International Journal of
Medicinal Chemistry & Analysis
www.ijmca.com
e ISSN 2249 - 7587
Print ISSN 2249 - 7595

SYNTHESIS, SCREENING & ANTIMICROBIAL ACTIVITY OF BENZIMIDAZOLE DERIVATIVES

G. Priyanka, P. Jayachandra Reddy, M. Alagu Sundaram, M. Sabareesh, V. Prabhakaran

Krishna Teja Pharmacy College, Chadalawada Nagar, Tirupathi-517506, Andhra Pradesh, India.

ABSTRACT

More than half of all therapeutic agents consists of heterocyclic compounds. The heterocyclic ring system in many cases comprises the very core of the active moiety or pharmacophore. Hundreds of thousands of new heterocyclic compounds are prepared annually throughout the world, and many of them are entering into pharmacological screenings to determine if they have useful biological activity. This process of random screening is inefficient, but it has resulted in identification of new compounds not produced naturally or imagined by chemists. Such lead compounds form the basis of a series of analogues intended to optimize the therapeutic activity. The antitubercular drug, Ethambutol, was developed in this way. More recently emphasis has been placed on rational design of new pharmaceuticals.

Keywords: Heterocyclic compounds, Therapeutic activity, Biological activity.

INTRODUCTION

This thesis deals with the investigation carried out by writer in the laboratory on the synthesis, characterization and antimicrobial screening of 3-[4-(1h-benzimidazol-2-yl) phenyl] substituted 1, 3-thiazolidine-4-ones. Before discussing the experimental procedures adopted and the results obtained, a brief introduction to therapeutic agents based on this ring and related moiety and in particular a literature survey on the investigations carried out by earlier workers on the synthesis and evaluation of the heterocyclic compounds based on the above ring moiety would be presented in this chapter [1].

The history of medicine starts with the history of man. The need to find out a solution for his health problems lead to the discovery of medicines. Man used drugs of natural origin from the primitive age onwards. As the science and technology developed his curiosity came down to the molecular level of the drug moieties. Knowledge of chemistry of drugs and the drug-receptor interaction changed the concept of discovery of medicines [2]. The question of "How" and "Why" these drugs acts leads to the answer of particular functional groups present in the drug. Medicinal chemistry emerged as a new branch of science, which played an important part in the discovery of new drugs.

Most of the drugs of natural origin contain heterocyclic rings. Those cyclic compounds which in addition to carbon, contain at least one atom of another element (hetero atom) in the ring, are called heterocyclic compounds or simply heterocyclic. The common heteroatom present in the carbon rings are O, N, and S. The heterocyclic compounds having the lesser common atoms such as Phosphorus, Tin, Boron, Silicon, Bromine etc., have been a subject of` much investigation in recent years [3-5].

Medicinal chemistry covers three critical steps

- A discovery step consisting of the identification and production of new active substances usually called lead compounds. Leads can originate from synthetic organic chemistry, from natural sources or from bio technological processes.
- An optimization step mainly deals with the synthetic modification of the lead structure in order to improve potency, selectivity and lessen toxicity. Its characteristics are: establishment and analysis of structure activity relation ships (SAR).

Corresponding Author: - **G. Priyanka** Email: priyankagangarapu@gmail.com

• Development step consist optimization of the synthetic route for bulk production.

Once a new pharmaceutical lead compound has been discovered, extensive and costly efforts usually are made to prepare a series of analogues in the hope that even better activity will be found. In an effort to improve the efficiency of analogue development, a variety of statistical methods have been introduced [6]. They range from Hansch approach, in which analysis of variance is used to derive an equation expressing the quantitative relationships between functional changes and biological activity, to pattern recognition and factor analysis methods. Nonquantitative methods, such as Topliss approach, are also popular. Computer aided design, including quantitative energy calculations and graphical methods, has been rapidly introduced in the pharmaceutical industry.

Although many natural products are used in pharmaceuticals in their original chemical structures, successful efforts have been made to improve their pharmaceutical and therapeutic properties by structural modifications. Some of these modifications are relatively simple, such as the formation of the phosphate esters of hydrocortisone. This derivative increases water solubility of the drug. Other modifications may be more substantial, as in the replacement of penicillin and cephalosporin side chains with new ones that modify the antibacterial spectrum [7].

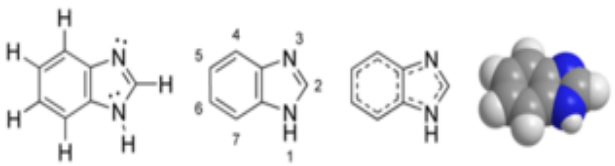
Another approach to improve the therapeutic properties is to identify that portion of a natural molecule responsible for its biological activity and synthesize new molecules that are based on it. This active portion is known as the essential structural unit. The development of cocaine is a prime example of this approach.

Intensive research in diverse heterocyclic derivatives continues to yield new many medicinal agents, such areas are "benzimidazoles" and "thiazolidines". In the present study, some new biologically active substituted Benzimidazole fused with Thiazolidine-4-ones were synthesized, purified and confirmed by IR, H^1 NMR, and Mass Spectra [8].

Benzimidazoles

A heterocyclic bicyclic aromatic Compound consists of fusion of benzene and imidazole. Most prominent benzimidazole in nature is N-ribosyl-dimethyl benzimidazole, which serves as an axial ligand for cobalt in Vitamin $B_{12.}$

Table 1. Properties of Thiazolidine

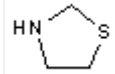


Chemical formula	$\mathbf{C_7H_6N_2}$
Molar mass	118.136 g mol^{-1}

Thiazolidines

Thiazolidines are heterocyclic five membered saturated ring possessing an amide group and a sulfide group at 1 and 3 positions in which the two heteroatoms are separated by one carbon atom and are sulfur analogs of Oxazolidines.

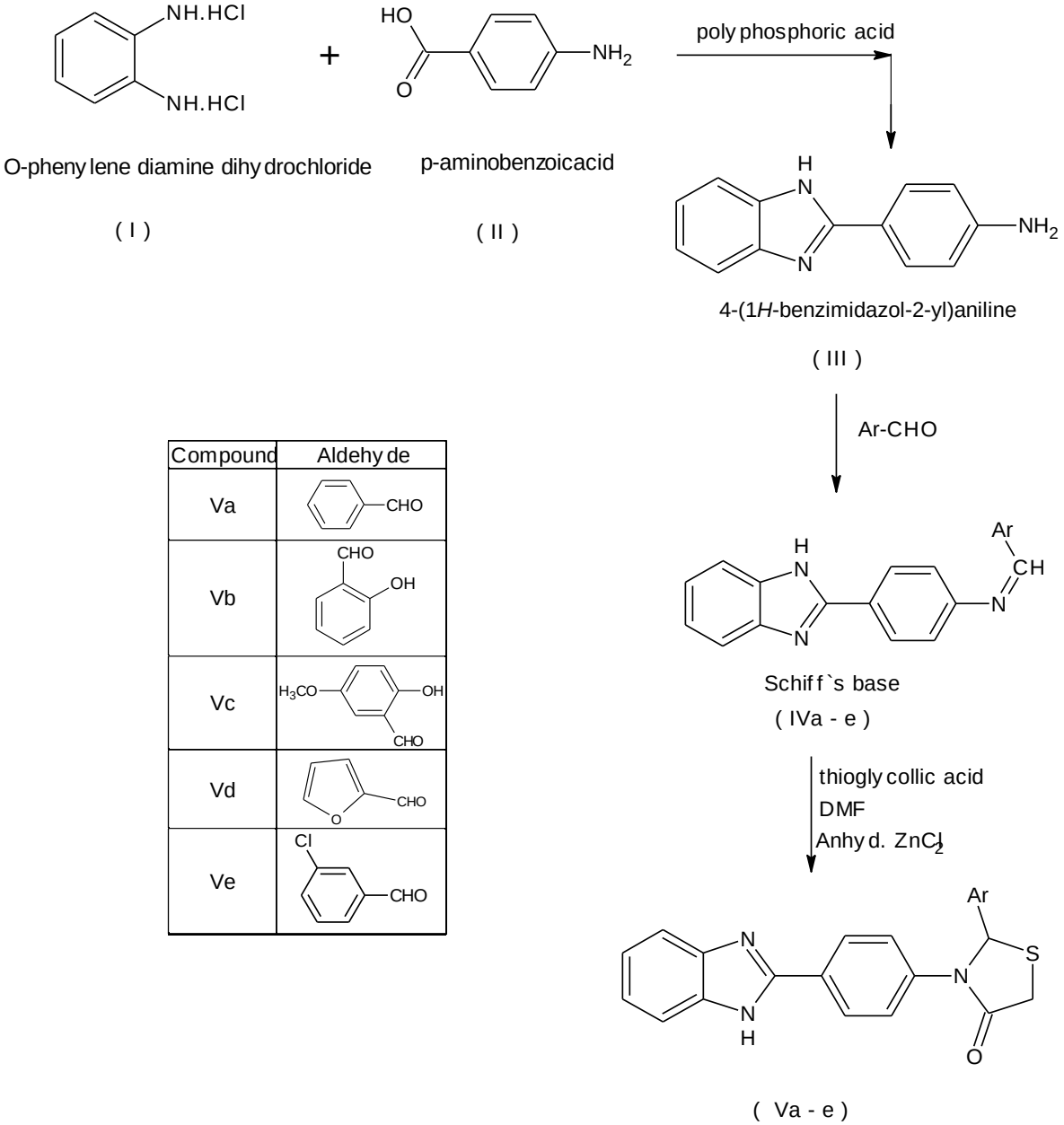
Table 2. Properties of Thiazolidine

Thiazolidine	
Chemical name	Thiazolidine
Chemical formula	C_3H_7NS
Molecular mass	89.15 g/mol
Melting point	°C
Boiling point	72-75 °C ,25 torr
Density	1.131 g/cm^3
	

Scope and Object

Chemical modifications of drug molecules of a series having optimal activity is widely used and continue to be an important factor in new drug discovery studies. In order to obtain new, effective and safe drugs has led today's researchers to improve the existing drugs by increasing their potency, duration of action and by decreasing the toxic side effects. Structure activity studies show that variations in ring system or minor group extend distinct pharmacological effect upon the drug molecules.
Benzimidazoles are biologically important group of compounds having activities like antiviral, antibacterial, antifungal, anti-inflammatory, insecticidal, anthelmintic, antitubercular and other biological activities. Thiazolidinone derivatives also reported to show a broad spectrum of biological activities. These include antibacterial, antifungal, antiviral, cardiac activity and anti-diabetic activities. Prompted by these reports, it was contemplated to synthesize new Benzimidazoles Thus an attempt was made to synthesize derivatives of "3-[4-(1H-benzimidazol-2-yl) phenyl]-2-substitued 1,3-thiazolidin-4-one" in this present study. It is likely that the new derivatives with some modification in their chemical structure may result in some profound change in the pharmacological response. It may increase, decrease or alter the nature of the response. The reaction, reagents and the condition of the reaction system are given in the scheme 1.

Scheme 1. Studies of antimicrobial activity of the synthesized compounds were performed, after confirming the structure from the IR, ^{1}H NMR and Mass spectral analysis



Compound	Aldehyde
Va	
Vb	
Vc	
Vd	
Ve	

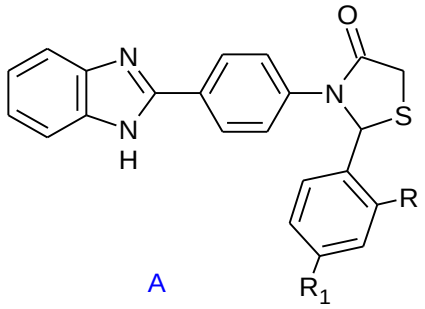
Methodology

Organic chemists are frequently facing the problem of characterizing and elucidating the structure of organic compounds. In the field of natural products has the prospect of isolating such compounds from their sources in a pure state and then determining their structure. On the other hand the synthetic organic chemists encounter new or unexpected compounds in the course of investigations into the applicability of new reagents or techniques or as by products of established reactions. All the reactions were carried out under prescribed laboratory conditions. All the reactions requiring anhydrous conditions were conducted in well-dried apparatus.

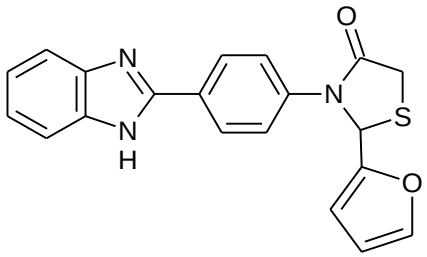
The solvents and reagents used in the synthetic work were of laboratory reagent grade and were purified by distillation and crystallization techniques wherever necessary and their melting points were checked with the available literature. Melting points of newly synthesized compounds were determined by open capillary method and were uncorrected. Micro TLC routinely checked purity of the compounds. The IR Spectra of compounds were recorded on THERMO NICOLET NEXUS 670

spectrometer using KBr pellet. NMR Spectra were recorded in AVANCE 300 MHz spectrometer using TMS as an internal standard. Mass Spectra were recorded in NCMS spectrometer.

Physical Data of Synthesized Compounds



A



B

Table 3. Physical Data of Synthesized Compounds

Compound	Molecular formula	R	R_1	Molecular wt in gms	% Yield	Melting point ^oC
Va	$C_{22}H_{17}N_3OS$ (A)	H	H	371.1	74%	223-225
V_b	$C_{22}H_{17}N_3O_2S$ (A)	OH	H	387.5	68%	234-236
V_c	$C_{23}H_{19}N_3O_3S$ (A)	OH	OCH_3	417.47	72%	280-281
V_d	$C_{20}H_{15}N_3O_2S$	B		356.41	64%	229-230
V_e	$C_{22}H_{16}N_3OSCl$ (A)	Cl	H	405.89	70%	265-268

Antimicrobial screening

Evaluation of antimicrobial activity

The antimicrobial activity can be evaluated by and disc diffusion test. Diffusion test used to determine the sensitivity of organism by measuring zone of inhibition.

Disc diffusion test

Modified Kirby-Bauer method[90] was used for the evaluation of microbial sensitivity of the synthesized compounds. Circular paper disks were impregnated with the specific amount of test compounds and were placed on suitable agar medium (Muller Hinton agar), which was inoculated with the test organism. After incubation, the petri dishes were observed for growth of inhibition zone around the disk. A "halo" or Zone of inhibition forms, where concentration of the diffused molecule is sufficient to inhibit microbial growth. The diameter of zone of inhibition is directly proportional to antimicrobial activity of the compound. The diameter of zone of inhibition was compared with that of standard antibiotics. The size of zone of inhibition depends on rate of antibiotic diffusion, rate of bacterial growth and incubation condition, concentration of organism [9,10].

Cultivation of microorganism

The following bacterial cultures were used for the study

1. *Bacillus subtilis* - Gram positive bacteria
2. *Staphylococcus aureous* - Gram positive bacteria
3. *Escherichia coli* - Gram negative bacteria
4. *Pseudomonas aeruginosa* - Gram negative bacteria

The following fungal cultures were used for the study

Aspergillus Niger
Candida albicans

Drugs control

Ciprofloxacin (antibacterial)
Clotrimazole (antifungal)

Concentration: All the test compounds were tested at 100 µg/ml.
Solvent: Dimethylformamide (DMF)

Table 4. Composition of Sabourauds dextrose agar medium

Beef infusion	300ml
Casein hydrolysate	16gm
Starch	1.5gm
Agar	15gm
Distilled water	1000ml
pH	7.2 ± 0.2

Table 5.

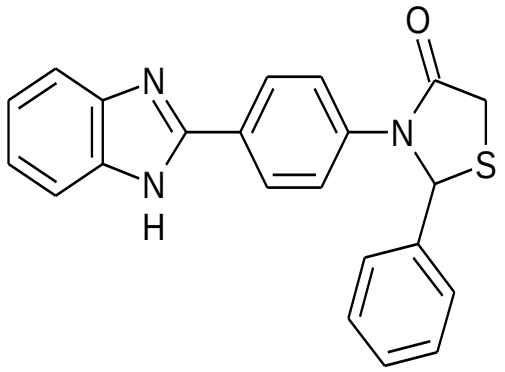
Dextrose	40gm
Peptone	10gm
Agar	20gm
Distilled water	1000ml
pH	5.6 ± 0.2

The medium was prepared by dissolving the specified quantity of the dehydrated medium in purified water and was dispersed in 20ml volumes in to test tubes. The test tubes were closed with cotton plugs and were sterilized by autoclaving at 121°C (15 lb psig) for 15 minutes. The contents of tubes were poured aseptically in to sterile petri plates (90mm diameter) and allowed to solidify [11].

Results and Discussion

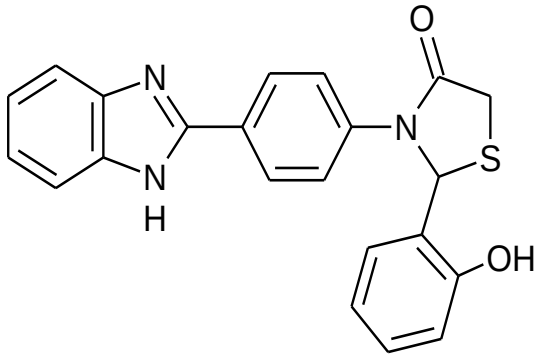
Spectral Data of Synthesized Compounds

(a) Spectral data of 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-phenyl-1, 3- thiazolidin-4-one (Va)



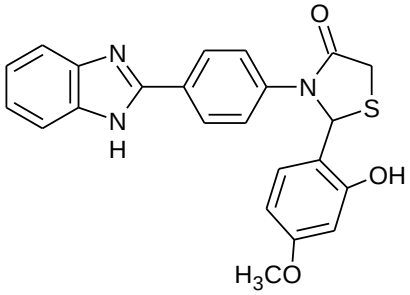
The IR spectrum of the compound was recorded on THERMONICOLET NEXUS-670spectrometer by KBr method is given in figure 1.

Spectral data of 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(hydroxyphenyl)-1, 3-thiazolidin-4-one (Vb)



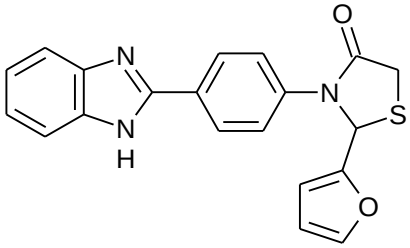
The IR spectrum of the compound was recorded on THERMONICOLET NEXUS-670spectrometer by KBr method is given in figure 2.

Spectral data of 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(hydroxyl-4-methoxyphenyl)-1,3-thiazolidin-4-one (Vc)



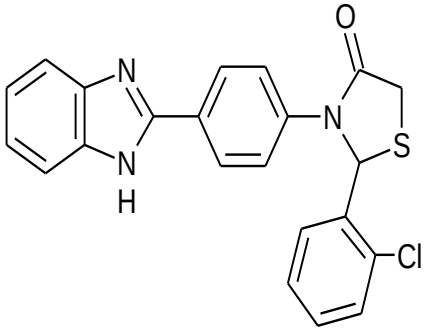
The IR spectrum of the compound was recorded on THERMONICOLET NEXUS-670spectrometer by KBr method is given in figure 3.

Spectral data of 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(furan-2-yl)-1, 3-thiazolidin-4-one (Vd)



The IR spectrum of the compound was recorded on THERMONICOLET NEXUS-670spectrometer by KBr method is given in figure 4.

Spectral data of 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(2-chlorophenyl)-1, 3-thiazolidin-4-one (Ve)



The IR spectrum of the compound was recorded on THERMONICOLET NEXUS-670spectrometer by KBr method is given in figure 5.

Table 6. IR frequencies: 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-phenyl-1, 3- thiazolidin-4-one

Compound	Types of vibration	Wave number [Cm^{-1}]
Va	N-H stretch	3324.79
	C-H Stretch (aromatic)	2924.62
	C=O (thiazolidone)	1601.68
	C=N stretch	1508.37
	C-S-C	695.41

Table 7. IR frequencies: 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(hydroxyphenyl)-1, 3-thiazolidin-4-one

Compound	Types of vibration	Wave number [Cm^{-1}]
Vb	O-H stretch	3256.66
	N-H stretch	3059.78
	C-H Stretch (aromatic)	2924.68
	C=O (thiazolidone)	1654.96
	C=N stretch	1601.08
	C-S-C	691.96

Table 8. IR frequencies: 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(hydroxyl-4-methoxyphenyl)-1, 3-thiazolidin-4-one

Compound	Types of vibration	Wave number [Cm^{-1}]
Vc	O-H stretch	3254
	N-H stretch	3058.93
	C-H Stretch (aromatic)	2925.05
	C=O (thiazolidone)	1655.08
	C=N stretch	1601.95
	C-S-C	693.08

Table 9. IR frequencies: 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(furan-2-yl)-1, 3-thiazolidin-4-one

Compound	Types of vibration	Wave number [Cm^{-1}]
Vd	O-H	3474.68
	N-H stretch	3375.26
	C-H Stretch (aromatic)	2922.19
	C-H stretch	2847.15
	C=O (thiazolidone)	1638.78
	C=N stretch	1603.28
	C-S-C	694.09

Table 10. IR frequencies: 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(2-chlorophenyl)-1, 3-thiazolidin-4-one

Compound	Types of vibration	Wave number [Cm^{-1}]
Ve	N-H stretch	3375.26
	C-H Stretch (aromatic)	2922.19
	C=O (thiazolidone)	2847.15
	C=N stretch	1604.78
	C-S-C	694.09
	C-Cl	744.51

Table 11. Antibacterial Activity

S.No.	Compound	Diameter of zone of inhibition (mm)			
		B.Subtilis	**E.Coli**	**S.Aureus**	**P.Aureginosa**
1	Va	14	12	11	12
2	Vb	20	15	21	15
3	Vc	16	14	18	13
4	Vd	19	18	19	15

S.No.	Compound	Diameter of zone of inhibition (mm)			
		B.Subtilis	**E.Coli**	**S.Aureus**	**P.Aureginosa**
5	Ve	12	09	10	11
6	Standard 1mg/ml	18	16	20	15
7	DMF	--	--	--	--

Standard Drug: Ciprofloxacin

Table 12. Anti fungal activity

S.No.	Compound	Diameter of zone of inhibition (mm)	
		C.Albicans	**A.Niger**
1	Va	12	13
2	Vb	15	14
3	Vc	16	12
4	Vd	19	18
5	Ve	21	19
6	Standard 1mg/ml	20	16
7	DMF	--	--

Standard Drug: Clotrimazole

Fig. 1. Spectral data of 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-phenyl-1, 3- thiazolidin-4-one

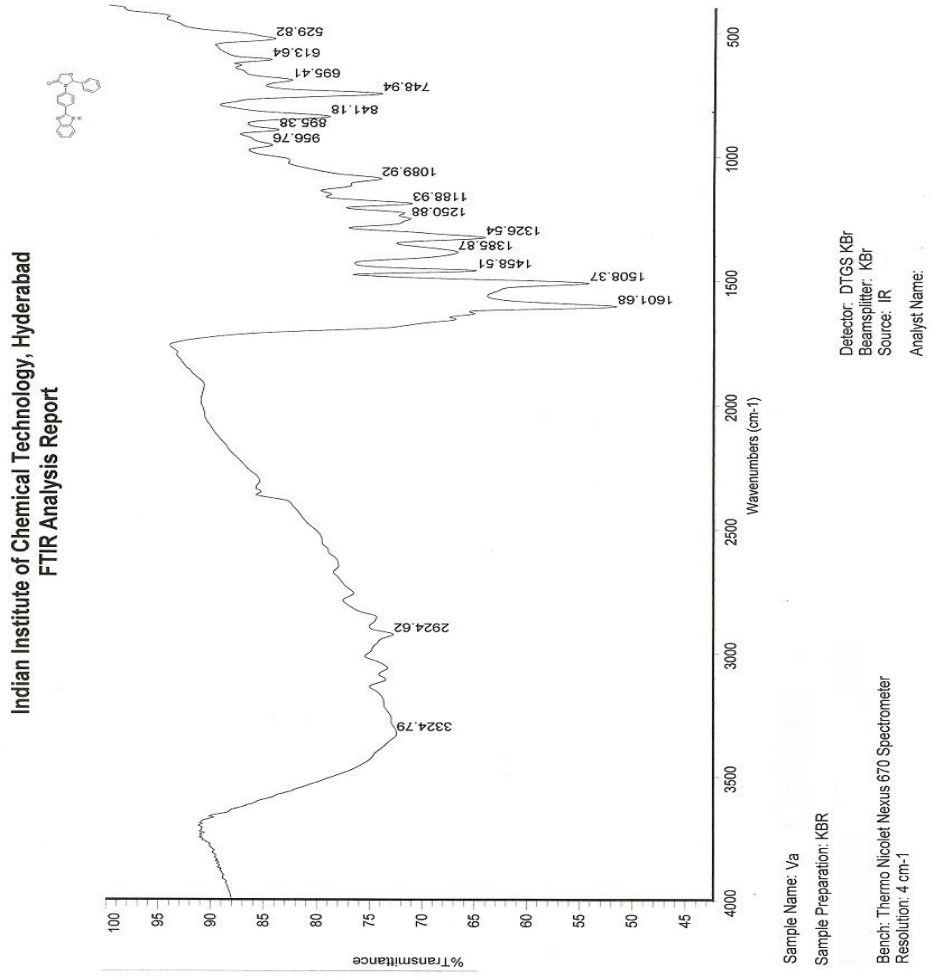


Fig. 2. Spectral data of 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(hydroxyphenyl)-1, 3-thiazolidin-4-one

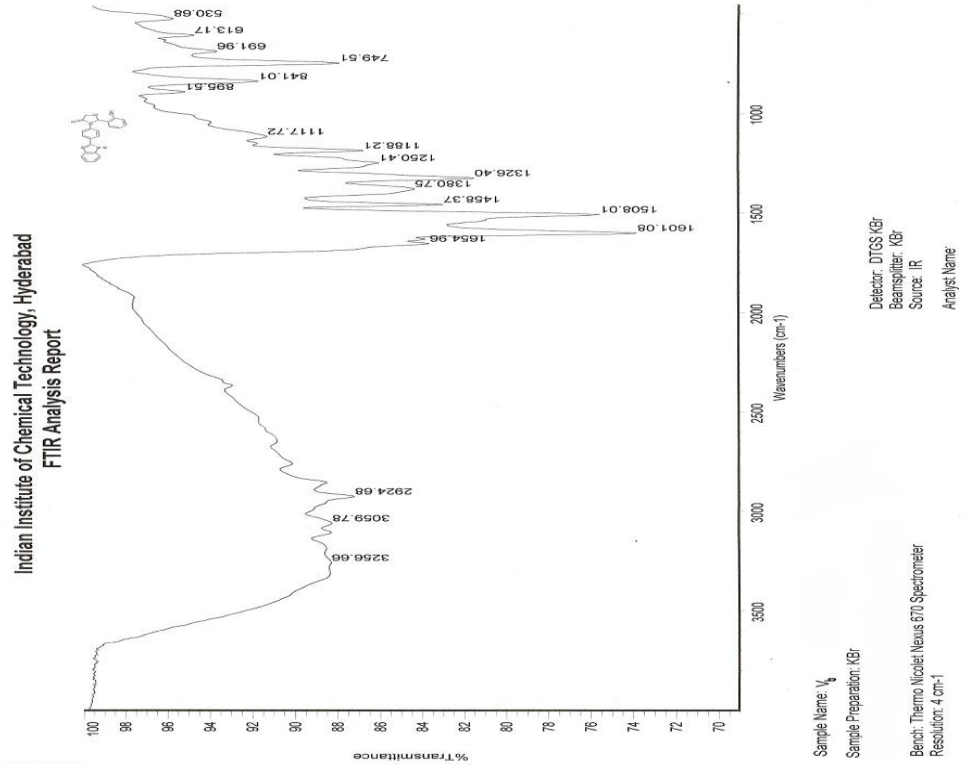


Fig. 3. Spectral data of 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(hydroxyl-4-methoxyphenyl)-1, 3-thiazolidin-4-one

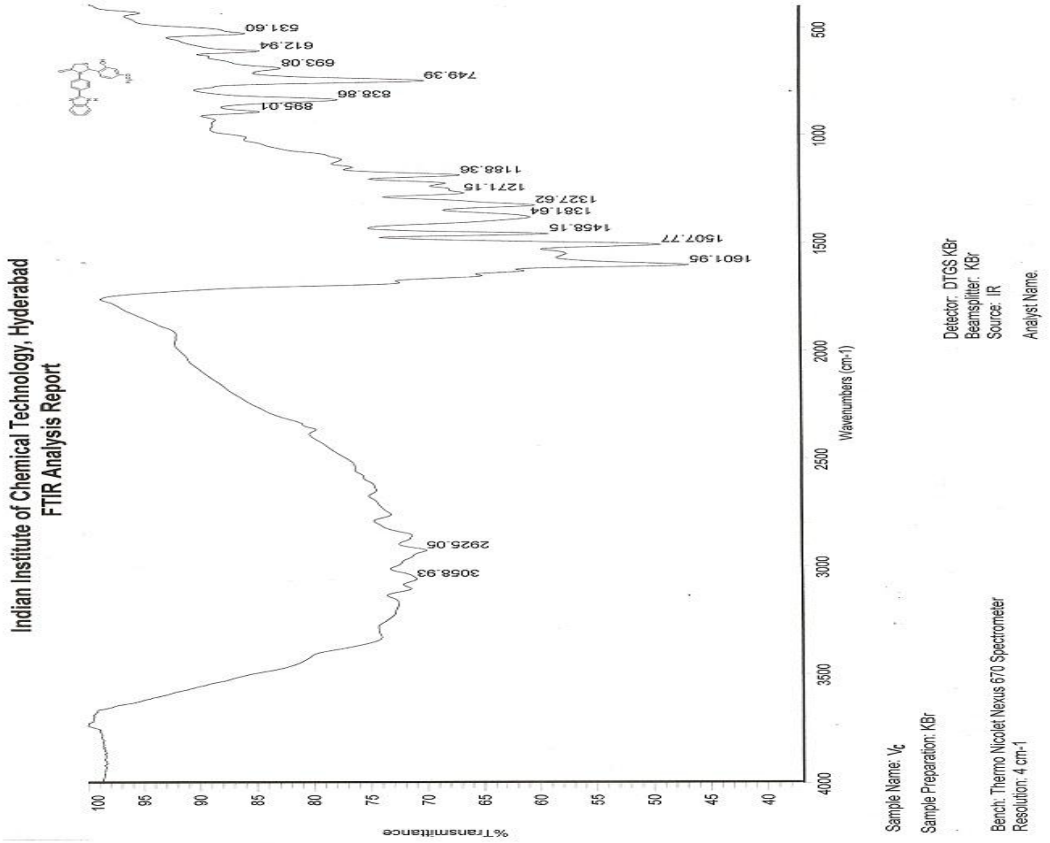


Fig. 4. Spectral data of 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(furan-2-yl)-1, 3-thiazolidin-4-one

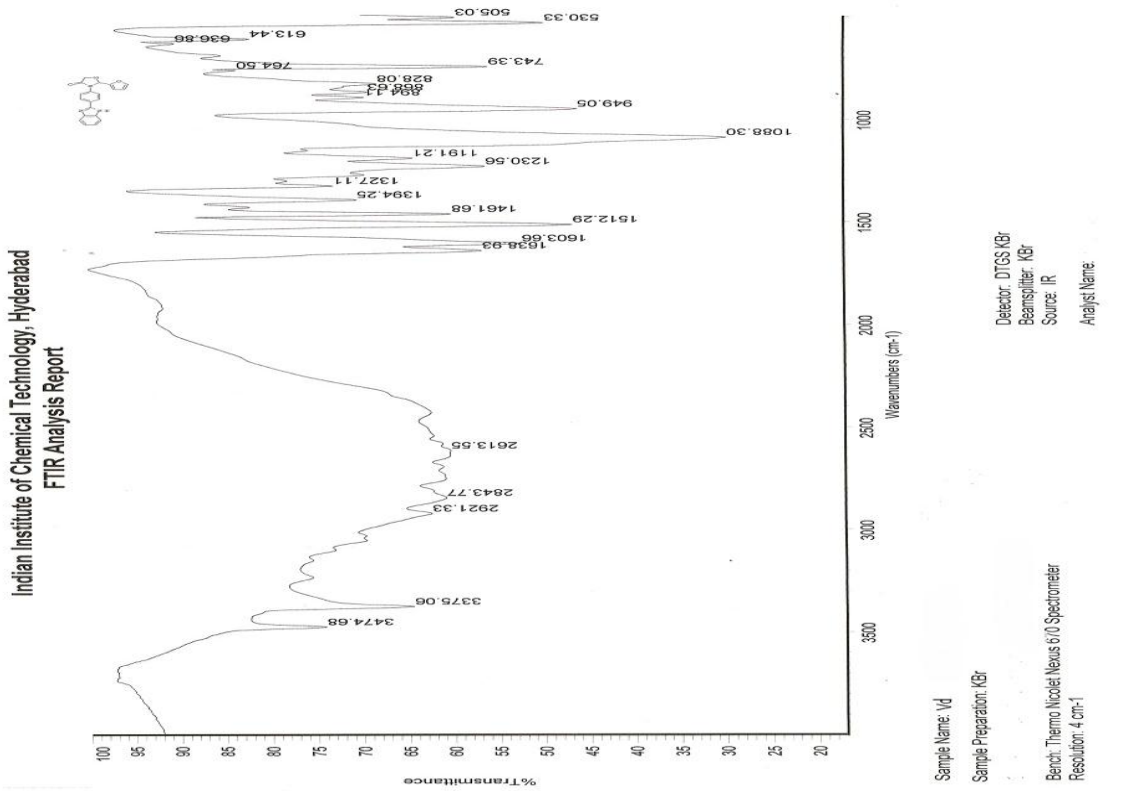
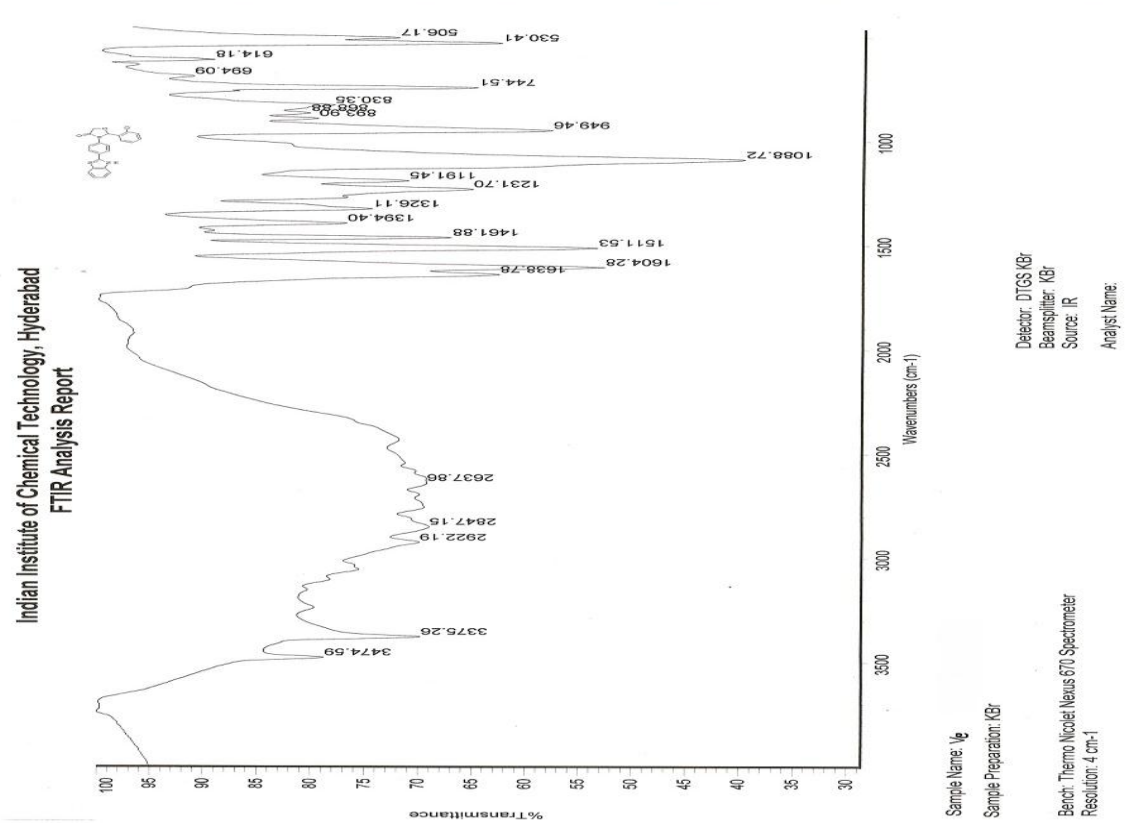


Fig. 5. Spectral data of 3-[4-(1H-benzimidazol-2-yl) phenyl]-2-(2-chlorophenyl)-1, 3-thiazolidin-4-one



Antimicrobial Screening

A drug which kills or inhibits the growth of microbes is known as antimicrobial agent. Invitro tests are used as screening procedure for new agents to test the susceptibility of individual isolates from infections to determine which of the available drugs might be useful therapeutically. Due to development of sulphonamides and penicillin's invitro measurement of susceptibility of microbes to chemotherapeutic agents have been used [12]. A drug is considered to be bacteriostatic or fungistatic when they inhibit the growth of bacteria or fungi respectively, and bactericidal or fungicidal due to its ability to kill bacteria or fungi. Important factors for antimicrobial activity are size of the inoculum, metabolic state of microbes, pH, temperature, and duration of interaction, concentration of inhibitor and presence of interference substances. The development of resistance among various pathogenic microbes towards antibiotics has increased the impetus for investigating new antimicrobial agents. When a compound was found to have positive therapeutic index, a new series of related compounds are synthesized in the hope that one of them would be more effective than the existing one. Antibacterial activity was carried out on four bacterial strains of which two are gram positive and other two are gram negative bacteria: *Bacillus Subtilis, Staphylococcus Aureus, Escherichia Coli,* and *Pseudomonas Aeruginosa.* Antifungal activity was carried out on two fungal strains:*Candida Albicans* and *Aspergillus Niger.*

Disc diffusion method

Antibacterial Activity

All the compounds synthesized have shown potent to weak antibacterial activity. Compound Vb shows good activity against *Bacillus Subtilis* and *Staphylococcus Aureus* when compared with standard [13]. Compound Vd shows good activity against *Pseudomonas Aeruginosa.* Compound Vc shows moderate antibacterial activity. Compounds Va and Ve show weak antibacterial activity when compared to standard. The results are given in the following table 11.

Anti fungal activity

From the studies it was evident that the synthesized compounds show potent to moderate anti fungal activity. Compounds Ve and Vd show good anti fungal activity against *Candida albicans* and *Aspergillus niger* when compared with standard. Compounds Vb and Vc show moderate antifungal activity [14,15]. Compound Va shows weak antifungal activity in comparison with standard. Results are given in the following table 12.

CONCLUSION

This thesis deals with the Synthesis, Characterization and antimicrobial screening of 3-[4-(1h-benzimidazol-2-yl) phenyl] substituted 1, 3-thiazolidine-4-ones. The synthesized compounds were characterized by IR, H^1 NMR and Mass Spectra. The first chapter of this thesis deals with a brief introduction to therapeutic agents based on "Benzimidazole" and "Thiazolidine" related moieties. The second chapter consists of particular literature survey on the investigation carried out by the earlier workers in the synthesis and evaluation of heterocyclic compounds based on "Benzimidazole" and "Thiazolidine" moieties.

The third chapter explains the scope and object of the present investigation in detail. It explains how benzimidazoles and thiazolidine-ones are important in their structural feature for biologically active compounds. Structure of "five" novel compounds proposed to be synthesized and investigated in the present work were presented. The fourth chapter explains in detail about the experimental procedures used in the synthesis of compounds, and their antimicrobial activity that are adopted in present investigation. The fifth chapter deals with the results obtained in the present study along with detailed discussion on results supported by reaction scheme, tables and figures.

Important aspects of the present study are out lined as:

1. Good yields of the synthesized compounds were obtained 64 - 74%
2. Compounds were confirmed by IR, H^1 NMR and Mass Spectra.
3. All synthesized compounds exhibited antibacterial and antifungal activities at various MIC levels.
4. Compounds Vb and Vd exhibited good, compound Vc showed moderate and compounds Va and Ve exhibited weak antibacterial activity.
5. Compound Ve shows excellent, Vb, Vc and Vd show moderate, and Va exhibits weak antifungal activities.
6. Compounds Va and Vc exhibited less activity on all bacterial and fungal strains when compared other synthesized compounds.

The synthesized compounds along with antimicrobial activity are believed to exhibit various other activities such as cardiac, antiviral, anti-inflammatory, insecticidal, anthelmintic, antitubercular and anti-diabetic activities. Further work is carried out for the evaluation of cardiac activity.

REFERENCES

1. Wickel JH, Paget CJ, Delong DC, Nelson JD, Wu CYE, RJ Chaney, MO Jones, Chamberrlin JW. *J. Med. chem*., 23, 1980, 368.
2. Rayemaekers AHM, Freyne EJE, Sanz GC. *Eur. patent Appl*., 260, 1988, 744.

3. Janssens F, Luyckx, M, Stokbroekx R, Torremans J. U.S. Patent: 1981, 4219559.
4. Lindberg P, Nordberg P, Alminger T, Braudstrom A, Wallmark B. *J.Med.Chem*., 29, 1986, 1327.
5. Kohl B, Strurm E, Sennn-Billfinger J, Simmon AW, Krueger U, Schaefer H, Rainer G, Figala V, Klemm K. *J.Med.Chem*., 35, 1992, 1049.
6. Actor PP, Pagano JF. Br. Patent: 1968, 1123317.
7. Van Gelder JLK, Rayemaekers AHM, Rovens LFC. *German Offen*, 1971, 2029637.
8. Gyurik RJ, Kingsbury WD. U.S. Patent: 1981, 4258198.
9. Grenda VJ, Jones RE, Gae G, Sletzinger M. *J.Org.Chem*., 30, 1959, 259.
10. Hoff DR, Fischer MH, Bochis RJ, Lusi A, Waksmunski JR, Egerton JR, Yakstis JJ, Cuckler AC, Campbell WC. *Experentia*., 26, 1970, 550.
11. Jerchee D, Fischer H, Kracht M. *Liebigs Annalen der Chemie*., 1952, 575, 173.
12. Hunger A, Kebrle J, Rossi A. Hoffmann K. *Experentia*., 13, 1957, 400.
13. Yu'eu YuK, Dyatlovitskaya SV, Bulavin LG, Zh Obshch. Khim. *Chem. Abstr*., 7c, 1958, 52.
14. Abrahamson S, Westerdhal A, Isaksson G, Sandstrom J. *Acta Chim. Scand*., 21, 1967, 442.
15. Turkerich NM, Petlichnaya LI, Metody Poulch Khim. *Reactive. Prep*., 13, 1971.