"Synthesis and Pharmacological Evaluations of 1,3,4-Thiadiazole, Triazole-5-thione and 1,3- Thiazolan-4-one Derivatives of Benzimidazole"

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CERTIFICATE

This is to certify that Mr. Kuldip Barot (08MPH404) has prepared his thesis entitled ***"Synthesis and Pharmacological Evaluations of 1,3,4-Thiadiazole, Triazole-5-thione and 1,3-Thiazolan-4-one Derivatives of Benzimidazole"****, in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under our guidance. He has carried out the work at the Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University.*

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DECLARATION

I declare that the thesis entitled ***"Synthesis and Pharmacological Evaluations of 1,3,4-Thiadiazole, Triazole-5-thione and 1,3 Thiazolan-4-one Derivatives of Benzimidazole"*** *under the guidance of* Prof. Manjunath Ghate *(Guide), Professor* and Mr. Kuntal Manna *(Co-guide), Assistant Professor, Department of Pharmaceutical Chemistry, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously in our institute and elsewhere.*

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DEDICATED

TO

GOD, FAMILY, TEACHERS

AND

FRIENDS

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Contents

ABSTRACT

A series of novel benzimidazole derivatives like 1, 3, 4-thiadiazole; 1, 2, 4-triazole-5-thione and 1, 3, -thiazolan-4-one derivatives containing benzimidazole heterocycle were synthesized by nucleophilic substitution reaction of 2-substituted -1*[H]* benzimidazole. The structures of the all synthesized compounds were elucidated by spectral analysis. All the synthesized compounds were evaluated *in vitro* antibacterial activity against three Gram-positive bacteria as *Bacillus cereus* (MTCC-430), *Enterococcus faecalis* (MTCC-493), *Staphylococcus aureus* (MTCC-737) and three Gram-negative bacteria as *Escherichia coli* (MTCC-1687), *Pseudomonas aeruginosa* (MTCC-2642) and *Klebsiella pneumoniae* (MTCC-109). Antifungal activity was evaluated against *Candida albicans* (MTCC-3017), *Aspergillus niger* (MTCC-1344) and *Fusarium oxyspora* (MTCC-1755). Some of the synthesized compounds showed good antibacterial and antifungal activities with 2.5 μ g/ml and 5.0 μ g/ml MIC (Minimum Inhibitory Concentration) respectively.

CHAPTER-1

INTRODUCTION

CHAPTER – 1

ABSTRACT & INTRODUCTION

1. Abstract

A series of novel benzimidazole derivatives like as 1, 3, 4-thiadiazole; 1, 2, 4-triazole-5-thione and 1, 3, -thiazolan-4-one derivatives containing benzimidazole heterocycle were synthesized by nucleophilic substitution of 2-substituted -1*[H]*benzimidazole.The structures of the all lead targeted synthesized compounds were evaluated by spectral and elemental methods of analysis. All the synthesized compounds were evaluated in vitro antibacterial activity against three Gram positive bacteria such as *Bacillus cereus* (MTCC-430), *Enterococcus faecalis* (MTCC-493), *Staphylococcus aureus* (MTCC-737) and three Gram negative bacteria such as *Escherichia coli* (MTCC-1687), *Pseudomonas aeruginosa* (MTCC-2642), *Klebsiella pneumoniae* (MTCC-109). Antifungal activity was evaluated against *Candida albicans* (MTCC-3017), *Aspergillus niger* (MTCC-1344), *Fusarium oxyspora* (MTCC-1755). Some of the synthesized compounds showed good antibacterial and antifungal activity with 2.5 μg/ml and 5.0 μg/ml MIC (Minimum Inhibitory Concentration) value respectively.

2. Introduction:

Antimicrobials are substances that kill or inhibit the growth of microorganisms such as bacteria[1], fungi[1] or protozoas[1]. Antimicrobial drugs kills microbes or prevent the growth of microbes.[1] The history of antimicrobials comes with the observations of Pasteur and Joubert[2], who discovered that one type of bacteria could prevent the growth of another.[2] Antimicrobials are substances that are produced by microorganism that kills or prevent the growth of another microorganism.[3] Microorganisms, especially bacteria and fungi are becoming resistant to antimicrobial agents.[4] Microorganisms are becoming resistant more quickly than new drugs are being available.[4] Thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials drugs.[5]

Antibacterial drugs are generally used to treat bacterial infections.[6] The toxicity to humans and other animals from antibacterial drugs is low.[6] Prolonged use of certain antibacterial drugs have a negative impact on health.[7] The term antibacterial originally described only those formulations derived from living organisms but is also applied to synthetic antibacterial such as the sulfonamides.[8] The discovery[9], development[9] and

clinical use[9] of antibacterial drugs during the 20th century has decreased from bacterial infections.[9] In 1980 the introduction of new antibacterial agents for clinical use is introduced for the development of testing new drugs.[10] Antibacterials are among the most commonly used drugs.[10] For example, 30% or more hospitalized patients are treated with one or more courses of antibiotic therapy.[11]

Antifungal drugs are used to treat fungal infections such as athlete's foot[12], ringworm[12], candidiasis[12], serious systemic infections such as cryptococcal meningitis[13] and others.[13] Antifungals works by differences between mammalian cells and fungal cells to kill fungal organism without dangerous effects on the host.[14] Fungi and humans are eukaryotes.[15] Thus, fungal and human cells are similar at the molecular level.[15] So, it is more difficult to find a target for an antifungal drug in the infected organism.[15] There are some side effects are observed with these drugs. Some of these side effects can be life-threatening if the drug is not used properly.[16]

Most of the earlier work was reported as various heterocyclic systems fused with benzimidazole.[17] 1, 3, 4-thiadiazole[18], triazole-5-thione[19] and 1, 3-thiazolan-4-one[20] moieties may be coupled with other heterocyclic ring system like benzimidazole by directly or through carbon and nitrogen bridges.[21] These types of heterocycles with benzimidazole as one of the compound have been of much of interest to explore biological activities.[21] Several research workers have tried to design and synthesize these types of molecules. [22]

1, 3, 4-thiadiazole[18] is reported for various biological activities such as antibacterial[23], antifungal[24], antiviral[25], anti-HIV[26], ant tubercular[27], anti-inflammatory[27], antidiabetics[27] etc. Other important heterocyclic moieties are 1, 2, 4-triazole-5-thione[19] and 1, 3-thiazolan-4-one[20], which are reported for the antitubercular[28], antimicrobial[28], herbicide[29], pesticide[29] and other activities.[29] The well known antibiotics ketoconazole[30], fluconazole[30], metronidazole[30], mebendazole[31], thiabendazole[31] and others carried benzimidazole ring systems as part of their structure.[32]

In view of the above facts, in our present study, we purpose to synthesize different 1, 3, 4-thiadiazole, triazole-5-thione and 1, 3-thiazolan-4-one derivatives as benzimidazole derivatives. A total of 15 compounds will be synthesized and they purposed to screen for antibacterial and antifungal activities.[33]

3. Purpose of the work

Chemical modification of 1, 3, 4-thiadiazole[34], triazole-5-thione[35] and 1, 3-thiazolan-4-one[36] heterocyclic ring systems containing benzimidazole[36] may provides versatile biological activities like antibacterial[37] and antifungal[37]. 1, 3, 4-thiadiazole, triazole-5-thione and 1, 3-thiazolan-4-one analogues are mostly reported as an antimicrobial agents[38]. Benzimidazole moiety leads to significant increase in their biological activity for providing potent antibacterial[39] and antifungal[39] activities. 1, 3, 4-thiadiazole, triazole-5-thione and 1, 3-thiazolan-4-one can easily binds with the allosteric site of enzyme. So, it decreases Koff value of allosteric site (Increases affinity for binding of drug with receptor). So, binding of drug with enzyme site will be optimum. So, it increases potency of drug.[40]

Certain heterocyclic systems can be fused with benzimidazole.[40] 1, 3, 4-thiadiazole[41], triazole-5-thione[42] and 1, 3-thiazolan-4-one[43] moieties may be coupled with other heterocyclic ring system like benzimidazole, thiadiazole, triazole by directly or through carbon and nitrogen bridges. These types of heterocycles with benzimidazole as one of the compound have been of much of interest to explore biological activities. Several research workers have tried to design and synthesize these types of molecules. Most of this type of work is come up during last two decades. The well known antibiotics ketoconazole[44], fluconazole[44], metronidazole[45], mebendazole[46], thiabendazole[47] and other carried benzimidazole[47] ring systems as part of their structure.[47]

Though there are good number of antimicrobial agents for the highely infectious diseases still there is need for newer drugs.[45] In view of the above regions, we have purticularly focused our work on antibacterial and antifungal properties.[47] As there is 1, 3, 4-

thiadiazole, triazole-5-thione and 1, 3-thiazolan-4-one derivatives, we have to studied for antibactrial and antifungal activities.[48]

4. Aim and Scope of the work

From certain years only two novel classes of antibiotics have reached the market to combat the clinical threat of multidrug resistant bacteria.[49] For many years, antibiotic research has focused on making new derivatives of these established classes of antibiotics, but many of these remain compromised by the pre-existing resistance mechanism generated by the various generations from the classes.[50] To provide sustainable antibiotics for the future, entirely novel antibiotics employing unexploited mechanism of action are needed.[50]

Not only semi synthetic antibiotics but synthetic antibiotics are also available such as fluoroquinolones.[50] But, these types of antibiotics also have some serious side effects like neuropathy[51], ototoxicity[51], skin rashes[51] and other CNS side effect[52]. Decades of antibiotics used are resulted in the development of widespread resistance to commonly prescribed antibacterial agents.[53] Nitrogen and mono sulphur or mono oxygen based heterocyclic compounds are very important in the field of medicinal chemistry.[54] The present 1, 3, 4-thiadiazole, triazole-5-thione and 1, 3-thiazolan-4-one heterocyclic ring systems containing benzimidazole can be prepared from N-(1H-benzimidazol-2-ylmethyl)-2-(pyridin-4-ylcarbonyl) hydrazine carbothioamide.[55]

5. Strategies of the work

1. N-((1H benzo[d]imidazol-2yl) methyl)-2-isonicotinyl hydrazine carbothiomide is synthesised from o-phenylene diamine and glycine.[56]
2. 1H-benzo[d]imidazol-2ylmethyl [5-(4-pyridyl)-1, 3, 4thiadiazol-2-yl] amine is synthesised from N-((1H benzo[d]imidazol-2yl) methyl)-2-isonicotinyl hydrazine carbothiomide.[57]
3. 4-(1H benzo [d]imidazol-2ylmethyl-3-(4-pyridyl)-4, 5-dihydro-1H-1, 2, 4-triazole-5-thione is synthesised from1H-benzo[d]imidazol-2ylmethyl [5-(4-pyridyl)-1, 3, 4thiadiazol-2-yl] amine.[58, 59]

4. 2-(1H-benzo[d]imidazol-2ylmethylimino) 3-(4-pyridyl carbonyl)-1, 3-thiazolan-4-one is synthesised from 4-(1H benzo [d]imidazol-2ylmethyl-3-(4-pyridyl)-4, 5-dihydro-1H-1, 2, 4-triazole-5-thione.[60]

6. Materials

The materials which are used for the proposed work was highlighting as follows.

Sr. No	Chemical	Company
1	O-phenylene diamine	CDH
2	Glycine	CDH
3	Hydochloric acid	CDH
4	P-amino benzoic acid (PABA)	CDH
5	Pyridine	S d fine
6	Carbondisulphide	S d fine
7	Iodine	CDH
8	Phenylacetic acid	CDH
9	Ethanol	CDH
10	Methanol	CDH
11	Sulphuric acid	CDH
12	Diethyl ether	CDH
13	Hydrazine hydrate	S d fine
14	Cinnamic acid	CDH
15	Isoniazide	S d fine
16	Sodium hydroxide	CDH
17	Sodium bicarbonate	CDH
18	Chloroform	CDH
19	Tolune	CDH
20	Ethyl acetate	CDH
21	Hexane	CDH
22	Acetonitrile	S d fine
23	Chloroacetyl chloride	S d fine

7. Methods

The proposed project work deals with the synthesis of 1, 3, 4-thiadiazole, triazole-5-thione and 1, 3-thiazolan-4-one that can be generated from diamine phenyl derivative precursor (o-phenylenediamine) and evaluated for various biological activities. The proposed work is classified as below:

1. Ordinary (orthodox) synthesis of 1, 3, 4-thiadiazole, triazole-5-thione and 1, 3 - thiazolan-4-one derivatives as benzimidazole deivatives.
2. Analysis by different spectrophotometric techniques.
    - FTIR spectra
    - Mass spectra
    - ^{1}H NMR spectra
3. Melting Point – Melting point apparatus
4. Thin Layer Chromatography (TLC) - Pre Coated TLC
5. Various antimicrobial screening of synthesized compounds

8. Analytical techniques [61]

- An infrared spectrum (FTIR) will record for all the compounds on Jasco-FTIR-6100 in KBr.
- ^{1}H-NMR spectra will record for the compounds on Advance Bruker (300MHz) instrument. Chemical shift is in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard.
- Mass spectra will record for the compounds on Qudrapole spectrophotometry. The elemental analyses may be performing on a Leco CHNS 930 analyzer and satisfactory result ± 0.4% of calculated values.
- Ultraviolet (UV)-visible absorption spectra will be measure with an UV 2100 spectrophotometer.
- The purity of all the compounds will confirm by thin layer chromatography (TLC) using silica gel G glass plates and pre coated TLC with various solvent systems. The spots will develop in iodine vapour and UV light to visualize the spots.

The methods followed to synthesize compounds are mentioned in experimental section.

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CHAPTER -2

LITERATURE REVIEW

CHAPTER -2.1

LITERATURE REVIEW OF BENZIMIDAZOLE DERIVATIVES

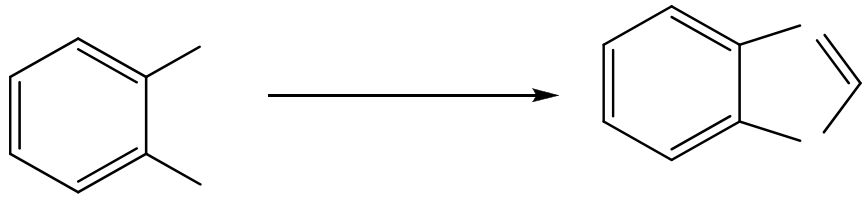
Heavy reliance on the benzimidazole since their introduction in the 1960's for the control of gastrointestinal parasites of livestock has led to widespread resistance in target parasite species.[1]The benzimidazole exerts their primary action by binding to tubulin, the major protein component of microtubules.[1] Although tubulin is a highly conserved protein present in both the host and the parasite, it demonstrates relatively low mammalian toxicity.[2] Significant differences between benzimidazole resistant parasite and tubulin from the host suggest the potential for the design of new generation benzimidazole active against 'Benzimidazole-resistant' parasites.[2]

Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole. The most prominent benzimidazole compound in nature is *N*-ribosyl-dimethylbenzimidazole[3], which serves as an axial ligand for cobalt in vitamin B12.[3, 4] Benzimidazole, in an extension of the well-elaborated imidazole system, has been used as carbon skeletons for N-heterocyclic carbenes (NHC).[4] The NHCs are usually used as ligands for transition metal complexes.[5] They are often prepared by deprotonating an N,N'-disubstituted benzimidazolium salt at the 2-position with a base.[5]

Benzimidazole has fungicidal properties.[6] It acts by binding to the fungal microtubules and stopping hyphal growth.[6] It also binds to the spindle microtubules and blocks nuclear division.[6] It also exerts different types of side effects. There are large number of research workers are working on derivatisation of benzimidazole for decreasing the side effects of benzimidazole.[7] Number of research works are completed for improving the efficacy and safety of the benzimidazole.[7]

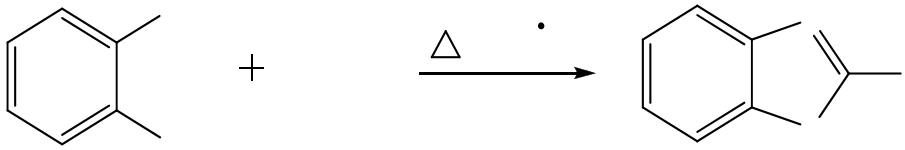
Different types of substitutions are carried out by the scientists for the improving the safety and quality of the derivatives of benzimidazole.[7] The derivatives of benzimidazole are substituted with different heterocyclic ring systems which improves the therapeutic index and quality of the drug.[8] 1st bezimidazole heterocycle was synthesized by E. C. Wagner and W. H. Millett **(1943)**.[8] The usual synthesis involves condensation of o-phenylenediamine with formic acid,or the equivalent trimethyl orthoformate.[8]

Michel jacobus, Maria gijsen and Michel Annajozef synthesized a series of 1*H*-benzo[d]imidazole [1] derivatives and evaluated those as potent antibacterial and antifungal agents.[9]



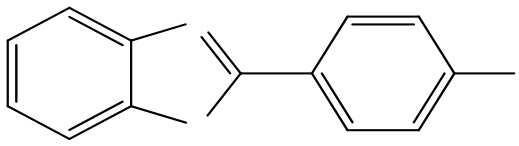
[1]

Uwe Greather konrad and Rainer F Martin synthesized a series of substituted 1*H*-benzimidazole [2] by the substitution with the different aminoacid derivatives and evaluated those as potent antibacterial agents.[10]



[2]

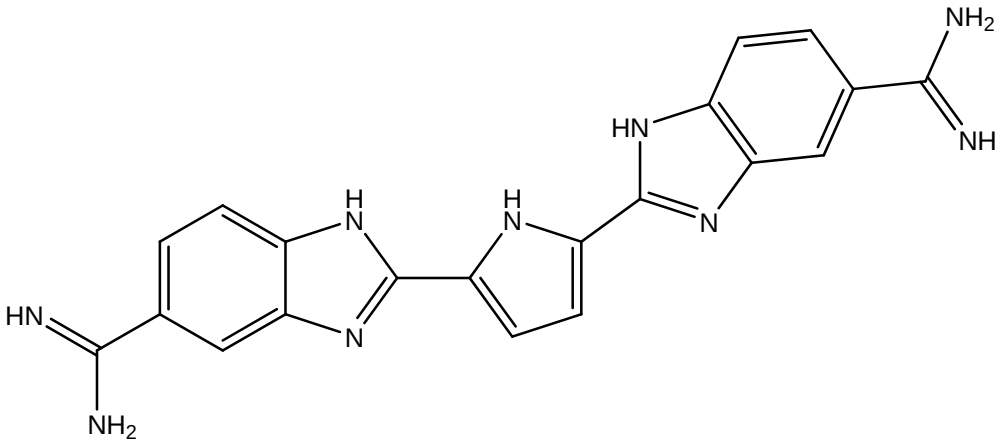
Martin Benson grogory and Hance Richter synthesized 4-(1*H*-benzo[d]imidazol-2-yl) aniline [3] derivatives and evaluated those as potent antibacterial agents and antifungal agents.[11]



[3]

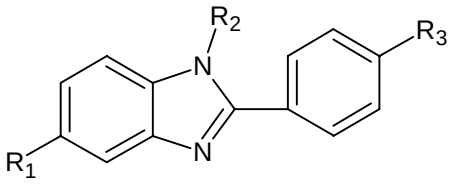
Hakan Goiiker synthesized 2-Substituted-phenyl-1*H*-benzimidazole- 5-carbonitriles [4] and evaluated as antifungal agent against *Candida Species.* New 2-substituted-phenyl-

1H-benzimidazole-5-carboxylic acids, ethyl-5-carboxylate, 5- carboxamides,-5-carboxaldehyde, -5-chloro, -5-trifluoromethyl , and -5-carbonitriles and -6-carbonitrile were prepared and evaluated in vitro against *Candida* species. The cyano substituted compounds exhibited the greatest activity with MIC values of 3.12 mg/mL, values similar to that of fluconazole.[12]



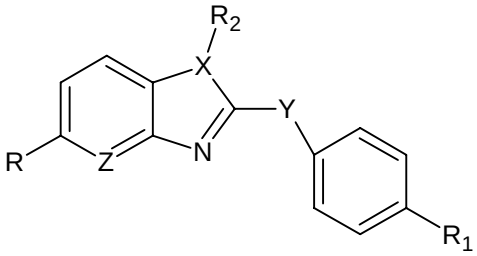
[4]

Claud A, Bern Hart synthesized different benzimidazole derivatives [5] from O-phenylenediamine and evaluated as potent antifungal agent against *Aspergillus niger.*[13]



[5]

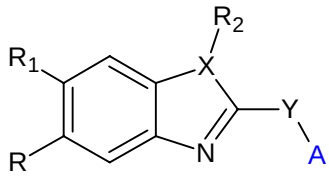
Oren K synthesized 2, 5 and or 6-substituted benzimidazole derivatives [6]. The synthesized compounds were tested in vitro against three Gram-positive, two Gram-negative bacteria and the yeast *Candida albicans* in comparison with several control drugs. 5-Chloro-2-(2- cyclohexylethyl) benzimidazole was found as the most active compound against the screened Gram-positive bacteria strains at a minimum inhibitory concentration (MIC) value of 12.5 mg/ ml.[14]



Z = CH, N
X = O, NH, S
Y = CH_2, CH_2CH_2, CH_2O, CH_2S
R = H, CH_3, Cl, NO_2, NH_2
R_1 = H, CH_3, C_2H_5, C $(CH_3)_3$, OCH_3,

[6]

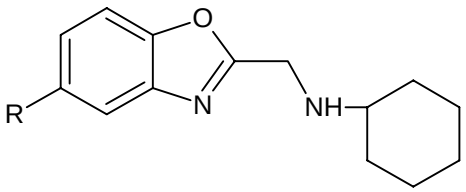
Canan KUS synthesized various benzimidazole derivatives [7] from O-phenylenediamine and substituted cyclohexyl and cyclopenyl derivatives. They are evaluated for potent antitubercular and anti-HIV activities.[15]



X = NH	Y = CH_2	A = Cyclopentyl,	R = H	R_1 = H
X = NH	Y = CH_2	A = Cyclopentyl,	R = Cl	R_1 = H
X = NH	Y =C_2H_4	A = Cyclopentyl,	R = H	R_1 = H
X = NH	Y =C_2H_4	A = Cyclopentyl	R = Cl	R_1 = H
X = NH	Y =C_2H_4	A = Cyclohexyl	R = H	R_1 = H

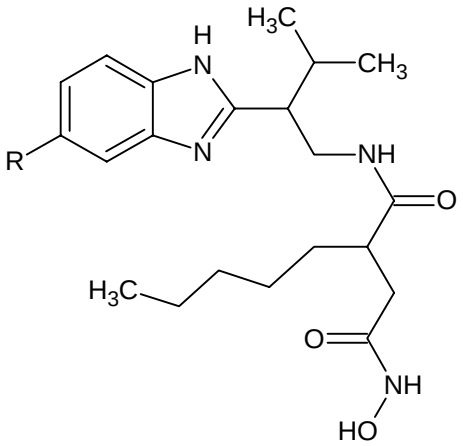
[7]

Rosa M.F. Batista synthesized various benzimidazole derivatives [8] and evaluated for potent antibacterial and antifungal activities. From these activities, potent activity against Aspergillus niger was observed.[16]



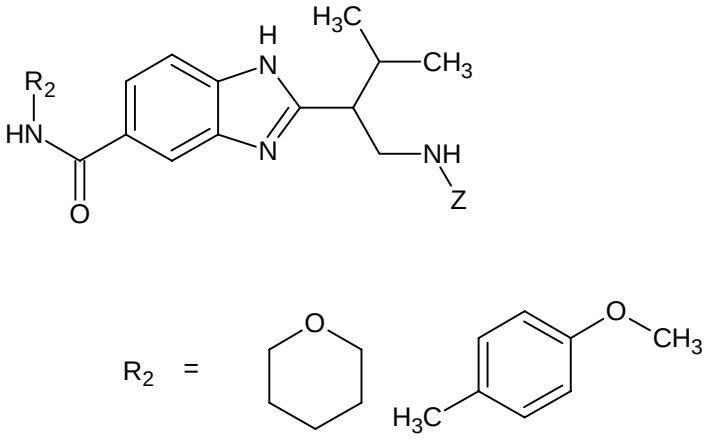
[8]

Alka Bali, Yogita Bansal synthesized actinonin derivatives [9] containing benzimidazole heterocycles and evaluated those as potent antibacterial agent. Potent antibacterial activity is observed against Staphylococcus aureus at a minimum inhibitory concentration (MIC) value of 5 mg/ ml.[17]



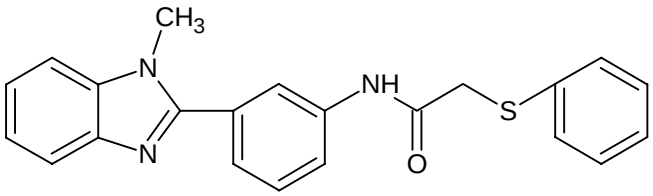
[9]

Sham M. Sondhi, Nirupma Singh synthesized substituted benzimidazole derivatives [10] by methoxy, cyclopentyl and cyclohexyl derivatives. They are evaluated for potent antitubercular and anti-HIV agent.[18]



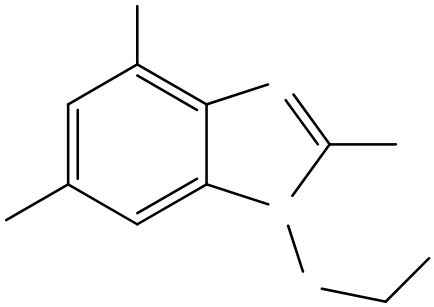
[10]

Özden Özel Güven syntehsized phenyl substituted benzimidazole derivative [11] and evaluated for potent antimicrobial and anti-HIV activities. It was shown that the some compound exhibited the potent activity against *B. subtilis, P. aeruginosa* and *C. albicans* at a minimum inhibitory concentration (MIC) value of 15 mg/ ml.[19]



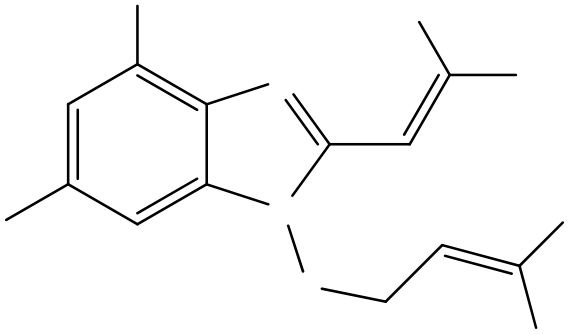
[11]

Jun Cheng, Jiangtao Xie synthesized substituted benzimidazole [12] and evaluated as potent anti-HIV agent. It was also shown the non nucleoside reverse transcriptase inhibitory activities.[20]



[12]

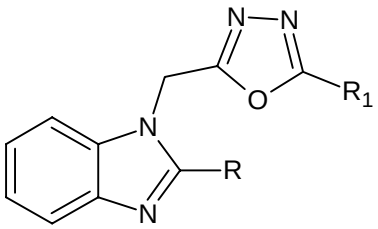
Sham M. Sondhi, Reshma Rani Evans synthesized substituted benzimidazole derivatives [13] and evaluated as potent antitubercular and anti-HIV agent. It was also shown the non nucleoside reverse transcriptase inhibitory activities.[21]



[13]

Zhan-Hui Zhang synthesized 2-methyl oxadiazole substituted benzimidazole [14]. All the synthesized compounds were screened for their antimicrobial activities. All of the derivatives showed good activity towards Gram-positive bacteria and negligible activity

towards Gram-negative bacteria. Some of the synthesized compounds showed moderate activity against tested fungi at a minimum inhibitory concentration (MIC) value of 3 mg/ml.[22]



[14]

CHAPTER -2.2

LITERATURE REVIEW OF 1, 3, 4-THIADIAZOLE DERIVATIVES

1, 3, 4-thiadiazole is also an important heterocyclic moiety under the class of derivatives of benzimidazole.[23] 1, 3, 4-thiadiazole heterocyclic system is also known to exhibit a wide range of biological properties such as antiinflammatory[23], antibacterial[24], antifungal[25], and antitumor[26] activities. The incorporation of heterocyclic ring into the benzimidazole ring would be more potent against multidrug resistant bacteria than benzimidazole ring alone.[26]

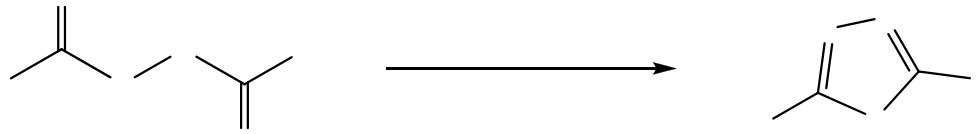
1, 3, 4-thiadiazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and thiazole.[27] The most prominent compound in nature is *N*-ribosyl-dimethy-1, 3, 4-thiadiazole[27]. 1, 3, 4-thiadiazole, in an extension of the well-elaborated thiadiazole system, has been used as carbon skeletons for N-heterocyclic carbenes (NHC). The NHCs are usually used as ligands for transition metal complexes.[27]

The antibacterial activity was screened against multidrug resistant *E. coli, P. aeruginosa, S. pneumoniae*, *S. agalactiae*, *S. typhi, S. aureus,* and *S. pyogenes* bacteria and carried out by *cylinder* and *well method.* The antifungal activity was screened against *Candida albicans* using agar couture medium.[28]

The antibacterial data revealed that all nitroimidazole derivatives showed interesting activity against tested Gram-positive bacteria (minimum inhibitory concentration, MIC=0.008–0.03 μg/ml) while they did not show good activity against Gram-negative organisms.[29] Despite the significant activity of nitroimidazole series, all nitrophenyl analogues were inactive against both Gram-positive and Gram-negative bacteria.[29]

Different types of substitutions are carried out by the scientists for the improving the safety and quality of the derivatives of 1, 3, 4-thiadiazole.[29] The derivatives of 1, 3, 4-thiadiazole are substituted with different heterocyclic ring systems which improves the therapeutic index and quality of the drug.[29]

W. J. Chambers synthesized 2, 5-disubstituted-1, 3, 4-thiadiazole [15] from Bis(polyfluoroalky1)thiadiazoles. The bis(polyfluoroalkyl)thiadiazoles, in which R = C_2F_5- and $H(CF_2)_4$-, were prepared by treating the bis(polyfluoroacy1)hydrazines or the hydrazinium fluorocarboxylates with phosphorus pentasulfide at 250-300 °C. They are evaluated as potent antimicrobial agents.[30]

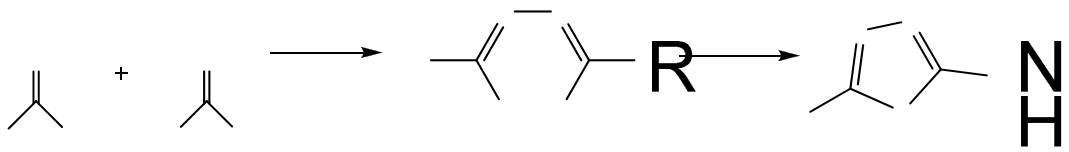


[15]

Jong Yup Kim, H. J. S synthesized 2, 5-dimethyl-1, 3, 4-thiadiazole [16] by the reaction of N'-acetylacetohydrazide with Lawesson's reagent will give 1, 5-disubstituted-1, 3, 4-thiadiazole. They are evaluated as potent antifungal agents against *Aspergillus niger*.[31]

[16]

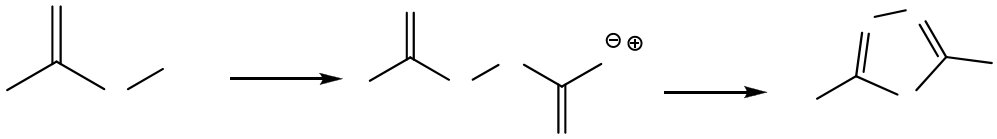
Christopher B Chapleo synthesized 1, 5-disubstituted-1, 3, 4-thiadiazole [17] by one pot-synthesis of 2, 5-dialkyl-1, 3, 4-thiadiazoles. It involves the reaction of hydrazine with aldehyde and elemental sulfur. The reaction proceeds via an intermediate which subsequently cyclized to 2, 5-dialkyl-1, 3, 4-thiadiazoles with an evolution of hydrogen sulfide involving the formation of C-S bond. They are evaluated as potent antibacterial agents against *E.coli* and *S.aureus*[32]



[17]

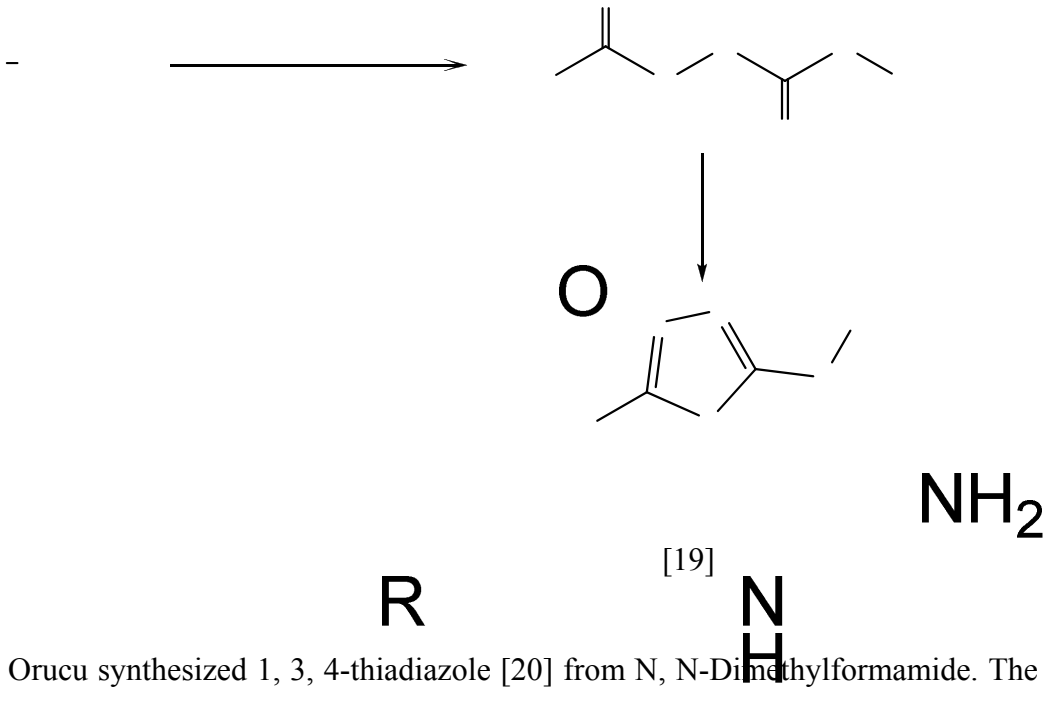
Bis(polyfluoroalky1)thi

Alireza Foroumadi synthesized 5-disubstituted-2-mercapto-1, 3, 4-thiadiazole [18] From Potassium dithiocarbazate. Reaction of appropriate hydrazide with carbon disulphide in alkaline condition yields dithiocarbazate, which on cyclization in acidic medium gives corresponding 1,3,4- thiadiazoles. It was observed that potent antibacterial activity is observed at a MIC of 10mg/ml.[33]



[18]

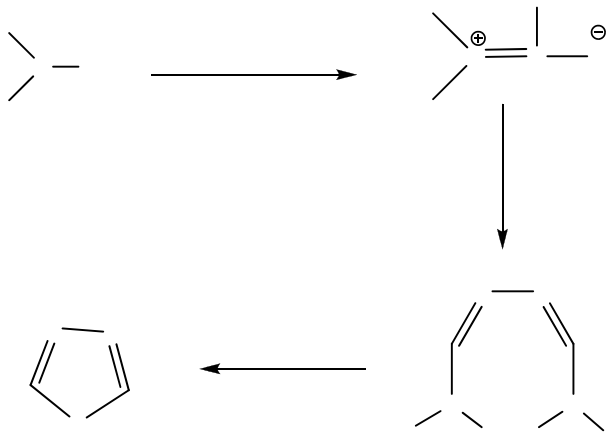
Alireza Foroumadi synthesized 2, 5-disubstituted-1, 3, 4-thiadiazole [19] from Reacting the suitable hydrazide with suitable aryl/alkyl isothicyanates results in formation of corresponding thisemicarbazides, which upon dehydartive cyclization under acidic medium using sulfuric acid, orthophosphoric acid, or polyphosphoric acid or phosphorous halides or methane sulphonic acid result in the formation of 5-Substituted-2-amino-1,3,4-thiadiazoles corresponding to thiosemicarbazide.[34]



[19]

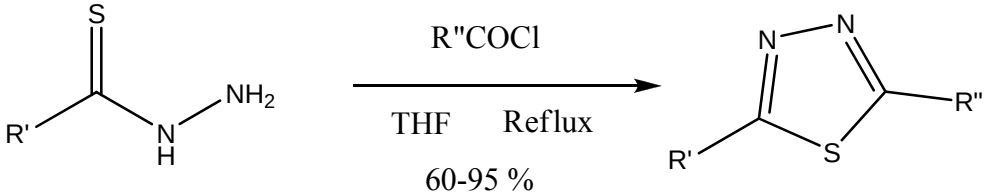
Elcuin E Orucu synthesized 1, 3, 4-thiadiazole [20] from N, N-Dimethylformamide. The reaction of DMF with thionyl chloride produces formamidoyl chloride which on

treatment with N, N-diformylhydrazine and with sodium ethoxide gives a free base. The free base obtained undergoes cyclization in the presence of hydrogen sulfide with the formation of parent 1, 3, 4-thiadiazole. It was shon potent antibacterial activity at a MIC 11mg/ml.[35]



[20]

S. Talath A. K. G synthesized 2, 5-disubstituted-1, 3, 4-thiosemicarbazide [21] from Substituted thiohydrazide. Substituted thiohydrazide when refluxed with substituted acid chloride in tetrahydrofuran will give corresponding 1, 3, 4-thiadiazole. They are evaluated as potent antifungal agents.[36]

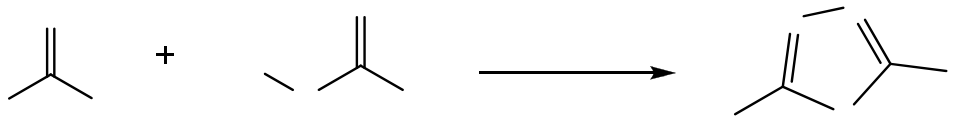


2,5-disubstituted-1,3,4-thiosemicarbazide

[21]

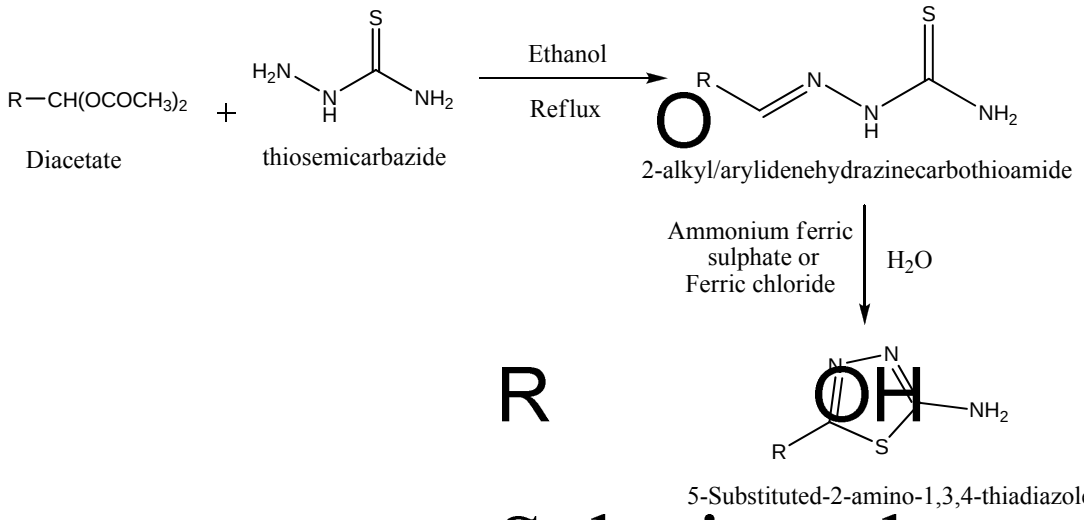
Kil-Joong Kim synthesized 5-Substituted-2-amino-1,3,4-thiadiazole [22] from Substituted acid and Thiosemicarbazide. Substituted acid in presence of polyphosphoric acid and

thiosemicarbazide will also give 5-Substituted-2-amino-1, 3, 4-thiadiazole. . They are evaluated as potent antitubercular and anti-HIV agents.[37]



[22]

Shinkai and P.J. Reider synthesized 5-Substituted-2-amino-1, 3, 4-thiadiazole [23] from Diacetate and Thiosemicarbazide. Diacetate when refluxed with thiosemicarbazide in presence of Ethanol gives imine intermediate, which on further reaction (reflux) with Ammonium ferric sulphate or ferric chloride in water gives 2-Substituted 5-amino-1, 3, 4-thiadiazole.[38]



[23]

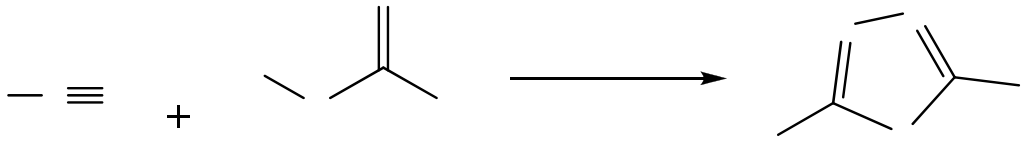
Substituted

acid

H_2N

thios

V. Arian, P. Goya synthesized 5-Substituted-2-amino-1, 3, 4-thiadiazole [24] from Thiosemicarbazide upon reaction with alkyl/aryl nitriles in presence of trifluoroacetic acid leads to the formation of 5-Substituted-2-amino-1, 3, 4-thiadiazoles.[39]



[24]

Márquez-Lucero synthesized 1, 3, 4-thiadiazole-2-amine [25] from Formic acid and Thiosemicarbazide. 2-amino-1, 3, 4-thiadiazole is obtained by heating thiosemicarbazide with a mixture of formic acid and hydrochloric acid. They are evaluated as potent anti-inflammatory agents.[40]

[25]

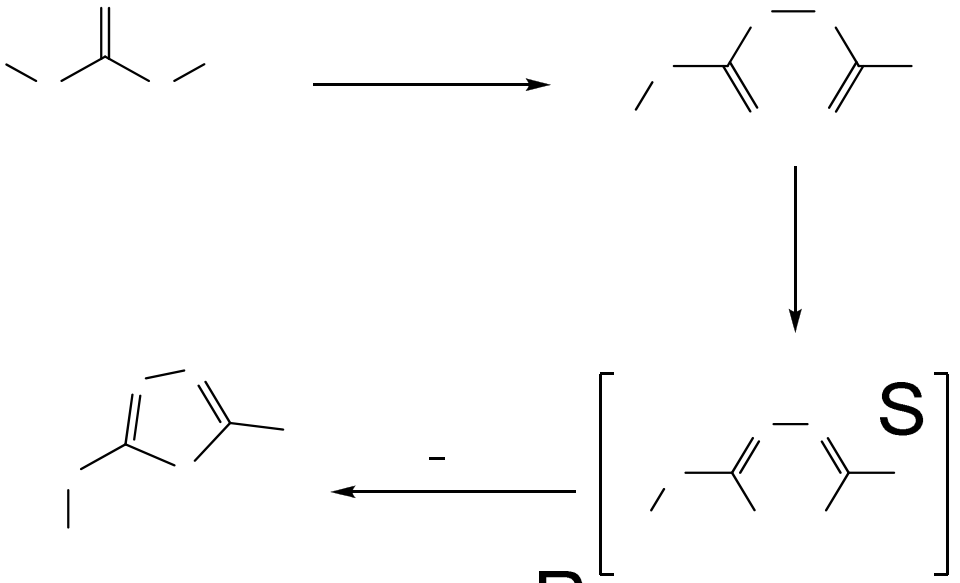
P. Strohriegl and J. Grazulevicius synthesized 2, 5-disubstituted 1, 3, 4-thiosemicarbazide [26] from Substituted Thiosemicarbazide. Acylation of substituted thiosemicarbazide in presence of anhydride produces acylated product which on further dehydration gives corresponding 1, 3, 4-thiadiazole. They are evaluated as potent antibacterial agents at a MIC if 13mg/ml.[41]

H_2N

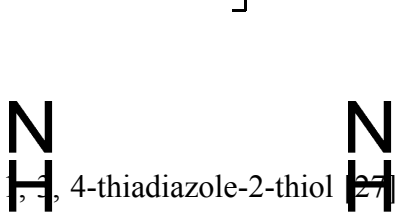
R C N

Nitrile

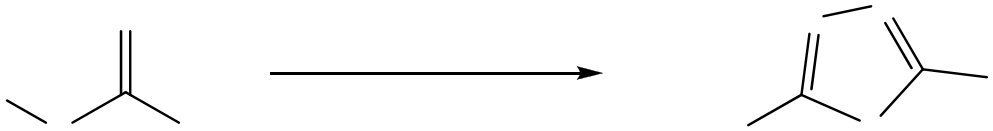
thio



[26]

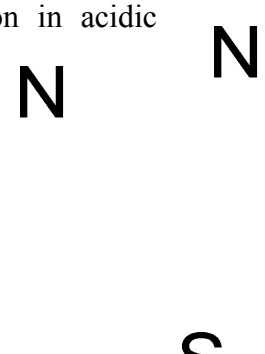


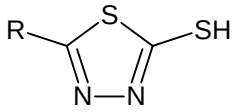
X.-G Duan, X.-L Duan, C.W. Rees synthesized 5-amino- 1, 3, 4-thiadiazole-2-thiol [27] from thiosemicarbazide. It is refluxed with carbon disulphide in ethanol; it gives 5-amino-2-mercapto-1, 3, 4-thiadiazole. It was shown potent anti HIV activities.[42]



[27]

Liu F, Luo X and Song B synthesized sulphoxide derivatives containing trimethoxy phenyl substituted 1, 3, 4-thiadiazole [28] and evaluated for potent antifungal agents from Potassium dithiocarbazate. Reaction of appropriate hydrazide with carbon disulphide in alkaline condition yields Potassium dithiocarbazate, which on cyclization in acidic medium gives corresponding 1, 3, 4-thiadiazoles.[43]

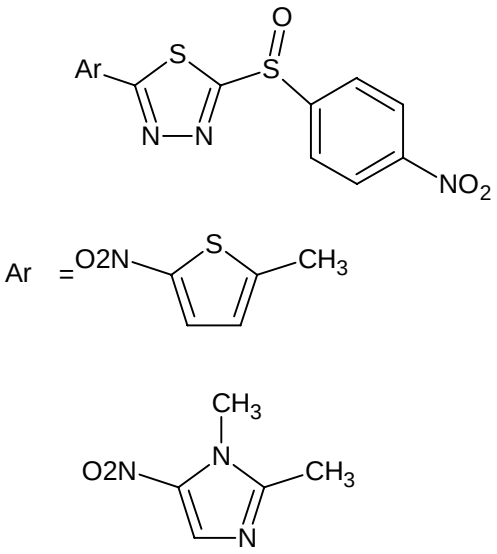




5-Ssubstituted-2-mercapto-1, 3, 4-thiadiazole

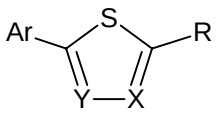
[28]

Foroumadi synthesized 1, 3, 4-thiadiazole from the nitro substituted thiazole or nitro substituted diazole [29] and evaluated as potent antitubercular activities. A series of 2-(nitroaryl)-5-(nitrobenzylsulfinyl and sulfonyl)-1, 3, 4-thiadiazole were synthesized and their antituberculosis activity were determined. The biological assay showed that all three active compounds belong to nitroimidazoles and sulfonyl compound was the most active analogue. [44]



[29]

Z. Kaleta, B. T Makowski synthesized 1, 3, 4-thiadiazole derivatives [30] by the Lawesson's Reagent. They are evaluated as potent antimicrobial agents against *Staphylococcus aureus* and *Candida albicans* at MIC value of 2mg/ml.[45]



[30]

CHAPTER -2.3

LITERATURE REVIEW OF 1, 2, 4-TRIAZOLE-5-THIONE DERIVATIVES

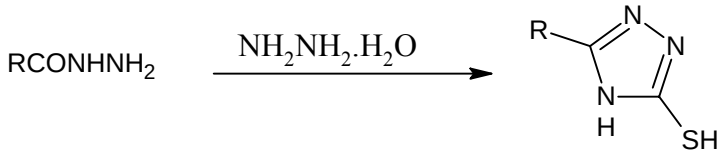
Triazole refers to either one of a pair of isomeric chemical compounds with molecular formula $C_2H_3N_3$, having a five-membered ring of two carbon atoms and three nitrogen atoms. [46] It has two isomers which are 1, 2, 3-triazole and 1, 2, 4-triazole. The triazole antifungal drugs include fluconazole, isavuconazole, itraconazole, voriconazole, pramiconazole, and posaconazole.[46] The triazole plant protection fungicides include epoxiconazole, triadimenol, propiconazole, metconazole, cyproconazole, tebuconazole, flusilazole and paclobutrazol.[46]

A large number of compounds containing 1,2,4-triazole system have been investigated as therapeutically interesting drug candidates because of their properties both as selective COX-2 inhibitors, anti-acetylcholinesterase, antimicrobial and antimycotic agents.[47] The efficacy of anastrozole and letrozole as aromatase inhibitors and their use as non-steroidal drugs for the treatment of estrogen-dependent cancer as well as the anticancer properties of ribavirine led to the investigation of many 1,2,4-triazole derivatives in laboratorial conditions for their anti-tumor activity.[47]

During the past decades, the human population affected with life-treating infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria increased an alarming level around the world.[48] Due to this reason, new classes of antibacterial agents with novel mechanisms are crucial need to combat with the multidrug-resistant infections.[48] In the past years, some azole derivatives were developed as new antimicrobial agents, for instance, Linezolid[48] and Eperezolid[48] are currently used for the treatment of multidrug-resistant Gram-positive infections. There are a number of antimicrobial compounds containing a 1, 2, 4-triazole ring in their structures such as Fluconazole, Itraconazole, Voriconazole, Ravuconazole and Posaconazole that are important antifungal drugs.[49]

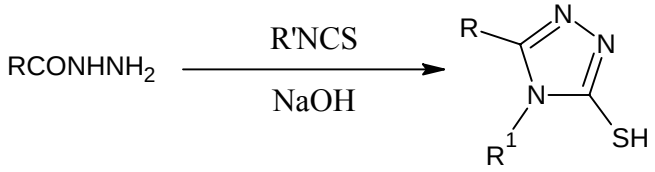
There are large number of inventions are carried by scientists for 1, 2, 4-triazole-5-thione with different types of substitutions, which improves the safety and therapeutic efficacy of the drug.[49] Some triazolothiadiazoles or triazolothiadiazines were obtained in our laboratories starting from 4-amino-5-mercapto-1, 2, 4-triazoles. [49]

R Pignatello, S Mazzone synthesized 4H-1, 2, 4-triazoles derivatives [31] from potassium dithiocarbazate and evaluated for antimicrobial and anti-HIV activities. The reaction of appropriate hydrazide with carbon disulphide in the presence of alkaline condition yields potassium dithiocarbazate. It undergoes cyclization in the presence of hydrazine hydrate gives corresponding 4H-1, 2, 4-triazoles.[50]



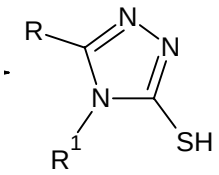
[31]

R. Subbaraman, H. Ghassemi synthesized 1, 2, 4- triazole derivatives [32] from From thiosemicarbazide and evaluated as potent antibacterial and antifungal agents. Reaction of suitable hydrazide with substituted alkyl/aryl isothiocynates results in formation of corresponding thiosemicarbazides, which upon cyclization under alkaline condition leads to formation of 1, 2, 4- trazoles corresponding to thiosemicarbazide.[51]



[32]

Xiao-Min Chen, Zhan-Jiang Li synthesized 1, 2, 4-triazole-5-thione [33] were by reaction of acid halides with a lead (II) thiocyanate and hydrazine hydrate (15%) in a solvent at -70 to + 200oC. They are evaluated as potent antimicrobial agent at a minimum inhibitory concentration (MIC) value of 5 mg/ ml. [52]



[33]

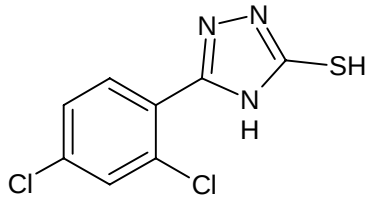
R and R^1 = (un)substituted alkyl,aryl, heteroaryl, aralkyl

Duen-Ren Hou, Safiul Alam, The 1, 2, 4-triazole-5-thiones [34] from the thermolysis of thiosemicarbazones. They are evaluated as potent antimicrobial agent against *Staphylococcus aureus* and *Candiada albicans.* Potent activity is observed at a minimum inhibitory concentration (MIC) value of 2 mg/ ml.[53]

R = CH_3, C_2H_5, C_6H_5, C6H5CH2, C_6H_5, 4-$CH_3C_6H_4$, 4-$CH_3OC_6H_4$, 4-ClC_6H_4

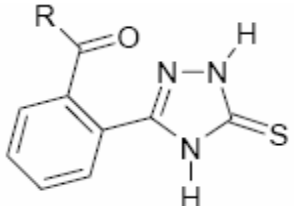
[34]

Zahra Rezaei, Soghra Khabnadideh, synthesized 1, 2, 4-triazole-5-thione [35] and reported that the oxidative cyclization of 1-(2, 4-dichloro-benzoyl)-thiosemicarbazide gives the 3-(2,4-dichlorophenyl)-1H-1,2,4-triazole-5-thione. They are evaluated as potent antimicrobial activities against *B. Cereus, E. coli and P. Salanarium* at a minimum inhibitory concentration (MIC) value of 7.5 mg/ ml.[54, 55]



[35]

Anelia Ts. Mavrova, Diana Wesselinova synthesized 1, 2, 4-triazole-5-thiones [36] by cyclization of the corresponding thiosemicarbazide which shown potent anti-inflammatory activity. It was shown that aliphatic substitution shows potent anti inflammatory activity.[56]



R = $H_3CCOOCH_2$, CH_3

[36]

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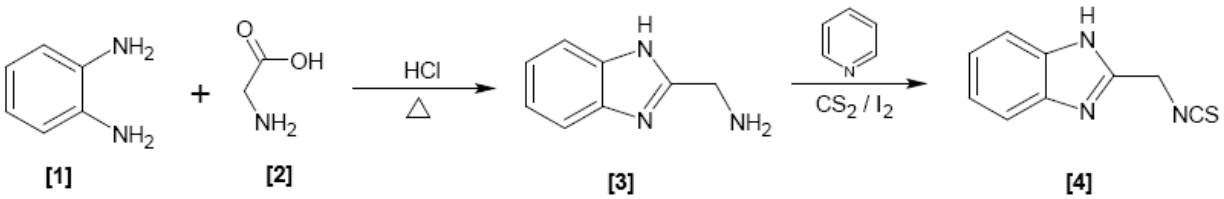
CHAPTER – 3

EXPERIMENTAL WORK – I

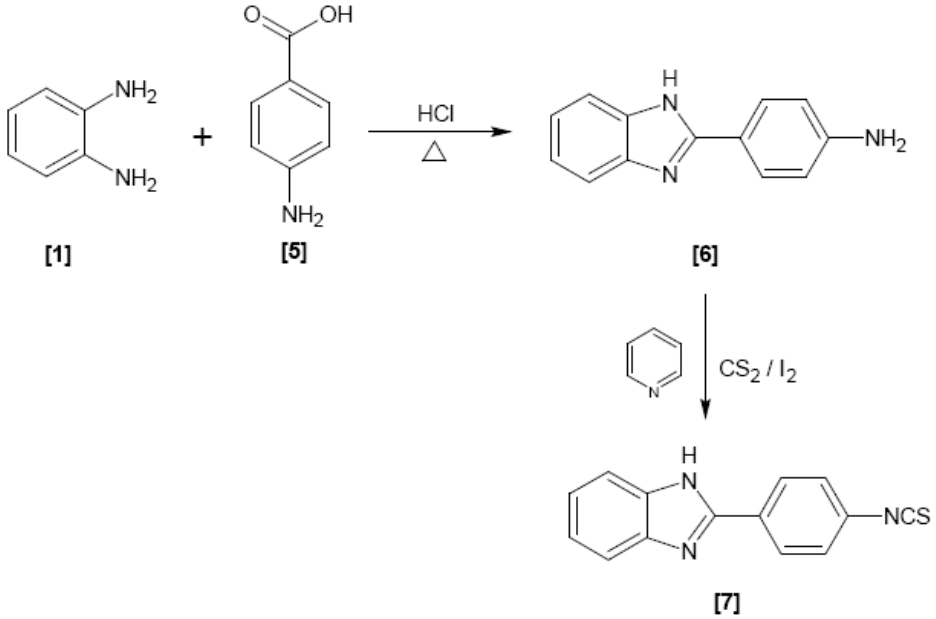
SYNTHESIS AND CHARACTERISATION OF 1, 3, 4-THIADIAZOLE, TRIAZOLE – 5-THIONE AND 1, 3-THIAZOLAN-4-ONE DERIVATIVES OF BENZIMIDAZOLE

Scheme - Ia



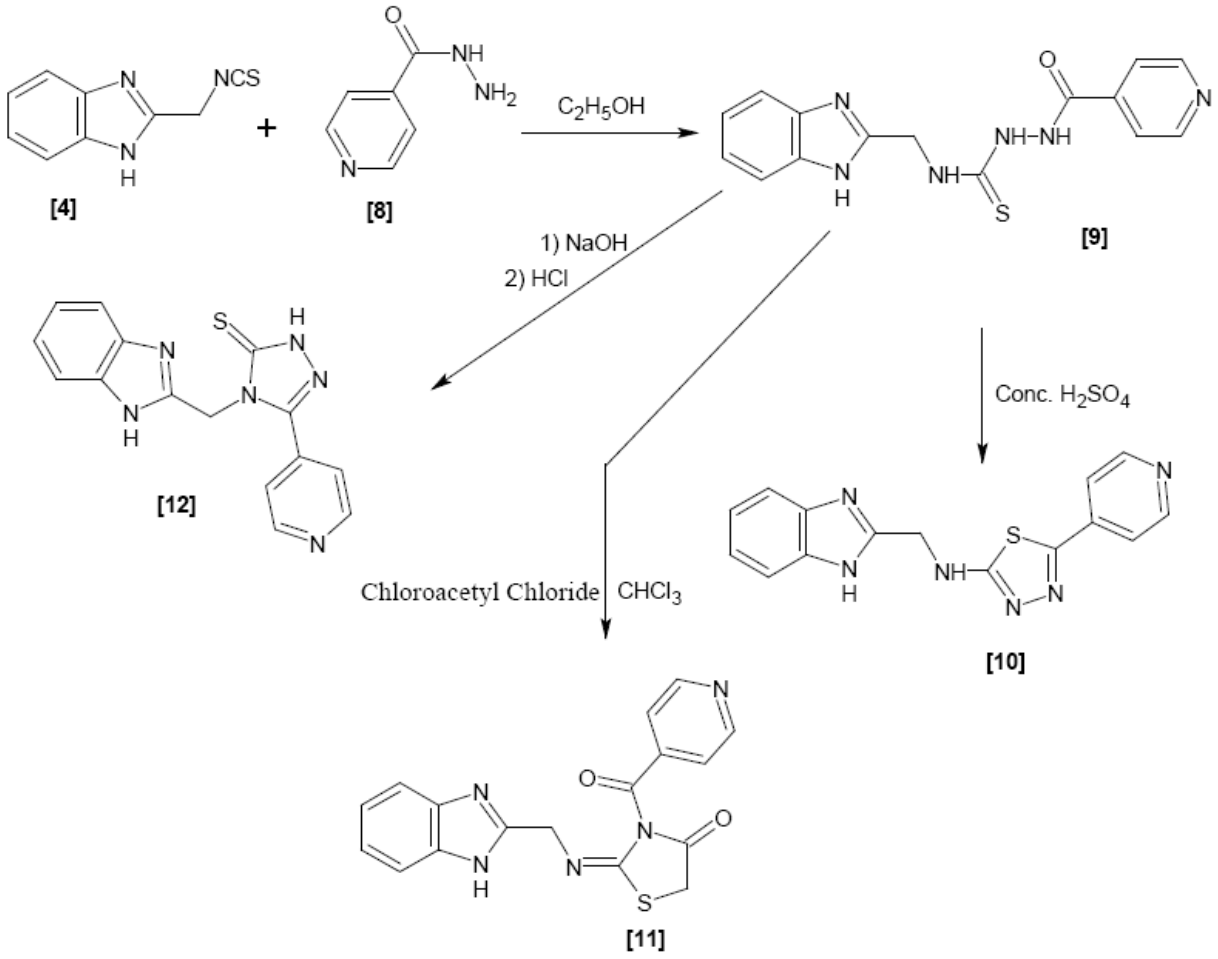
[1] [2] [3] [4]

Scheme - Ib

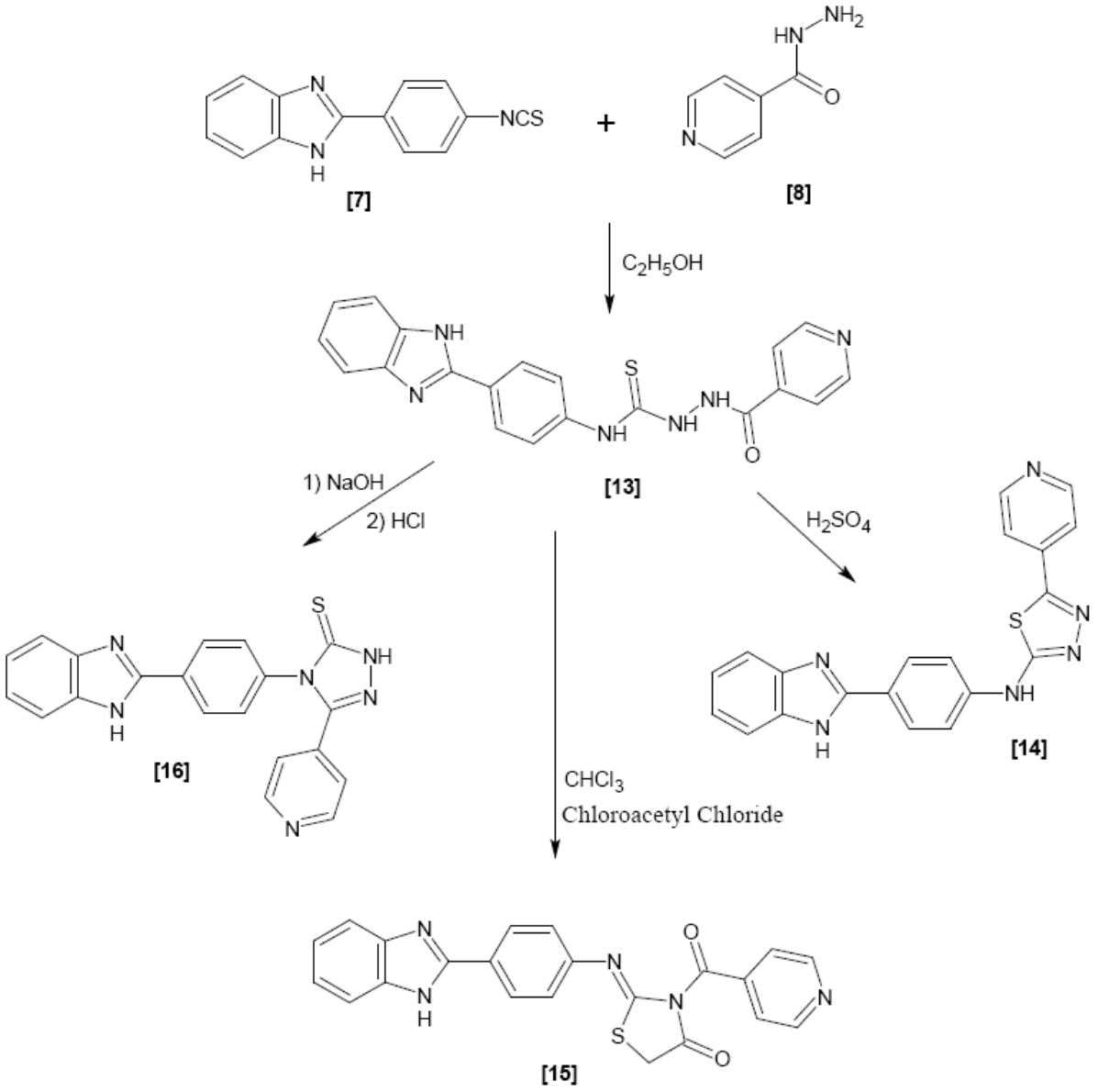


[1] [5] [6] [7]

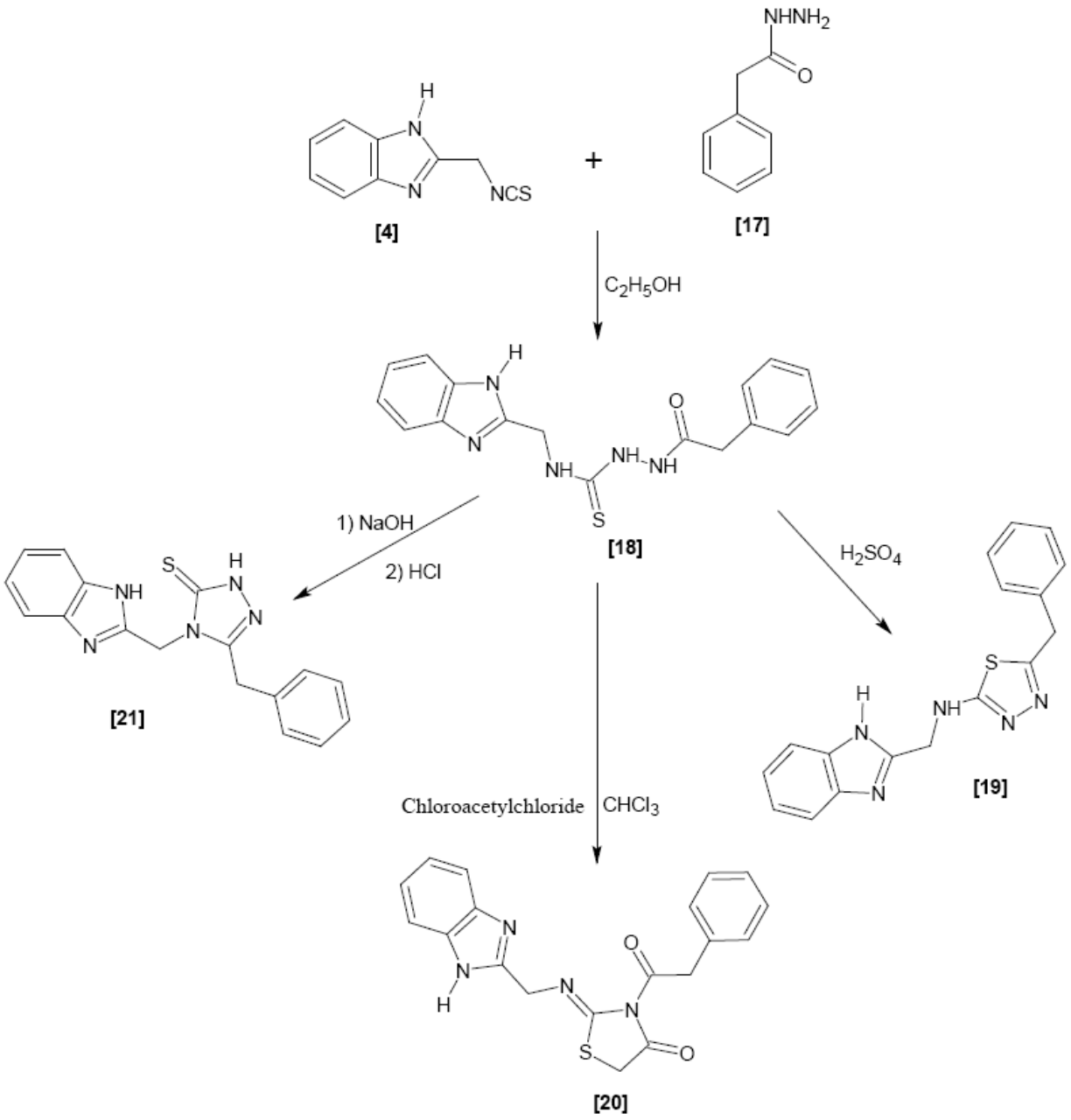
Scheme - II


NCS
[4]
[8]
C_2H_5OH
[9]
1) NaOH
2) HCl
[12]
Conc. H_2SO_4
[10]
Chloroacetyl Chloride
$CHCl_3$
[11]

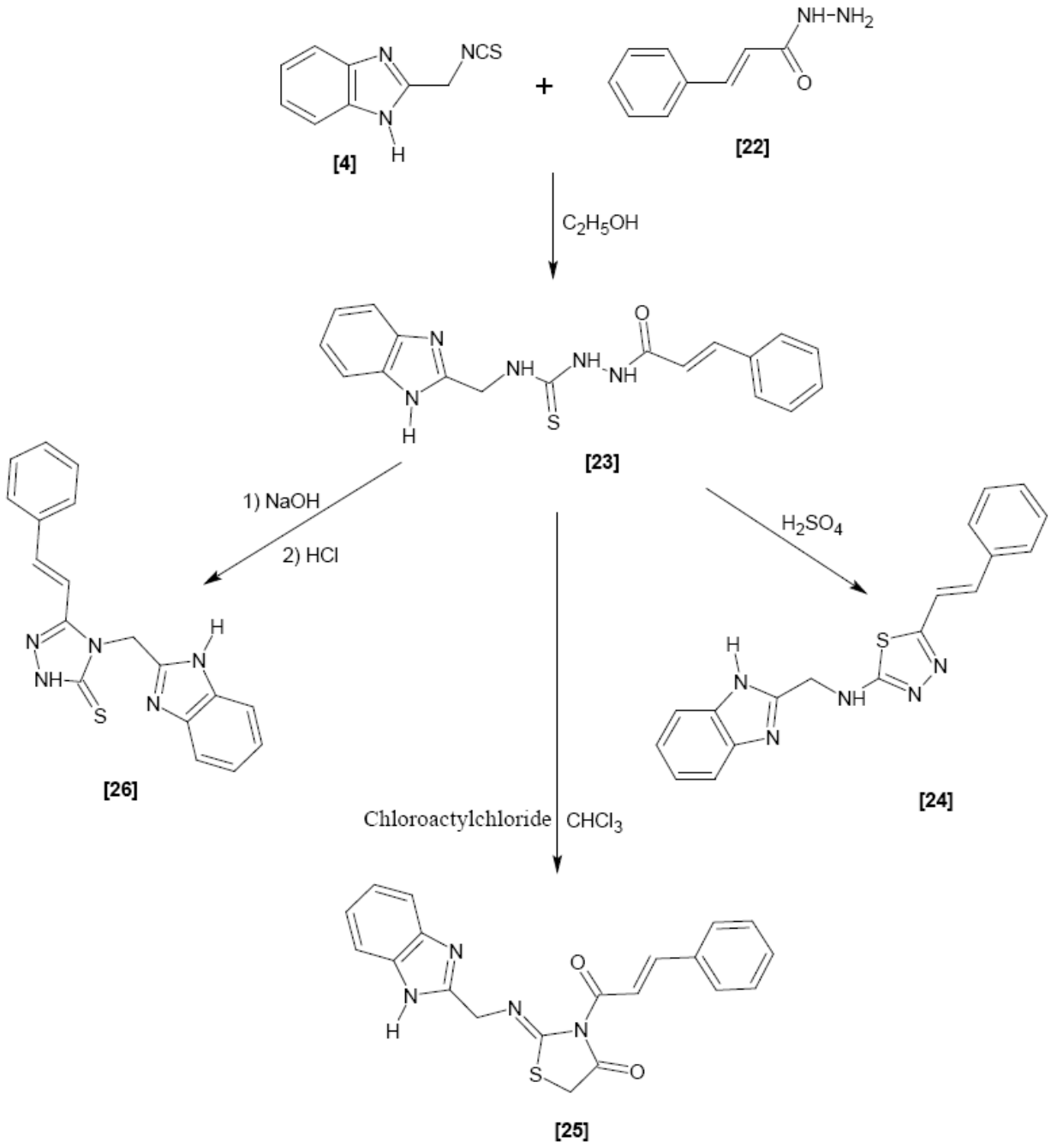
Scheme - III


NCS
[7]
[8]
C_2H_5OH
[13]
1) NaOH
2) HCl
H_2SO_4
[16]
[14]
$CHCl_3$
Chloroacetyl Chloride
[15]

Scheme - IV


NHNH2
O
H
N
N
NCS
+
[4]
[17]
C2H5OH
O
NH
NH
NH
S
[18]
1) NaOH
2) HCl
H2SO4
S
H
N
N
NH
N
[21]
S
N
N
NH
N
[19]
Chloroacetylchloride
CHCl3
O
N
N
H
N
O
S
[20]

Scheme - V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O
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2) HCl
H2SO4
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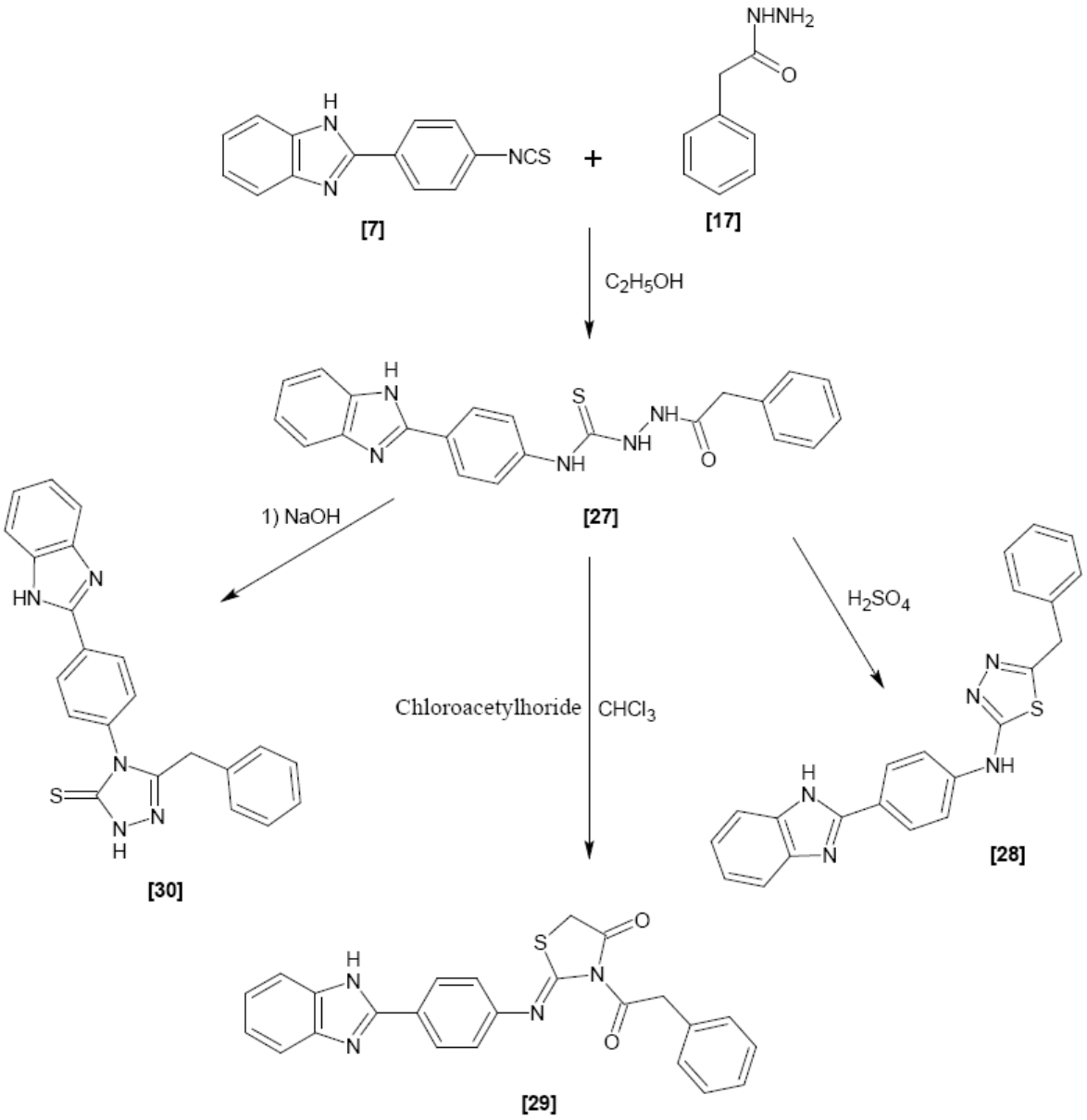
Chloroactylchloride
CHCl3
[25]

Scheme - VI


NHNH2
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H
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N
NCS
+
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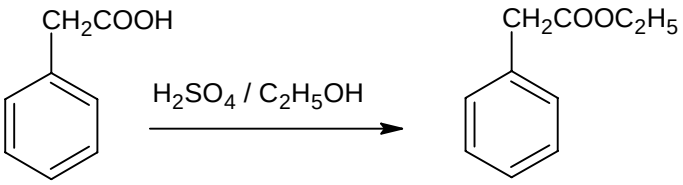
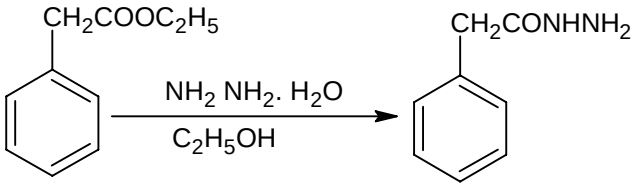
[17]
C2H5OH
S
NH
NH
O
[27]
1) NaOH
HN
N
S
N
N
H
[30]
Chloroacetylhoride
CHCl3
H2SO4
N
N
S
NH
H
N
N
[28]
S
O
N
O
[29]

Scheme – VII



[31] **[32]**



[32] **[33]**

3. Procedure for the synthesis of 1, 3, 4-thiadiazole, 1, 2, 4-triazole-5-thione and 1, 3-thiazolan-4-one derivatives of benzimidazole:

3.1 Synthesis of 2-aminomethyl-1*[H]* benzimidazole (3)

Required amount of o-phenylenediamine **(1)** (5.0gm, 0.046mol) was dissolved in 20 ml water with slight heating with continuous stirring. It was mixed with 20 ml HCl. Then, it was mixed with required amount of glycine **(2)** (3.45gm, 0.046mol). It was refluxed for 45 minute. After reflux, the resulting reflux mixture was cooled by water. Concentrated ammonia solution was added (Basify) drop wise for the precipitation of the product. It was added upto the neutralization point. Precipitates was comes out at the neutralization point. It was filtered by vacuum filtration. Crude product was recrystalized by ehanol. Percentage yield (81.5%), Meting point (175-177^{0}C) and R_f value (0.75) was found. (Mobile Phase: Benzene: ethyl acetate – 4:1)

3.2 Synthesis of 2-aminophenyl-1*[H]* benzimidazole (6)

Required amount of o-phenylene diamine **(1)** (5.0gm, 0.046mol) was dissolved in 20 ml water with slight heating with continuous stirring. It was mixed with 20 ml HCl. Then, it was mixed with required amount of p- aminobenzoicacid (PABA) **(5)** (6.3gm, 0.046mol). The resulting content was stirred for 30 minute. It was refluxed for 45 minute. After reflux, the resulting reflux mixture was cooled by water. Concentrated ammonia solution was added (Basify) drop wise for the precipitation of the product. It was added upto the neutralization point. Precipitates was comes out at the neutralization point. It was filtered by vacuum filtration. Crude product was recrystalized by ethanol. Percentage yield (77.0%), Meting point (170-175^{0}C) and R_f value (0.71) was found. (Mobile Phase: Benzene: ethyl acetate – 4:1)

3.3 Synthesis of 2-methylsulphonitrile-1*[H]*benzimidazole (4)

Solution of required amount of iodine (8.62gm, 0.034mol) in carbon disulfide (2.58ml, 0.034mol) was added drop wise to a suspension of 2-methyl amino-1*[H]* benzimidazole **(3)** (5.0gm, 0.034mol) in pyridine (2.69ml, 0.034mol) at 0^{o}C. The resulting content was stirred at 0^{o}C for 4 hours. The reaction mixture was distilled until a trace of Carbon

disulphide and Pyridine are removed. Residue was treated with excess amount of dilute HCl and solid product was separated out by vacuum filtration. Crude product was recrystalized by ethanol. Percentage yield (68.7%), Meting point (182-185^{0}C) and R_f value (0.78) was found. (Mobile Phase: Hexane: ethyl acetate – 3:1)

3.4 Synthesis of 2-phenylsulphonitrile-1*[H]*benzimidazole (7)

Solution of required amount of iodine (5.83gm, 0.023mol) in carbon disulfide (1.75ml, 0.023mol) was added drop wise to a suspension of 2-phenylamino-1*[H]* benzimidazole **(6)** (5.0gm, 0.023mol) in pyridine (1.8ml, 0.023mol) at 0^{o}C. The resulting content was stirred at 0^{o}C for 4 hours. The reaction mixture was distilled until a trace of Carbon disulphide and Pyridine are removed. Residue was treated with excess amount of dilute HCl and solid product was separated out by vacuum filtration. Crude product was recrystalized by ethanol. Percentage yield (67.1%), Meting point (170-173^{0}C) and R_f value (0.71) was found. (Mobile Phase: Hexane: ethyl acetate – 3:1)

3.5 Synthesis of Phenylaceticacid ethyl ester (32)

Required quantity of Phenylaceticacid **(31)** (13.6gm, 0.1mol) was dissolved in ethanol (45ml) with stirring followed by addition of H_2SO_4 (4ml, 0.04mol) in 250 ml round bottom flask. Then, reaction mixture was refluxed for 10 hr on a water bath. The reaction was monitored by TLC using silica gel G plate and appropriate solvent system. Excess of alcohol was distilled off under reduced pressure and reaction mixture was poured into cold water. Excess of acid was neutralized by drop wise addition of $NaHCO_3$ solution. It was extracted with diethyl ether (2*50ml). The ether layer was collected using separating funnel. The ether layer was distilled off under reduced pressure to obtain Phenylaceticacid ethyl ester. Purity was checked using TLC. Percentage yield (73.5%), Meting point (200-202 ^{O}C) and R_f value (0.76) was found. (Mobile Phase – Chloroform: Methanol – 9:1)

3.6 Synthesis of Phenylaceticacid hydrazide (33)

Phenylaceticacid ethyl ester **(32)** (15ml, 0.091) and hydrazine hydrate (4.5ml, 0.0898mol) was added in ethanol (45ml) with stirring in 100ml round bottom flask. Then, reaction

mixture was refluxed for 8 hr on a water bath. The reaction was monitored by precoated TLC. Excess of alcohol was distilled off under reduced presure. The resulting content was added in cold water. After cooling, solid was filtered under vaccum filtration. It was dried and re crystallized using ethanol to obtain pure phenylaceticacid hydrazide. The purity was checked using pre coated TLC. Percentage yield (83.5%), Meting point (108-110^{O}C) and R_f value (0.64) was found. (Mobile Phase – Chloroform: Methanol – 9: 1).

3.7 Synthesis of N-((1H-benzo[d]imidazol-2-yl) methyl)-2-(2-phenylacetyl) hydrazine carbothioamide (18) (Scheme – IV)

Required amount of 2-methyl sulphonitrile-1*[H]*benzimidazole **(4)** (3gm, 0.0158mol) was dissolved in water and required amount of phenylaceticacid hydrazide **(17)** (2.25gm, 0.0158) was added into it. It was stirred for 1 hr. The resulting reaction mixture was refluxed with 40 ml ethanol (40ml) for 3 hr. Then, the reaction mixture was cooled at 0^0C for 24 hr. Precipitates are collected by filtration. It was dried and re crystallized using ethanol. The purity of the final product was checked by pre coated TLC. Percentage yield (61.2%), Meting point (127^{O}-129^{O} C) and R_f value (0.60) was found. (Mobile Phase – Tolune: Ethylacetate –2:1)

3.8 Synthesis of N-((1H-benzo[d]imidazol-2-yl) methyl)-5-benzyl-1, 3, 4-thiadiazol-2-amine (19) (Scheme – IV)

Required amount of N-((1H-benzo[d]imidazol-2-yl) methyl)-2-(2-phenylacetyl) hydrazine carbothioamide **(18)** (2.5gm, 0.0073mol) was taken into a beaker. 50% H_2SO_4 (0.7ml, 0.0073mol) was added drop wise to above acid carbazide. Then, resulting mixture was stirred at room temperature for 3 hr. It was poured into the ice cold water. Solid precipitation was observed and collected by the vacuum filtration. It was washed with Na_2CO_3 followed by cold water. It was recrystallized by ethanol. Percentage yield (48.6%), Meting point (145-148^{0}C) and R_f value (0.65) was found. (Mobile Phase – Hexane: Ethyl acetate –3:1)

3.9 Synthesis of 4-((1H-benzo[d]imidazol-2-yl) methyl)-3-benzyl-1H-1, 2, 4-triazole-5(4H)-thione (21) (Scheme – IV)

Required amount of N-((1H-benzo[d]imidazol-2-yl) methyl)-2-(2-phenylacetyl) hydrazine carbothioamide **(18)** (2.5gm, 0.0073mol) was dissolved in 2^{M}NaOH (0.3ml) solution. It was heated under reflux for 6 hr. after cooling; the solution was acidified with hydrochloric acid (0.3ml). Precipitated crude product was filtered by vacuum filtration. It was washed with cooled water. The solid thus separated was dried and re crystallized in chloroform and petroleum ether. %yield, melting point and R_f value of the re crystallized compound was found. Percentage yield (45.1%), Meting point (151-153^{0}C) and R_f value (0.69) was found. (Mobile Phase –Toluene: Ethyl acetate –1:1)

3.10 Synthesis of 2-((1H-benzo[d]imidazol-2-yl) methyl imino)-3-(2-phenylacetyl) thiazolidin-4-one (20) (Scheme – IV)

Required amount of N-((1H-benzo[d]imidazol-2-yl) methyl)-2-(2-phenylacetyl) hydrazine carbothioamide **(18)** (2.5gm, 0.0073mol) was dissolved in chloroacetylchloride (0.8ml, 0.073mol). Then, resulting mixture was refluxed in chloroform (0.8ml, 0.0073mol) for 6 hr. Excess solvent was removed by distillation under reduced pressure. The solid product obtained was filtered by vacuum filtration. It was washed cooled water and followed by ethanol. The solid crude product was re crystallized in mixture of Dimethyl formamide (DMF) and water to obtain thiazolidinone. Percentage yield (41.3%), Meting point (161-163^{0}C) and R_f value (0.71) was found. (Mobile Phase – Benzene: Ethyl acetate –4:1)

3.11 Synthesis of N-(4-(1*H*-beno[d]imidazol-2-yl) phenyl)-2-(2-phenylacetyl) hydrazinecarbothiomide (27) (Scheme – VI)

Required amount of 2-phenyl sulphonitrile-1*[H]*benzimidazole **(7)** (3.0gm, 0.0119mol) was dissolved in water. Required amount of Phenylaceticacid hydrazide **(17)** (1.7gm 0.011mol) was added into it. It was stirred for 1 hr. The resulting reaction mixture was refluxed with 40 ml ethanol for 3 hr. The reaction was monitored using pre coated TLC. Then, the reaction mixture was cooled at 0^{0}C for 24 hr. Precipitates are collected by filtration. It was dried and re crystallized using ethanol. The purity of the final product

was checked by pre coated TLC. Percentage yield (65.9%), Meting point (131-133^{O} C) and R_f value (0.75) was found. (Mobile Phase – Hexane: Ethylacetate –3:1)

3.12 Synthesis of N-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-5-benzyl-1, 3, 4-thiadiazol-2-amine (28) (Scheme – VI)

Procedure:

Required amount of N-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-2-(2-phenylacetyl) hydrazine carbothioamide **(27)** (2.5gm, 0.0062mol) was taken into a beaker. 50% H_2SO_4 (0.6ml, 0.0062mol) was added drop wise to above acid carbazide. Then, resulting mixture was stirred at room temperature for 3 hr. It was poured into the ice cold water. Solid precipitation was observed and collected by the vacuum filtration. It was washed with Na_2CO_3 followed by cold water. It was recrystallized by ethanol. Percentage yield (46.7%), Meting point (147-150^{0}C) and R_f value (0.63) was found. (Mobile Phase – Hexane: Ethyl acetate –3:1)

3.13 Synthesis of 4-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-3-benzyl-1H-1, 2, 4-triazole-5(4H)-thione (30) (Scheme – VI)

Required amount of N-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-2-(2-phenylacetyl) hydrazine carbothioamide **(27)** (2.5gm, 0.0062mol) was dissolved in 2^{M}NaOH (0.2ml, 0.0062mol) solution. It was heated under reflux for 6 hr. after cooling; the solution was acidified with hydrochloric acid (0.2ml, 0.0062mol). Precipitated crude product was filtered by vacuum filtration. It was washed with cooled water. The solid thus separated was dried and re crystallized in chloroform and petroleum ether. %yield, melting point and R_f value of the re crystallized compound was found. Percentage yield (43.4%), Meting point (154-156^{0}C) and R_f value (0.67) was found. (Mobile Phase –Toluene: Ethyl acetate –1:1)

3.14 Synthesis of 2-(4-(1H-benzo[d]imidazol-2-yl)phenylimino)-3-(2-phenylacetyl)thiazolidin-4-one (29) (Scheme – VI)

Required amount of N-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-2-(2-phenylacetyl) hydrazine carbothioamide **(27)** (2.5gm, 0.0062mol) was dissolved in chloroacetylchloride

(0.8ml, 0.073mol). Then, resulting mixture was refluxed in chloroform (0.8ml, 0.0073mol) for 6 hr. Excess solvent was removed by distillation under reduced pressure. The solid product obtained was filtered by vacuum filtration. It was washed cooled water and followed by ethanol. The solid crude product was re crystallized in mixture of Dimethyl formamide (DMF) and water to obtain thiazolidinone. Percentage yield (46.1%), Meting point (165-168^{0}C) and R_f value (0.75) was found. (Mobile Phase – Benzene: Ethyl acetate –4:1)

3.15 Synthesis of N-((1H-benzo[d]imidazol-2-yl) methyl)-2-isonicotinoylhydrazinecarbothioamide (13) (Scheme – III)

Required amount of 2-phenylsulphonitrile-1*[H]*benzimidazole **(7)** (3gm, 0.0158mol) was dissolved in water and required amount of isoniazide **(8)** (2.16gm, 0.0158mol)) was added into it. It was stirred for 1 hr. The resulting reaction mixture was refluxed with 40 ml ethanol for 3 hr. Then, the reaction mixture was cooled at 0^{0}C for 24 hr. Precipitates are collected by filtration. It was dried and re crystallized using ethanol. The purity of the final product was checked by pre coated TLC. Percentage yield (64.2%), Meting point (138^O-140^O C) and R_f value (0.69) was found. (Mobile Phase – Tolune: Ethylacetate – 2:1)

3.16 Synthesis of N-((1H-benzo[d]imidazol-2-yl) methyl)-5-(pyridin-4-yl)-1, 3, 4-thiadiazol-2-amine (14) (Scheme – III)

Required amount of N-((1H-benzo[d]imidazol-2-yl) methyl)-2-isonicotinoylhydrazinecarbothioamide **(13)** (2.5gm, 0.0076mol) was taken into a beaker. 50% H_2SO_4 (0.6ml, 0.0062mol) was added drop wise to above acid carbazide. Then, resulting mixture was stirred at room temperature for 3 hr. It was poured into the ice cold water. Solid precipitation was observed and collected by the vacuum filtration. It was washed with Na_2CO_3 followed by cold water. It was recrystallized by ethanol. Percentage yield (43.5%), Meting point (151-153^{0}C) and R_f value (0.70) was found. (Mobile Phase – Hexane: Ethyl acetate –3:1)

3.17 Synthesis of 4-((1H-benzo[d]imidazol-2-yl) methyl)-3-(pyridin-4-yl)-1H-1, 2, 4-triazole-5(4H)-thione (16) (Scheme – III)

Required amount of N-((1H-benzo[d]imidazol-2-yl) methyl)-2-isonicotinoylhydrazinecarbothioamide **(13)** (2.5gm, 0.0076mol) was dissolved in 2^{M}NaOH (0.2ml, 0.0062mol) solution. It was heated under reflux for 6 hr. after cooling; the solution was acidified with hydrochloric acid (0.2ml, 0.0062mol). Precipitated crude product was filtered by vacuum filtration. It was washed with cooled water. The solid thus separated was dried and re crystallized in chloroform and petroleum ether. %yield, melting point and R_f value of the re crystallized compound was found. Percentage yield (48.2%), Meting point (160-162^{0}C) and R_f value (0.73) was found. (Mobile Phase – Toluene: Ethyl acetate –1:1)

3.18 Synthesis of 2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-isonicotinoylthiazolidin-4-one (15) (Scheme – III)

Required amount of N-((1H-benzo[d]imidazol-2-yl) methyl)-2-isonicotinoylhydrazinecarbothioamide **(13)** (2.5gm, 0.0076mol) was dissolved in chloroacetylchloride (0.85ml, 0.0076mol). Then, resulting mixture was refluxed in chloroform (0.9ml, 0.0076mol) for 6 hr. Excess solvent was removed by distillation under reduced pressure. The solid product obtained was filtered by vacuum filtration. It was washed cooled water and followed by ethanol. The solid crude product was re crystallized in mixture of Dimethyl formamide (DMF) and water to obtain thiazolidinone. Percentage yield (51.3%), Meting point (171-173^{0}C) and R_f value (0.78) was found. (Mobile Phase –Benzene: Ethyl acetate –4:1)

3.19 Synthesis of N-(4-(1H-benzo[d]imidazol-2-yl) methyl)-2-isonicotinoyl hydrazine carbothioamide (9) (Scheme – II)

Required amount of 2-methylsulphonitrile-1*[H]*benzimidazole **(4)** (3gm, 0.0119mol) was dissolved in water and required amount of isoniazide **(8)** (1.6gm, 0.0119mol)) was added into it. It was stirred for 1 hr. The resulting reaction mixture was refluxed with 40 ml ethanol for 3 hr. Then, the reaction mixture was cooled at 0^{0}C for 24 hr. Precipitates are collected by filtration. It was dried and re crystallized using ethanol. The purity of the

final product was checked by pre coated TLC. Percentage yield (61.8%), Meting point (141^{O}-143^{O} C) and R_f value (0.78) was found. (Mobile Phase – Tolune: Ethylacetate – 2:1)

3.20 Synthesis of N-(4-(1H-benzo[d]imidazol-2-yl) methyl)-5-(pyridin-4-yl)-1, 3, 4-thiadiazol-2-amine (10) (Scheme – II)

Required amount of N-(4-(1H-benzo[d]imidazol-2-yl) methyl)-2-isonicotinoyl hydrazine carbothioamide **(9)** (2.5gm, 0.0064mol) was taken into a beaker. 50% H_2SO_4 (0.6ml, 0.0064mol) was added drop wise to above acid carbazide. Then, resulting mixture was stirred at room temperature for 3 hr. It was poured into the ice cold water. Solid precipitation was observed and collected by the vacuum filtration. It was washed with Na_2CO_3 followed by cold water. It was recrystallized by ethanol. Percentage yield (51.4%), Meting point (160-162^{0}C) and R_f value (0.72) was found. (Mobile Phase – Hexane: Ethyl acetate –3:1)

3.21 Synthesis of 4-(4-(1H-benzo[d]imidazol-2-yl) methyl)-3-(pyridin-4-yl)-1H-1, 2, 4-triazole-5(4H)-thione (12) (Scheme – II)

Required amount of N-(4-(1H-benzo[d]imidazol-2-yl) methyl)-2-isonicotinoyl hydrazine carbothioamide **(9)** (2.5gm, 0.0064mol) was dissolved in 2^{M}NaOH (0.25ml, 0.0064mol) solution. It was heated under reflux for 6 hr. after cooling; the solution was acidified with hydrochloric acid (0.23ml, 0.0064mol). Precipitated crude product was filtered by vacuum filtration. It was washed with cooled water. The solid thus separated was dried and re crystallized in chloroform and petroleum ether. %yield, melting point and R_f value of the re crystallized compound was found. Percentage yield (53.6%), Meting point (168-171^{0}C) and R_f value (0.72) was found. (Mobile Phase –Toluene: Ethyl acetate –1:1)

3.22 Synthesis of (Z)-2-(4-(1H-benzo[d]imidazol-2-yl) methylimino)-3-isonicotinoyl thiazolidin-4-one (11) (Scheme – II)

Required amount of N-(4-(1H-benzo[d]imidazol-2-yl) methyl)-2-isonicotinoyl hydrazine carbothioamide **(9)** (2.5gm, 0.0064mol) was dissolved in chloroacetylchloride (0.72ml, 0.0064mol). Then, resulting mixture was refluxed in chloroform (0.76ml, 0.0064mol) for

6 hr. Excess solvent was removed by distillation under reduced pressure. The solid product obtained was filtered by vacuum filtration. It was washed cooled water and followed by ethanol. The solid crude product was re crystallized in mixture of Dimethyl formamide (DMF) and water to obtain thiazolidinone. Percentage yield (55.7%), Meting point (178-180^{0}C) and R_f value (0.76) was found. (Mobile Phase –Benzene: Ethyl acetate –4:1)

3.23 Synthesis of N-((1H-benzo[d]imidazol-2-yl) methyl)-3-cinnamoyltriazane-1-carbothioamide (23) (Scheme – V)

Required amount of 2-methylsulphonitrile-1*[H]*benzimidazole **(4)** (3gm, 0.0158mol) was dissolved in water and required amount of Cinnamicacid hydrazide **(22)** (2.5gm, 0.0158mol)) was added into it. It was stirred for 1 hr. The resulting reaction mixture was refluxed with 40 ml ethanol for 3 hr. Then, the reaction mixture was cooled at 0^{0}C for 24 hr. Precipitates are collected by filtration. It was dried and re crystallized using ethanol. The purity of the final product was checked by pre coated TLC. Percentage yield (65.3%), Meting point (153^O-155^O C) and R_f value (0.68) was found. (Mobile Phase – Tolune: Ethylacetate –2:1)

3.24 Synthesis of N-((1H-benzo[d]imidazol-2-yl) methyl)-5-styryl-1, 3, 4-thiadiazol-2-amine (24) (Scheme – V)

Required amount of N-((1H-benzo[d]imidazol-2-yl) methyl)-3-cinnamoyltriazane-1-carbothioamide **(23)** (2.5gm, 0.0071mol) was taken into a beaker. 50% H_2SO_4 (0.7ml, 0.0071mol) was added drop wise to above acid carbazide. Then, resulting mixture was stirred at room temperature for 3 hr. It was poured into the ice cold water. Solid precipitation was observed and collected by the vacuum filtration. It was washed with Na_2CO_3 followed by cold water. It was recrystallized by ethanol. Percentage yield (55.7%), Meting point (170-172^{0}C) and R_f value (0.78) was found. (Mobile Phase – Hexane: Ethyl acetate –3:1)

3.25 Synthesis of 4-((1H-benzo[d]imidazol-2-yl) methyl)-3-styryl-1H-1, 2, 4-triazole-5(4H)-thione (26) (Scheme – V)

Required amount of N-((1H-benzo[d]imidazol-2-yl) methyl)-3-cinnamoyltriazane-1-carbothioamide **(23)** (2.5gm, 0.0071mol) was dissolved in 2^{M}NaOH (0.28ml, 0.0071mol) solution. It was heated under reflux for 6 hr. after cooling; the solution was acidified with hydrochloric acid (0.25ml, 0.0071mol). Precipitated crude product was filtered by vacuum filtration. It was washed with cooled water. The solid thus separated was dried and re crystallized in chloroform and petroleum ether. %yield, melting point and R_f value of the re crystallized compound was found. Percentage yield (55.6%), Meting point (175-178^{0}C) and R_f value (0.76) was found. (Mobile Phase –Toluene: Ethyl acetate –1:1)

3.26 Synthesis of 2-((1H-benzo[d]imidazol-2-yl) methylimino)-3-(2-oxo-4-phenylbut-3-enyl) thiazolidin-4-one (25) (Scheme – V)

Required amount N-((1H-benzo[d]imidazol-2-yl) methyl)-3-cinnamoyltriazane-1-carbothioamide **(23)** (2.5gm, 0.0071mol) was dissolved in chloroacetylchloride (0.72ml, 0.0064mol). Then, resulting mixture was refluxed in chloroform (0.76ml, 0.0064mol) for 6 hr. Excess solvent was removed by distillation under reduced pressure. The solid product obtained was filtered by vacuum filtration. It was washed cooled water and followed by ethanol. The solid crude product was re crystallized in mixture of Dimethyl formamide (DMF) and water to obtain thiazolidinone. Percentage yield (60.3%), Meting point (1181-183^{0}C) and R_f value (0.78) was found. (Mobile Phase –Benzene: Ethyl acetate –4:1)

3.27 Result and Discussion

In order to obtain effective antibacterial and antifungal agents, 1, 3, 4-thiadiazole, triazole-5-thione and 1, 3-thiazolan-4-one derivatives of benzimidazole were successfully synthesized. A series of total 15 compounds were prepared having 1, 3, 4-thiadiazole, trazole-5-thione and 1, 3-thiazolan-4-one moiety which are shown as following.

3.27.1 Table-1 List of the synthesized compounds with IUPAC name

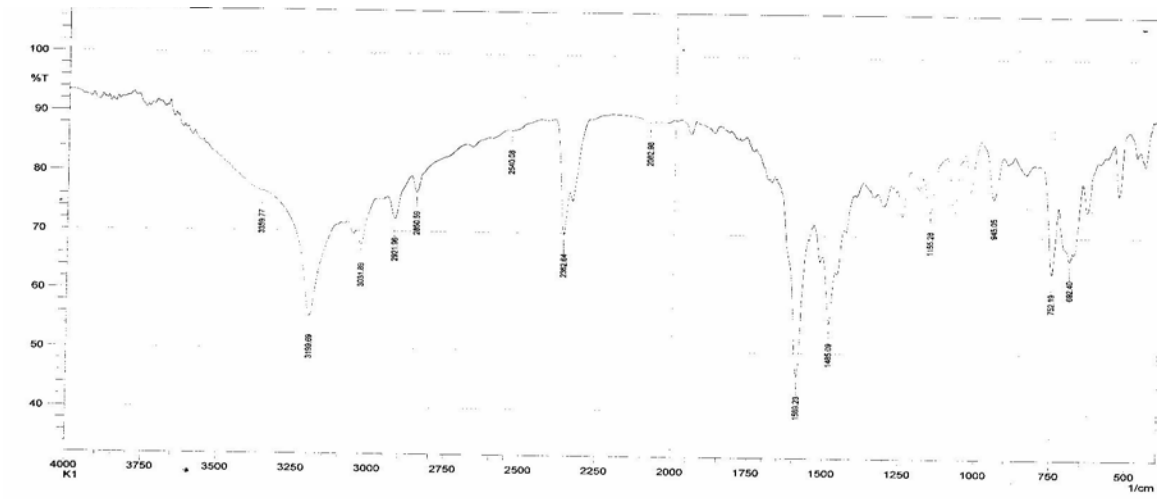
Compound Code	IUPAC Name	Molecular Formula
KPB-1	N-((1H-benzo[d]imidazol-2-yl)methyl)-5-benzyl-1,3,4-thiadiazol-2-amine	$C_{17}H_{15}N_5S$
KPB-2	4-((1H-benzo[d]imidazol-2-yl)methyl)-3-benzyl-1H-1,2,4-triazole-5(4H)-thione	$C_{17}H_{15}N_5S$
KPB-3	2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-(2-phenylacetyl)thiazolidin-4-one	$C_{19}H_{16}N_4O_2S$
KPB-4	N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-5-benzyl-1,3,4-thiadiazol-2-amine	$C_{22}H_{17}N_5S$
KPB-5	4-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-benzyl-1H-1,2,4-triazole-5(4H)-thione	$C_{22}H_{17}N_5S$
KPB-6	2-(4-(1H-benzo[d]imidazol-2-yl)phenylimino)-3-(2-phenylacetyl)thiazolidin-4-one	$C_{24}H_{18}N_4O_2S$
KPB-7	N-((1H-benzo[d]imidazol-2-yl)methyl)-5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine	$C_{15}H_{12}N_6S$
KPB-8	4-((1H-benzo[d]imidazol-2-yl)methyl)-3-(pyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione	$C_{15}H_{12}N_6S$
KPB-9	2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-isonicotinoylthiazolidin-4-one	$C_{17}H_{13}N_5O_2S$
KPB-10	N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine	$C_{20}H_{14}N_6S$

KPB-11	4-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-(pyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione	$C_{20}H_{14}N_6S$
KPB-12	(Z)-2-(4-(1H-benzo[d]imidazol-2-yl)phenylimino)-3-isonicotinoylthiazolidin-4-one	$C_{22}H_{15}N_5O_2S$
KPB-13	N-((1H-benzo[d]imidazol-2-yl)methyl)-5-styryl-1,3,4-thiadiazol-2-amine	$C_{18}H_{15}N_5S$
KPB-14	4-((1H-benzo[d]imidazol-2-yl)methyl)-3-styryl-1H-1,2,4-triazole-5(4H)-thione	$C_{18}H_{15}N_5S$
KPB-15	2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-(2-oxo-4-phenylbut-3-enyl)thiazolidin-4-one	$C_{21}H_{18}N_4O_2S$

3.27.2 Table-2 Summary of the physical data of target synthesized compounds

Compound Code	Molecular Formula	Percentage yield	Melting Point	R_f Value
KPB-1	$C_{17}H_{15}N_5S$	48.6%	145-148^{0}C	0.65
KPB-2	$C_{17}H_{15}N_5S$	45.1%	151-153^{0}C	069
KPB-3	$C_{19}H_{16}N_4O_2S$	41.3%	161-163^{0}C	0.71
KPB-4	$C_{22}H_{17}N_5S$	46.7%	147-150^{0}C	0.63
KPB-5	$C_{22}H_{17}N_5S$	43.4%	154-156^{0}C	0.67
KPB-6	$C_{24}H_{18}N_4O_2S$	46.1%	165-168^{0}C	0.68
KPB-7	$C_{15}H_{12}N_6S$	43.5%	151-153^{0}C	0.67
KPB-8	$C_{15}H_{12}N_6S$	48.2%	160-162^{0}C	0.76
KPB-9	$C_{17}H_{13}N_5O_2S$	51.3%	171-173^{0}C	0.78
KPB-10	$C_{20}H_{14}N_6S$	51.4%	160-162^{0}C	0.70
KPB-11	$C_{20}H_{14}N_6S$	53.9%	168-171^{0}C	0.72
KPB-12	$C_{22}H_{15}N_5O_2S$	55.7%	170-172^{0}C	0.73
KPB-13	$C_{18}H_{15}N_5S$	55.7%	170-172^{0}C	0.73
KPB-14	$C_{18}H_{15}N_5S$	55.1%	175-177^{0}C	0.73
KPB-15	$C_{21}H_{18}N_4O_2S$	51.6%	181-183^{0}C	0.71

3.28 I. R spectra of synthesized compound KPB – 1



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K1

No. of Scans;
Resolution;
Apodization;

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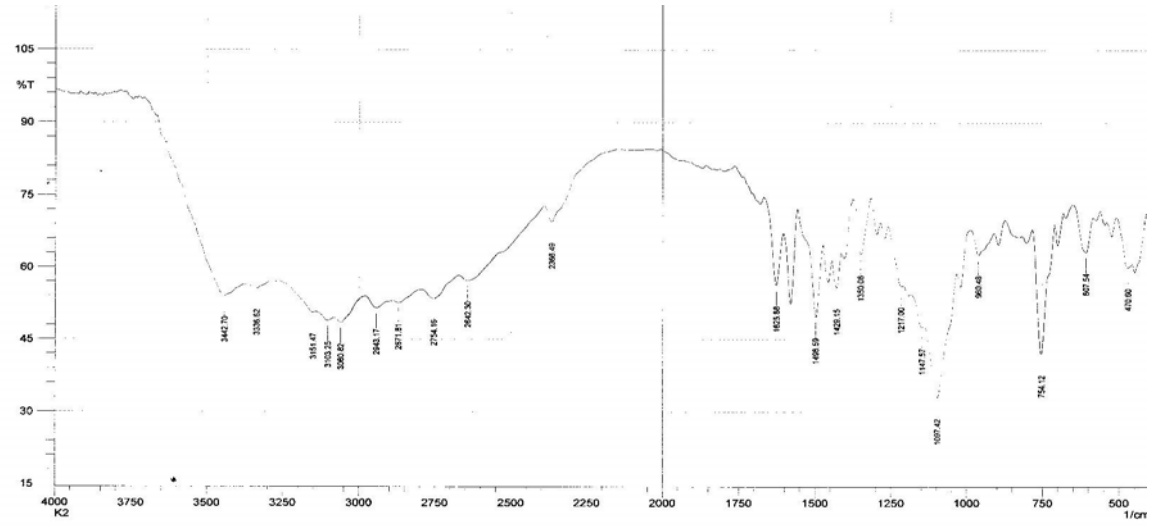
User; $(IRsolution|Vishranti) LMCP

3.29 I. R spectra of synthesized compound KPB – 2



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K2

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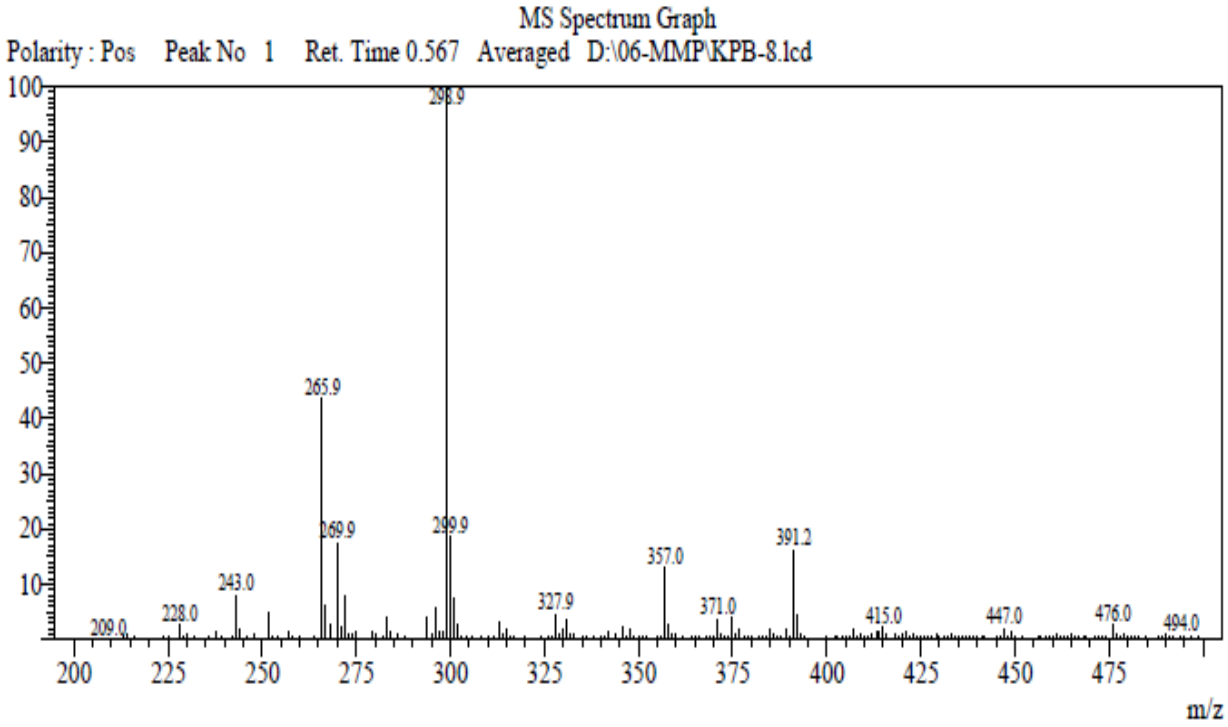
3.30 Mass spectra of synthesized compound KPB – 8



3.31 Mass spectra of synthesized compound KPB

3.32 ^{1}H NMR spectra of synthesized compound KPB

3.33 ^{1}H NMR spectra of synthesized compound KPB

3.34 I. R Spectral data of synthesized compounds

Compound Code	Spectral Data (KBR, cm^{-1})
KPB - 1	
KPB – 2	
KPB – 3	
KPB – 4	
KPB – 5	
KPB – 6	
KPB – 7	
KPB – 8	
KPB – 9	
KPB – 10	
KPB – 11	
KPB – 12	
KPB – 13	
KPB – 14	
KPB – 15	

3.35 Spectral characterization of synthesized compounds by ^{1} H NMR and Mass Spectroscopy

Compound Code (Mol. Wt)	^{1} H NMR (DMSO – D_6, ppm)	ESI – MS (m/z)
KPB - 1		
KPB - 2		

CHAPTER – 4

EXPERIMENTAL WORK – II

ANTIBACTERIAL AND ANTIFUNGAL EVALUATION OF 1, 3, 4-THIADIAZOLE, TRIAZOLE – 5-THIONE AND 1, 3-THIAZOLAN-4-ONE DERIVATIVES OF BENZIMIDAZOLE

4. ANTIBACTRIAL AGENTS

4.1 Introdution

Pharmacological evaluation is a one most factor for the determination of activity of compounds. Evaluation part of the work should be variable and easy to perform. Since last few years, prevalence of infectious diseases has increased to a great extent.[1] Antimicrobial agents are the most commonly used to treat the different types of infectious diseases.[1] Literature review revealed that 1, 3, 4-thiadiazole, triazole-5-thione and 1, 3-thiazolan-4-one derivatives of benzimidazole shows different types of biological activities. These compounds are also evaluated for their antimicrobial activities from last few years. There are various in vivo and in vitro methods are available for evaluation of antibacterial and antifungal activity. Synthesized compounds are were evaluated for their antibacterial and antifungal activity against three gram +ve organisms like *B. cereus, E. faecalis, S. aureus* and three gram -ve organisms like *E. coli, P. aeruginosa, K. pneumonia.* [1]

4.2 Methods of antimicrobial susceptibility testing

Several methods are available for the determination of bacterial sensitivity to the antibacterial agents. The most commonly used methods include.[2]

(A) Diffussion Test [2]

- Agar disc diffusion method
- Agar well diffusion method

(B) Dilution Test [3]

- Agar dilution method
- Broth dilution method

(C) Diffusion and Dilution method – E test method [4]

4.2.1 Diffusion Test [2]

Diffusion test for susceptibility testing is currently recommended by the FDA. It is a slight modification of the procedure developed by Baur, Kirly and Sherris and Turck in 1966 which is highly standard technique. The agar diffusion test or the Kirby-Bauer disk-diffusion method is a means of measuring the effect of an antimicrobial agent against

bacteria grown in culture.A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. Thus, the size of the zone of inhibition is a measure of the compound's effectivenes. The larger the clear area around the filter disk, the more effective the compound.

4.2.2 Dilution Test [3]

Dilution test for the antimicrobial activity can determines the succeptibility of the organisms. It is followed by the serial dilution method for the antimicrobial activity determination. Serial dilutions of tested compounds are followed by this test with dimethyl sulphoxide (DMSO) as a solvent.

4.2.3 Diffusion and Dilution method – E test method [4]

The Epsilometer test (Etest) is a laboratory test used to determine specific strain of bacterium or fungus is susceptible to the action of a specific antibiotic. This is most commonly used in the setting of medicine, where a particular organism has been found to infect a patient, and the doctor treating the patient is seeking guidance on what antibiotic is suitable. The principle of the epsilometer test was first described in 1988 and was introduced commercially in 1991 by AB Biodisk. The Etest is basically an agar diffusion method. The E test utilises a rectangular strip that has been impregnated with the drug to be studied. A lawn of bacteria is inoculated onto the surface an agar plate and the E test strip is laid on top; the drug diffuses out into the agar, producing an exponential gradient of the drug to be tested. There is an exponential scale printed on the strip. After 24 hours of incubation, an elliptical zone of inhibition is produced and the point at which the ellipse meets the strip gives a reading for the minimum inhibitory concentration (MIC) of the drug.

4.3 Experimental protocol

In the present study, in vitro antibactrial activity of synthesized compounds was assessed against a panel of three gram possitive and three gram negative bacterial species by the agar well diffusion method for the preliminary in vitro antibacterial activity using Soayabean Casein Digest Agar media as follows.[5]

Ingredients	**Quantity (gm/l)**
Casein enzymic hydrolysate	15.0
Peptic digest of soyabean meal	5.0
Sodium chloride	15.0
Agar	15.0

Bacterial strain used were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH) Chandhigargh. The strains used are following.

1. Gram possitive bacteria
    - *Bacillus cereus* (MTCC-430)
    - *Enterococcus faecalis* (MTCC-493)
    - *Staphylococcus aureus* (MTCC-737)
2. Gram negative bacteria
    - *Escherichia coli* (MTCC-1687)
    - *Pseudomonas aeruginosa* (MTCC-2642)
    - *Klebsiella pneumoniae* (MTCC-109)

4.4 Procedure

4.4.1 Preparation of Soyabean casein digest agar media

Agar was prepared by the directions of manufacturer which is 35 gm of nutrient agar was suspended in 1000 nl of distilled water and heated to boiling to dissolve powder agar.It was mixed with the dye Tetrazolium red (57 mg/L). The melted agar medium was filled in 20 ml test tubes. The media was then sterillized by autoclavingat 15 lb pressure at 121^0C for 15min. All media containing test tubes were preserved at -20^0C in the deep freezer and used as required.

4.4.2 Preparation of test and Standard compounds

All the synthesized compounds (KPB-1 to KPB-15) were soluble in dimethyl sulphoxide (DMSO). The solutions of required conentration 10 μ g/ml, 20 μ g/ml and 30 μ g/ml were prepared by dissolving in DMSO. All the test compounds were prepared from the stock solution of 10mg/10ml.

Ofloxacine and Metronidazole (Zydus Cadial Pvt. Ltd) were taken as reference standard drug. 10 μ g/ml of concentration was made by dissolving 2mg in 1ml. Other concentrations were also made by this way.

4.4.3 Preparation of bacterial culture

From the stock solution of bacterial culture, 0.1 ml was taken and dilluted to 10 ml with distilled water. So, 100times dilltion was done. From the dilluted cultures, 0.1 ml was taken into petri dish conataining solid agar and spread properly. After 24 hr of incubation at 37^0C colony forming unit was checked. All colonies were counted by digital colony counter. (Toshiba, EIE-1901)

4.4.4 Dterminations of zone of inhibitions [5]

Agar well diffusion method (Cup plate method) using soybean casein digest agar was used to study antibacterial activity of the synthesized compounds against three gram positive and three gram negative bacterial species.

All Petri dishes were sterilized by hot air oven at 180^0C for 30min. Agar media was sterilized and about 20ml agar media was poured into sterilized Petri dishes. Petridishes were allowed to cool and solidify. Diluted bacterial culture was applied on the plates by micro pipette. After spreading, wells were prepared on agar surface by sterile cork borer of 6 mm diameter. The reference standard 10 μ g/ml and test compounds 10 μ g/ml, 20 μ g/ml and 30 μ g/ml were loaded into the well with the help of micropipette. They were kept aside to allow the solution to diffuse totally in the medium. The plates were incubated at 37^0C for 24 hr in Biological Oxygen Demand (BOD) incubator (EIE Instruments Pvt Ltd). The zone of inhibition was measured in mm in all the incubated

plates. DMSO was taken as a control. Same procedure was applied for the standard drug (Ofloxacine and Metronidazole). All the experiments were performed in triplicates.

4.5 Observation

Antibacterial activity of test compounds were recorded in terms of zone of inhibition (in mm) shown by each compound against various bacterial species

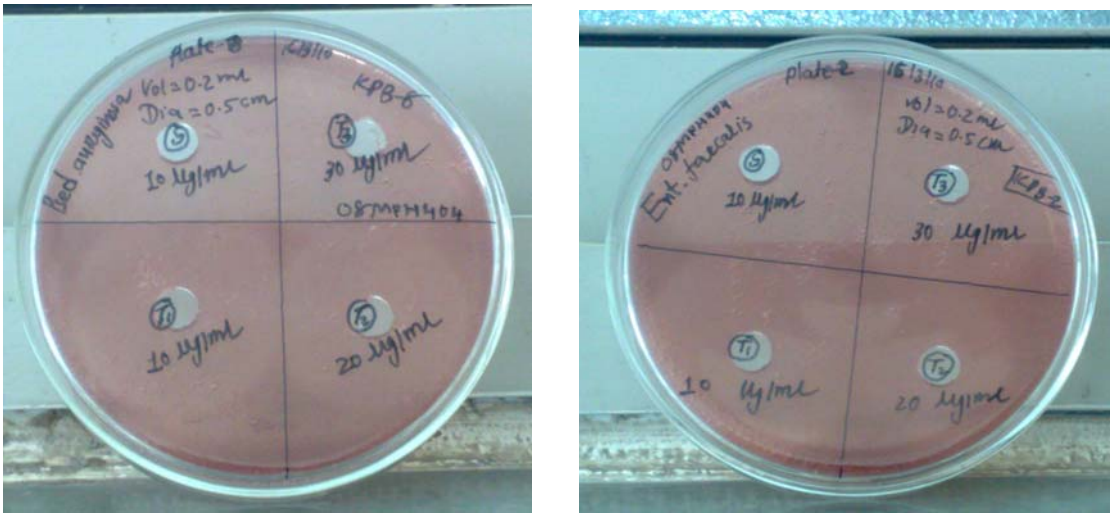


Fig.1 Zone of inhibition of synthesized compound KPB-8 and KPB-2 against *Pseudomonas aeruginosa* and *Enterococcus faecalis* respectively.

4.6 Minimum Inhibitory Concentration (MIC)

The lowest concentration of drug inhibition growth is MIC.

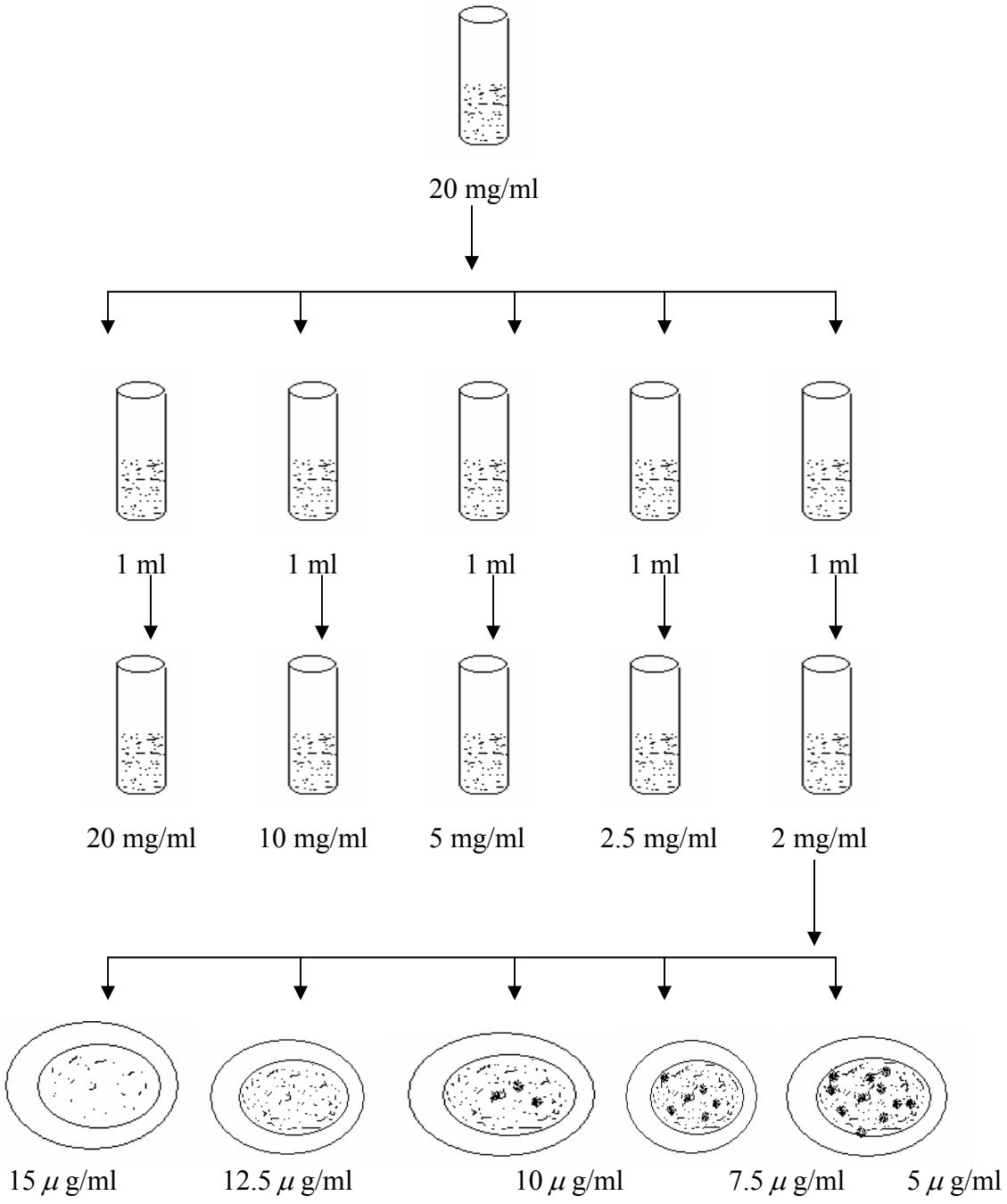


Fig. 2 The lowest concentration of drug inhibition growth is MIC (Minimum Inhibitory Concentration)

4.7 Antibacterial activities measured by zone of inhibition in mm and MICs measured by µg/ml of all target synthesized compounds.

Table - 1

Name of Compd.	Concentration	Gram +ve Bacteria						Gram -ve Bacteria					
		B. cereus (MTCC – 430)		*E. faecalis* (MTCC – 493)		*S. aureus* (MTCC-737)		*E. coli* (MTCC-1687)		*P. aeruginosa* (MTCC-2642)		*K. pneumonia* (MTCC-109)	
		mm	MIC (μg/ml)	mm	MIC (μg/ml)	mm	MIC (μg/ml)	mm	MIC (μg/ml)	mm	MIC (μg/ml)	mm	MIC (μg/ml)
KPB -1	10	9	5.5	8	12.5	13	12.5	0	NP	4	12.5	0	NP
	20	12	7.5	8	10.	13	7.5	0	NP	8	10	0	NP
	30	17	7.5	10	5.5	17	2.5	0	NP	10	10	0	NP
KPB -2	10	9	5.5	8	10	8	12.5	9	12.5	9	12.5	8	10
	20	11	10	13	7.5	12	12.5	13	10	11	7.5	11	7.5
	30	13	10	15	7.5	13	7.5	17	2.5	17	5	16	7.5
KPB -3	10	10	7.5	8	10	11	10	0	NP	0	NP	8	12.5
	20	12	10	11	7.5	12	5	0	NP	0	NP	12	7.5
	30	17	2.5	17	2.5	18	2.5	0	NP	0	NP	16	2.5
KPB -4	10	0	12.5	0	NP	0	15	7	12.5	12	10	0	NP
	20	3	7.5	0	NP	7	12.5	11	7.5	14	5	0	NP
	30	5	5.5	0	NP	7	12.5	16	5	18	2.5	0	NP
KPB -5	10	8	12.5	8	12.5	11	10	6	12.5	9	10	0	NP
	20	10	7.5	11	7.5	13	7.5	7	10	13	7.5	0	NP
	30	13	5.5	14	2.5	15	7.5	11	10	16	2.5	0	NP
KPB -6	10	9	15	6	7.5	11	10	0	NP	0	NP	8	12.5
	20	13	7.5	11	5	14	5	0	NP	0	NP	11	7.5
	30	17	5.5	17	2.5	17	5.5	0	NP	0	NP	16	2.5
KPB -7	10	0	NP	0	NP	0	NP	6	12.5	8	15	0	NP

	20	0	NP	0	NP	1	NP	12	10	12	7.5	0	NP
	30	0	NP	0	NP	2	NP	16	7.5	17	5	0	NP
KPB -8	10	0	NP	0	NP	0	NP	6	15	9	10	10	7.5
	20	0	NP	0	NP	0	NP	12	12.5	14	7.5	13	5
	30	0	NP	0	NP	0	NP	16	5	17	5	16	5
KPB -9	10	9	12.5	8	12.5	10	7.5	0	NP	0	NP	9	12.5
	20	11	5.5	12	7.5	13	7.5	0	NP	0	NP	11	5
	30	16	2.5	16	2.5	16	2.5	0	NP	0	NP	16	2.5
KPB -10	10	0	NP	0	NP	0	NP	6	15	9	15	0	NP
	20	0	NP	0	NP	0	NP	6	10	12	5	0	NP
	30	0	NP	0	NP	0	NP	13	7.5	14	5	0	NP
KPB -11	10	0	NP	0	NP	0	NP	3	12.5	0	NP	0	NP
	20	0	NP	0	NP	3	NP	4	12.5	0	NP	0	NP
	30	0		0	NP	6	NP	6	10	0	NP	0	NP
KPB -12	10	9	7.5	9	12.5	11	10	0	NP	0	NP	0	NP
	20	13	7.5	11	7.5	12	7.5	0	NP	0	NP	0	NP
	30	15	2.5	17	5.5	16	2.5	0	NP	0	NP	0	NP
KPB -13	10	0	NP	3	12.5	0	NP	8	12.5	10	12.5	2	15
	20	0	NP	5	10	0	NP	11	7.5	12	7.5	3	15
	30	0	NP	7	10	0	NP	16	5	16	5	6	12.5
KPB -14	10	9	12.5	9	12.5	0	15	10	10	9	7.5	8	12.5
	20	11	10	13	10	3	12.5	13	7.5	12	7.5	11	7.5
	30	14	2.5	14	7.5	7	12.5	13	7.5	14	5	14	7.5
KPB -15	10	0	NP	0	NP	0	NP	0	NP	0	NP	0	NP
	20	0	NP	0	NP	0	NP	0	NP	0	NP	0	NP
	30	0	NP	0	NP	0	NP	0	NP	0	NP	0	NP
Ofloxacin		16	2.5	17	2.5	18	2.5	19	2.5	18	2.5	17	2.5
Metronidazole		16	2.5	18	5	18	2.5	18	5	18	2.5	16	2.5

Where,

mm = Zone of inhibitions.

MIC = Minimum Inhibitory Concentration. (μ g/ ml)

NP = Not Performed

>10 μ g/ml = MIC value at 10 μ g/ml

>20 μ g/ml = MIC value at 20 μ g/ml

>30 μ g/ml = MIC value at 30 μ g/ml

4.8 Result and Discussion

The synthesized compounds show good antibacterial activity against certain species of bacteria. Synthesized compounds have shown good antibacterial activity against three gram positive bacteria like *B. cereus* (MTCC – 430), *E. faecalis* (MTCC – 493), and *S. aureus* (MTCC-737) and three gram negative bacteria like *E. coli* (MTCC-1687), *P. aeruginosa* (MTCC-2642) and *K. pneumonia* (MTCC-109) but still they are less potent than standard.

For the development of the more effective and less toxic novel lead structures with antibacterial activity, novel 1, 3, 4-thiadiazole, 1, 2, 4-triazole-5-thione and 1, 3-thiazolan-4-one derivatives of benzimidazole were successfully synthesized and evaluated for their antibacterial activity by agar well diffusion method at different concentration 10 μ g/ml, 20 μ g/ml and 30 μ g/ml. The results of the antibacterial activity were reported as zone of inhibitions (mm) and MIC (minimum Inhibitory Concentration) against different bacteria was also founded. Ofloxacin (10 μ g/ml) and Metronidazole (20 μ g/ml) were used as standard drugs.

KPB-3 (2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-(2-phenylacetyl)thiazolidin-4-one), KPB-6 (2-(4-(1H-benzo[d]imidazol-2-yl)phenylimino)-3-(2-phenylacetyl)thiazolidin-4-one), KPB-9 (2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-isonicotinoylthiazolidin-4-one) and KPB-12 ((Z)-2-(4-(1H-benzo[d]imidazol-2-yl)phenylimino)-3-isonicotinoylthiazolidin-4-one) exhibited good degree of inhibitory effect against *S.aureus* (MTCC-737) and *E. faecalis* (MTCC – 493). KPB-3, KPB-6 and KPB-9 exhibited good antibacterial activity against *S.aureus* (MTCC-737), *E. faecalis* (MTCC – 493) and *B. cereus* (MTCC – 430). But other compounds showed very less antibacterial activity against these gram positive bacterial.

KPB-2 (4-((1H-benzo[d]imidazol-2-yl)methyl)-3-benzyl-1H-1,2,4-triazole-5(4H)-thione), KPB-4 (N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-5-benzyl-1,3,4-thiadiazol-2-amine), KPB-5 (4-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-benzyl-1H-1,2,4-triazole-5(4H)-thione), KPB-7 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-(pyridin-4-yl)-1,3,4-

thiadiazol-2-amine), KPB-8 (4-((1H-benzo[d]imidazol-2-yl)methyl)-3-(pyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione) and KPB-13 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-styryl-1,3,4-thiadiazol-2-amine) showed good antibacterial activity against *E. coli* (MTCC-1687) and *P. aeruginosa* (MTCC-2642) where as KPB-2 exhibited potent antibacterial activity against *E. coli* (MTCC-1687), *P. aeruginosa* (MTCC-2642) and *K. pneumonia* (MTCC-109). But other compounds showed very less antibacterial activity against these gram negative bacterial.

4.9 ANTIFUNGAL ACTIVITY

4.9.1 Introduction [7]

Most of the fungal infections are treatable and result in minimal complications such as redness, itching and discomfort, though complication may be severe or fatal if left untreated in certain populations. In immunocompetent persons, fungal infection is usually a very localized infection of the skin or mucosal membranes, including the oral cavity, the pharynx or esophagus, the gastrointestinal tract, the urinary bladder, or the genitalia. Candidiasis is a very common cause of vaginal irritation or vaginitis, and can also occur on the male genitals.[7] In immunocompromised patients, *Candida* infections can affect the esophagus with the potential of becoming systemic, causing a much more serious condition, a fungemia called candidemia. Children, mostly between the ages of three and nine years of age, can be affected by chronic mouth fungal infections.The genus *Candida* and species *C. albicans* was described by botanist Christine Marie Berkhout.[7]

4.9.2 Methods

Following methods are used for the In vitro evaluation of the antifungal activity of the synthesized compounds

(1) Dillution Tests [2]

- Broth dilution method
- Agar dilution method

(2) Diffusion methods [3]

- Disk diffsion method
- Agar well diffusion method

(3) E test [4]

(4) Bio-autographic methods [4]

Total 15 derivatives of 1, 3, 4-thiadiazole, triazole-5-thione and 1, 3-thiazolan-4-one derivatives of benzimidazole were screened for antifungal activity against *Candida albicans* (MTCC-3017), *Aspergillus niger* (MTCC-1344) and *Fusarium oxyspora* (MTCC-1755) by the use of Rose Bengal agar medium.

4.9.3 Experimental Protocol

In the present work, the synthesized compounds were evaluated for in vitro antifungal activity using agar well diffusion method against several fungal starins using Rose Bengal Agar medium (HIMIDIA). Composition of the Rose Bengal Agar medium ia as follows.

Ingredients	**Quantity**
Peptone	5.0
Dextrose	10
Monopotassium Phosphate	1
Magnesium sulphate	0.05
Rose Bengal Agar	15

Final pH at 25^0C : 7 – 7.4

The fungal strains used were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh which are given below.

(1) *Candida albicans* (MTCC-3017)

(2) *Aspergillus niger* (MTCC-1344)

(3) *Fusarium oxyspora* (MTCC-1755)

4.9.4 Procedure

4.9.4.1 Preparation of Rose Bengal Agar medium

Agar was prepared according to directions provided by manufacturer which is 31.5 gm of media was suspended in 1000 ml of distilled water and heated to boiling to dissolve the medium completely. The media was then sterilized by autoclaving (Indofos) at 15 lbs pressure at 121^0C for 15 min.after that, it was allowed to cooled to 45^0C, mixed throuhly and poured into sterilized petridishes.

4.9.4.2 Preparation of test and Standard compounds

All the synthesized compounds (KPB-1 to KPB-15) were soluble in dimethyl sulphoxide (DMSO). The solutions of required conentration 10 μ g/ml, 20 μ g/ml and 30 μ g/ml were prepared by dissolving in DMSO. All the test compounds were prepared from the stock solution of 10mg/10ml. Fluconazole (Zydus Cadial Pvt. Ltd) were taken as reference

standard drug. 10 μ g/ml of concentration was made by dissolving 2mg in 1ml. Other concentrations were also made by this way.

4.9.4.3 Preparation of bacterial culture

From the stock solution of bacterial culture, 0.1 ml was taken and dilluted to 10 ml with distilled water. So, 100 times dilltion was done. From the dilluted cultures, 0.1 ml was taken into petri dish conataining solid agar and spread properly. After 24 hr of incubation at 37^0C colony forming unit was checked. All colonies were counted by digital colony counter (Toshiba, EIE-1901).

4.9.4.4 Dterminations of zone of inhibitions [5]

Agar well diffusion method (Cup plate method) using Rose Bengal agar medium was used to study antifungal activity of the synthesized compounds against three gram positive and three gram negative bacterial species.

All Petri dishes were sterilized by hot air oven at 180^0C for 30min. Rose Bengal agar media was sterilized and about 20ml agar media was poured into sterilized Petri dishes. Petri dishes were allowed to cool and solidify. Diluted fungal culture was applied on the plates by micro pipette. After spreading, wells were prepared on agar surface by sterile cork borer of 6 mm diameter. The reference standard 10 μ g/ml and test compounds 10 μ g/ml, 20 μ g/ml and 30 μ g/ml were loaded into the well with the help of micropipette. They were kept aside to allow the solution to diffuse totally in the medium. The plates were incubated at 37^0C for 24 hr in Biological Oxygen Demand (BOD) incubator (EIE Instruments Pvt Ltd). The zone of inhibition was measured in mm in all the incubated plates. DMSO was taken as a control. Same procedure was applied for the standard drug (Fluconazole). All the experiments were performed in triplicates.[5]

4.9.5 Minimum Inhibitory Concentration (MIC)

The lowest concentration of drug inhibition growth is MIC.

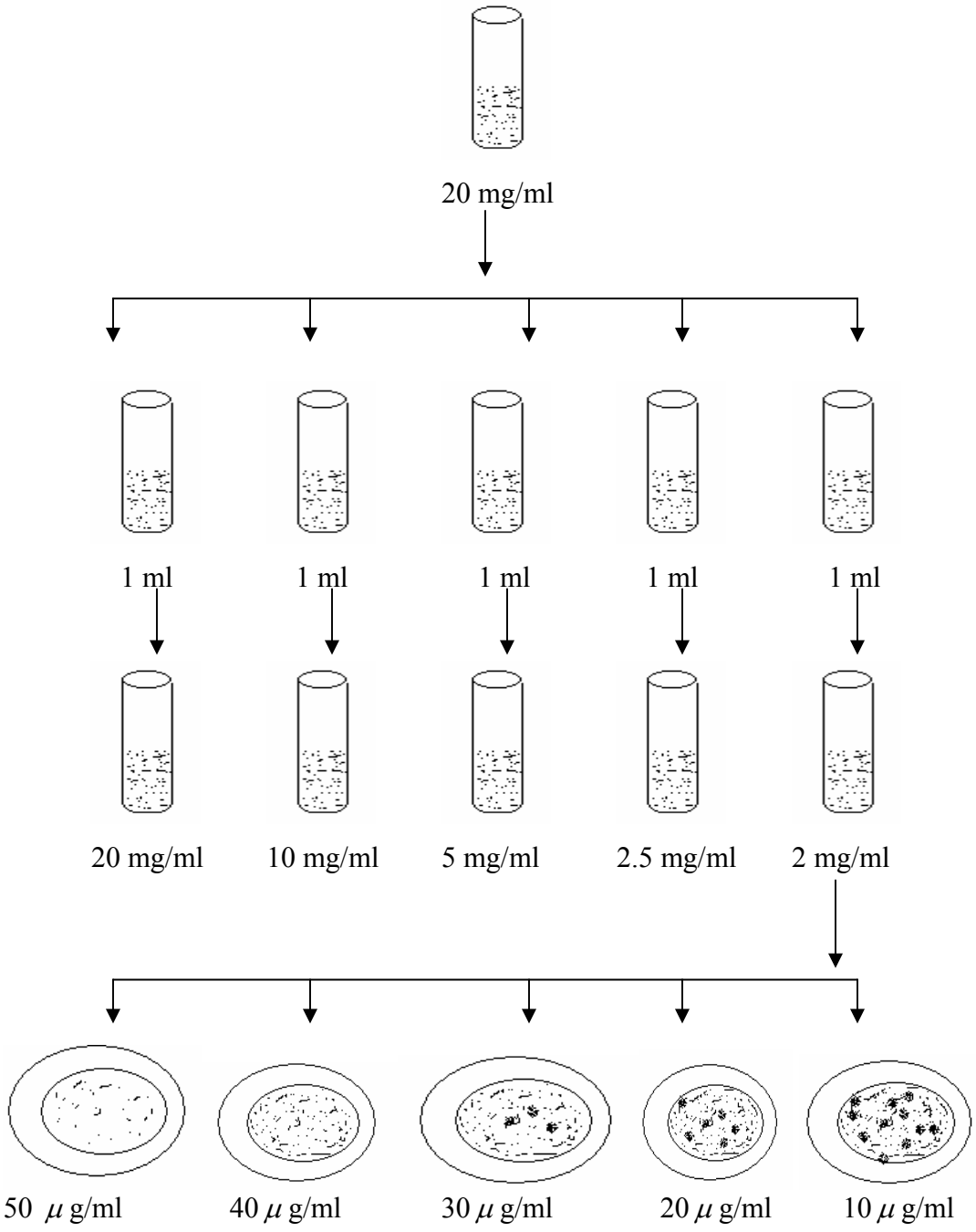


Fig.3 The lowest concentration of drug inhibition growth is MIC (Minimum Inhibitory Concentration)

4.9.6 Antifungal activities measured by zone of inhibition in mm and MICs measured by µg/ml of all target molecules.

Table – 2

Name of Compd.	Concentration	Fungal Organism					
		Candida albicans (MTCC-3017)		*Aspergillus niger* (MTCC-1344)		*Fusarium oxyspora* (MTCC-1755)	
		mm	MIC	mm	MIC	mm	MIC
KPB -1	10	0	NP	0	NP	2	12.5
	20	0	NP	0	NP	6	12.5
	30	0	NP	0	NP	9	10
KPB -2	10	0	NP	8	15	0	NP
	20	0	NP	11	10	0	NP
	30	0	NP	16	10	0	NP
KPB -3	10	11	7.5	0	NP	9	12.5
	20	13	5	0	NP	12	10
	30	16	2.5	0	NP	17	10
KPB -4	10	0	NP	0	NP	0	NP
	20	0	NP	0	NP	0	NP
	30	0	NP	0	NP	0	NP
KPB -5	10	0	NP	0	NP	7	12.5
	20	0	NP	0	NP	11	10
	30	0	NP	0	NP	17	7.5
KPB -6	10	10	10	0	NP	2	15
	20	13	10	0	NP	2	15
	30	16	2.5	0	NP	6	10
KPB -7	10	0	NP	8	12.5	9	10
	20	0	NP	10	7.5	13	7.5
	30	0	NP	15	7.5	16	2.5
KPB -8	10	0	NP	5	12.5	0	NP

	20	0	NP	8	12.5	0	NP
	30	0	NP	12	10	0	NP
KPB -9	10	9	15	0	NP	0	NP
	20	12	7.5	0	NP	0	NP
	30	17	2.5	0	NP	0	NP
KPB -10	10	0	NP	0	NP	3	12.5
	20	0	NP	0	NP	3	12.5
	30	0	NP	0	NP	7	10
KPB -11	10	0	NP	0	NP	6	12.5
	20	0	NP	0	NP	8	12.5
	30	0	NP	0	NP	12	7.5
KPB -12	10	0	NP	0	NP	0	NP
	20	0	NP	0	NP	0	NP
	30	0	NP	0	NP	0	NP
KPB -13	10	0	15	8	12.5	0	NP
	20	3	12.5	10	10	0	NP
	30	7	12.5	14	7.5	0	NP
KPB -14	10	7	10	0	NP	4	12.5
	20	9	10	0	NP	8	10
	30	13	7.5	0	NP	14	5
KPB -15	10	0	NP	3	12.5	0	NP
	20	0	NP	5	7.5	0	NP
	30	0	NP	9	7.5	0	NP
Fluconazole		19	2.5	16	2.5	17	2.5

Where,
mm = Zone of inhibitions, MIC = Minimum Inhibitory Concentration. (μ g/ ml)

NP = Not Performed

>10 μ g/ml = MIC value at 10 μ g/ml

>20 μ g/ml = MIC value at 20 μ g/ml

>30 μ g/ml = MIC value at 30 μ g/ml

4.9.7 Result and Discussion

In search for more effective and less toxic novel lead target structure with antifungal activity, novel 1, 3, 4-thidiazole, 1, 2, 4-triazole-5-thione and 1, 3-thiazolan-4-one derivatives of benzimidazole were synthesized and evaluated for in vitro antifungal activity by agar well diffusion technique at the different concentration (10 μ g/ml, 20 μ g/ml and 30 μ g/ml) of the synthesized compounds.

The results of the antifungal activity of the synthesized compounds were reported as zone of inhibition (mm) against fungal strains such as *Candida albicans* (MTCC-3017), *Aspergillus niger* (MTCC-1344) and *Fusarium oxyspora* (MTCC-1755).

KPB-3 (2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-(2-phenylacetyl)thiazolidin-4-one), KPB-6 (2-(4-(1H-benzo[d]imidazol-2-yl)phenylimino)-3-(2-phenylacetyl)thiazolidin-4-one) , KPB-9 (2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-isonicotinoylthiazolidin-4-one and KPB-14 (4-((1H-benzo[d]imidazol-2-yl)methyl)-3-styryl-1H-1,2,4-triazole-5(4H)-thione exhibited maximum inhibitory activity against *Candida albicans.*

KPB-2 (4-((1H-benzo[d]imidazol-2-yl) methyl)-3-benzyl-1H-1, 2, 4-triazole-5(4H)-thione), KPB-7 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine) and KPB-13 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-styryl-1,3,4-thiadiazol-2-amine) exhibited maximum inhibitory activity against *Aspergillus niger*

KPB-1 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-benzyl-1,3,4-thiadiazol-2-amine), KPB-3 (2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-(2-phenylacetyl)thiazolidin-4-one), KPB-5 (4-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-benzyl-1H-1,2,4-triazole-5(4H)-thione), KPB-7 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine) and KPB-14 (4-((1H-benzo[d]imidazol-2-yl)methyl)-3-styryl-1H-1,2,4-triazole-5(4H)-thione) exhibited maximum inhibitory activity against *Fusarium oxyspora*

KPB-3 and KPB-14 demonstrated highest inhibitory activity against both *Candida albicans* and *Fusarium oxyspora.* KPB-7 shows maximum inhibitory activity against both *Aspergillus niger* and *Fusarium oxyspora.* Moreover, antifungal activities of the other compounds tested against fungal strains were very weak.

4.10 Refernces

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CHAPTER – 5
SUMMARY

The different types of the antibiotics were recognized to be therapeutic benefit from1940 to 1975. Despite of this, the major therapeutic group of agents has become identified from the natural products screening in the last quarter of a century. Decades of the antibiotic use have resulted in the development of widespread resistance to commonly prescribed antibiotic agents.

Currently available antimicrobial agents like polyenes, azoles and recently introduced echinocandins classes of drugs have been recognized as important therapeutic agents for treatment of a variety of systemic and superficial microbial infections. These antimicrobial agents have established a new development of the pharmacotherapy of microbial infections.

The treatments of microbial infections have become major problems due to various demerits occurred with the known antimicrobial agents. They bind extensively to plasma proteins and are not considered optimally bioavailable. Moreover, the growth of resistant microbial strains has become the major factor. Antimicrobial activities of the most of the antimicrobial agents are directed towards inhibition of ergosterol either directly or indirectly.

There are some needs for the identification of the novel structural lead that can be used to design new, potent and less toxic antimicrobial agents. With the help of the novel technologies like genomics and proteomics, high throughput screening assays, discovery of novel targets and novel molecules has been made. Some of these molecules have advanced to successive phases of the clinical trials and some of these compounds are in pre clinical development stage.

Intense investigation is being carried out on 1, 3, 4-thiadiazole, 1, 2, 4-triazole-5-thione and 1, 3-thiazolan-4-one containing compounds giving wide spectrum of antimicrobial activity Different classes of thiadiazole compounds are 1, 2, 4-thiadiazole, 1, 2 5-thiadiazole and 1, 3, 4-thiadiazole . Among the different thiadiazole, 1, 3, 4-thiadiazole represents the most therapeutically active classes of compound and has wide range of

antimicrobial activities. Literature review which is given and also other literature work shows that 1, 3, 4-thiadiazole, 1, 2, 4-triazole-5-thione and 1, 3-thiazolan-4-one containing compounds gives optimum antimicrobial activities. Based on these observations, as a part of this present study aimed for the developing the new biologically active 1, 3, 4-thiadiazole, 1, 2, 4-triazole-5-thione and 1, 3-thiazolan-4-one derivatives of benzimidazole were synthesized according to the steps reported in scheme and all the reaction steps were optimized in context of the present study.

The structures of the synthesized compounds were established by IR, Mass and ^{1}H NMR spectroscopic techniques. The data of the physical characterizations of the compounds are given below.

Table.1 Summary of the physical data of target synthesized compounds

Compound Code	Molecular Formula	Percentage yield	Melting Point	R_f Value
KPB-1	$C_{17}H_{15}N_5S$	48.6%	145-148^{0}C	0.65
KPB-2	$C_{17}H_{15}N_5S$	45.1%	151-153^{0}C	069
KPB-3	$C_{19}H_{16}N_4O_2S$	41.3%	161-163^{0}C	0.71
KPB-4	$C_{22}H_{17}N_5S$	46.7%	147-150^{0}C	0.63
KPB-5	$C_{22}H_{17}N_5S$	43.4%	154-156^{0}C	0.67
KPB-6	$C_{24}H_{18}N_4O_2S$	46.1%	165-168^{0}C	0.68
KPB-7	$C_{15}H_{12}N_6S$	43.5%	151-153^{0}C	0.67
KPB-8	$C_{15}H_{12}N_6S$	48.2%	160-162^{0}C	0.76
KPB-9	$C_{17}H_{13}N_5O_2S$	51.3%	171-173^{0}C	0.78
KPB-10	$C_{20}H_{14}N_6S$	51.4%	160-162^{0}C	0.70
KPB-11	$C_{20}H_{14}N_6S$	53.9%	168-171^{0}C	0.72
KPB-12	$C_{22}H_{15}N_5O_2S$	55.7%	170-172^{0}C	0.73
KPB-13	$C_{18}H_{15}N_5S$	55.7%	170-172^{0}C	0.73
KPB-14	$C_{18}H_{15}N_5S$	55.1%	175-177^{0}C	0.73
KPB-15	$C_{21}H_{18}N_4O_2S$	51.6%	181-183^{0}C	0.71

The 1, 3, 4-thiadiazole, 1, 2, 4-triazole-5-thione and 1, 3-thiazolan-4-one derivatives of benzimidazole were evaluated for their in vitro antibacterial and antifungal activity by agar well diffusion technique using Soyabean Casein Digest Agar media and Rose bengal agar media respectively. Antibacterial activity of the synthesized compounds were performed against three gram positive bacteria (*B. cereus* (MTCC – 430), *E. faecalis* (MTCC – 493) and *S. aureus* (MTCC-737)) and three gram negative bacteria (*E. coli* (MTCC-1687), *P. aeruginosa* (MTCC-2642) and *K. pneumonia* (MTCC-109)). Antifungal activities of the synthesized compounds were performed against *Candida albicans* (MTCC-3017), *Aspergillus niger* (MTCC-1344) and *Fusarium oxyspora* (MTCC-1755). Ofloxacin (10 μg/ml) and Metronidazole (10 μg/ml) were used as reference standard drug for the antibacterial activity. Fluconazole (10 μg/ml) was used as reference standard drug for the antifungal activity. The bacterial and fungal strains were procured from the Institute of Microbial Technology (IMTECH), Chandigarh.

Some of the compounds have shown comparable antibacterial and antifungal activity as that of standard but at the higher concentration. KPB-1 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-benzyl-1,3,4-thiadiazol-2-amine), KPB-3 (2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-(2-phenylacetyl)thiazolidin-4-one), KPB-6 (2-(4-(1H-benzo[d]imidazol-2-yl)phenylimino)-3-(2-phenylacetyl)thiazolidin-4-one) and KPB-9 (2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-isonicotinoylthiazolidin-4-one)and KPB-12 ((Z)-2-(4-(1H-benzo[d]imidazol-2-yl)phenylimino)-3-isonicotinoylthiazolidin-4-one) show higher degree of inhibitory activity against all the gram positive bacteria but others are not much active against gram positive bacteria.

KPB-2 (4-((1H-benzo[d]imidazol-2-yl)methyl)-3-benzyl-1H-1,2,4-triazole-5(4H)-thione), KPB-5 (4-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-3-benzyl-1H-1,2,4-triazole-5(4H)-thione), KPB-7 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine), KPB-8 (4-((1H-benzo[d]imidazol-2-yl)methyl)-3-(pyridin-4-yl)-1H-1,2,4-triazole-5(4H)-thione) and KPB-13 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-styryl-1,3,4-thiadiazol-2-amine) show higher degree of inhibitory activity against all the gram negative bacteria but others are not much active against gram negative bacteria.

KPB-3 (2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-(2-phenylacetyl)thiazolidin-4-one), KPB-6 (2-(4-(1H-benzo[d]imidazol-2-yl)phenylimino)-3-(2-phenylacetyl)thiazolidin-4-one) and KPB-9 (2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-isonicotinoylthiazolidin-4-one) show higher degree of inhibition activity against *Candida albicans* (MTCC-3017). KPB-2 (4-((1H-benzo[d]imidazol-2-yl)methyl)-3-benzyl-1H-1,2,4-triazole-5(4H)-thione), KPB-7 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine) and KPB-13 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-styryl-1,3,4-thiadiazol-2-amine) exhibited the higher degree of inhibition against *Aspergillus niger* (MTCC-1344). KPB-3 (2-((1H-benzo[d]imidazol-2-yl)methylimino)-3-(2-phenylacetyl)thiazolidin-4-one) and KPB-7 (N-((1H-benzo[d]imidazol-2-yl)methyl)-5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine) exhibited potent inhibitory activity against *Fusarium oxyspora* (MTCC-1755).

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