Synthesis and biological evaluation of Substituted 2-Alkyl/Aryl Benzimidazole Derivatives

Ashish Singh* Dr.Reetesh Yadav[1], Dr.Deepak Patel[2], Dilend Patle[3]
Shri Ram Institute of Pharmacy Jabalpur, Madhya Pradesh, India

Abstract- Benzimidazole and its derivatives represent an important class of heterocyclic compounds due to their wide range of pharmacological activities. In the present study, a series of novel substituted 2-alkyl/aryl benzimidazole derivatives were designed and synthesized to explore their biological potential. The target compounds were synthesized via condensation of *o*-phenylenediamine with appropriate alkyl/aryl carboxylic acids or their derivatives under suitable reaction conditions. The synthesized compounds were purified and structurally characterized using spectroscopic techniques such as FT-IR, 1H NMR, 13C NMR, and mass spectrometry.

The biological evaluation of the synthesized benzimidazole derivatives was carried out to assess their antimicrobial activity using standard in-vitro assay methods. Several compounds exhibited significant biological activity when compared with standard reference drugs, indicating that substitution at the 2-position of the benzimidazole nucleus plays a crucial role in enhancing biological efficacy. The results suggest that these newly synthesized 2-alkyl/aryl benzimidazole derivatives may serve as promising lead compounds for further optimization and development of novel therapeutic agents.

Keywords- Benzimidazole derivatives; 2-alkyl/aryl substitution; Heterocyclic compounds; Synthesis; Spectral characterization; Biological evaluation; Structure–activity relationship

I.INTRODUCTION

Benzimidazole and its derivatives have attracted significant scientific interest for over a century due to their diverse biological activities. This interest was further intensified following the discovery that 5,6-dimethylbenzimidazole is a structural component of vitamin B_{12}. Although vitamin B_{12} promotes bacterial growth, it was later found that the benzimidazole nucleus and many of its derivatives exhibit antibacterial properties. This observation led to extensive research aimed at developing new benzimidazole-based antimicrobial agents, resulting in several derivatives being successfully commercialized as active pharmaceutical ingredients.
Benzimidazole derivatives possess a wide spectrum of pharmacological activities, including anthelmintic, antithrombotic, antipsychotic, analgesic, antihypertensive, antifungal, antihistaminic, antiemetic, antiulcerative, anticancer, antiviral, antimicrobial, anti-HIV, anti-inflammatory, and antitumor activities. Among them, benzimidazol-2-thiol and 2-mercapto benzimidazole derivatives have received considerable attention due to their potent anti-ulcer, antimicrobial, anti-inflammatory, anticancer, and antihyperlipidemic activities.

Antifungal and Antibacterial Significance
Benzimidazoles constitute one of the most important classes of systemic fungicides currently used for the control of fungal diseases. Compounds such as benzimidazole, 2-methylbenzimidazole, thiabendazole, and carbendazim have demonstrated strong activity against pathogenic fungi including *Candida albicans* and *Aspergillus fumigatus*. The incidence of fungal infections has increased markedly, particularly among immunocompromised patients such as those undergoing chemotherapy, organ transplantation, or suffering from HIV/AIDS. The emergence of resistant fungal strains and the limited efficacy of existing antifungal agents necessitate the development of new and more effective antifungal drugs.
Similarly, the rapid emergence of antibiotic-resistant bacteria, including multidrug-resistant strains such as MRSA and VRE, poses a serious global health threat. Resistance mechanisms such as target modification, enzymatic drug inactivation, efflux pumps, and ribosomal protection have reduced the effectiveness of many existing antibiotics. Consequently, there is an

urgent need for novel antimicrobial agents with new mechanisms of action. Benzimidazole derivatives have shown promise in this regard, including inhibition of bacterial biofilm formation and interference with bacterial cell division proteins such as FtsZ.

Antitubercular Potential

Tuberculosis remains one of the leading causes of death from infectious diseases worldwide, aggravated by drug resistance and co-infection with HIV. The lack of new antitubercular drugs over recent decades highlights the urgent need for novel therapeutic agents. Studies have demonstrated that substituted benzimidazoles, particularly those bearing 2-substituents and halogenated benzene rings, exhibit significant antimycobacterial activity, making the benzimidazole scaffold a promising platform for antitubercular drug development.

Introduction to Antioxidants

Free radicals are highly reactive species generated during normal metabolic processes or introduced through environmental factors such as pollution and tobacco smoke. These reactive oxygen species can damage lipids, proteins, enzymes, and DNA, contributing to aging and various diseases. Although the body possesses endogenous antioxidant defense mechanisms, excessive free radical production can overwhelm these systems, leading to oxidative stress.

IIMATERIALS AND METHODS

Materials

o-Phenylenediamine, substituted aliphatic and aromatic carboxylic acids aldehydes, acetic acid, solvents such as ethanol, methanol, chloroform, dichloromethane, ethyl acetate, and petroleum ether were obtained from commercial suppliers and used without further purification. Analytical grade reagents and solvents were used throughout the study.

Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated plates and visualized under UV light (254 nm). Melting points were determined using an open capillary method and are uncorrected.

Procedure for the Synthesis of Substituted 2-Alkyl/Aryl Benzimidazole Derivatives

A mixture of *o*-phenylenediamine (1.0 mmol) and the appropriate alkyl or aryl carboxylic acid (1.1 mmol) was taken in a round-bottom flask containing polyphosphoric acid (or 4 N HCl / glacial acetic acid as catalyst). The reaction mixture was heated under reflux at 120–150 °C for 3–5 h with continuous stirring.

The progress of the reaction was monitored by TLC. After completion, the reaction mixture was allowed to cool to room temperature and poured onto crushed ice with constant stirring. The resulting solid was neutralized using aqueous sodium bicarbonate solution, filtered, washed with cold water, and dried.

The crude product was purified by recrystallization from ethanol or by column chromatography using silica gel and suitable solvent systems to afford the pure substituted 2-alkyl/aryl benzimidazole derivatives.

III.CHARACTERIZATION

Spectral Analysis

1. IR spectral analysis Infrared

(IR) spectroscopy was employed to identify functional groups and molecular structures of the synthesized compounds. The sample was prepared by grinding 1–2 mg of the solid sample with 100 mg of dry KBr powder (Sigma-Aldrich, FTIR grade), which was then compressed into a thin, transparent pellet using a hydraulic press (PerkinElmer, model 25T). For liquid samples, a thin film was applied onto an ATR crystal (PerkinElmer, Diamond/ZnSe) or between two KBr discs (Sigma-Aldrich). The FTIR spectrometer (PerkinElmer Spectrum Two) was set to a resolution of 4 cm^{-1}, and the background spectrum was collected before sample measurement. The IR spectrum was analyzed in the range of 4000–400 cm^{-1} to identify characteristic absorption bands corresponding to functional groups such as hydroxyl (-OH), carbonyl (-C=O), and amines (-NH2).

2. 1 H NMR spectral analysis

Proton nuclear magnetic resonance (1H NMR) spectroscopy was used to determine the structure and dynamics of the synthesized compounds. The sample was prepared by dissolving 10–15 mg of the compound in 0.5–1 mL of a deuterated solvent such as $DMSOd_6$ (Sigma-Aldrich, 99.9% D) or $CDCl_3$ (Cambridge Isotope Laboratories, 99.8% D). The solution was transferred to a clean NMR tube (Norell, 5 mm), and the spectrum was acquired using a high-field NMR spectrometer (Bruker Avance III 400

MHz). The spectrometer parameters were set to a frequency of 400 MHz, a sweep width of 12 ppm, a pulse angle of 90°, a relaxation delay of 1 seconds, and 32 scans for a good signal-to-noise ratio. The chemical shifts, splitting patterns, and integration of the NMR signals were analyzed to deduce the molecular structure and identify functional groups.

3. 13C NMR spectral analysis

Carbon-13 nuclear magnetic resonance (13C NMR) spectroscopy was employed to investigate the structure and composition of the synthesized compounds. The sample was prepared by dissolving 10–20 mg of the compound in 0.5–1 mL of a deuterated solvent such as $CDCl_3$ (Cambridge Isotope Laboratories, 99.8% D) or DMSO-d_6 (Sigma-Aldrich, 99.9% D). The spectrum was acquired using a high-field NMR spectrometer (Bruker Avance III 400 MHz), with parameters set to a frequency range of 0–200 ppm, a pulse angle of 90°, a relaxation delay of 2 seconds, and 2000 scans for adequate signal-to-noise ratio. The chemical shifts were analyzed to identify distinct carbon environments and functional groups within the molecule.

4. Method and Procedure for UV-Vis Spectroscopy:

To conduct UV-Vis spectroscopy for 1,3-benzoxazole and its derivatives, first, prepare a solution of the compound by dissolving 5–10 mg of the sample in an appropriate solvent such as ethanol, methanol, or acetonitrile, ensuring the concentration is approximately 10^{-3} to 10^{-5} M. This concentration ensures that the absorbance falls within the linear range of the spectrophotometer, typically between 0.1 and 1.0 absorbance units. Transfer the solution into a clean, dry quartz cuvette, which is placed into the UV-Vis spectrophotometer. The spectrophotometer should be set to scan a wavelength range from 200 to 400 nm, or extended up to 800 nm if studying derivatives with extended conjugation. Prior to measurement, blank the instrument using the same solvent to account for any absorbance from the solvent. Start the scan, allowing the spectrophotometer to record the absorption spectra. The system will generate an absorption vs. wavelength plot, where peaks are observed due to electronic transitions, particularly π-π* transitions in the conjugated aromatic system. Throughout the experiment, the solution should be stirred continuously, and the cuvette should be handled carefully to avoid contamination or air bubbles that might interfere with the scan.

Antioxidant Activity Assays

Antimicrobial Activity

The synthesized compounds were screened for their antimicrobial activity against selected Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungal strains (*Candida albicans*, *Aspergillus niger*) using the agar well diffusion method.

Test compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain a concentration of 1 mg/mL. Standard drugs such as ciprofloxacin (for antibacterial activity) and fluconazole (for antifungal activity) were used as positive controls, while DMSO served as the negative control. Zones of inhibition were measured after incubation at 37 °C for bacteria (24 h) and 28 °C for fungi (48 h).

IV.RESULTS AND DISCUSSION

Synthesis and Characterization

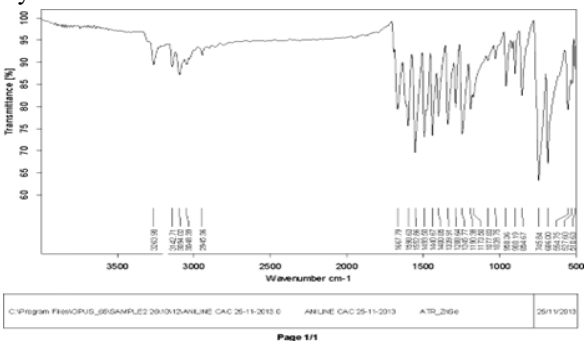


Figure 1.1 IR spectra of 2-chloro-N-phenylacetamide

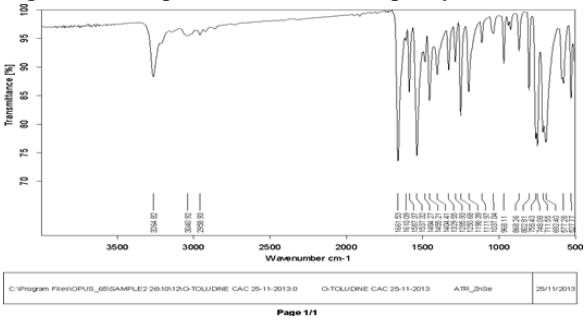


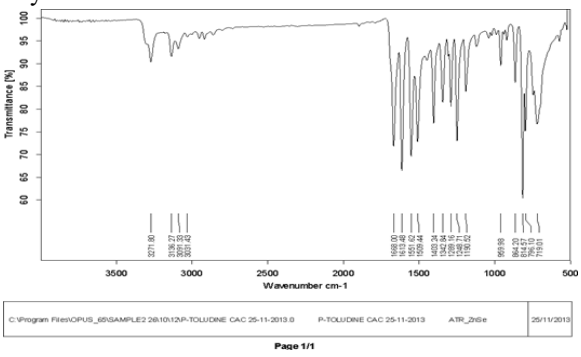
Figure 1.2 IR spectra of 2-chloro-N-o-tolylacetamide

Table 1.1 IR interpretation of 2-chloro-N-phenylacetamide

I.R.frequency(cm^{-1})	Remarks	I.R.frequency(cm^{-1})	Remarks

1667.79	C=O(-NHCO) stretching	1661.53	C=O(-NHCO) stretching
3048.39	C-Hstretching	3040.92	C-Hstretching
3263.98	N-H stretching	3264.82	N-H stretching

Table 1.2 IR interpretation of 2-chloro-N-o-tolylacetamide



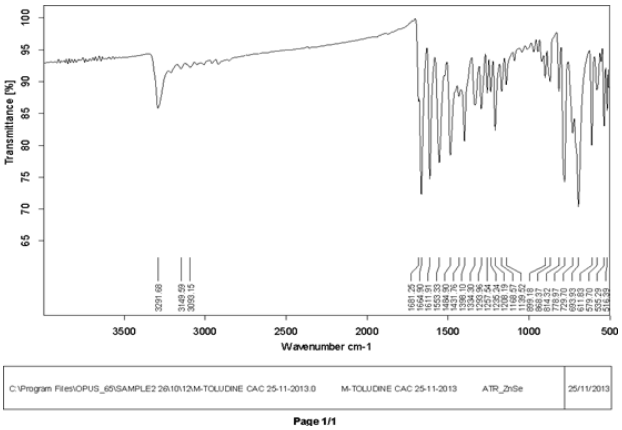


Figure 1.3 Masss pectra

Mass Spectra-m/z 313.9(M^+)

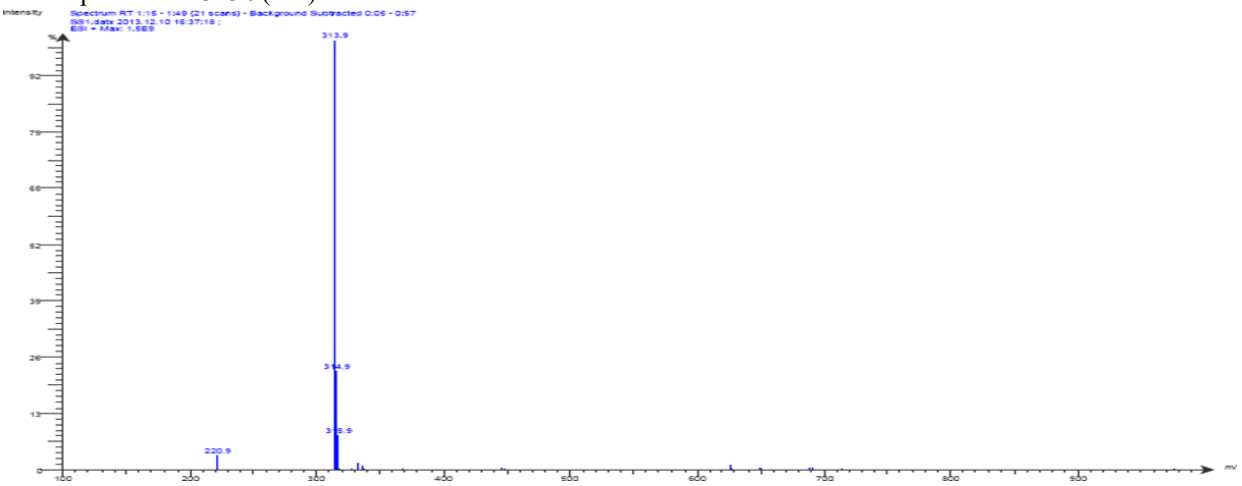
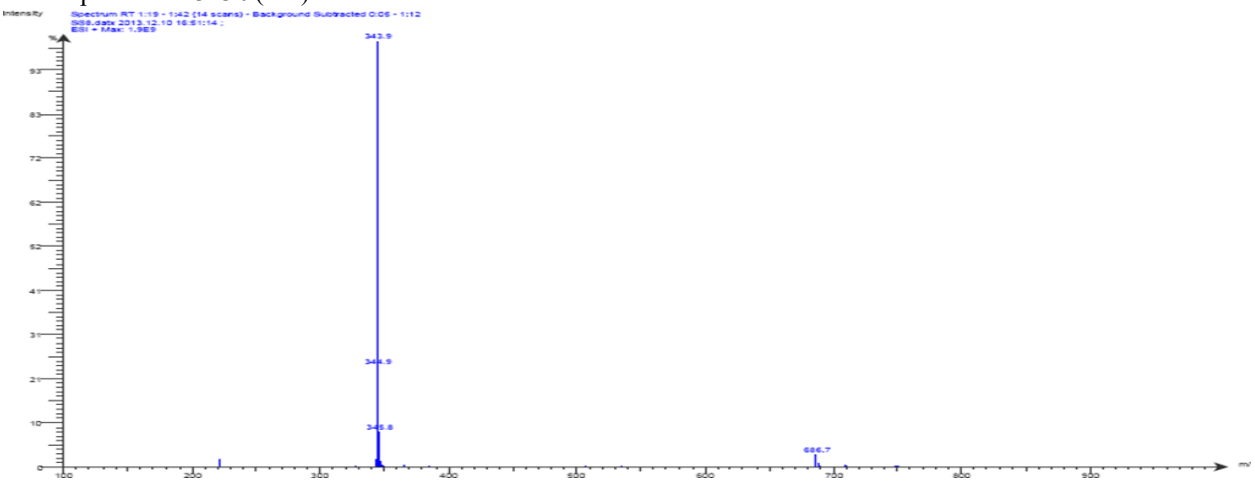


Figure 1.4 Masss pectra

Mass Spectra-m/z343.9(M^+)



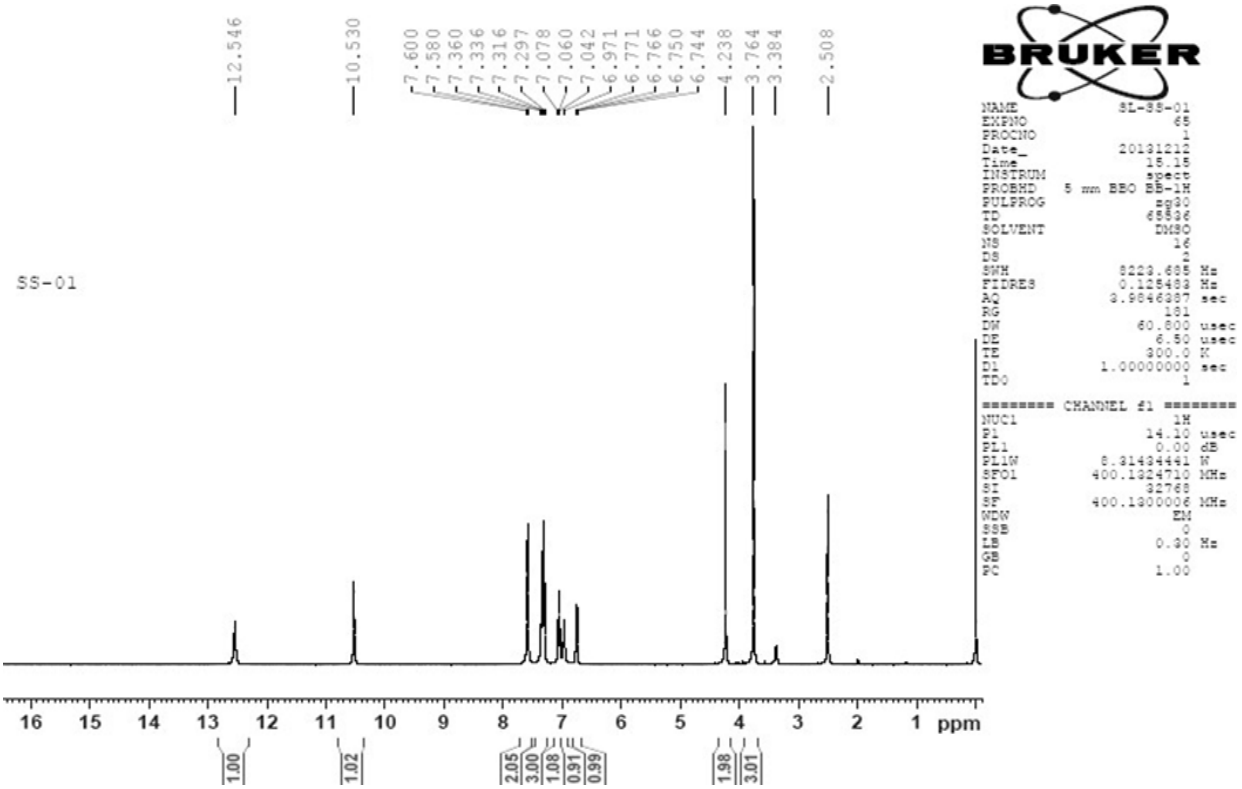


Figure 1.5 NMR spectra

^{1}HNMR(CDCl3,ppm):3.775(s,3H,OCH3),3.859(s,3H,OCH3),3.932(s,2H,CH_2), 9.999(s,1H,NH),10.677(s,1H,RingNH),6.831-7.563(m,7H,ArH)

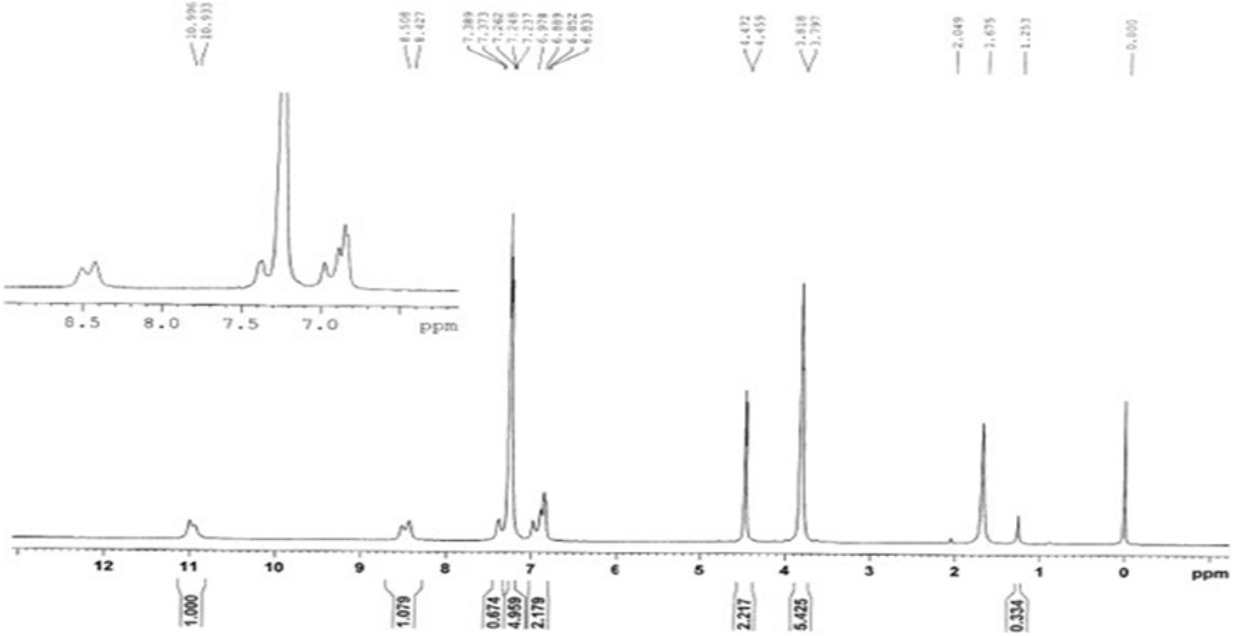


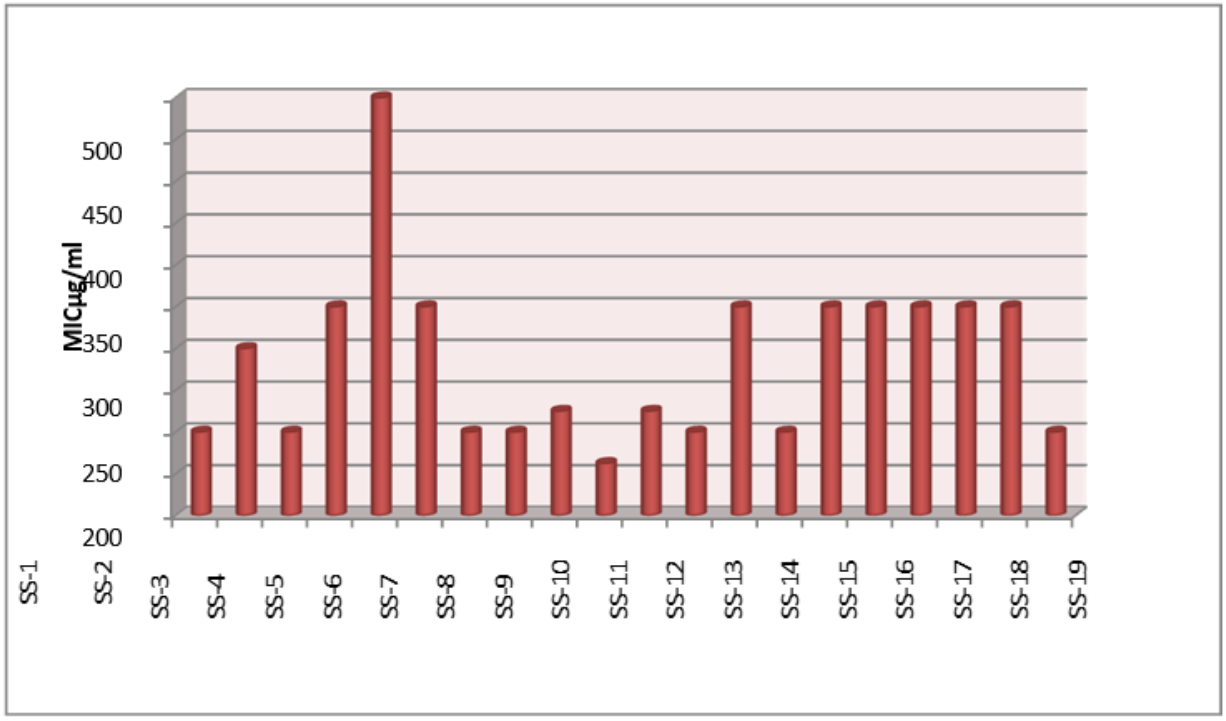
Figure 1.6 NMR spectra

^{1}HNMR(CDCl3,ppm):3.797(s,3H,OCH3),3.818(s,2H,CH_2),4.459(s,2H,CH_2),8.508(s,1H,NH),10.996 (s,1H,RingNH),6.833-7.389(m,8H,ArH)

Anti microbial activity

MINIMALFUNGICIDALCONCENTRATION(µg/ml)					
CODE NO.	C. albicans	A. niger	T. rubrum	E. floccosum	Penicillium spp.
	MTCC 227	MTCC 282	MTCC 296	MTCC7880	WILD STRAIN
SS-1	250	500	500	500	1000
SS-2	62.5	100	125	100	100
SS-3	1000	1000	250	500	1000
SS-4	500	1000	500	1000	250

Figure 1.7 Anti bacterial activity of SS1 against *E.coli* MTCC443



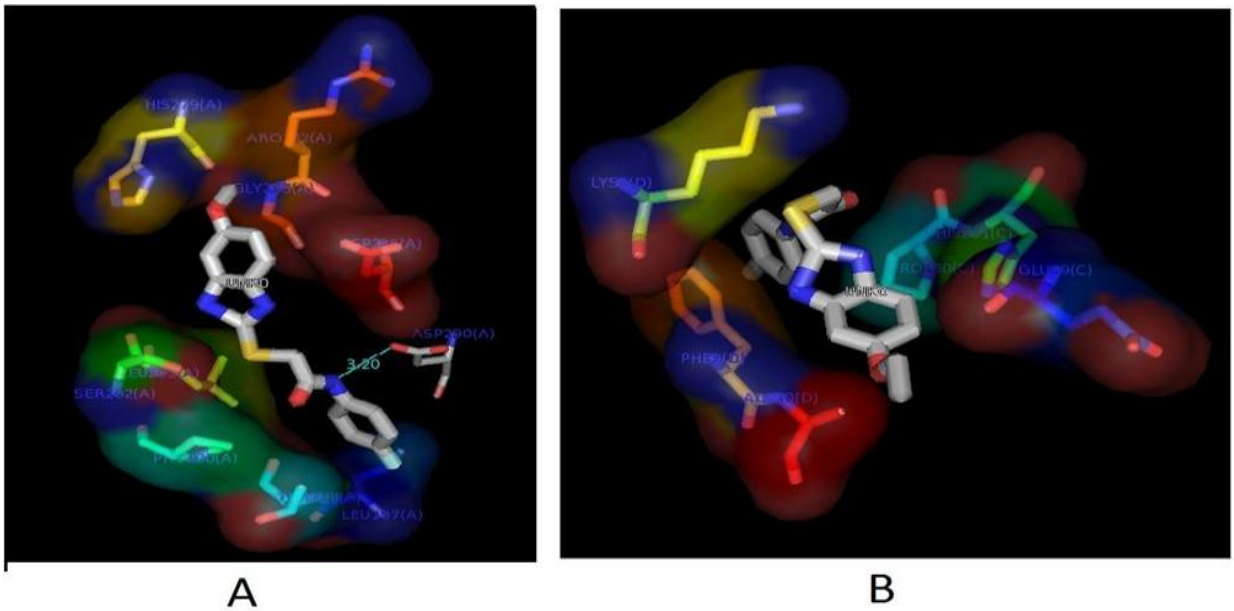


Figure 1. 8 Interaction between synthesized compounds with 1KPI (A) and 1KPG (B)

V.CONCLUSION

In the present work benzimidazole-2-thiol derivatives were synthesized using conventional method for synthesis and were obtained in good to excellent yield. The compound SS2 with 4-nitro substitution on phenyl ring had very good activity against all bacterial strain tested. Compounds with 4-methoxy substitution in the phenyl also had excellent antibacterial activity.

It can also be concluded that 4-methoxy substitution on the phenyl ring ofhas an important role in antimicrobial activities.

Compounds possessing promising activity against fungi implicate the importance of nitro and methoxy group for this activity.

Docking studies showed that binding energy for most of the molecules is lower than that of DDDMAB indicating better affinity for the targets.

Anti-mycobacterial activity of compounds was found to be less than that of Isoniazid and Rifampicin.

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