسة اثر التعديلات المدخلة على تلك الخصائص. معتمدين في هذه التعديلات على حلقات عطرية ,بشكل اساسي الثيازول, وكذلك البنزين و البيريدين و البينزايميدازول, هذه الحلقات لها دور في اضفاء مزيد من الخصائص الدهنية و القدرة على اختراق الاغشية الخلوية والنووية لهذه المركبات. كما اخذ بعين الاعتبار الحفاظ على كتلة جزيئية معقولة لهذه المركبات حتى تزيد من فرصة الحصول على مركبات لها خصائص تؤهلها للتطوير الى ادوية محتملة لعلاج حالات السرطان و الالتهابات البكتيرية او الفطرية او الفيروسية.