



Synthesis and in Vitro Evaluation of Coupled Mercaptobenzimidazole Derivatives Used as a Potent Biological Agent

Deepak Kardile *, Mrunal Shirsat

Department of pharmaceutical sciences, Madhav University, Pindwara, Rajasthan, India.

ABSTRACT

In the present study, coupled mercaptobenzimidazole derivatives were efficiently synthesized, which were further characterized and authenticated by means of TLC, and different spectral data such as IR and ¹H NMR. The synthesized compounds DPK2d1 to DPK2d8 were screened for their *in vitro* antimicrobial and antitubercular activities. The result showed that the titled compounds exhibited potent to moderate antimicrobial activities against *Bacillus subtilis*, *Escherichia coli* (antibacterial) and *Aspergillus niger* (antifungal) as compared to ciprofloxacin and fluconazole, respectively. Compounds DPK2d1, DPK2d2, and DPK2d3 exhibited potent to moderate antitubercular activities against *Mycobacterium tuberculosis* as compared to pyrazinamide, ciprofloxacin, and streptomycin.

Key Words: Coupled mercaptobenzimidazole, antimicrobial activity, antitubercular activity.

eIJPPR 2020; 10(1):127-133

HOW TO CITE THIS ARTICLE: Deepak Kardile, Mrunal Shirsat (2020). "Synthesis and in Vitro Evaluation of Coupled Mercaptobenzimidazole Derivatives Used as a Potent Biological Agent", International Journal of Pharmaceutical and Phytopharmacological Research, 10(1), pp.127-133.

INTRODUCTION

In the 20th century, chemotherapy has revolutionized the treatment of infective diseases since the innovation of antibacterial dyes by Paul Ehrlich and covered the way to a great victory for human health and long life. The development of resistance against currently used antimicrobial drugs led to an invigorated curiosity of the researchers in infective diseases to develop new chemical entities to battle them [1-3]. Patient morbidity, costs of treatment, rates of hospitalization, and use of broad-spectrum agents are remarkably increased by antimicrobial resistance [4-6].

Tuberculosis is a deadly disease usually caused by *Mycobacterium tuberculosis*. It has killed an estimated one billion people over the last two spans and even remains among the top ten causes of death in the world. According to the 2018 report of WHO, 558,000 people developed rifampicin-resistant (RR TB), multidrug-resistant tuberculosis (MDR-TB) or extensively drug-resistant TB (XDR TB) in the world. Therefore, it is essential to develop rational chemotherapeutic agents to delay the emergence of

resistance and, ideally, shorten the duration of therapy of this infection [7-9].

Benzimidazole is a lead molecule for most of the biological agents used in the pharmaceutical industry. It consists of a fused benzene ring with heterocyclic aromatic imidazole. The existence of imidazole creates it resourceful heterocycles with an extensive range of biological activities such as antiulcer (Gastric H⁺/K⁺-ATPase inhibitors), antihypertensive, anti-inflammatory, anticonvulsant, analgesic, antiprotozoal, antitrichinellosis, antidiabetic, anti-HIV, antimicrobial, antitubercular, anticancer, antihistaminic, antioxidant, antiviral, antiparasitic agents, diuretic, and DNA binding activities [10-23].

Encouraged by the upstairs findings and in the persistence of our work on coupled mercaptobenzimidazole derivatives, we herein report the synthesis and in vitro evaluation of coupled mercaptobenzimidazole derivatives as a potent biological agent.

MATERIALS AND METHODS:

The chemicals of analytical grade required for the synthesis of coupled mercaptobenzimidazole derivatives were

Corresponding author: Deepak Kardile

Address: Department of pharmaceutical sciences, Madhav University, Pindwara, Rajasthan, India.

E-mail: ✉ kardiledeepak@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 November 2019; **Revised:** 23 February 2020; **Accepted:** 17 February 2020



purchased from Sigma-Aldrich and SD fine chemicals (India). Synthesized compounds were determined for their melting points with the help of precision melting point apparatus and were uncorrected. Completion of the reaction was confirmed by TLC on silica gel-G plates and the spots were visualized in the UV chamber or iodine chamber. IR spectra of intermediates and derivatives were recorded by using on KBr pellets on a Jasco FTIR-460 plus spectrophotometer and vibrational frequencies expressed in cm^{-1} . ^1H NMR spectra were recorded on BRUKER 400 MHz spectrometer in deuterated DMSO using tetramethylsilane (TMS) as internal standard and chemical shifts were recorded as δ (parts per million).

General Procedure:

General procedure for the synthesis of Mercaptobenzoxazole (Compound I):

2-amino phenol (10.91g, 0.1 moles) treated with carbon disulfide (7.67g, 0.1 moles) in the presence of potassium hydroxide (5.65g, 0.1 moles), 100 ml of 95% ethanol and 15 ml of water used as a solvent in a round bottom flask was refluxed on a water bath for three hours. After the completion of the reaction, the reaction mixture was cooled

and filtered. After that, 1-1.5g of activated charcoal was added carefully in the filtrate and refluxed on the water bath for 10 minutes; the activated charcoal was removed by filtration. The filtrate was treated with 100ml of warm water at 60-70°C for 10 minutes. Dilute acetic acid was poured into the reaction mixture for acidification with gentle agitation to yield shiny crystals as a product, which is further kept in a refrigerator for three hours to allow the complete crystallization process. The obtained solid product was separated through the Buchner funnel and dried at 40°C overnight and recrystallized from the ethanol.

General procedure for the synthesis of substituted Mercaptobenzimidazole (DPK2B1-DPK2B4):

Mercaptobenzoxazole (5.5g, 0.1 moles) was treated with primary aromatic amine (2.7g, 0.1 moles) and was dissolved in 15 ml of glacial acetic acid. The above reaction mixture was refluxed on a water bath for 6 hours. The completion of the reaction was ascertained by TLC (Toluene: Ethyl acetate/ 1:4). After that, the reaction mixture was cooled to room temperature and filtered. The obtained solid crystals were separated through Buchner funnel and the dried product was recrystallized from the ethanol.

Table 1: Physicochemical properties of substituted mercaptobenzimidazole (DPK2B1-DPK2B4):

Sr.No.	Compound code	Molecular Formula	Mol. wt.	M.P.(°C)	Percentage Yield	Recrystallization Solvent
1	DPK2B1	$\text{C}_{14}\text{H}_{12}\text{N}_2\text{S}$	240.32	240-245 °C	81	Ethanol
2	DPK2B2	$\text{C}_{13}\text{H}_{10}\text{N}_2\text{S}$	226.29	235-240 °C	77	Ethanol
3	DPK2B3	$\text{C}_{13}\text{H}_9\text{ClN}_2\text{S}$	260.42	251-256 °C	80	Ethanol
4	DPK2B4	$\text{C}_{13}\text{H}_9\text{N}_3\text{O}_2\text{S}$	271.29	263-268 °C	83	Ethanol

General procedure for the synthesis of coupled mercaptobenzimidazole derivatives (DPK2d1-DPK2d8):

Substituted Mercaptobenzimidazole (5g, 0.02 moles) (DPK2B1-DPK2B4) treated with 2.3g (0.01 moles) dibromoalkane (1, 2- dibromoethane or 1, 3- dibromopropane) was dissolved in 60ml of ethanol. Further 5g (0.01 moles) anhydrous potassium carbonate was added as a deacidifying agent. The above reaction mixture was

refluxed on a water bath for 14 hours. The completion of the reaction was ascertained by TLC (Ethyl acetate: n-hexane/4:6 or Benzene: Methanol/4:2). After that, the reaction mixture was cooled to room temperature and neutralized with 2N aqueous solution of sodium hydroxide and filtered. The obtained solid crystals were separated through the Buchner funnel and the dried product was recrystallized from the ethanol.

Table 2: Physicochemical properties of coupled mercaptobenzimidazole derivatives (DPK2d1-DPK2d8):

Sr.No	Compound code	Molecular Formula	Mol. wt.	M.P.(°C)	Percentage Yield	Recrystallization Solvent
1	DPK2d1	$\text{C}_{30}\text{H}_{26}\text{N}_4\text{S}_2$	506.7	325-330 °C	60	Ethanol
2	DPK2d2	$\text{C}_{31}\text{H}_{28}\text{N}_4\text{S}_2$	520.7	345-350 °C	71	Ethanol
3	DPK2d3	$\text{C}_{28}\text{H}_{22}\text{N}_4\text{S}_2$	478.6	328-333 °C	80	Ethanol
4	DPK2d4	$\text{C}_{32}\text{H}_{29}\text{N}_5\text{O}_2\text{S}_2$	579.7	335-340 °C	75	Ethanol
5	DPK2d5	$\text{C}_{28}\text{H}_{20}\text{Cl}_2\text{N}_4\text{S}_2$	547.5	352-357 °C	65	Ethanol
6	DPK2d6	$\text{C}_{32}\text{H}_{27}\text{Cl}_2\text{N}_5\text{O}_2\text{S}_2$	648.6	360-365 °C	72	Ethanol
7	DPK2d7	$\text{C}_{28}\text{H}_{20}\text{N}_6\text{O}_4\text{S}_2$	568.6	342-344 °C	74	Ethanol
8	DPK2d8	$\text{C}_{29}\text{H}_{22}\text{N}_6\text{O}_4\text{S}_2$	582.7	355-360 °C	68	Ethanol

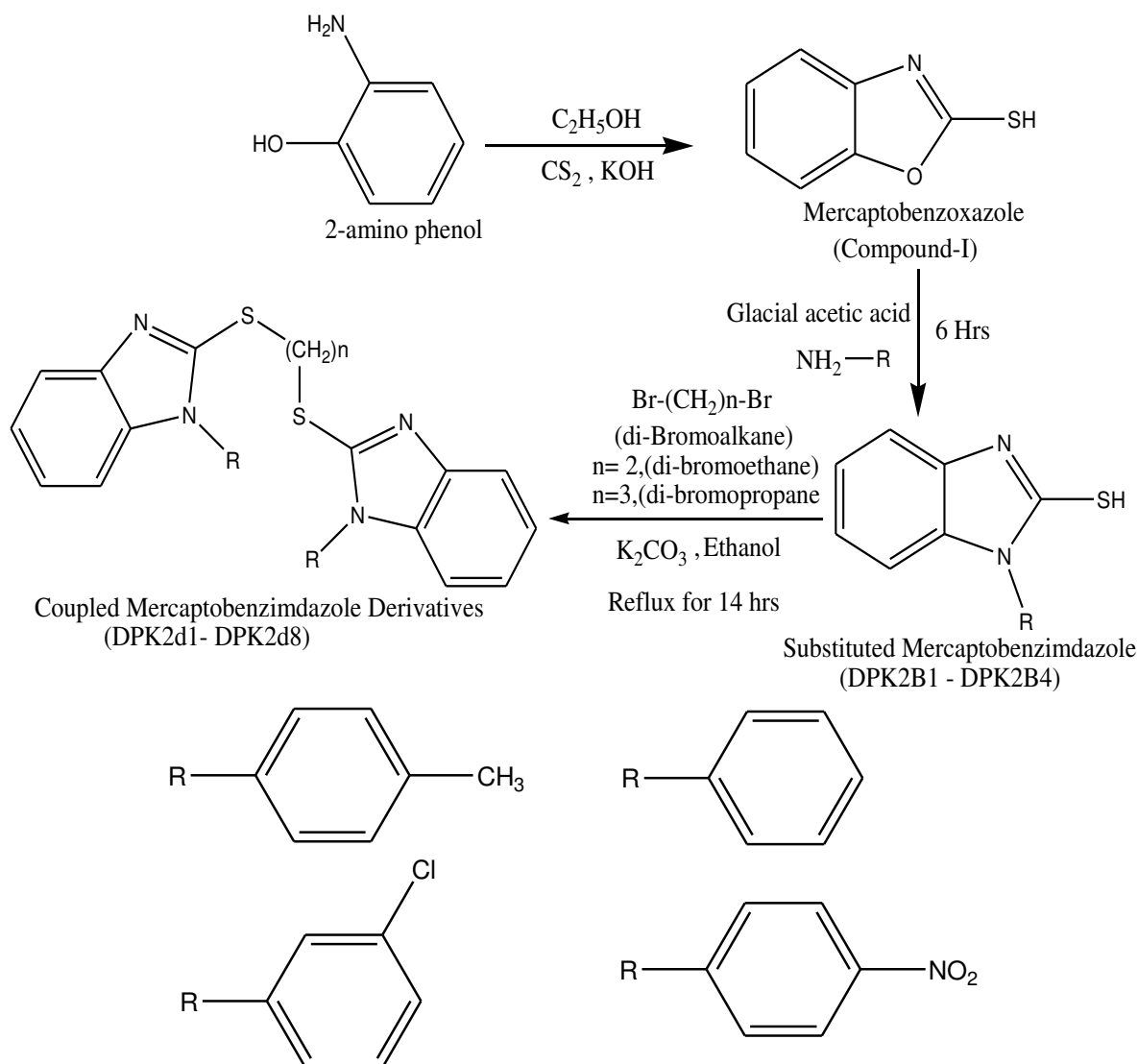


Fig.1: Synthesis of coupled mercaptobenzimidazole derivatives.

Spectral data:

Spectral data of Compound I: FTIR (KBr, cm^{-1}): 2916 (C-H, Ar), 1651 (C=N), 1550 (C=C), 1242 (NH), 1111 (C-O-C), 779 (C-S).

Spectral data of substituted mercaptobenzimidazole:

DPK2B1: FTIR (KBr, cm^{-1}): 3394 (NH, Benzimidazole), 3232 (C-H, Ar), 2924 (C-H, Aliphatic), 2638 (SH), 1527 (C=C), 1319 (C-N), 678 (C-S),

DPK2B2: FTIR (KBr, cm^{-1}): 3394 (NH, Benzimidazole), 3070 (C-H, Ar), 2337 (SH), 1604 (C=C), 1319 (C-N), 694 (C-S).

DPK2B3: FTIR (KBr, cm^{-1}): 3394 (NH, Benzimidazole), 3039 (C-H, Ar), 2507 (SH), 1573 (C=C), 1319 (C-N), 720 (C-S), 875 (C-Cl).

DPK2B4: FTIR (KBr, cm^{-1}): 3333 (NH, Benzimidazole), 3248 (C-H, Ar), 2476 (SH), 1688 (C=C), 1535 (NO_2), 1388 (C-N), 717 (C-S).

Spectral data of coupled mercaptobenzimidazole derivatives:

DPK2d1: FTIR (KBr, cm^{-1}): 3296 (NH, Benzimidazole), 3063 (C-H, Ar), 2962 (C-H, Aliphatic), 1512 (C=C), 1265 (C-N), 609 (C-S). $^1\text{H NMR}$ (500MHz, DMSO-d₆): δ 9.862 (bs, 1H, NH), 7.071-7.461 (m, 3H, Ar-H), 2.015-3.403 (m, 1H, CH).

DPK2d2: FTIR (KBr, cm^{-1}): 3271 (NH, Benzimidazole), 3070 (C-H, Ar), 2939 (C-H, Aliphatic), 1512 (C=C), 1265 (C-N), 609 (C-S). $^1\text{H NMR}$ (500MHz, DMSO-d₆): δ 9.841 (bs, 1H, NH), 7.073-7.470 (m, 3H, Ar-H), 2.335-3.382 (m, 1H, CH).

DPK2d3: FTIR (KBr, cm^{-1}): 3402 (NH, Benzimidazole), 3063 (C-H, Ar), 2924 (C-H, Aliphatic), 1550 (C=C), 1265 (C-N), 756 (C-S). $^1\text{H NMR}$ (500MHz, DMSO-d₆): δ 9.993 (bs, 1H, NH), 7.004-7.584 (m, 3H, Ar-H), 2.039-3.377 (m, 1H, CH).

DPK2d4: FTIR (KBr, cm^{-1}): 3425 (NH, Benzimidazole), 3063 (C-H, Ar), 2994 (C-H, Aliphatic), 1525 (C=C), 1265

(C-N), 756 (C-S). ¹H NMR (500MHz, DMSO-d₆): δ 9.941 (bs, 1H, NH), 7.005-7.588 (m, 3H, Ar-H), 2.043-3.401 (m, 1H, CH).

DPK2d5: FTIR (KBr, cm⁻¹): 3264 (NH, Benzimidazole), 3124 (C-H, Ar), 2924 (C-H, Aliphatic), 1535 (C=C), 1280 (C-N), 871 (C-Cl), 756 (C-S). ¹H NMR (500MHz, DMSO-d₆): δ 10.141 (bs, 1H, NH), 7.073-7.824 (m, 3H, Ar-H), 2.0362-3.381 (m, 1H, CH).

DPK2d6: FTIR (KBr, cm⁻¹): 3305 (NH, Benzimidazole), 3070 (C-H, Ar), 2800 (C-H, Aliphatic), 1535 (C=C), 1280 (C-N), 793 (C-Cl), 702 (C-S). ¹H NMR (500MHz, DMSO-d₆): δ 10.138 (bs, 1H, NH), 7.075-7.826 (m, 3H, Ar-H), 2.064-3.394 (m, 1H, CH).

DPK2d7: FTIR (KBr, cm⁻¹): 3225 (NH, Benzimidazole), 3078 (C-H, Ar), 2893 (C-H, Aliphatic), 1597 (C=C), 1311 (NO₂), 1188 (C-N), 840 (C-S). ¹H NMR (500MHz, DMSO-d₆): δ 9.9941 (bs, 1H, NH), 6.589-7.825 (m, 3H, Ar-H), 2.123-3.362 (m, 1H, CH).

DPK2d8: FTIR (KBr, cm⁻¹): 3483 (NH, Benzimidazole), 3039 (C-H, Ar), 2939 (C-H, Aliphatic), 1651 (C=C), 1427 (NO₂), 1149 (C-N), 879 (C-S). ¹H NMR (500MHz, DMSO-d₆): δ 9.9841 (bs, 1H, NH), 6.586-7.954 (m, 3H, Ar-H), 2.498-3.347 (m, 1H, CH).

BIOLOGICAL STUDIES:

In Vitro Antimicrobial Evaluation:

In vitro antimicrobial activity of synthesized coupled mercaptobenzimidazole derivatives were evaluated by the tube dilution method against *Escherichia coli* ATCC 25922 (Gram-negative bacteria), *Bacillus subtilis* ATCC 6051 (Gram-positive bacteria), and *Aspergillus niger* ATCC 6275 (fungal strain) using Ciprofloxacin and fluconazole as standard antibacterial and antifungal drugs, respectively. All the synthesized derivatives had nine-time dilutions to be done with brain heart infusion (BHI) for minimum inhibitory concentration (MIC). Initially, 20 µL of the drugs in the initial tube was added into the 380 µL of BHI broth. For dilution, 200µL BHI broth was added into the next nine tubes separately. Further, 200µL broth from the initial tube was transferred to the first tube to make 10⁻¹ dilution. 10⁻² dilution was prepared from 10⁻¹ diluted tube, (200 µL was transferred to the second tube). The sequential dilution was reiterated up to 10⁻⁹ dilution for each drug. 5 µL was taken from the preserved stock cultures of required organisms and was added into 2 µL of BHI broth. 200µL of the above culture suspension was added to each sequentially diluted tube. The turbidity of cultures was checked after 24 hours of incubation. The minimum inhibitory concentration was the highest dilution of the synthesized compounds (DPK2d1- DPK2d8) with no visually detectable bacterial or fungal growth.

In Vitro Antitubercular Evaluation:

In vitro antitubercular activity of synthesized coupled mercaptobenzimidazole derivatives were evaluated by the Microplate Alamar Blue Assay (MABA) against *Mycobacterium tuberculosis* (H37Rv strain, ATCC 27294) using Pyrazinamide, Ciprofloxacin, and Streptomycin, as standard drugs. This assay procedure was non-toxic, and used thermally stable chemical reagent. MABA method exhibited an agreeable connection with BACTEC radiometric and proportional method. During incubation, 200 µL of sterile deionized water was added to all outer perimeter wells of the sterile 96-well plate for minimizing the evaporation of the medium in the test wells. The 96-well plate in the columns received 100 µL of the Middlebrook 7H9 broth. 100 to 0.2 µg/ml of drug concentration were added to the 96-well plate. Parafilm was used to cover and sealed the plates. After that, the plates were incubated at 37°C for five days. 25 µL of a freshly prepared mixture of Almar blue reagent and 10% tween 80 (1:1) was added to the plates and were reincubated at 37°C for 24 hours. The blue colour of the well indicates that no mycobacterial growth and pink colour was counted as mycobacterial growth for synthesized compounds (DPK2d1-DPK2d8).

RESULT AND DISCUSSION:

From the literature survey, benzimidazole has been reported to develop several molecules with various potent pharmacological activities. In this research study, we have reported synthesized coupled mercaptobenzimidazole derivatives and screened for their in vitro antimicrobial and antitubercular activities. The purity and homogeneity of the coupled mercaptobenzimidazole derivatives were preliminarily checked by their physical constants. These compounds were characterized by various spectral studies such as IR, and ¹H NMR for structural elucidation, and studies showed satisfactory results.

In Vitro Antimicrobial Activity:

The results of the MIC values of synthesized compounds (µg/ml) against *B. subtilis*, *E. coli*, and *A. niger* are summarized in Table 3. Some of the coupled Mercaptobenzimidazole derivatives were found to be highly efficient as antimicrobial agents in comparison to the standard drug ciprofloxacin (CPFX) and fluconazole (FCZ) as they represented by their low MIC values compared to standard drugs. Amongst the synthesized compounds DPK2d1 to DPK2d8 showed significant and potent activity against *B. subtilis* compared with the standard Ciprofloxacin. DPK2d5 showed a moderate effect against *B. subtilis* compared with standard Ciprofloxacin. Also, DPK2d1 to DPK2d8 showed significant and potent antifungal activity against *A. niger* compared with fluconazole. Whereas, DPK2d1 to DPK2d8 were found to

have an average activity against *E. coli* compared with standard Ciprofloxacin.

Table 3: Antimicrobial activity and MIC values of synthesized compounds.

Sr. No.	Compound Code	MIC in µg/ml		
		Antibacterial activity		Antifungal activity
		B. subtilis	E. coli	A.niger
1	DPK2d1	0.8	100	0.8
2	DPK2d2	0.4	100	1.6
3	DPK2d3	0.8	100	1.6
4	DPK2d4	0.4	100	1.6
5	DPK2d5	3.12	50	3.12
6	DPK2d6	0.4	50	0.8
7	DPK2d7	0.8	50	1.6
8	DPK2d8	0.4	50	0.8
9	Ciprofloxacin	2	2	-
10	Fluconazole	-	-	8
11	Control (DMF)	-	-	-

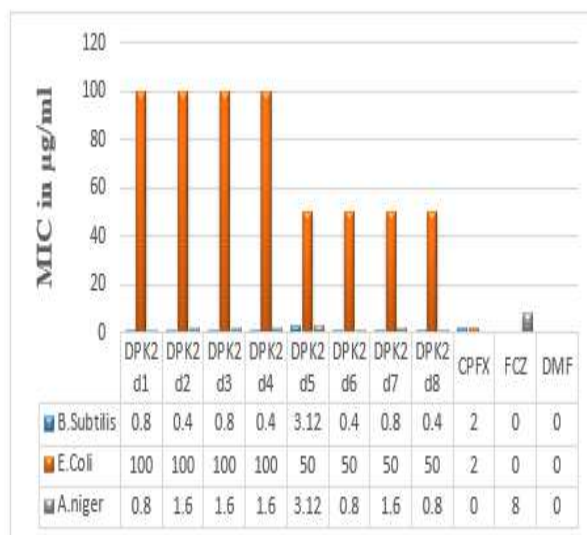


Fig. 2: Graphical representation of antimicrobial activity and MIC values of synthesized compounds.

In Vitro Antitubercular Activity:

The results of the MIC values of synthesized compounds (µg/ml) against *M. tuberculosis* (H37Rv) are summarized in Table 4. It is evident that among the eight compounds out of three compounds such as DPK2d1, DPK2d2, and DPK2d3 showed potent antitubercular activity with MIC of 1.6 µg/ml compared with standard antitubercular drugs such as Pyrazinamide, Ciprofloxacin, and Streptomycin. The rest of the synthesized compounds (DPK2d4 to DPK3d8) revealed moderate to good antitubercular activity.

Table 4: Antitubercular activity and MIC values of synthesized compounds.

Sr.No.	Compound Code	MIC in µg/ml	Sr. No.	Compound Code	MIC in µg/ml
1	DPK2d1	1.6	7	DPK2d7	6.25
2	DPK2d2	1.6	8	DPK2d8	6.25
3	DPK2d3	1.6	9	Pyrazinamide	3.12
4	DPK2d4	6.25	10	Ciprofloxacin	3.12
5	DPK2d5	6.25	11	Streptomycin	6.25
6	DPK2d6	6.25			

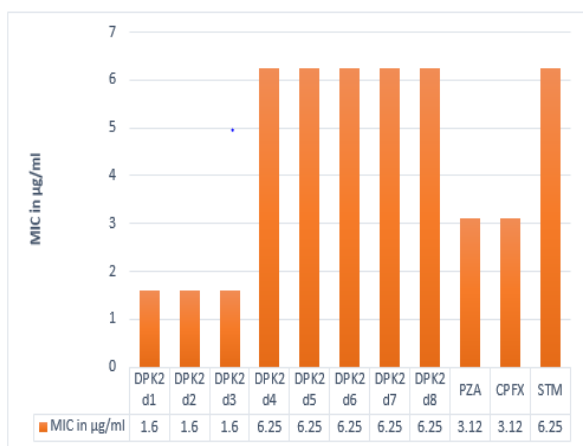


Fig. 3: Graphical representation of antitubercular activity and MIC values of synthesized compounds.

Structure-activity relationship of coupled mercaptobenzimidazole derivatives:

From the comparison of antimicrobial and antitubercular activities of synthesized coupled mercaptobenzimidazole derivatives, the following SAR may be assumed.

1. Antimicrobial activity of the synthesized coupled mercaptobenzimidazole derivatives compared to the standard drug ciprofloxacin and fluconazole conclude that there should be slight structural modifications to develop affinity of the drug to the binding of a molecule to the target site.
2. The antitubercular activity of the synthesized coupled mercaptobenzimidazole derivatives compared to the standard drugs such as Pyrazinamide, Ciprofloxacin, and Streptomycin may draw attention that the

synthesized compounds have a very good interaction with target sites and require supplementary in vivo studies to confirm the antitubercular activity.

- The above results also indicated a fact that different structural requirements are essential for a compound to show different activities. The structure-activity relationship amongst the coupled mercaptobenzimidazole derivatives outcomes are summarized as follows:

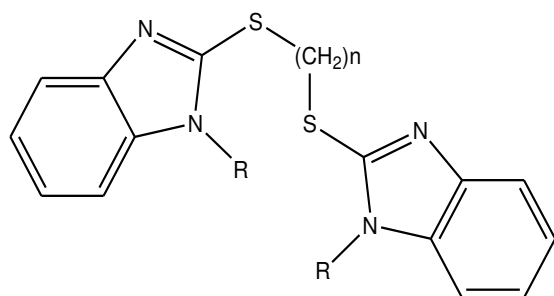


Fig. 4. SAR of coupled mercaptobenzimidazole Derivatives.

- Presence of benzimidazole ring essential for antimicrobial and antitubercular activities.
- Presence of alkyl side chain (ethyl or propyl side chain) essential for antimicrobial and antitubercular activities.

At position R:

- The presence of aromatic rings such as benzene rings is important for antimicrobial and antitubercular activities.
- Substitution of $-NO_2$ (Nitro group) as well as $-CH_3$ (Methyl group) in the benzene ring at para position shows potent to moderate antimicrobial and antitubercular activities.
- Substitution of $-Cl$ (Chloro group) at meta position of the benzene ring, shows potent to moderate antimicrobial and antitubercular activities.

CONCLUSION:

Coupled mercaptobenzimidazole derivatives were efficiently synthesized and screened for their in vitro antimicrobial and antitubercular activities. Amongst the synthesized compounds DPK2d1 to DPK2d8 showed significant and potent activity against *B. subtilis* and *A. niger* compared with the standard Ciprofloxacin and Fluconazole. DPK2d5 showed a moderate effect against *B. subtilis* compared with standard Ciprofloxacin. Also, coupled mercaptobenzimidazole derivatives such as DPK2d1, DPK2d2, and DPK2d3 showed potent antitubercular activity with MIC values of 1.6 μ g/ml compared with the standard Pyrazinamide, Ciprofloxacin, and Streptomycin.

ACKNOWLEDGMENTS:

The authors would like to acknowledge Savitribai Phule Pune University, Maharashtra, India for providing a good spectral data analysis facility. We are also thankful to Dr. Kishore G. Bhat, Maratha Mandal's Central Research Laboratory Belgaum, for the antimicrobial and antitubercular screening of the synthesized compounds.

Conflict of interest:

The authors declare that they have no competing interests.

REFERENCES

- [1] Morse S.S. Factors in the Emergence of Infectious Diseases. *Emerging Infectious Disease*, 1995; 1, 7-15.
- [2] Matsos A., Johnston I.N. Chemotherapy-induced cognitive impairments: A systematic review of the animal literature. *Neuroscience and Biobehavioral Reviews*. 2019; 102, 382-399.
- [3] Saeed S., Rashid N, Jones P.G., Ali M., Hussain R. Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. *European Journal of Medicinal Chemistry*, 2010; 45, 1323-1331.
- [4] Taramian, S., Mahfoozi, L., Soodmand M. Antibiotic Resistance Trend of Uropathogens in a Tertiary Teaching Hospital in Rasht, Iran, a Longitudinal Study. *International Journal of Pharmaceutical and Phytopharmacological Research*, 2019; 9(1), 98-102.
- [5] Randhawa, M. A., Bawadekji, A., Al Ali, M., Oueslati, M. H., Jamith, B. W. Antimicrobial Effects of Methanolic Extract of *Salvia Officinalis* L, Including MRSA and Multidrug Resistant *Acinetobacter Baumannii*. *International Journal of Pharmaceutical and Phytopharmacological Research*, 2018; 8(4), 1-5.
- [6] Salehi Nobandegani, A., Motamedifar, M. Antibiotic Sensitivity Profile of the Bacterial Isolates from the Blood Samples of the Patients in Different Wards of A Major Referral Hospital, Shiraz, Iran 2015-2016. *Pharmacophore*, 2019; 10(2), 30-36.
- [7] Mohanty S.K., Khuntia A., Yellasubbaiah N., Ayyanna C., Sudha B.N., Harika M.S. Design, synthesis of novel azo derivatives of benzimidazole as potent antibacterial and antitubercular agents. *Beni-Suef University Journal of Basic and Applied Sciences*, 2018; 7, 646-651.
- [8] Knudson S.E., Kumar K., Awasthi D., Ojima I., Slayden R.A. In vitro-in vivo activity relationship of substituted benzimidazole cell division inhibitors with activity against *Mycobacteria tuberculosis*. *Tuberculosis*, 2014; 94 (3), 271-276.

- [9] Yoon Y.K., Ali M.A., Wei A.C., Choon T.S. Synthesis and evaluation of antimycobacterial activity of new benzimidazole aminoesters. *European Journal of Medicinal Chemistry*, 2015; 93, 614-624.
- [10] Yajnanarayana H.R. Micmel A.G., Joseph S. X-ray crystal structure and reactions of 2-cyano-1, 3-dibenzoyl-2, 3-dihydrobenzimidazole, a novel reassert compound. *Tekahedmn Letters*, 1991; 32, 2997-3000.
- [11] Chen J. Qu J. Zhang Y. Chen Y. Liu N., Chen B. Metal-free construction of tricyclic or tetracyclic compounds-acid-promoted synthesis of benzo[4,5]imidazo[2,1-a]isoindole and 1,2-dialkyl-2,3-dihydrobenzimidazoles. *Tetrahedron*, 2013; 69, 316-319.
- [12] Chen G.F., Dong X.Y. Facile and Selective Synthesis of 2-Substituted Benzimidazoles Catalyzed by FeCl₃/Al₂O₃. *European Journal of Medicinal Chemistry*, 2012; 9(1), 289-293.
- [13] Keri R.S., Hiremathad A., Budagumpi S., Nagaraja B.M. Comprehensive Review in Current Developments of Benzimidazole – Based Medicinal Chemistry. *Chem Bio Drug Des*, 2015; 86(1), 19-65.
- [14] Sridevi C.H., Balaji K., Naidu A., Sudhakaran R. Synthesis of some phenylpyrazolo benzimidazole quinoxaline derivatives as potent antihistaminic agents. *European Journal of Medicinal Chemistry*, 2010; 7, 234-238.
- [15] Ranjith P.K., Rajeesh P., Haridas K.R., Susanta N.K., Row T.N. Design and synthesis of positional isomers of 5 and 6-bromo-1-[(phenyl) sulfonyl]-2-[(4-nitrophenoxy) methyl]-1H-benzimidazoles as possible antimicrobial and antitubercular agents. *Bioorg Med Chem Lett*, 2013; 23, 5228–5234.
- [16] Arora R.K., Kaur N., Bansal Y., Bansal G. Novel coumarin-benzimidazole derivatives as antioxidants and safer anti-inflammatory agents. *Acta Pharm Sin B*, 2014; 4, 368–375.
- [17] Pan T., He X., Chen B., Chen H., Geng G., Luo H. Development of benzimidazole derivatives to inhibit HIV-1 replication through protecting APOBEC3G protein. *European Journal of Medicinal Chemistry*, 2015; 95, 500-513.
- [18] Vasantha K., Basavarajaswamy G., Rai M.V., Boja P., Pai V.R., Shruthi N. Rapid 'one-pot' synthesis of a novel benzimidazole-5-carboxylate and its hydrazine derivatives as potential anti-inflammatory and antimicrobial agents. *Bioorg Med Chem Lett*, 2015; 25, 1420-1426.
- [19] Gaba M., Gaba P., Uppal D., Dhingra N., Bahia M.S., Silakari O. Benzimidazole derivatives: search for GI-friendly anti-inflammatory analgesic agents. *Acta Pharm Sin B*, 2015; 5, 337-342.
- [20] Wen J., Luo Y., Zhang H., Zhao H., Zhou C., Cai G. A green and convenient approach toward benzimidazole derivatives and their antimicrobial activity. *Chin Chem Lett*. 2016; 27, 391–394.
- [21] Bansal Y., Silakari O. The therapeutic journey of benzimidazoles: a review. *Bioorganic and Medicinal Chemistry*, 2012; 20, 6208-6236.
- [22] Haque R.A., Budagumpi S., Choo S.Y., Choong M.K., Lokesh B.E., Sudesh K. Nitrilefunctionalized Hg (II) - and Ag (I)-N-heterocyclic carbene complexes: synthesis, crystal structures, nuclease and DNA binding activities. *Appl Organometal Chem*, 2012; 26, 689-700.
- [23] Ansari K.F., Lal C. Synthesis physicochemical properties and antimicrobial activity of some new benzimidazole derivatives. *European Journal of Medicinal Chemistry*, 2009; 44, 4028-4033.