Synthesis and evaluation of the antimicrobial activity of newly synthesized benzothiazole derivatives

By

Bahaa Shafiq Ismail Mustafa

Supervisor

Dr. Elham Al-Kaissi

Co-supervisor

Prof. Zuhair Muhi-eldeen

University of Petra

MSc. Thesis

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Examination committee Signature:

1- Dr. Elham Al-Kaissi ----------

2- Prof. Zuhair Muhi-eldeen ----------

3- Dr. Ibrahim Al-Adham ----------

4- Dr. Ghadeer Suaifan ----------

Abstract

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Benzothiazole and its derivatives are the most important heterocyclic compounds, which are common and integral feature of a variety of natural products and pharmaceutical agents. Benzothiazole moiety has been very small yet; benzothiazole and its derivatives have fascinated the scientists by exhibiting different biological activities. One of the most important biological activities that benzothiazole and its derivatives showed is the antimicrobial activity. The project involved the synthesis of new benzothiazole derivatives, in which aminoacetylenic side chains have been incorporated, with the aim that the target compounds may show good antimicrobial activity. Structures of the newly synthesized compounds have been deduced on the basis of elemental analysis and spectral data. *In-vitro* antimicrobial activity evaluation was done by two methods: Agar diffusion method and Broth dilution test. *In-vitro* antimicrobial activity evaluation was

done against *S. aureus* ATCC 6538p, *C. albicans* ATCC 10231, *P. aeruginosa* ATCC 9027, *E. coli* ATCC 8739, and *B. subtilis* ATCC 6633. MIC and MBC determination was carried out for the newly synthesized compounds **(BZ2-BZ7)**. The results of antimicrobial testing were compared to two positive control drugs Ciprofloxacin 5mcg/ml and Fluconazole 500 mcg/ml. Compound 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole **BZ5** showed the highest antibacterial activity against *S. aureus* among all the compounds with MIC value of 15.62 mcg/ml while; Compound 2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ7** exhibited the highest antibacterial activity against *P. aeruginosa* with MIC value of 31.25 mcg/ml. Compounds 2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ2** and 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole **BZ5** showed the highest antifungal activity against *C. albicans* with MIC value of 15.62 mcg/ml (for both). The results obtained are sufficient to indicate that these compounds have good antimicrobial activity and each one of them had a different degree of antimicrobial activities on different types of microorganisms tested.

Acknowledgments

I am using this opportunity to express my gratitude to everyone who supported me throughout my master study. I am thankful for their aspiring guidance, invaluably constructive criticism and friendly advice during the project work. I am sincerely grateful to them for sharing their truthful and illuminating views on a number of issues related to the project.

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A special thanks to my family. Words cannot express how grateful I am with my mother, my father, my brothers and sisters: **Alaa, Ahmad, Ola, Doaa** and **Hanin** for all of the sacrifices that you've made and for the great support in hard moments.

I would also like to thank all of my friends: **Abood, Hamood, Hasson and Turki**, who supported me, and incited me to strive towards my goal.

Last but not least I want to deeply thank my beloved wife, **Amal** for her patience, encouragement, pure love and amazing moments she gave me through my study. Without you my angel I could not finish this thesis.

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KSCN	Potassium Thiocyanate
PABA	Para aminobenzoic acid
A. niger	*Aspergillus niger*
E. coli	*Escherichia coli*
P. aeruginosa	*Pseudomonas aeruginosa*
S. aureus	*Staphylococcus aureus*
B. subtilis	*Bacillus subtilis*
C. albicans	*Candida albicans*
MRSA	Methicillin-resistant *Staphylococcus aureus*
TMS	Tetramethylsilane
DMSO	Dimethyl sulfoxide
D.W	Distilled water
IR	Infrared Spectra
FT-IR	Fourier transform infrared spectroscopy
KBr	Potassium bromide
NMR	Nuclear magnetic resonance
BZ1	2-(prop-2-yn-1-yl)-1,3-benzothiazole
BZ2	2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole
BZ3	2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole
BZ4	2-[4-(piperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole
BZ5	2-[4-(azepan-1-yl)but-2-yn-1-yl]-1,3-benzothiazole
BZ6	2-[4-(4-methylpiperazin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole
BZ7	2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole
DSC	Differential Scanning Calorimetry
MHz	Megahertz
WHO	World Health Organization
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
CFU	Colony Forming Unit
ATCC	American Type Culture Collection
m.p	Melting Point
SAR	Structure Activity Relationship

Chapter one

1.1 Introduction:

One of the aims of medicinal chemistry is to develop antibacterial and antifungal drugs for the treatment of various bacterial and fungal infections. Benzothiazole derivatives represent a class of heterocyclic compounds that possess various biological properties **(Figure 1.1)** (Chaudhary *et al.*, 2010). Benzothiazole derivatives represent a class of molecules capable of binding to multiple receptors (Keri *et al.*, 2015). Consequently, 1,3 - benzothiazole moiety has attracted a great deal of interest in research. Benzothiazole analogs, most commonly found in various marine and terrestrial natural products which have shown useful biological properties (Yadav *et al.* , 2011; Yamazaki *et al.* , 2005; Yalcin *et al.* ,1992). They were part of the structure of firefly luciferin and are also known as aroma constituents of tea leaves and cranberries or flavor compounds produced by the fungi *Aspergillus clavatus* and *Polyporus frondosus* (Keri *et al.*, 2015). It exhibits a unique scaffold for the synthesis of unique novel compounds. Hofmann A.W. (1887), was the first scientist who synthesized 2-substituted benzothiazole, because of diversified activity as well as simple cyclization mechanism, numbers of chemical routes have been adopted as reported by (Keri *et al.*, 2015). 1,3-benzothiazole as an aromatic heterocyclic compound consists of five-membered 1,3-thiazole ring fused to a benzene ring. Its aromaticity makes it relatively stable heterocycle, it has reactive sites, which allow for functionalization (Ali and Siddiqui, 2013). Benzothiazole is a colorless, slightly viscous liquid with a melting point of 2°C and a boiling point of 227-228°C. The density of benzothiazole is 1.24 g/ml, and its

molecular mass is 135.19 $gmol^{-1}$. Benzothiazole has no household use (Ali and Siddiqui, 2013). It is used in industry and research. It has application in industry as anti-oxidant and vulcanization accelerator (Yadav *et al.*, 2011).

The broad spectrum of the pharmacological activity in individual Benzothiazole derivative indicates that, this series of compounds is of a great interest (Keri *et al.*, 2015). Benzothiazole derivatives possess a wide spectrum of biological applications such as antitumor (Joseph *et al.*, 2010; Chaudhary *et al.*, 2010; Xuejiao *et al.*, 2013), antimicrobial (Gilani *et al.*, 2012; Chaudhary *et al.*, 2010), anti-inflammatory (Gurupadayya *et al.*, 2008; Chaudhary *et al.*, 2010), anticonvulsant (Siddiqui *et al.*, 2009), antidiabetic (Diaz *et al.*, 2008; Mariapan *et al.*, 2012), antipsychotic (Arora *et al.*, 2012), H_3 antagonist (Muhi-Eldeen *et al.*, 2014) and diuretic (Yar & Ansari 2009). Riluzole (2- amino-6-trifluoromethoxybenzothiazole) is marketed by Rhone-Poulenc (Rilutek) for treatment of amyotrophic lateral sclerosis (Keri *et al.*, 2015).

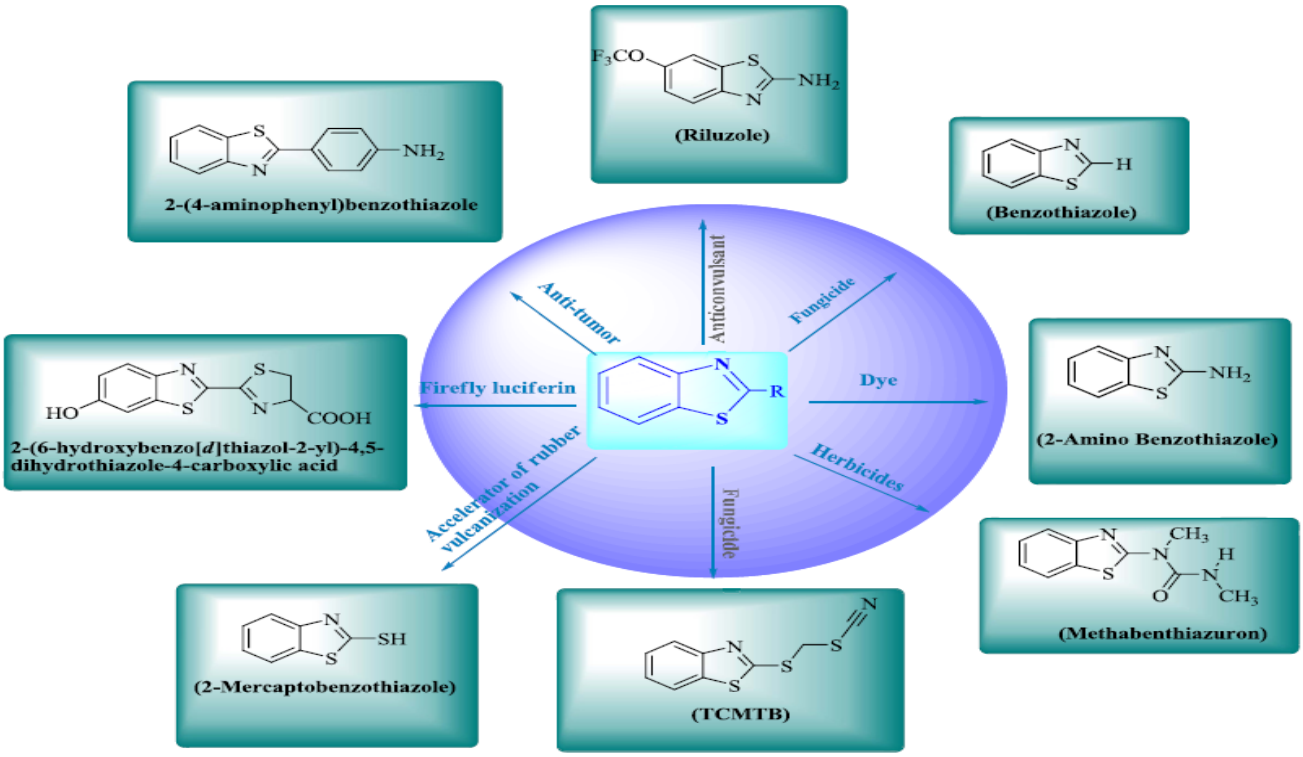


Figure (1.1): Benzothiazole, a multifunctional nucleus (Keri *et al.*, 2015).

Many literatures revealed that benzothiazole derivatives may serve as an important model as a potent antimicrobial agent (Franchini *et al.*, 2009). The related research and developments in benzothiazole-based medicinal chemistry have become a rapidly developing and increasingly active topic (Keri *et al.*, 2015). One research involved the synthesis of novel heterocyclic 4-thiazolidinone derivatives (compound **a**) **Figure 1.2**. Compounds were prepared by first condensation of various substituted 2-amino benzothiazole with 1-(4-methyl Phenyl)-3-methyl-5- pyrazolone to give various substituted Schiff bases, which then react with thioglycolic acid and thiolactic acid to yield the desired series of compounds. The synthesized compounds were screened for their in-vitro antibacterial activity against *S. aureus* and *E. coli* using agar diffusion method. Among the synthesized compounds nitro, methoxy and hydroxyl derivatives showed good antibacterial activity against tested microorganisms (Mistry and Desai, 2004).

Additionally, 4-oxothiazolidines and their 5-arylidenes derivatives of benzothiazole (compound **b**) **Figure 1.2** were synthesized by Srivastava *et al.* in 2004. All the synthesized compounds were evaluated for their antibacterial activity against *E. coli*, *B. subtilis* and *Salmonella typhimurium*. Screening of antifungal activity was done against *C. albicans, A. niger and Fusarium heterosporium*. Amongst the synthesized compounds bromophenyl derivatives showed higher antimicrobial activity.

Group of researchers involved in the synthesis of 6-fluoro-2-[4-formyl-3-(substituted phenyl) pyrazol-1-yl] benzothiazoles (compound **c**) **Figure 1.2**. The synthesized compounds were evaluated for their antibacterial activity. The results showed that the compounds with chloro substitution showed good antibacterial activity (Sandhya *et al.*, 2005).

Another study involved in the synthesis of polyfluorinated 2-benzylthiobenzothiazoles 3a-l compounds (compound **d**) **Figure 1.2**. The compounds were prepared via a microwave-assisted, one-pot procedure. The antifungal activity of the compounds was evaluated against *Rhizoctonia solani, Botrytis cinereapers,* and *Dothiorella gregaria* species. Compounds with substitution at RF^1 with 4-F and at RF^2 with 3,4-F showed higher antifungal activity than others . Bioassay results revealed that polyfluorinated 2-benzylthiobenzothiazol derivatives can be used as lead compound for developing novel fungicides (Huang *et al.*, 2006).

Another promising antifungal compounds were 2-(6-fluorobenzothiazole-2′yl amino)-4-(phenylthioureido)-6-(substituted thioureido)-1,3,5-triazine were synthesized (compound **e**) **Figure 1.2**. The compounds were evaluated for their antifungal activity against *Alternaria alternate, A. niger and Macrophomina* using the agar diffusion technique. Results revealed that compounds with Fluoro substitution showed maximum antifungal activity (Sareen *et al.*, 2006).

Furthermore, 2-Heteroarylimino-5-benzylidene-4-thiazolidinones analogues of 2-thiazolylimino-5-benzylidene-4-thiazolidinones were synthesized (compound **f**) **Figure 1.2**. The synthesized Compounds were assayed *in vitro* for their antimicrobial activity against Gram positive and Gram negative bacteria, yeasts and mould. Most of the benzo[d]thiazole analogues display good inhibition of the growth of Gram positive bacilli and staphylococci, including methicillin-resistant Staphylococcus strains. The results showed that the replacement of the thiazole nucleus for the benzo[d]thiazole bicyclic system in the parent 2-(benzo[d]thiazol-2-ylimino) thiazolidin-4-one leads to significant antifungal properties (Vicini *et al.*, 2008).

Another study in which new 2-styryl benzothiazolium salts substituted on the heterocyclic ring have been synthesized by the condensation of 3-alkyl-2-methylbenzothiazolium halides with 4-substituted benzaldehydes (compound **g**) **Figure 1.2**. Intramolecular charge transfer from the double bond substituent to the benzothiazolium ring is a typical feature for the prepared compounds. *In vitro* antibacterial activity was evaluated against *S. aureus, B. subtilis, Micrococcusluteus* and *Enterococcus faecalis*. Antifungal screening against *Saccharomyces cerevisiae*, *Hansenula anomala*, *C. albicans* CCY 29-3-112, *C. albicans* 271 was also carried out. According to the results obtained, the antimicrobial activity of the compounds with electron acceptor NO_2 substituent is not significant neither against Gram positive bacteria nor yeast. Also compounds that contain electron-donor substituent –NH-CO-CH_3 was inactive against yeasts , while compounds 7c **table (1)** seems to be the most active one against Gram-positive bacteria (Sigmundova *et al.*, 2008).

Khan *et al.* in 2009, carried out a research which involved the synthesis of 1,3,4-thiadiazole and imidazolinone derivatives. Two subgroups of compounds were synthesized 2-aryl-5-(6′-chloro-1′,3′-benzothiazole-2-yl-amino)-1,3,4-thiadiazoles (compound **h**) **Figure 1.2** and 4-(4′-arylidene) -2-phenyl-1-(6′-chloro-1′,3′-benzothiazol-2 -yl-thiourido)-4,5-dihydroimidazolin-5-ones (compound **i**) **Figure 1.2**. The synthesized compounds were evaluated for their antifungal activity against *A. niger* and *C. albicans* in addition to antibacterial activity against *E. coli, S. aureus* and *P. aerugenosa* using cup-plate agar diffusion method. Based on the results obtained, only 4-chlorophenyl derivatives showed high antibacterial activity while, 4-chlorophenyl and 3-indolyl imidazolinone derivatives showed significant antifungal

activity. It was concluded from the screening results that 1,3,4-thidiazole derivatives were most effective against all the tested microorganisms.

Recent study involved the synthesis of novel benzothiazole and benisoxazole derivatives (compound **j**) **Figure 1.2**. The newly synthesized compounds were screened for their antibacterial activity against *E. coli* (ATTC- 25922), *S. aureus* (ATTC-25923), *P. aeruginosa* (ATTC-27853) and *B. subtilis* (recultured) bacterial stains by the disk diffusion method. Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus* (NICM No. 524), *C. albicans* (NCIM No. 3100), *Aspergillus fumigatus* (NCIM No. 902) and *Penicillium marneffei* (recultured) in DMSO by the serial plate dilution method. The results showed that, dibromopropane, dibromohexane, dibromobutane and dibromopentane derivatives exhibit significant antimicrobial activity when compared to that of other derivatives (Kumbhare *et al.*, 2009).

Argyropoulou *et al.* in 2009 carried out a project for the synthesis of several thiazoles and benzothiazoles carrying a benzenesulfonamide moiety at position 2 of the heterocyclic nucleus (compound **k**) **Figure 1.2**. The synthesized compounds were assayed *in vitro* for their antimicrobial activity against a panel of selected Gram positive (*B. subtilis and S. aureus*) and Gram negative bacteria (*E. coli*), yeasts (*Saccharomyces cerevisiae, Candida tropicalis*) and mould (*A. niger*). MIC values for the synthesized compounds were also detected. Nitro substituted sulfonamides of benzothiazole showed good activity against Gram positive bacteria. No inhibition of Gram negative bacteria and fungi is detected up to the concentration of 100 mcg/ml.

Additionally, a study involved the synthesis of new 2-Amino substituted-benzothiazole (compound **l**) **Figure 1.2** by treating with Potassium thiocyanate (KSCN) in presence of glacial acetic acid and with different substituted aniline. The anti-microbial activity of the synthesized compound was evaluated by disc diffusion method. According to the results, compounds with nitro substitution showed higher antimicrobial activity than others (Malik *et al.*, 2009).

In 2009 Bondock *et al.*, carried out a research which included the synthesis of polyfunctionally substituted heterocycles (e.g. pyrazoles, isoxazole, pyrimidines, thiazolo[3,2-a]pyrimidine, tetrazolo[1,5-a]pyrimidine, pyrido[1,2-a]pyrimidine, 1,5-benzodiazepine, and pyrazolo[1,5-a]pyrimidine) (compound **m**) **Figure 1.2**. Representative compounds of the synthesized products were tested and evaluated for antimicrobial activity. Among these compounds, 6a and 6b compounds showed good antimicrobial activity against *B. subtilis*.

Makrandi in 2009, carried out a research in which 2-(Benzofuran-2-yl)-7-(substituted)imidazo[2,1-b]benzothiazoles (compound **n**) **Figure 1.2** were prepared by condensation of 2-(2-bromoacetyl) benzofurans and various 2-amino-7-(substituted) benzothiazoles under normal thermal condition as well as microwave irradiations. The synthesized compounds were evaluated for their antimicrobial activity by paper disc method against *S. aureus, E. coli, P. aeruginosa* and *C. albicans*. Some of the synthesized compounds showed good antimicrobial activity when compared to that of standard drugs Ciprofloxacin and Fluconazole.

Another study in 2009 by Nagarajan *et al.,* has involved the synthesis of novel benzothiazole substituted thiazolidinone derivatives (compound **o**) **Figure 1.2**. The synthesized compounds were evaluated for their antimicrobial activity against *Proteus mirabilis*, *S. aureus, Salmonella typhi* and *Klebsiella pneumonia*. Amongst the tested compounds, fluoro substituted compound showed significant antimicrobial activity against tested bacteria namely *Proteus mirabilis*.

Another study at 2010 by Sreenivasa *et al.,* involved the synthesis of various 2(5'-substituted phenyl 1',3',4' oxadiazol-2'-yl) amino-6-fluoro-7-substituted (1,3)-benzothiazoles (compound **p**) **Figure 1.2**. These Fluoro substituted Benzothiazoles were screened for their anthelmentic activity by using earthworms. Compounds with substitutions such as p-nitro, PABA, and diphenyl amine showed higher anthelmentic activity than others.

Another interesting study in 2012 involved the synthesis of new 2-(5-substituted-1,3,4-oxadiazole-2-yl)-1,3- benzothiazole (compound **q**) **Figure 1.2**. Compounds were synthesized by refluxing benzothiazolyl carboxyhydrazide with different aryl acids in phosphoryl chloride. The antimicrobial activity of the synthesized compounds was evaluated against *B. subtilis, E. coli, P. aeruginosa and Bacillus pumilis* by disc diffusion method. Among the synthesized compounds 3b, 3d compounds were found to possess a broad spectrum activity (Karaarslan *et al.*, 2012).

Later study at 2013 by Catalano *et al.,* was conducted involving the synthesis of new series of 6-substituted 2-aminobenzothiazole derivatives (compound **r**) **Figure 1.2**. The synthesized compounds were evaluated for their antibacterial activity against Gram-positive and Gram-negative bacteria belonging to the ATCC collection (*S. aureus* 29213*, E. faecalis* 29212, *E. coli* 25922). Antifungal screening was done against a panel of fungi strains (*C. albicans* 102231, *Candida parapsilosis* 22019, *Candida krusei* 6258, *Candida tropicalis* 750) belonging to the ATCC collection. All the 6-substituted 2-amino-1,3-mercapto benzothiazole derivatives showed slight to high antifungal activity against all the Candida species tested. Compounds with halogen substitution such as chlorine and fluorine atom at 6-position of benzothiazole moiety showed weak antifungal activity. Compounds that have phenoxy and benzyloxy substitution were more potent than others. Based on the results obtained, the antifungal activity enhanced with the increase of steric hindrance at position 6 of the heterocycle.

Recently in 2014 by Maru *et al.*, a study was started involving the synthesis of some novel benzothiazole derivatives (compound **s**) **Figure 1.2** by condensation of N-(4-Acetylphenyl)-2- (benzothiazole-2-ylsulfanyl)-acetamide with different substituted aniline in the presence of catalytic amount of acetic acid. The new compounds were examined for antibacterial and antifungal effects against different strains of bacteria and fungi using conventional broth-dilution method. N-(4-Acetylphenyl)-2-(benzothiazole-2-ylsulfanyl)-acetamide with different substituted anilines were reported as good antimicrobial agents.

According to the literature review, molecules with benzimidazole, benzoxazole and benzothiazoles moieties are attractive targets for synthesis since they often exhibit diverse and important biological properties (Padalkar *et al.*, 2012). These heterocycles have shown different pharmacological activities such as Gram-positive antibacterial agents, antiparasitic, anti-inflammatory, elastase inhibitors, anti-stress and anti-cancer agents (Padalkar *et al.*, 2012).

Another study by Alp, 2005, involved the synthesis of a series of novel 1,2-disubstituted-1-H-benzimidazole-N-alkylated-5-carboxamidine derivatives was done (compound **t**) **Figure 1.2**. The reaction involved the introduction of aromatic amidine groups into the benzimidazole system. The synthesized compounds were evaluated for their antibacterial activity against *S. aureus*, methicillin resistant *S. aureus* (MRSA)*, E. coli* and *Enterococcus faecalis* and for antifungal activity against *C. albicans* by macro-broth dilution assay. Compound [1-(2,4-dichlorobenzyl)- N-2-(diethylaminoethyl)-1-H-benzimidazole-5-carboxamidine] exhibited the greatest antimicrobial activity against *S. aureus* , MRSA and the fungus *C. albicans*.

An interesting study was done in 2006 by Balasubramanian *et al.,* involved the synthesis of some novel benzimidazol /benzoxazolylethoxypiperidoneoximes (compound **u**) **Figure 1.2** was conducted. The synthesized compounds were evaluated for their antibacterial activity against *S. aureus* (NCIM-2492), *B. subtilis* (NCIM-2439), *E. coli* (NCIM-2345) and *P. aeruginosa* (NCIM-2035) by the twofold serial dilution method. Antifungal activity screening was carried out against *C. albicans* (NCIM-C27), *Candida-6* (NCIM-C27), *Candida-51* (NCIM-C27), *A. niger* (NCIM-590) and *Aspergillus flavus* (NCIM-539). All the tested compounds expressed a remarkable activity against *E. coli* and *Candida-51*. The results

revealed that, the oximes may be used as templates to generate better drugs to combat bacterial and fungal infections.

Another research involved the synthesis of a new class of antimicrobial agents was conducted in 2012 in which, a series of 2-(1H-benzimidazol -yl)-5-(diethylamino) phenol, 2-(1,3-benzoxazol-2-yl)-5-(diethylamino)phenol, 2-(1,3-benzothiazol-2 yl)-5-(diethylamino) phenol and their derivatives (compound **v**) **Figure 1.2** were synthesized. All the synthesized compounds were evaluated for *in vitro* antibacterial activity against *E. coli* and *S. aureus* strains and *in vitro* antifungal activity against *C. albicans* and *A. niger* strains using serial dilution method. Benzimidazole and benzoxazole showed excellent results against bacterial strain and benzothiazole against fungal strain (Padalkar *et al.*, 2012).

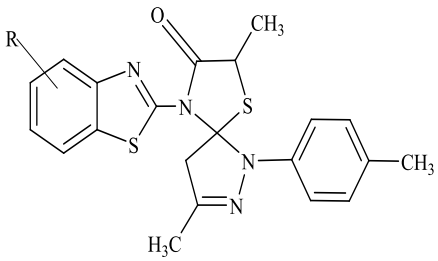
In 2012 Taher *et al.*, carried out a research which involved the synthesis of a series of 2-substituted-1H-benzimidazole derivatives (compound **w**) **Figure 1.2**. These derivatives are hybrids between 2-aminobenzimidazole pharmacophore and certain substituted pyrazoles possessing antimicrobial activities. The synthesized compounds were evaluated for their antimicrobial activity. The results showed that, all the tested compounds showed potent antimicrobial activity against some species of Gram-negative bacteria (*P. aeruginosa, E. coli, Salmonella typhi)* and fungi *C. albicans*. In contrast, all the tested compounds were inactive against Gram-positive bacteria *S. aureus.*

Further study was done by Singh *et al.* in 2013, involved the synthesis of interesting benzoxazole derivatives (compound **x**) **Figure 1.2**. The reaction of 2-aminophenol and p-amino benzoic acid yielded 2-(4-aminophenyl)benzoxazole in the presence of

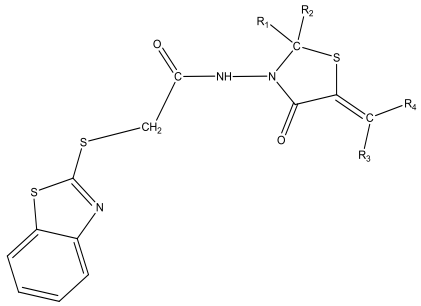
Polyphosphoric acid which was further condensed with different aromatic aldehydes offered Schiff bases. The antimicrobial activity of the resulting compounds was evaluated. The results revealed that, the synthesized compounds exhibited significant antimicrobial activity.

Al-Mohammed *et al.*, 2013 carried out another research in which novel imidazole and benzimidazole sulfonamide compounds were synthesized (compound y) **Figure 1.2**. The strategy of synthesis combines two or more pharmacologically compatible moieties in one molecule by attaching a sulfonamide moiety to an imidazole, benzimidazole or other sulfonamide moiety. The evaluation of the antibacterial activity of the synthesized compounds was done against standard strains of six Gram positive and four Gram negative bacteria using the microbroth dilution technique. Results revealed that Gram positive bacteria strains seem to be more sensitive to the synthesized compounds than Gram negative bacteria strains.

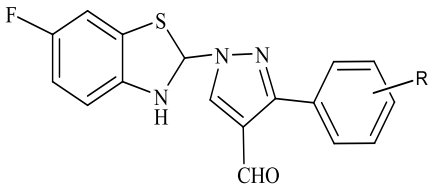
The research done by Reddy *et al.* in 2014, in which a new class of amido-linked pyrazolyl benzoxazoles, benzothiazoles and benzimidazoles (compound **z**) **Figure 1.2** was prepared. Compounds were tested for their antimicrobial activity against some Gram positive bacteria, Gram negative bacteria and fungi. The results, showed that chloro-substituted pyrazole benzothiazole derivative was most potent against *B. subtilis*, while chloro-substituted benzimidazole derivative was most potent against *A. niger*.



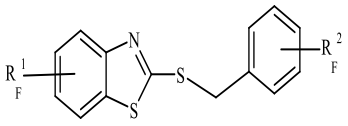
Compound **a**



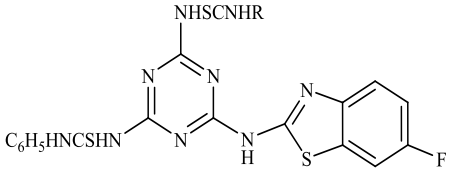
Compound **b**



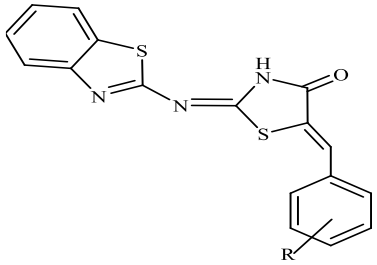
Compound **c**



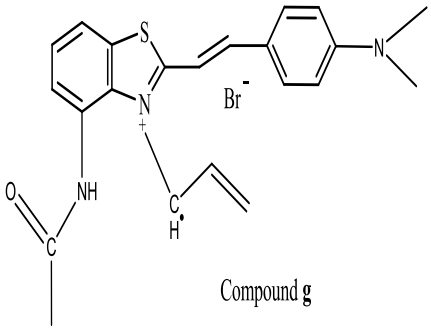
Compound **d**



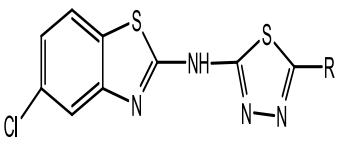
Compound **e**



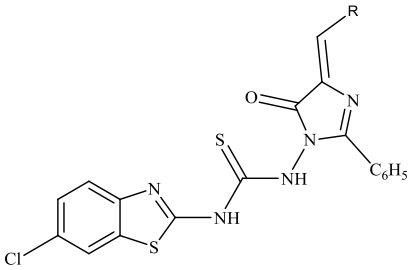
Compound **f**



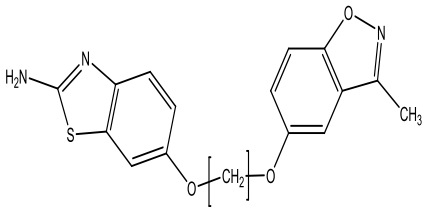
Compound **g**



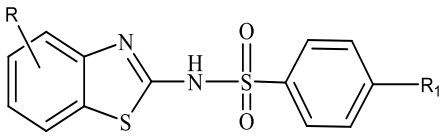
Compound **h**



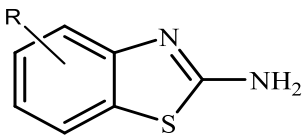
Compound **i**



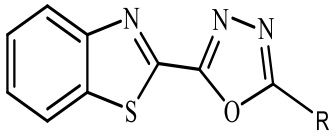
Compound **j**



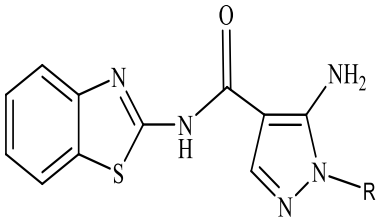
Compound **k**



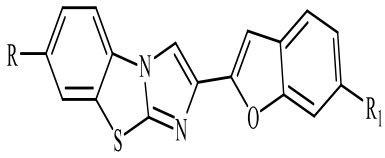
Compound **l**



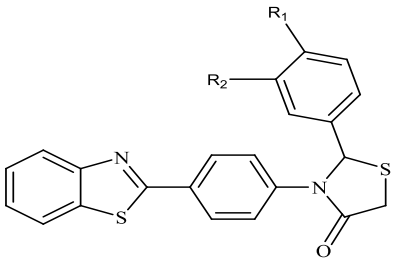
Compound **m**



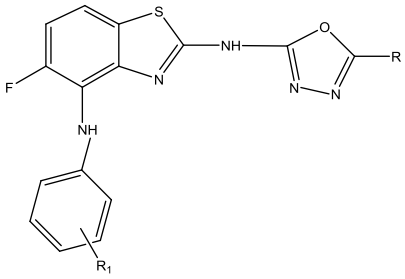
Compound **n**



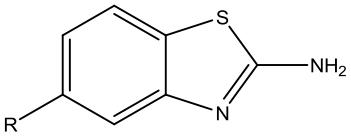
Compound **o**



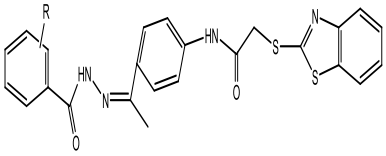
Compound **p**



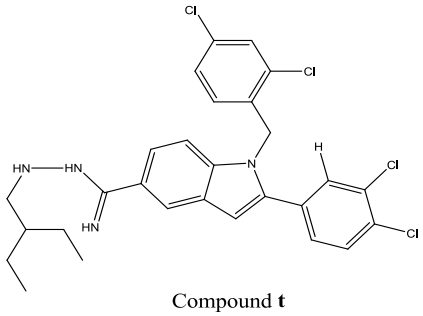
Compound **q**



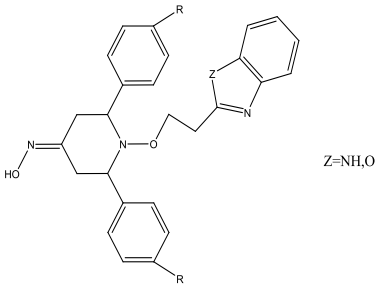
Compound **r**



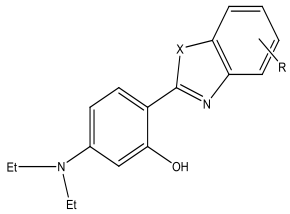
Compound **s**



Compound **t**



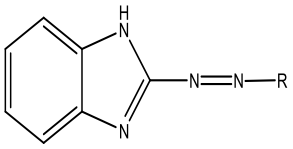
Compound **u**



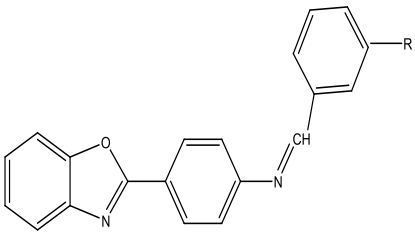
Compound **v**

R=H,NH_2

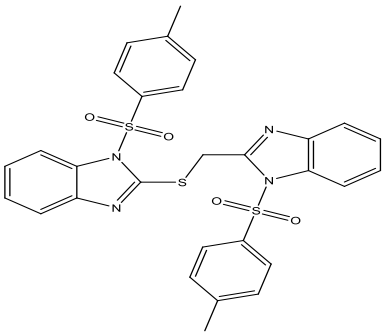
X=NH,S,O



Compound **w**



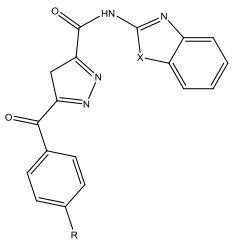
Compound **x**



Compound **y**

R=H,CH_3,Cl

X = O,S,NH



Compound **z**

Figure **1.2**: Different **benzothiazole, benzoxazole and benzimidazole** derivatives (compounds **a-z**).

The successful treatment with any therapeutic agent is compromised by the potential development of tolerance or resistance to that compound from the time it is first employed (Davies *et al.*, 2010). Antimicrobial agents are among the most used and misused of all drugs used for treatment (Frieden 2013). The use of antibiotics is the single most important factor leading to antibiotic resistance around the world (Frieden 2013). This misuse leads to the emergence of antibiotic-resistant pathogens, fueling an increased need for new drugs. Bacterial resistance also imposes a significant financial burden on world economies, with the USA alone spending an estimated $35 billion per annum on resistant infection problem (Frieden 2013). A wide range of biochemical and physiological mechanisms may be responsible for the resistance **Figure 1.3**. In the specific case of antimicrobial agents, the complexity of the processes that contribute to the emergence and dissemination of resistance cannot be overemphasized, and the lack of basic knowledge on these topics is one of the primary reasons that there has been so little significant achievement in the effective prevention and control of resistance development (Davies *et al.*, 2010). Antibiotic resistance has been isolated in virtually all environments (Perron *et al.*, 2015). The total diversity of resistance genes in the wild is hard to estimate (Nesme *et al.*, 2014). The Comprehensive Antibiotic Resistance Database lists over 1600 annotated resistance genes, a number that is constantly increasing (McArthur *et al.*, 2013). Also the emerging of new pathogens contributes to this interest (Gilani *et al.*, 2012). Many of the bacterial pathogens associated with epidemics of human infections have evolved into multidrug-resistant subsequent to antibiotic use (Davies *et al.*, 2010). Antibacterial resistance is a serious public health problem and the prevalence of multidrug- and pandrug-resistant organisms in tertiary medical institutions has compounded concerns for patients (Xiao *et al.*, 2015). The significance and impact of antibiotic resistance on

human health are widely recognized (Spellberg *et al.*, 2008; Rogers *et al.*, 2012). Therefore, there could be a rapidly growing global crisis in the clinical management of life-threatening infectious diseases caused by multidrug-resistant strains of the Gram-positive pathogens like *Streptococcus, Enterococcus,* and *Staphylococcus,* and Gram-negative pathogens like *Escherichia, Salmonella,* and certain *Pseudomonas* strains. Especially the emergence of multidrug-resistant strains of Gram-positive bacterial pathogens such as methicillin-resistant *S. aureus* and *Staphylococcus epidermis* and vancomycin-resistant *Enterococcus* is an alarming problem of ever increasing significance (Yilmaz *et al.*, 2013). Bacteria that are resistant to large numbers of antibiotics are popularly designated as superbugs (Tortora *et al.*, 2013). The containment of microbial drug resistance has become a global priority, and in 2011 the World Health Organization (WHO) highlighted concerns with their Combat Drug Resistance: No Action Today No Cure Tomorrow policy. The WHO recommended more extensive surveillance of drug resistance, catching up with the dynamic development of drug resistance, and improved management strategies (Anon 2014). New forms of antibiotic resistance can cross international boundaries and spread between continents with ease (Frieden 2013). World health leaders have described antibiotic resistant microorganisms as “nightmare bacteria” that “pose a catastrophic threat” to people in every country in the world (Frieden 2013). Almost 250,000 people each year require hospital care for *Clostridium difficile (C. difficile)* infections. In most of these infections, the use of antibiotics was a major contributing factor leading to the illness. At least 14,000 people die each year in the United States from *C. difficile* infections. Many of these infections could have been prevented (Frieden 2013). A survey in rural Bangladesh showed that only 8% of antibiotics had been prescribed by a physician. In much of the world, antibiotics are sold to treat headaches and for other inappropriate uses

(Tortora *et al.*, 2013). Even when the use of antibiotics is appropriate, dose regimens are usually shorter than needed to eradicate the infection, thereby encouraging the survival of resistant strains of bacteria (Tortora *et al.*, 2013). Based on 2007 data, outpatient care costs were estimated at about EUR 10 million and productivity losses due to absence from work of infected patients were estimated at more than EUR 150 million, each year productivity loss due to patients who died from their infection were estimated at about EUR 450 million each year. Overall societal costs of infections due to the selected antibiotic-resistant bacteria were estimated at about EUR 1.5 billion each year (Report 2009). Antibiotic resistance is costly in many ways beyond those that are apparent in higher rates of disease and mortality. Developing new drugs to replace those that have lost effectiveness is costly (Tortora *et al.*, 2013).

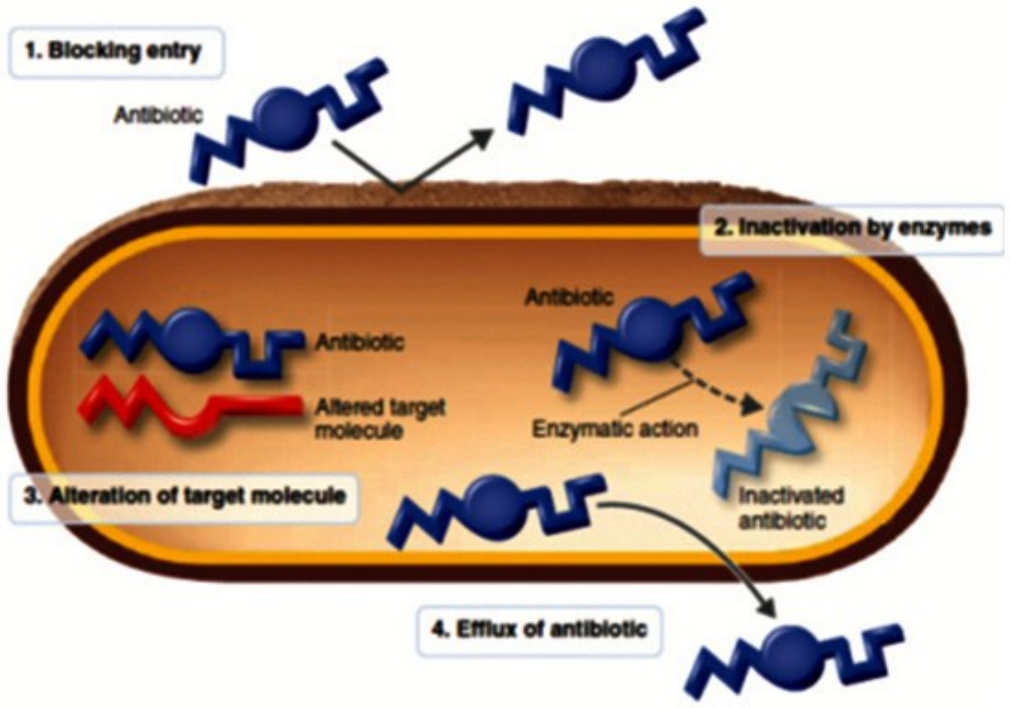


Figure (1.3): Mechanisms of microbial resistance to antimicrobial agents (Tortora et al., 2013).

Figure (1.4): Timeline of Key Antibiotic Resistance Events (Frieden 2013).

More than 100 years ago Christian Gram (1884) developed a staining procedure that allowed him to classify nearly all bacteria into two large groups, and this eponymous stain is still in widespread use (Silhavy *et al.*, 2010). Gram stain is one of the most useful procedures for classification of bacteria into two large groups: Gram-positive and Gram-negative (Tortora *et al.*, 2013). The differences were resting in the structure of their cell wall as shown in **Figure 1.5**. The cell wall of Gram-positive bacteria is host to a wide variety of molecules and serves a multitude of functions, most of which are critical to the viability of the bacterial cell (Navarre *et al.*, 1999). The cell wall of Gram-positive bacteria is a peptidoglycan macromolecule with attached accessory molecules such as teichoic acids, teichuronic acids, polyphosphates, or carbohydrates (Navarre *et al.*, 1999). The chemical structure of the peptidoglycan in Gram-positive bacteria are similar to that in Gram-negative bacteria in that it is composed of a disaccharide-peptide repeat coupled through glycosidic bonds to form linear glycan strands which are cross linked into a meshlike frame work through the peptide stems attached to the disaccharide repeat (Silhavy *et al.* , 2010). The major difference between Gram-positive and Gram-negative bacterial peptidoglycan involves the thickness of the layers surrounding the plasma membrane. Whereas Gram-negative peptidoglycan is only a few nanometers thick, representing one to a few layers, Gram-positive peptidoglycan is 30-100 nm thick and contains many layers (Silhavy *et al.*, 2010). The teichoic acids which are present in Gram positive bacteria cell wall consist primarily of an alcohol and phosphate. There are two classes of teichoic acids: lipoteichoic acid and wall teichoic acid.Because of their negative charge (from phosphate groups), teichoic acids may bind and regulate the movement of cations into and out of the cell (Tortora *et al.*, 2013). The surfaces of Gram-positive bacteria are decorated with a variety of proteins, some of which are analogous to proteins found in the periplasm

of Gram-negative bacteria (Dramsi *et al.*, 2008). Conversely, as mentioned before the cell wall of Gram-negative bacteria consist of one or a very few layers of peptidoglycan with outer membranes. The outer membrane is a distinguishing feature of Gram negative bacteria; Gram-positive bacteria lack this organelle. The outer membrane does contain phospholipids; they are confined to the inner leaflet of this membrane. The outer leaflet of the outer membrane is composed of glycolipids principally lipopolysaccharide (Kamio and Nikaido 1976). The outer membrane plays a major role in protecting Gram-negative bacteria from the environment by excluding toxic molecules and providing an additional stabilizing layer around the cell (Silhavy *et al.*, 2010). Gram-negative bacterial cell walls do not contain teichoic acids. Because the cell walls contain only a small amount of peptidoglycan, they are more susceptible to mechanical breakage (Tortora *et al.*, 2013). The outer membrane of the Gram-negative bacteria consists of lipopolysaccharide, lipoproteins and phospholipids. Its strong negative charge is an important factor in evading defenses of the host like phagocytosis (Tortora *et al.*, 2013). The outer membrane also provides a barrier to certain antibiotics and digestive enzymes. Part of the permeability of outer membrane is due to the presence of proteins in the membrane, called porins. Porins permit the passage of molecules such as nucleotides, peptides and vitamins (Tortora *et al.*, 2013). The composition and structure of bacterial cell walls, and their variation as a function of different environmental variables (e.g., pH solution and substrate composition), is responsible for most surface interactions of bacteria (Jiang *et al.*, 2004).

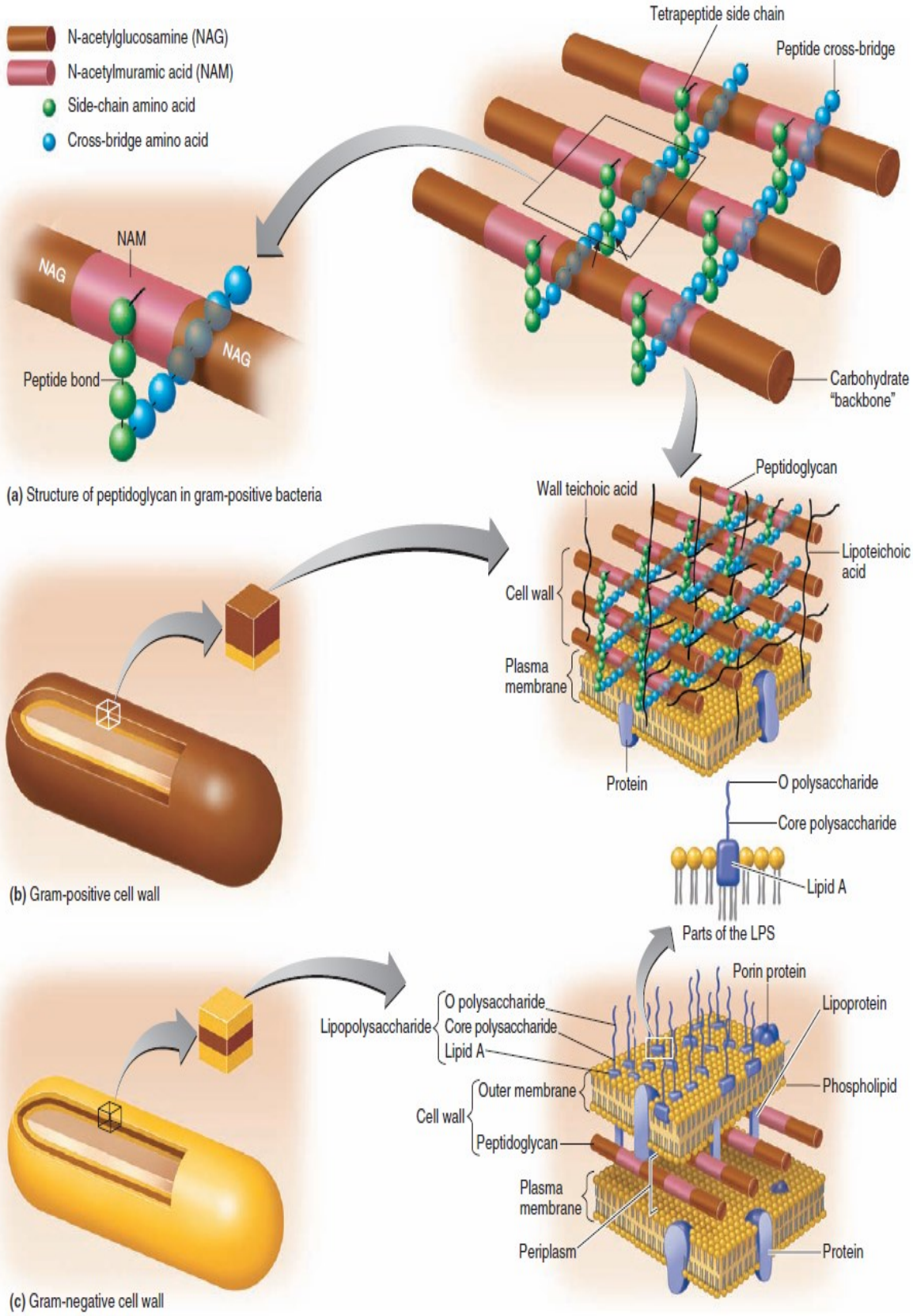


Figure (1.5): Cell wall of Gram positive and Gram negative bacteria (Tortora et. al., 2013)

Antimicrobial agents interfere with specific processes that are essential for bacterial growth and/or division (Harold and Gootz, 1996). They reduce or completely block the growth and multiplication of bacteria **Figure 1.6**. Cell wall synthesis is inhibited by ß-lactam antibiotics, such as penicillins and cephalosporins, which inhibit peptidoglycan polymerization, and by vancomycin, which combines with cell wall substrates while; Polymyxins disrupt the plasma membrane, causing bacterial leakage. The plasma membrane sterols of fungi are attacked by polyenes (amphotericin) and imidazoles. Quinolones bind to the bacterial complex of DNA and DNA gyrase, blocking DNA replication. Nitroimidazoles damage the DNA. Rifampin blocks RNA synthesis by binding to DNA directed RNA polymerase. Aminoglycosides, tetracycline, chloramphenicol, erythromycin, and clindamycin all interfere with ribosome function. Sulfonamides and trimethoprim block the synthesis of the folate needed for DNA replication (Harold and Gootz, 1996).

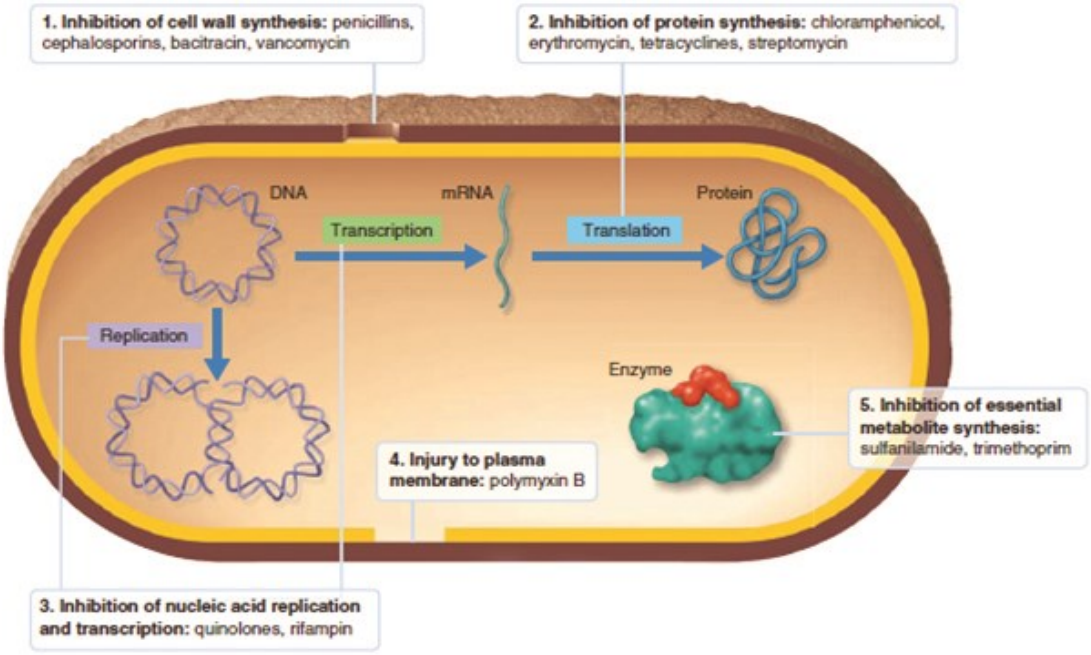


Figure (1.6): Major Modes of action of antimicrobial drugs (Tortora *et al.*, 2013)

For approximately four decades (from the 1940s up to the 1970s) the pharmaceutical industry provided a steady flow of new antibiotics **Figure 1.7**, including several with new mechanisms of action that circumvented the problems caused by bacterial resistance to earlier agents (Report 2009). The chosen set of species for in-vitro antimicrobial activity evaluation (*S. aureus* ATCC 6538p*, P. aeruginosa* ATCC 9027, *E. coli* ATCC 8739, *C. albicans* ATCC 10231 and *B. subtilis* ATCC 6633) provides a good model for screening of newly synthesized chemical compounds for antimicrobial activity. They differ in cell wall structure, mechanism of the pathogenicity and susceptibility to antimicrobial drugs. These microorganisms are the cause of many hospital acquired infections (Krawiecka *et al.*, 2013). *S. aureus is a* Gram-positive coccus. It is found in the nose and skin of humans and animals (Tortora *et al.*, 2013). *S. aureus* infections usually cause purulent skin and food poisoning through the production of toxins (Krawiecka *et al.*, 2013). *E. coli* is a Gram negative bacillus, which is the part of the physiological bacterial flora of the colon (Tortora *et al.*, 2013). It may cause urinary tract infections, abscesses, nosocomial infections and food poisoning (Krawiecka *et al.*, 2013). *C.albicans* is a fungus (a form of yeast). It causes many opportunistic infections in the oral cavity and genitals in humans. Systemic fungal infections (fungemias) have emerged as important causes of morbidity in immunocompromised patients (Krawiecka *et al.*, 2013). Under normal circumstances, *C. albicans* lives in 80% of the human population with no harmful effects, although overgrowth results in candidiasis (Krawiecka *et al.*, 2013). In the last few years it was reported that benzothiazole, its bioisosters and derivatives had antimicrobial activities against Gram-negative, Gram-positive bacteria (e.g., *Enterobacter, P. aeruginosa, E. coli,* and *Staphylococcus epidermidis* etc.) and the yeast e.g. *C. albicans* (Yadav *et al.*, 2011).

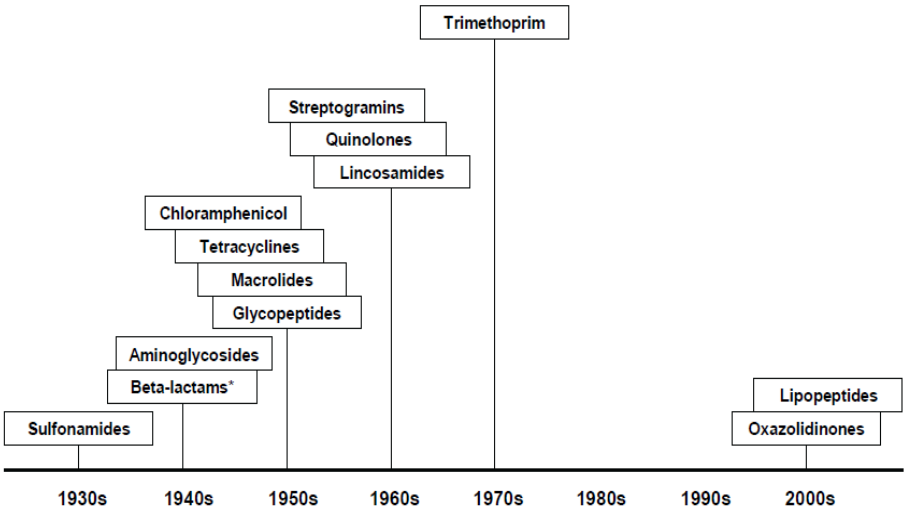
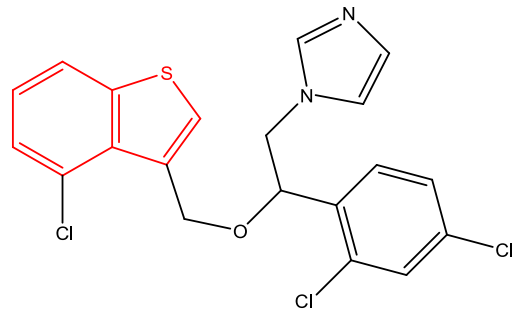
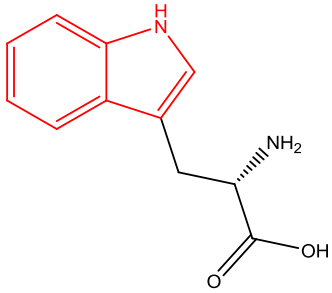


Figure (1.7): Discovery of new classes of antibiotics (Report 2009).

Sertaconazole(1-(2-((4-chlorobenzo[b]thiophen-3-yl)methoxy)-2-(2,4-dichlorophenyl) ethyl)-1H-imidazole) is a broad spectrum antifungal agent with excellent activity against yeasts, dermatophytes and opportunistic fungi. Sertaconazole owns a benzothiophene ring in its structure. The role of Benzothiazole may imitate the chloro benzothiophene role in exerting antifungal activity as an analogue to amino acid tryptophan found on the fungal membrane through changes in the pores opening, and these changes in pore size lead to the loss of its intracellular content mainly ATP, sugars and minerals. The fungus lacks energy and dies (Muhi- El deen, 2011).



Sertaconazole :1-(2-((4-chlorobenzo[*b*]thiophen-3-yl)methoxy)-2-(2,4-dichlorophenyl)ethyl)-1*H*-imidazole



Tryptophan

Figure 1.8 : Structure similarity between benzothiophene ring in Sertaconazole and Tryptophan.

1.2 Aim of investigation:

The development of a clean procedure for the preparation of heterocyclic compounds is a major challenge in modern heterocyclic chemistry in view of the environmental, practical and economic issues. The structure–activity relationships (SAR) of previously synthesized compounds indicated that the fused heterocyclic nucleus was important for the antimicrobial activity. Benzothiazole and its derivatives are the most important heterocyclic compounds, which are common and integral feature of a variety of natural products and pharmaceutical agents (Keri *et al.*, 2015). The literature review revealed that many interesting derivatives are still lacking which may represent a novel approach to generate more potent antibacterial and antifungal agents namely aminoacetylenic side chain at position number two of benzothiazole. This unique aminoacetylenic side chain provides additional forces of interaction with the microorganism such as cell wall or essential enzymes. Also the aminoacetylenic side chain is important to the activity against Gram positive bacteria, Gram negative bacteria and fungi. Our novel compounds provide the benzothiazole heterocyclic ring that represents the basic fractional analysis directed towards bacteria and fungi. Then aminoacetylenic side chain provides the critical groups to provide greater potency as exemplified by acetylenic group for electrostatic interaction, and cyclicamine to provide ionic interaction, in addition to the appropriate distance between the basic cyclic amine and benzothiazole. All these new and unique groups are expected to generate effective antibacterial and antifungal agents.

Chapter two

2. Experimental:

2.1 Material and methods:

2.1.1 The following chemicals and materials were used:

- Benzothiazole, Piperidine, Propargyl bromide, 2-Methylpiperidine, 2,6-Dimethyl-piperidine, Pyrrolidine, 1-Methyl-piperazine, Hexamethylinimine, DMSO (Sigma-Aldrich, U.S.A).

- Potassium carbonate anhydrous, Chloroform, Ethanol (Finland Chemical Company, U.K).

- Cuprous Chloride, 1,4-Dioxane (Full Time Chemicals, China).

- Acetonitrile (SDFCL Chemicals, India).

- Paraformaldehyde (BDH Chemicals, U.K).

2.1.2 Instruments:

- Analytical balance with a precision of 0.01mg (Sartorius, Germany)

- Hot plate with magnetic stirrer (Stuart Scientific, U.K)

- Heidolph WB 200 Rotary evaporator (Germany).

- G24 Environmental incubator shaker (New Brunswick Scientific Co., USA).

- Static incubator (Memmert, West Germany).

- Trade Raypa Steam Sterilizer (Spain).

- Vortex mixer (Labinco, the Netherlands).

- Micropipette (Edvotek, U.S.A).

- Varian 300 MHz spectrometer (U.S.A).

- Gallenkamp Melting Point Apparatus (U.S.A).

- Bruker FT-IR spectrophotometer (U.S.A).

- Euro EA elemental analyser (Italy) .

The structures of the synthesized compounds were confirmed by IR, ^{1}HNMR, ^{13}C-NMR and elemental analysis. Melting points were determined on Gallenkamp Melting point Apparatus. Infrared (IR) spectra were recorded on Bruker FT-IR spectrophotometer, using KBr discs and values were represented in cm-1. ^{1}H NMR and ^{13}C-NMR spectra were measured on a Varian 300 MHz spectrometer and DMSO-d_6 as solvent with TMS (Tetramethylsilane) as the internal standard. ^{1}H data are reported in order: multiplicity (br, broad; s, singlet; d, doublet; t, triplet, m, multiplet). The elemental analysis were performed for C, H, N using (Euro EA elemental analyser , University of Jordan).The results obtained had a maximum deviation of (+2.59 to -2.95) from the theoretical value, which is considered within the acceptable variation range in results (±4.4 %) . This variation range is set according to the accuracy of Euro EA Elemantal Analyser device.

2.1.3 Synthesis of of 2-(prop-2-yn-1-yl)-1,3-benzothiazole(BZ1) :

A mixture of Propargyl bromide (1.88g,15.8mmol) in Acetonitrile (10ml) was added to mixture of Benzothiazole (1.93g, 13.2mmol) and Potassium carbonate anhydrous (2.18g,15.88mmol) in Acetonitrile (20ml). The reaction mixture was heated and stirred under reflux for 80 min. After cooling, the insoluble residue was removed by filtration, and then the solvent was removed under reduced pressure. After that, 30 ml chloroform and 20 ml distilled water (D.W) was added and the filtrate was extracted using a separatory funnel. The organic layer was concentrated by the removal of chloroform under reduced pressure to afford the desired orange powder compound **BZ1 (Figure 2.1)**.

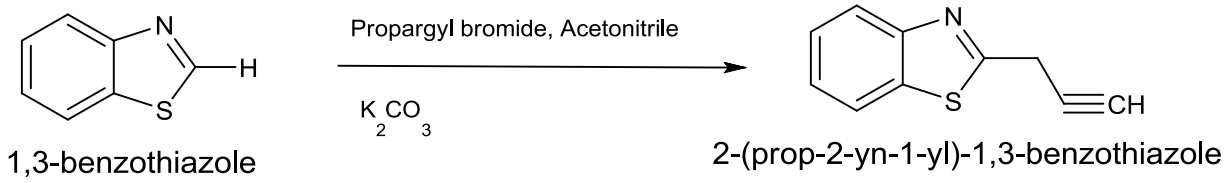


Figure (2.1): Synthesis of 2-(prop-2-yn-1-yl)-1,3-benzothiazole **(BZ1)**

2.1.4 Synthesis of 2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7) :

A mixture of 2-(prop-2-yn-1-yl)-1,3-benzothiazole **BZ1** (1.5g,0.01 mol) , Paraformaldehyde (0.5g,0.015 mol) , the cyclic amine around (0.01mol), and catalytic amount of Cuprous chloride (0.03gm) , in Peroxide-free 1,4-Dioxane(30ml) was stirred and refluxed for 1 hour. After cooling, the insoluble residue was removed by filtration then the solvent was distilled off under reduced pressure. Ethanol (30ml) was added to the residue, and then ethanol was evaporated under reduced pressure. The residue left was dissolved in the least amount of ethanol, precipitated with water, filtered and dried to afford Mannich bases **BZ2, BZ3, BZ4, BZ5, BZ6, BZ7 (Figure 2.2)**. Physical and spectral data for the resulting compounds **(BZ1-BZ7)** are listed below in the results.

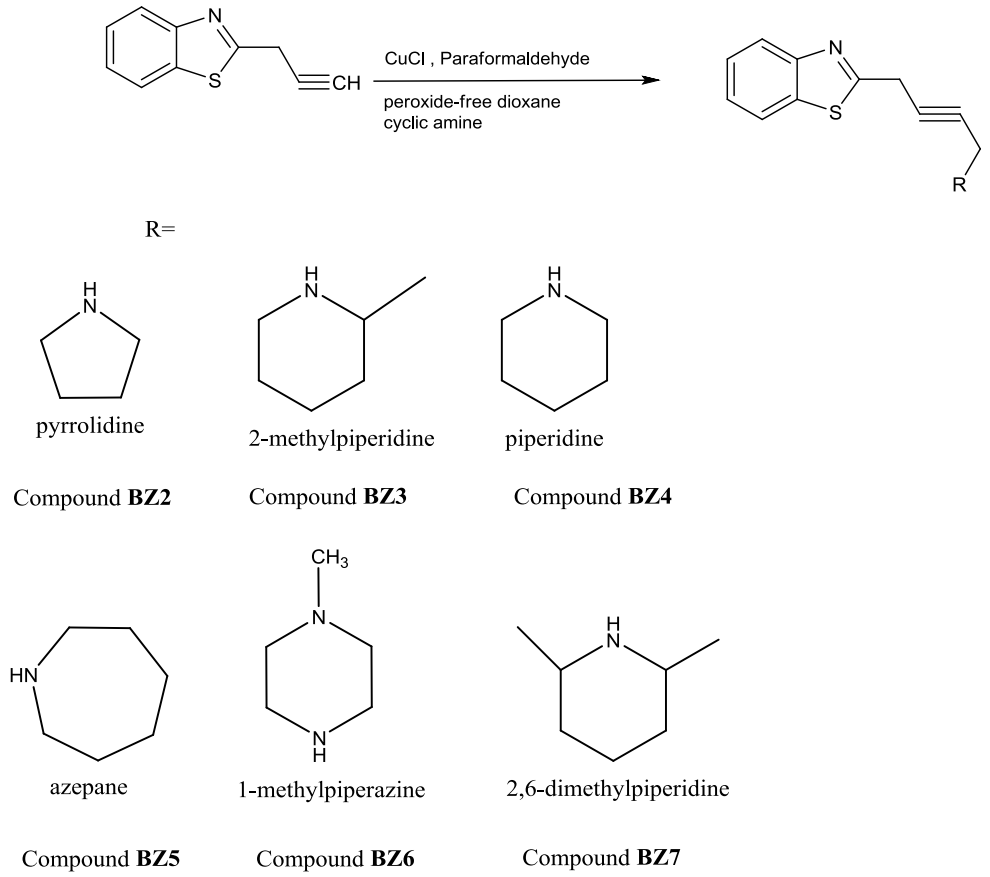


Figure (2.2): Synthesis of t-amino [but-2-yn-1-yl]-1,3-benzothiazole derivatives **(BZ2-BZ7)**

2.2 Antimicrobial Activity (Materials and equipments):

2.2.1 Culture media:

Muller Hinton Agar CM0337 (MHA), Muller Hinton Broth CM0405 (MHB), Nutrient Agar CM0003 (NA), Sabourauds Dextrose Agar 1461507 (SDA), and Sabourauds Dextrose Broth 1260675 (SDB) were obtained from Oxoid Laboratories, U.S.A.

2.2.2 Preparation and Sterilization of Culture Media:

All culture media were prepared according to the manufacturer's directions indicated on the culture media bottles. After dissolving the medium in distilled water (D.W), the pH was adjusted to the specified pH for each culture medium with the addition of either 0.1M NaOH or 15% v/v H_3PO_4. The medium was then sterilized by autoclaving at 121 °C, 15 psi for 15 to 20 minutes.

2.2.3 Test Microorganisms:

Staphylococcus aureus ATCC 6538p*, Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231 and *Bacillus subtilis* ATCC 6633 were kindly obtained from Dar Al Dawa (Na'ur, Jordan).

2.3 Method for obtaining pure cultures:

2.3.1 Streak Plate method:

The isolation method most commonly used to get pure cultures is the streak plate method (Tortora *et al.*, 2013). The streak plate technique was chosen to obtain separate colonies of microorganism, as it is the simplest and, most commonly used method to obtain pure cultures (Tortora *et al.*, 2013). In this technique, a loopful (0.1µl) of overnight culture was streaked across the surface of a sterile solidified nutrient agar plate for bacteria and Sabouraud Dextrose agar for fungi. The plates were incubated at 37^{o}C for 24 hrs for bacteria and at 20 ^{o}C for 48 hrs for fungi. The principle of streaking method ensured the separation of microorganisms in a concentration gradient manner across the surface of the agar, in which confluent growth of microorganisms occurred on part of the plate where there was no sufficient separation. On the other hand, individual isolated colonies were obtained in other parts of the plate where only few numbers of bacteria were deposited to form separate colonies that were easily seen and distinguished by the naked eye. Each isolated colony was created from a single bacterium cell or fungal cell and therefore, represented a clone of the pure original culture. A subculture of one isolated colony onto NA for bacteria and SDA for fungi by streak method gave pure cultures which were used as the original microorganism cultures for further investigations.

2.3.2 Bacterial and Fungal Cultures:

The direct colony suspension method is the most convenient method for inoculums preparation. This method can be used with most organisms (Clsi, 2012). Cultures of *S. aureus* (ATCC 6538p), *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027) and *B. subtilis* (ATCC 6633) were maintained and stored on nutrient agar plates in a dark place at a temperature of 20 ±2 °C after being incubated at 37°C for 24 hours. Cultures of *C. albicans* (ATCC 10231) were maintained and stored on Sabouraud Dextrose agar plates and kept in the same condition after being incubated at 20 °C for 48 hours. Fresh cultures from each species mentioned above were prepared every week and their morphological characteristics confirmed by macroscopic and microscopic examination.

2.3.3 Viable Count Procedure:

The direct viable count (DVC) is one of the most reliable methods for the counting of bacterial and fungal cells (Tortora *et al.*, 2013). This method involves a serial dilution of an overnight culture of bacteria or fungi, from each dilution a measured amount (0.1ml) is uniformly spread with the sterile bent glass rod over the surface of an appropriate solid growth medium, nutrient agar for bacteria or Sabouraud Dextrose agar for fungi. The plates are incubated at 37°C for 18 to 24 hrs for bacteria, while for fungi, at 20 °C for 24 to 36 hrs, in order to allow bacteria and fungi to grow into observable colonies. It is assumed that each colony arises from a single bacterium or fungal cell. Thus, counting the numbers of observable colonies that develop and multiplying it by the dilution factors will give the original concentration of bacteria or fungi as colony forming units (CFU). Plates with number of colonies less than 30 CFU are not acceptable statistically, while plates with more than 300 CFU are more likely to produce colonies that are too close to be

distinguished as separate individual CFU (Roszak and Colwell 1987; Dupray, *et al.* 1993; Salvesen and Vadstein 2000).

Viable count is calculated as:

$$CFU=N\ (1/DF)$$

Where N is the number of colonies per plate and DF is the dilution factor. A count of 1.0×10^6 CFU/ml was used.

2.4 Statistical Methods:

All experiments for detection of antimicrobial activities were designed to allow for statistical analysis and were performed in triplicates. Experimental data presented in this study represent the mean of those triplicate data sets.

2.5 Procedure for antimicrobial activity evaluation:

Benzothiazole is an bicyclic ring system, made from thiazole ring fused with benzene ring. Thiazole is an aromatic five membered ring featuring both sulfur and nitrogen atoms. New compounds were synthesized incorporating benzothiazole moiety with aminoacetylenic side chain. Benzothiazole compounds were known from long ago to have varied biological activities (Kaur *et al.*, 2010; Prabhu *et al.*, 2011; Yadav *et al.*, 2011). In this study, we examined substituted derivatives of 2-(prop-2-in-1-yl)-1,3-benzothiazole, our aim was to estimate the antimicrobial activity and the MIC values for each compound against five microorganisms, *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis* and *C. albicans*. All the newly synthesized compounds **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)** were tested for in vitro antimicrobial activity by using two methods: the first method was the agar well diffusion method, by measuring the diameter of inhibition zone. The second method is the determination of minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) against the selected microorganisms. The synthesized compounds were screened for antimicrobial activity against two Gram negative microorganisms ***E. coli*** (ATCC 8739) and ***P. aeruginosa*** (ATCC 9027) and two Gram positive microorganisms ***S. aureus*** (ATCC 6538p) and ***B. subtilis*** (ATCC 6633) on Muller Hinton agar medium at 200 ,100 ,50 ,25 mcg/ml concentrations. Screening of antifungal activity was done against ***C. albicans*** (ATCC 10231) on Sabourauds dextrose agar medium at 200 , 100 ,50 , 25 mcg/ml. Stock solutions of the compounds were first prepared by dissolving them in a solution of

(30% DMSO in water). Then the solutions were diluted serially in the media (Muller Hinton Broth for bacteria and Sabourauds Dextrose Broth for fungi) so as to achieve concentrations of the compound ranging from 25-200 mcg/ml. A solution of 30% DMSO in distilled water (D.W) was employed as solvent control.

2.5.1 Agar Diffusion Method:

The diffusion method is the most widely used method to test microbial susceptibility because of its simplicity (Tortora *et al.*, 2013). A petri dish containing MHA medium was inoculated (seeded) uniformly over its entire surface with 0.1ml (1.0×10^5 CFU/ml) of one of the tested bacteria suspension (*E. coli, S. aureus, P. aeruginosa and B. subtilis*) using sterile bent glass rod (spreader). A stainless steel cork borer was used to bore the wells. The wells were filled serially with 0.2 ml of the compound solutions **(BZ2-BZ7)** which have four serial dilutions 200,100,50,25 mcg/ml with the help of micropipette and sterile tips as shown in **(table 2.1).** The plates were allowed to stand for 15 minutes to allow the compounds to diffuse into the medium. Then the plates were incubated at 37^o C for 18 hrs. In case of fungi the same procedure was done, however, SDA was used as culture media and the incubation period was 48 hrs at 37^{o}C. If the compound is effective, a zone of inhibition forms around the well. The diameter of the zone was measured and compared to the diameter formed around the well containing the positive controls (Ciprofloxacin disk containing 5mcg and Fluconazole 500mcg/ml dissolved in 30% DMSO in D.W). A negative control was also included containing 30% DMSO in sterile D.W. The test for each compound was done in triplicate, the mean diameter was calculated.

Table (2.1): Preparation of serial concentrations of the synthesized compounds **(BZ2-BZ7)** for agar diffusion method.

Test tube no.	Content	Concentration
1 (Stock solution)	10mg of compound powder dissolved up to 5ml in 30% DMSO in D.W	**2000 mcg/ml**
2	1ml taken from test tube no.1 then add 30%DMSO in D.W was added up to 10ml	**200 mcg/ml**
3	1ml taken from test tube no.2 then add to 1ml 30% DMSO in D.W.	**100 mcg/ml**
4	1ml taken from test tube no.3 then add to 1ml 30% DMSO in D.W.	**50 mcg/ml**
5	1ml taken from test tube no.4 then add to 1ml 30% DMSO in D.W.	**25 mcg/ml**
6	6 ml DMSO then add D.W up to 20 ml	**30% DMSO in D.W (Negative Control)**

2.5.2 Broth Dilution Test for the MIC determination:

A broth dilution test is often useful in determining the MIC and the MBC of an antimicrobial drug (Tortora *et al.*, 2013). It is very useful test to check if the compounds are bactericidal not just bacteriostatic (Tortora *et al.*, 2013). Minimum inhibitory concentrations (MICs) are considered the 'gold standard' for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing (Andrews, 2001). The range of antibiotic concentrations used for determining MICs is universally accepted to be in doubling dilution steps up and down from 1 mg/L as required (Andrews, 2001). First, prepare antimicrobial agent stock solutions at concentrations of at least 1000 mcg/ml or 10 times the highest concentration to be tested, whichever is greater (Clsi, 2012). So a stock solution of 1mg/ml of each compound **(BZ2-BZ7)** was prepared in 30% v/v DMSO in water, a sequence of decreasing concentrations of the compounds **(BZ2-BZ7)** in Muller Hinton broth were prepared in a series of test tubes to get a final concentrations ranging from (500-7.8) mcg/ml. MHB is recommended as the medium of choice for susceptibility testing of commonly isolated, rapidly growing aerobic or facultative microorganisms (Clsi,2012). The final volume in each tube was 5 ml. 0.1 ml (1.0×10^5 cfu/ml) of testing microorganisms (*E. coli, S. aureus, P. aeruginosa and B. subtilis*) suspension was added to each of the tubes. Positive control tube with 5ml sterile MHB and 0.1ml of each tested microorganism suspension was included. In addition, a negative control tube with 5ml of the tested compound dilution in MHB without any microorganism was included as shown in **(table 2.2)**. The test tubes were incubated at 37^o C for 24hrs. Similarly, MIC determination of the tested compounds was performed against *C.albicans* however, SDB was used instead of

MHB and the test tubes were incubated at 37^{o}C for 48 hrs. The test for each compound was performed in triplicate. The MIC was determined by finding the test tube with the lowest concentration of the compound in which no turbidity was observed. Unfortunately, the synthesized compounds have characteristic colors of their solutions so; plate method was used to determine the MIC of the synthesized compounds. 0.1 ml of the suspected MIC tube were subcultured on MHA with uniformed spreading for each of the compounds, then colony count was done , MIC tube should give colonies count less than 1 x 10^4 (control), and the suspected ones are correct.

The MBC is the lowest concentration of antibiotic required to kill a particular bacterium (Yilmaz *et al.*, 2012). All of the susceptibility test methods commonly performed by clinical microbiology laboratories (e.g., disk diffusion, broth dilution, and agar dilution) measure the inhibitory activity (MIC) of an antimicrobial agent (Clsi, 1999). On occasion, it may be necessary to achieve bactericidal activity with an antimicrobial agent. This need has been well documented for endocarditis and has been suggested by some for meningitis, for osteomyelitis, as well as for infections in immunocompromised patients. The clinical occurrence of tolerance may on rare occasion necessitate bactericidal testing (Clsi, 1999). MBC determination was done to identify if each compound has a bactericidal or bacteriostatic effect. Similarly MFC determination was done to identify if each compound has a fungicidal or fungistatic effect. In our determination of the MBC, it was the concentration of the compound that gave no colonies when 0.1ml from the MIC tubes which contain higher concentrations of the compound were subcultured into MHA for bacteria for 24 hrs and into SDA for fungi for 48 hrs (for MFC determination). The first tube which gave no visible colonies was the MBC or MFC.

Table (2.2): Serial dilution of the synthesized compounds **(BZ2-BZ7)** for MIC testing.

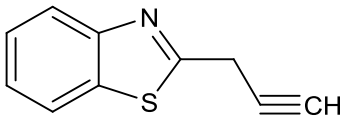
Test tube no.	Content	Final Concentration
1 (Stock Solution)	10 mg of the compound powder **(BZ2-BZ7)** dissolved up to 10ml 30% DMSO in water solution and mixed.	**1mg/ml**
2	5ml taken from test tube no.1 and added to 5ml of MHB or SDB and mixed.	**500mcg/ml**
3	5ml taken from test tube no.2 and added to 5ml of MHB or SDB and mixed.	**250mcg/ml**
4	5ml taken from test tube no.3 and added to 5ml of MHB or SDB and mixed.	**125mcg/ml**
5	5ml taken from test tube no.4 and added to 5ml of MHB or SDB and mixed.	**62.5mcg/ml**
6	5ml taken from test tube no.5 and added to 5ml of MHB or SDB and mixed.	**31.25mcg/ml**
7	5ml taken from test tube no.6 and added to 5ml of MHB or SDB and mixed.	**15.625mcg/ml**
8	5ml taken from test tube no.7 and added to 5ml of MHB or SDB and mixed.	**7.8mcg/ml**
9 (Negative control)	5ml taken from test tube no.8 and added to 5ml of MHB or SDB , mixed then 5ml discarded.	**-**
10 (Positive control)	5ml MHB or SDB with 0.1 ml tested microorganism suspension.	**-**

Chapter three

3. Results:

3.1 Chemistry:

3.1.1 2-(prop-2-yn-1-yl)-1,3-benzothiazole **(BZ1)** :



2-(prop-2-yn-1-yl)-1,3-benzothiazole

Melting point (m.p**)**: (120-121^{o}C)

Yield: 1.4 gm, 80%.

IR (KBr cm-1): 3200 (acetylenic ≡C-H stretch), 2900 (C-H stretch,Ar), 2150 (C≡C stretch), 1600 (Ar C=C stretch), 1465 (-S-,thio group), 780 (Ar C-H bend) **Figure 3.1**.

^{1}H-NMR (DMSO- d_6): δ 2.49 (s, 1H, C≡ CH), 3.38 (s, 2H, CH_2-C≡), 6.8-7.1 (m, 4H, ArH) **Figure 3.2**.

Elemental analysis for **$C_{10}H_7NS$**:

Table (3.1): Elemental analysis for compound **BZ1**.

	C	H	N
Calculated	63.47%	3.73%	7.40%
Found	63.24%	3.51%	7.31%

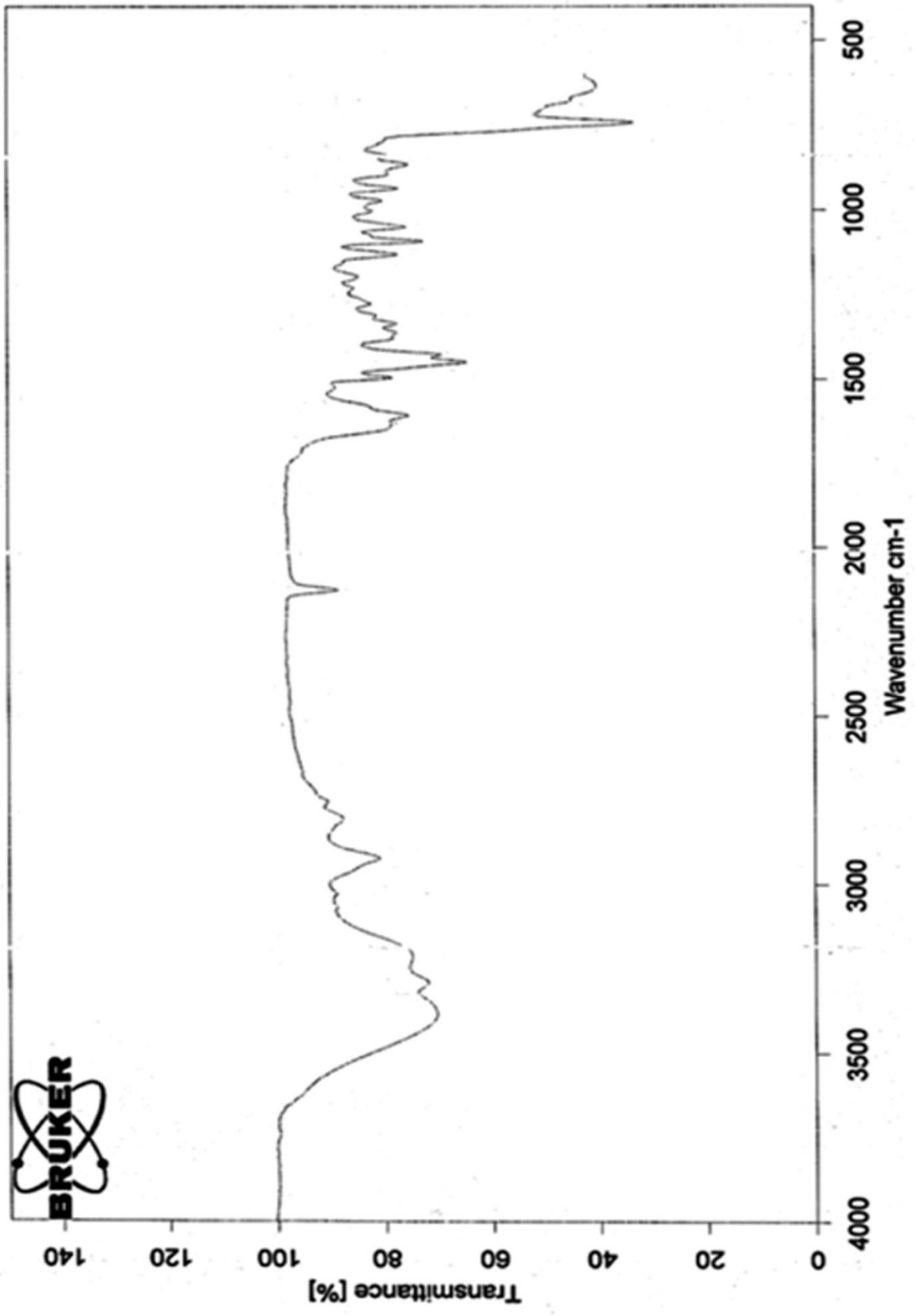


Figure (3.1): IR spectrum of **BZ1.**

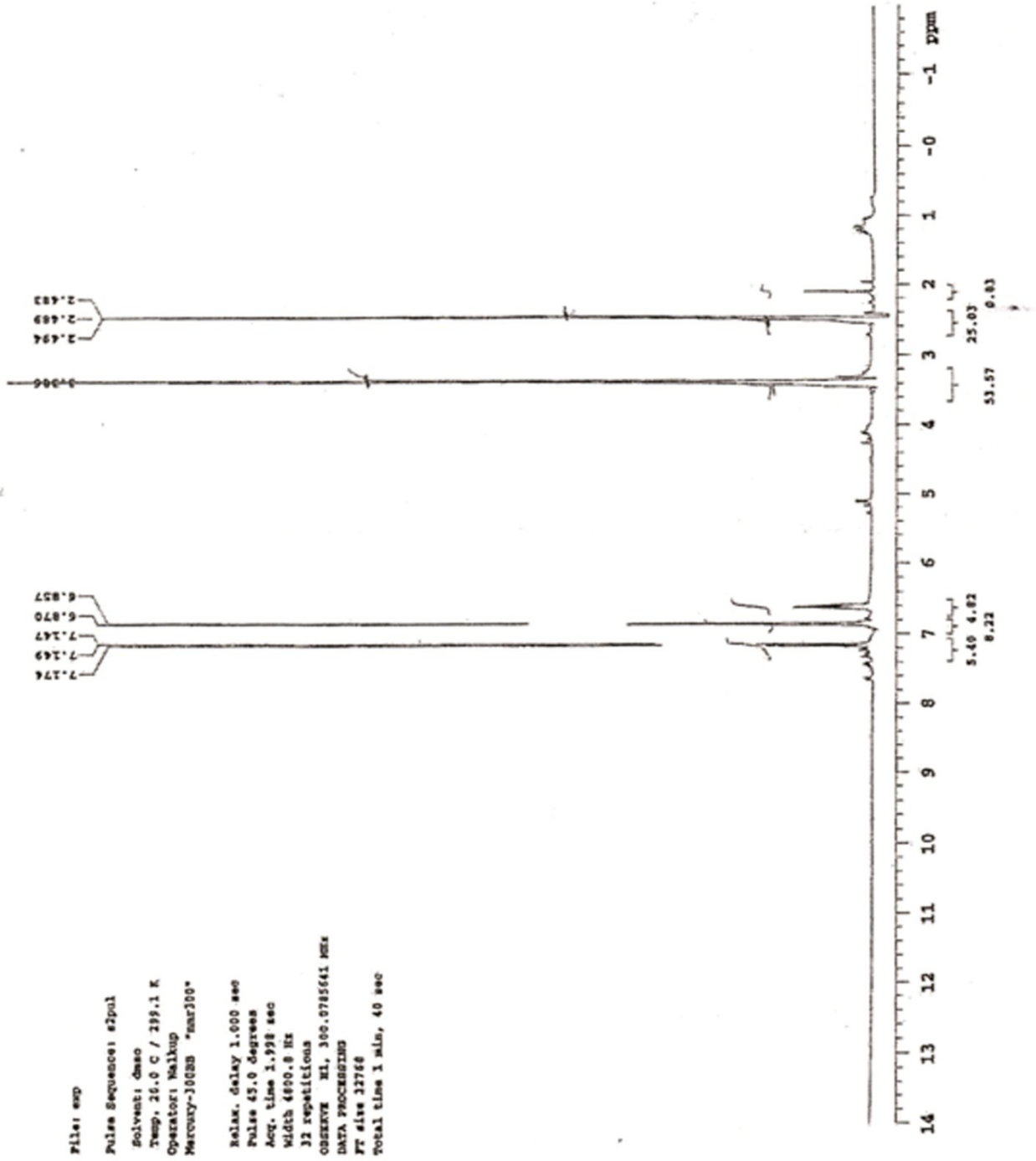


Figure (3.2): [1]HNMR spectrum of **BZ1**.

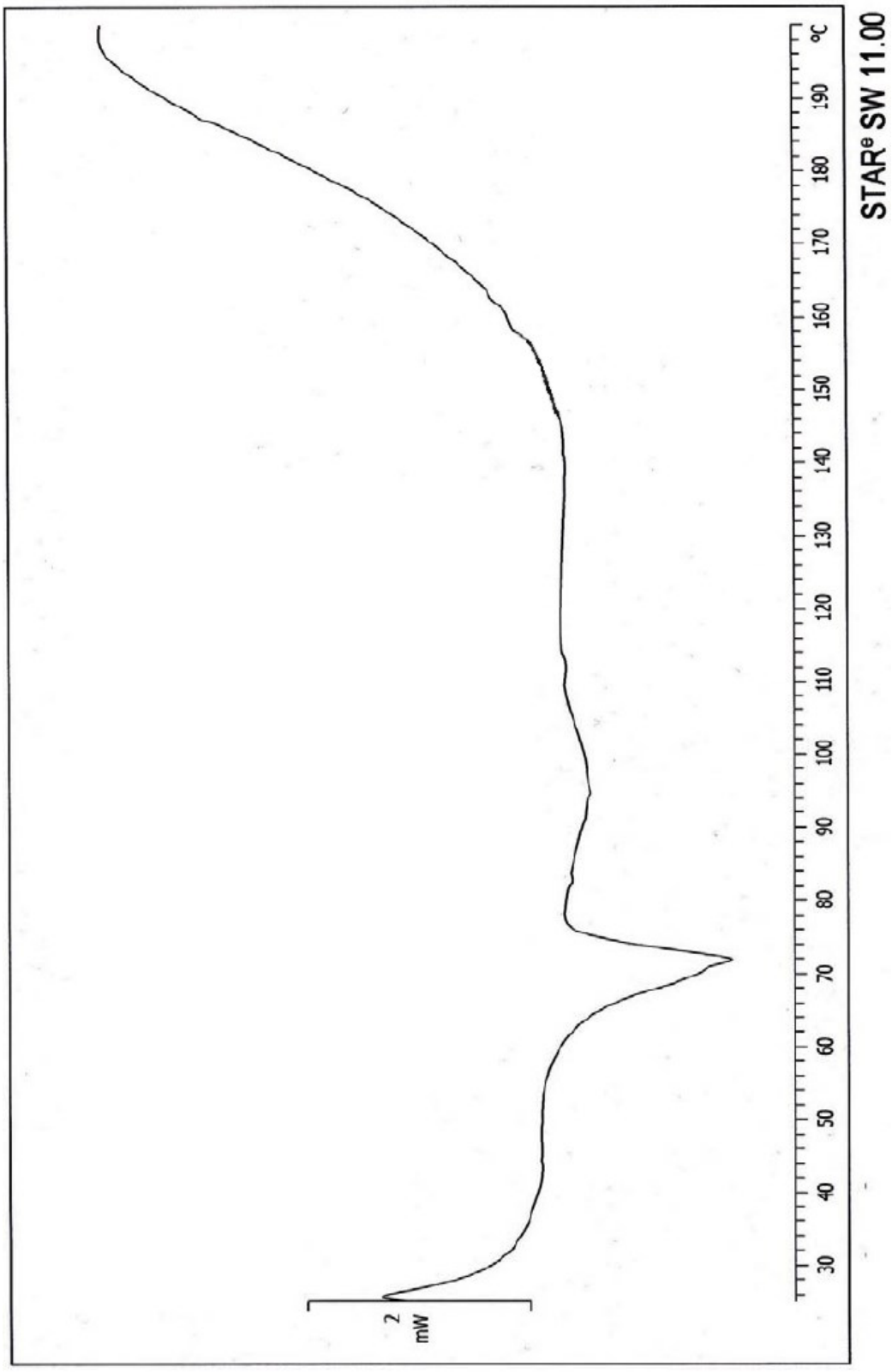
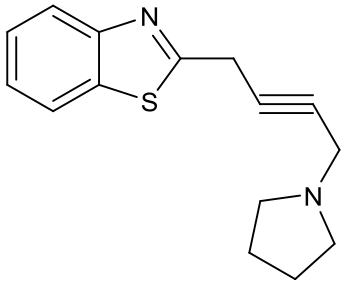


Figure 3.3: DSC thermogram of compound **BZ1**.

3.1.2 2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ2)**:



2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole

m.p: (157-159 ^{o}C).

Yield: 1.4 gm, 54%.

IR (KBr cm-1): 2915 (Ar C-H stretch), 2800 (Ar C-H stretch), 1600 ,1480 (Ar C=C stretch), 1250 (C-N stretch), 1025 (C-N stretch), 850 (Ar C-H bend), 750 (Ar C-H bend) **Figure 3.4**.

^{1}H-NMR (DMSO- d_6): δ, 1.6-1.7 (m, various proton of cyclic amine), 2.4 (s, 2H, C-CH_2-N), 3.8(s, 2H, CH_2-C), 6.6-7.2(m, 4H, ArH) **Figure 3.5**.

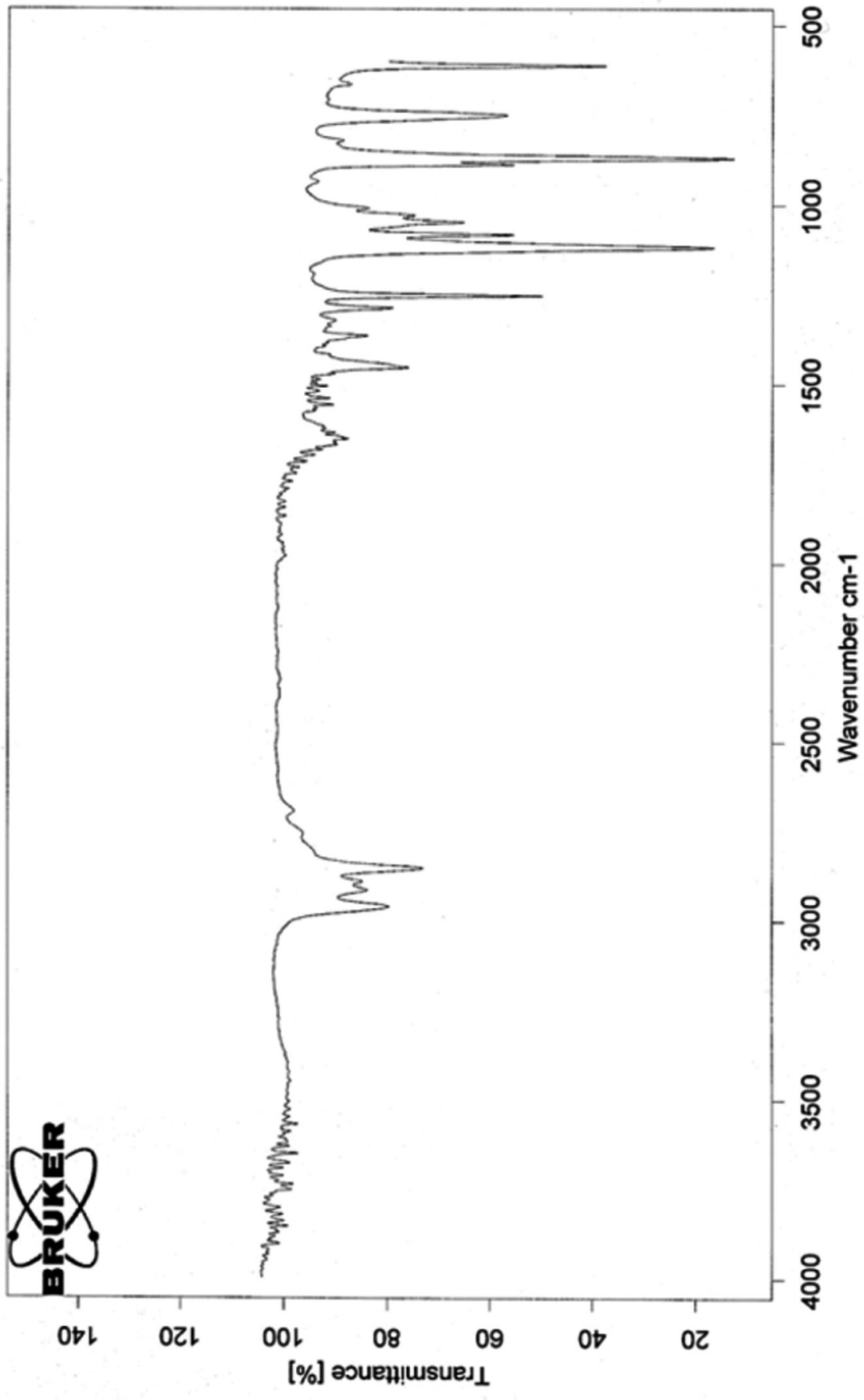


Figure (3.4): IR spectrum of BZ2.

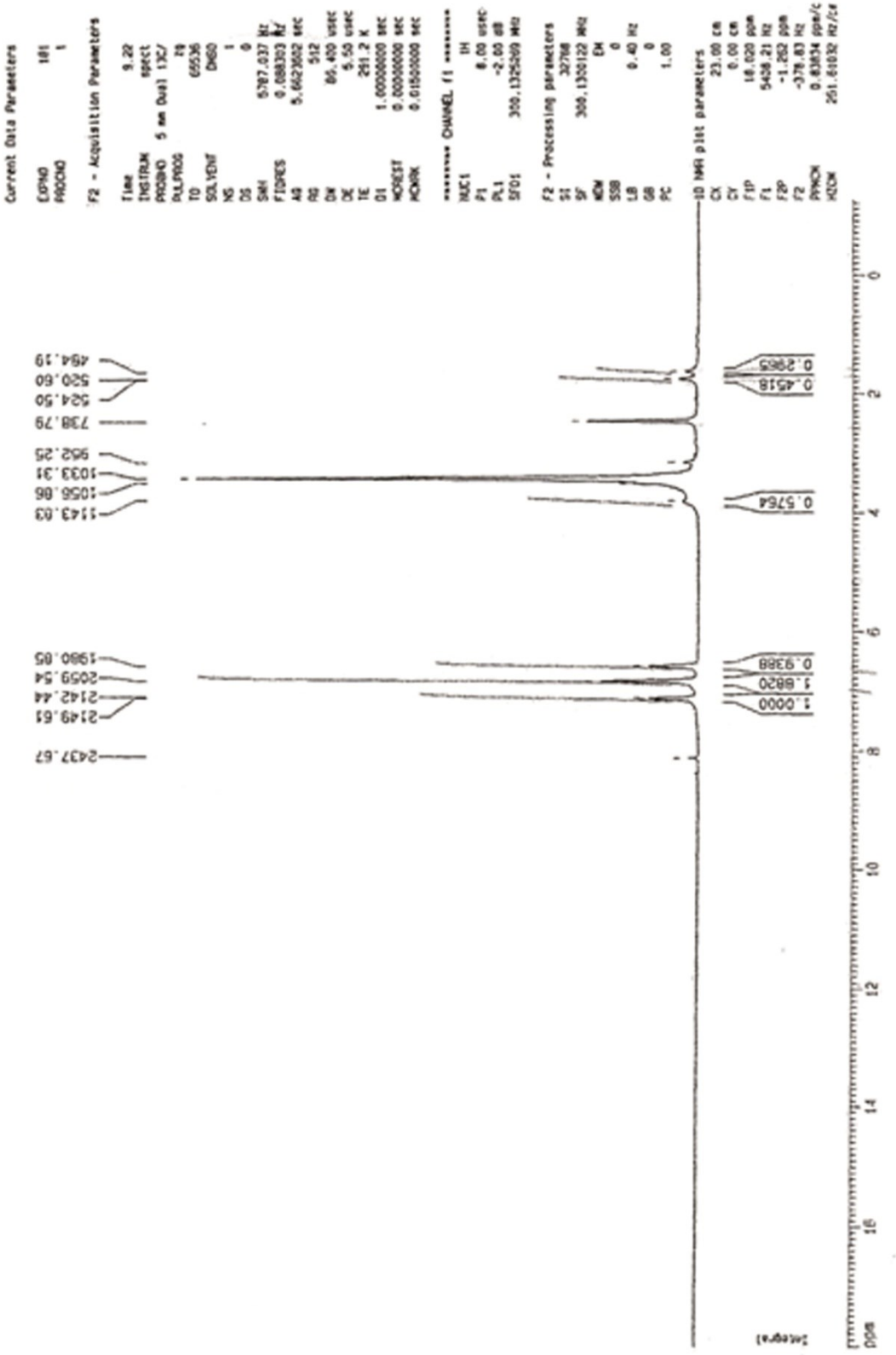


Figure (3.5): ^{1}HNMR spectrum of **BZ2**.

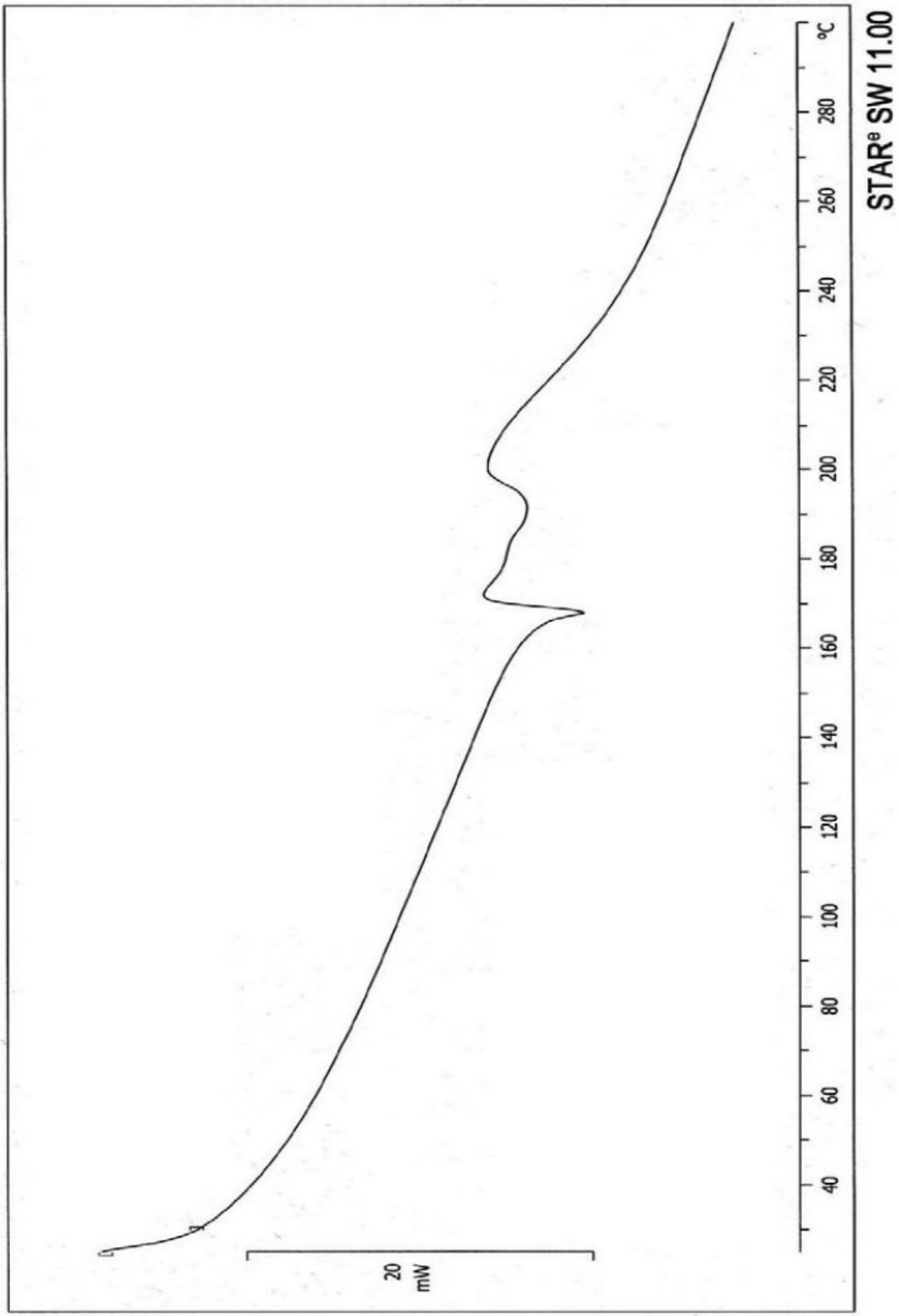
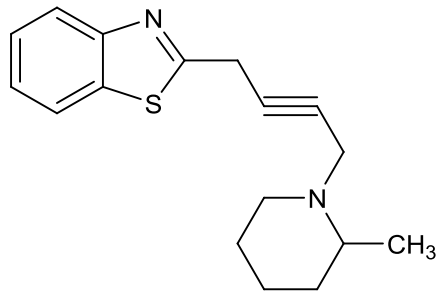


Figure (3.6): DSC thermogram of compound **BZ2.**

3.1.3 2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ3)**:



2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole

m.p : (130-134 ^{o}C).

Yield: 1.5gm, 52%.

IR (KBr cm-1): 2915 (Ar C-H stretch), 2800 (Ar C-H stretch), 1480 (Ar C=C stretch), 1250 (Ar C-N stretch), 1025 (Ar C-N stretch), 850 (Ar C-H bend), 750 (Ar C-H bend) **Figure 3.7**.

^{1}H-NMR (DMSO- d_6): δ, 1.1 (d, 3H, CH- CH_3), 1.34, 1.5, (m, various proton of cyclic amine), 3.4 (s, 2H, C-CH_2-N), 3.7 (s, 2H, CH_2-C), 6.6-7.2(m, 4H, ArH) **Figure 3.8**.

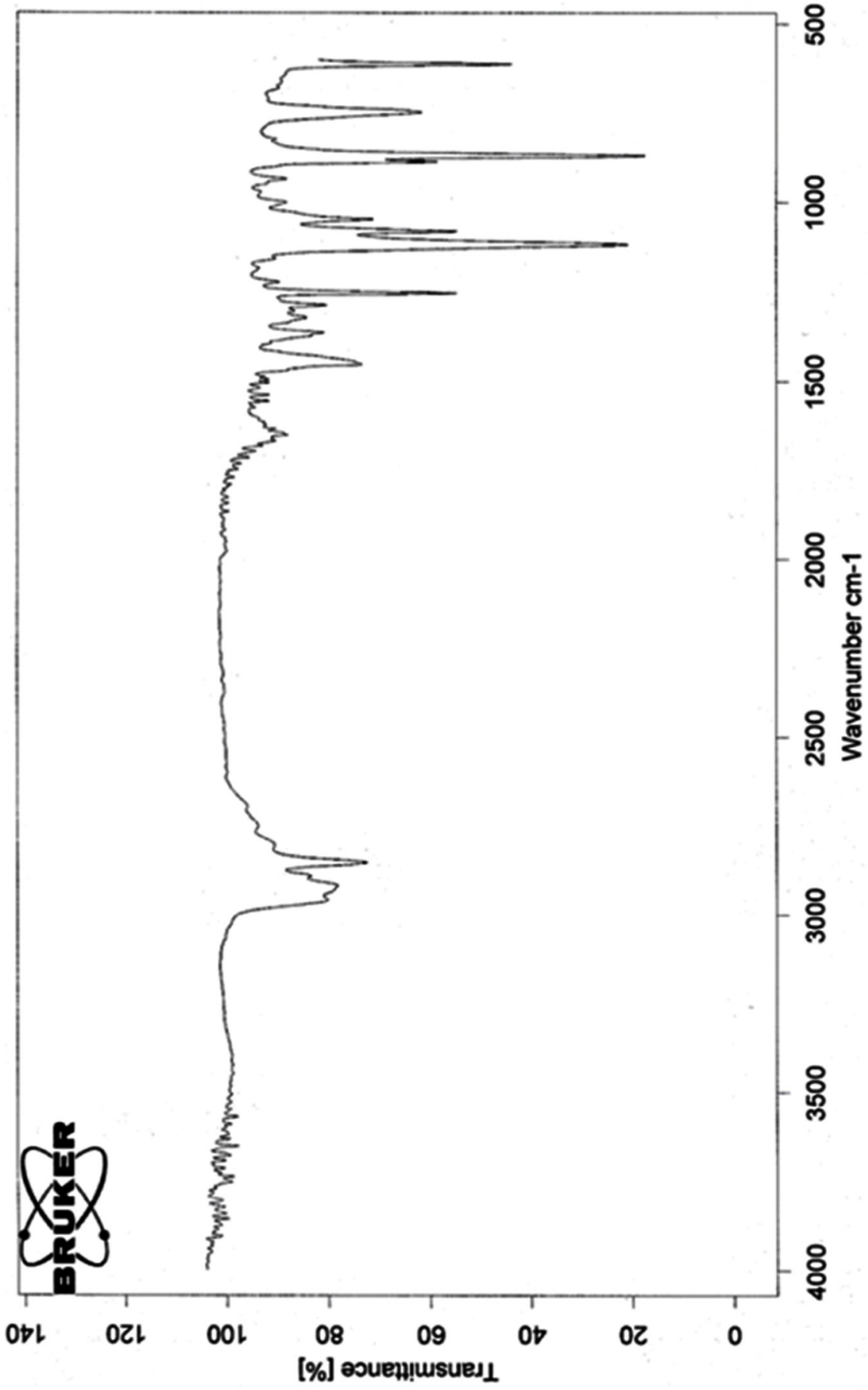


Figure (3.7): IR spectrum of **BZ3**.

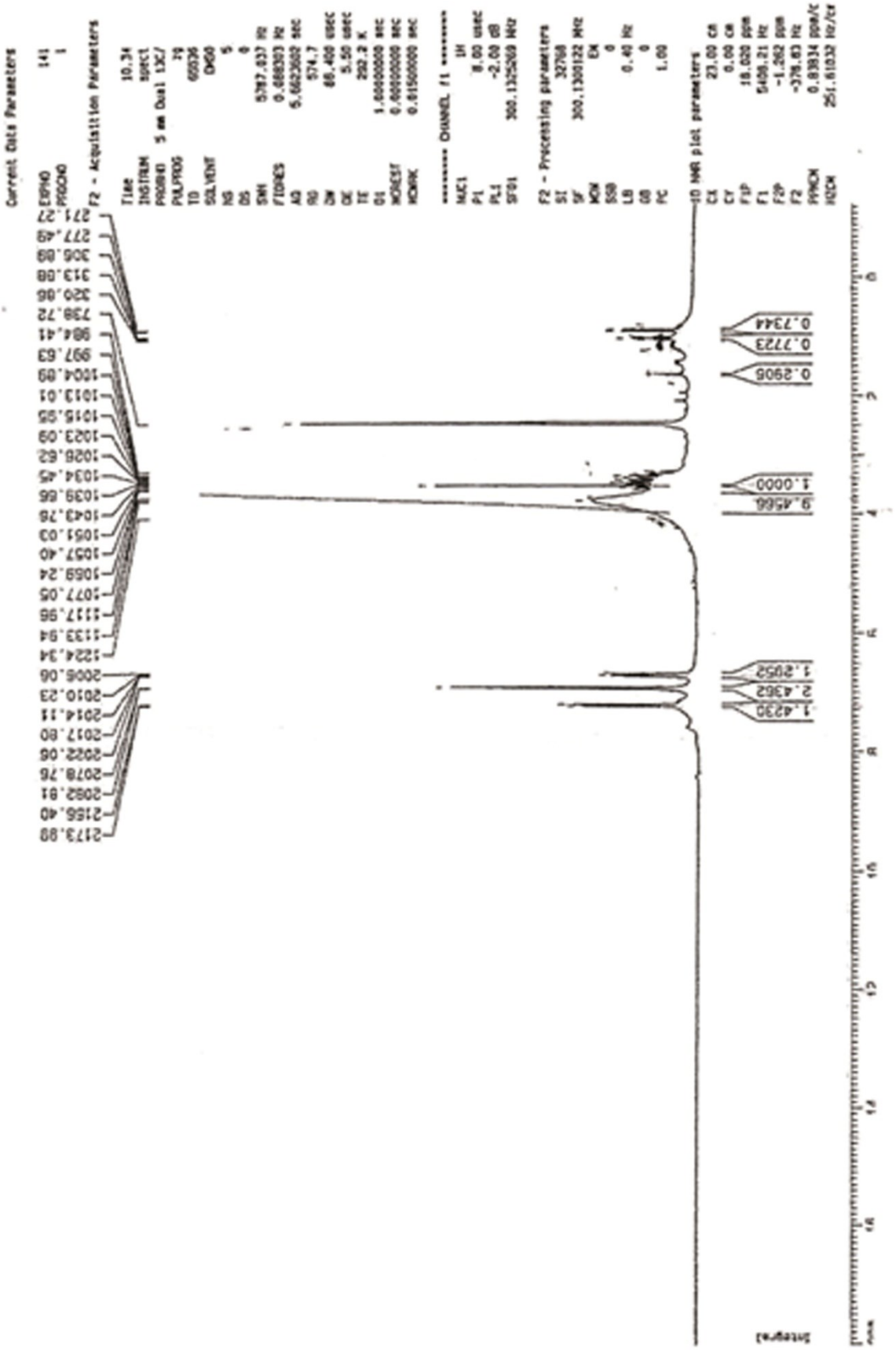
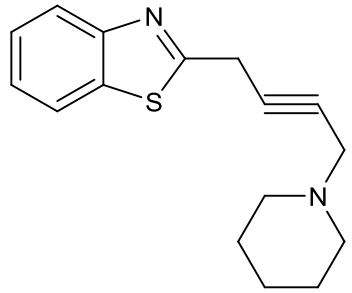


Figure (3.8):[1]HNMR spectrum of **BZ3**.

3.1.4 2-[4-(piperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ4)** :



2-[4-(piperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole

m.p**:** (148-150 ^{o}C).

Yield: 1.73gm, 64%.

IR (KBr cm-1): 2915 (Ar C-H stretch), 2800 (Ar C-H stretch), 1480 (Ar C=C stretch), 1250 (Ar C-N stretch), 1025 (Ar C-N stretch), 850 (Ar C-H bend), 750 (Ar C-H bend) **Figure 3.9**.

^{1}H-NMR (DMSO- d_6): δ, 1.5 (m, various proton of cyclic amine), 2.8(s, 2H, C-CH_2-N), 3.7(s, 2H, CH_2-C≡), 6.6-7.2 (m, 4H, ArH) **Figure 3.10**.

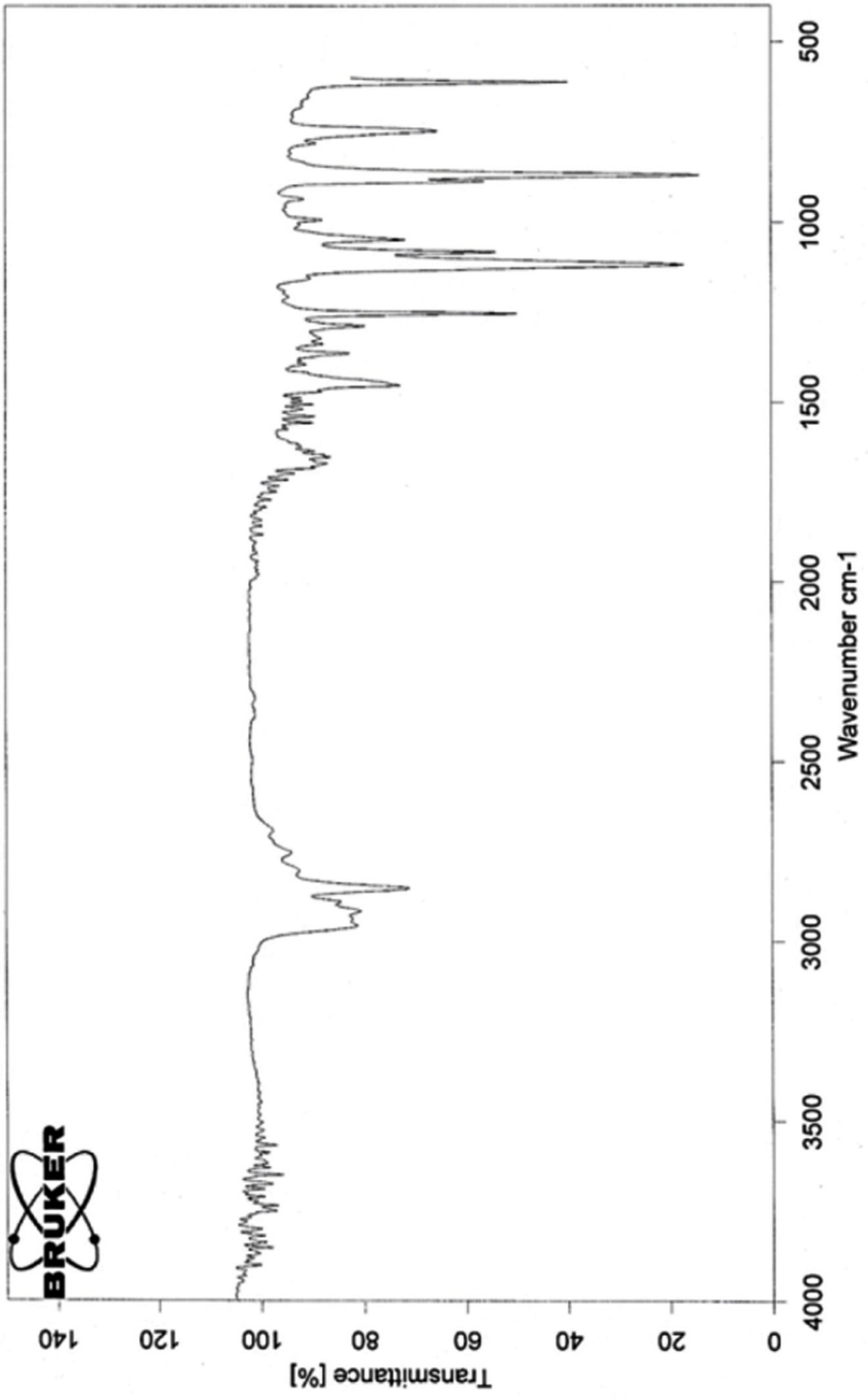


Figure (3.9) : IR spectrum of **BZ4**.

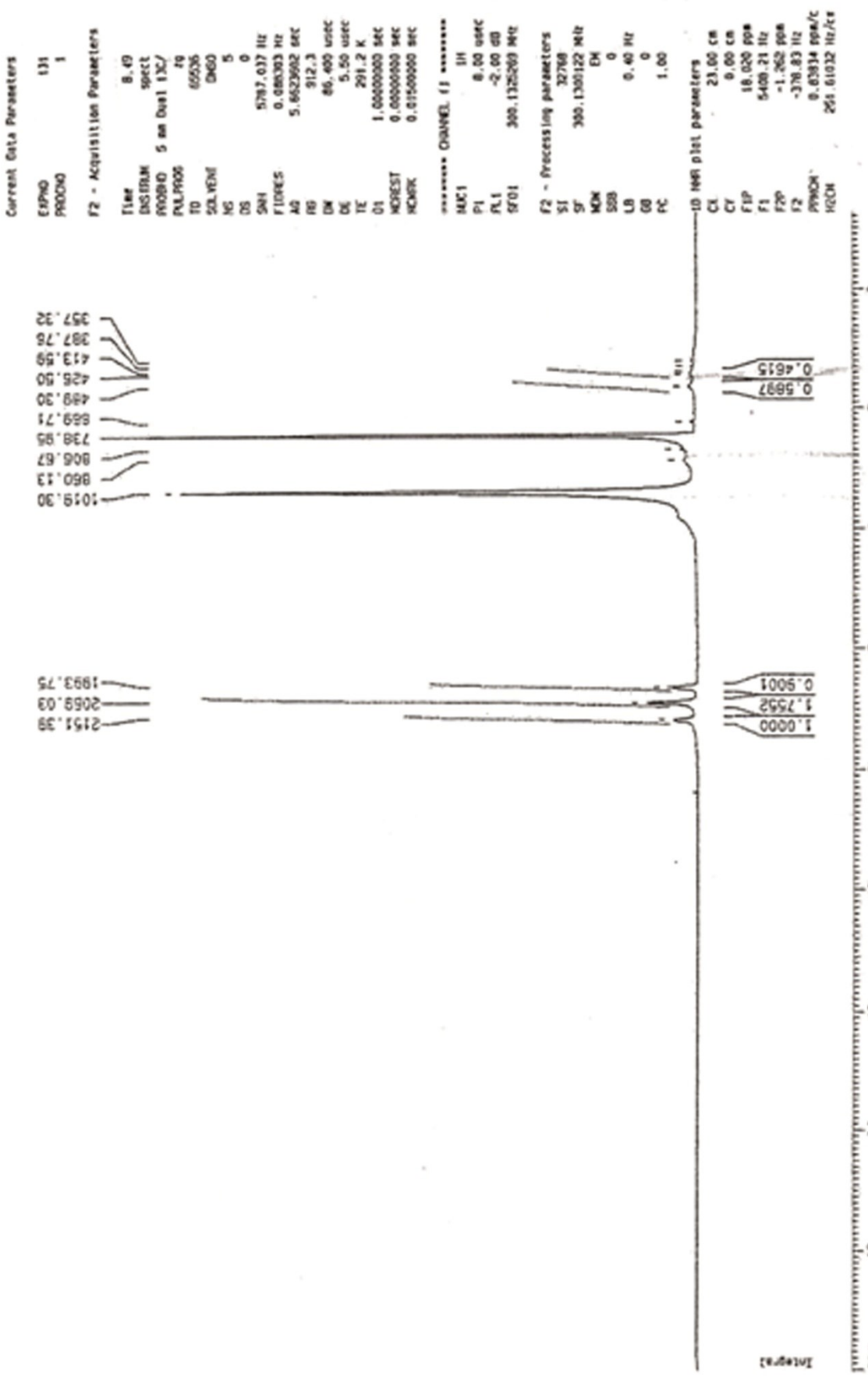


Figure (3.10): [1]HNMR spectrum of **BZ4**.

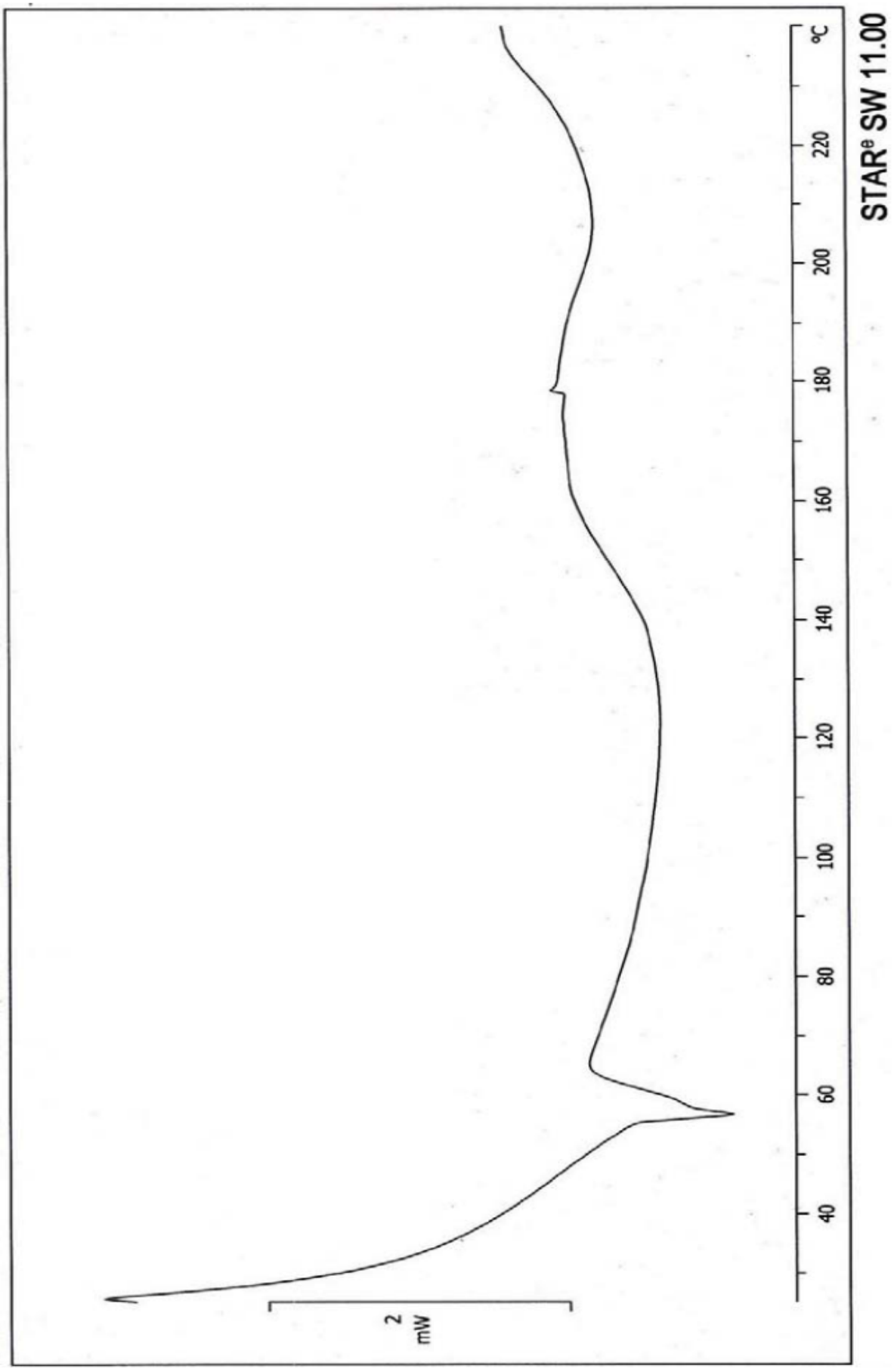
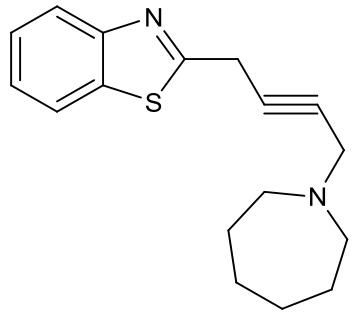


Figure (3.11): DSC thermogram of compound **BZ4**.

3.1.5 2-[4-(azepan-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ5)**:



2-[4-(azepan-1-yl)but-2-yn-1-yl]-1,3-benzothiazole

m.p : (122-125^{o}C).

Yield: 1.44 gm, 50%.

IR (KBr cm-1)**:** 2915 (Ar C-H stretch), 2800 (Ar C-H stretch), 1480 (Ar C=C stretch), 1250 (Ar C-N stretch), 1025 (Ar C-N stretch), 850 (Ar C-H bend), 750 (Ar C-H bend) **Figure 3.12**.

^{1}H-NMR (DMSO- d_6): δ, δ 1.6-1.7 (m, various proton of cyclic amine), 2.4 (s, 2H, C-CH_2-N), 3.6 (s, 2H, CH_2-C), 6.6-7.2(m, 4H, ArH) **Figure 3.13**.

^{13}C-NMR (DMSO- d_6)**:** δ, 26 (C^{20}) , 28(C^{19}) ,32 ($C^{17,18}$), 66 ($C^{13,15,16}$), 86 ($C^{11,12}$), 114 (C^{3}),117(C^{4}) , 120 (C^{6}),123(C^{5}), 132(C^{2}), 153(C^{1}), 174(C^{8}), 55(C^{10}) **Figure 3.15**.

Elemental analysis for **$C_{17}H_{20}N_2S$**:

Table (3.2) : Elemental analysis for compound **BZ5**.

	C	H	N
Calculated	67.97%	6.71%	9.32%
Found	64.3%	3.89%	6.3%

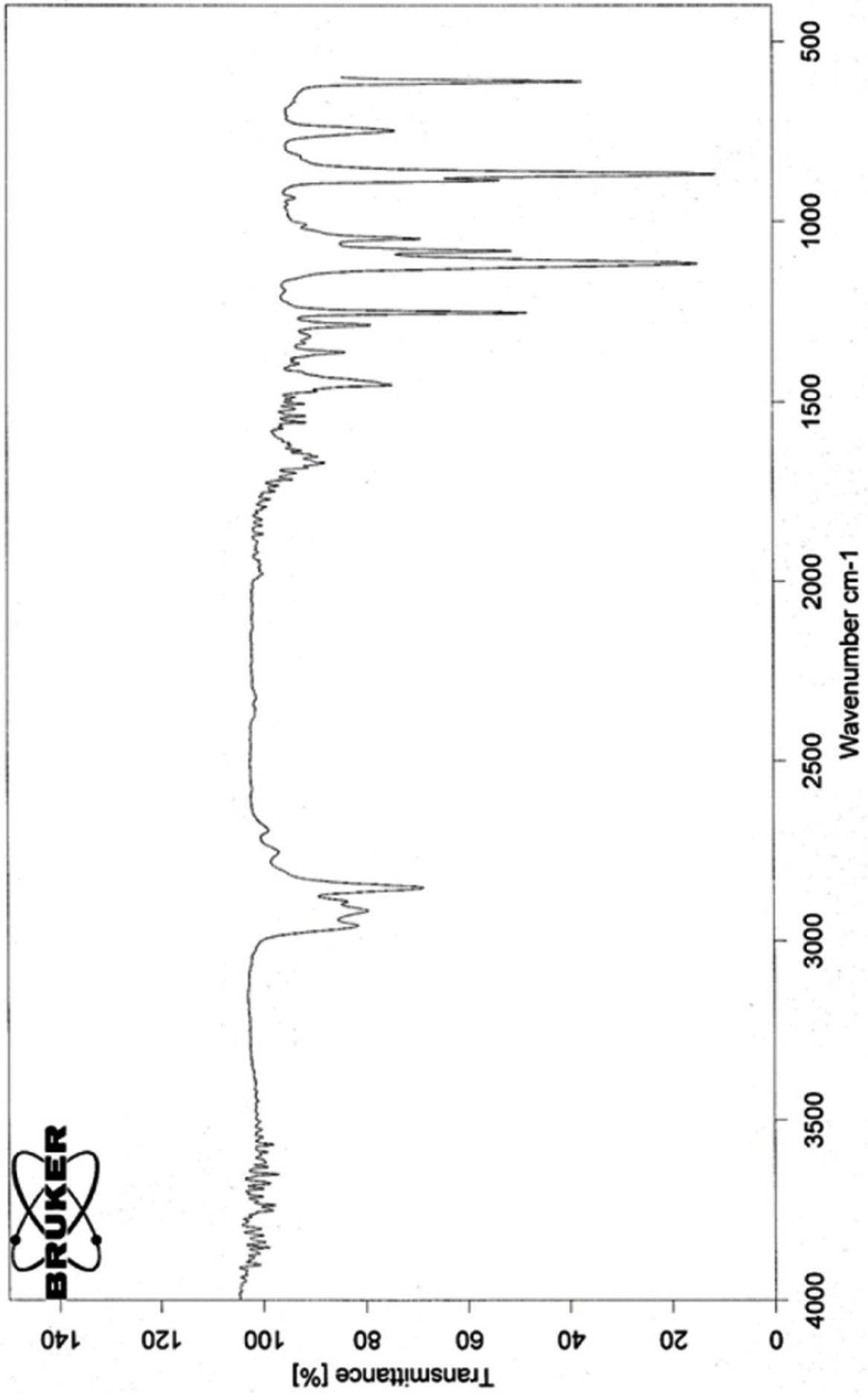


Figure (3.12): IR spectrum of **BZ5.**

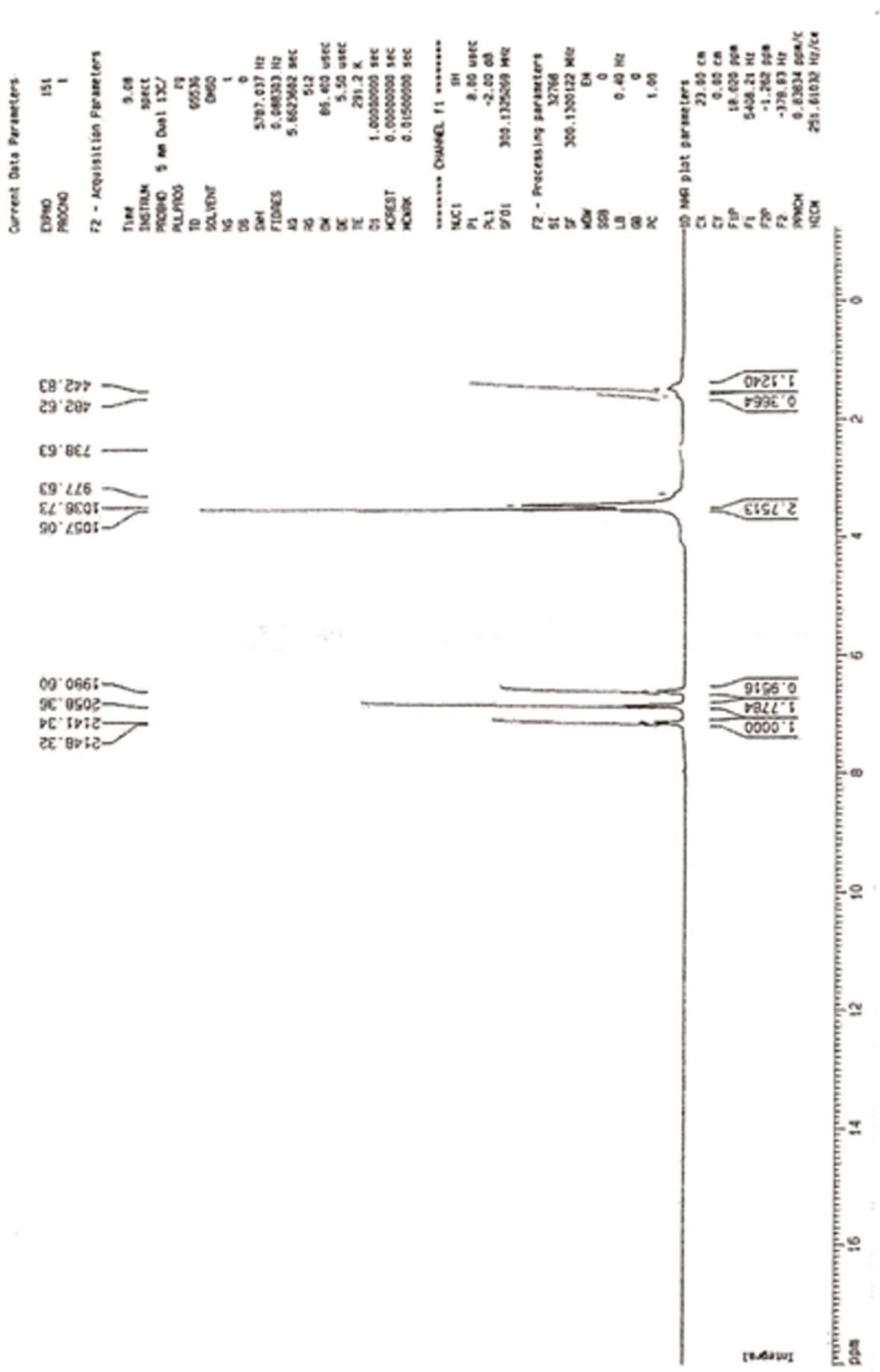


Figure (3.13): [1]HNMR spectrum of **BZ5**.

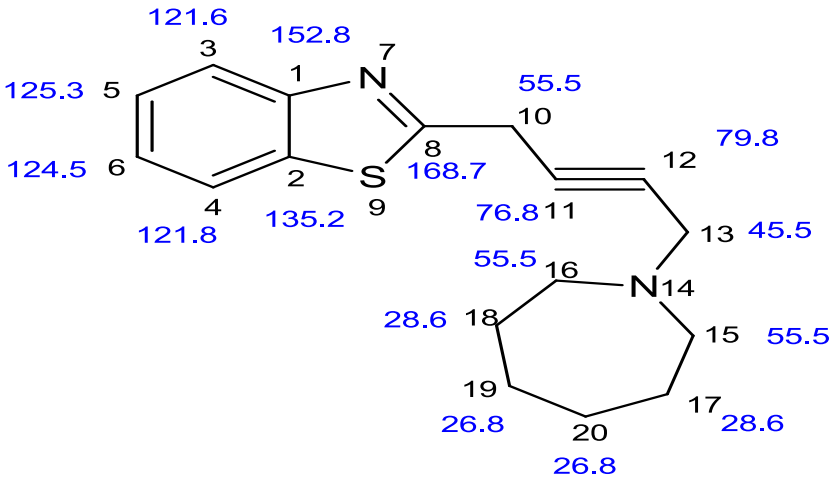


Figure (3.14): Estimation of ^{13}C-NMR for compound **BZ5**.

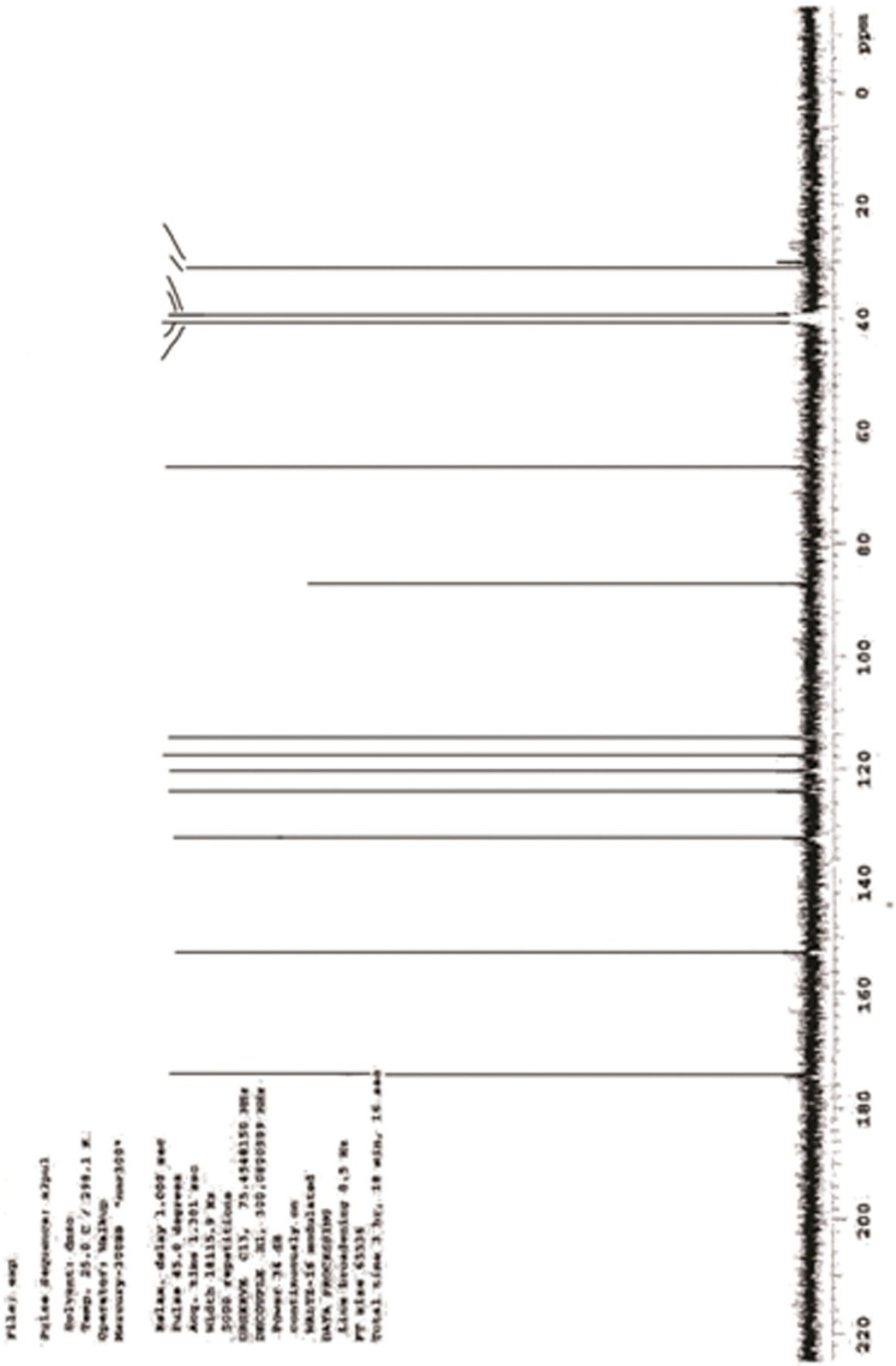
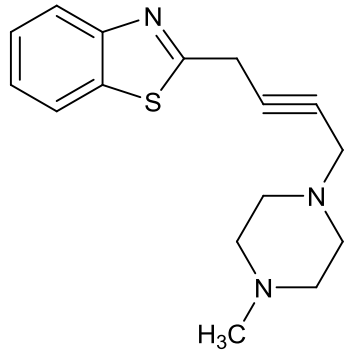


Figure (3.15): ^{13}C-NMR spectrum of **BZ5**.

3.1.6 2-[4-(4-methylpiperazin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ6)** :



2-[4-(4-methylpiperazin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole

m.p: (166-170 ^{o}C).

Yield: 1.75 gm, 64%.

IR (KBr cm-1)**:** 2915 (Ar C-H stretch), 2800 (Ar C-H stretch), 1480 (Ar C=C stretch), 1250 (Ar C-N stretch), 1025 (Ar C-N stretch), 850 (Ar C-H bend), 750 (Ar C-H bend) **Figure 3.16**.

^{1}H-NMR (DMSO- d_6): δ, 1.8-2.1 (m, various proton of cyclic amine), 2.5 (s, 2H, C-CH_2-N), 3.7(s, 2H, CH_2-C), 6.6-7.2(m, 4H, ArH) **Figure 3.17**.

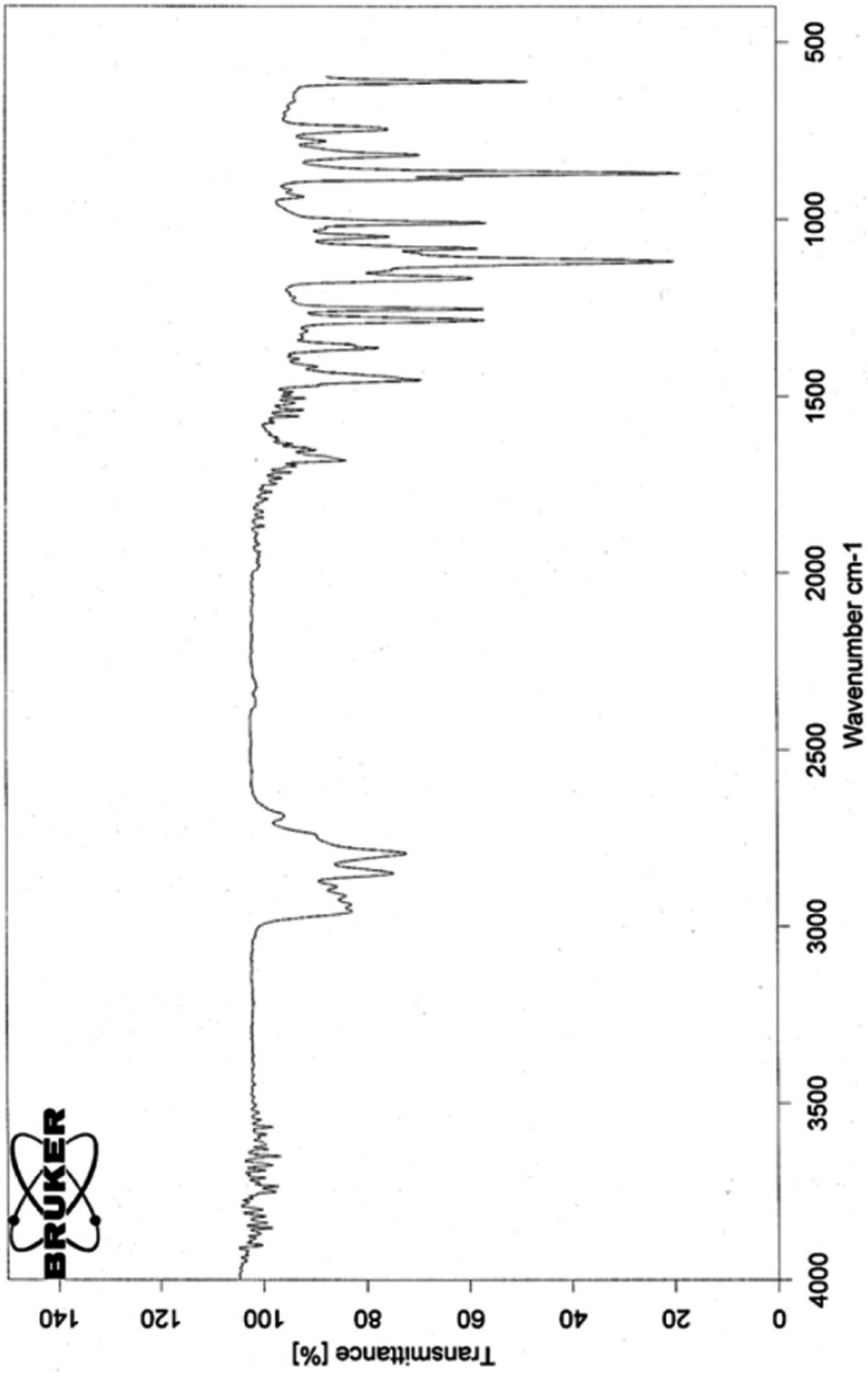


Figure (3.16): IR spectrum of **BZ6**.

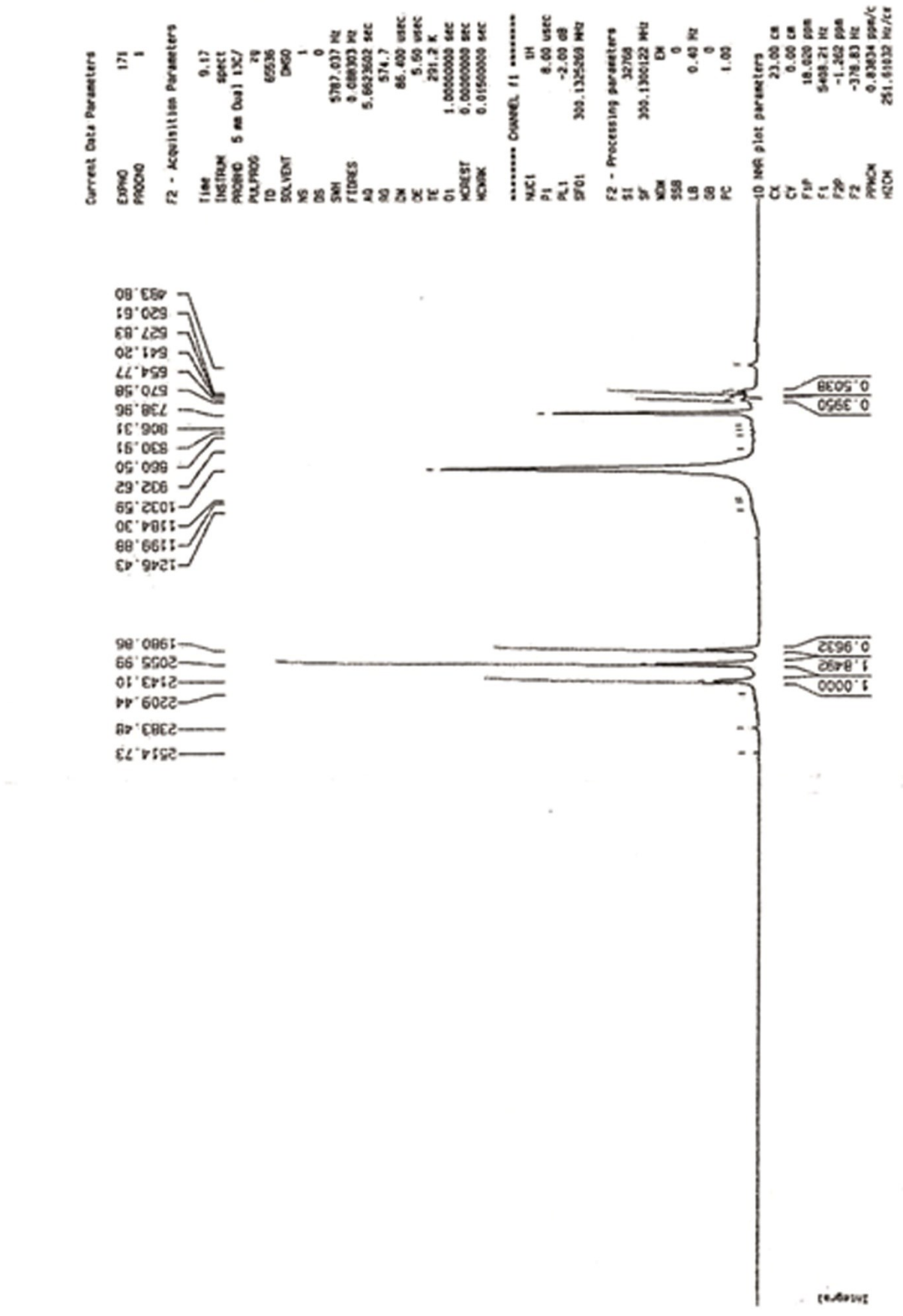
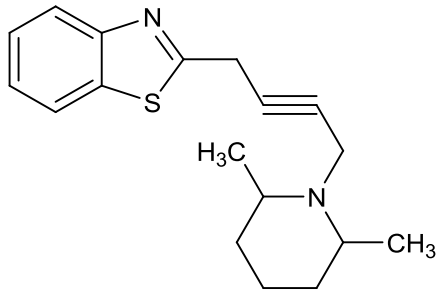


Figure (3.17): [1]HNMR spectrum of **BZ6**.

3.1.7 2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ7)**:



2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole

m.p **:(**160-163 ^{o}C).

Yield:1.83gm, 63%.

IR (KBr cm-1)**:** 2915 (Ar C-H stretch), 2800 (Ar C-H stretch), 1480 (Ar C=C stretch), 1250 (Ar C-N stretch), 1025 (Ar C-N stretch), 850 (Ar C-H bend), 750 (Ar C-H bend) **Figure 3.18**.

^{1}H-NMR (DMSO- d_6): δ, 1.1 (d, 6H,CH- CH_3), 1.4, 1.5, 1.7, 1.8, 1.9 (m,various proton of cyclicamine), 3.4(s,2H, C-CH_2N), 4.2 (s, 2H, CH_2-C), 7-7.6 (m, 4H, ArH) **Figure 3.19**.

^{13}C-NMR (DMSO- d_6): δ, 23, 25, 35 (various C of cyclic amine), 66 (C-N), 80, 81 (C≡C), 114, 117, 120, 123, 132, 153 (Ar C), 174 (N=C-S) **Figure 3.21**.

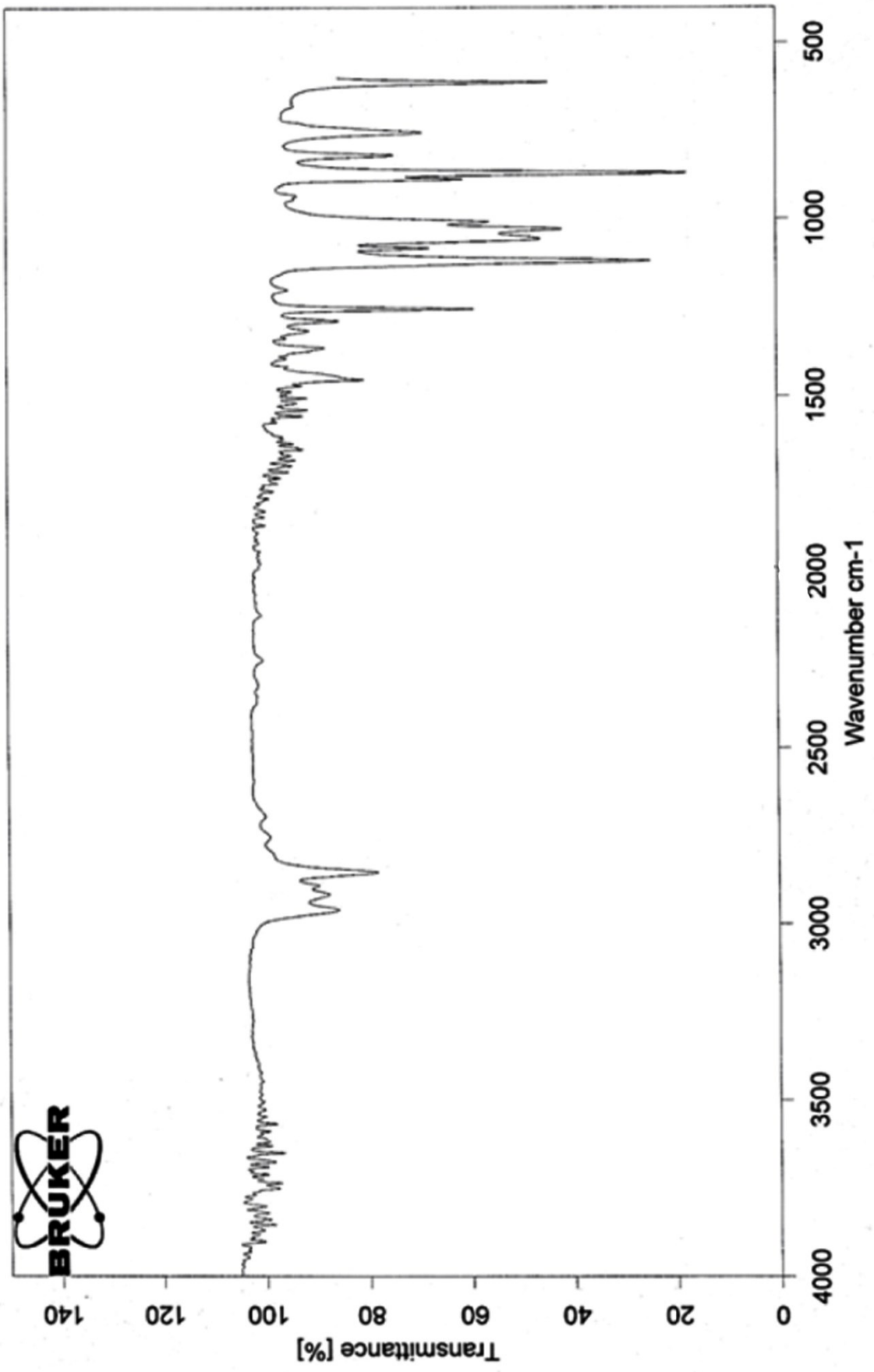


Figure (3.18): IR spectrum of **BZ7**.

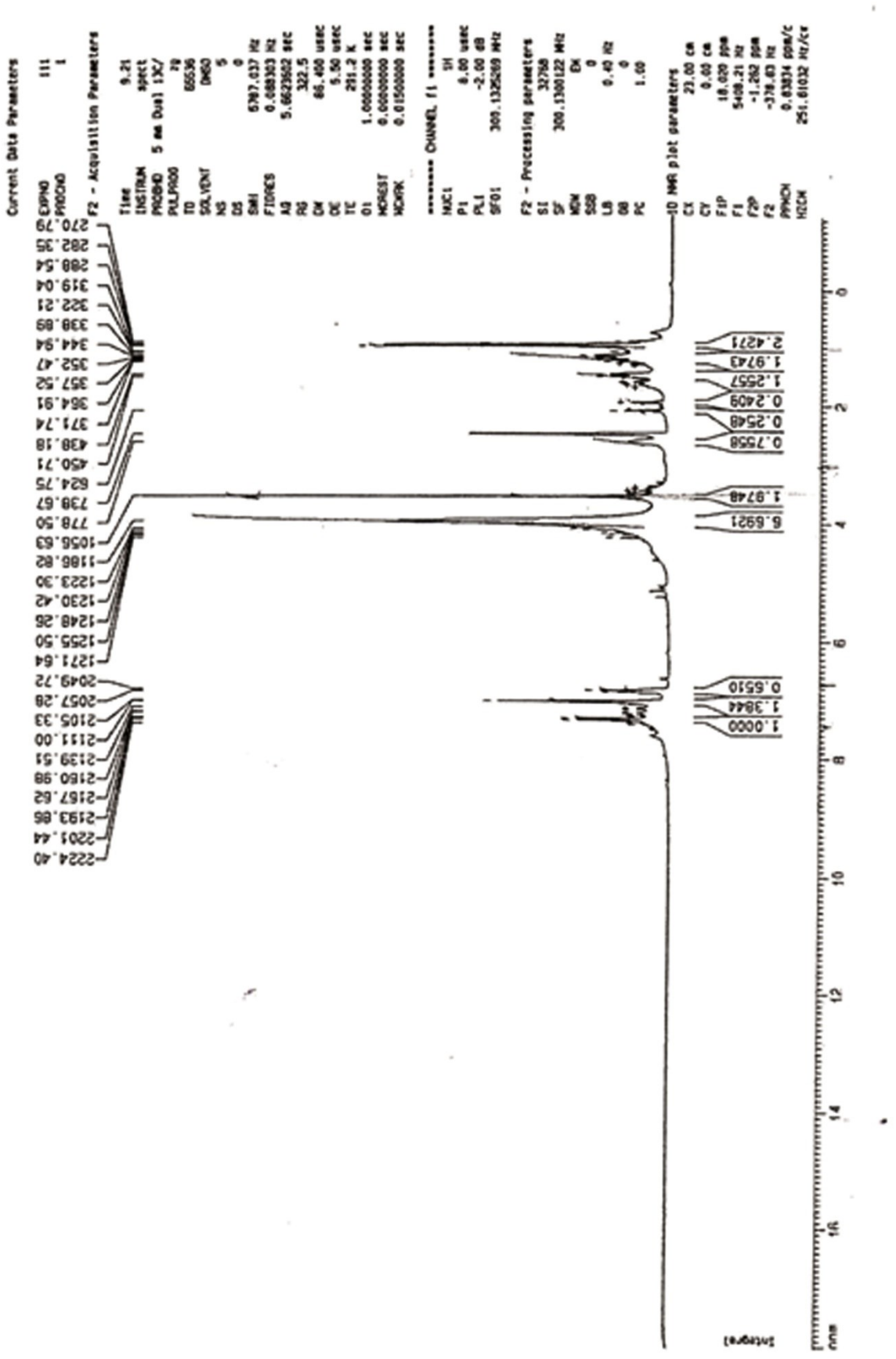


Figure (3.19): ^{1}H-NMR spectrum of **BZ7**.

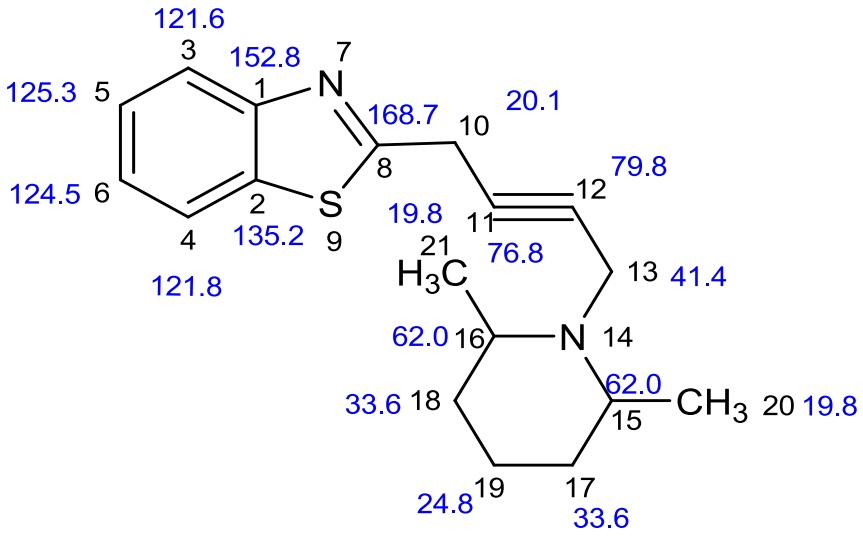


Figure (3.20): ^{13}C-NMR spectrum estimation of compound **BZ7.**

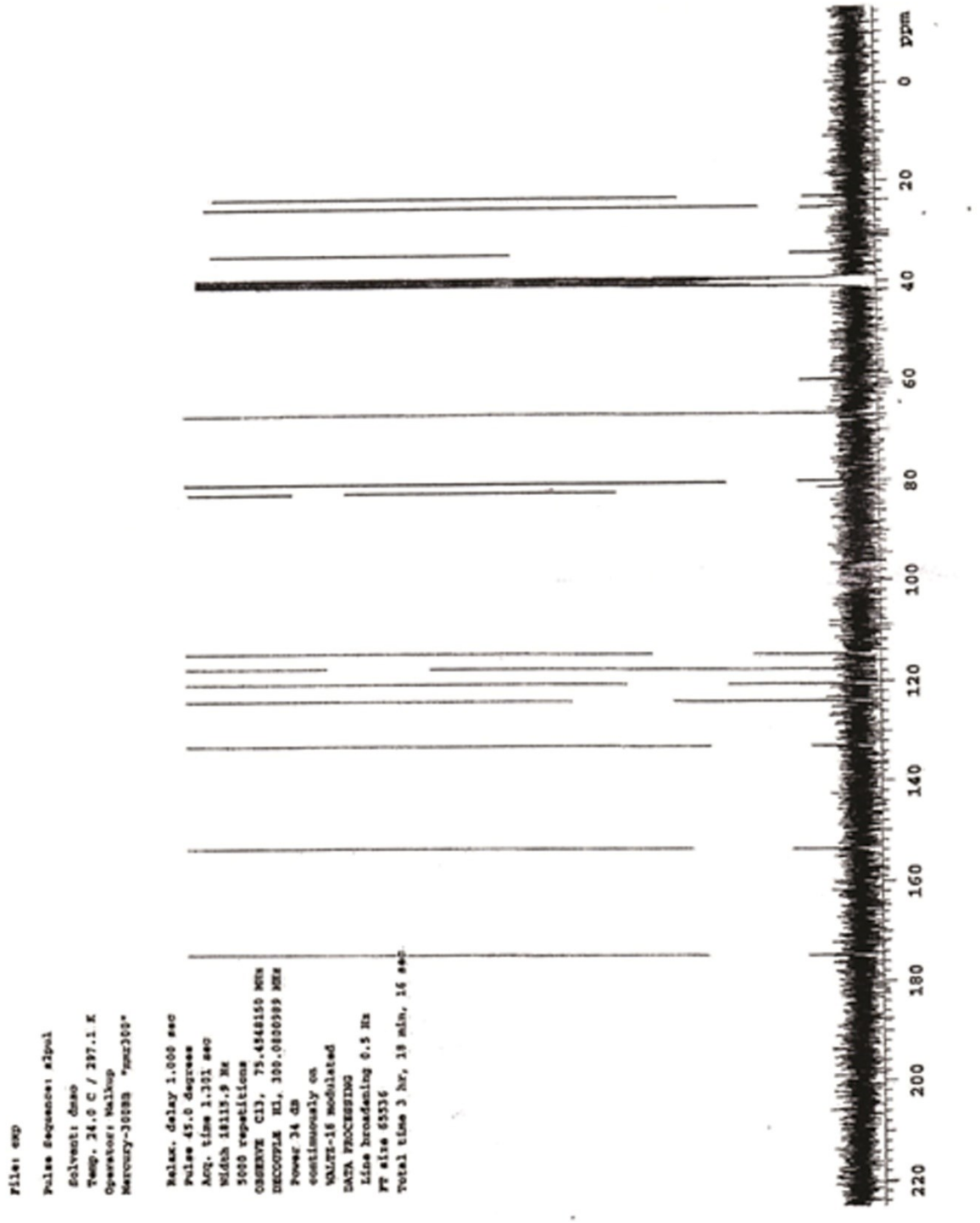


Figure (3.21): ^{13}C-NMR spectrum of **BZ7.**

3.2 Antimicrobial activity evaluation results:

The new compounds were tested for their antibacterial and antifungal activity using two methods as outlined in the experimental part **(Page 44-49)**. In the first method, the agar diffusion method was used (Barry, 1976), the in-vitro antimicrobial evaluation was done against *S. aureus* ATCC 6538p, *P. aeruginosa* ATCC 9027, *E. coli* ATCC 8739 , *C. albicans* ATCC 10231 and *B. subtilis* ATCC 6633 as shown in the experimental part. The results of antimicrobial testing are reported and compared with those of standard drugs Ciprofloxacin 5 mcg/ml and Fluconazole 500 mcg/ml.

The newly synthesized compounds showed activity against each type of the tested microorganisms after 24 hrs incubation at 37 ^{o}C for bacteria and after 48 hrs incubation at 37 ^{o}C for fungi, the diameter of zone of inhibition were measured and the results are indicated in **(table3.3)**. The diameter of zone of inhibition (in mm) for different concentrations of the newly synthesized compounds **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives BZ2-BZ7** ranging from 200-25 mcg/ml are indicated in the table. Compound 2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ2)** showed high antimicrobial activity against *P. aeruginosa* with zone of inhibition diameter of 30,27,20 mm for concentrations of 200,100,50 mcg/ml respectively in comparison with the positive control ciprofloxacin 5 mcg/ml which gave 21mm. Also the same compound **BZ2** gave antimicrobial activity in concentration of 200mcg/ml similar to the positive control ciprofloxacin 5 mcg/ml against *E. coli* with zone of inhibition diameter of 30mm. This compound **BZ2** also showed higher antimicrobial activity against *B. subtilis* with zone of inhibition diameter of 23mm in concentration of 200mcg/ml in comparison with the positive control ciprofloxacin 5mcg/ml which gave 21 mm. All the other concentrations of the compound **BZ2** showed less antimicrobial activity against *B. subtilis*. All the concentrations of compound **BZ2** gave less antimicrobial activity against *S. aureus* than the positive control ciprofloxacin 5 mcg/ml.

Table (3.3): The diameter of the zone of inhibition (in mm) of **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)** at 200,100,50,25 mcg/ml concentrations.

Compound	*S. aureus*				*B. subtilis*				*E. coli*				*P. aeruginosa*			
Concentration	***200***	***100***	***50***	***25***	***200***	***100***	***50***	***25***	***200***	***100***	***50***	***25***	***200***	***100***	***50***	***25***
BZ2	27	21	17	13	23	20	16	12	30	14	13	11	30	27	20	12
BZ3	25	22	18	13	24	21	18	14	27	18	15	11	28	26	19	13
BZ4	28	23	17	14	26	20	15	12	25	17	11	9	26	20	17	14
BZ5	27	24	17	15	24	20	18	15	18	11	9	7	24	17	15	13
BZ6	19	12	9	5	20	14	9	4	17	10	7	0	18	13	9	5
BZ7	17	11	8	4	19	13	8	0	18	11	9	4	19	12	8	4
Ciprofloxacin 5mcg/ml	30				21				30				21			
Negative control	0				0				0				0			

Compound 2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ3)** showed high antimicrobial activity against *P. aeruginosa* with zone of inhibition diameter of 28,26 mm in concentrations of 200,100 mcg/ml respectively in comparison with the positive control ciprofloxacin 5 mcg/ml which gave 21mm. Compound **BZ3** showed higher antimicrobial activity against *B. subtilis* with a zone of inhibition diameter of 24 in comparison with ciprofloxacin 5 mcg/ml which gave 21 mm. This compound showed higher antimicrobial activity at 100 mcg/ml with a zone of inhibition of 26 mm in comparison to the positive control ciprofloxacin 5 mcg/ml with a zone of inhibition diameter of 21mm against *P. aeruginosa*. All the concentrations of **BZ3** showed less antimicrobial activity than the positive control against *E. coli* and *S. aureus*.

Compound 2-[4-(piperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ4)** in concentration of 200mcg/ml showed higher antimicrobial activity against *B. subtilis* with zone diameter of 26mm in comparison to ciprofloxacin 5mcg/ml which gave 21mm. All other concentrations showed less antibacterial activity than the positive control against *B. subtilis*. Compound **BZ4** at a concentration of 200mcg/ml showed higher antimicrobial activity against *P. aeruginosa* with zone diameter of 26mm in comparison to the control drug ciprofloxacin 5 mcg/ml which gave 21mm. Compound **BZ4** showed less antimicrobial activity than ciprofloxacin 5 mcg/ml against *E. coli* and *S. aureus*.

Compound 2-[4-(azepan-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ5)** showed high antimicrobial activity against *B. subtilis* and *P. aeruginosa* with a diameter of inhibition zone of 24mm in comparison to the control ciprofloxacin 5 mcg/ml which gave 21mm. All other concentrations gave less antimicrobial activity than the positive control. Compounds 2-[4-(4-methylpiperazin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ6)** and 2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ7)** showed less antimicrobial activity than the positive control ciprofloxacin 5 mcg/ml against all tested microorganisms.

The MIC is the lowest concentration of an antimicrobial agent that inhibits microorganism growth, as determined visually after a standard incubation period (usually 18 to 24 hrs) (Peterson *et al.* ,1992). In order to determine the MIC of the newly synthesized compounds against the five standard microorganisms, an inoculation size of $1x10^5$ CFU/ml of each microorganism was prepared, different concentrations of the compounds **(BZ2-BZ7)** were prepared in series of test tubes ranging from 500-7.8 mcg/ml respectively. Muller Hinton broth was added accordingly to achieve the desired final concentration in each test tube as shown in **(table 2.2)**. The final volume of the compound along with the medium in each test tube was 5ml. The test tubes were incubated at 37° C for 24 hours (for bacteria), and incubated at 37 ° C for 48 hrs (for fungi). The MIC was determined by finding the test tube with lowest concentration of the compound in which no turbidity was observed.

The MIC values for the newly synthesized compounds **BZ2-BZ7** are indicated in **(table3.4)** Compound **BZ2** has the lowest MIC value which was 31.25 mcg/ml against *E. coli*, while against *S. aureus , B. subtilis and P. aeruginosa* the MIC value was 62.5 mcg/ml. Compound **BZ3** has the lowest MIC value which was 31.25 mcg/ml against *B. subtilis* and *E. coli*, while against *S. aureus* and *P. aeruginosa* the MIC value was 62.5 mcg/ml. Compound **BZ4** has the lowest MIC value which was 31.25 mcg/ml against *B. subtilis*, while it has MIC value of 62.5 mcg/ml against both *E. coli* and *S. aureus*. Compound **BZ4** showed weaker activity against *P. aeruginosa* with MIC value of 125 mcg/ml. Compound **BZ5** has the lowest MIC value against *S. aureus*, which was 15.62 mcg/ml which was less than the positive control ciprofloxacin 5mcg/ml which has an MIC value of 50 mcg/ml , while against *E. coli* and *P. aeruginosa* the MIC value was 62.5 mcg/ml. This compound showed also moderate activity against *B. subtilis* with a MIC value of 31.25 mcg/ml. Compound **BZ6** has an MIC value of 62.5 mcg/ml against *P. aeruginosa* and *B. subtilis*, while against *E. coli* and *S. aureus* it has a higher MIC value of 125mcg/ml. Compound **BZ7** has an MIC value of 31.25 mcg/ml against *P. aeruginosa* and *B. subtilis*, while having higher MIC value of 125 mcg/ml against *E. coli*

and *S. aureus*. Based on the results obtained, if we compare the MIC values of compounds **BZ2-BZ7** with the MIC values of the positive control ciprofloxacin 5mcg/ml, we find that the MIC value of compound **BZ5** against *S. aureus* is less than the MIC value of the positive control (15.62, 50 mcg/ml respectively). The MIC of compound **BZ7** against *P. aeruginosa* is less than that of the positive control ciprofloxacin 5 mcg/ml (31.25, 50 mcg/ml respectively).

Table (3.4): Minimum inhibitory concentration (MIC) of **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)** in mcg/ml against *S. aureus, B. subtilis, E. coli and P. aeruginosa.*

Compound	***S. aureus***	***B. subtilis***	***E. coli***	***P. aeruginosa***
	MIC (mcg/ml)	**MIC (mcg/ml)**	**MIC (mcg/ml)**	**MIC (mcg/ml)**
BZ2	62.5	62.5	31.25	62.5
BZ3	62.5	31.25	31.25	62.5
BZ4	62.5	31.25	62.5	125
BZ5	15.62	31.25	62.5	62.5
BZ6	125	62.5	125	62.5
BZ7	125	31.25	125	31.25
Ciprofloxacin 5 mcg/ml	50	25	25	50
Negative control	-	-	-	-

The minimal concentration of drug needed to kill most (99.9%) of the viable microorganisms after incubation for a fixed length of time (generally 24 hrs) under a given set of conditions is the most common estimation of bactericidal activity and is known as either minimum bactericidal concentration (MBC) or minimal lethal concentration (MLC) (Clsi,1999). The MBC values for the newly synthesized compounds **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)** are indicated in **(table 3.5)**. Compound **BZ2** has the lowest MBC value which was 62.5 mcg/ml against *E. coli* ,while against *S. aureus , B. subtilis and P. aeruginosa* the MBC value was 125 mcg/ml. Compound **BZ3** has the lowest MBC value which was 62.5 mcg/ml against *B. subtilis* and *E. coli*, while against *S. aureus* and *P. aeruginosa* the MBC value was 125 mcg/ml. Compound **BZ4** has the lowest MBC value which was 62.5 mcg/ml against *B. subtilis*, while it has MBC value of 125 mcg/ml against both *E. coli* and *S. aureus* . Compound **BZ4** showed weak activity against *P. aeruginosa* with MBC value of 250 mcg/ml. Compound **BZ5** has the lowest MBC value against *S. aureus*, which was 31.25 mcg/ml, while against *E. coli* and *P. aeruginosa* has an MBC value of 125 mcg/ml. This compound showed also moderate activity against *B. subtilis* with MBC value of 62.5 mcg/ml. Compound **BZ6** has an MBC value of 125 mcg/ml against *P. aeruginosa* and *B. subtilis*, while against *E. coli* and *S. aureus* it has higher MBC value of 250 mcg/ml. Compound **BZ7** has an MBC value of 62.5 mcg/ml against *P. aeruginosa* and *B. subtilis*, while having higher MIC value of 250 mcg/ml against *E. coli* and *S. aureus*.

Table (3.5): Minimum bactericidal concentration (MBC) of **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)** in mcg/ml against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* strains.

Microorganism	***S. aureus***	***B. subtilis***	***E. coli***	***P. aeruginosa***
Compound	**MBC (mcg/ml)**	**MBC (mcg/ml)**	**MBC (mcg/ml)**	**MBC (mcg/ml)**
BZ2	125	125	62.5	125
BZ3	125	62.5	62.5	125
BZ4	125	62.5	125	250
BZ5	31.25	62.5	125	125
BZ6	250	125	250	125
BZ7	250	62.5	250	62.5

Antifungal susceptibility testing has been a recognized diagnostic tool for over 20 years (Fothergill, 2012). To test the antifungal activity of the newly synthesized compounds of **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)** , the agar diffusion method was done as mentioned before using *C. albicans* as the tested microorganism. The diameter of zone of inhibition (in mm) for different concentrations of the newly synthesized compounds **(BZ2-BZ7)** ranging from 200-25 mcg/ml against *C. albicans* are indicated in **(table 3.6)**. Compound **BZ2** showed higher antifungal activity with a zone of inhibition diameter of 32 mm at a concentration of 200mcg/ml in comparison to positive control Fluconazole 500 mcg/ml which has a diameter of zone of inhibition of 30 mm. Compound **BZ3** showed similar antifungal activity in comparison to Fluconazole 500 mcg/ml with diameter of zone of inhibition of 30mm. All the other concentrations showed less antifungal activity. Compounds **BZ4, BZ5, BZ6, and BZ7** showed less antifungal activity than Fluconazole 500 mcg/ml in all concentrations.

Table (3.6): The diameter of the zone of inhibition (in mm) of **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)** at 200,100,50,25 mcg/ml concentrations against *C. albicans*.

Compound	***C. albicans***			
Concentration (mcg/ml)	**200**	**100**	**50**	**25**
BZ2	32	22	16	12
BZ3	30	23	17	13
BZ4	27	21	18	11
BZ5	21	14	11	8
BZ6	19	12	8	0
BZ7	20	13	9	4
Fluconazole 500 mcg/ml	30			
Negative control	0			

To determine the MIC values of the newly synthesized compounds of **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)** against *C. albicans*, an inoculation size of (1.0 x 10^5cfu/ml) of *C. albicans* was prepared, different concentrations of the compounds **(BZ2-BZ7)** were prepared in a series of test tubes ranging from 500-7.8 mcg/ml respectively. Sabourauds dextrose broth was added accordingly to achieve the desired final concentration in each test tube as shown in **(table 2.2)**. The final volume of the compound along with the medium in each test tube was 5ml. 0.1 ml of *C. albicans* suspension is added to each test tube. The test tubes were incubated at 37^o C for 48 hours. The MIC values for the newly synthesized compounds **BZ2-BZ7** against *C. albicans* are indicated in **(table 3.7)**. Compounds **BZ2** and **BZ5** both have the lowest MIC value of 15.62 mcg/ml among other compounds. Compounds **BZ3** and **BZ6** have an MIC value of 31.25 mcg/ml. Compounds **BZ4** and **BZ7** showed the highest MIC value of 62.5 mcg/ml. In comparison with positive control Fluconazole 500 mcg/ml which has an MIC value of 8mcg/ml, all the newly synthesized compounds **BZ2-BZ7** have MIC values higher than that of Fluconazole 500 mcg/ml.

Table (3.7): Minimum inhibitory concentration (MIC) of **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7**) in mcg/ml against *C. albicans.*

Compound	***C. albicans***
	MIC (mcg/ml)
BZ2	15.62
BZ3	31.25
BZ4	62.5
BZ5	15.62
BZ6	31.25
BZ7	62.5
Fluconazole (500 mcg/ml)	8
Negative Control	-

The MFC values for the newly synthesized compounds **BZ2-BZ7** against *C. albicans* are indicated in **(table 3.8)**. Compounds **BZ2** and **BZ5** both have the lowest MFC value of 31.25 mcg/ml among the compounds. Compounds **BZ3** and **BZ6** have an MFC value of 62.5 mcg/ml. Compounds **BZ4** and **BZ7** showed the highest MFC value of 125 mcg/ml.

Table (3.8): Minimum fungicidal concentration (MFC) of **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)** in mcg/ml against *C. albicans* strain.

Compound	***C. albicans***
	MFC (mcg/ml)
BZ2	31.25
BZ3	62.5
BZ4	125
BZ5	31.25
BZ6	62.5
BZ7	125

A comparison among the antimicrobial activity of the newly synthesized compounds based on the above results, compound 2-[4-(azepan-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ5** have the highest activity against Gram positive bacteria *S. aureus* followed by compounds 2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole(**BZ2),**2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,3 benzothiazole(**BZ3)** and 2-[4-(piperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole**(BZ4)** then compounds 2-[4-(4-methylpiperazin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ6** and 2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ7**. Among all the tested compounds, compound **BZ6** showed the weakest activity against Gram positive bacteria. Compound **BZ7** has the highest antibacterial activity against *P. aeruginosa* among all the tested compounds. Compound **BZ4** has the weakest antibacterial activity against *P. aeruginosa*. Compounds **BZ2** and **BZ3** have high activity against Gram negative bacteria *E. coli*. When we compare the antifungal activity of the compounds **BZ2-BZ7** with the positive control Fluconazole 500 mcg/ml, we found that compounds **BZ2** and **BZ5** have the higher antifungal activity followed by compounds **BZ3** and **BZ6**. Compounds **BZ4** and **BZ7** have the lowest antifungal activity, among other compounds.

Chapter four

Discussion:

The importance and value of antimicrobial agents cannot be overestimated, we are totally dependent on them for the treatment of infectious diseases, and they should never be considered mere commodities. In addition to their use in the treatment of infectious diseases, antimicrobial agents are critical to the success of advanced surgical procedures, including organ and prosthetic transplants.

Not forgetting all best efforts to control antimicrobial usage, there is little doubt that the situation with respect to antimicrobial resistance is grim. Resistance problems are pandemics and create an enormous clinical and financial burden on health care systems worldwide. The antimicrobial resistance necessitates the need to find novel antibacterial and antifungal drugs to be designed and synthesized.

The amino acid Tryptophan can be found on the fungal membrane. Sertaconazole is an antifungal drug, which contains benzothiophene ring, that may take place of tryptophan because it mimics tryptophan. Pores and holes are formed on the fungal cell membrane. When the pore forms, the fungal cell loses its intracellular content mainly ATP. Benzothiazole ring may mimic the role of benzothiophene ring in fungal cell membrane (Muhi- el deen, 2011).

The newly synthesized compounds were prepared through the following sequence of reaction: Alkylation and Mannich reaction. Alkylation reaction, in which 2-(prop-2-yn-1-yl)-1,3-benzothiazole **BZ1** was generated through nucleophilic displacement of the bromine located at 3-bromoprop-1-yne by two proposed mechanisms outlined in **(scheme 4.1)**.

The second step involved Mannich reaction, in which 2-(prop-2-yn-1-yl)-1,3-benzothiazole **BZ1** react with paraformaldehyde, appropriate cyclic amine and catalytic amount of cuprous

chloride to generate the desired compounds **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)**.The proposed mechanism for Mannich reaction, involved the formation of a reactive immonium cations intermediates from the condensation of the formaldehyde and the appropriate amines (Schiff base formation) in order for the reaction to proceed. The attack of the carbanion in 2-(prop-2-yn-1-yl)-1,3-benzothiazole cuprous salt on the Schiff base generates the desired mannich products **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)** as shown in **(scheme 4.2)**. The IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis were consistent with the assigned structures as illustrated in the results.

The newly synthesized compounds were screened for their antimicrobial activity against *S. aureus* ATCC 6538p, *P. aeruginosa* ATCC 9027, *E. coli* ATCC 8739, *C. albicans* ATCC 10231 and *B. subtilis* ATCC 6633 by measuring the diameter of the zone of inhibition surrounding the compound, the compound with the largest zone of inhibition indicate good antimicrobial activity. The investigation of antimicrobial screening data revealed that **2-{4-(t-amino)-2-(but-2-yn-1-yl)}-1,3-benzothiazole derivatives (BZ2-BZ7)** with promising results. It was clear that there is variability in the susceptibilities of the different microorganisms to the different tested compounds and variability in the antibacterial activity, according to the method of investigation (agar diffusion method or broth dilution method). The agar diffusion method probably is the most widely used one; although not necessarily the best (Tortora *et al.*, 2013). Many factors may influence the results, of these is the diffusion of the compound into the agar medium, which affected by the solubility of the compound (Tortora *et al.*, 2013) and the molecular weight of the compound, compound with poor water solubility diffuse into the agar medium with difficulty giving smaller zone of inhibition reported as low antimicrobial activity, the results obtained are often inadequate for comparison with the results of broth dilution test which lacks those difficulties (Tortora *et al.*, 2013). The results revealed that there is a possible relation between the solubility and the molecular weight of the compounds

and its effect on the diffusion rate of compound solution through the agar media. As the molecular weight of the compound increases, the diameter of the zone of inhibition formed around was decreased as outlined in **table 4.1**. For example, the molecular weight of the majority of the compounds **BZ2-BZ7** increases from compound BZ2 to BZ7, however the diameter of inhibition zone for the compounds decreases with the same order against *P. aeruginosa* at 100mcg/ml which may indicate that, the solubility and the molecular weight of the compound have a direct effect on the results.

The MIC of the newly synthesized compounds **(BZ2-BZ7)** was determined by the broth dilution test against the set of previously mentioned microorganisms. Among these compounds, 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole **(BZ5)** showed the highest antibacterial activity against *S. aureus*. The MIC value of compound **BZ5** was lower than that of the positive control Ciprofloxacin 5 mcg/ml (15.62, 50 mcg/ml respectively) as shown in **table 3.4**. This compound also showed good antibacterial activity against other Gram positive bacteria *B. subtilis*. Also compound 2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ3** showed good antibacterial activity against the Gram positive bacteria *B. subtilis*. A distinct feature of the cell wall of Gram positive bacteria is the thick layers of peptidoglycan, while Gram negative bacterial cell wall is thinner as it consist of a few layers of peptidoglycan. The good antibacterial activity of the compounds 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole **BZ5** and 2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ3** against Gram positive bacteria is attributed to the lipophilicity and steric effect due to the large size of azepan and methylpiperidine respectively. The steric effect may exert physical pressure on the cell wall of Gram positive bacteria and the lipophilicity effect may facilitate the diffusion of the compound through the cell wall. Compounds 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole **BZ5** and 2-[4-(piperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ4)** showed better antibacterial activity against Gram positive bacteria than Gram negative bacteria, this may be attributed to the flexibility of these amines. On the other

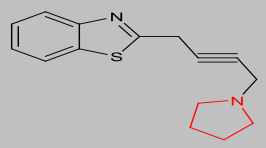
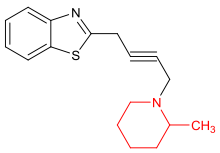
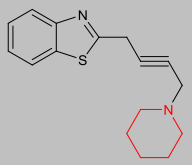
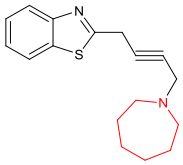
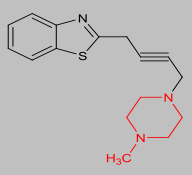
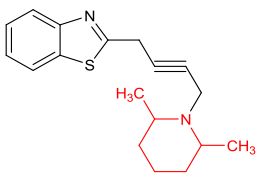
hand, compound 2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **(BZ7)** showed the highest antibacterial activity against Gram negative *P. aeruginosa* among the synthesized compounds. The MIC value of compound **BZ7** was lower than that of the positive control Ciprofloxacin 5 mcg/ml (31.25, 50 mcg/ml, respectively) as shown in **table 3.4** against the commonly resistant bacteria *P. aeruginosa*. Compound 2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ3** showed good antibacterial activity against Gram negative bacteria specially *E. coli*. The outer membrane of the Gram negative bacteria which characterize them from Gram positive bacteria consists mainly of lipopolysaccharide, lipoproteins and phospholipids **(Figure 1.5)** and the lipophilicity facilitated the entry of the compound into the microorganism which related to its antibacterial activity against Gram negative bacteria. Substitution on the ortho-position of the piperidine ring with methyl group lead to enhanced antimicrobial activity against Gram negative bacteria (compound 2-[4-(2-methylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole (**BZ3**) when compared to compound **BZ4**. Also disubstitution on ortho position of the piperidine ring with a methyl group increased the antibacterial activity (compound 2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ7**) in comparison with compound 2-[4-(piperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ4** against *P. aeruginosa*. Other researchers also investigate the MIC values against the same set of Gram positive bacteria and Gram negative bacteria and the values were between 6.25-50 mcg/ml (Abdellatif et al., 2015)

The pattern of the results of antifungal screening was different from that of antibacterial evaluation. The results of antifungal screening showed good antifungal activity of all the synthesized compounds against *C. albicans*. Compound 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole **BZ5** also showed the highest antifungal activity among the synthesized compounds due to steric and lipophilicity effects of the large azepan group. Also compound 2-[4-(pyrrolidin-1-yl) but-2-yn-1-yl]-1,3-benzothiazole **(BZ2)** showed good activity as compound 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole **BZ5** against *C. albicans*.

Compounds **BZ2** and **BZ5** had the lowest MIC value of 15.62 mcg/ml (for both) among all the other compounds as shown in table **3.7**. The positive control Fluconazole showed a MIC value of 8 mcg/ml against *C. albicans* while other researchers investigate the MIC value of Fluconazole and it was between 0.125-64 mcg/ml (Fothergill 2012).

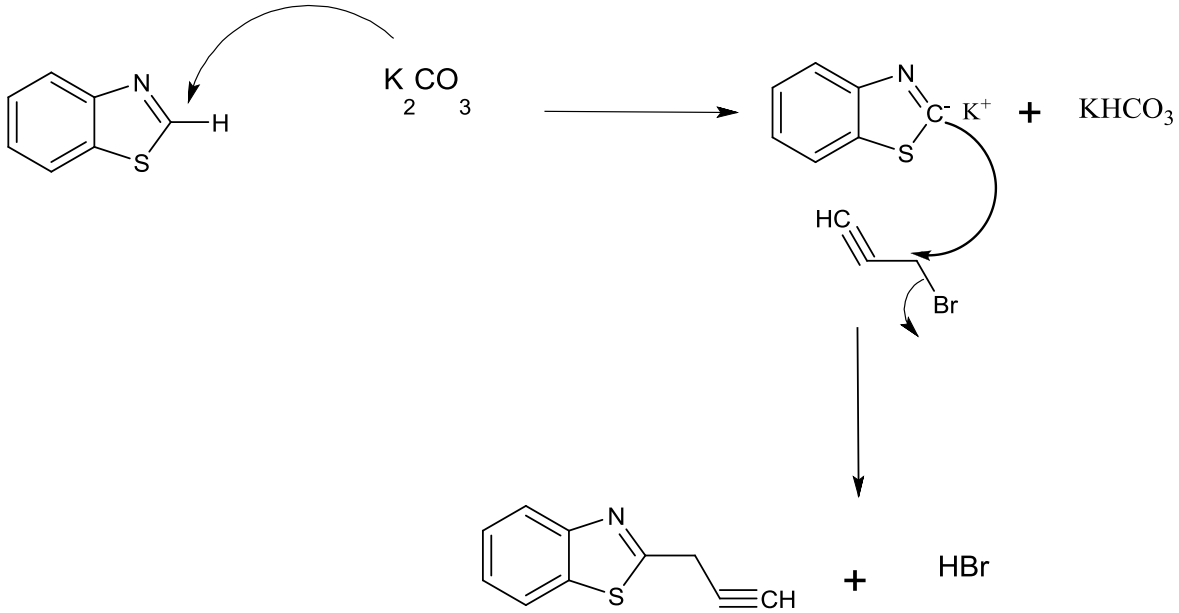
The compounds possessed a broad spectrum of antimicrobial activity against the tested microorganisms and displayed similar antimicrobial activity against fungi, as bacteria demonstrated by similar MIC values against the tested microorganisms. What we stated in regard to the antibacterial or antifungal activities with the structure activity relation represent at most possible rationale. All these compounds represent a novel and new approach in the area of antimicrobial and antifungal agents.

Table 4.1 : Proposed effect of molecular weight of the compounds on the diameter of inhibition zone.

Aminoacetylenic Compound	Molecular weight	Zone of inhibition at 100 mcg/ml against *P. aeruginosa*	Zone of inhibition at 100 mcg/ml against *S.aureus*	Zone of inhibition at 100 mcg/ml against *E.coli*	Zone of inhibition at 100 mcg/ml against *B.subtilis*
BZ2 	261 g/mol	27 mm	21mm	14 mm	20 mm
BZ3 	284 g/mol	26 mm	22mm	18 mm	21 mm
BZ4 	270 g/mol	20 mm	23mm	17 mm	20 mm
BZ5 	284 g/mol	17 mm	24mm	11 mm	20 mm
BZ6 	285 g/mol	13 mm	12mm	10 mm	14 mm
BZ7 	298 g/mol	12 mm	11mm	11 mm	13 mm

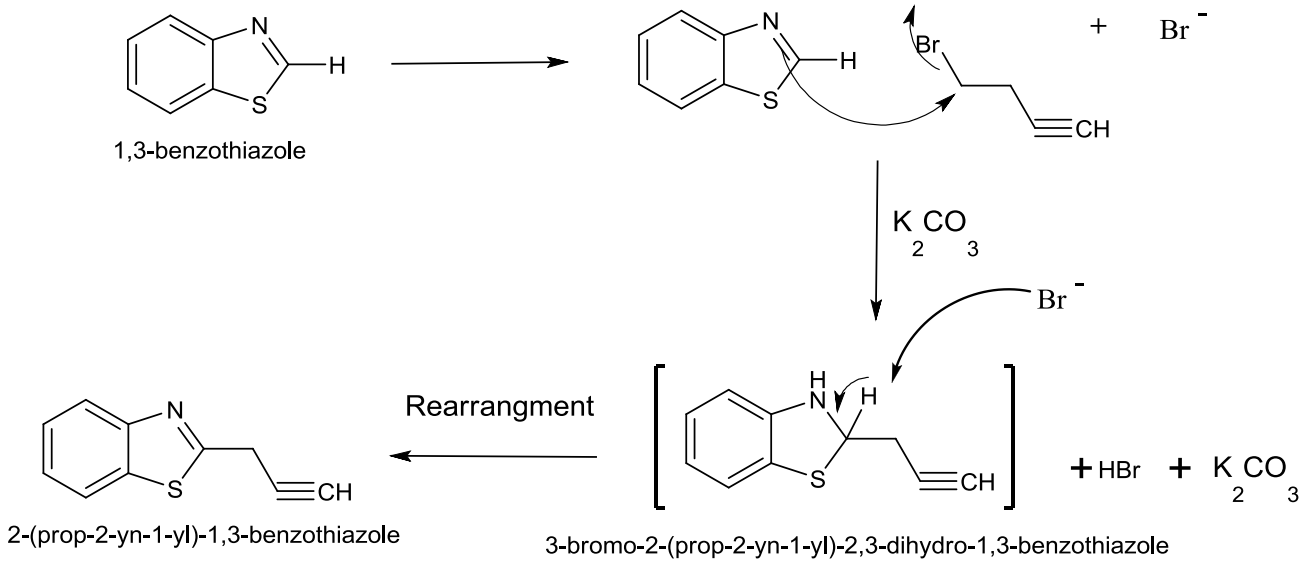
Possible alkylation reaction mechanism by two proposed reactions:

Reaction 1:



2-(prop-2-yn-1-yl)-1,3-benzothiazole

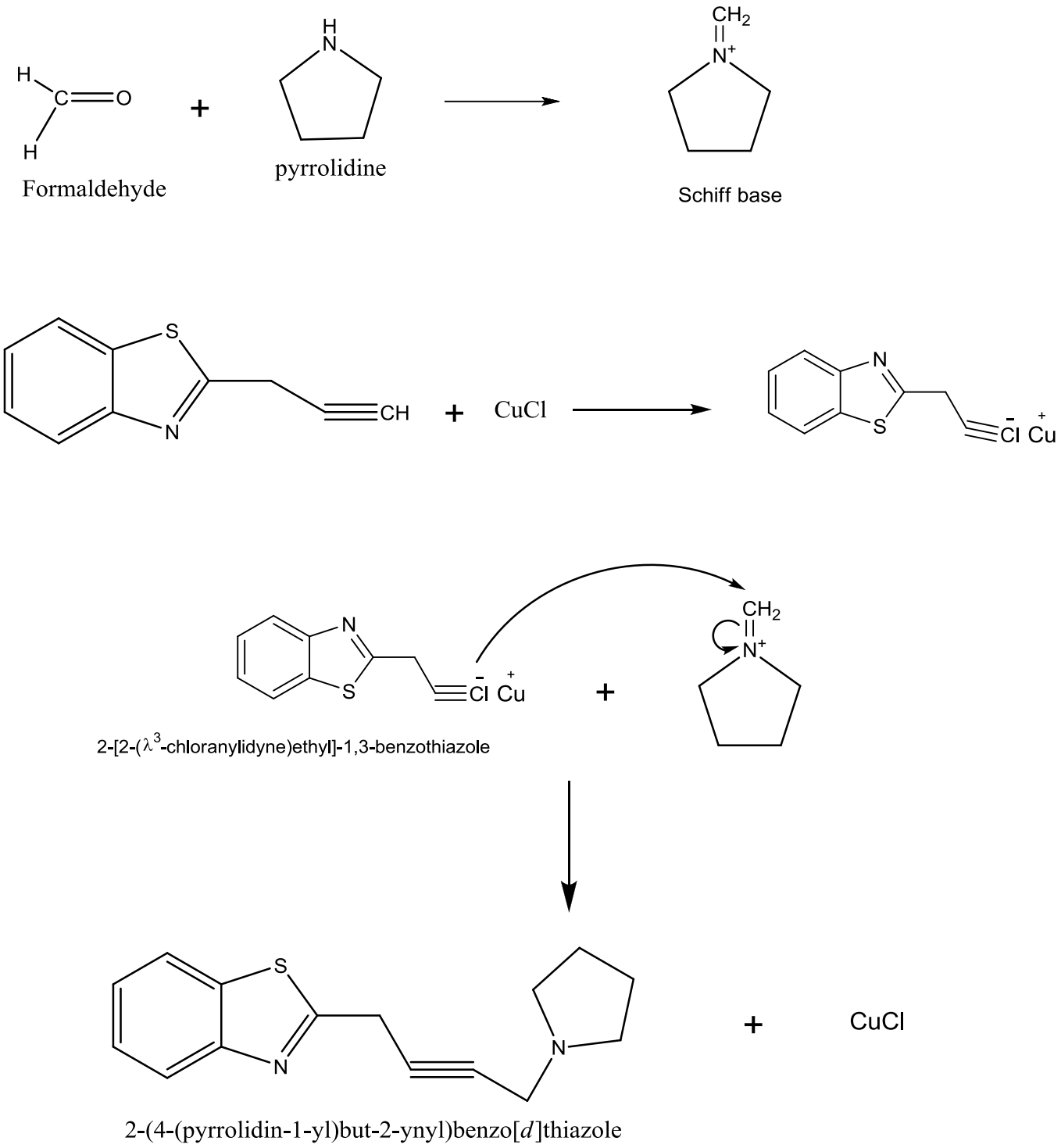
Reaction 2 :



2-(prop-2-yn-1-yl)-1,3-benzothiazole

3-bromo-2-(prop-2-yn-1-yl)-2,3-dihydro-1,3-benzothiazole

Scheme (4.1) : Alkylation reaction of benzothiazole moiety.



Scheme (4.2): Proposed Mannich reaction.

Conclusion:

In conclusion, we have reported the synthesis of novel series of aminoacetylenic benzothiazole derivatives. A unique aminoacetylenic side chain provides additional forces of interaction with the microorganism. All the novel above mentioned compounds were evaluated for their antimicrobial activity. These aminoacetylenic benzothiazole derivatives showed promising activity against Gram positive bacteria, Gram negative bacteria and fungi. Compound 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole **BZ5** showed the highest antibacterial activity against *S. aureus* among all the compounds with MIC value of 15.62 mcg/ml while; Compound 2-[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ7** exhibited the highest antibacterial activity against *P. aeruginosa* with a MIC value of 31.25 mcg/ml. This potency may be attributed to the steric effect and lipophilicity effect in azepan cyclic amine and 2,6-dimethylpiperidne group. Compounds 2-[4-(pyrrolidin-1-yl)but-2-yn-1-yl]-1,3-benzothiazole **BZ2** and 2-[4-(azepan-1-yl) but-2-yn-1-yl]-1,3-benzothiazole **BZ5** showed the highest antifungal activity against *C. albicans* with MIC value of 15.62 mcg/ml (for both). The results obtained from this project merit further studies to generate potential drug candidate derived from benzothiazole in the development of novel antimicrobial agents to resolve the problem of bacterial resistance.

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ملخص

تصنيع و دراسة الفعالية ضد الميكروبات المرضية لمشتقات بنزوثيازول حديثة الصنع

إعداد :

بهاء شفيق مصطفى

المشرف الرئيسي :

د.إلهام القيسي

المشرف الثاني :

أ.د زهير محي الدين

Benzothiazole ومشتقاته هي من المركبات الحلقية غير المتجانسة الأكثر أهمية، التي لها سمة مشتركة ومتكاملة من مجموعة متنوعة من المنتجات الطبيعية والمستحضرات الصيدلانية. Benzothiazole هي شاردة صغيرة ومع ذلك ، Benzothiazole ومشتقاته قد شغلت العلماء عن طريق عرض الأنشطة البيولوجية المختلفة. واحدة من الأنشطة البيولوجية الأكثر أهمية التي أظهرتها مجموعة Benzothiazole ومشتقاته هو النشاط المضاد للميكروبات. تضمن المشروع تركيب مشتقات Benzothiazole الجديدة، التي أدرجت المجاميع الجانبية aminoacetylenic، بهدف أن المركبات المقترحة قد تظهر فعالية جيدة ضد الميكروبات المرضية غالبا. تم استنتاج هياكل المركبات المحضرة حديثا على أساس تحليل العناصر والبيانات الطيفية. في المختبر تم تقييم الفعالية ضد الميكروبات بواسطة طريقتان هما : الانتشار خلال أجار و طريقة التخفيف في وسط زراعي سائل . في المختبر قد تم تقييم الفعالية ضد *S.aureus* ATCC 6538p ، *C. albicans* ATCC 10231، *P. aeruginosa* ATCC 9027 ، *E. coli* ATCC 8739، *B. subtilis* ATCC 6633 . تم تحديد MIC و MBC للمركبات التي تم تصنيعها حديثا في هذا البحث. وتمت مقارنة نتائج اختبار فعالية المركبات ضد الأحياء الدقيقة مع عقارين سيبروفلوكساسين5 ميكروجرام/ مل والفلوكونازول 500 ميكروجرام/ مل. مركب BZ5 أظهر أعلى فاعلية ضد *S. aureus* من بين كل المركبات مع قيمة MIC تساوي 15.62 ميكروجرام/مل. مركب BZ7 أظهر أعلى فاعلية ضد *P. aeruginosa* من بين كل المركبات مع قيمة MIC تساوي 31.25 ميكروجرام/مل.

مركبات BZ2 وBZ5 أظهرا أعلى فاعلية ضد *C.albicans* مع قيمة MIC تساوي 15.62 ميكروجرام/مل (على حد سواء) .النتائج التي تم الحصول عليها كافية لتشير إلى أن هذه المركبات لها فعالية جيدة ضد الأحياء الدقيقة التي تسبب الخمج ,و كان لكل واحد من هذه المركبات المصنعة في هذا البحث فعالية ضد الميكروبات على درجة مختلفة عند معاملتها على أنواع مختلفة من الكائنات الحية الدقيقة التي تم اختبارها.