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Synthesis and in vitro Evaluation of Pyridincarbonitrile Mercaptobenzimidazole Derivatives as a potent biological agent

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ABSTRACT:
In this present investigation to study of novel pyridincarbonitrile mercaptobenzimidazole derivatives was efficiently synthesized, which is further characterized and authenticated by using TLC, different spectral data such as IR and ^{1}HNMR. The newly synthesized derivatives were tested against in vitro antimicrobial and antitubercular activities. The result shows that the titled compounds shown average antibacterial activity against gram-positive *Bacillus Subtilis,* gram-negative *Escherichia Coli* as compared to standard ciprofloxacin. Compounds DPK4B1d2 and DPK4B1d7 shown potent antifungal activity against *Aspergillus Niger* as compared to standard fluconazole. Antitubercular activity tested against *mycobacterium tuberculosis*, compound DPK4B1d2 showed potent activity and also, rest of the compounds showed moderate activities in the comparison of standard such as Pyrazinamide, Ciprofloxacin, and Streptomycin.

KEYWORDS: Pyridincarbonitrile Mercaptobenzimidazole, 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 causes 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.

Therefore, it is essential to develop rational chemotherapeutic agents to deferral the development of resistance and, ideally, shorten the duration 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 evaluation of pyridincarbonitrile mercaptobenzimidazole derivatives as a potent biological agents.

MATERIALS AND METHODS:

The chemicals of analytical grade required for the synthesis of pyridincarbonitrile mercaptobenzimidazole derivatives were purchased from Sigma-Aldrich and SD fine chemicals (India). The newly 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, chemical structures 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:

General procedure for synthesis of Mercaptobenzimidazole:

O-phenylenediamine (10.8g, 0.1moles) treated with carbon disulfide (7.67g, 0.1moles) in the presence of potassium

hydroxide (5.65g, 0.1moles), 100 ml of 95% ethanol and 15ml 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 60-70^{0}C 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 40^{0}C overnight and recrystallized from the ethanol.

General procedure for Synthesis of N-Acetyl mercaptobenzimidazole:
Mercaptobenzimidazole (5.5g, 0.1moles) was treated with acetic anhydride (5ml, 0.1moles) in presence of glacial acetic acid (5ml, 0.1moles) 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 Pyridincarbonitrile Mercaptobenzimidazole derivatives (DPK4B1d1-DPK4B1d7):
A series of N-Acetyl mercaptobenzimidazole incorporating pyridine nucleus linked at first position. Pyridincarbonitrile Mercaptobenzimidazole derivatives were prepared by direct one pot reaction of N-Acetyl mercaptobenzimidazole (2g, 0.1moles) with the suitable aromatic aldehydes (1.5g, 0.1moles) in the presence of malononitrile (5ml, 0.1moles), ammonium acetate (2g, o.1moles) and 10ml of ethanol used as a solvent in a round bottom flask was refluxed on water bath for four hours. The completion of reaction was ascertained by TLC. After that, the reaction mixture was cooled to room temperature. The obtained solid crystals were separated through Buchner funnel and dried product was recrystallized from the ethanol.

Figure 1: Scheme for synthesis of pyridincarbonitrile mercaptobenzimidazole derivatives.

Table 1: Attachment of different aromatic aldehyde.

Compound Code	Ar-CHO	Compound Code	Ar-CHO
DPK4B1d1		DPK4B1d5	
DPK4B1d2		DPK4B1d6	
DPK4B1d3		DPK4B1d7	
DPK4B1d4			

Table 2: Characterization of Pyridincarbonitrile Mercaptobenzimidazole derivatives.

Compound code	Molecular Formula	M.P. (^{o}C)	R_f Value	% Yield	IR (KBr cm-1)	NMR) (δ ppm)
DPK4B1d1	$C_{17}H_{11}N_5OS$	350-355 ^{o}C	0.62	66	3664, 3495, 3095, 2916, 2515, 2322, 1519, 1249, 1018, 671.	6.682-8.863, 3.361, 2.501-2.515.
DPK4B1d2	$C_{21}H_{15}N_5S$	365-370 ^{o}C	0.55	68	3448, 3348, 3185, 3047 2385, 2222 1597,1226, 710.	7.249-8.098,4.533, 3.345, 2.500-2.525.
DPK4B1d3	$C_{19}H_{12}N_6O_2S$	358-363 ^{o}C	0.68	59	3495, 3402, 3063, 2924, 2612, 2214, 1575, 1483, 1350, 1250 752.	6.680-8.306, 3.41, 2.434-2.716.
DPK4B1d4	$C_{19}H_{12}ClN_5S$	345-350 ^{o}C	0.56	67	3618, 3464, 3055, 2839, 2337, 2214, 1550, 1250, 825,771.	6.752-7.951, 3.356, 2.445-2.508.
DPK4B1d5	$C_{19}H_{13}N_5S$	355-360 ^{o}C	0.59	55	3387, 3294, 3086, 2916, 2515, 2322, 1519, 1249, 671.	7.463-7.950, 3.346-4.993, 2.499-2.507.
DPK4B1d6	$C_{19}H_{13}N_5OS$	348-353 ^{o}C	0.78	68	3641, 3302, 3194, 3047, 2931, 2337, 2137,1527, 1249, 646.	6.333-7.308, 3.435-3.803, 2.498-2.526.
DPK4B1d7	$C_{19}H_{12}ClN_5S$	347-352 ^{o}C	0.66	70	3271, 3171, 3047, 2939, 2337, 2229, 1545, 1219, 896, 702.	7.529-8.687, 3.357-3.526, 2.501-2.530.

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 pyridincarbonitrile mercaptobenzimidazole derivatives. These newly synthesized derivatives were tested against in vitro antimicrobial and antitubercular activities. Synthesized derivatives having purity and homogeneity preliminarily checked by their physical constants and spectral studies such as IR, ^{1}HNMR for structural elucidation and studies showed satisfactory results.

In vitro antimicrobial activity:

In vitro antimicrobial activity of synthesized pridincarbonitrile mercaptobenzimidazole derivatives were evaluated 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 pridincarbonitrile mercaptobenzimidazole derivatives were found to be highly efficient as antimicrobial agents. All the synthesized compounds showed average antibacterial activity against *Bacillus Subtilis* and *Escherichia coli* as compared to standard ciprofloxacin. Compounds DPK4B1d2 and DPK4B1d7 showed potent antifungal activity with MIC value of 0.4µg/ml and 0.8µg/ml respectively 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	**DPK4B1d1**	50	25	1.6
2	**DPK4B1d2**	50	50	0.4
3	**DPK4B1d3**	50	50	6.25
4	**DPK4B1d4**	100	25	6.25
5	**DPK4B1d5**	100	50	1.6
6	**DPK4B1d6**	50	50	3.12
7	**DPK4B1d7**	100	50	0.8
8	**Ciprofloxacin**	2	2	-
9	**Fluconazole**	-	-	8

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

In vitro antitubercular activity:

In vitro antitubercular activity of synthesized pridincarbonitrile mercaptobenzimidazole derivatives were evaluated 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. Compound DPK4B1d2 showed potent activity and also, rest of the compounds showed 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	DPK4B1d1	1.6	6	DPK4B1d6	1.6
2	DPK4B1d2	0.8	7	DPK4B1d7	1.6
3	DPK4B1d3	1.6	8	Pyrazinamide	3.12
4	DPK4B1d4	1.6	9	Ciprofloxacin	3.12
5	DPK4B1d5	1.6	10	Streptomycin	6.25

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

Structure-activity relationship of pyridincarbonitrile mercaptobenzimidazole derivatives:

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

1. From the results of antimicrobial activities of the pyridincarbonitrile mercaptobenzimidazole 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 pyridincarbonitrile 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 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 pyridincarbonitrile mercaptobenzimidazole derivatives outcomes are summarized as follows:

Figure 4: Basic nucleus of pyridincarbonitrile mercaptobenzimidazole derivatives.

- Presence of benzimidazole as well as pyridine ring essential for antimicrobial and antitubercular activities.
- Presence of–SH (Mercapto group), -NH_2 (Amino group) and –CN (Cyano group) groups are essential for antimicrobial

and antitubercular activities.

At position Ar:

- Presence of aromatic ring such as benzene 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.
- Presence of Furan ring essential for antimicrobial and antitubercular activities.

CONCLUSION:

Pyridincarbonitrile mercaptobenzimidazole derivatives were efficiently synthesized. These newly synthesized derivatives were tested against in vitro antimicrobial and antitubercular activities. Amongst the synthesized compounds DPK4B1d2 and DPK4B1d7showed significant and potent activity against A. *niger* as compared with the standard fluconazole. DPK4B1d1to DPK4B1d7 showed an average antibacterial activity against *B. subtilis* and *E. coli* compared with standard Ciprofloxacin. Also, pyridincarbonitrile mercaptobenzimidazole derivatives such as DPK4B1d2 showed potent activity in the comparison of standard antitubercular drugs such as Pyrazinamide, Ciprofloxacin, and Streptomycin.

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CONFLICTS OF INTEREST:

No.

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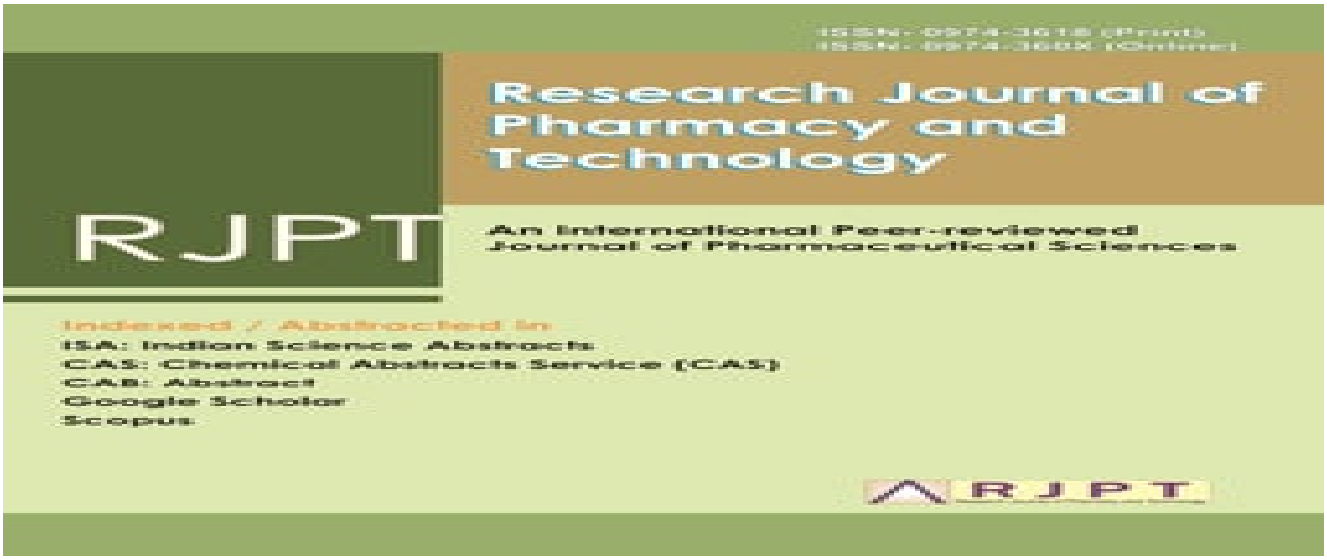

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