International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: https://doi.org/10.7439/ijbar
CODEN: IJBABN **Original Research Article**

Synthesis and biological evaluation of benzimidazole derivatives as an antitubercular and antimicrobial agents

Kale Sachin C.[*1,2] and Kale M.K.[3,]

[1]*M.J.R.P.University, Jaipur, Rajasthan, India*
[2]*Anuradha College of Pharmacy, Chikhli, Dist. Buldhana, Maharashtra, India*
[3]*K.G.R.D. College of Pharmacy and Research Institute, Karjat, Raigad, India*

QR Code

***Correspondence Info:**
Mr. Sachin C. Kale
Assistant Professor,
Anuradha College of Pharmacy,
Chikhli, Dist-Buldhana, Maharastra State, India

***Article History:**
Received: 15/01/2018
Revised: 21/01/2018
Accepted: 21/01/2018
DOI: https://doi.org/10.7439/ijbar.v9i1.4585

Abstract

Heterocyclic Compounds especially with Benzimidazole, Thiophene & Piperazine moieties were synthesized and investigated for their biological activities. The Increasing global tuberculosis burden due to the curse of HIV, MDR and XRD TB has led to the search of newer therapeutic agents to tackle the menance. The benzimidazole, Thiophene, Piperazine are a heterocyclic compounds. It is an important pharmacophores and privileged structure in medicinal chemistry. It plays a very important role with useful therapeutic activity such as antitubercular activity. Literature shows that the benzimidazole, Thiophene, Piperazine derivatives are outstandingly effective compounds and a large number of reviews available for biochemical and pharmacological studies conformed that their molecules are useful against a wide variety of micro-organisms. Because of their importance, the methods for their synthesis have become a focus of synthetic organic chemists. Therefore in the present work study of chemistry of different derivative of substituted benzimidazole, Thiophene, Piperazine as well as various Antifungal, Antibacterial, Antitubercular activities). The structures of these compounds were established by means of IR, 1H-NMR, C^{13} NMR and elemental analysis. All compounds were evaluated for antibacterial, antifungal and antitubercular activities. Most of the compounds have shown significant antibacterial, antifungal and antitubercular activity when compared with the standard drug. QSAR studies were done by using Schrodinger software.

Keywords: Piperazine,Thiophene,Benzimidazole,Antitubercular.

1. Introduction

Tuberculosis is an infectious disease characterized by the growth of nodules (tubercles) in the tissues, especially in the lungs. Bacillus *Mycobacterium tuberculosis* is the etiological agent of TB belonging to the genus *Mycobacterium*, which is presumed to have originated more than 150 million years ago. Tuberculosis (TB) is one of the leading causes of death due to a single infectious organism in the world. It typically affects the lungs (pulmonary TB) but can affect other sites as well (extrapulmonary TB). The probability of developing TB is much higher among people infected with the Human Immunodeficiency Virus (HIV).

The benzimidazole, Thiophene, Piperazine are a heterocyclic compounds. It is an important pharmacophore and privileged structure in medicinal chemistry. It plays a very important role with useful therapeutic activity such as antitubercular activity. Literature shows that the benzimidazole, Thiophene, Piperazine derivatives are outstandingly effective compounds and a large number of reviews available. [1-10]

1.1 Medicinal Chemistry of Benzimidazole

Benzimidazole is an important pharmacophore and a privileged structure in medicinal chemistry. This compound is bicyclic in nature which consists of the fusion of benzene and imidazole. The most prominent benzimidazole compounds in nature are N-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin B12 Benzimidazole is a heterocyclic

aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole. Heterocyclic compounds are occupied prominent place among various classes of aromatic organic compounds. Benzimidazole are having a variety of therapeutic uses including antitumor, antifungal, antiparasitic, analgesics, antiviral, antihistamine, as well as use in cardiovascular disease, neurology, endocrinology, and ophthalmology and Antitubercular activity was studied by Shingalapur *et al.* Synthesized series of novel 5-(nitro/bromo)-styryl-2-benzimidazoles derivatives and screened for *in-vitro* anti-tubercular activity against Mycobacterium tuberculosis, and these compounds showed good antitubercular activities. Streptomycin was used as reference drug. [2]

1.2. Medicinal Chemistry of Piperazine.

Piperazine consists of a six-member ring containing the two opposing nitrogen atoms was originally named because of its chemical similarities with piperidine, a constituent of piperine in the black pepper plant (*Piper nigrum*). It is a weak base with pKa of 4.19 and freely soluble in water and ethylene glycol but insoluble in diethyl ether.

Linas *et al* was studied on, A convenient synthetic route for preparation of various 4-[4-(1H-indol-3-yl)butyl]piperazines bearing heterocyclic and aliphatic substituents in position 1 has been developed. During this work some synthetic possibilities of common precursor, 4-[4-(1H-indol-3-yl) butyl] piperazine, were studied and evaluated. [3-4]

1.3 Medicinal Chemistry of Thiophene

Thiophene is a heterocyclic compound aromatic in nature consisting of four carbon atom and one sulfur atom in a five member ring compound analogues to thiophene includes furan, and pyrrole.

Sharda *et al* Synthesized 1, 2, 5, selenadiazolo (3, 4-e) benzo (b) Thiophenes and its 7-nitro derivatives. [5-7]

1.4 Flux Balance Analysis of Mycolic Acid Pathway: Targets for Anti-Tubercular Drugs

Karthik *et al* [3] in 2005 was studied on; Mycobacterium tuberculosis is the focus of several investigations for design of newer drugs, as tuberculosis remains a major epidemic despite the availability of several drugs and a vaccine. Mycobacteria owe many of their unique qualities to mycolic acids, which are known to be important for their growth, survival, and pathogenicity. Mycolic acid biosynthesis has therefore been the focus of a number of biochemical and genetic studies.

1.5 Current & Future Developments [4]

The worldwide problem caused by TB and the lack of new drugs in the market makes it imperative to have new drugs to fight efficiently against the rapid spread of multi-drug resistant TB strain against all major antituberculosis drugs in the market. In this context, there is an urgent need for TB drugs with fewer toxic side effects, improved pharmacokinetics properties, extensive and potent activity against Gram-positive and Gram-negative bacteria, including resistant strains and drugs able to reduce the total duration of treatment.

1.6 Anti-TB Drugs

Nowadays, there are different classes of compounds under research and development to obtain new drugs against TB. For example, thiolactomycin and analogs, ethambutol analogs, mefloquine and analogs, deazapteridines, 9-benzylpurines, benzoxazines, diterpenoids, imidazo (4,5-c)pyridines, tryptanthrin and analogs, clofazimine and others phenazines, 1,2,4 triazoles, isoniazid analogs, fulleropyrroli-dines, toluidine derivatives, saccharides, quinolones, oxazolidinones and miconazole analogues, as well as the natural product calanolide. Also new classes with TB activity, such as pyrrolidine-2, 5-dione and piperidine-2, 6-dione derivatives, sulpho compounds, halogenated *p*-aminosalicylic acid and thioacetazone and *p*-guanidinosalicylate sodium hydrochloride.

Table 1: Essential Antituberculosis Drugs

Drugs Abbreviations	Recommended dosage (dose range) in mg/Kg Daily	Brand Names
Isoniazid (I)	05 (4-6)	3Fd Tablet, 4D
Rifampicin (R)	10 (8-12)	Rimactane (150, 300, 450 mg)
Pyrazinamid (P)	25 (20-30)	Actizid (1000mg)
Ethambutol (E)	15 (15-20)	Myambutol

2. Material and Methods [11,12]

Melting points were determined in open capillary method and are uncorrected. IR spectra were recorded on Thermo Nicolet IR 200 spectro-photometer using KBr disc method. The 1H-NMR spectra were recorded on sophisticated multinuclear FT-NMR Spectrometer model Avance-III (Bruker), using dimethylsulfoxide-d6 as solvent and tetramethylsilane as internal standard.

A] Procedure for Scheme 1

i) General method for preparation of substituted-cinnamic acids {CA1-CA8}: (Doebners Method) {Common Procedure for Scheme I & Scheme II}

Differently Substituted Aromatic aldehyde (0.02 mole) and malonic acid (0.42 mole) was dissolved in a mixture of dry pyridine (75 ml) and piperidine (1.3 ml) was added. The reaction mixture was heated under reflux for 2 hrs. A rapid evolution of carbon dioxide took place. Cooled, poured into excess of water containing hydrochloric acid (1N) to combine with pyridine. The solid that separated was filtered and recrystallized from hot water [10-14]. Yield -91%, m.p. -134-135^{0}C.

ii) Differently substituted Synthesis of 2-(2-Phenyl ethenyl) – 1 H- benzimidazole: {Common Procedure for Scheme I & Scheme II}

A mixture of subtituted aryl cinnamic acid (0.05 mol) and substituted o-Phenylenediamine (0.05 mol) was treated with 4 N Hydrochloric acid and stirred at room temperature for 1hour, Until it goes into solution. The reaction mixture refluxed further for 4-6 hours cooled and neutralized with dilute ammonia. The precipitate that separated was filtered and washed with water and crystallized from methanol to get solid crystal of pure (BZ1) [11-15] yield - 96%, m.p. –119-120^{0}C.

iii) Synthesis of substituted 2-(2-Phenyl ethenyl) – 1 carboxy- benzimidazole {BC1-BC8}: {Procedure for Scheme 1}

A mixture of differently substituted 2 (2-Phenyl ethenyl)–1H benzimidazole (0.01 mol) and chloroacetic acid (0.01mol) in 30 ml of dry benzene was refluxed for 7-8 hour using dean stark apparatus. The residue was washed with Sodium bicarbonate solution and the product was washed with water thoroughly and crystallized from alcohol to get solid crystals of pure (BC1-BC8) [12-13]. Yield-82%, m.p. – 125-126^{0}C.

iv) Synthesis of differently substituted 5-(1 carboxy-benzimidazole-2yl)-4-phenyl dihydrothiophen 3(2H) one {BT1-BT8}

A mixture of differently substituted 2 (2-Phenyl ethenyl)–1 Carboxy benzimidazole (0.01 mol) and thioglycolic acid (0.01mol) in 30 ml of dry benzene and pinch of anhydrous Zinc chloride added and was refluxed for 7-8 hour using dean stark apparatus. The residue was washed with Sodium bicarbonate solution and the product was washed with water thoroughly and crystallized from alcohol to get solid crystals of pure (BT1-BT8) .Yield - 80%, m.p.142-143 ^{0}C.

B] Procedure for Scheme 2

i) General method for preparation of substituted-cinnamic acids {CA1-CA8}: (Doebners Method) {Common Procedure for Scheme I & Scheme II}.

ii) Differently substituted Synthesis of 2-(2-Phenyl ethenyl) – 1 H- benzimidazole:{Common Procedure for Scheme I & Scheme II}.

iii) Synthesis of 5-(1H-benzo[d]imidazol-2yl)-4-phenyldihydrothiophen-3(2H)-one {BTU1-BTU8} synthesized by addition of Thioglycolic acid to previous step compounds as per SchemeI.

iv) Synthesis of 5-(1-(2-(piperazin-1-yl) ethyl-1H benzo[d]imidazol-2 yl)-4-phenyl dihydrothiophen benzo[d]imidazol-2-yl)-4-phenyl dihydrothiophen 3(2H)-one {BTP1-BTP8}: Synthesized by addition of piperazine and 1-bromo-3-chloro ethane to previous step compounds as per Scheme I.

v) Compounds Coded **(BTPB1-BTPB4)** were synthesized by using Starting material as 5-(1-(2-(piperazin-1-yl) ethyl-1H benzo[d]imidazol-2 yl)-4-phenyl dihydrothiophen benzo[d]imidazol-2-yl)-4-phenyl dihydrothiophen 3(2H)-one. Where differently substituted aromatic benzene were added as per Scheme 1.and Compound coded **(BTPB5)** was synthesized from 5-(5-fluoro-1-(2-(piperazin-1-yl) ethyl-1H benzo[d]imidazol-2 yl)-4-(4-phenyl) dihydrothiophen 3(2H)-one. As starting material and addition of aromatic benzene as per scheme I.

Spectral Data of (BTPB1-BTPB5):

BTPB1 : IR(KBr) cm^{-1}: 3005(Ar-Cl), 3223(-NH Str), 2919(-Ar-C-H Str), 1672(C=0 Str), 1448 (C-N Str), 949(-Ar).461(CH_2 def).1H NMR ($CDCl_3$) : 4.29-4.42 (2H Methine), 3.44-3.83(6H of Methylene), 7.50-7.59 (4H of Benzimidazole), 7.27-7.40(5H of Benzene). C_{13} NMR: 129.1(C_1 Aromatic), 134.7, (C_4 Aromatic), 67.1(C_5 of Methine), 36.1(C_6 of Methine), 43.1(C_7 Aliphatic), 207.1 (C_8 of Carbonyl), 141.5(C_9 of Benz), 123.4(C_{12} of Benz.), 43.9 (C_{13} Methylene), 61.9(C_{15} of Cyclohexane), 129.9 (C_{20} Aromatic ring).

m.p. 180-182°C, yield 67%, mol. Wt. 544.45, $C_{29}H_{25}N_4ClOS$ (Found C: 63.90 H:4.52 N: 10.20 required C : 63.91 H: 4.59 N : 10.28).

BTPB2: IR(KBr) cm^{-1}: 3223 (-NH Str), 2919(Ar-C-H Str), 1672(-C=O Str), 1448(-C-N Str), 1005(-Ar), 461 CH_2 def), 1H NMR ($CDCl_3$): 4.29-4.40(2H of Methine), 2.86-3.83(6H of Methylene), 7.22-7.59(4H of Benzimidazole), 6.79-7.27(5H of Benzene). m.p. 276-277°C, yield 69%, mol. Wt. 510, $C_{29}H_{26}N_4$ O S. (Found C: 68.20 H:5.02 N:10.92 required C : 68.23 H: 5.09 N : 10.98).

BTPB3: IR (KBr) cm^{-1}: 3223(-NH Str), 2919(-Ar-C-H Str), 1530(N-O Str), 1672(-C=O Str), 1448(-C-N Str), 1340(-N-O Str), 749(-Ar), 461(CH_2 def). 1H NMR ($CDCl_3$): 4.29-4.42(2H of Methine), 2.97-3.83(6H of Methylene), 7.22-7.59 (4H of Benzimidazole), 7.02-8.08(4H of Benzene). m.p. 287-288°C, yield 69%, mol. Wt. 523., $C_{29}H_{25}N_5O_3S$ (Found C: 66.50 H:4.75 N:11.42 required C: 66.53 H: 4.78 N: 11.47).

BTPB4: IR (KBr)cm^{-1}: 3223(-NHStr), 2919(-Ar-C-HStr), 1672(C=OStr), 1448(C-NStr), 980(-Ar), 461(CH_2def) .

1H NMR ($CDCl_3$): 4.29-4.42(2H of Methine), 2.97-3.83(6H of Methylene), 7.22-7.59 (4H of Benzimidazole), 7.02-8.08(4H of Benzene). m.p. 273-274°C, yield 71%, mol. Wt. 478. $C_{29}H_{26}N_4OS$. (Found C: 72.75 H: 5.42 N: 11.69 required C: 72.80 H: 5.43 N: 11.71).

BTPB5: IR(KBr) cm^{-1}: 4352(-OH Str), 3207(NH Str), 2920(Tert. nitrogen), 1552(C-NO_2 Str), 1433(-C-N Str), 1150(-C-F), 970(-Ar), 461(-CH_2 def), 1H NMR: 4.29-4.42 (2H of Methine), 2.87-3.83 (6H of Methylene), 6.97-7.57(3H of Benzimidazole),6.54-7.27 (5H of Benzene). M.P.291-292°C, yield 73%, mol. Wt. 496. $C_{29}H_{25}N_4FOS$ (Found C: 70.10 H: 5.0 N: 11.21 required C: 70.16 H: 5.04 N: 11.29).

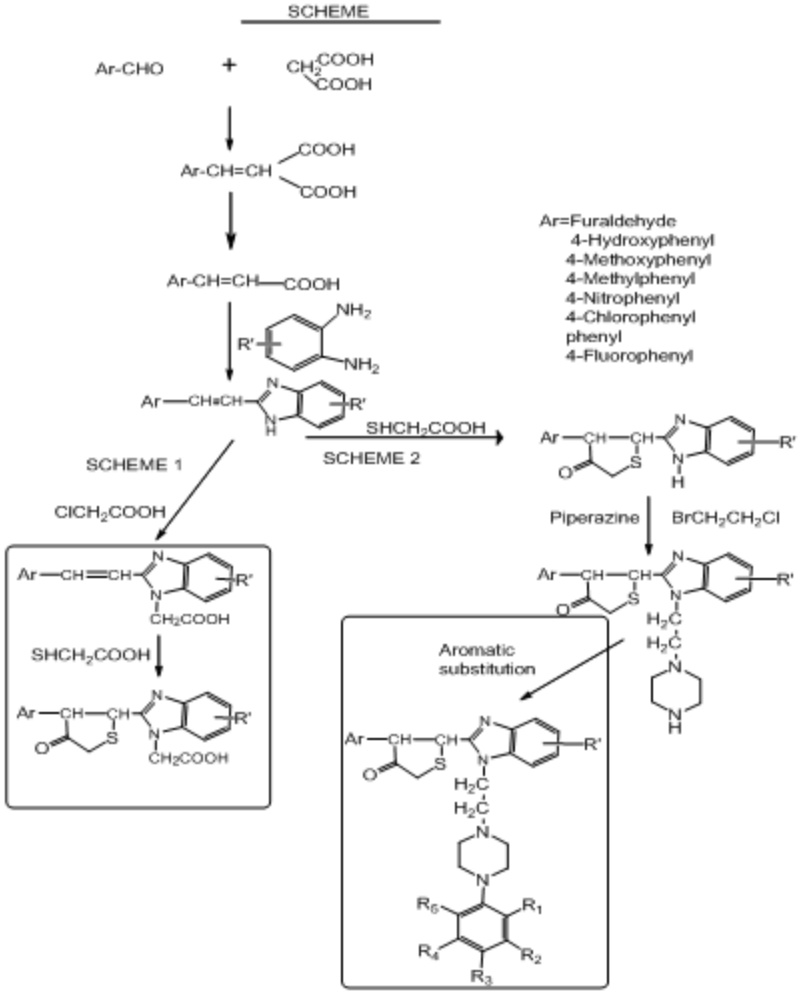


Figure 1: Scheme I and Scheme II

2.1 Antimicrobial activity

The antimicrobial activity of the synthesized compounds was determined by cup-plate method. The antibacterial activity was determined against gram-positive organism *Staphylococcus aureus* and gram-negative organism *Escherichia coli* at 50-mcg/ml and 75mcg/ml concentration of sample compounds. Dimethyl Formanide was used as control. The bacteria were subcultured on nutrient agar broth and incubated at 37^0C for 18-24 hrs. Standard antibacterial drug Ciprofloxacin was also screened under similar conditions at 50-mcg/ml and 75mcg/ml concentration for comparison. The antifungal activity was carried out against the fungi *Candida albicans* and *A. fumigatus* at 50 mcg/ml and 75mcg/ml concentration of sample compounds. The fungi were subcultured in Sabourod's dextrose agar medium. The fungal susceptibility testing was done by cup-plate method using Fluconazole (50 mcg/ml and 75mcg/ml concentration) as standard. The petri dishes were incubated a 37^0C for18-24 hrs.

2.2 Antitubercular evaluation

The antitubercular screening of synthesized compounds was carried out by middle brook 7H9 broth base (M198) medium against. H37Rv strain at100 mcg/ml, 125 mcg/ml and 250mcg/ml. L J Medium containing standard drug as well as control. Middle brook 7H9 broth base (M198) medium was inoculated with mycobacterium tuberculosis of H37Rv strain. The inoculated medium was incubated for 370 C for 6 weeks. At the end of 6 weeks the growth of mycobacterium tuberculosis was read. Streptomycin (100 mcg/ml, 125 mcg/ml and 250mcg/ml) was used as a standard drug.

3. Result and Discussion [13-18]

The title compounds (BC^1-$BTPB^5$) (Thirty seven compounds) were prepared from 2(2-substituted phenyl ethenyl) 1H benzimidazole by various steps. The structures of the compounds were confirmed by spectra and analytical studies. All the compounds synthesized matched with spectral data. The compounds synthesized were screened mainly for the antitubercular activity by using middle brook 7H9 broth base media method using H37Rv strain. Compounds BC_2, BC_4, BC_5, BC_6, BC_8BT_2, BT_4, BT_5, BT_6, BT_8, BTU_2, BTU_4, BTU_5, BTU_6, BTU_8, BTP_2, BTP_4, BTP_5, BTP_6, BTP_8, $BTPB_{2\text{-}5,}$ have shown Very -significant antitubercular activity. Whereas other compounds shown moderate antitubercular activity. H_{37}Rv strain was used as

standard tubercular organism. Streptomycin was used as standard drug.

Compounds BC_5, BC_7, BC_8 and BT_5, BT_8, BTU_5, BTU_6, BTU_8, BTP_4, BTP_5, BTP_6, BTP_8, $BTPB_{1-5}$ have shown significant anti bacterial activity against E-coil, while other compound showed moderate activity. Ciprofloxacin was used as standard drug. Compounds BC_4, BC_6, BC_8, BT_5, BT_6, BTU_4, BTU_5, BTU_6, BTP_7, BTP_8, $BTPB_{1-5}$ have shown significant antifungal activity against *C. albicans*, while other compounds show moderate activity. Fluconazole was used as standard drug. It was noted that the compound which showed antitubercular activity were not good antibacterial and antifungal agents this may be explained on the basis of organism mycobacterial tuberculosis has different cell wall structure compared to bacteria and fungi. All compounds found to be very good antitubercular agents & present synthesized compound can definitely as lead compound for future molecular manipulation studies.

QSAR studies were carried out by using Schrondinger Software a) Bioluminate 2014 b) Maestro 2014 c) Material Science Suit 2014.

Figure 2a: *In-vitro* testing of compounds for their antibacterial activity against *Escherichia coli* (NCTC 10418).

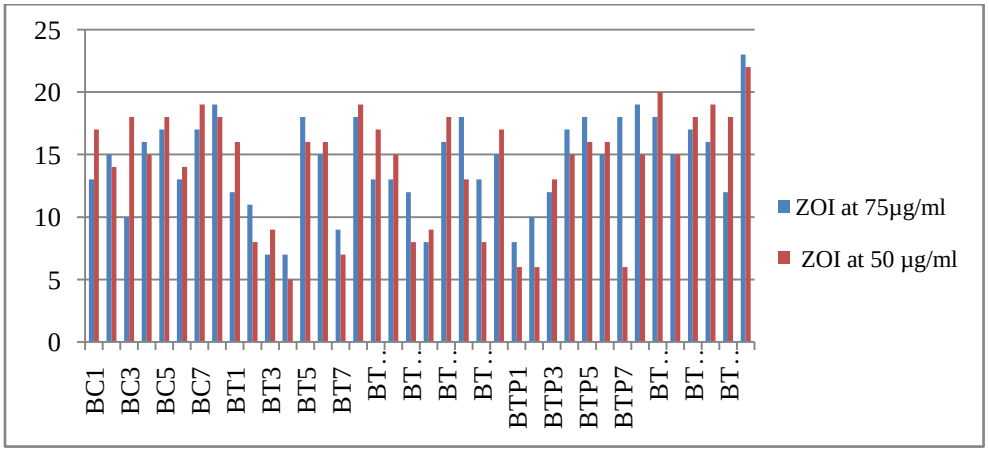


Figure 2b: *In-vitro* testing of compounds for their antibacterial activity against *Staphylococcus aureus* (NCTC 6571)

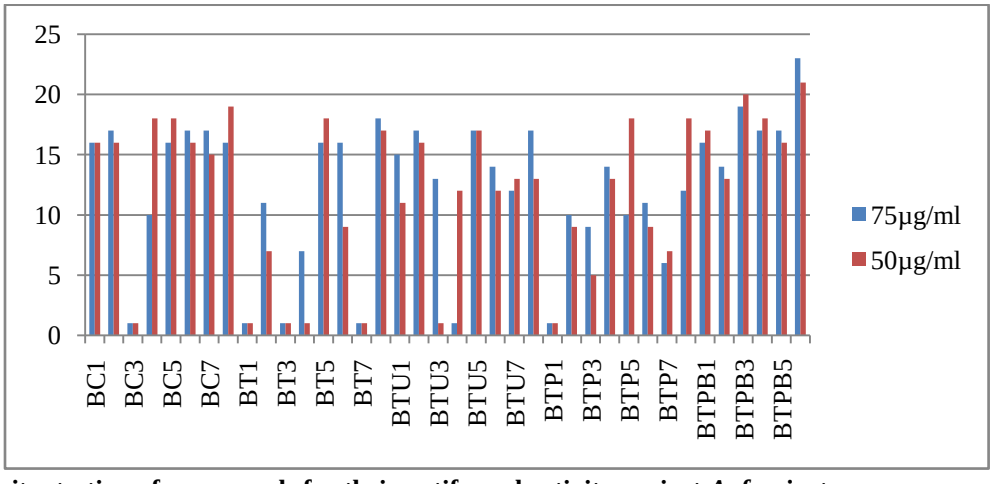


Figure 2c: *In-vitro* testing of compounds for their antifungal activity against *A. fumigatus*

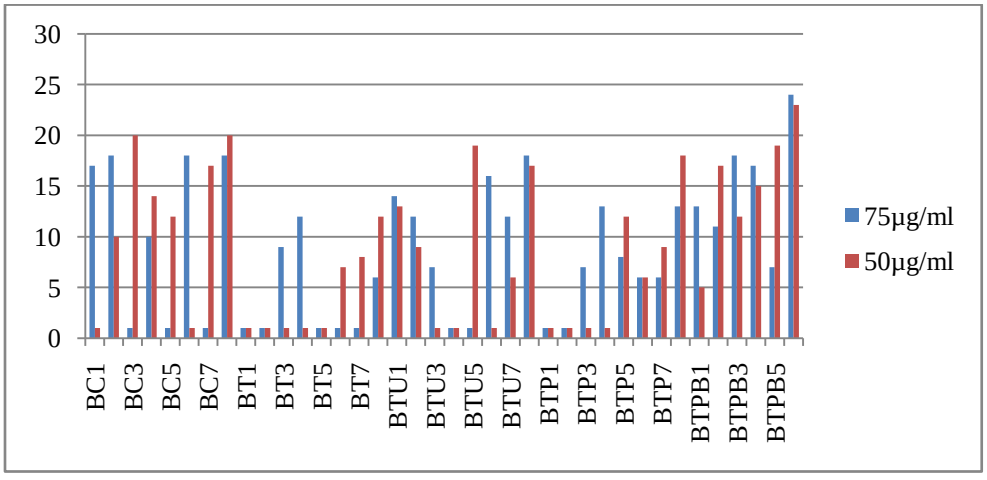


Figure 2d: *In-vitro* testing of compounds for their antifungal activity against *Candida albicans* (ATCC 10231).

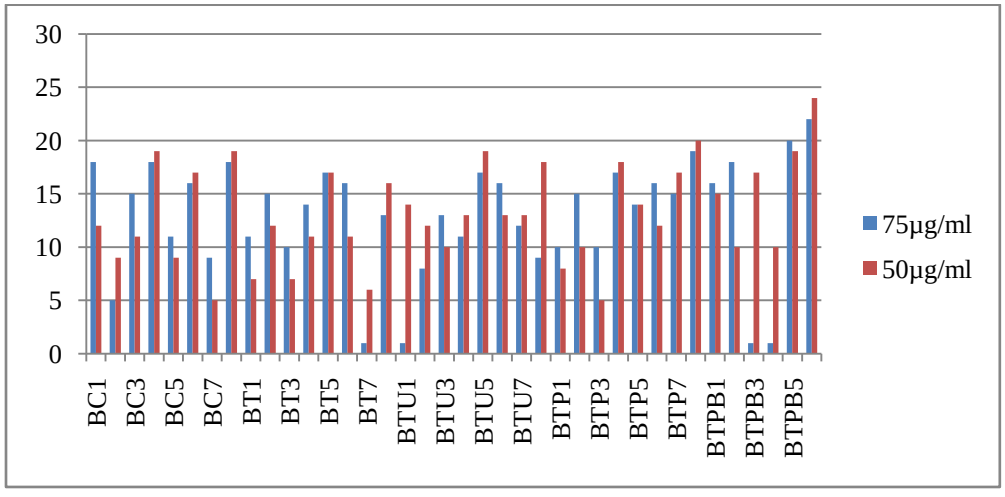
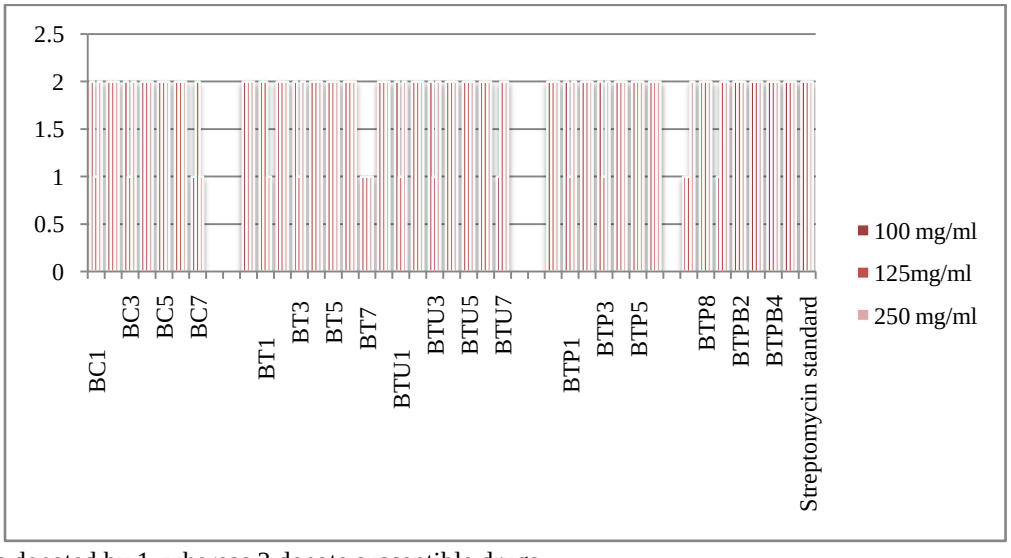
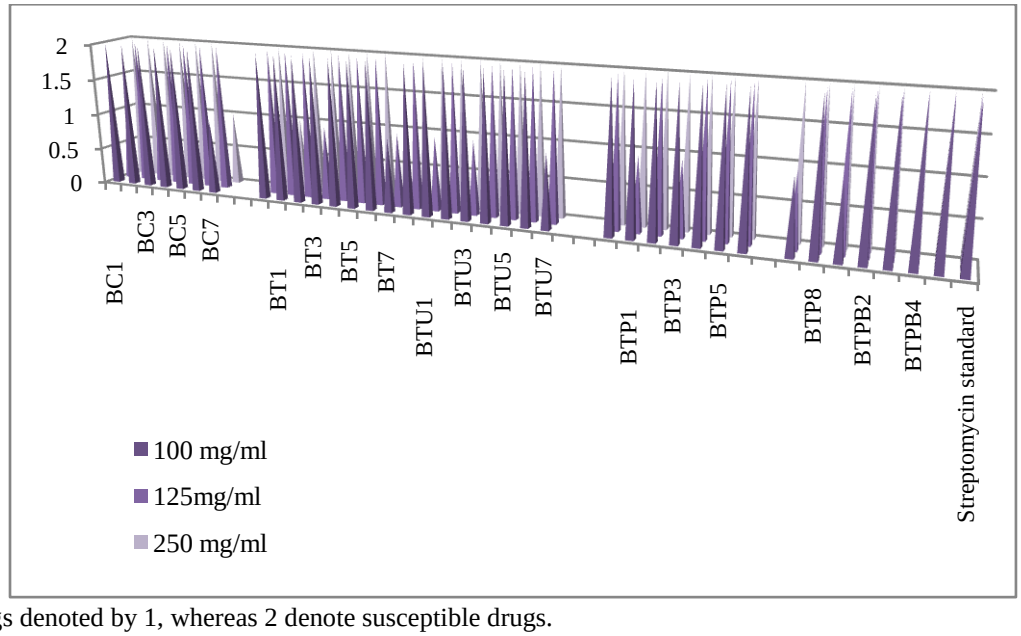


Figure 3a: Antitubercular Activity of Synthesized Compounds



Resistant drugs denoted by 1, whereas 2 denote susceptible drugs.

Figure 3b: Antitubercular Activity of Synthesized Compounds



Resistant drugs denoted by 1, whereas 2 denote susceptible drugs.

QSAR Study using Software (Schrondinger)

A) Bioluminate 2014

B) Maestro 2014

C) Material Science Suit 2014.

Figure 4a: Material Science study

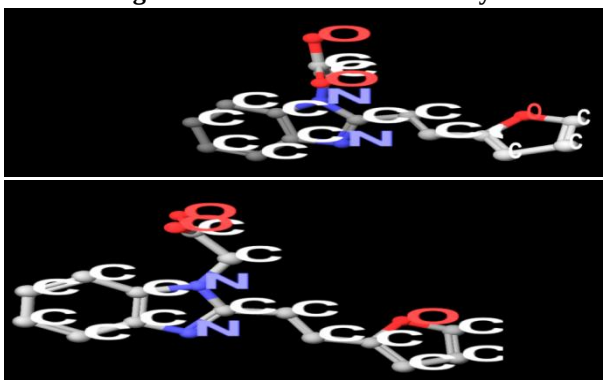


Figure 4b: Bioluminate compound BT

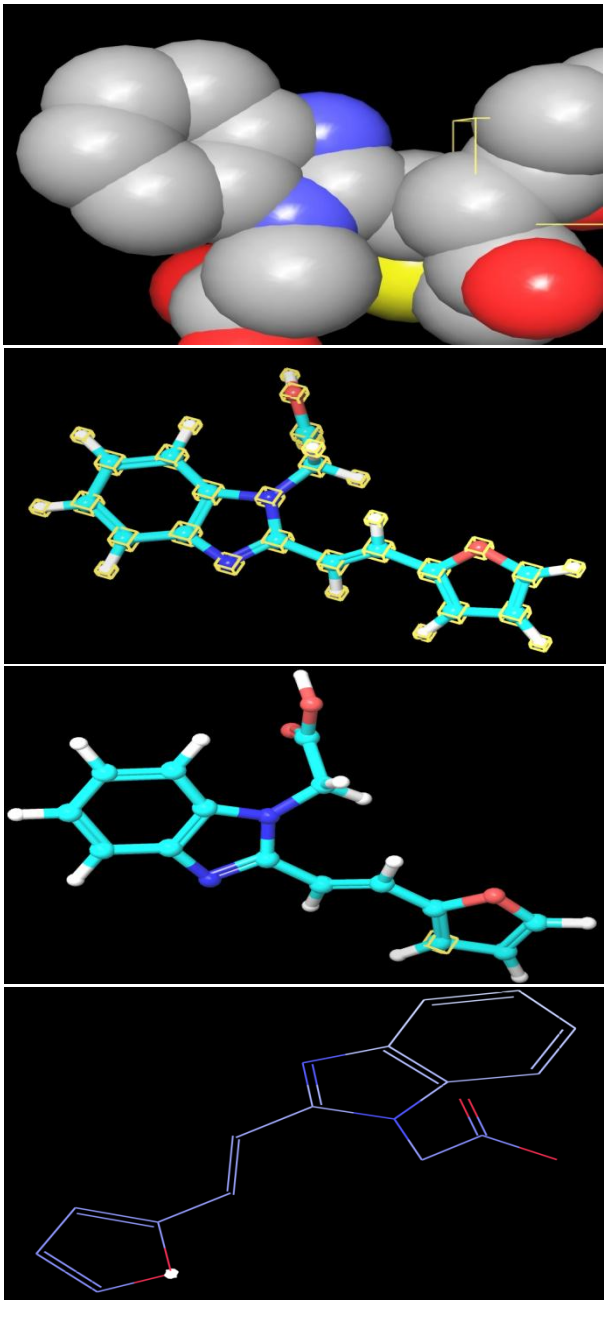


Figure 5: Ramchandran Plot for BT1

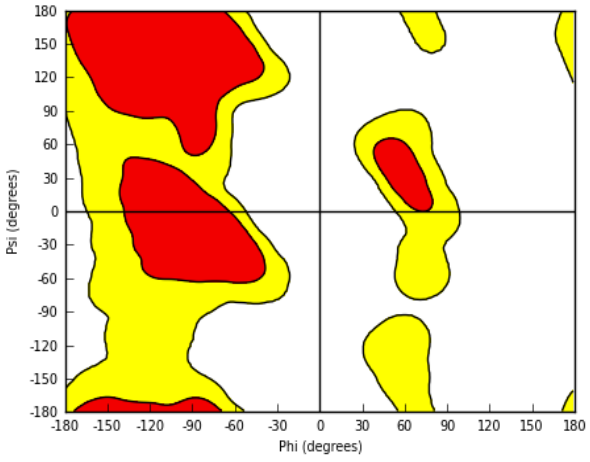


Figure 6a: BC 1 Compound Stereo View- numbered

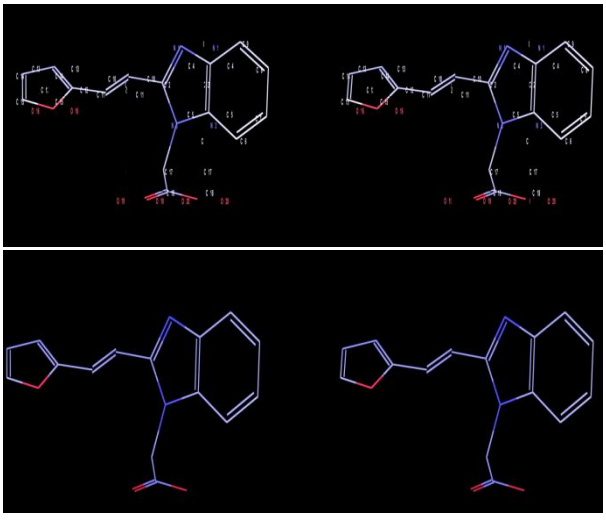


Figure 6b: BC1 (3D Structure Scupling)

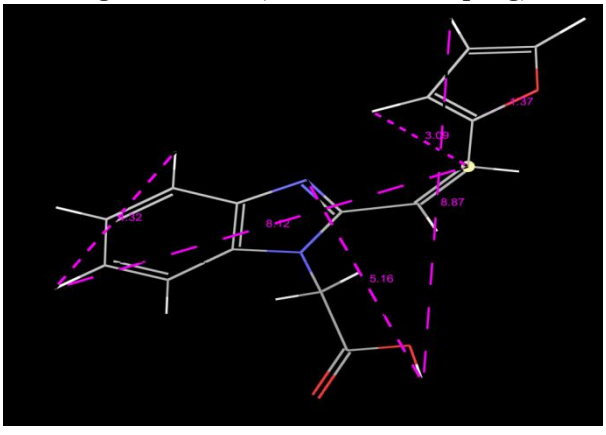
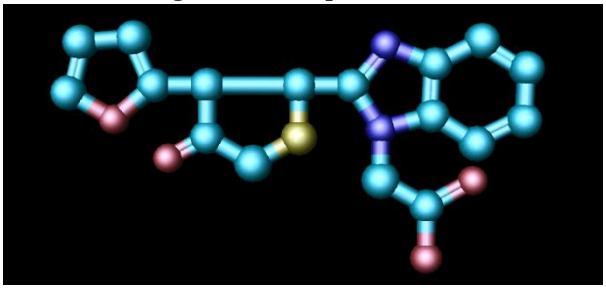


Figure 6c: Compound BT1



4. Conclusion [19-22]

Literature survey also reveals that benzimidazole ring containing molecules are potent growth inhibitors over a wide range of bacteria and fungi. Synthesis and evaluation of differently substituted benzimidazole analogs resulted in the discovery of some important drugs viz. omeprazole, lansoprazole, rabeprazole and pantoprazole.

It is also observed that benzimidazole derivatives are found to possess anti-HIV, anthelmintic, antimycobacterial, antidiabetic and antioxidant properties. Several biological properties exhibited by different benzimidazole scaffolds include antiallergic, analgesic and anti-hypertensive activity. The literature survey helped to understand the versatility of benzimidazole and its high potency as a therapeutic agent.

Benzimidazole analogs bearing electron-withdrawing as well as electron-donating substituent were synthesized, in order to achieve bioactive molecules with significant antimicrobial property. The desired compounds were prepared by multi-step synthesis process. The formation of intermediates and their corresponding derivatives was confirmed by spectral characterization such as ^{1}H NMR, C13 NMR, IR and elemental analysis. The compounds were screened for their antimicrobial properties. From the SAR studies data; it was observed that the derivatives with electron-withdrawing functional groups were more bio-active than that with electron-donating functional groups.

References

[1]. Maruthamuthu, Shameela Rajam, Christina Ruby Stella P., Bharathi Dileepan A. G. *et al.* 'The chemistry and biological significance of imidazole, benzimidazole, benzoxazole, tetrazole and quinazolinone nucleus'; *JCPR* 2016; 8(5): 505-526.

[2]. Linas Labanauskas, Rita Mazeikaite, Gintaras Urbelis, Olga Gedrimaite *et al.* 'Synthesis of 1- substituted 4-[4-(1H-indol-3-yl) butyl]piperazines'. *ARKIVOC* 2013; (iv): 363-376.

[3]. Karthik Raman, Preethi Rajagopalan, Nagasuma Chandra *et al.* Flux Balance Analysis of Mycolic Acid Pathway: Targets for Anti-Tubercular Drugs, *PLoS Computational Biology*, October 2005; 1 (5): 349-358.

[4]. Marcus Vinícius Nora de Souza *et al.* 'Promising Drugs against Tuberculosis. Recent Patents on Anti-Infective Drug Discovery'. *Current Organic Synthesis* 2006; 1: 33-44.

[5]. Sharada K, Makrandi JK, Goel VK., synthesis of 1, 2, 5-selenadiazolo [3,4-c] benzo [b] thiophene and its derivatives. *Ind. J. Hetero Chem*, 2003; (13): 181-182.

[6]. Jadhav G.R., Shaikh M.V, Kale R.P. *et al.* SAR study of clubbed [1,2,4]-triazolyl with fluorobenzimidazoles as antimicrobial and antituberculosis agents. *Eur. J. Med. Chem* 2008; 32:1-16.

[7]. Tripathi KD. Essential of Medical Pharmacology 5th edition, Jaypee publishers. New Delhi 2003:698 - 699.

[8]. Rajendra Prasad,Management of Multi-Drug Resistant Tuberculosis: Practitioner's view point. *Ind. J. Tuber.*, 2007; 54:3-11.

[9]. Yadav R, Srivastava SD, Srivastava SK., Synthesis antimicrobial and anti-inflammatory activities of 4-oxothiazolidines and their 5-arylidines. *Ind. J. Chem* 2005; 44(B): 1262-1266.

[10]. Fatma E.Goda, Atif S, *et al.* Synthesis, biological evaluation and molecular modeling investigation of new benzimidazole analogs as antiviral agents. *Saudi Pharm. Journal* 2008; 16:103-111.

[11]. A R Prasad, A Narashima Rao, fused thiazoles: synthesis and antifungal activity of 2-thiazolo[3',2':1,2]imidazo[5,4-b] pyrindin-3(2H)-ones, 2-arylidenethiazolo-[3,2-a] benzimidazol-3(2H)-ones and 6-arylidenethiazolo[3,2-b]-s-triazol-5(6H)-ones. *Ind. J. Chem* 1986; 25 B: 776-778.

[12]. Agarwal Alka, Agarwal Shiv K., Bhankuni D. S. antiparasitic agents: Part XVI -synthesis of 5(6)-substituted benzimidazole-2-carbamates as anthelminitic agents. *Ind J. Chem* 1993; 32B: 413-456.

[13]. A. L. Barry, "The antimicrobial susceptibility test: principle & practices", edited by Illus Leu & Febiger, Philadelphia, Pa. USA, 180; *Biol. Abstr* 1976; 64: 25783.

[14]. Elmer W K, Stephen D A, William M J, Paul C S, Washing Jr C W, Text Book of Diagnostic Microbiology, 5th ed ; Lippincot- publishers ; 2002.

[15]. Agrawal RK, Sharma S., A new synthesis of substituted benzimidazole-2-ones. *Ind. J. Chem*, 1982; 21 (B): 967-968.

[16]. Vijayender KR, Mogilaiah K, Sreenivasulu B., Synthesis of 2-(2-amino-2-pyridyl) benzimidazoles. *Ind. J. Chem*, 1984; 23 (B): 866-867.

[17]. Katoch VM. New generation methods for drug susceptibility testing for tuberculosis. *Ind. J.* Tuberc. 2008; 55:61-63.

[18]. Kokare, C. C. In Pharmaceutical Microbiology, 4th Edn, Nirali Prakashan, 2007: 1.1- 1.11, 2.1.

[19]. Robert Fd. Wilson and Gisvolds Text book of organic medicinal and pharmaceutical chemistry, 8th Edn, JB Lippincott Company, Philadelphia 1996: 354-375.

[20]. Goodman Gilman, Alfred and McGraw-Hill, the Pharmacological Basis of Therapeutics, McGraw-Hill Medical Publishing Division, 2001: 1284.

[21]. Bhat A. R, Kale S.C. *et al* synthesis of some new thiophene derivatives and evaluation for their antimicrobial and antitubercular activities. *Indian Drugs* 2009; 46(11): 51-55.

[22]. Sandhyali M.S, Mohan S, Kale R, synthesis of some -2-substitute amino 3(N-P- tolyl Carboxamido)-4,5, trimethylene thiophene as potent NSAIDS *Ind. J. Hetero. Chem.* 2004; (13); 193-196.