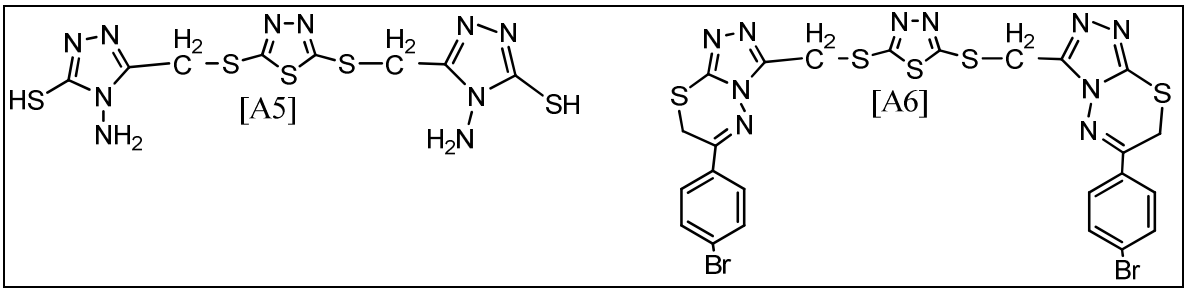
Abstract:

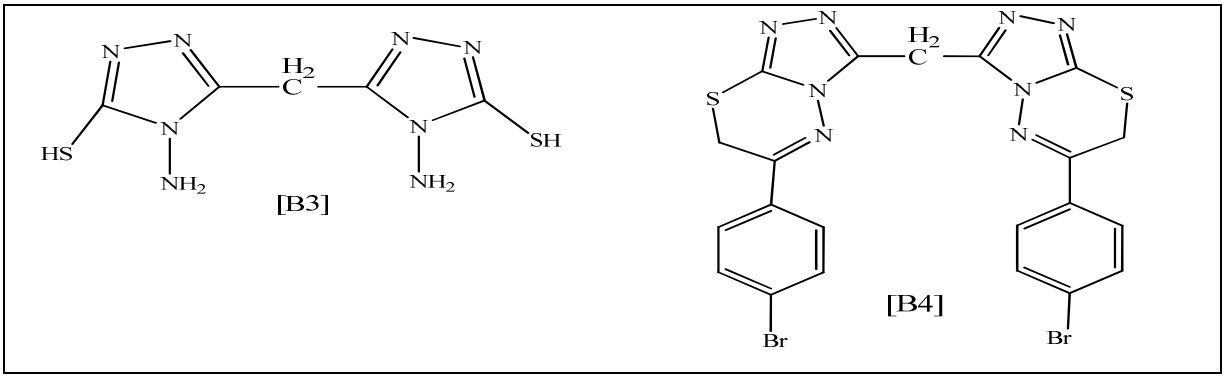
In this study, some of five membered and fused heterocyclic derivatives containing 1,2,4- Triazole, 1,3,4- Thiadiazole and triazolo [3,4-b] thiadiazine were synthesized by several cyclization steps.

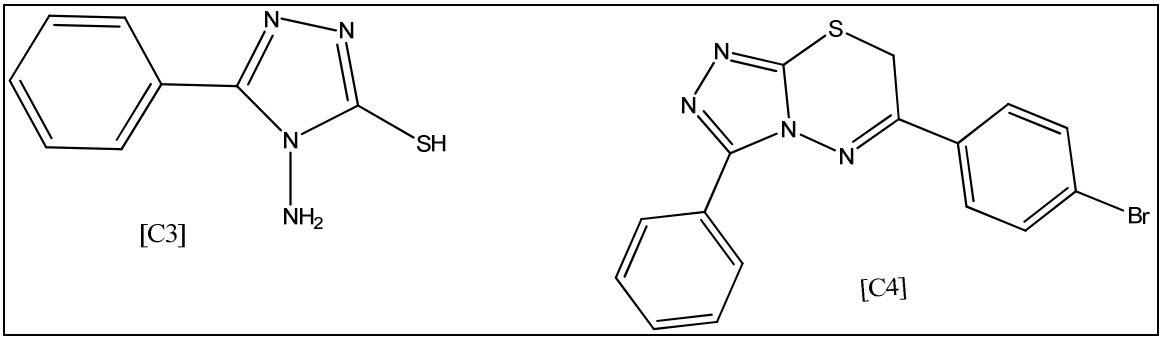
2, 5-dimercapto-1, 3, 4-thiadiazole [A1] was obtained by the reaction of hydrazine hydrate with carbon disulfide. Compound [A1] was reacted with Ethyl chloro acetate in presence of alkali ethanol to give 2, 5-bis (thioethylacetate 2, 2', diyl)1,3,4 thiadiazole [A2]. 2, 5-bis (thioacetiohydrazide-2-yl) - 1, 3, 4 -thiadiazole [A3] was obtained from the reaction of compound [A2] with hydrazine hydrate. bis-potassium dithiocarbazinate [A4] was obtained by reacting of compound [A3] with CS_2 in alkali ethanol. 2,5-bis(4-amino-3-thiol-5-thiomethyl-1,2,4 triazole-5,5'yl)- 1,3,4-thiadizole [A5] was obtained by the cyclization reaction of compound [A4] with hydrazine hydrate. The last step in this study involved preparation of 2,5-bis((6-(4-bromophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl-thiomethyl) -1,3,4-thiadiazole [A6] by the cyclization reaction of compound [A5] with p-Bromo phenacylbromide.



Compounds 5, 5'-methylene bis-(4-amino-4H-1,2,4-triazole-3-thiol) [B3], bis (6-(4-bromophenyl)-7H-[1,2,4]triazolo[3,4 b][1,3,4]thiadiazin-3-yl)methane [B4], 4[amino]-5-phenyl-4H-1, 2, 4-triazole-3-thiol [C3] and 6- (4-bromophenyl) - 3-phenyl - 7H - [1,2,4] triazolo[3,4-b] [1,3,4] thiadiazine [C4] were synthesized by

the same chemical reactions as these were used in the synthesis of compounds [A5] and [A6] using Ethyl Maolnate and Methyl Benzoate as starting materials instead of compound [A2].





The biological activity of compounds [A5], [A6], [B3], [B4], [C3] and [C4] were studied against four types of bacteria, (two of them were gram negative (*Escherichia Coli* and *Klepsiala pneomonia*), the others were gram positive (*Staphylococcus Aureus* and *Enterococcus Faecalis*). The fungus *Candida Albicans* was used in the antifungal study.

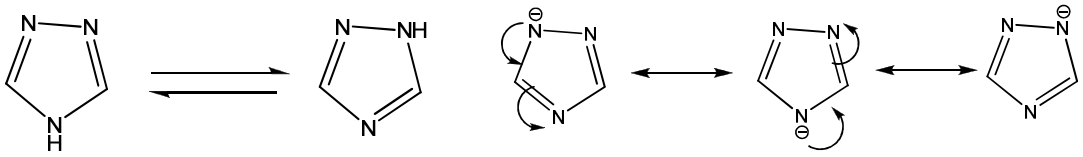
The results showed that most of these compounds have a high biological activity as antibacterial and antifungal agent against these types of microorganisms in comparison with the standard antibiotics and antifungal (Ceftriaxone, Amoxicillin and Fluconazole) as references.

1. Introduction

1.1 Triazoles:

Triazoles are five membered heterocyclic compounds containing three nitrogen and two carbon atoms [1]. There are two triazoles, and each has one pyrrole-like nitrogen and two pyridine-like nitrogens. Both triazoles have the possibility of tautomerism (in 1, 2, 3-triazole the tautomers are identical) and both give rise to a single anion.

1,2,3-triazole delocalized anion



1,2,4-triazole delocalized anion

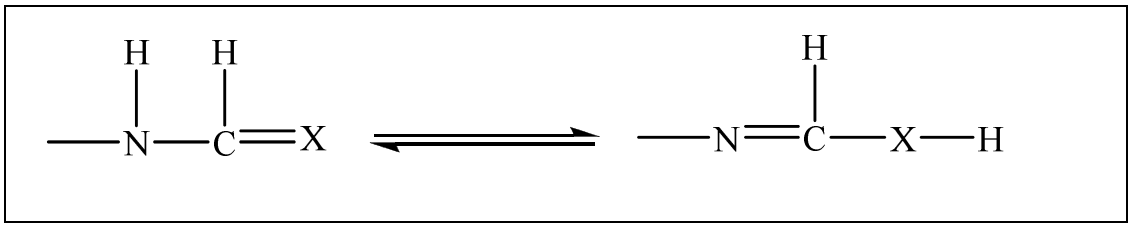
The 1,2,4-triazole is more important because it is the basis of the best modern agricultural fungicides as well as drugs for fungal diseases in humans. The extra nitrogen atom makes it more like pyridine and so more weakly basic, but it increases its acidity so that the anion is now

easy to make [2]. Many triazole compounds possess good fungicidal and plant growth regulating activity [3].

The 1, 2, 4-triazole is an ubiquitous feature of many pharmaceutical and agrochemical products. The substituted 1, 2, 4-triazole nucleus is particularly common, and can be found in marketed drugs such as fluconozole, terconazole, and rizatriptan alperazolam [4].

As drugs, triazole compounds are highly efficient, low poisonous and inward –absorbent. The studies on triazole derivatives are mainly concentrated on compounds with the triazole as the only active group, the reports of triazole compounds that contain both triazole group and other active group in the single molecule has rarely been found [5].

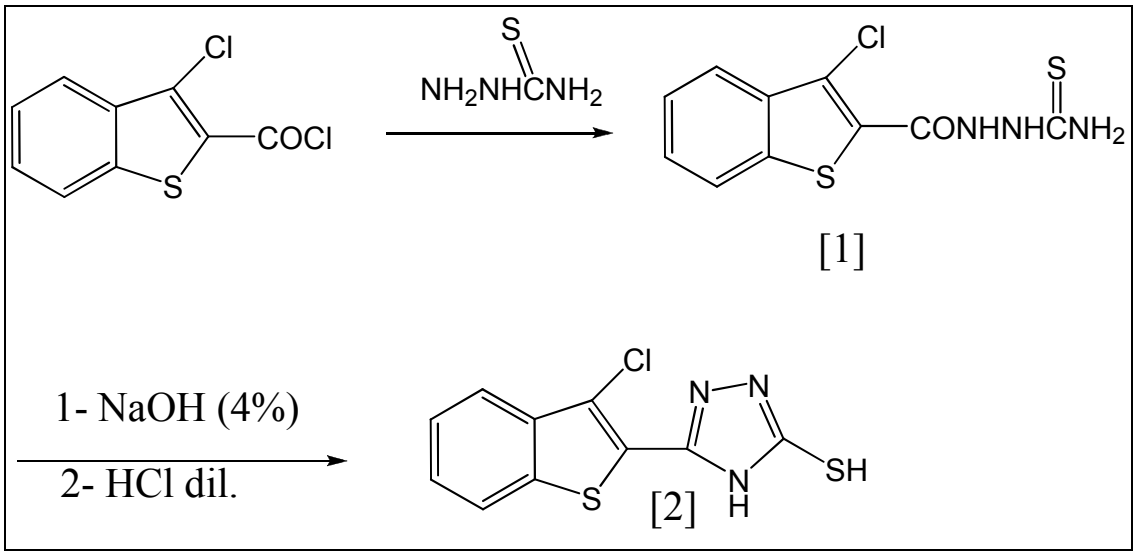
An important and versatile class of well-established biologically active compounds are those containing the moiety X, (X=N, O, S), which can exist in two tautomeric forms [6].



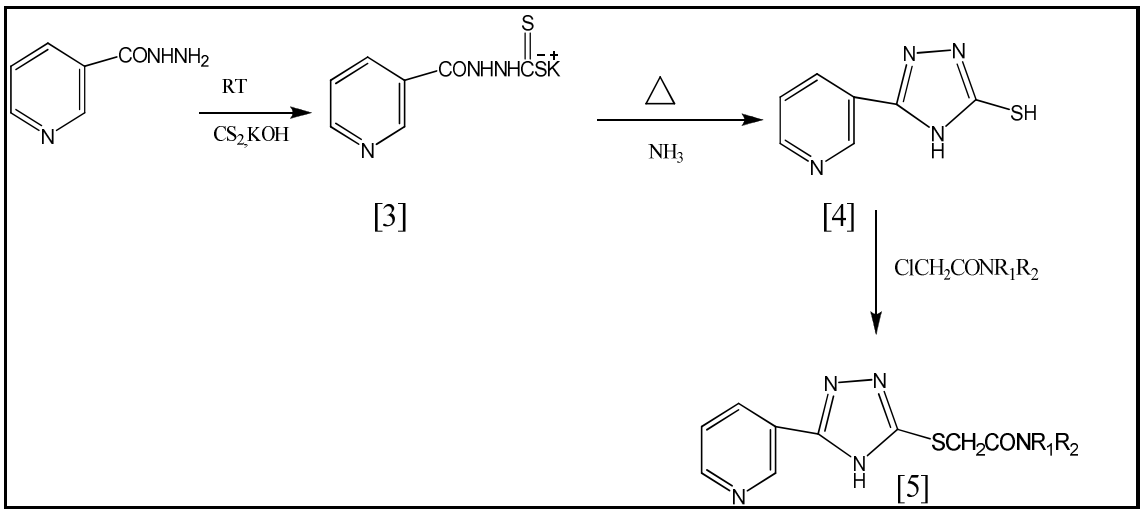
1.1.1 Synthesis of 1,2,4-triazoles:

Several methods have been used to synthesize 1,2,4-triazoles that explore the possibility of obtaining biologically useful compounds that contain the ring system, it was interesting for many scientists everywhere to synthesize numerous derivatives of these compounds.

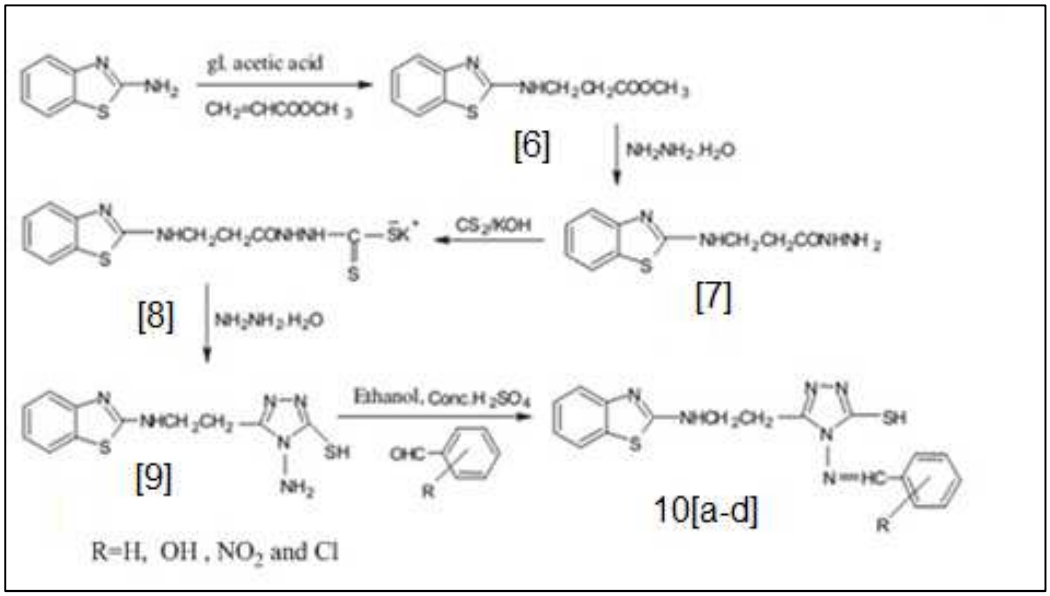
Sharba et al. [7], prepared 1, 2, 4-triazole derivative [2] by cyclization of compound [1] with dil. NaOH, the Compound [2] was obtained by acidification with dil. HCl.



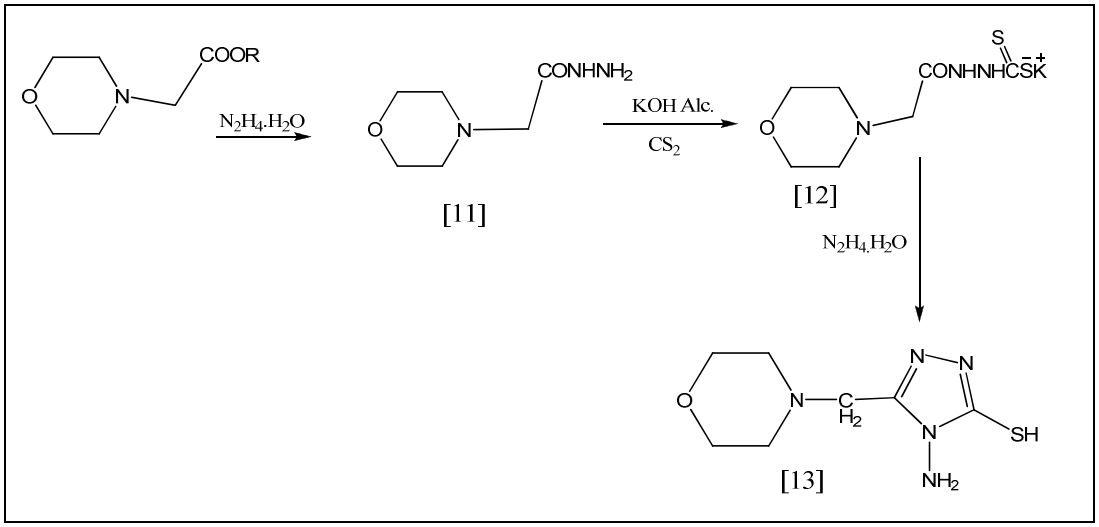
Mali R.K et al. [8], prepared the 3, 5-disubstuted triazole derivative [4] by Reaction of 3- (3'-pyridyl) -1, 2, 4-triazole-5-thiol [4] with appropriately N-substituted-α–chloroacetanilides in aqueous potassium hydroxide yielded corresponding 5-(N-substituted carboxamidomethylthio)–3-(3'-pyridyl) -1, 2, 4-triazoles [5].



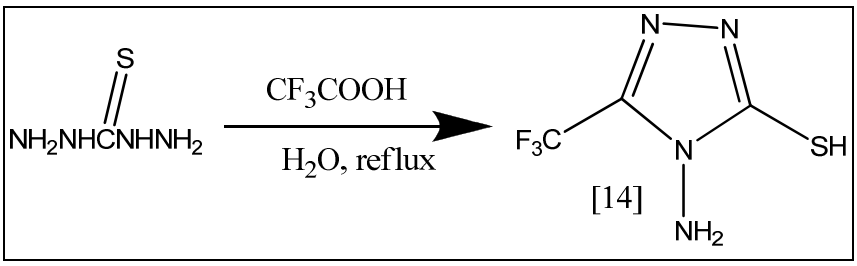
Soni B. et al. [9], prepared 1, 2, 4- trizole derivative [9] from cyclization of acid hydrazide salt with hydrazine hydrate to obtain the required triazole [9].



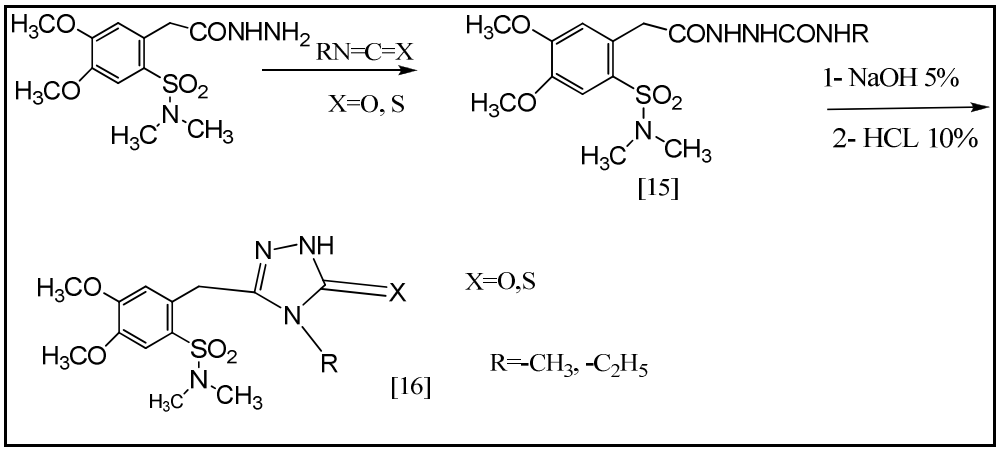
Sudhir N. et al. [10], prepared 1, 2, 4-triazoles derivative [13] by treating morpholine hydrazide with alcoholic potassium hydroxide, carbon disulfide and hydrazine hydrate.



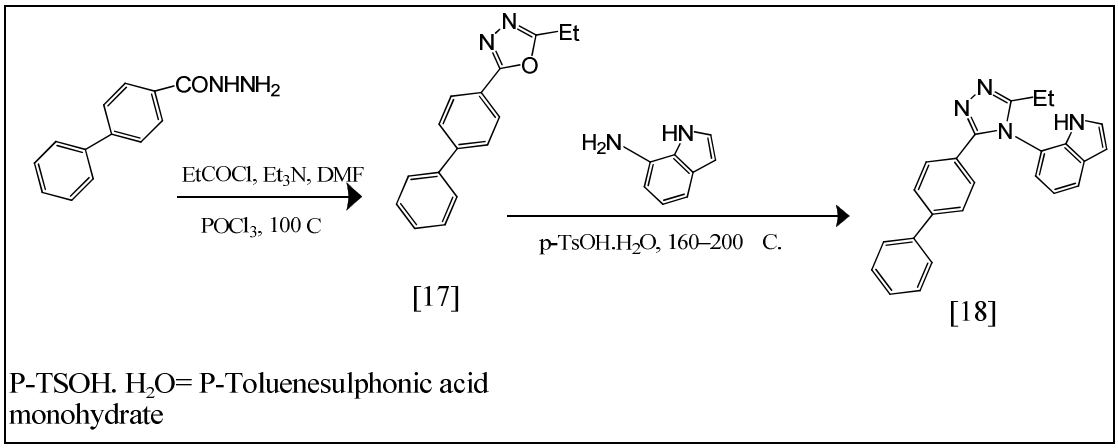
Chen M. et al. [11], prepared (4-amino-5-(trifluoromethyl)-4H-1,2,4-triazole-3-thiol) [14] by reaction of trifluoroacetic acid (TFA) with thiocarbohydrazide in refluxing water for 5 hrs.



Zoumpoulakis P. et al. [12], prepared 1, 2, 4-triazole derivative [15] by cyclization of compound [14] with NaOH % and acidified with HCl 10%.



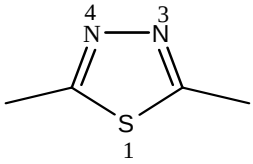
Takashi S., et al. [13], prepared 7-(3-Biphenyl -5-ethyl-4H-1, 2, 4-triazol-4-yl)-1H-indole [18] by reaction of compound [17] with aromatic amine under acidic conditions.



P-TSOH. H_2O= P-Toluenesulphonic acid monohydrate

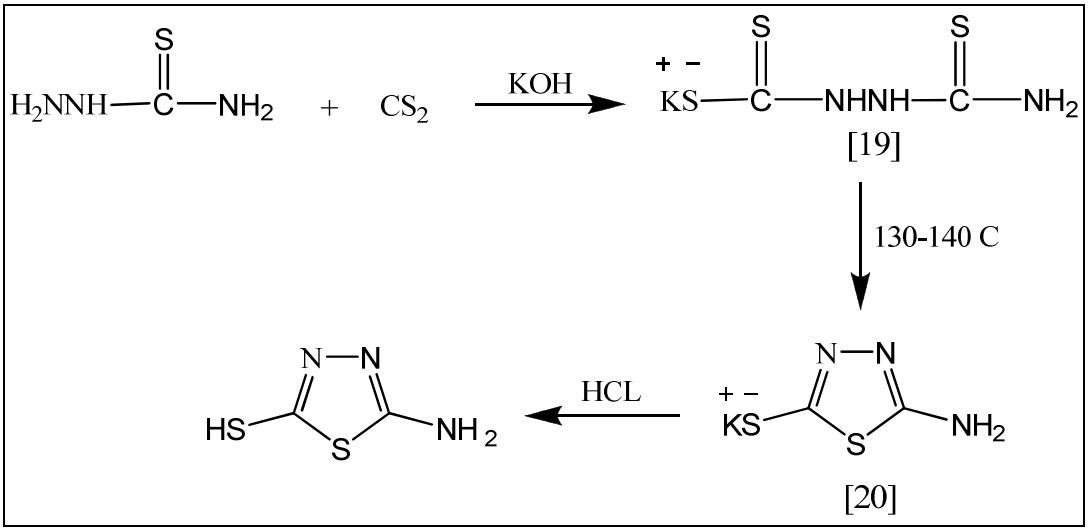
1.2. Thiadiazole

Thiadiazole is a 5-membered ring system containing two nitrogen and one sulphur atom. They occur in nature in four isomeric forms which were 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole and 1,3,4-thiadiazole. Thiadizoles have occupied an important place in drug industry. 1,3,4-thiadiazole have wide application in many fields the earliest uses were in the pharmaceuticals area as antibacterial with known sulphonamides drugs [14], some of the later used as antitumor [15],[16], anti-inflammatory [17], antimicrobial [18],[19], antihypertensive [14], antidepressant [14] and anti-diabetic [14] agents. These activities are probably due to –N=C–S– moiety. Furthermore, great number of variously substituted 1, 3, 4-thiadiazoles have been synthesized and tested for their different activities [20].

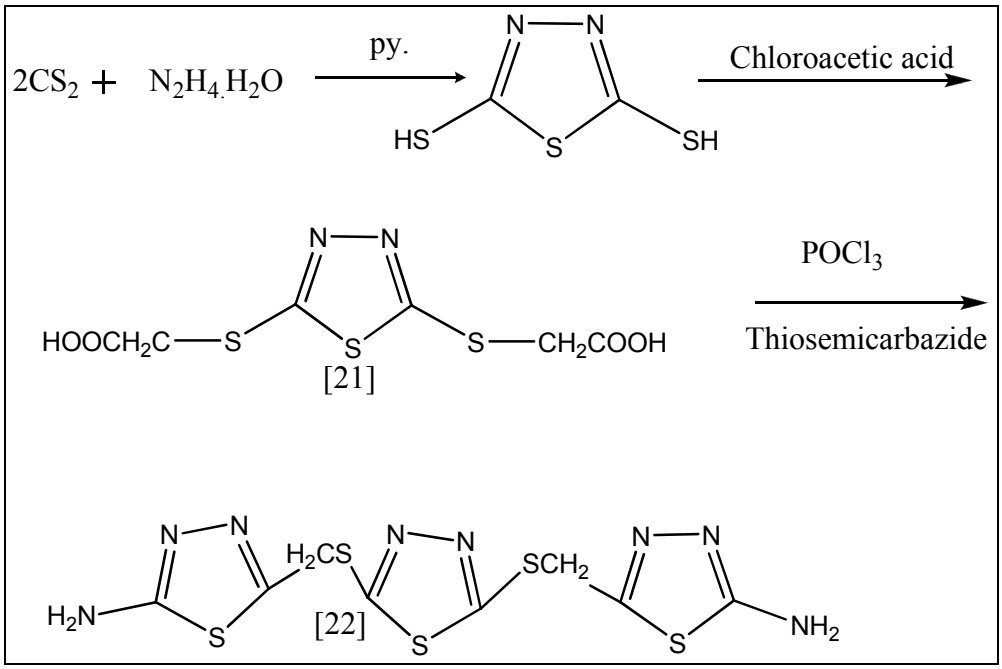


1.2.1 Synthesis of 1, 3, 4- thiadiazole

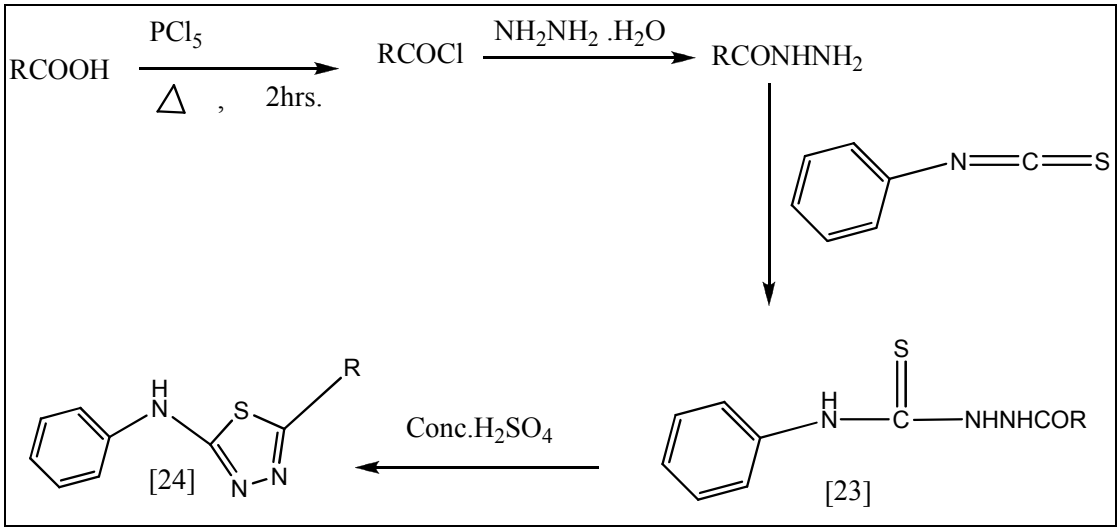
Since thiadiazoles have a variety of potential biological activities and utilities as technologically useful materials, a number of methods for the preparation have been developed. A useful preparative method for 2-amino-5-mercapto-1,3,4-thiadiazole was developed by *Guha* [21]



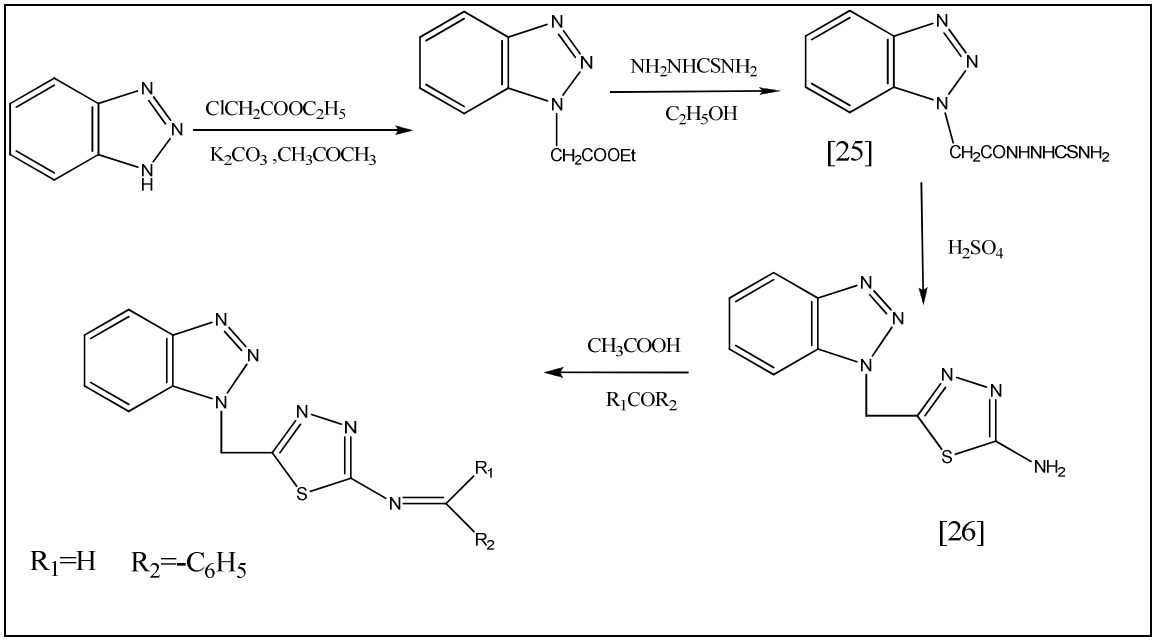
Salimon et al. [22], synthesized 1, 3, 4-Thiadiazole derivative [22] by cyclization of 2, 5-Dithioacetic acid-1,3,4-thiadiaozle [21] by phosphorus oxy chloride and thiosemicarbazide to obtain the required compound.



Muthukumar A. et al. [23], prepared 2-[phenyl] amino-5-substituted-1,3,4-thiadiazoles [24] by cyclization of substituted thiosemicarbazides [23] with sulfuric acid .

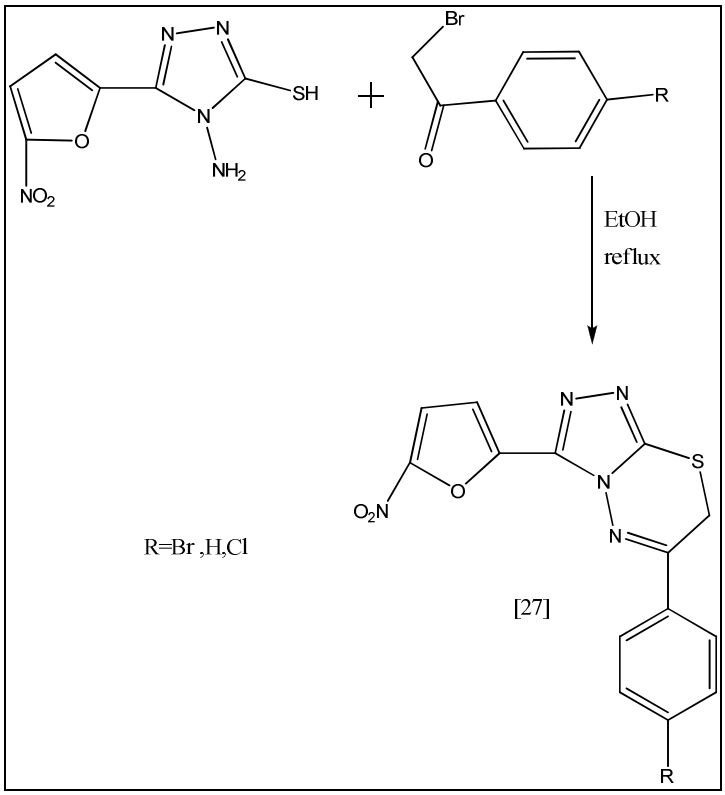


Singh T. et al. [24], Synthesized 1, 3, 4-thiadiazole derivative [26] by cyclization of N-Benztrizole acetyl thiosemicarbazide [25] with H_2SO_4.

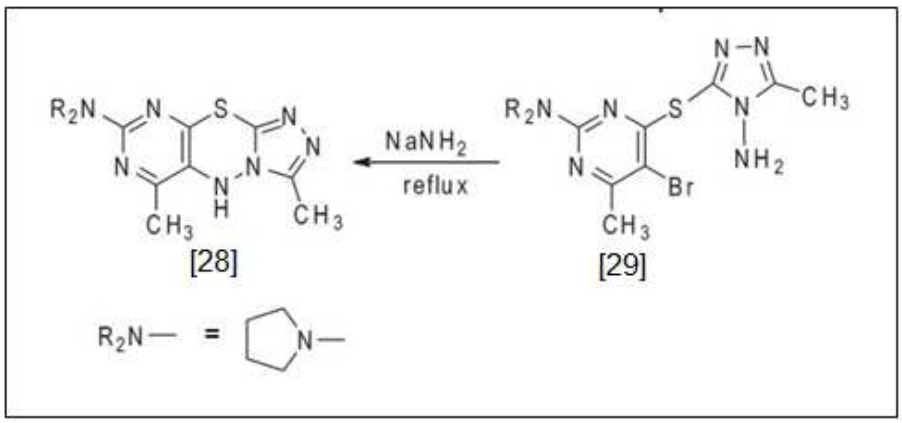


1.2.2 Synthesis of thiadiazine

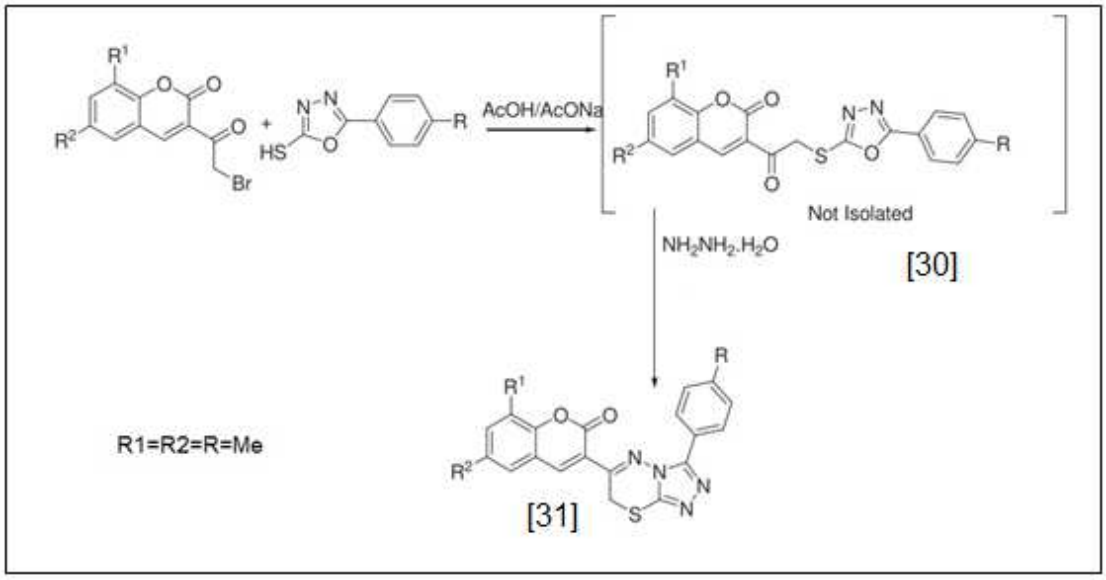
Badr S. et al. [25], prepared [1,3,4] thiadiazine derivative [27] by cyclization of 4-amino-5-(5-nitrofuran-2-yl)-4H-1,2,4-triazole-3-thio with 4-substituted phenacyl bromides as shown below.



Bakavolia et al [26]. prepared [1,3,4] thiadiazine derivative [29] by cyclization of compound [28] with $NaNH_2$.



Rao et al [27]. prepared [1,3,4]thiadiazine derivative [31] by cyclization of compound [30] with hydrazine hydrate.



1.3 Biological activity

1.3.1 Antimicrobials:

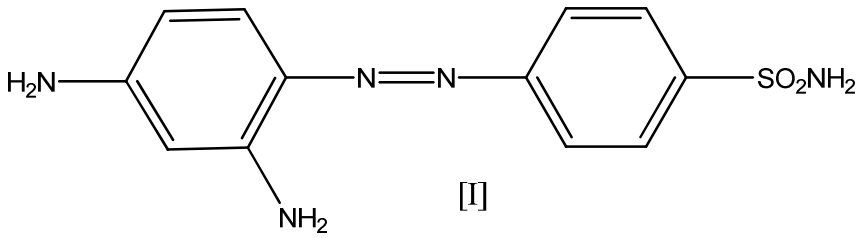
Antimicrobial chemotherapeutic agents are chemical agents that are used to treat diseases. Chemo therapeutic agents destroy pathogenic microorganisms or inhibit their growth at concentration low enough to avoid undesirable damage to the host [28].

Most of these agents are antibiotics their derivatives that can kill susceptible microorganisms or inhibit their growth. The modern era of chemotherapy began with the work of the German physician *Paul Ehrlich* (1854-1915) who began experimenting with dyes, *Ehrlich*'s successes in the chemotherapy of sleeping sickness and syphilis established his concept of the selective toxicity and led to testing of hundreds of compounds of their therapeutic potential.

German chemical industry in 1927 began a long-term search for the chemotherapeutic agents under the direction of *Gerhard Domagk* who

discovered that prontosil red [I] A new dye of staining leather, was nontoxic for animals and completely protected mice against pathogenic bacteria *streptococci* and *staphylococci*. In 1929 the Scottish physician *Alexander Fleming* found that broth from a pencillium culture contained penicillin and that the antibiotic could destroy a number of pathogenic bacteria. The discovery of penicillin stimulated the search for other antibiotics [28].

This was led to broad screening programs that were set up to find agents which would be effective in the treatment of infections that had been resistant to chemotherapeutic agent, as well as to provide safer and more rapid therapy for infections. As a result large numbers of antibiotics were discovered such as Incomycin [29], Gentamycin [30] and the researches were continued in order to discover new antibiotics.



[I]

2`,4`-diamino azobenzen-4-sulfonamide (prontosil red)

1.3.2. Mechanism of resistance to antimicrobial agent

Sooner or later bacteria develop resistance to virtually any antibiotic agents. Resistance has many consequences and also requires the use of more toxic or more expensive alternative drugs[31]. Resistance thus effects the antibiotic options available to every practitioner and is no less a problem in the developing world [32]. Despite the versatility of antimicrobial agents and the evaluation of new ones, bacteria have a limited number of mechanisms of antimicrobial resistance; including:

The production of detoxifying enzyme like β-lactamase which destroy β-lactam antibiotics, or production of chloramphenicol acetyl-transferase in gram- negative chloramphenicol resistant bacteria[33].

In some cases the alteration in the target for the drug; which includes both reduction of receptor affinity and the substitution of an alternative pathway[34]. For example, resistance for some penicillins and cephalosporins May be a function of the loss or alternation of the pencillin binding proteins (PBPs) of bacterial ribosome [35].

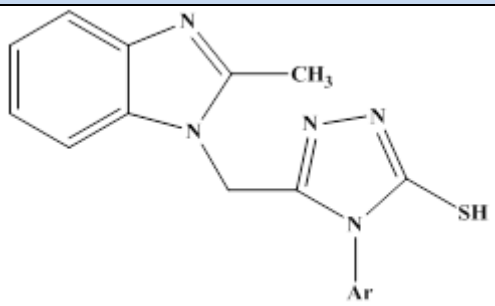
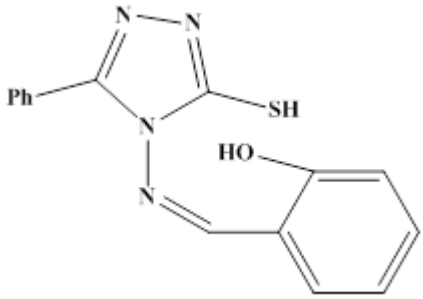
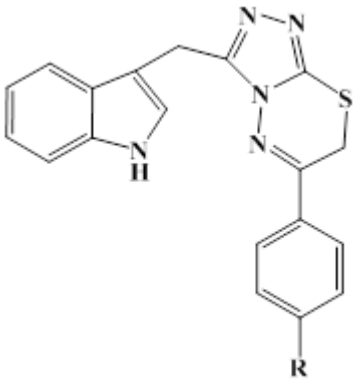
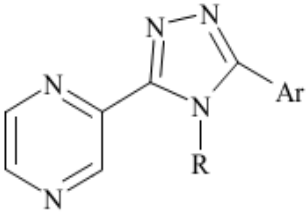
Also decreased antibiotic uptake; which occurs through either diminished permeability or an active afflux system [33], in *pseudomonas astreruginosa*, 50 to 85% of resistant strains produce chloramphenicol acetyl transferase CAT [35]. In the remainder resistance is caused by decreased outer member permeability [36],[37] reported that the active efflux of tetracycline is mediated by new membrane transport system *pseudomonas astreruginosa* can also become resistant specifically to imipenem through the loss of an outer membrane protein (porin) that provides a channel for the entry of imipenem [38]. At last the over expression, that chromosomal β-lactamase of *E.Coli* is made in low amounts that is does not contribute appreciably to β-lactam resistance However, clinical strains have been isolated that make large amount of β-lactamase because the promoter for it's gene has been altered to allow more efficient expression [39].

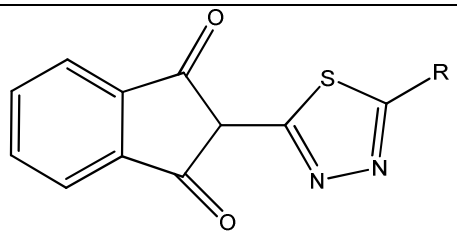
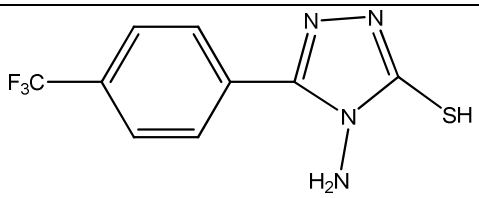
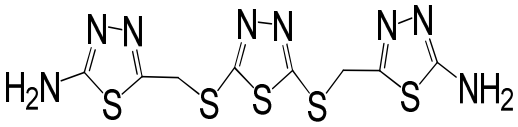
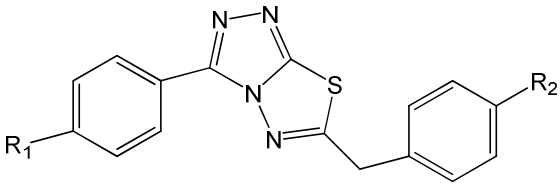
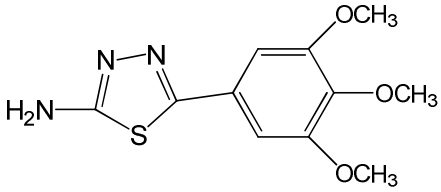
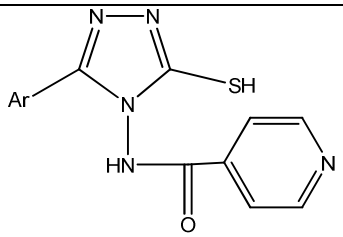
1.3.3-Biological activity of 1, 2, 4-triazole and 1, 3, 4-thiadiazole

In the past decades, the problem of multi drug resistant micro-organisms has reached on alarming level around the world, and the synthesis of new anti-infective compounds has become an urgent need for the treatment of microbial infections. The 1, 2, 4-triazole nucleus has been incorporated into a wide variety of therapeutically important agents, which mainly displaying antimicrobial activities [40-44].

Organic compounds incorporating heterocyclic ring systems continue to attract considerable interest due to their wide range of biological activities. Among different five-membered heterocyclic systems 1, 2, 4-triazoles and 1, 3, 4-thiadiazoles and their derivatives have gained importance as they constitute the structural features of many bioactive compounds. It is known that triazole rings are included in the structure of various drugs [45-49]. From these classes of heterocyclic compounds, the synthesis of new derivatives of 1,2,4-triazole-3-thiones has been attracting considerable attention because of various biological properties such as: antibacterial [50,51], antifungal [52-54], anti-tubercular [55-56], antiviral [55,56], antioxidant [57,58], antitumoral [59-61], anti-inflammatory [62-64], anticonvulsant [65-67] etc. Table (1-1) Summarizes structures and biological activity of some 1, 2, 4-triazole and 1, 3, 4-thiadiazole compounds.

Table (1-1) biological activity of some 1, 2, 4-triazole and 1, 3, 4-thiadiazole compounds

No.	Compound name	structure	Biological activity
1	4-aryl-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-4H-1,2,4-triazole-3-thiols		Antibacterial and antifungal
2	2-[(3-Mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)iminometh-yl]phenol		Antibacterial and antifungal
3	3-[(1H-Indol-3-yl)methyl]-6-aryl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines		Antibacterial and antifungal
4	2-(4,5-disubstituted -4,5-dihydro-3H-1,2,4-triazol-3-yl) pyrazine		Antibacterial and antifungal

5	2-(5- pentadecyl-1,3,4-thiadiazole-2-y l)isoindoline-1,3-dione		Antibacterial and antifungal
6	4-Amino-5-(4-(trifluoromethyl)phenyl)-4H-1,2,4-triazole-3-thiol		Antifungal
7	2,5-Di[5-amino-1,3,4-thiadiazol-2-thiomethyl]-1,3,4-thiadiazole		Antibacterial activity
8	3-aryl-6-arylamino-1,2,4-triazolo[3,4-b]1,3,4-thiadiazoles		Antibacterial activity
9	2-amino-5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole		Antibacterial and antifungal
10	N-(3-aryl-5-mercapto-4H-1,2,4-triazol-4-yl)isonicotinamide		Antimicrobial and anti-inflammatory

1.3.4. Mechanism of action of antifungal agent

There are three general mechanisms of action for the antifungal agents:

1-Cell membrane disruption

2-Inhibition of cell division

3-Inhibition of cell wall formation

1.3.4.1 Cell membrane disruption

Antifungal agents that disrupt the cell membrane do so by targeting Ergosterol, either by binding to the sterol, forming pores and causing the membrane to become leaky (as with polyene antifungals), or inhibiting Ergosterol biosynthesis (as seen with azole antifungal agents). Ergosterol is similar to mammalian cholesterol, thus agents binding Ergosterol may have a cytotoxic effect in the host tissue. Ergosterol has two conjugated double bonds that are lacking in mammalian sterols.

1.3.4.1.1 Azole and Triazole antifungal agents

Azole antimycotics were first developed in the 1960s [77]. Azoles are classified by the number of nitrogen atoms located in the azole, nitrogen heterocyclic ring: the imidazoles have two nitrogens, and include ketoconazole, miconazole, and clotrimazole; the triazoles have three nitrogens and include fluconazole [77]. The structure of fluconazole is shown in Figure (1.1). Fluconazole is prescribed for certain systemic fungal infections, and is administered per orally[77]. As of 1999, fluconazole had been used to treat over 16 million patients.

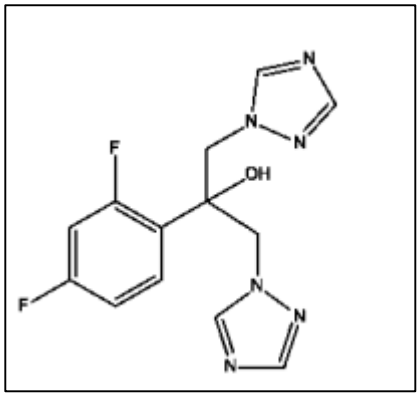
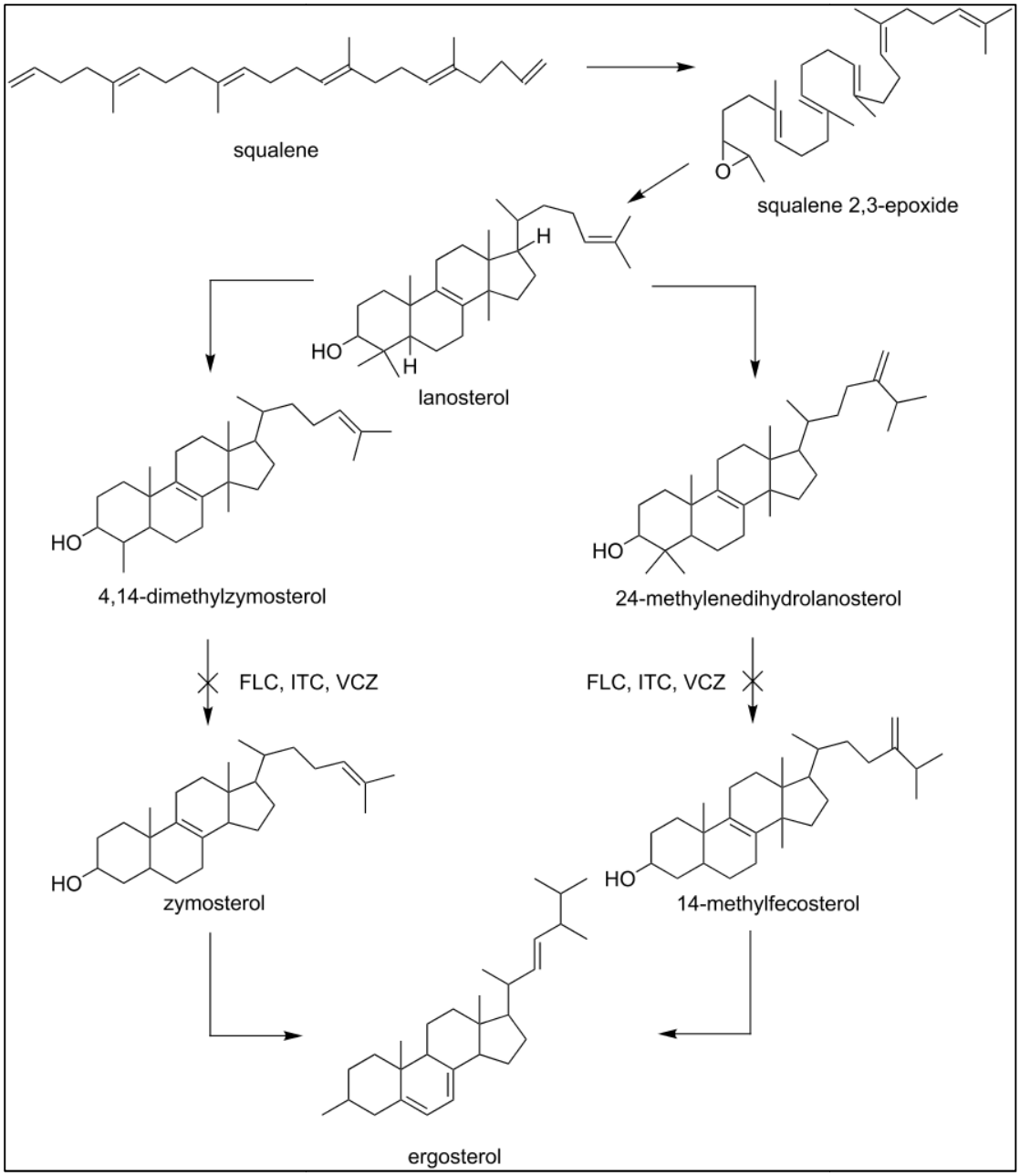


Figure (1.1) Molecular structure of fluconazole.

1.3.4.1.1.1 Mechanism of action of triazole

The antifungal triazoles (fluconazole , itraconazole ,voriconazole) are synthetic compounds that have one or more azole rings with three nitrogen atoms in a five membered ring. They act by inhibition of the cytochrome P450- dependent conversion of lanosterol to ergosterol [(78)]. Triazoles act as cytochrome P450 14α-demethylase inhibitors. This enzyme is involved in the conversion of lanosterol to ergosterol which is helpful in the cell membrane synthesis. In this mechanism the basic nitrogen of the azole ring is tightly bound to the heme iron of the fungal cytochrome P450 preventing substrate and oxygen binding. Inhibition of the 14α-demethylase results in accumulation of sterols and causes permeability change and malfunction of membrane proteins. The ergosterol biosynthesis inhibitor pathway is shown in Figures (1.2) and (1.3) [(79)].



(FLC) Fluconazole, (ITC) Itraconazole, (VCZ) Voriconazole.

Figure (1.2) Ergosterol biosynthesis inhibitor pathway.

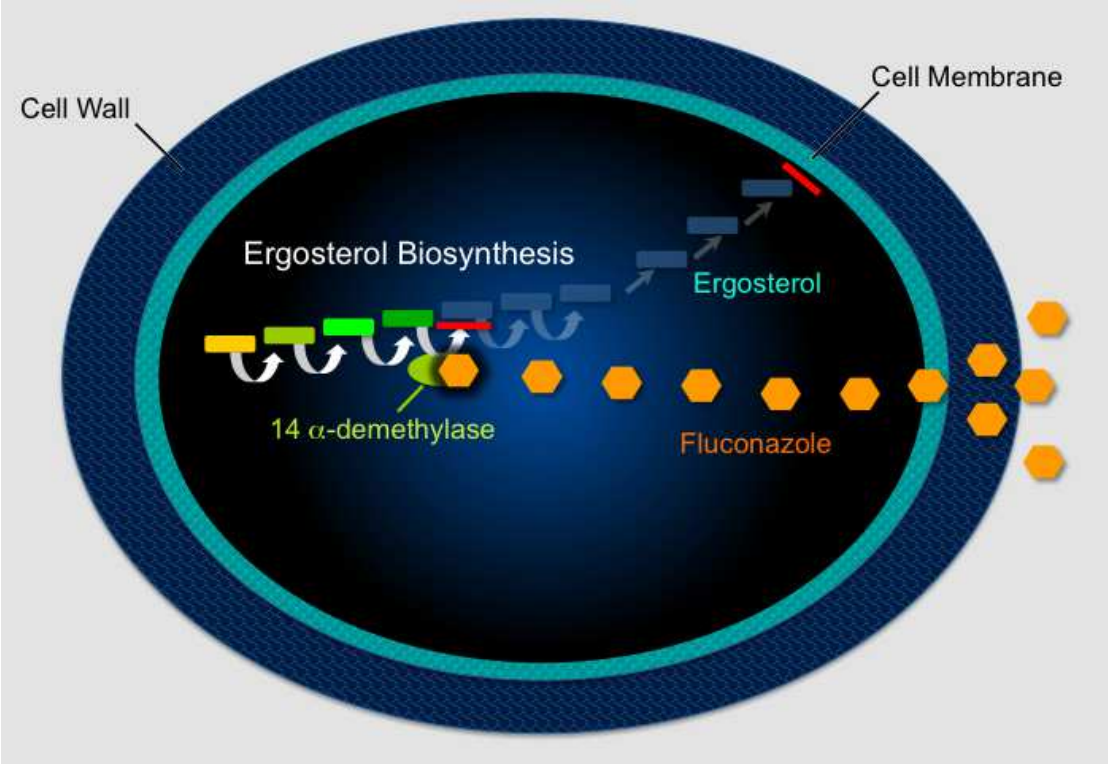


Figure (1.3) Ergosterol biosynthesis inhibitor pathway.

1.4 Pathogenic bacteria

The pathogenesis of bacterial infection includes initiation of the infectious process and the mechanisms that lead to development of signs and symptoms of disease. Characteristics of bacteria that are pathogens include transmissibility, adherence to host cell invasion of host cells and tissues. Toxigenicity and ability to evade the host's immune system. Many infections caused by bacteria that are commonly considered to be pathogens are in apparent or asymptomatic. Disease occurs if the bacteria or immunologic reactions to their presence cause sufficient harm to the person [80].

١.4.1 Staphylococci

They are gram positive spherical cell. Usually arranged in grape like irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of normal flora of skin and mucous membranes of humans, a variety of pyogenic infections. And even fatal septicemia. The pathogenic *staphylococci* often hemolyse blood. Coagulate plasma and produce a variety of extra cellular enzymes and toxins. The most common type of food poisoning is caused by a heat-stable *staphylococci* rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems.

S. aureus is a major pathogen for human. Almost every person will have some type of *S. aureus* infection during a poisoning or minor skin infection to serve life-threating infections. *S. aureus* infection can also result from direct contamination and a wound like the post-operative staphylococcoal [80].

1.4.2 Escherichia coli

They are gram negative cells, part of normal flora and incidentally caused disease, while others, the *salmonellae* and *shigellae*, are regularly pathogenic for humans. *E. coli* cause diarrhea are extremely common world-wide. These *E. coli* are classified by characteristics of their virulence properties and each group caused disease by different mechanism. Enteropathogenic *E. coli* (EPCE*)* is an important cause of diarrhea in infants especially in developing countries. EPCE adhere to the mucosal cell small bowel [80].

1.4.3 Klebsiella pneumonia

K. pneumonia is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines [81]. *K. pneumonia* contains a capsule around its cell. Known as K antigen, it is to protect the bacteria from phagocytosis [82].

K. pneumonia is commonly found in the gastrointestinal tract and hands of hospital personnel [83]. The reason for its pathogenicity is the thick capsule layer surrounding the bacterium. It is 160 nm thick of fine fibers that protrudes out from the outer membrane at right angles [84], [85]. Another site on the human body that this bacteria can be found is the nasopharynx. Its habitat is not limited to humans but is ubiquitous to the ecological environment. This includes surface water, sewage, and soil [86]. *K. pneumonia* is an important cause of human infections. Infections or diseases are usually nosocomial or hospital-acquired. In 1998, *K. pneumonia* and *K. oxytoca* accounted for 8% of nosocomial bacterial infections in the United States and in Europe. Diseases include urinary tract infections, pneumonia, septicemias, and soft tissue infections [87] *K.pneumoniae* can cause the disease Klebsiella pneumonia. They cause destructive changes to human lungs inflammation and hemorrhage with cell death (necrosis) that sometimes produces a thick, bloody, mucoid sputum (currant jelly sputum). The diseases caused by *K. pneumonia* can result in death for patients who are immune deficient [87]. CPS and LPS O side chain are two of the most important virulence factors of K. pneumoniae [88]. They serve to protect the bacterium from phagocytosis by the host. Treatment is done by antibiotics such as clinafloxacin[89]. But, there are an increasing amount of antibiotic-resistance strains. Ciprofloxacin is an antibiotic that is becoming less effective [90].

1.4.4 Enterococcus faecalis

Enterococcus faecalis is a Gram-positive, commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals [81] Like other species in the genus *Enterococcus*, *E. faecalis* can cause life-threatening infections in humans, especially in the nosocomial (hospital)environment, where the naturally high levels of antibiotic resistance found in *E. faecalis* contribute to its pathogenicity [81]. *E. faecalis* has been frequently found in root canal-treated teeth in prevalence values ranging from 30% to 90% of the cases [91]. Root canal-treated teeth are about nine times more likely to harbor *E. faecalis* than cases of primary infections [92]. *E. faecalis* can cause endocarditis and bacteremia, urinary tract infections (UTI), meningitis, and other infections in humans [93].

1.5 Pathogenic Fungi:

Fungi cause direct harm to human and animals by means of toxins or inducing allergic reactions, or by progressive infection (mycoses). About one – fifth of the world's population, suffer or have suffered from mycoses. In human, most infection involves the skin, hair and nails (Superficial mycoses). These mycoses are unpleasant and can be difficult to cure, but will not normally be lethal. Infections inside the body (deep – seated mycoses) are much more dangerous. They may become generalized and be fatal unless treated. The agents of most superficial mycoses are a group known as dermatophyton, belonging to genera. *Microsporium, Epidermophyton and trichophyton*. The various different dermatophytes usually cause virtually identical lesion if the infection involve the same part of the body [94]. Dermatophytoses refers to

infections of the skin, nail or hair that are caused by classified as dermatophytes.

Normally, it is not considered that dermatophytic fungi can systemic disease, in fact, with only one minor exception; none of these fungi can grow at $37C^0$. The dermatophytic fungi include numerous species of fungi which contained in three major genera. These organisms occur world-wide, mainly in soil and on certain animals. Within a given locale one never sees all of these organisms causing diseases.

Dermatophytoses are some of the most common diseases of man. Although incidences of infection vary greatly, at least 10 – 20% of the world's population may be infected with these organisms. An outstanding feature of these organism is that they are all keratinophilic which means that love keratin [95].

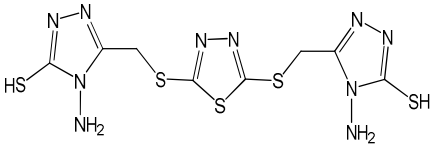
1.5.1 Candida albicans:

Candida albicans is ubiquitous, dimorphous yeast; it has been known for many centuries and has the potential to cause human diseases under specific circumstances and condition. The actual taxonomy of Candida albicans and related species has only been confirmed within the twentieth century. The oral carriage rate for the organism is high, with nearly one half of the healthy population harboring the organism. Numerous predisposing factors for oral condidiasis have been recognized including metabolic, dietary, mechanical and iatrogenic factors. Multiple clinical forms of the disease including acute, chronic and mucocutaneous presentation. Although rarely fatal in the absence of other serious underlying disease, oral candidiasis may serve as a useful clinical marker for the presence of significant predisposing condition [(96)].

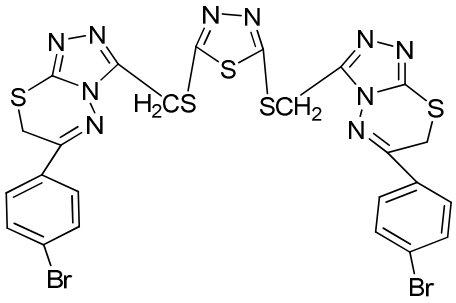
Candida albicans is a component of the normal skin flora and also the chief cause of the mucocutaneous fungal disease in humans [(97)]. Candida can also infect fingernail, producing onychomycosis and paronychia and is more common with advanced HIV disease [(98)]. *Candida albicans* is the yeast pathogen most frequently isolated from patients with fungemia: Oropharyngeal candidiasis is the most common opportunistic infection immunodeficiency virus infected patients [(99)].

1.6 Aim of the work

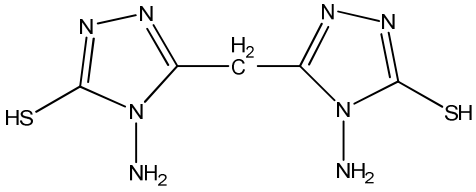
1-Synthesis of heterocyclic derivatives containing 1,2,4-triazole, 1,3,4-thiadiazole and triazolo [3,4-b] thiadiazine (A5, A6, B4, B5, C4 and C5) derived from 2, 5-Dimercaptothiadiazole, Ethyl Malonate and Methyl Benzoate having the following structural formula:



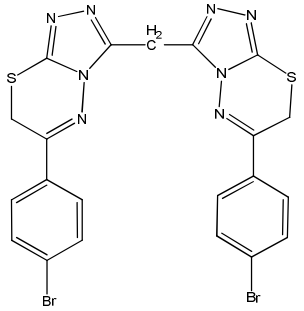
[A5]



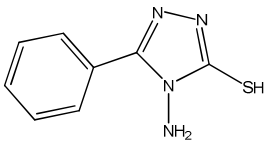
[A6]



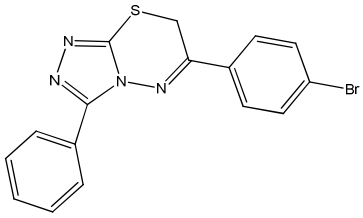
[B3]



[B4]



[**C3**]



[C4]

2- Evaluation the antibacterial and antifungal activities of the synthesized compounds.

2. Experimental

2.1 Instruments

- Melting points were determined on electro thermal capillary apparatus and were uncorrected, *Gallenkamp*, England.
- Infrared spectra were recorded on FT.IR-8300 Fourier transforms infrared spectrophotometer Shimadzu as potassium bromide disc in the (400-4000) cm^{-1} spectral range. (AlNahrain University & AlMustansirya University).

- ^{1}H NMR spectra were obtained with Bruker spectrophotometer model ultra shield at 300 MHz in DMSO- d6 solution with the TMS as internal standard. (AL-Albait University/ Amman/ Jordan).

  Note: for all ^{1}H NMR spectra, the peaks at 2.5 and 3.33 are for the solvent (DMSO-d6) and dissolved water in (DMSO-d6) respectively.
- Microorganisms were incubated on *Unive* 2011, Incubator/ Baghdad University/ Biology Department.

2.2 Materials

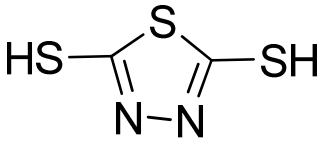
All chemicals employed were of analytical reagent grade and were used without further purification.

Table (2-1) shows all the utilized chemicals in the experimental course of the thesis.

Table (2-1) Chemicals and their manufacturers

Number	Materials	Company
1	Abs. ethanol	Scharlau
2	Amoxicillin	Bilim pharmaceuticals
3	Anhydrous sodium acetate	Fluka
4	Carbon-di-sulfide	Scharlau
5	Ceftriaxone	Bilim pharmaceuticals
6	Chloro ethyl acetate	Fluka
7	Conc. HCl	Thomas BAKER
8	Di-Ethyl malonate	Fluka
9	Dimethyl Formamide	BDH
10	Dimethyl sulfoxide	Fluka
11	Ethyl Ether	BDH
12	Fluconazole	Bilim pharmaceuticals
13	Hydrazine hydrate 80%	Thomas BAKER
14	Lead acetate	Fluka
15	Methyl benzoate	Fluka
16	p-bromophenacyl bromide	Fluka
17	Potassium hydroxide	Fluka
18	Pyridine	Fluka

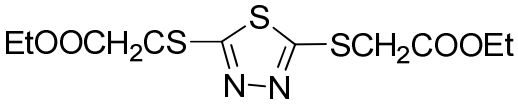
2.3.1 Preparation of 2, 5-dimercapto-1, 3, 4-thiadiazole: [A1]



A mixture of (80%) hydrazine hydrate (0.1 mol, 4.5 mL) and carbon disulfide (0.2 mol, 20mL) in (30 mL) dry pyridine was refluxed for (5 hrs.) and the resulting solid was separated out by adding (25 mL) of water and (5 mL) of Conc. hydrochloric acid. The mixture was then filtered and the solid was recrystallized from ethanol.

yield 77.6 %, Color : yellow, m.p: (162-164) °C, reported١٦٤ -166°C (100), (22)

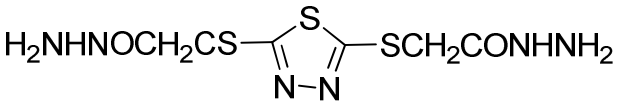
2.3.2 Synthesis of 2, 5-bis (thio ethylacetate 2, 2', diyl)1,3,4 thiadiazole[101]. [A2]



To a solution of 2,5-dimercapto-1,3,4-thiadiazole (0.1 mol, 15 g) in 50 mL of absolute ethanol, (0.2 mol, 12g) of potassium hydroxide was added. The solution was stirred for 30 minutes, and then (0.2mol, 25mL) ethyl chloroacetate was added drop wise to the solution. The reaction mixture was refluxed for 4-5 hrs; cooled to room temperature and poured into 100 mL of ice water. The precipitated solid was filtered off, washed with water and crystallized from ethanol.

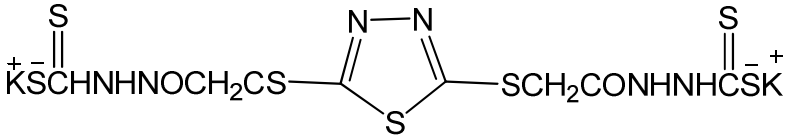
m.p: (47-48) C° yield 79%., color : white

2.3.3 Synthesis of 2, 5-bis (thio acetiohydrazide-2-yl) - 1, 3, 4 - thiadiazole [A3]



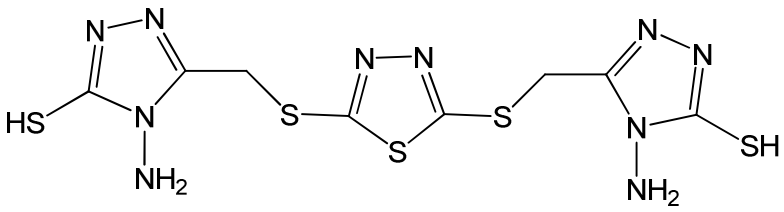
A Suspention (0.02 mol, 6.4 g) of compound [A2] with 20 mL of absolute ethanol was stirred for 15 min at 40 C until the ester was dissolved after that (0.04 mol, 2.2mL 2 g) hydrazine hydrate (80 %) was added and stirred for 30 min . Cooling the solution, filtered and washed with ethanol. m.p = (140-142) C°, yield 74 %. , color: white

2.3.4 Synthesis of bis-potassium dithiocarbazinate [102]. [A4]



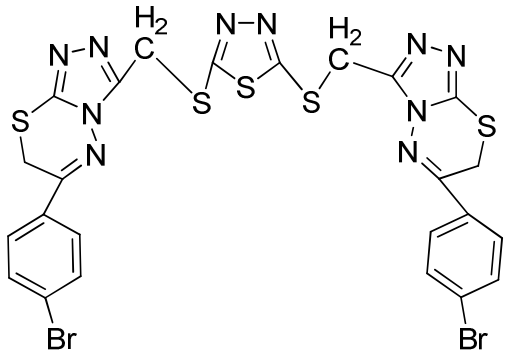
(0.03 mol, 1.68 g) of Potassium hydroxide was dissolved in (25 mL) absolute ethanol. The solution was cooled in ice bath and (0.01 mol, 2.94g) of the compound [A3] was added with stirring. To this mixture (0.05 mol, 5 mL) carbon disulfide was added in small portions with constant stirring. The reaction mixture was agitated continuously for 18 h at room temperature. Cold ethanol (20 mL) and dry di-ethyl ether (20mL) was added to the solution and then evaporated. The potassium salt thus obtained was used in the next step without further purification.Yield 73 %., color: yellow.

2.3.5 Synthesis of 2,5-bis(4-amino-3-thiol-5-thiomethyl-1,2,4 triazole-5,5'yl)- 1,3,4-thiadizole [103]. [A5]



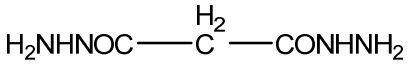
A suspension of (3 mmol, 1.56g) compound [A4] in (4 mL) water and hydrazine hydrate (80%, 9 mmol, 0.45mL) was refluxed for 18– 20 hrs. The color of the reaction mixture changed into green with the evolution of hydrogen sulfide. The reaction mixture was cooled to room temperature and diluted with (5mL) cold water. On acidification with HCl the required triazole was precipitated out, which was recrystallized from DMF–water mixture. m.p = (198-200) C°, Yield= 52%, color: white

2.3.6 Synthesis of 2,5-bis((6-(4-bromophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)thiomethyl)-1,3,4-thiadiazole [102]. [A6]



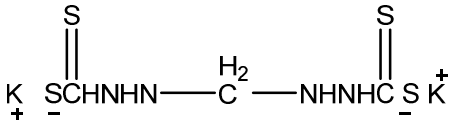
A suspension of (0.5 mmol, 0.4 g) compound [A5] and (1.5mmol, 0.4g) p-bromophenacyl bromide in (10 mL) absolute ethanol was heated under reflux for 3 hrs., then (1.5mmol, 0.15 g) of anhydrous sodium acetate was added. The reaction mixture was heated for an additional 1hr., then cooled and poured onto ice-cold water. The solid product was crystallized from ethanol. m.p = (192-194) C°, yield =71%, color: yellow.

2.3.7 Synthesis of Malonohydrazide [104]. [B1]



Ethyl malonate (0.12 mol, 19 mL) in 25 mL of ethanol absolute is taken in a round bottom flask. To that (80 %) (0.24 mol, 14mL) hydrazine hydrate was added and refluxed for 4 hrs. The total volume of the solution is reduced to half and it was cooled. The solid was recrystallised from ethanol. M.p. (133-135) °C, yield 76.5 %, color: white

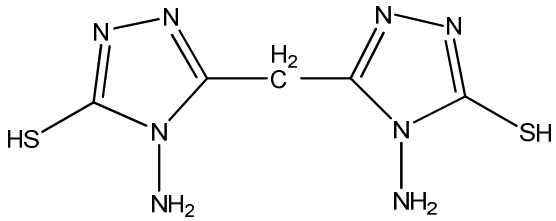
2.3.8 Synthesis of bis-potassium dithiocarbazinate [102]. [B2]



the compound [B2] was synthesized according to the procedure that was described in 2.3.4 using compound [B1] instead of compound [A3].

yield 74%., color: yellow

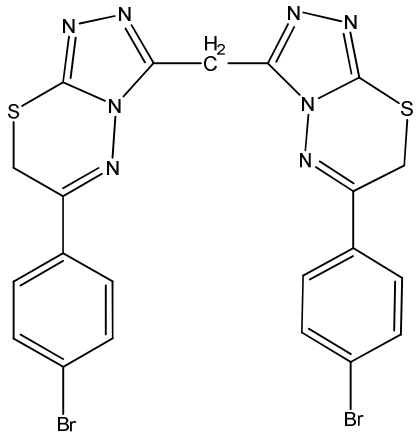
2.3.9 Synthesis of 5, 5'-methylene bis-(4-amino-4H-1,2,4-triazole-3-thiol) [103]. **(B3)**



The compound [B3] was synthesized according to the procedure that was described in 2.3.5 using compound [B2] instead of compound [A4].

m.p: (190-193) °C yield 52%, color: white

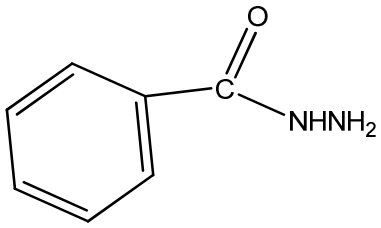
2.3.10 Synthesis of bis (6-(4-bromophenyl)-7H-[1,2,4]triazolo[3,4 b][1,3,4]thiadiazin-3-yl)methane[102]. (B4)



the compound [B4] was synthesized according to the procedure that was described in 2.3.6 using compound [B3] instead of compound [A5].

m.p (158-160) C° yield 69%, color : yellow

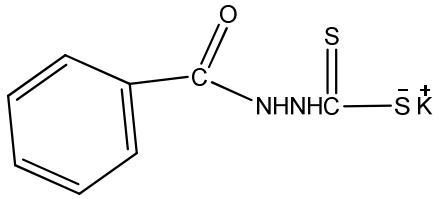
2.3.11 preparation of benzo hydrazide [C1] (104)



The compound [C1] was synthesized according to the procedure that was described in 2.3.7 using Methyl benzoate instead of ethyl malonate.

m.p (111-113) °C., Reported (112-114) C° yield 71 %, color: white

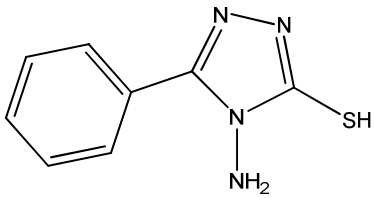
2.3.12 preparation of potassium dithiocarbazinate, [C2] (102)



The compound [C2] was synthesized according to the procedure that was described in 2.3.4 using compound [C1] instead of compound [A4].

yield 66%, color: yellow

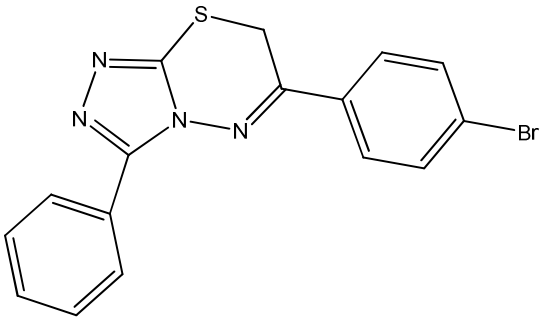
2.3.13 Synthesis of 4[amino]-5-phenyl-4H-1, 2, 4-triazole-3-thiol, [C3] (103)



The compound [C3] was synthesized according to the procedure that was described in 2.3.5 using compound [C2] instead of compound [A4].

Recrystallized from ethanol. m.p (195-197) C° reported (195) C° (105) yield: 60 %, color : white

2.3.14 synthesis of 6- (4-bromophenyl) - 3-phenyl - 7H - [1,2,4] triazolo[3,4-b] [1,3,4] thiadiazine (102). [C4]



The compound [C4] was synthesized according to the procedure that was described in 2.3.6 using compound [C3] instead of compound [A5].

m.p (213-215) °C, yield 72%., color : yellow.

2.4 Biological activity

2.4.1 Antimicrobial activity

In this study, some of the synthesized compounds were evaluated for their in vitro antimicrobial activity against the pathogenic bacteria and pathogenic fungal, four bacterial species were used: two of them were gram positive bacteria which were Staphylococcus aureus and *Enterococuus faecalis* the others were gram negative bacteria, which were *Escherichia Coli* and *Klepsialla Pneumonia* and one fungus was used which was candida albicans. All of these microorganisms were obtained from, Department of biology/ college of Science/ University of Baghdad.

2.4.2 Culture Media:

Some liquid and solid media are used and prepared according to methods. These media are:

1- Nutrient agar

2- Nutrient broth

2.4.3 Preparation of nutrient broth and nutrient agar

Nutrient broth and nutrient agar were prepared by dissolving 28 gm for each liter of distilled water [106]. Sterilization was achieved by autoclaving under pressure of (1.5 atm) and temperature of (115C^{o}) for (15-20 minutes). The medium was cooled to 50C^{o}. Then it was poured into the plates and left in room temperature to dry so that the plantation medium will be solid, a semi-solid gelatinous layer was generated [107].

2.4.4 Activation of microorganisms

3 – 5 colonies of studied bacteria and fungi were transported by using a loop to a test tube containing (5 mL) of sterilized nutrient broth. The tube was shaken well and incubated at 37C^{o} for 24 hours. The loop was sterilized by a flame before using so that the planted bacteria were not killed.

2.4.5 Antibacterial and antifungal evaluation

Medium Inoculated bacteria and fungi suspension were diluted by 1/100 by using normal-saline liquid with concentration of (0.85%) to prevent crowded growth. (0.1 mL) of bacteria diluted suspension were transported to each plate and spread by using sterilized cotton spreader on test medium surface. The plates were left for 15-20 minutes at 37^{o}C to make absorption.

In the solidified medium suitably spaced apart holes were made all 6mm in diameter. These holes were filled with 0.1mL of the prepared compounds. Four concentrations for each compound were prepared, (25,50,100and 200 µg/mL)), Amoxicillin and ceftriaxone were used as references antibiotic drugs and fluconazole as reference antifungal drug. DMSO was used as a solvent for these compounds.

one of these holes were filled with DMSO as control, to see the effect of solvent for each type of bacteria and fungi, These plates were incubated at 37C° for 24hrs.

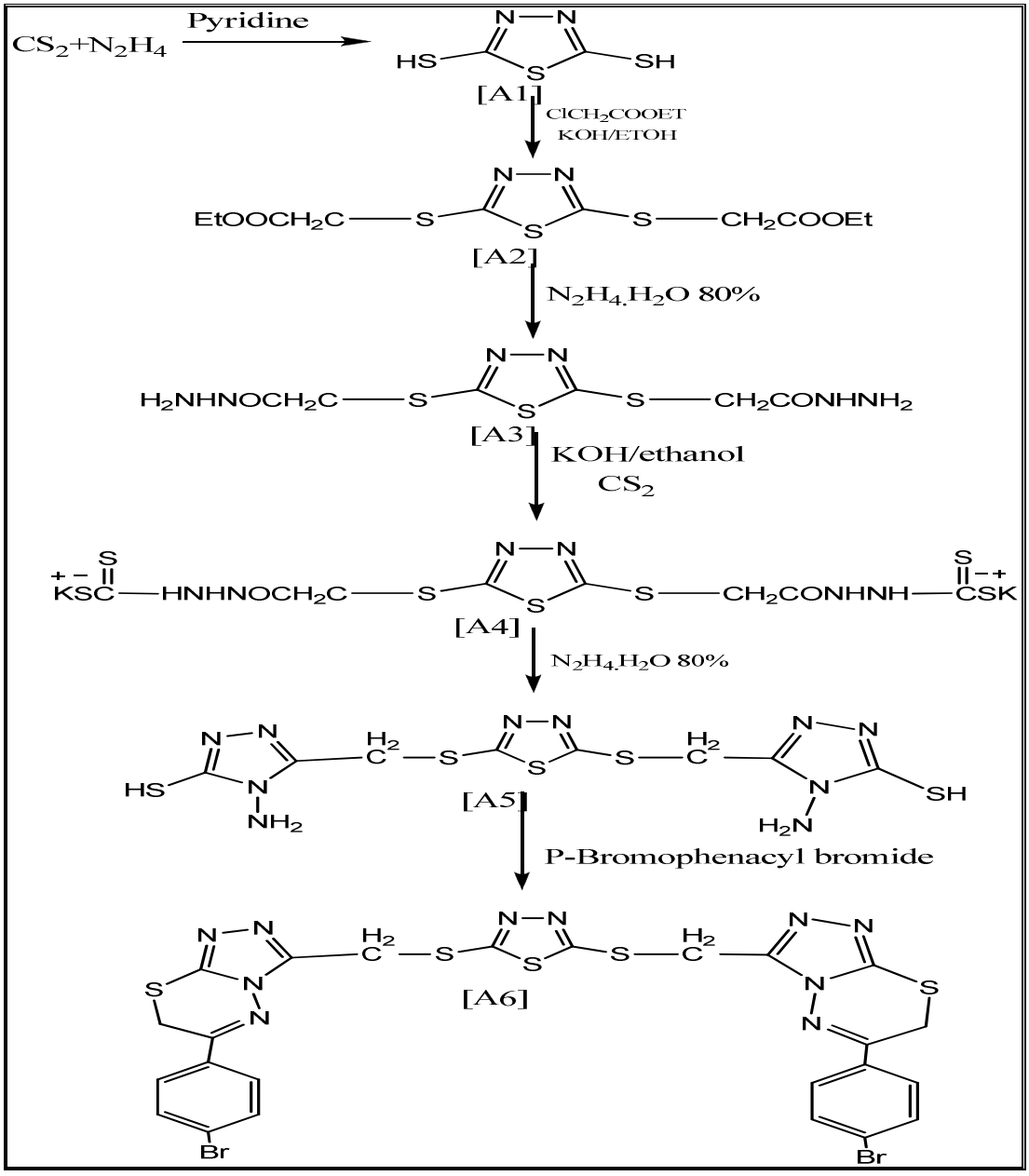
These procedure was used of another attempt using the concentration 25 µg/mL only as a suitable concentration which was the less concentration to kill bacteria and fungi with a clear inhibition zone.

After the incubation, then measurement of Inhibition Zone the transparency area which surrounds the hole, including the radius of hole which represents the area which had no bacterial or fungal growth, was measured. This area is called inhibition zone by using a mm ruler with digital caliper. The bacteria and fungi were considered sensitive, mean-sensitive or resistant depending on inhibition zone.

3- Results and Discussion:

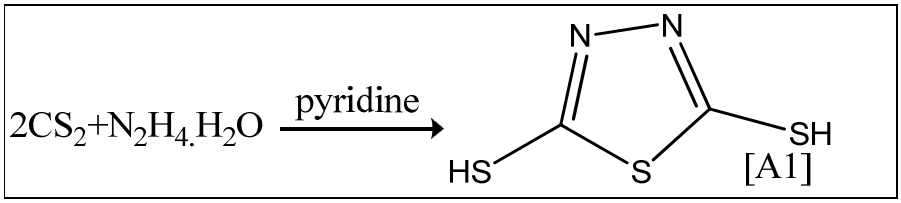
3.1 Chemistry:

The chemical steps for the synthesis of compounds [A1-A6] are shown in scheme (3-1).

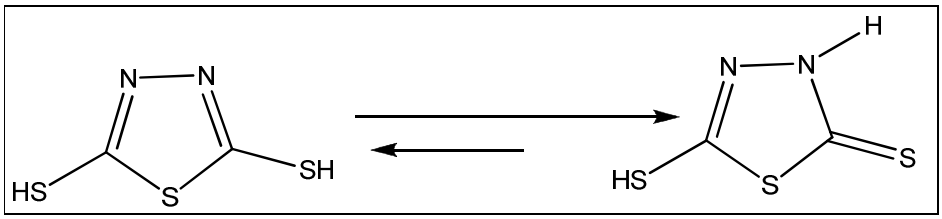


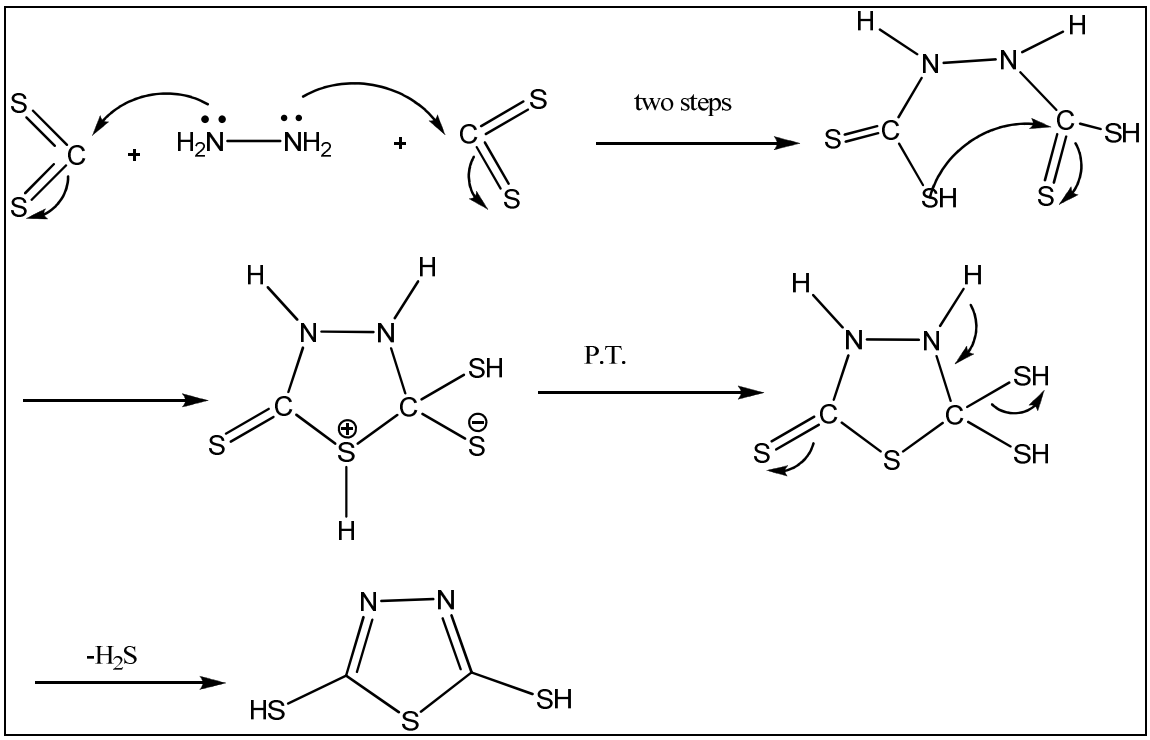
Scheme (3-1): The chemical steps for synthesis of compounds [A1-A6].

3.1.1 Preparation of 2, 5-dimercapto-1, 3, 4-thiadiazole: [A1]



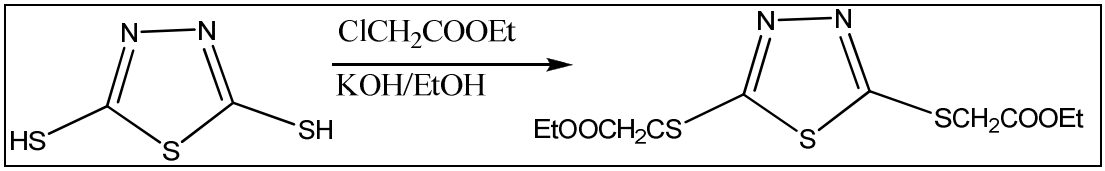
The mechanism reaction for the synthesis of compound [A1] was illustrated in scheme (3-2) [100]. Compound [A1] was characterized by its melting point and by IR spectroscopy. Melting point was recorded (162-164) C° and the reported m.p was (164-166) C° [100]. The FTIR spectrum of compound [A1] showed a medium stretching vibration band at 1624 cm^{-1} that corresponds to (C=N) bond in the vicinity of 1,3,4-thiadiazole ring (Fig. 3-1). In this spectrum there are two other characteristic bands at 3055.35cm^{-1} and 2733.22 cm^{-1} due to (N-H, thione form) and (S-H) stretching vibrations, respectively. That means compound [A1] is existing in the thiol and thione form.





Scheme (3-2): Reaction mechanism for preparation of compound [A1].

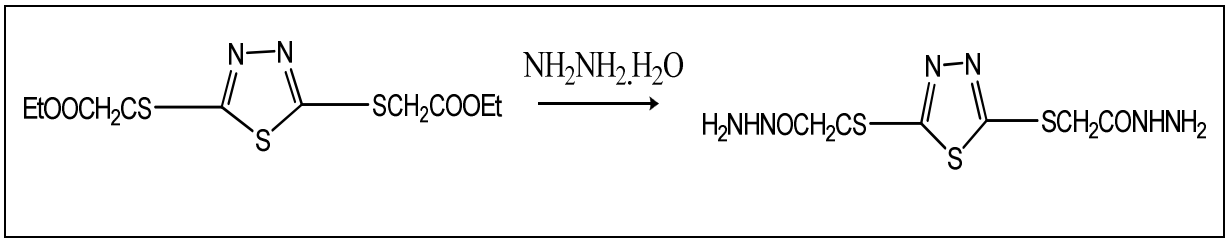
3.1.2 Synthesis of 2, 5-bis (thio ethylacetate 2, 2', diyl)-1,3,4 thiadiazole [A2]



Compound [A2] was characterized by FTIR and ^{1}H NMR spectroscopy, FTIR spectrum, (Fig. 3-2), shows strong absorption band at 1741 cm^{-1} due to carbonyl group for ester, bands at 2987 cm^{-1} and 2939 cm^{-1} for aliphatic (CH_2) group and characteristic bands for (C-O-C) bond at 1165 cm^{-1} Symmetrical, 1199 cm^{-1} asymmetrical. Disappearance of S-H and N-H absorption bands confirmed the formation of the compound [A2].

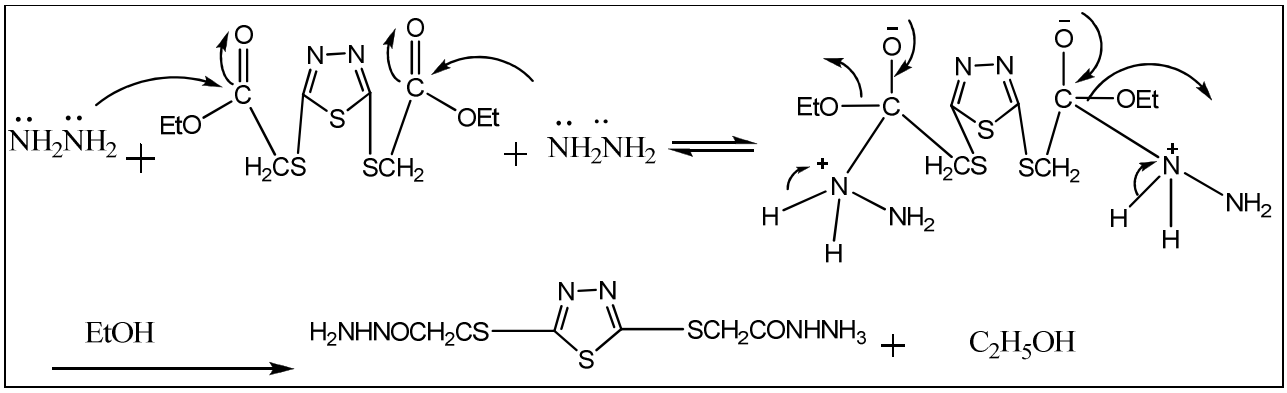
The ^{1}H NMR spectrum of compound [A2] showed a triplet signal at δ= (1.16-1.21) ppm belongs to (CH_3), quartet signals at δ =(4.09-4.16)ppm belong to (CH_2) and singlet signal at δ= 4.21 ppm belong to (SCH_2) (Fig. 3-6).

3.1.3 Synthesis of 2, 5-bis (thio acetiohydrazide-2-yl) - 1, 3, 4 -thiadiazole [A3]



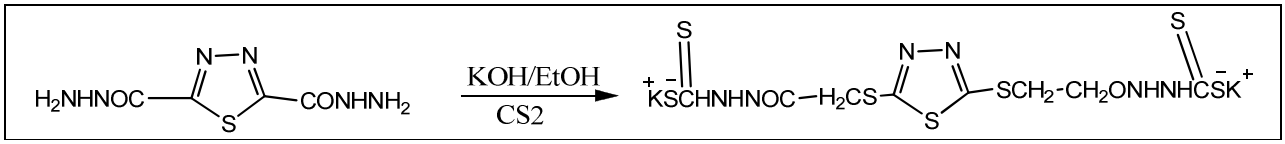
The mechanism reaction [108] for the synthesis of compound [A3] was illustrated in scheme (3-3).

FTIR spectrum of compound [A3] shows characteristic absorption bands at 3319 cm^{-1} for N-H and (3292-3269) cm^{-1} for (NH_2) group, the shifting of the stretching absorption bands of C=O from 1741 to 1693 cm-1 confirmed the transformation reaction from ester to amide . Disappearance of C-O band was also seen (Fig. 3-3).



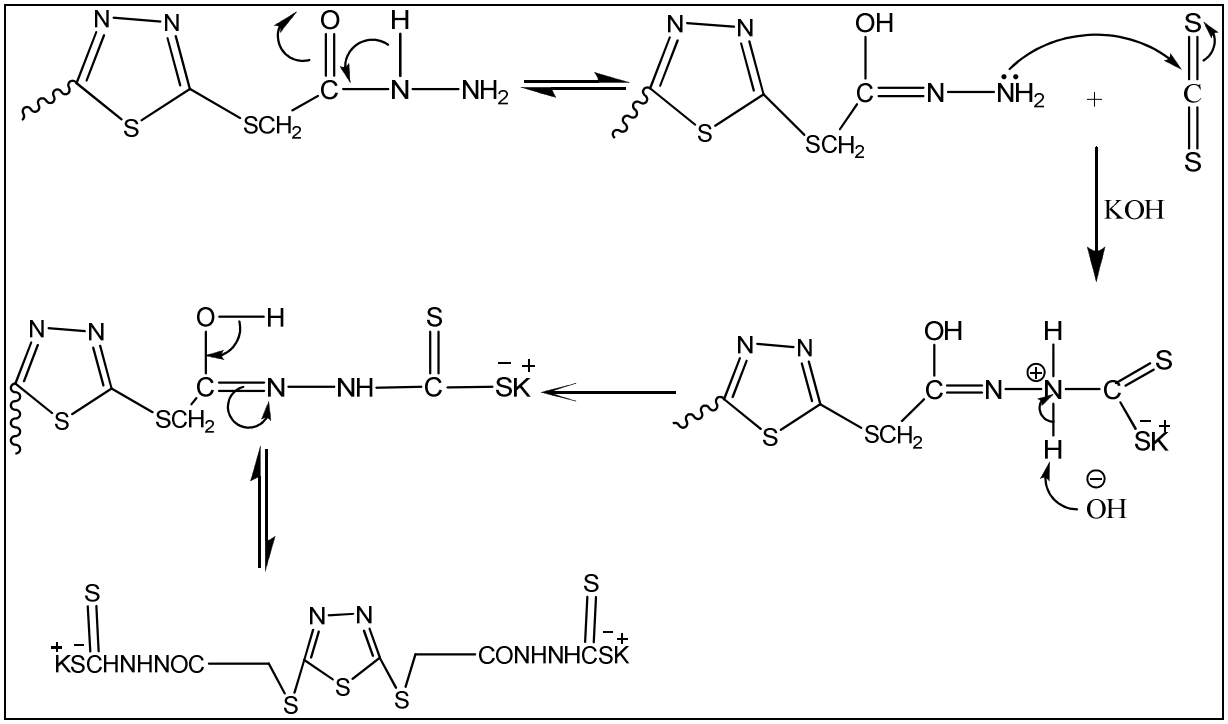
Scheme (3-3): Reaction mechanism for the preparation of compound [A3].

3.1.4 Synthesis of bis-potassium dithiocarbazinate [A4]



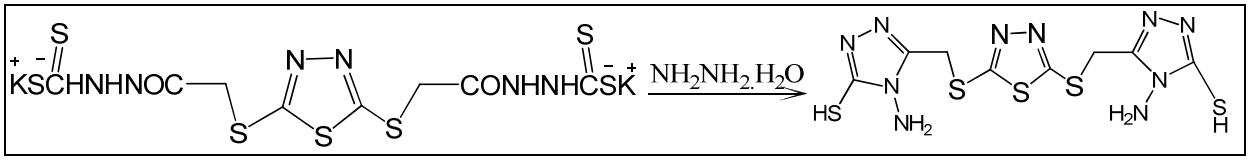
The mechanism of reaction [6] for the synthesis of compound [A4] was illustrated in scheme (3-4).

Compound [A4] is such a semi oily compound, IR spectroscopy and melting point could not be achieved.



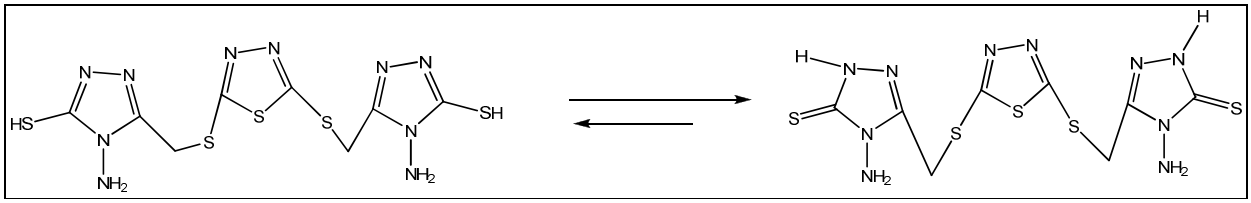
Scheme (3-4): Reaction mechanism for the preparation of compound [A4].

3.1.5 Synthesis of 5, 5'-(1, 3, 4-thiadiazole-2, 5 diyl) bis(sulfanediyl) bis (methylene) bis(4-amino-4H-1,2,4-triazole-3 thiol) [A5]

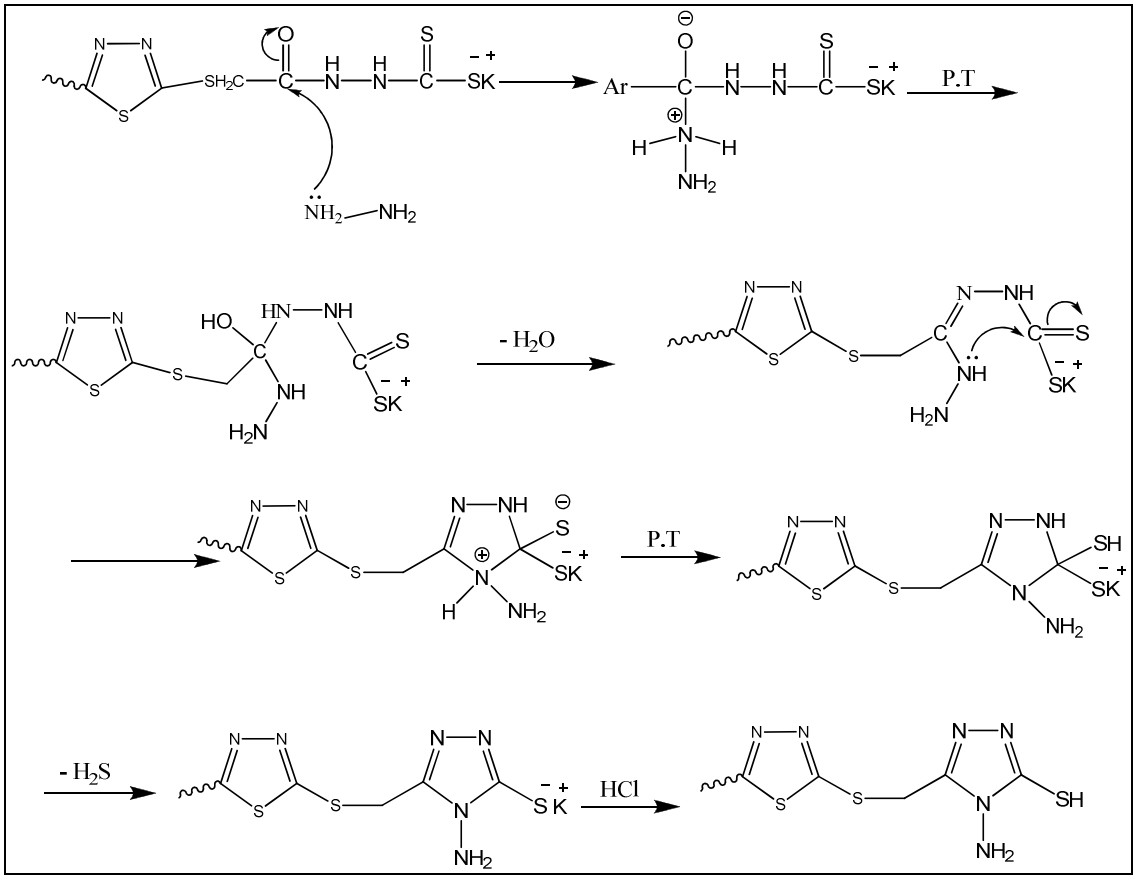


The mechanism of reaction for the synthesis of compound [A5] was illustrated in scheme (3-5).

FTIR spectrum of compound [A5] shows disappearance of the absorption band for carbonyl group. And characteristic bands at 3211, 3267 for NH_2 were also seen. There are two characteristic bands at 3456 cm^{-1} and 2804 cm^{-1} due to (N-H) and (S-H) stretching vibration bands, respectively. That indicates that the compound [A5] exists in the thiol and thione form (Fig. 3-4).

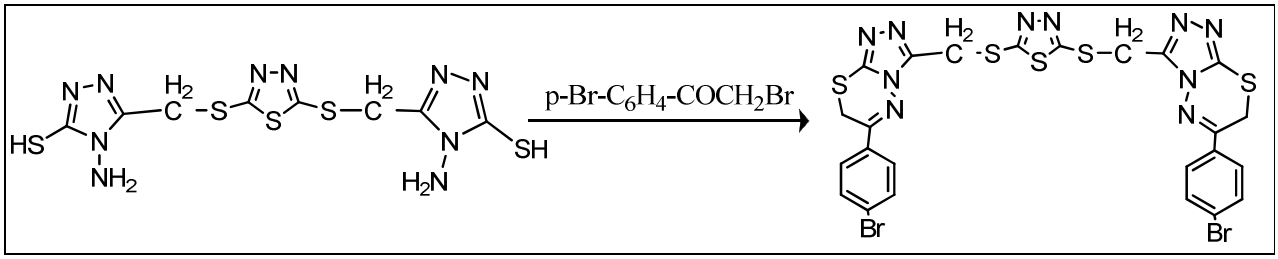


The ^{1}H NMR spectrum shows a singlet signal at 4.26 ppm due to SCH_2, singlet signal at 5.27 ppm due to NH_2 and singlet signal at 12.8 ppm due to S-H, (Fig. 3-7).



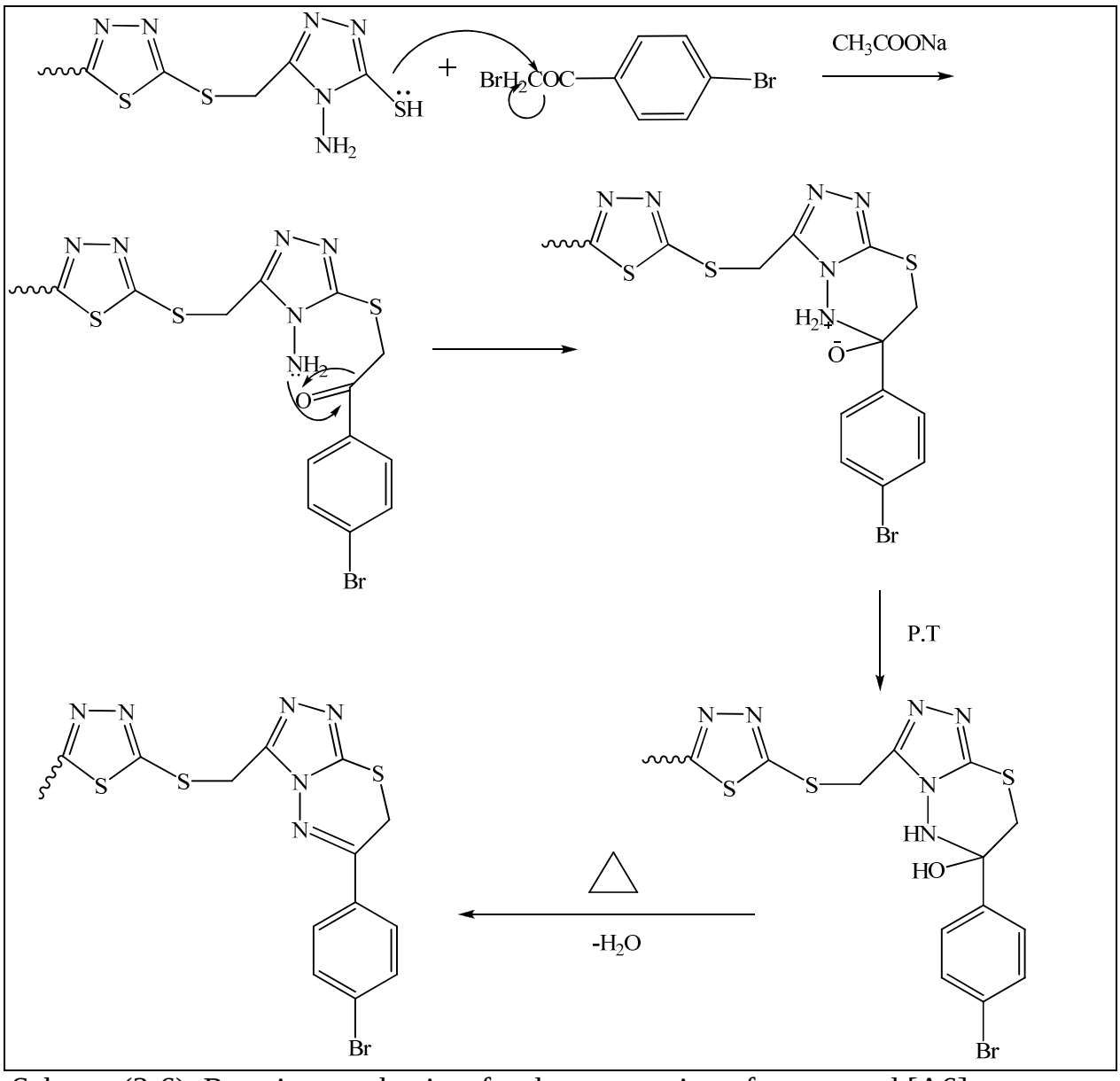
Scheme (3-5): Reaction mechanism for the preparation of compound [A5].

3.1.6 Synthesis of 2,5-bis (6-(4-bromophenyl)-7H-[1,2,4]triazolo [3,4-b][1,3,4] thiadiazin-3-yl) methyl)-1,3,4-thiadiazole [A6].

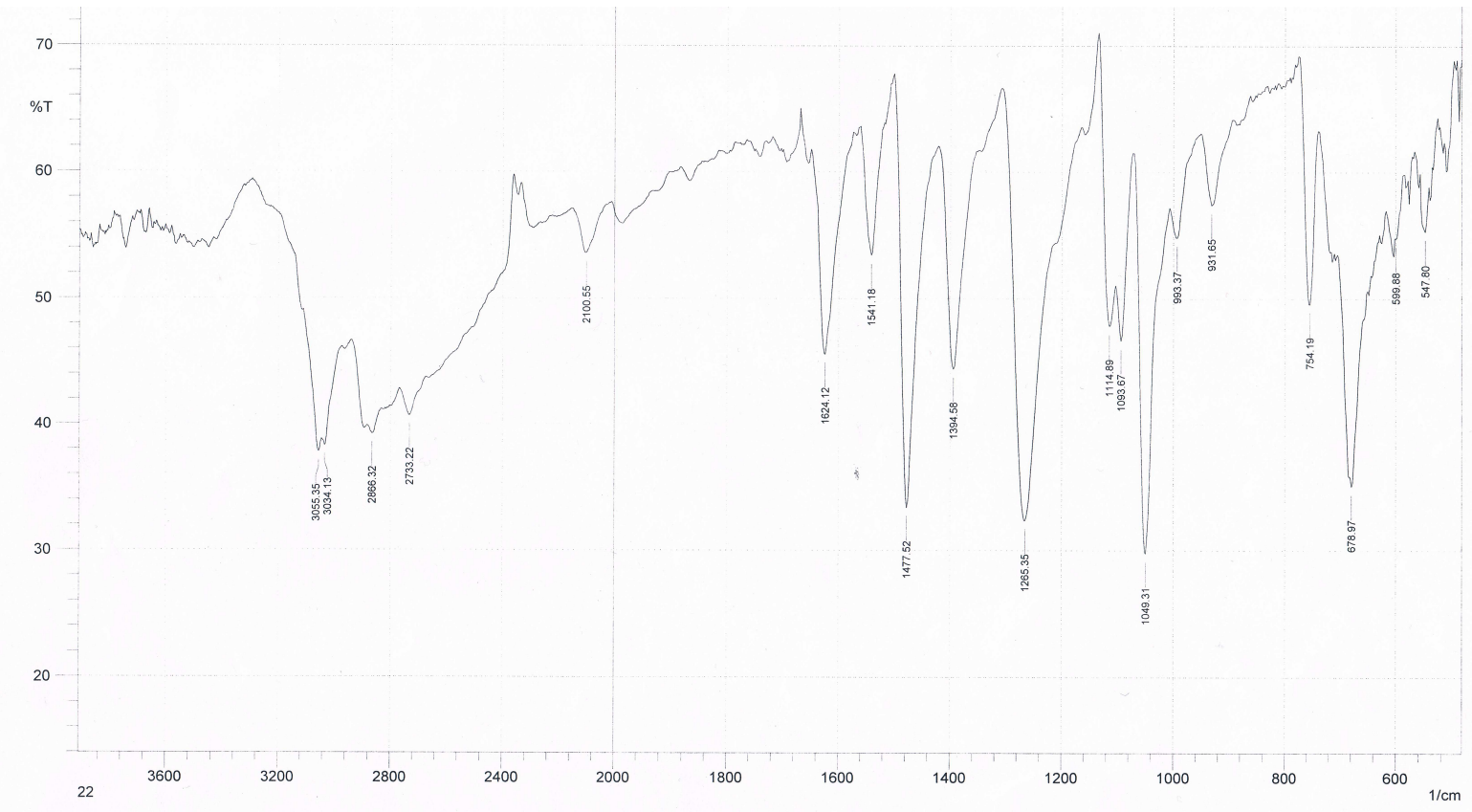


The mechanism of reaction for the synthesis of compound [A6] was illustrated in scheme (3-6). The formation of compound [A6] is indicated by the disappearance of NH_2 bands, NH band and S-H bands, and appearance of C-H aromatic absorption band was also seen in the FTIR spectrum (Fig.3-5).

The ^{1}HNMR spectrum, (Fig.3-8), shows a singlet signal at 4.15 ppm due to (SCH_2) , singlet signal at 4.80 for (SCH_2) in thiadiazine ring doublet signal at (7.78 -7.80) belongs to (2H) in benzene ring and doublet signal at (7.12 -7.29) belongs to (2H) in benzene ring ortho to bromine.



Scheme (3-6): Reaction mechanism for the preparation of compound [A6].



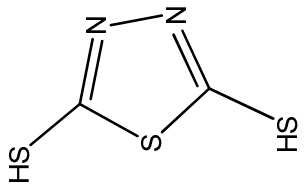
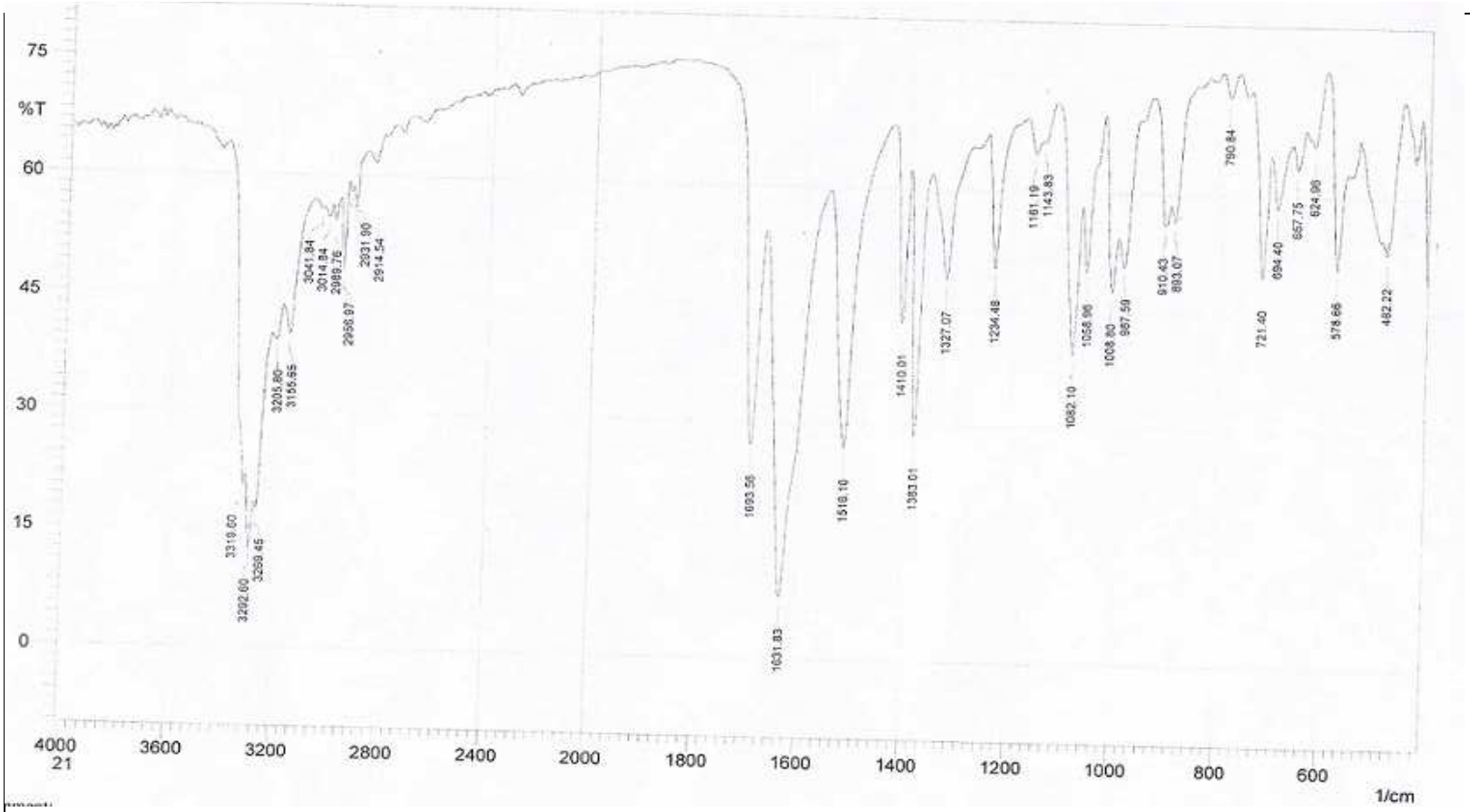
Structure:	**Physical properties :**	FTIR, KBr disk, cm^{-1}
	Color = yellow	ν C=N = 1624 cm^{-1}
	Yield=77.6 %	ν S-H = 2733 cm^{-1}
	m.p= (162 - 164) C°	ν N-H = 3055 cm^{-1}
	M.Wt. = 150.25 $gmol^{-1}$	

Fig. (3-1) FTIR spectrum for compound [A1].

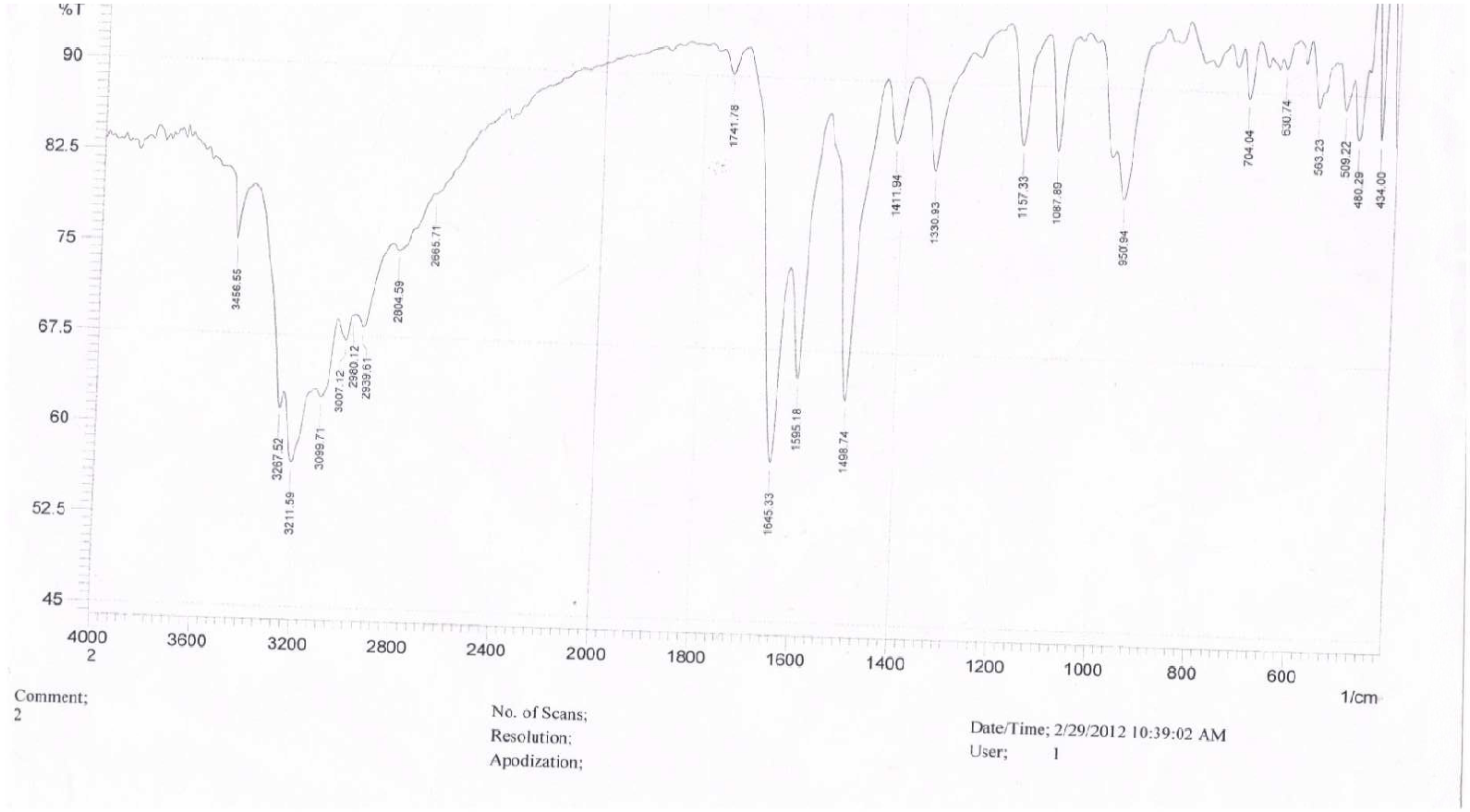
	Physical properties : Color = White Yield= 79 % m.p= (47 - 48) C° M.Wt. =322.42 g mol^{-1}	**FTIR ,KBr disk ,cm^{-1}** ν C=O (ester)= 1741 cm^{-1} ν C-H aliph. =(2987-2939) cm^{-1} ν C=N = 1638 cm^{-1} ν C-O-C = 1165 cm^{-1} Sym., 1199 cm^{-1}

Fig. (3-2) FTIR spectrum for compound [A2].



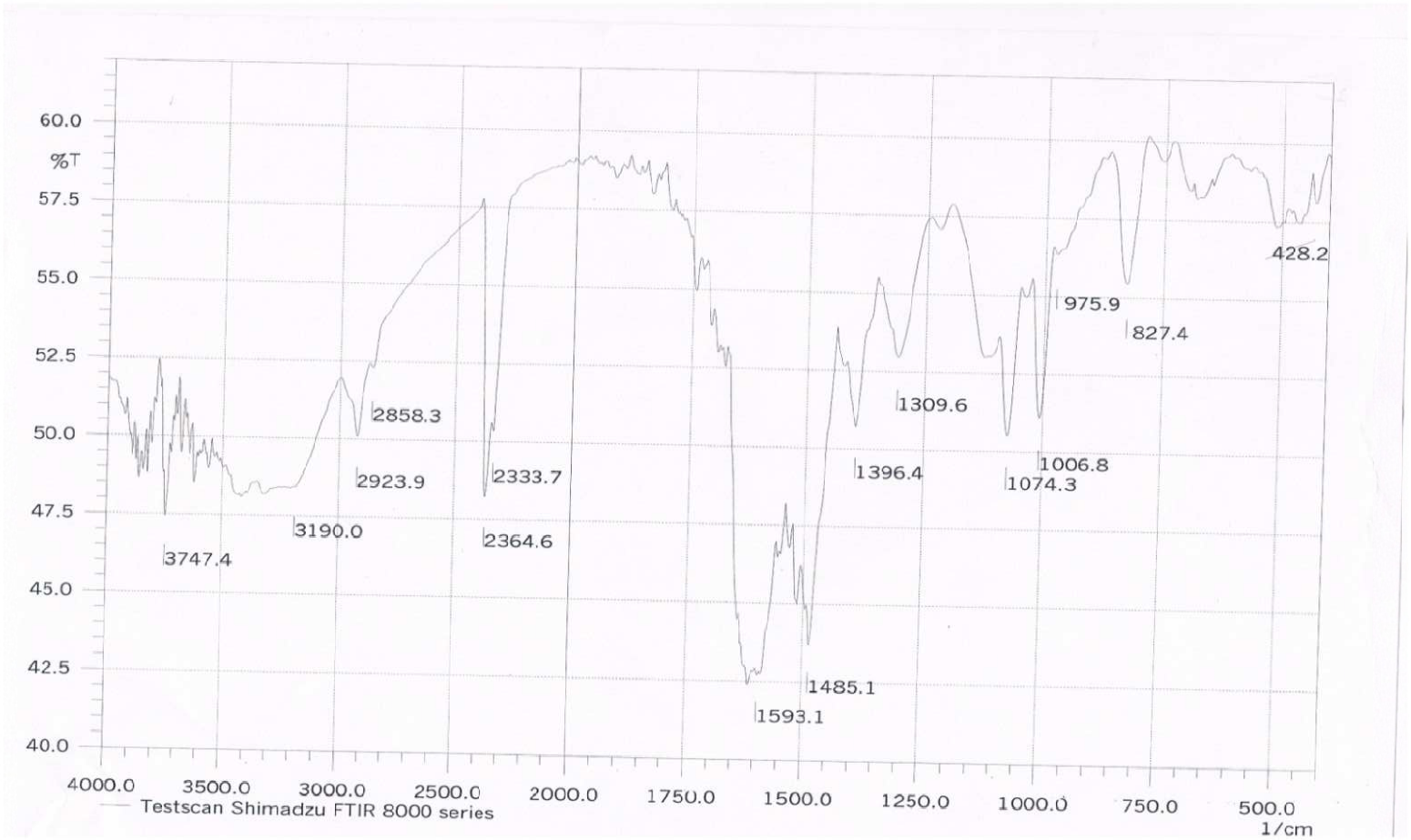
	Physical properties : Color = white , Yield=74% m.p= (140- 142) C° M.Wt. =294 $gmol^{-1}$	**FTIR, KBr disk ,cm^{-1}** ν N-H =3319 cm^{-1} ν NH_2 = (3292.60-3269.45) cm^{-1} ν C=O = 1693 cm^{-1} ν C=N = 1631 cm^{-1}

Fig. (3-3) FTIR spectrum for compound [A3].



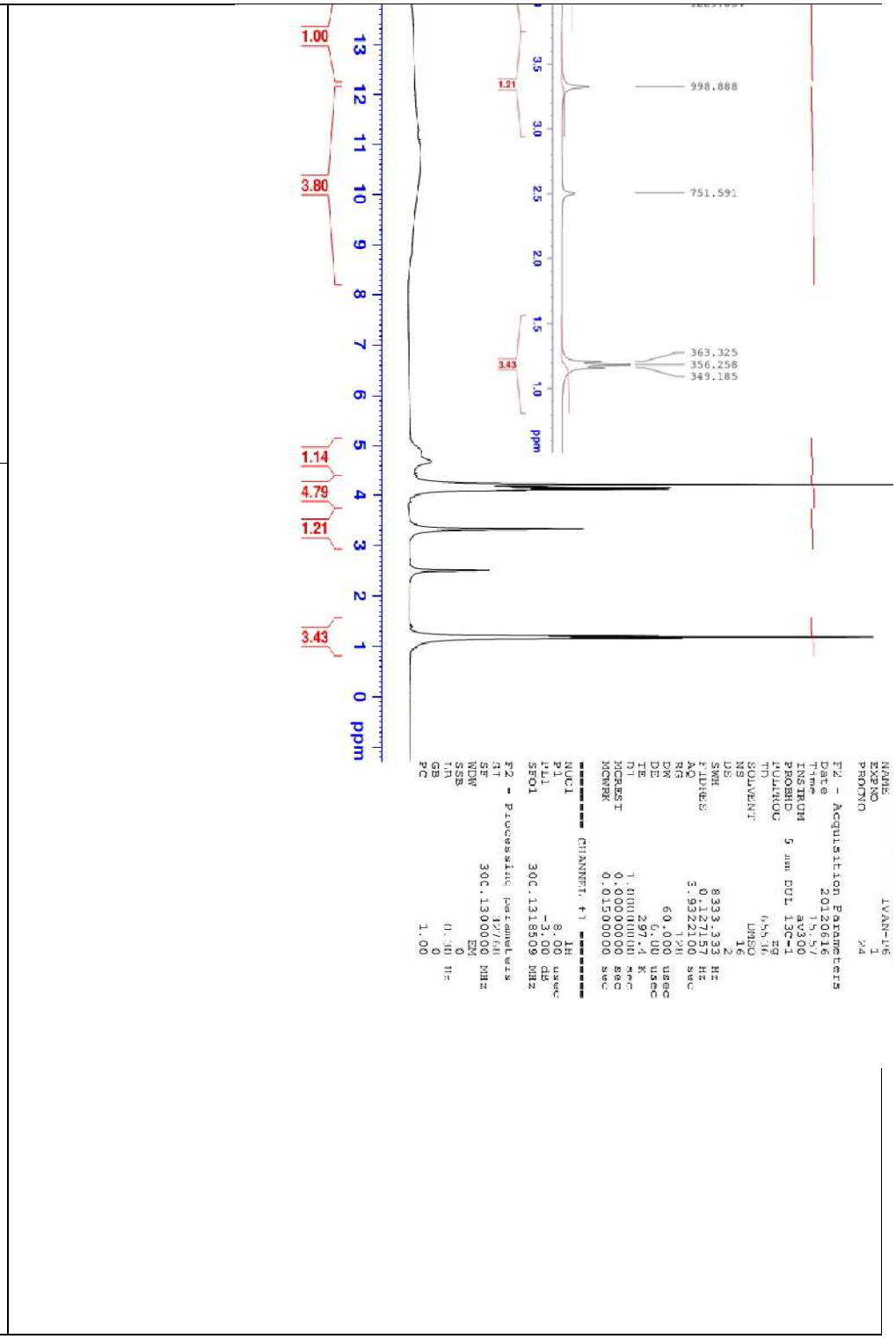
	Physical properties :	**FTIR ,KBr disk ,cm^{-1}**
	Color = White	ν S-H = 2804 cm^{-1}
	Yield=52%	ν N-H = 3456.55 cm^{-1}
	m.p= (198 -200) C°	ν NH_2= (3267-3211) cm^{-1}
	M.Wt. =406 $gmol^{-1}$	ν C=N = 1645 cm^{-1}

Fig. (3-4) FTIR spectrum for compound [A5].



	Physical properties : Color = yellow Yield= 71% m.p= (192- 194) C° M.Wt. = 700 $gmol^{-1}$	**FTIR ,KBr disk ,cm^{-1}** ν C-H aromatic= 3190 cm^{-1} ν C-H aliphatic= (2923-2885) cm^{-1} ν C=N = 1593 cm^{-1}

Fig. (3-5) FTIR spectrum for compound [A6].



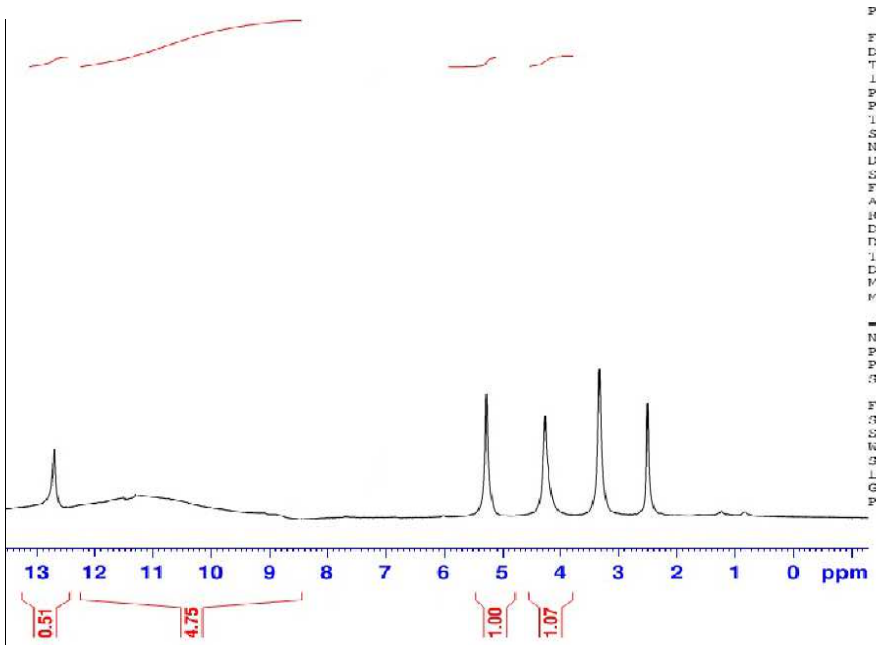
^{1}HNMR :

SCH_2 = 4.215 ppm

CH_2 = 4.168 ppm

CH_3 = 1.22 ppm

Fig. (3-6) ^{1}HNMR spectrum for compound [A2].

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F2 - Acquisition Parameters
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Time                 15.57
INSTRUM              av300
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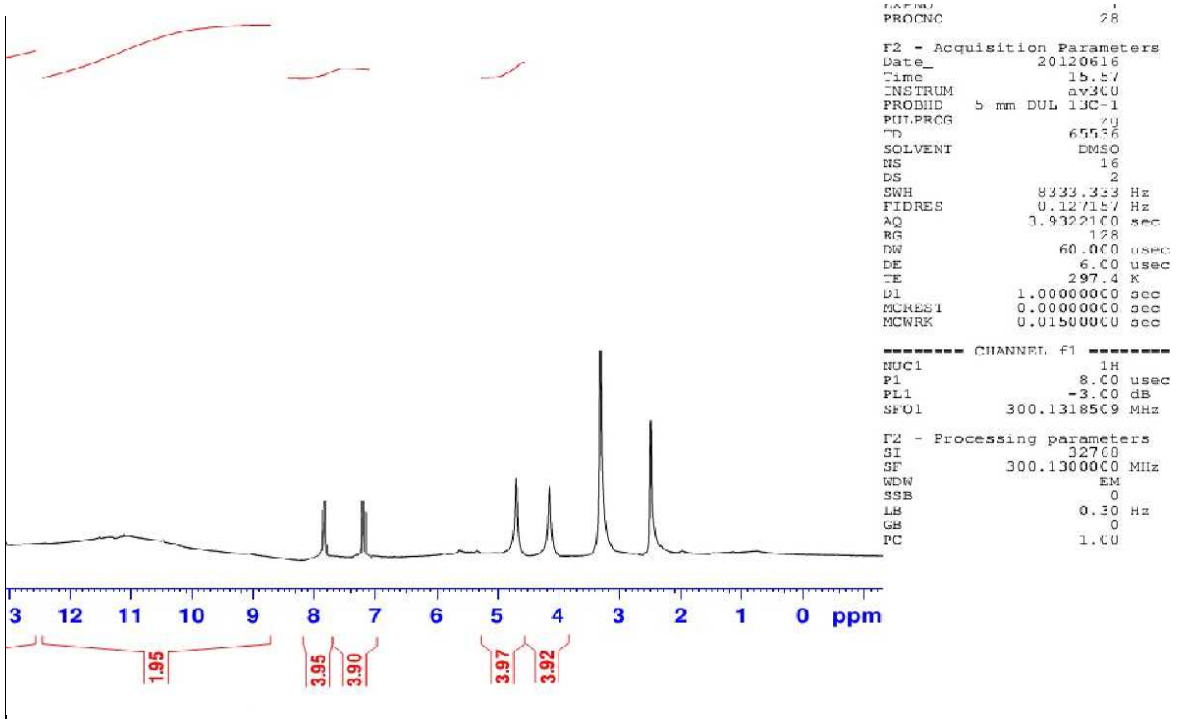
PULPROG                 zg
TD                  655536
SOLVENT               DMSO
NS                      16
DS                       2
SWH              8333.333 Hz
FIDRES           0.127157 Hz
AQ               3.9322100 sec
RG                     128
DW                60.000 usec
DE                 6.00 usec
TE                297.4 K
D1           1.00000000 sec
MCREST       0.00000000 sec
MCWRK        0.01500000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   8.00 usec
PL1                 -3.00 dB
SFO1          300.1318509 MHz

F2 - Processing parameters
SI                   32768
SF           300.1300000 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

	^{1}HNMR: SCH_2 = 4.285 ppm NH_2 = 5.278 ppm S-H= 12.8 ppm

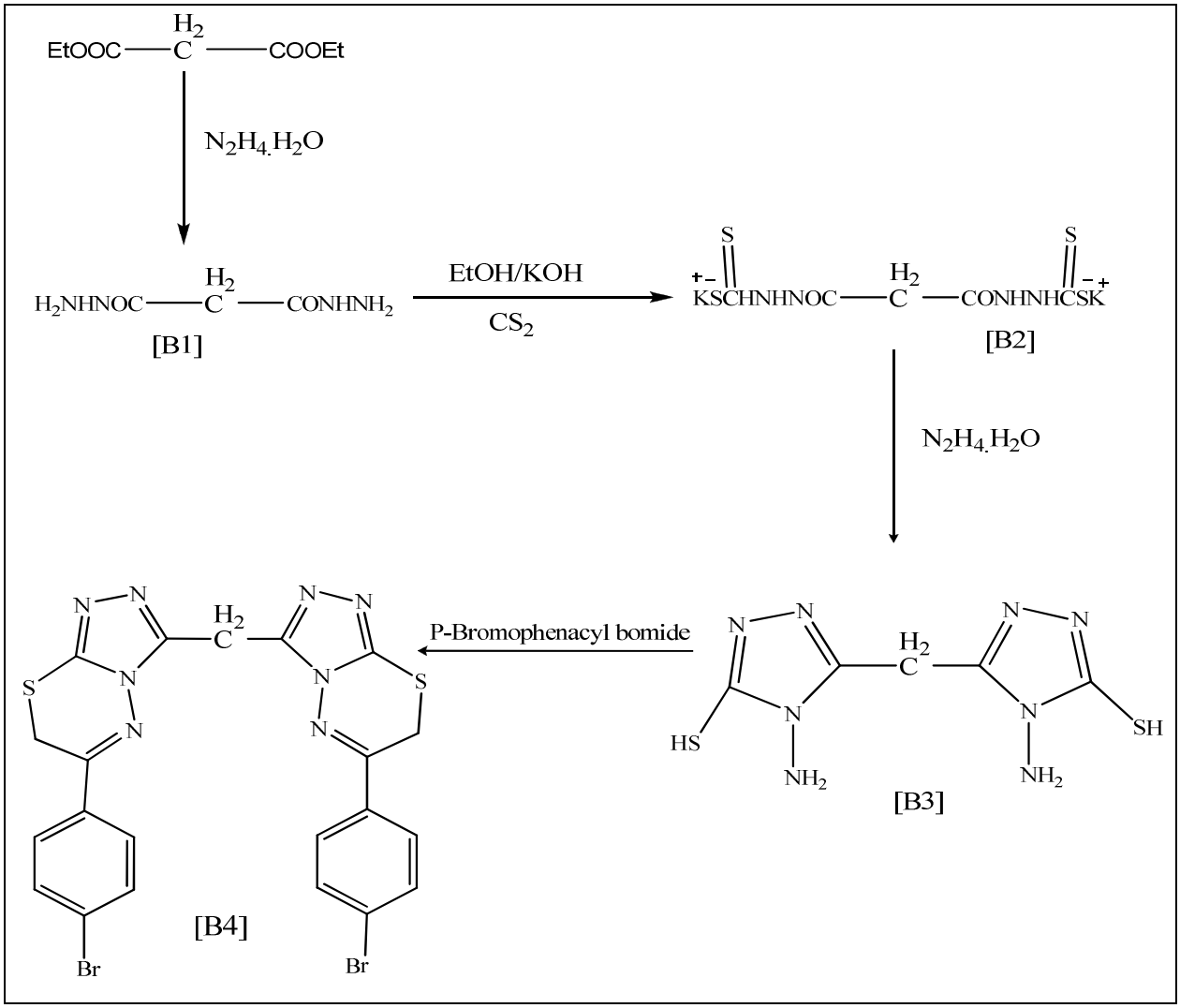
Fig. (3-7) ^{1}HNMR spectrum for compound [A5].



	[1]HNMR: 4.16 ppm for SCH_2 4.80 ppm for SCH_2 in ring CH_2 benz. (7.77-7.80) ppm CH_2 benz. Ortho to bromo group (7.12-7.29) ppm

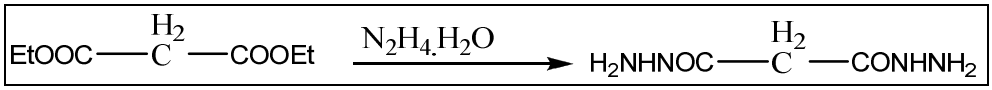
Fig. (3-8) [1]HNMR spectrum for compound [A6].

Chemical steps for synthesis of compounds [B1-B4] are shown in (Fig. 3-7).



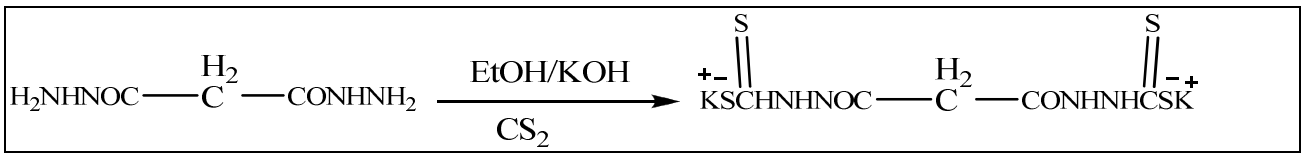
Scheme (3-7): Chemical steps for synthesis of compounds [B1-B4].

3.1.7 Synthesis of Malonohydrazide [B1]



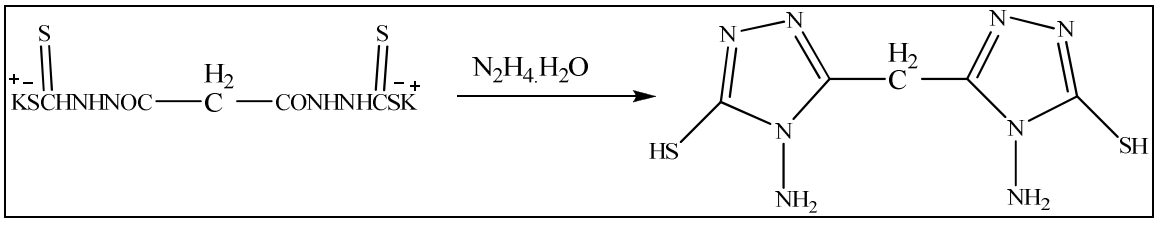
The FTIR spectrum, Fig (3-9), shows characteristic absorption bands at 3300 cm^{-1} for N-H and (3220.60-3132) cm^{-1} for (NH_2) group, and absorption band at 1666cm^{-1} due to carbonyl group.

3.1.8-Synthesis of bis-potassium dithiocarbazinate [B2]



Compound [B2] is such a semi oily compound, IR spectroscopy and melting point could not be achieved.

3.1.9 Synthesis of 5, 5'-methylenebis (4-amino-4H-1, 2, 4-triazole-3-thiol) [B3]

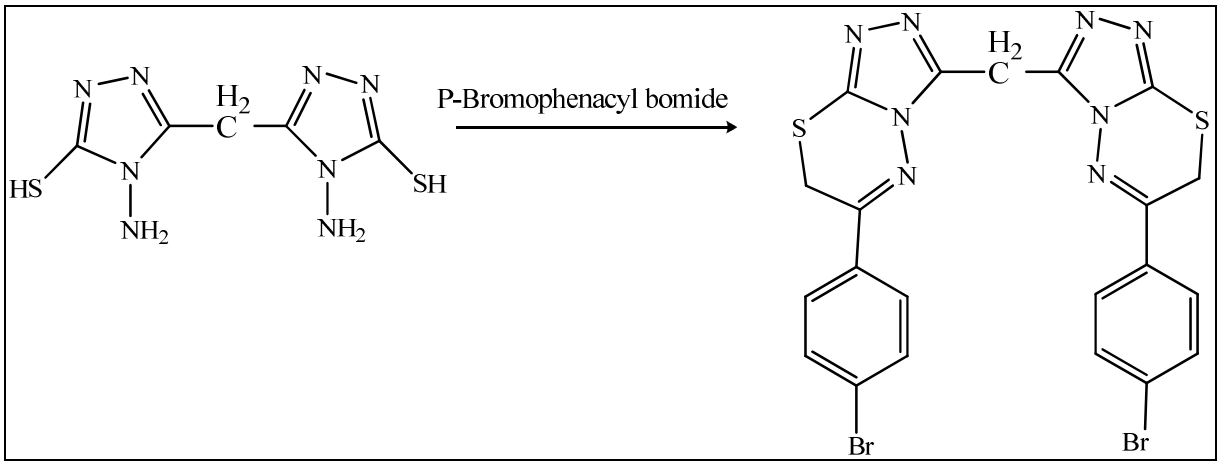


The FTIR spectrum, (Fig. 3-10), of compound [B3] shows the disappearance of the absorption band for carbonyl group, There are two other characteristic bands at

(3210-3265) cm^{-1} and 2790 cm^{-1} due to (NH_2) and (S-H) stretching vibrations, respectively.

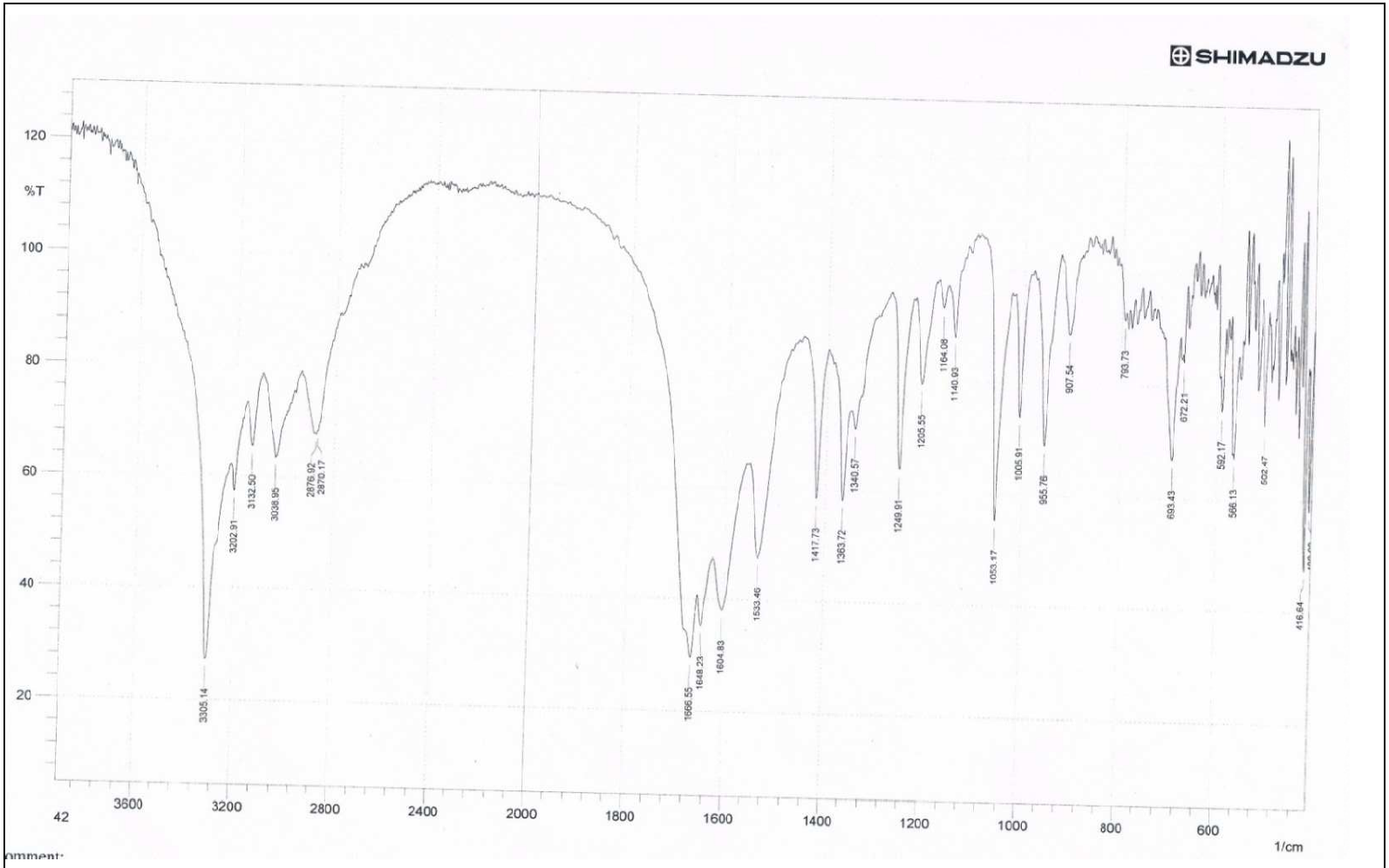
The ^1H NMR spectrum, (Fig.3-12), shows a singlet signal at 3.868 ppm due to CH_2, singlet signal at 5.27 ppm due to NH_2 and singlet signal at 13.3 ppm due to S-H

3.1.10 Synthesis of bis(6-(4-bromophenyl)-7H-[1,2,4]triazolo[3,4 b][1,3,4]thiadiazin-3-yl)methane [B4]



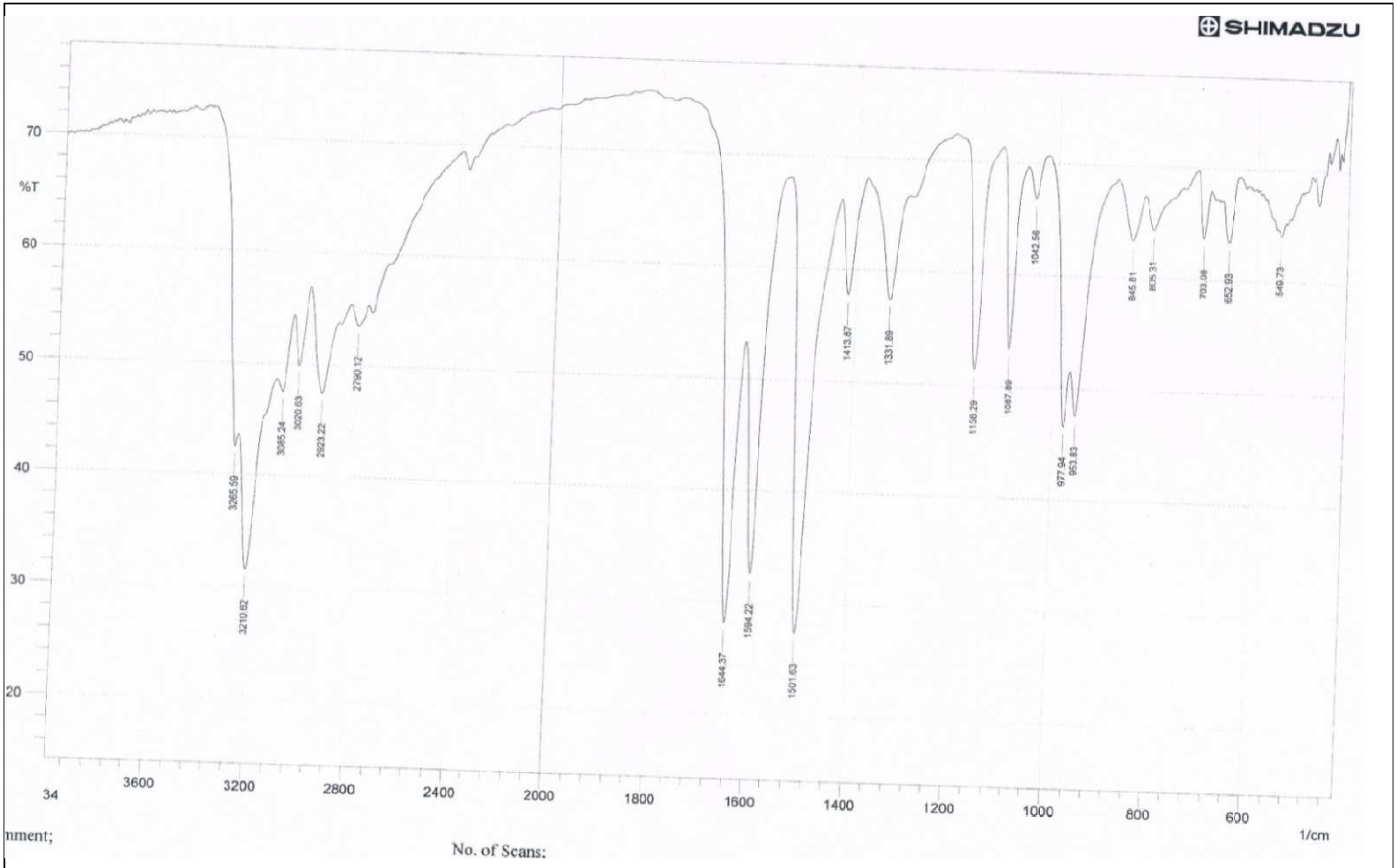
The FTIR spectra, fig (3-11), for compound [B4] show that disappearance of NH_2 and S-H stretching absorption band bands.

The ^1H NMR spectrum, (Fig. 3-13), shows a singlet signal at 3.85 ppm due to (CH_2), singlet signal at 4.37 ppm for (SCH_2) in thiadiazine ring, doublet signal at (7.83 -7.90) belongs to (2H) in benzene ring and doublet signal at (7.22 -7.39) belongs to (2H) in benzene ring ortho to bromine.



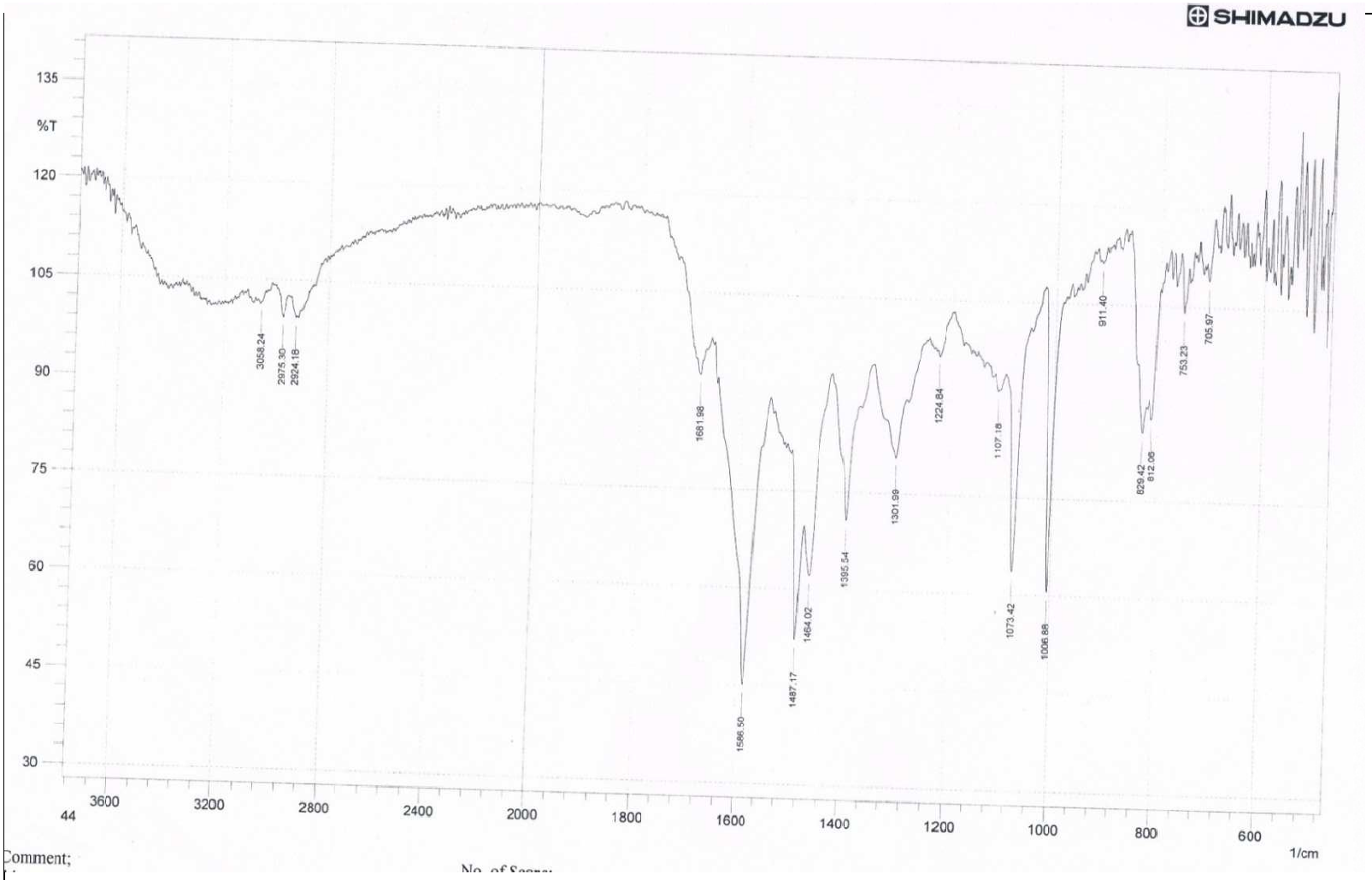
	Physical properties :	**FTIR ,KBr disk ,cm^{-1}**
	Color = white	ν N-H = 3300 cm^{-1}
	Yield= 76.5%	ν NH_2 (3220-3132) cm^{-1}
	m.p= (133- 135) C°	ν C=O 1666 cm^{-1}
	M.Wt. =132.12$gmol^{-1}$	ν C-H Aliph. =(2876-2870) cm^{-1}

Fig. (3-9) FTIR spectrum for compound [B1].



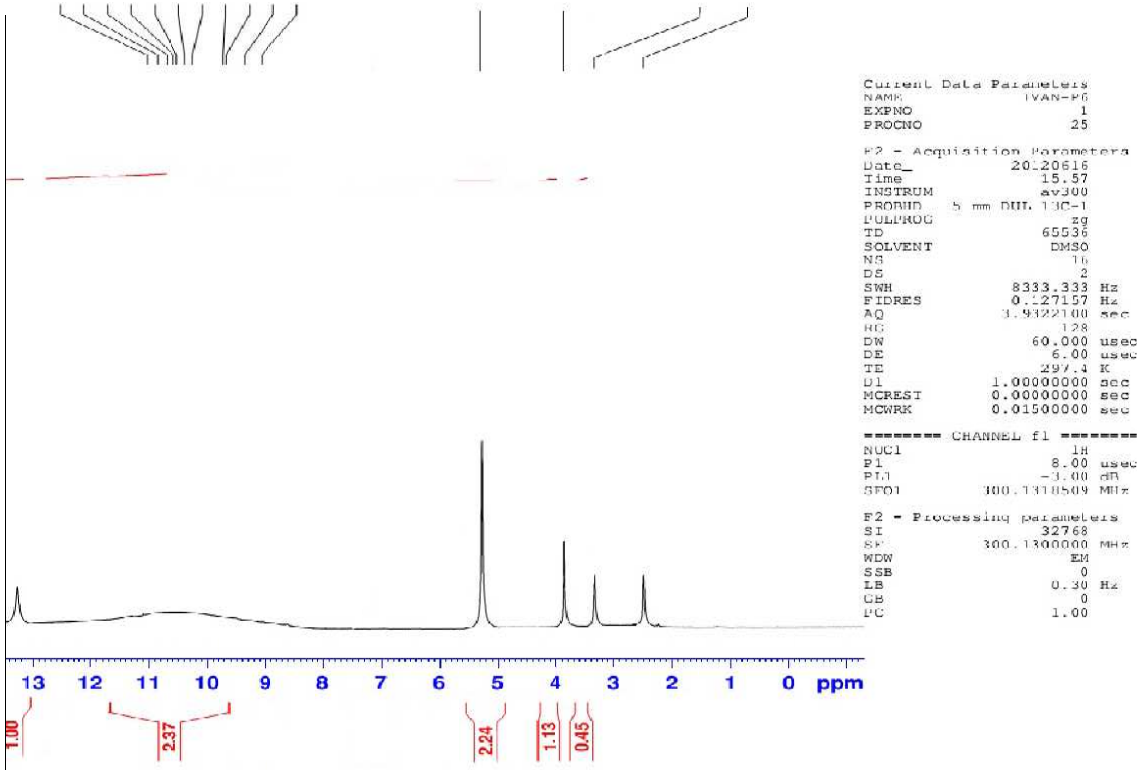
	Physical properties : Color = white Yield= 52% m.p= (190- 193) C° M.Wt. =244.30 $gmol^{-1}$	**FTIR ,KBr disk ,cm^{-1}** ν NH_2= (3210-3265) cm^{-1} ν S-H = 2790 cm^{-1} ν C=N = 1644 cm^{-1}

Fig. (3-10) FTIR spectrum for compound [B3].



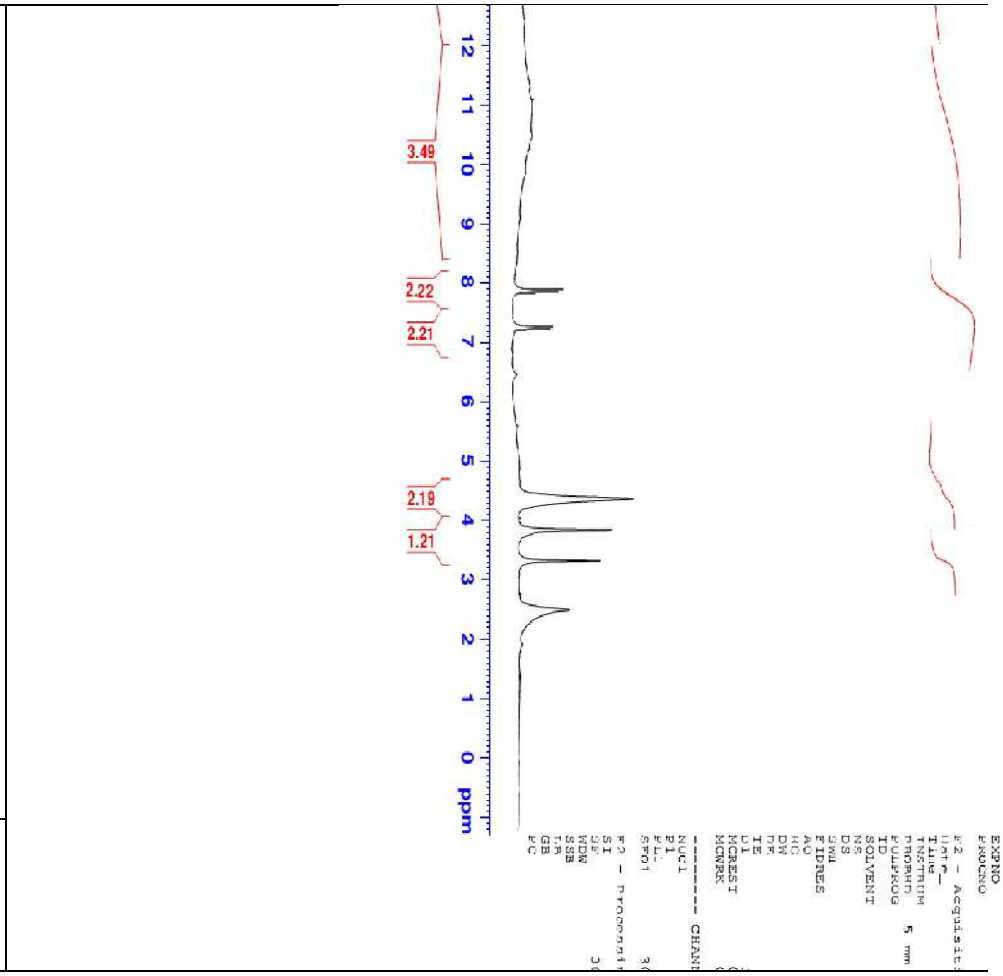
	Physical properties : Color = Yellow Yield= 69 % M.P= (158- 160) C° M.Wt. =602.33 $gmol^{-1}$	**FTIR ,KBr disk ,cm^{-1}** ν C-H arom. = 3058 cm^{-1} ν C=N = 1586.5 cm^{-1} ν C-H alip. = (2975-2924) cm^{-1}

Fig. (3-11) FTIR spectrum for compound [B4].



	^{1}HNMR S-H = 13.3 ppm N-H_2 = 5.27 ppm

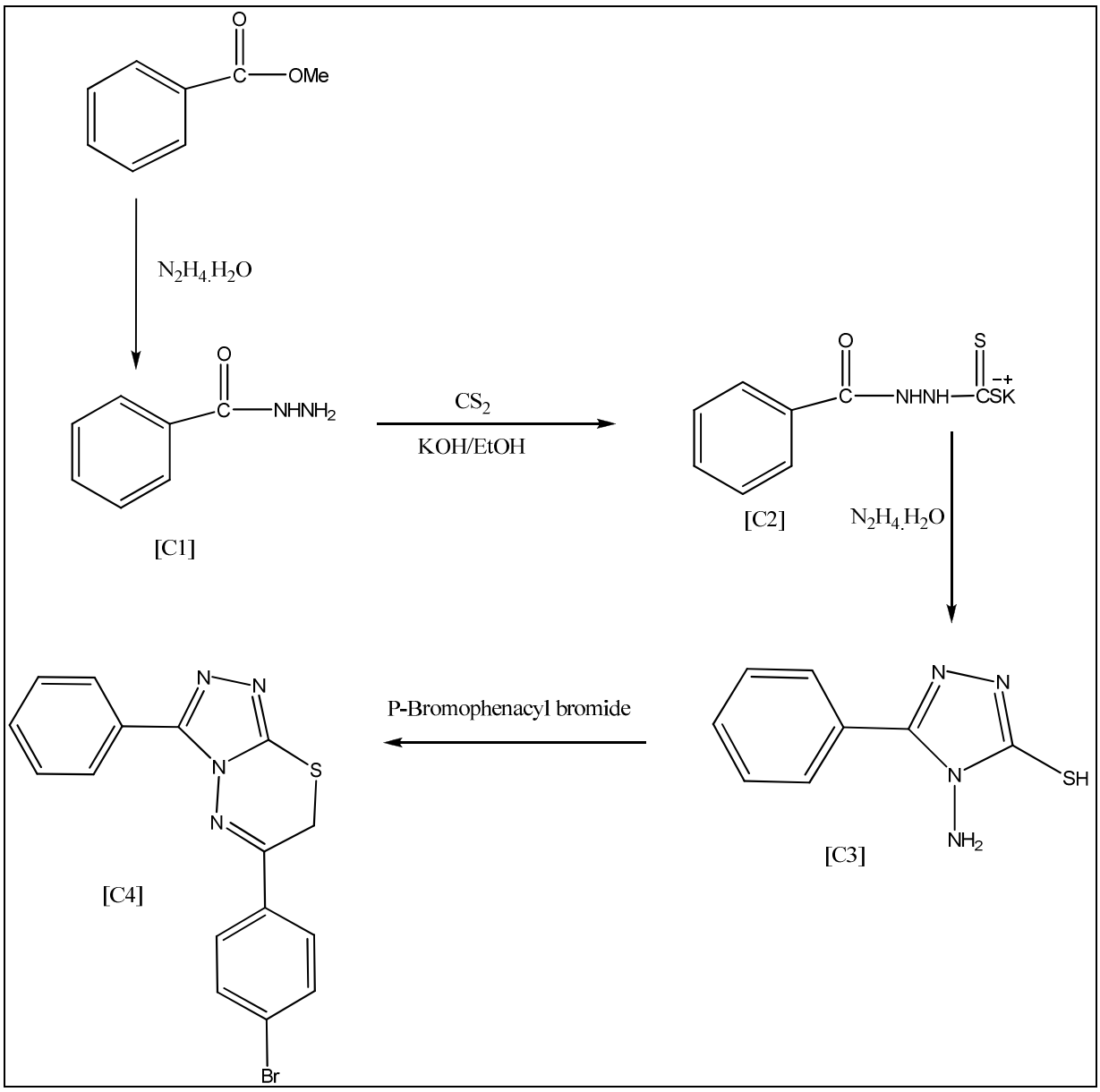
Fig. (3-12) ^{1}HNMR spectrum for compound [B3].



^{1}HNMR CH_2 =3.85 ppm	

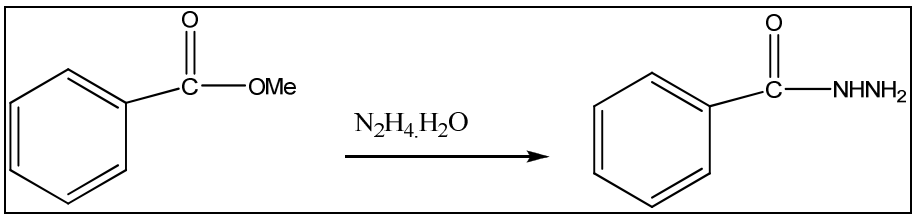
Fig. (3-13) ^{1}HNMR Spectrum for

Chemical steps for synthesis of compounds [C1-C4] are shown in scheme (3-8).



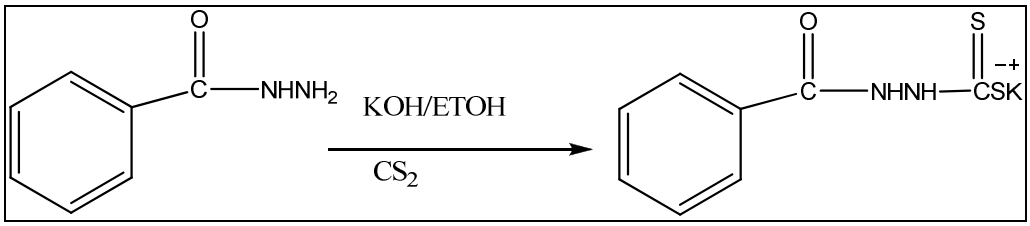
Scheme (3-8): Chemical steps for synthesis of compounds (C1-C4).

3.1.11 preparation of benzo hydrazide [C1]



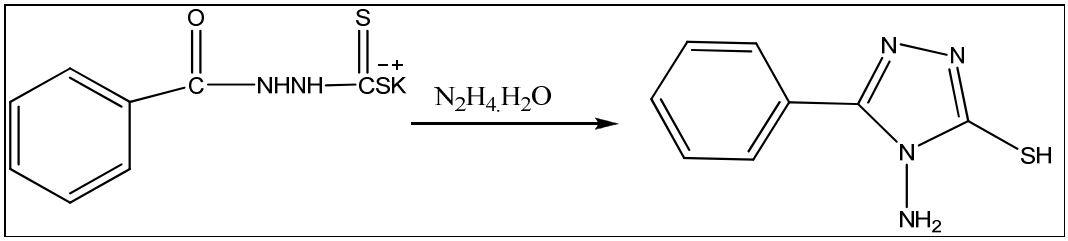
Compound [C1] was characterized by melting point, And FTIR spectrum, the FTIR spectrum, (Fig. 3-14), shows appearance of two stretching bands of NH_2 asymmetric and symmetric at (3301 and 3214cm^{-1}), carbonyl of Amide group was also seen at 1661 cm^{-1}.

3.1.12 preparation of potassium dithiocarbazinate [C2].



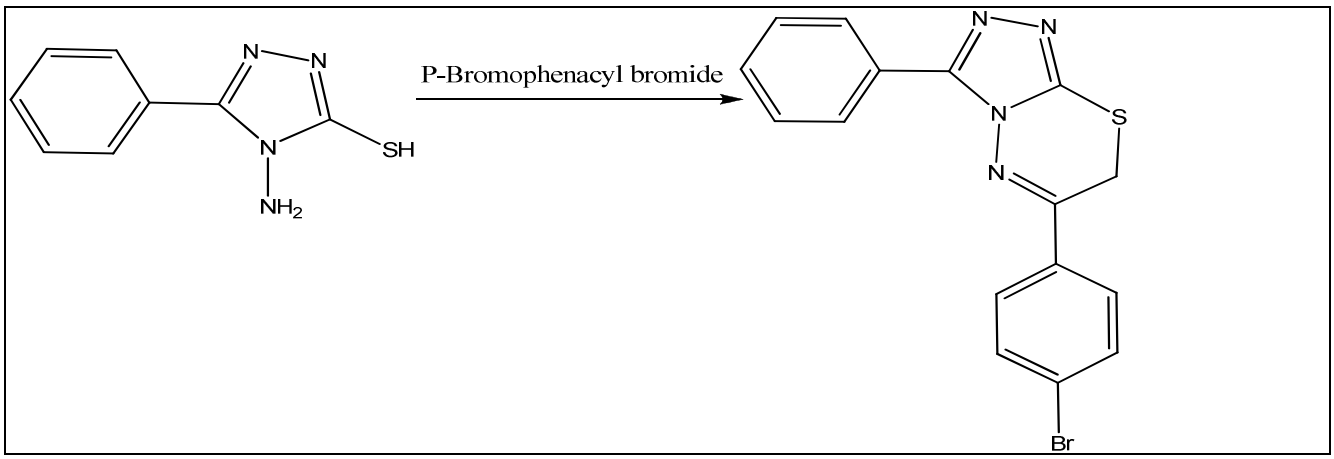
The FTIR spectrum shows a shifting in carbonyl group to 1631 cm^{-1} and appearance of C-S band at 600 Cm^{-1} (Fig. 3-15).

3.1.13 Synthesis of 4[amino]-5-phenyl-4H-1, 2, 4-triazole-3-thiol [C3]

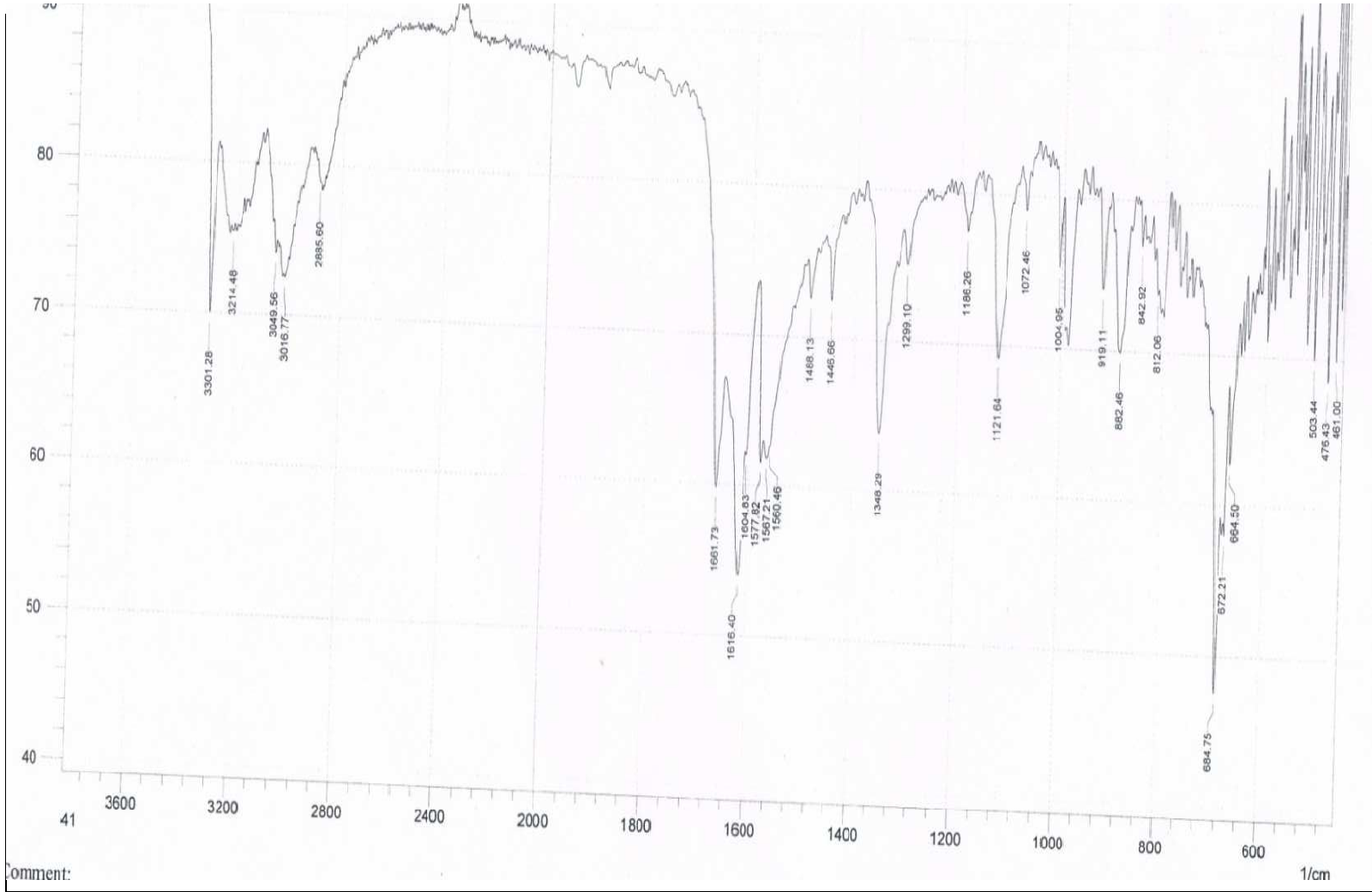


This compound was characterized by melting point, and FTIR spectrum, the FTIR spectrum, (Fig. 3-16), showed absorption band at (3200-3118) Cm^{-1} due to NH_2 and another characteristic band at 2720 cm^{-1} belong to S-H.

3.1.14 synthesis of 6-(4-bromophenyl)-3-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine [C4].

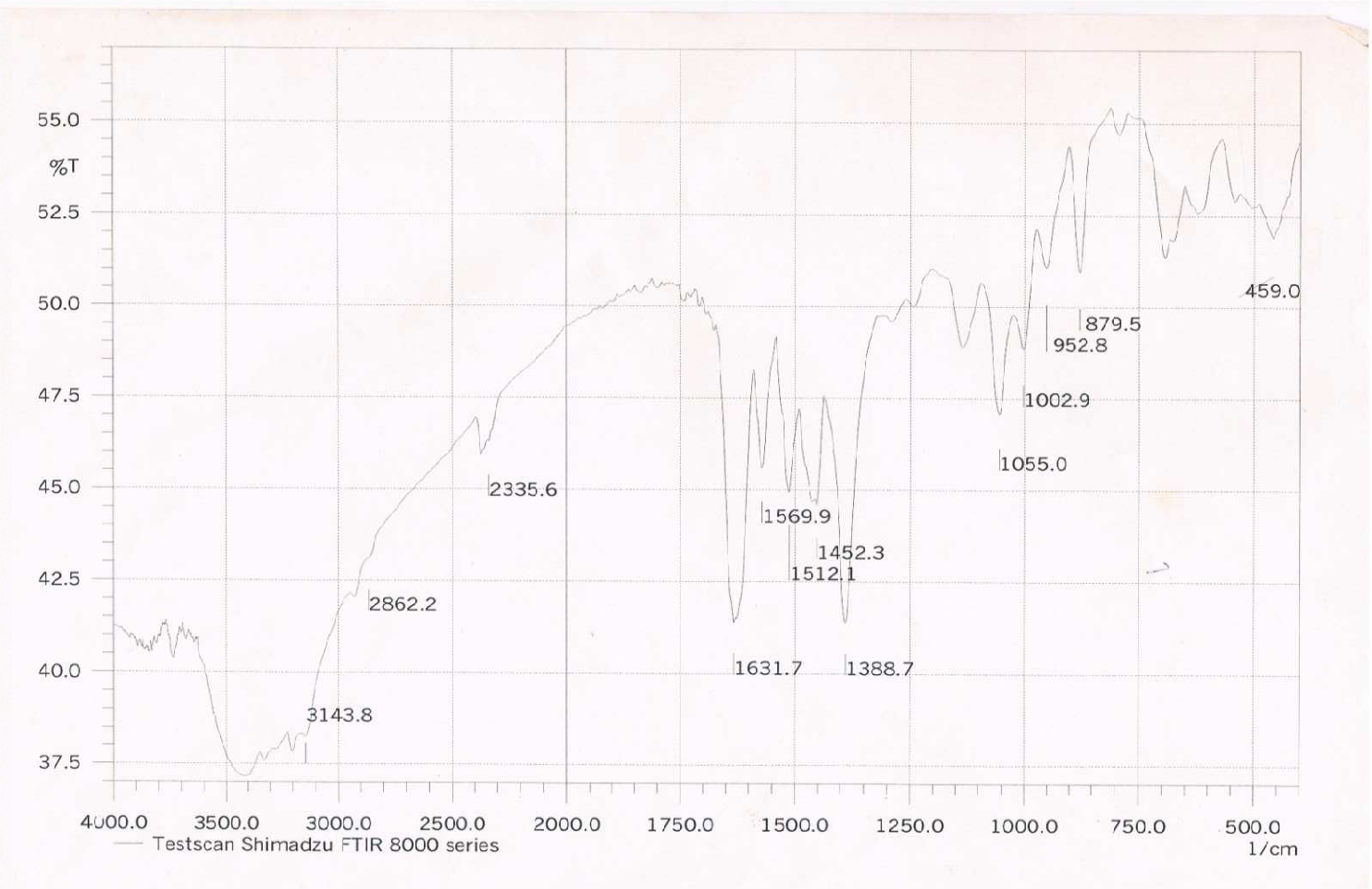


The FTIR spectrum of this compound shows the disappearance of absorption band for NH_2 and S-H , (Fig. 3-17).



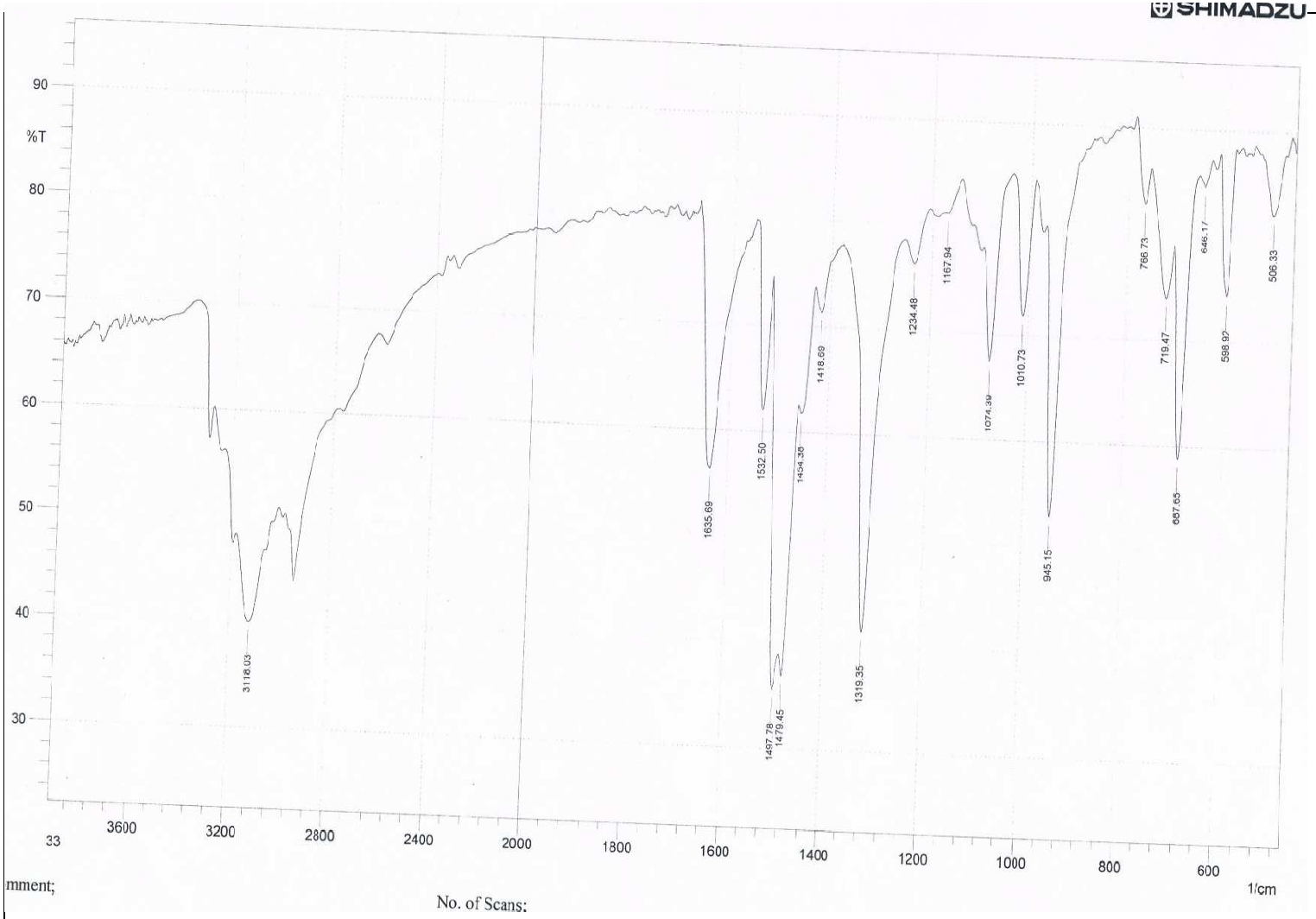
	Physical properties :	**FTIR ,KBr disk ,cm^{-1}**
	Color = white ,Yield= 71 %	ν NH-NH_2 (3301 - 3214) cm^{-1}
	m.p= (111 -113) C°	ν (C=O amide) =1661 cm^{-1}
	M.Wt. = 136.5 $gmol^{-1}$	ν C-H arom. =3016 cm^{-1}, ν C=C arom. =1616 cm^{-1}

Fig. (3-14) FTIR spectrum for compound [C1].



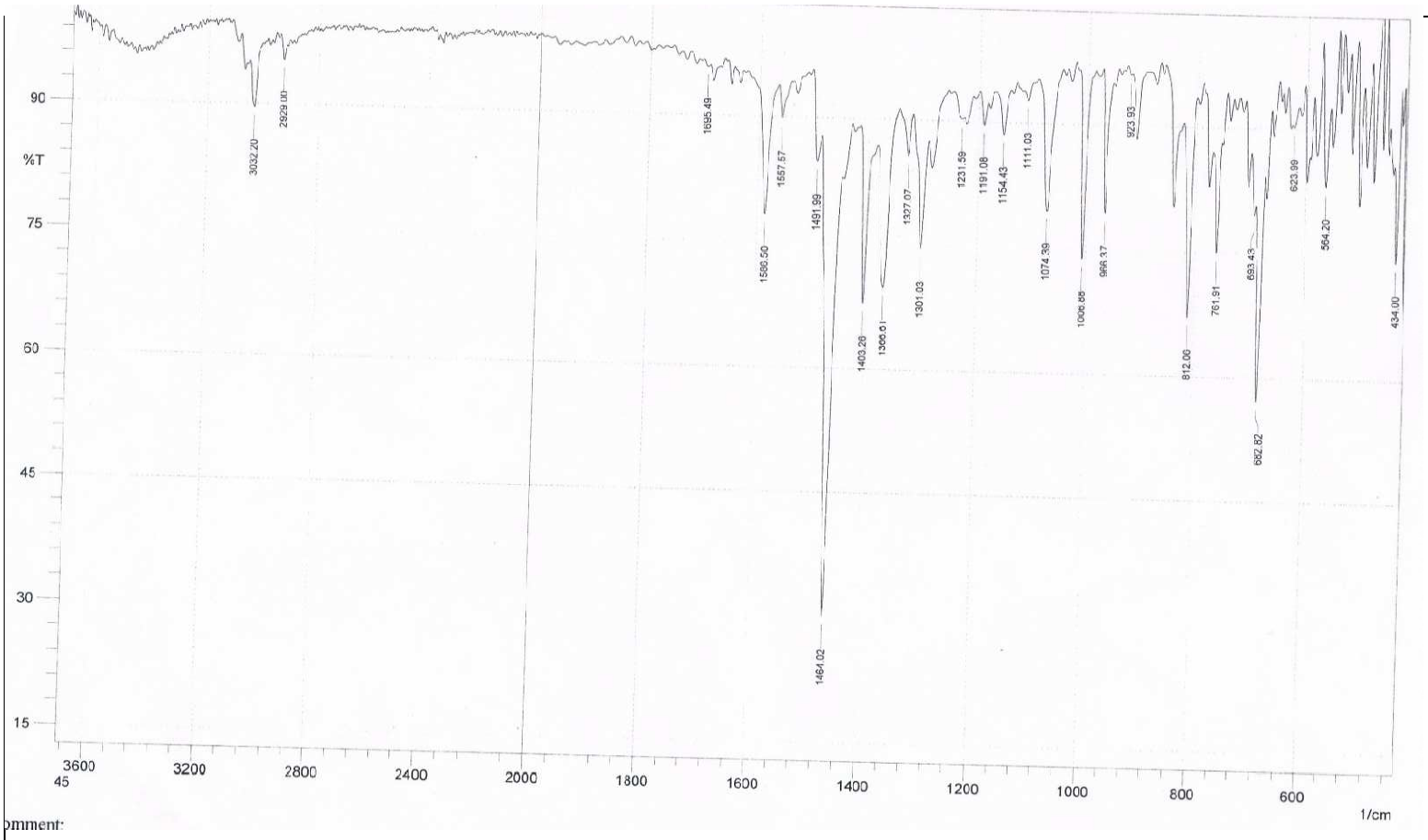
	Physical properties : Color = yellow Yield= 66 % M.Wt. = 250 $gmol^{-1}$	**FTIR ,KBr disk ,cm^{-1}** ν C-S = 600 cm^{-1}, ν C=C arom.=1569 ν C-N = 1452.3 cm^{-1} ν C=O amide=1631 cm^{-1}

Fig. (3-15) FTIR spectrum for compound [C2].



	Physical properties :	**FTIR ,KBr disk ,cm^{-1}**
	Color = White , Yield= 60	ν NH_2 =(3200-3118) cm^{-1}
	m.p=(195-197) C°	ν S-H = 2270 cm^{-1}
	M.Wt. = 192 $gmol^{-1}$	ν C=N = 1635 cm^{-1}

Fig. (3-16) FTIR spectrum for compound [C3].



	Physical properties :	**FTIR ,KBr disk ,cm^{-1}**
	Color = yellow	C-H arom. = 3032 cm^{-1}
	Yield= 72 %	ν C=N = 1586 cm^{-1}
	m.p= (213-215) C°	ν C-Br = 682cm-1
	M.Wt. = 371 $gmol^{-1}$	ν C-S = 761 cm-1

Fig. (3-17) FTIR spectrum for compound [C4].

3.2 Biological activity:

3.2.1 Antibacterial activity:

The inhibition zones caused by the various compounds on the four types of bacteria were examined. (**25** µg / ml concentration for all of these compounds). The results

Were listed in Table (3-1), fig. (3-19)- Fig. (3-27)

Table (3-1) The inhibition zones in mm for compounds (A5, A6, B3, B4, C3 and C4) and antibiotics against the four types of bacteria

	Inhibition zone in mm				
Compound number	Concentration µg/ml	Gram positive		Gram negative	
		E.faecalis	*S.aureus*	*E.coli*	*K.Pneumonia*
A5=E	25	16.5	18.33	21.44	16.8
A6=F	25	18.8	16.9	17.57	13.3
B3=C	25	15.8	18.94	18.9	17.2
B4=D	25	16.77	17.58	15.39	16.17
C3=A	25	15.5	17.9	16.22	15.9
C4=B	25	19.3	18.26	23.77	16.8
Amoxicillin = (Amox.)	25	14.1	13.4	15.11	12.9
Ceftriaxone= (Cef.)	25	18.2	21.3	20.28	20.93
DMSO		-	-	-	-

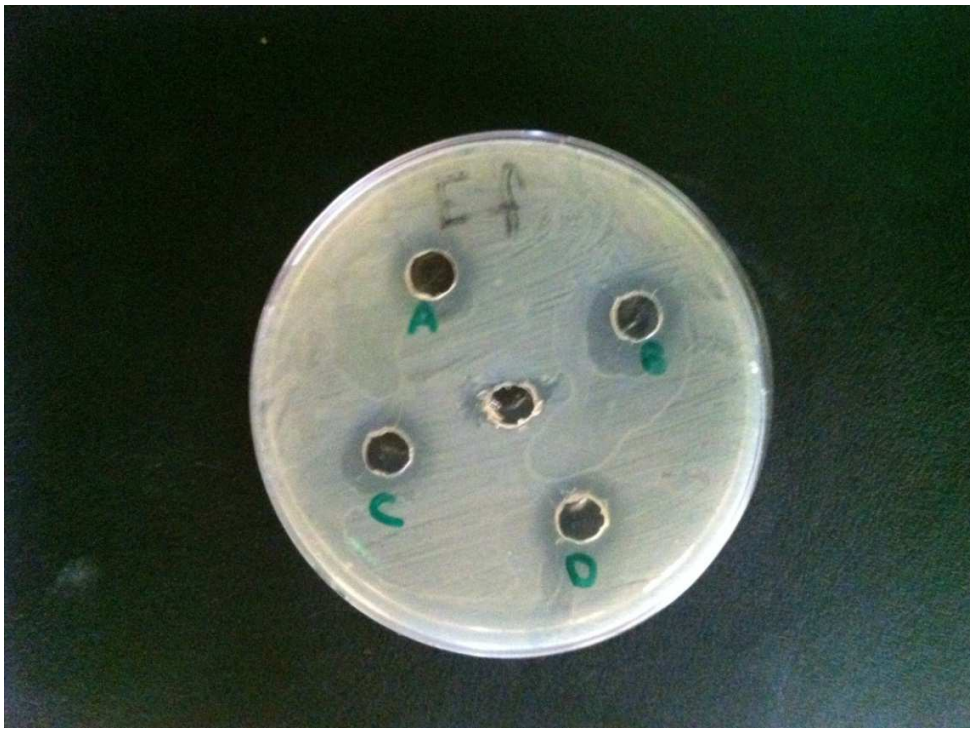


Fig. (3-19) The effect of compounds [B3 (C)], [B4 (D)], [C3 (A)] and [C4 (B)] on *Enterococcus Fecalis.*

Fig. (3-20) The effect of compounds [A5 (E)], [A6(F)], Cef. Amox. And DMSO on *Enterococcus Faecalis.*

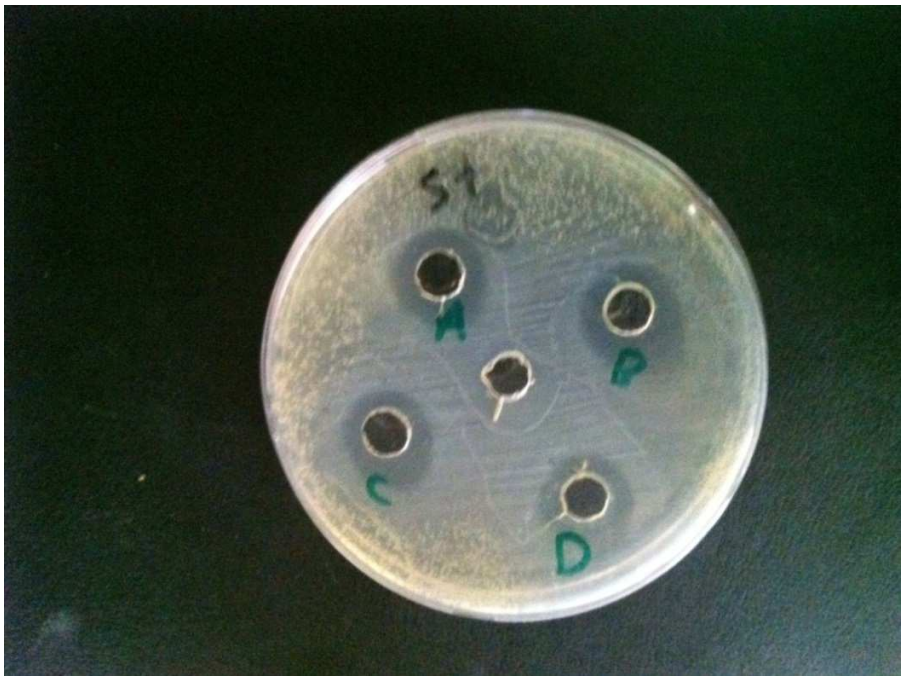


Fig. (3-21) The effect of compounds [B3 (C)], [B4 (D)], [C3 (A)] and [C4 (B)] on *S.aureus.*

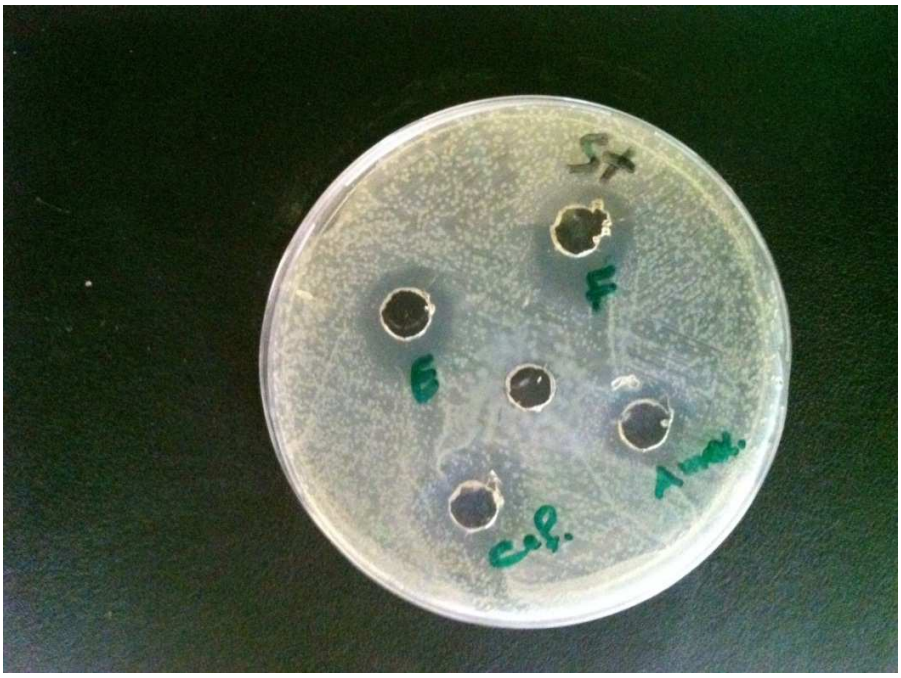


Fig. (3-21) The effect of compounds [A5 (E)], [A6 (F)], Cef., Amox. and DMSO on *S.Aureus*.

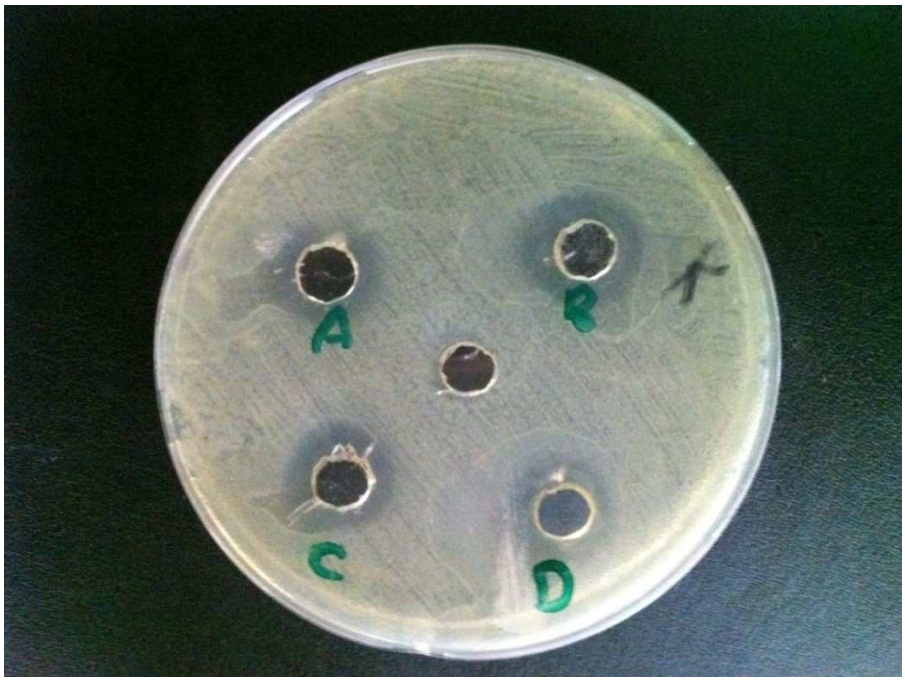


Fig. (3-22) The effect of compounds [B3(C)], [B4 (D)], [C3 (A)] and [C4 (B)] on *K.pmeumonia.*

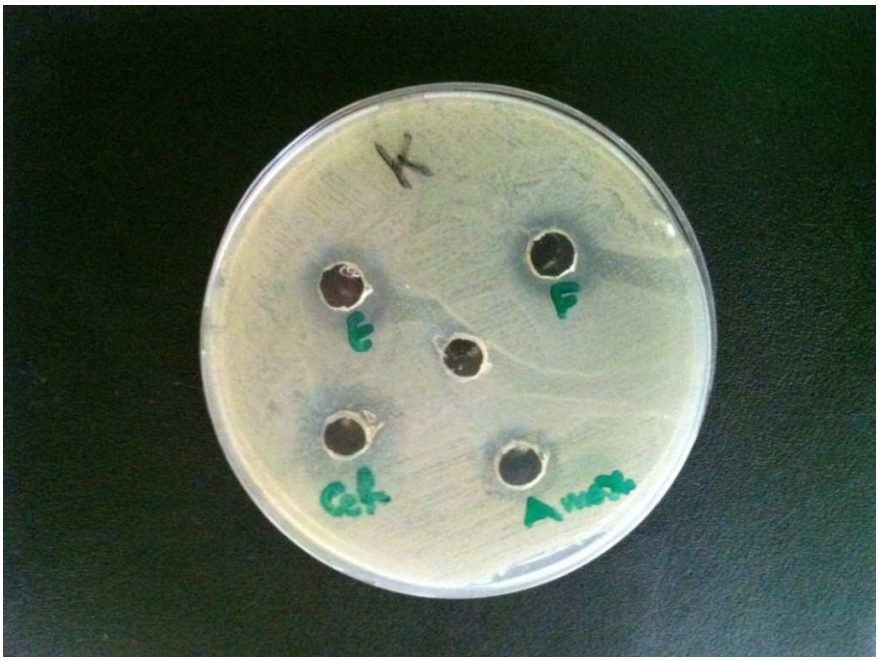


Fig. (3-23) The effect of compounds [A5 (E)], [A6 (F)], Cef., Amox.and DMSO on *S.Aureus.*

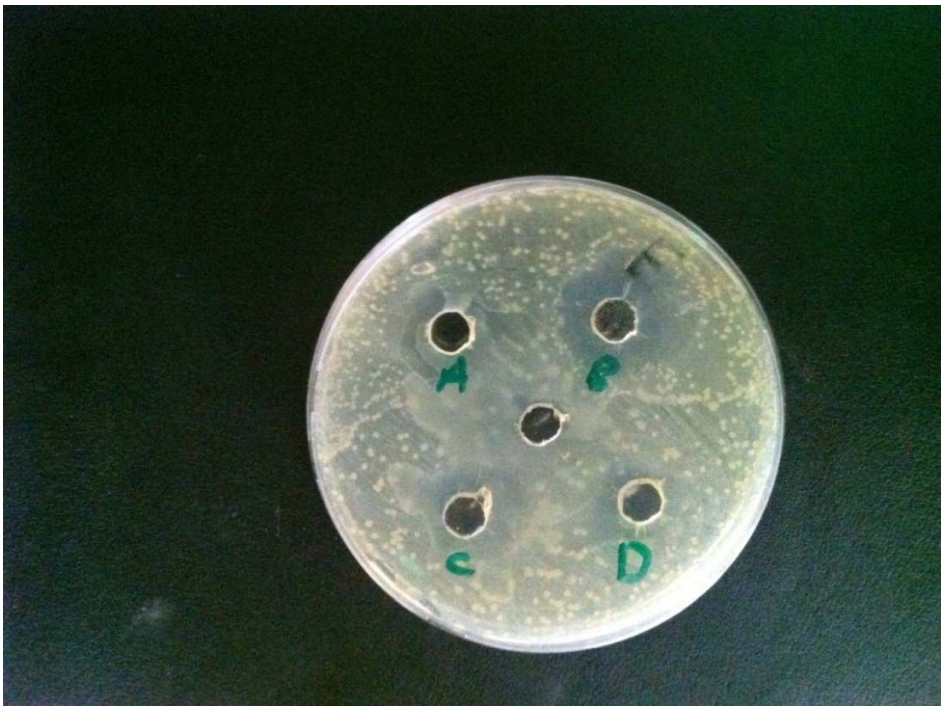


Fig. (3-25) The effect of compounds [B3(C)], [B4 (D)], [C3 (A)] and [C4(B)] on *E.Coli*

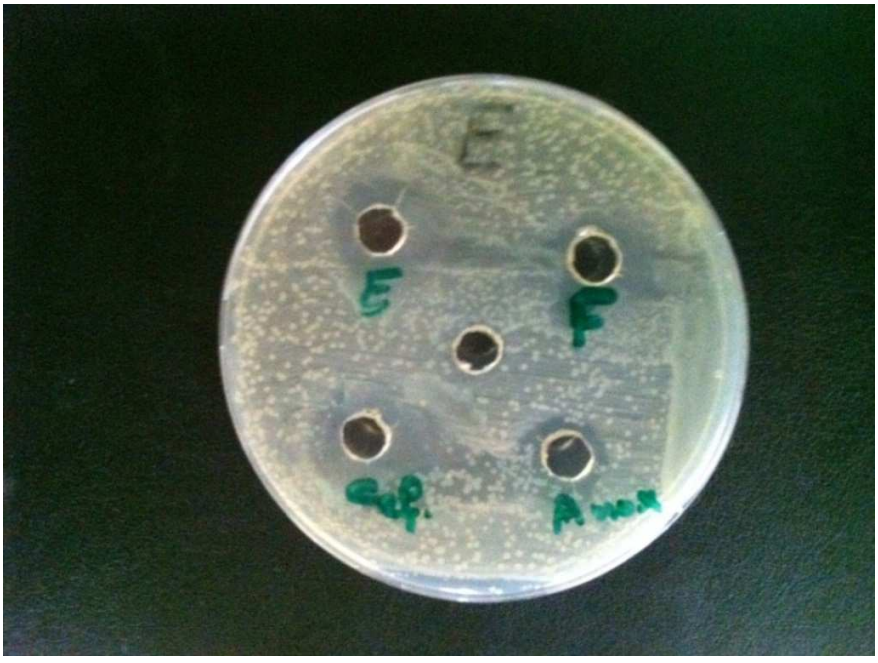


Fig. (3-26) The effect of compounds [A5(E)], [A6(F)], Cef., Amox.and DMSO on *E.Coli.*

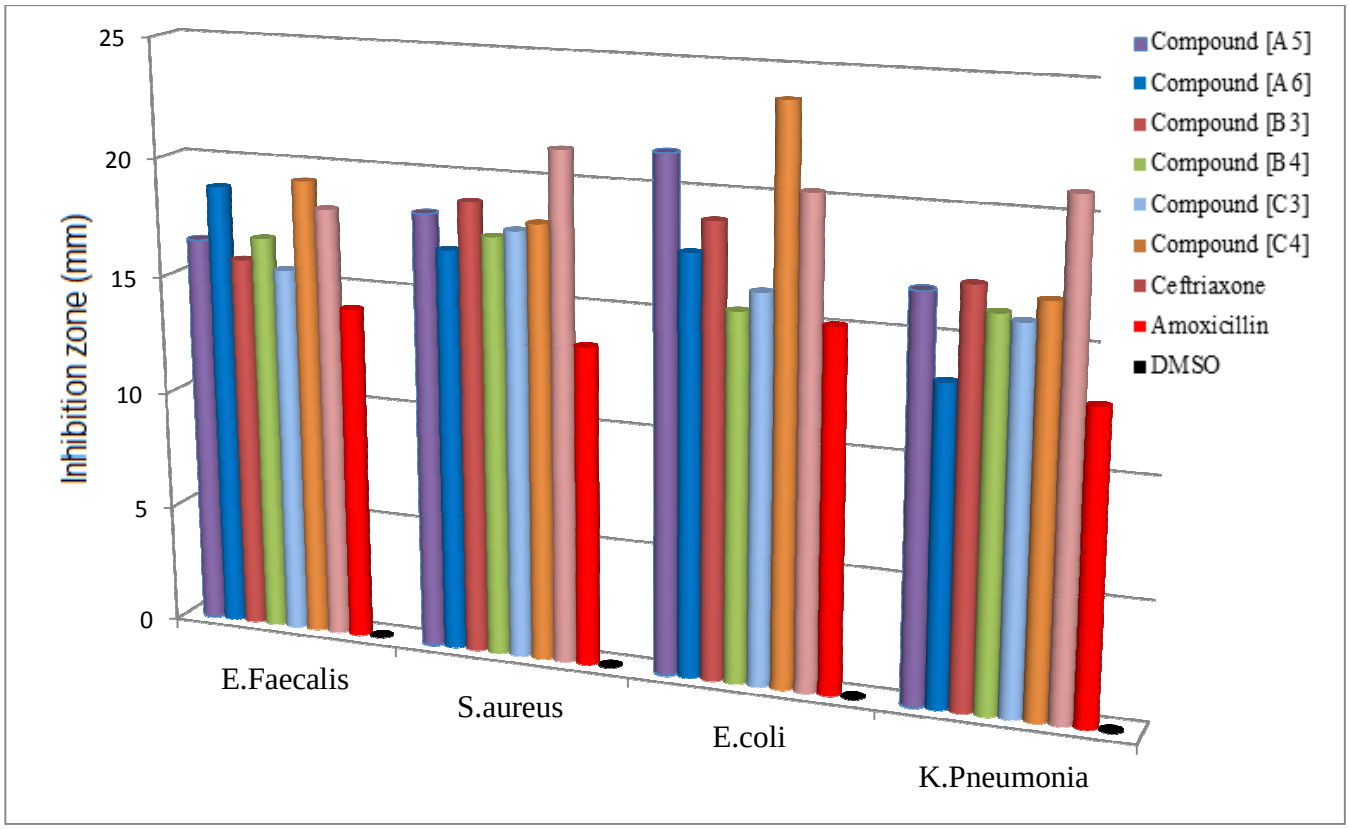


Fig. (3-27). The effect of compounds (A5, A6, B3, B4, C3 and C4) and (Ceftriaxone, Amoxicillin) on *E.faicalis*, *S.aureus*, *E.coli* and *K.pneumonia* in concentration (25µg/ml) dissolved in DMSO at 37C° for 24 hours.

The compounds (A5, A6, B3, B4, C3 and C4) have higher biological activity as antibacterial agent than Amoxicillin on these four types of bacteria.

Compounds [A6], [C4] have a higher biological activity than Ceftriaxone (third generation of *cephalosporin*) on the *E. Faecalis*, this may due to Thiadiazine ring. Compounds [A5], [B3], [B4] and [C3] have a biological activity a little less than Ceftriaxone on *E. faecalis.*

Ceftriaxone showed a higher biological activity than all of these compounds against the bacteria *S. Aureus.* Compounds [A5] and [B3] have a biological activity a little less than Ceftriaxone on *S. Aureus.*

Compounds [A5] and [C4] have a higher biological activity than Ceftriaxone when they were tested against *E. Coli*. Compounds [A6] and [B3] have a biological activity a little less than Ceftriaxone on the bacteria *E. Coli*. While the compounds [B4] and [C3] have a lower biological activity than Ceftriaxone but higher than Amoxicillin.

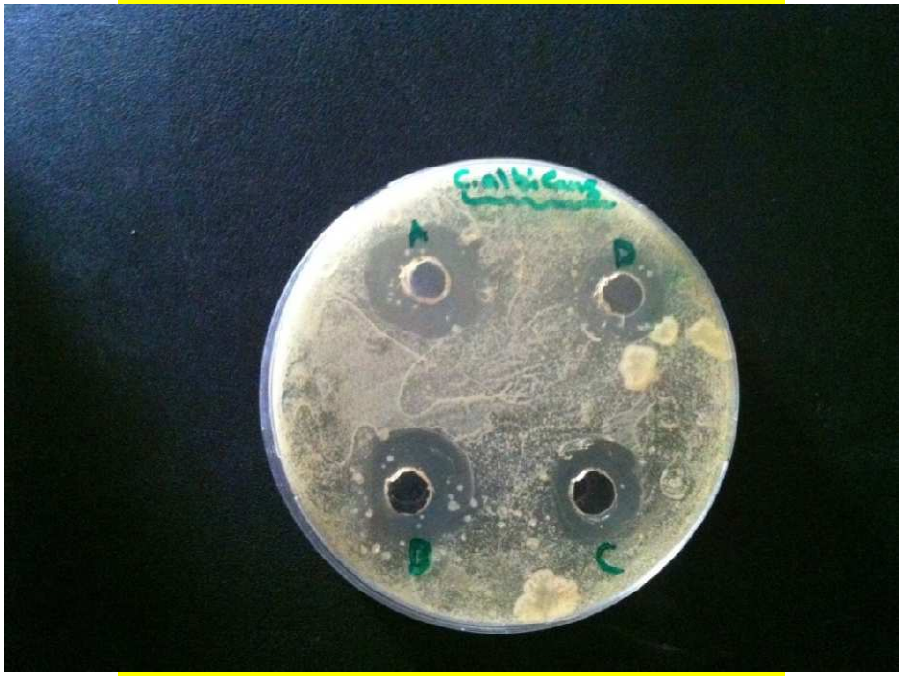
In *K. Pneumonia*, Ceftriaxone showed a higher biological activity than all of the tested compounds against this bacterium. (Fig. 3-27).

3.2.2 Antifungal Activity

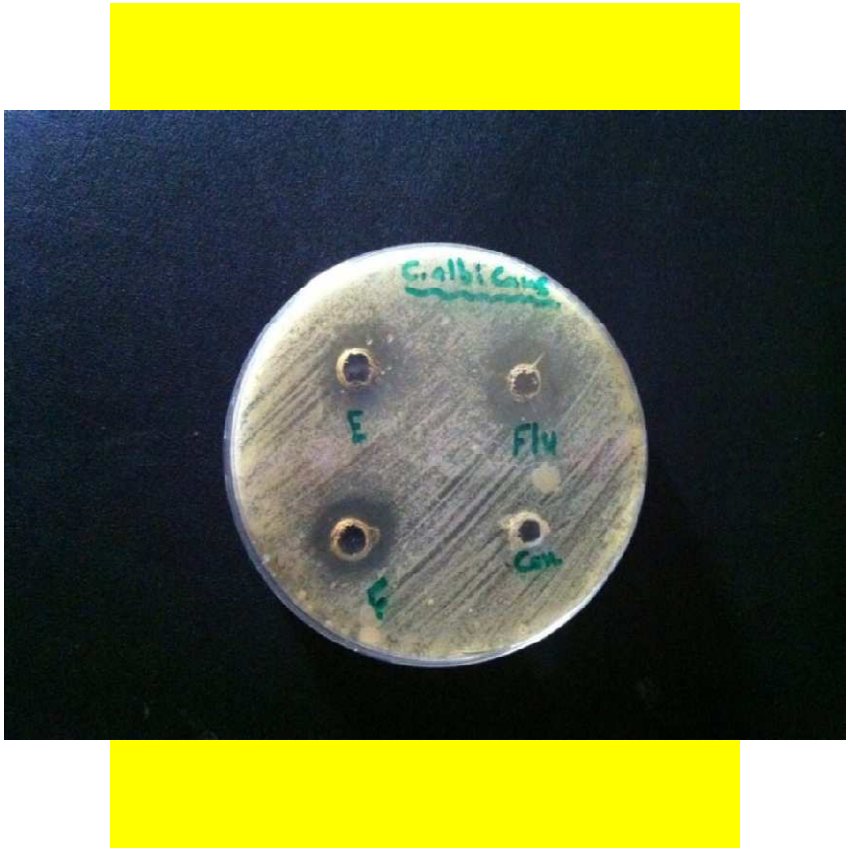
The inhibition zones caused by the various compounds on the fungi (*Candida albicans*) were examined. (**25** µg/ml concentration for all of these compounds). The results were listed in Table (3.2), Fig. (3-28), Fig. (3-2 9) and Fig. (3-30).

Table (3.2) The Inhibition zones of compounds [A5, A6, B3, B4, C3 and C4] and fluconazole on the fungi *Candida albicans*

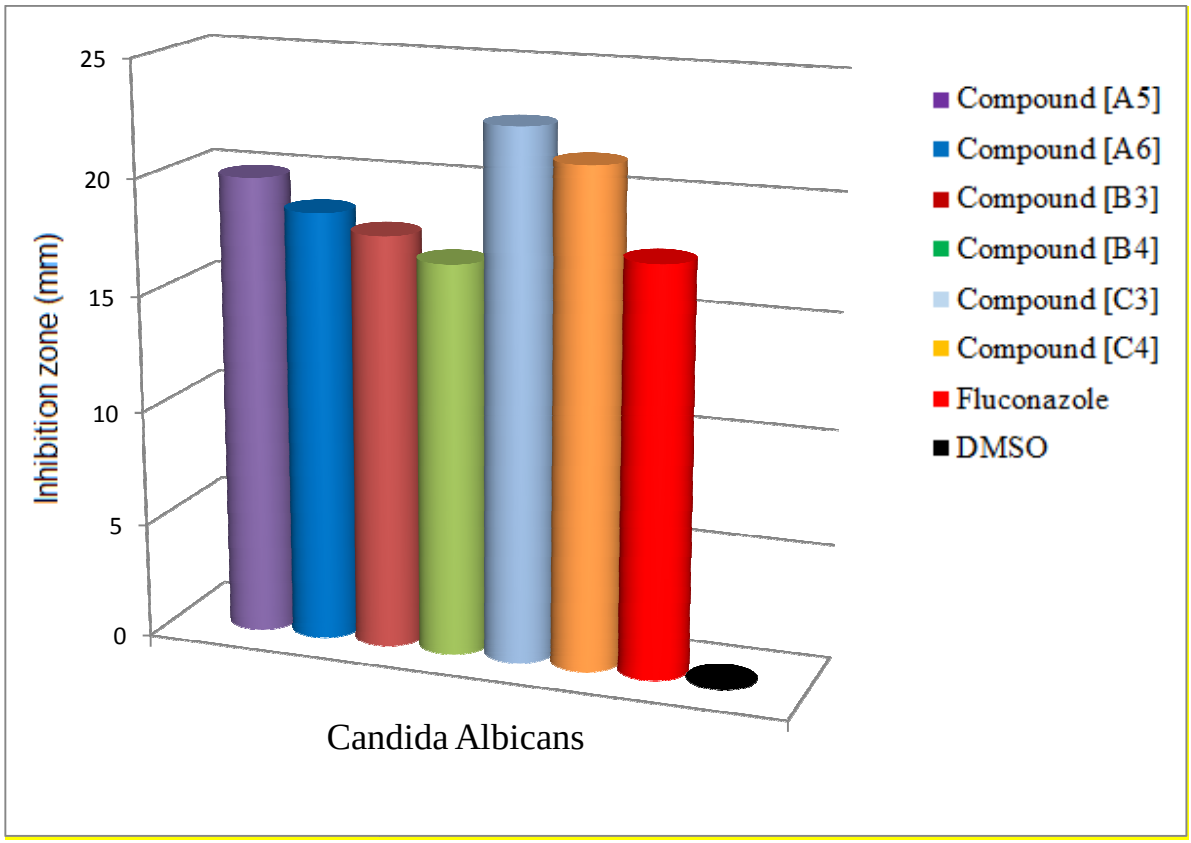
Compound Number	Concentration µg/ml	Inhibition Zone in mm
		Candida Abicans
A5=E	25	19.9
A6=F	25	18.61
B3=C	25	17. 85
B4=D	25	16.89
C3=A	25	22.73
C4=B	25	21.35
Fluconazole = (Flu.)	25	17.58
DMSO= (con.)	-	-



(Fig. 3-28) The effect of compounds [B3(C)], [B4 (D)], [C3 (A)] and [C4(B)] on *Candida albicans.*



(Fig. 3-29) The effect of compounds [A5 (E)], [A6 (F)], Fluconazole (Flu.) and DMSO (con.) On *Candida Albicans.*



(Fig.3-30) the effect of compounds [A5, A6, B3, B4, C3 and C4] and Fluconazole on *Candida Albicans* in concentration (25µg/ml) dissolved in DMSO at 37C° for 24 hours.

The compounds [A5, A6, C3 and C4] have a biological activity against *Candida Albicans* higher than fluconazole (reference antifungal).

Compound [B3) have a biological activity a little higher than fluconazole, while compound [B4] have biological activity a little lower than fluconazole, (Fig. 3-30).

3.2.3 Conclusions

From the results, the compounds (A5, A6, B3, B4, C3 and C4) that containing 1,2,4-triazole, 1,3,4-thiadiazole and triazolo [3,4-b] thiadiazine rings showed antibacterial and antifungal agents as expected when they compared with the references antimicrobials (Ceftriaxone, Amoxicillin and fluconazole) against four types of bacteria two of these bacteria were gram positive (E. *Faecalis* and *S. Aureus*) and the others were gram negative (*E. Coli* and *K. Pneumonia*) and one fungus *candida albicans* was used.

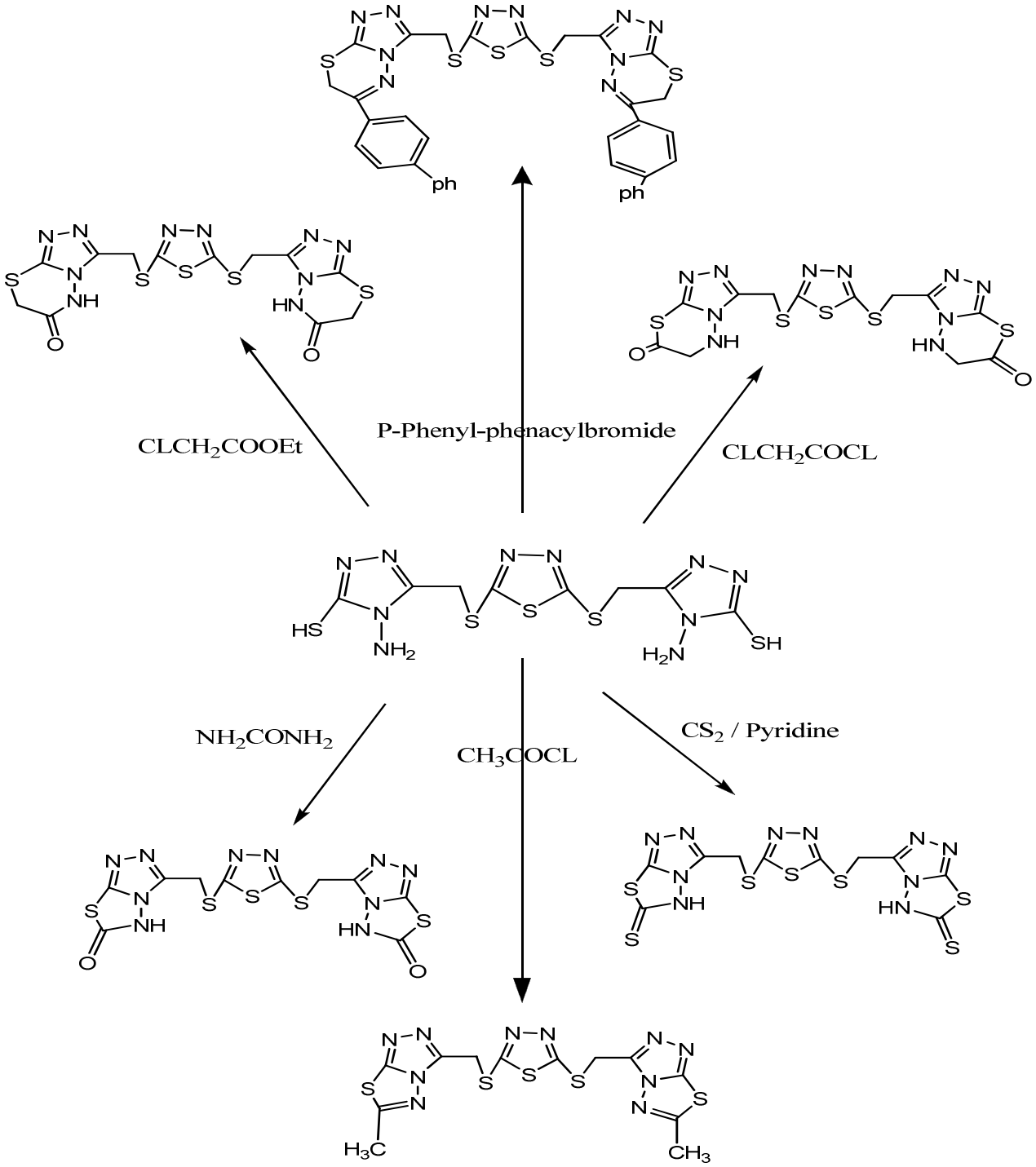
Suggestion for further work:

Our extensive program directed towards the synthesis of novel heterocyclic compounds of biological activity applications can be continued in further as follows.

1- The synthesized compounds can be test in the future and possible use as antitumor agents.

2- Also we suggest evaluating the cytotoxic activity of the synthesized compounds in vitro.

3-Cyclization between the two functional groups (SH and NH_2) of compounds [A5, B3 and C3] by using different cyclization reagents to form various heterocyclic rings. As shown:


ph
ph
CLCH2COOEt
P-Phenyl-phenacylbromide
CLCH2COCL
NH2CONH2
CH3COCL
CS2 / Pyridine

ACKNOWLEDGEMENT

Above all else, I want to express my great thanks to ***Allah*** *for his uncountable gifts and for helping me to present this thesis.*

I wish to express my sincere gratitude and great appreciation to my Supervisors ***Dr. Alaa H. Jawad*** *and* ***Dr. Jawad K, Shneine*** *for their guidance and their encouragement throughout this work . I ask Allah to bless both of them.*

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Mustafa Al-Kadhimi

CHAPTER ONE

INTRODUCTION

CHAPTER TWO

EXPERIMENTAL

CHAPTER THREE

RESULTS & DISCUSSION

Committee Certification

We, the examining committee, certify that we have read this thesis and have examined the student **"Mustafa Mohammed Abdulrasool"** in its contents and that in our opinion it is adequate with (**Excellent**) standing as a thesis for the degree of Master of Science in chemistry.

Signature:

Name:

Scientific Degree: Professor

Date: /5/2013

(Chairman)

Signature:

Name:

Scientific Degree: Assistant Professor

Date: /5/2013

(Member)

Signature:

Name:

Scientific Degree: Lecturer

Date: /5/2013

(Member)

Signature:

Name:

Scientific Degree: Assistant Professor

Date: /5/2013

Member (Supervisor)

Signature:

Name:

Scientific Degree: Lecturer

Date: /5/2013

Member (Supervisor)

I, hereby certify upon the decision of the examining committee.

Signature:
Name: Dr. Khulood Waheeb AL-Samarrae
Scientific Degree: Professor
Title: Dean of the College of Science
Date:

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جُـــمهورية العـــراق
وزارة التعليم العالي والبحث العلمي
جامعة النـهرين
كلية العلوم
قسم الكيمياء

تحضير، تشخيص ودراسة الفعالية البايولوجية لبعض مشتقات الحلقات الخماسية والملتحمة غير المتجانسة

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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF BIOLOGICAL ACTIVITY FOR SOME FIVE MEMBERED AND FUSED HETEROCYCLIC DERIVATIVES

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Supervisor Certification

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
وَأَنْ لَيْسَ لِلْإِنْسَانِ إِلَّا مَا
سَعَى *
وَأَنَّ سَعْيَهُ سَوْفَ يُرَى *
ثُمَّ يُجْزَاهُ الْجَزَاءَ الْأَوْفَى *
صدق الله العظيم
سورة النجم
الايـــــة (39-41)

الاهـــــــــــداء

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إلى من سعى وشقى لأنعم بالراحة والهناء الذي لم يبخل بشئ من أجل دفعي في طريق النجاح الذي علمني أن أرتقي سلم الحياة بحكمة وصبر إلى

والدي العزيز.

إلى من حبهم يجري في عروقي ويلهج بذكراهم فؤادي إلى

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