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RESEARCH ARTICLE

In vitro Antimicrobial and Antitubercular screening of newly synthesized Mercaptobenzimidazole –clubbed chalcone derivatives

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ABSTRACT:
In this present investigation to studies of mercaptobenzimidazole-clubbed chalcone derivatives was efficiently synthesized, which is further characterized with the help of thin layer chromatography, spectroscopy techniques such as FTIR and ^{1}HNMR. The newly synthesized derivatives were examined against in vitro antimicrobial and antitubercular activities. The result revealed that the titled compounds an average antibacterial activity against gram-positive *Bacillus subtilis*, gram-negative *Escherichia coli* as compared to standard ciprofloxacin. Compounds DPK4B2d2, DPK4B2d3, DPK4B2d4 and DPK4B2d6 shown potent antifungal activity against *Aspergillus niger* as compared to standard fluconazole. Antitubercular activity of the synthesized compounds examined against *Mycobacterium tuberculosis*, compounds DPK4B2d1 and DPK4B2d2 shown potent activity in the comparison of standard such as Pyrazinamide, Ciprofloxacin, and Streptomycin.

KEYWORDS: Mercaptobenzimidazole, Chalcone, Antimicrobial, Antitubercular.

INTRODUCTION:
In the 20th century chemotherapy has revolutionized the treatment of infective diseases since the innovation of antibacterial dyes by Paul Ehrlich, 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 beyond the preceding two decade and even remains the top ten reasons of death in the world. According to the 2018 report of WHO, 5,58,000 people developed rifampicin-resistant (RR TB), multidrug-resistant tuberculosis (MDR-TB) or extensively drug-resistant (XDR TB) in the world.

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Therefore, it is essential to develop rational chemotherapeutic agents to deferral the development of resistance and, ideally, shorten the period of therapy of this infection[7-9].

Benzimidazole is a lead molecule for the most of the biological agent use in the pharmaceutical industry. It consists of fused benzene ring with heterocyclic aromatic imidazole. The existence of imidazole creates it a 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 synthesis and in vitro antimicrobial and antitubercular screening of newly synthesized mercaptobenzimidazole –clubbed chalcone derivatives.

MATERIALS AND METHODS:
The chemicals of analytical grade required for the synthesis of mercaptobenzimidazole–clubbed chalcone derivatives were purchased from Sigma-Aldrich and SD fine chemicals (India). The procured novel synthesized

compounds having purity and homogeneity preliminarily checked by determining melting points and were uncorrected. Progress of chemical reaction was authenticated by the thin layer chromatography study and spots were visualized in UV chamber or iodine chamber. FTIR spectra of intermediate and derivative compounds were recorded with help of pressed pellet technique on Jasco FTIT-460 plus spectrophotometer and vibrational frequencies expressed in cm^{-1}. Also, skeleton structure of synthesized derivatives confirmed with help of ^{1}HNMR study by using BRUCKER 400 MHz spectrometer in deuterated DMSO, TMS as internal standard and chemical shifts were recorded as δ (parts per million).

General procedure for synthesis of Mercaptobenzimidazole:

O-phenylenediamine (10.8g, 0.1 moles) treated with carbon disulfide (7.67g, 0.1 moles) in the presence of potassium hydroxide (5.65g, 0.1 moles), 100ml of 95% ethanol and 15 ml of water used as solvent in a round bottom flask was refluxed on water bath for three hours. After the completion of reaction, reaction mixture was cooled and filtered. After that, 1-1.5g of activated charcoal was added carefully in the filtrate. Further, filtrate refluxed for 10 minutes; the activated charcoal was removed by filtration. Filtrate was treated with 100ml of warm water at 60-70ºC for 10 minutes. Dilute acetic acid was poured into the reaction mixture for acidification with gentle agitation to yield shiny crystals as product, which is further kept in a refrigerator for three hours to allow the complete crystallization process. The obtained solid product was separated through Buchner funnel and dried at 40ºC overnight and recrystallized from the ethanol.

General procedure for Synthesis of N-Acetylmercaptobenzimidazole:

Mercaptobenzimidazole (5.5g, 0.1 moles) was treated with acetic anhydride (5ml, 0.1 moles) in the presence of glacial acetic acid (5ml, 0.1 moles) and 5ml of pyridine used as acetylating agent. Prepared solution mixture refluxed on sand bath for 10-15 minutes. After the completion of reaction, reaction mixture was cooled and filtered. Filtrate pours it slowly in 100ml of ice-cold water and stirring with help of glass rod. The obtained solid product was separated through Buchner funnel and recrystallized from the ethanol. The completion of reaction was ascertained by TLC (Benzene: Methanol/ 5:1).

General procedure for Synthesis of mercaptobenzimidazole–clubbed chalcone derivatives (DPK4B2d1-DPK4B2d1):

N-Acetylmercaptobenzimidazole (2g, 0.01 moles) treated with aromatic aldehyde (1.5g, 0.01 moles), 10ml of 95% ethanol used as solvent in a round bottom flask equipped with a magnetic stirrer. Then, 10ml of 0.1N sodium hydroxide was added drop wise to the reaction mixture on vigorous stirring for 30 minutes at 20-25ºC. The completion of reaction was ascertained by thin layer chromatography. After that, reaction mixture was neutralized by 0.1 N Hydrochloric acid whereby the precipitation occurred. The obtained crude chalcone was separated through Buchner funnel and were dried in air and recrystallized from the ethanol.

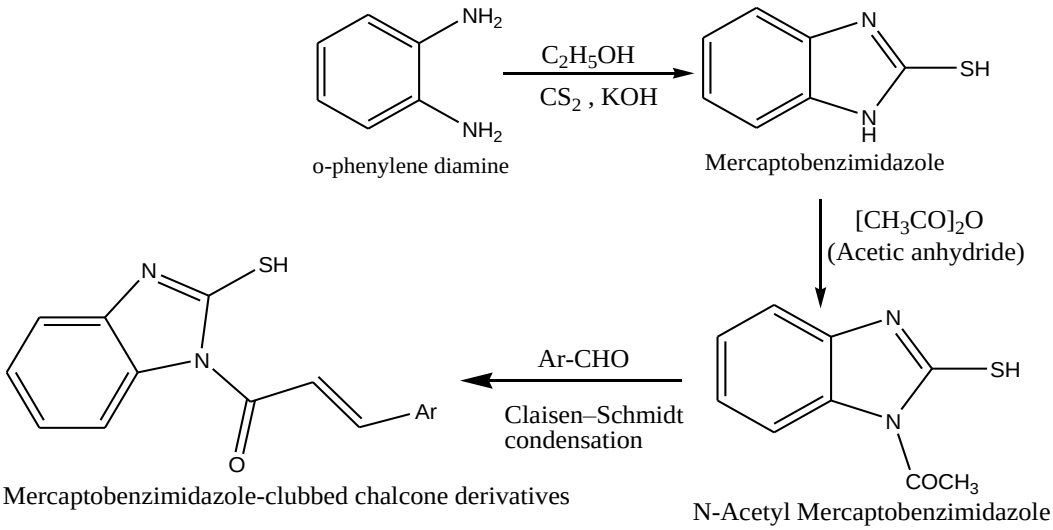


Figure 1: Scheme for synthesis of mercaptobenzimidazole–clubbed chalcone derivatives.

Table 1: Attachment of different aromatic aldehyde.

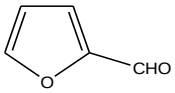
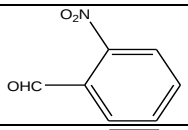
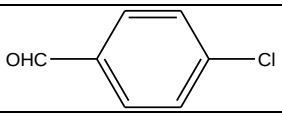
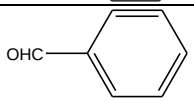
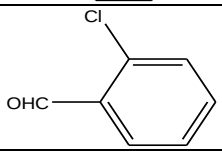
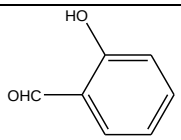
Compound Code	Ar-CHO	Compound Code	Ar-CHO
DPK4B2d1		DPK4B2d4	
DPK4B2d2		DPK4B2d5	
DPK4B2d3		DPK4B2d6	

Table 2: Characterization of mercaptobenzimidazole–clubbed chalcone derivatives.

Compound code	Molecular Formula	M.P.(ºC)	R_fValue	% Yield	FTIR (KBr cm-1)	NMR) (δ ppm)
DPK4B2d1	$C_{14}H_{10}N_2O_2S$	320-325ºC	0.56	68	3047, 2908, 2337, 1257, 1666, 1566, 1512, 1188, 696	6.4-8.5, 3.3- 6.3, 2.3-2.6
DPK4B2d2	$C_{16}H_{11}ClN_2OS$	346-351ºC	0.60	72	3147, 2965, 2299, 1319, 1689, 1620, 1512, 709, 696	7.114-10.016, 3.386-4.492, 2.377-2.652
DPK4B2d3	$C_{16}H_{11}ClN_2OS$	348-353ºC	0.65	65	3055, 2965, 2453, 1365, 1689, 1627, 1512, 709, 648	7.114-10.352, 3.364, 2.511-2.645
DPK4B2d4	$C_{16}H_{11}N_3O_3S$	355-360ºC	0.67	55	3063, 2931, 2360, 1257, 1674, 1625, 1519, 1565, 1442, 678	7.514-8.046, 3.340-5.556, 2.503
DPK4B2d5	$C_{16}H_{12}N_2OS$	300-305ºC	0.68	62	3063, 2965, 2453, 1265 ,1697, 1620, 1512, 655	7.222-8.529, 3.350-4.489, 2.360-2.641
DPK4B2d6	$C_{16}H_{12}N_2O_2S$	321-326ºC	0.58	63	3680, 3115, 2993, 2453, 1357, 1712, 1645, 1512, 655	7.108-8.018, 3.012-3.386, 2.369-2.642

RESULT AND DISCUSSION:

From the literature survey, it was revealed that benzimidazole has been reported to develop number of molecules have exposed various potent pharmacological activities. In this research study, we have reported synthesized mercaptobenzimidazole–clubbed chalcone derivatives. These newly synthesized derivatives were screened against in vitro antimicrobial and antitubercular activities. Synthesized derivatives having purity and homogeneity preliminarily checked by their physical constants and spectral studies such as FTIR, ^{1}HNMR for structural elucidation and studies showed satisfactory results.

In vitro antimicrobial activity:

In vitro antimicrobial activity of synthesized mercaptobenzimidazole-clubbed chalcone derivatives were screened by the tube dilution method against *Escherichia Coli* (Gram-negative bacteria/ATCC 25922), *Bacillus Subtilis* (Gram-positive bacteria/ATCC 6051), and *Aspergillus Niger* (fungal strain/ATCC 6275). Synthesized compounds having observed MIC values are showed in Table 3. Some of the mercaptobenzimidazole-clubbed chalcone derivatives were found to be highly efficient as antimicrobial agents. All the synthesized compounds showed average antibacterial activity as compared to standard ciprofloxacin. Compounds DPK4B2d2, DPK4B2d3, DPK4B2d4 and DPK4B2d6 shown potent antifungal activity with MIC value of 0.8µg/ml in the comparison of standard fluconazole.

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

Sr. No.	Compound Code	MIC in µg/ml		
		Antibacterial activity		Antifungal activity
		B. subtilis	***E. Coli***	***A. niger***
1	DPK4B2d1	100	100	1.6
2	DPK4B2d2	100	50	0.8
3	DPK4B2d3	100	50	0.8
4	DPK4B2d4	100	50	0.8
5	DPK4B2d5	100	100	1.6
6	DPK4B2d6	100	6.25	0.8
7	Ciprofloxacin	2	2	-
8	Fluconazole	-	-	8

In vitro antitubercular activity:

In vitro antitubercular activity of synthesized mercaptobenzimidazole-clubbed chalcone derivatives were screened by the Microplate Alamar Blue Assay (MABA) against *Mycobacterium tuberculosis* (H37Rv strain, ATCC 27294). Synthesized compounds having observed MIC values are showed in Table 4.

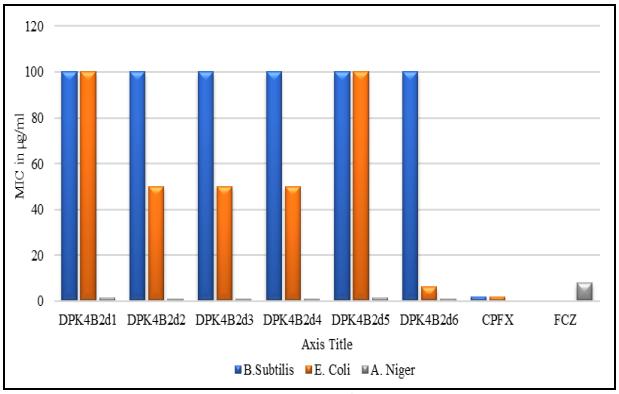


Figure 2: Graphical representation of antimicrobial activity, MIC values of synthesized compounds

Compounds DPK4B2d1 and DPK4B2d2 showed potent activity and also, rest of the compounds shown moderate activities in the comparison of standard antitubercular drugs such as Pyrazinamide, Ciprofloxacin, and Streptomycin.

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

Sr. No.	Compound Code	MIC in µg/ml	Sr. No.	Compound Code	MIC in µg/ml
1	DPK4B2d1	0.8	6	DPK4B2d6	12.5
2	DPK4B2d2	0.8	7	Pyrazinamide	3.12
3	DPK4B2d3	1.6	8	Ciprofloxacin	3.12
4	DPK4B2d4	3.12		Streptomycin	6.25
5	DPK4B2d5	6.25			

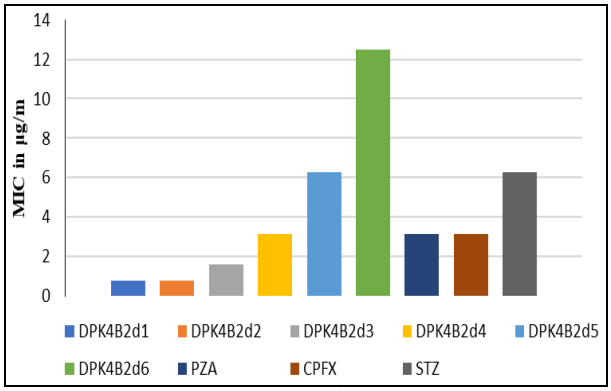


Figure 3: Graphical representation of antitubercular activity, MIC values of synthesized compounds.

Structure - activity relationship of mercaptobenzimidazole–clubbed chalcone derivatives:

From the comparison of antimicrobial and antitubercular activities of synthesized mercaptobenzimidazole–clubbed chalcone derivatives, the following SAR may be assumed:

1. From the results of antimicrobial activities of the mercaptobenzimidazole–clubbed chalcone derivatives compared to the standard drug ciprofloxacin and fluconazole conclude that, there should be slight structural modifications to develop affinity of drug to the binding of a molecule to the target site.
2. From the results of antitubercular activities of the mercaptobenzimidazole–clubbed chalcone 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 has need of supplementary in vivo studies to confirm the antitubercular activity.
3. 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 mercaptobenzimidazole–clubbed chalcone derivatives outcomes are summarized as follows:

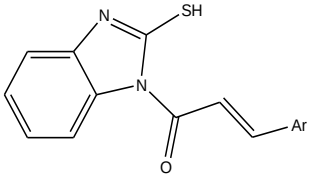


Figure 4: Basic nucleus of mercaptobenzimidazole–clubbed chalcone derivatives.

- Presence of mercaptobenzimidazole ring essential for antimicrobial and antitubercular activities.
- Presence of–SH (Mercapto group), C=O (Carbonyl group) and ethylene side chain are essential for antimicrobial and antitubercular activities.

At position Ar:

- Presence of aromatic ring such as benzene as well as furan ring important for antimicrobial and antitubercular activities.
- Substitution of -Cl (Chloro group) in the benzene ring at para as well ortho position shows potent to moderate antimicrobial and antitubercular activities.
- Substitution of $–NO_2$ (Nitro group) and –OH (Hydroxy group) in the benzene ring at para as well ortho position shows moderate antimicrobial and potent antitubercular activities.

CONCLUSION:

Novel series of mercaptobenzimidazole-clubbed chalcone derivatives were efficiently synthesized. These newly synthesized derivatives were examined against in vitro antimicrobial and antitubercular activities. Amongst the synthesized compounds DPK4B2d2, DPK4B2d3, DPK4B2d4 and DPK4B2d6 shown significant and potent activity against *A. Niger* as compared with the standard fluconazole. Compounds DPK4B2d1 and DPK4B2d2 showed potent antitubercular activity in the comparison of standard drugs such as Pyrazinamide, Ciprofloxacin, and Streptomycin.

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CONFLICTS OF INTEREST:

No.

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