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Synthesis and Characterization of Benzimidazole Derivatives for Antimicrobial Property

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ABSTRACT: Benzimidazoles are an important class of compounds with a wide spectrum of biological activity ranging from anti-hypertensive, anti-viral, anti-microbial, antitumor and anthelmintic activity. Benzimidazole rings are the most important nitrogen-containing heterocycles, which are widely explored and utilized by the pharmaceutical industry for drug discovery. Due to their special structural features and electron-rich environment, Benzimidazole containing drugs bind to a variety of therapeutic targets, thereby exhibiting a broad spectrum of bioactivities. Numerous benzimidazole based drugs have been extensively used in the clinic to treat various types of diseases with high therapeutic potential.
The main objective of present work was to study the anti-microbial activity of the Benzimidazole derivatives. A series of benzimidzole derivatives have been synthesized and identified. The compounds were synthesized by using ethyl acetate and benzene as starting material. The series of 1, 2-disubstituted benzimidazoles containing pyrimidine and other functional groups was prepared which provides advantages such as, easy workup and high yield. All reagents used for synthesis were of synthetic grade. Purification of all compounds was done by thin layer chromatography using silica gel G as absorbent on glass plate using acetate: benzene (6:4 v/v %), Toluene: Acetone (8:2 v/v %) and Ethyl Acetate: n- Hexane (6:4 v/v %) as mobile phase. Compounds were detected by using iodine vapor as detecting agent. All compounds show single spot. All the newly synthesized compounds were characterized by IR spectral study. The compounds were investigated for their antimicrobial activity against clinical standard drug Ciprofloxacin. The anti-microbial study of the synthesized derivative was done Broad panels of bacterial and fungal strains were used for testing the antimicrobial properties of the synthesized molecules **III1-13**. The compounds **III_1** (m-NO_2), **III_2** (p-NO_2), **III_3** (m-Cl), **III_4** (3-F-4-Cl) and **III_9** (p-OCH_3) showed excellent activity (62.5 µg/ml), even better than ciprofloxacin.
Keywords: Benzimidazole, Derivatives, Anti-microbial Activity, Ciprofloxacin, Spectral data.

INTRODUCTION

Benz imidazole is an amphoteric compound as they possess both acidic and basic characteristics. The NH group present in Benz imidazole is relatively strongly acidic and weakly basic. Benz imidazole makes an important constituent of naturally occurring Vit-$B12^{56}$ and is a structural unit of nucleotide, due to which it easily interacts with the biopolymers of living system and this interaction is responsible for its numerous biological aspects, since proteases have been linked with several disease states Benz imidazole exhibits numerous biological aspects like antihelminthic, antifungal, anti-allergic, antimicrobial,

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antiviral, antineoplastic, antioxidant, anticancer, antihypertensive, anti-inflammatory, analgesic, antiprotozoal, anti-hepatitis B virus, antiulcer and anticonvulsant[1] activities. Benzimidazoles with their two ring systems bearing different functional substituents leads to essential modification of the physico-chemical metabolic and pharmacokinetic properties of these drugs. In the past few decades, the incorporation of Benz imidazole nucleus have received much attention due to their Anti-microbial values.

Microbial disease are responsible for a significant of death worldwide and according to the world health organization cancer is increasing world's largest problem. Unfortunately, however a number of the current clinically efficacious anticancer agents are becoming less active because of development of anticancer resistance. A long standing scientific research has shown that Benz-imidazole shows important biological properties such as anti-cancer, anti-malarial, anti-microbial, antibacterial and anti-oxidant activities. There is an urgent need for the discovery or optimization of novel anti-microbial agents.

The present work is aimed to find heterocyclic linked Benz imidazole as antimicrobial agents.

MATERIALS AND METHOD

MATERIALS

The marketed formulation of ciprofloxacin tablet manufactured by Cipla was procured from local market. All the chemicals used were analytical grade and were purchased from Merck Specialties Private Limited, Mumbai, India.

INSTRUMENTATION:

FTIR spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Standard and sample drugs were weighed by using Denver electronic chemical balance (SI-234) and Melting point was determined by using Melting point apparatus BTI. Apparatus-Reflux condenser by WENSAR is also used.

EXPERIMENTALS

EXPERIMENTAL PROCEDURE FOR THE SYNTHESIS OF BENZIMIDAZOLE DERIVATIVES:

A. Synthesis of 5-methoxy-1-H-benzo[d]imidazole-2-thiol I (step 1)

Ethanol (40 ml) and potassium hydroxide (0.01 mol, 56.11 gm/mol, 0.56 gm in 2 ml H_2O) were taken in a dry round bottom flask. 4-methoxybenzene-1,2diamine (0.01 mol, 138.08 gm/mol, 1.38gm) was added to it and stirred well to get a clear solution. Carbon disulfide (0.02 mol, 76.14 gm/mol, 1.2 ml) was added to the clear solution obtained above and refluxed for 12 -15 h. The ethanol was distilled off and then cooled to room temperature. The content was poured into water and acidified with diluted

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HCl till the precipitates were separated. The separated solid was washed with cold water and dried to get the desired product.

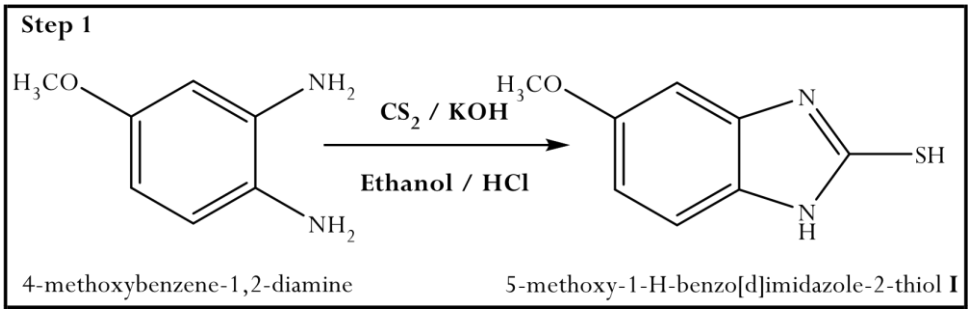

Fig.1 Synthesis of 5-methoxy-1-H-benzo[d]imidazole-2-thiol I

B. Synthesis of 5-(difluoromethoxy)-1H-benzo[d]imidazole-2-thiol I' (step 1)

Ethanol (40 ml) and potassium hydroxide (0.01 mol, 56.11 gm/mol, 0.56 gm in 2 ml H_2O) were taken in a dry round bottom flask. 4-(difluoromethoxy) benzene-1,2diamine (0.01 mol, 174.14gm/mol, 1.74 gm) was added to it and stirred well to get a clear solution. Carbon disulfide (0.02 mol, 76.14 gm/mol, 1.2 ml) was added to the clear solution obtained above and refluxed for 12 -15 h. The ethanol was distilled off and then cooled to room temperature. The content was poured into water and acidified with diluted HCl till the precipitates were separated. The separated solid was washed with cold water and dried to get the desired product.

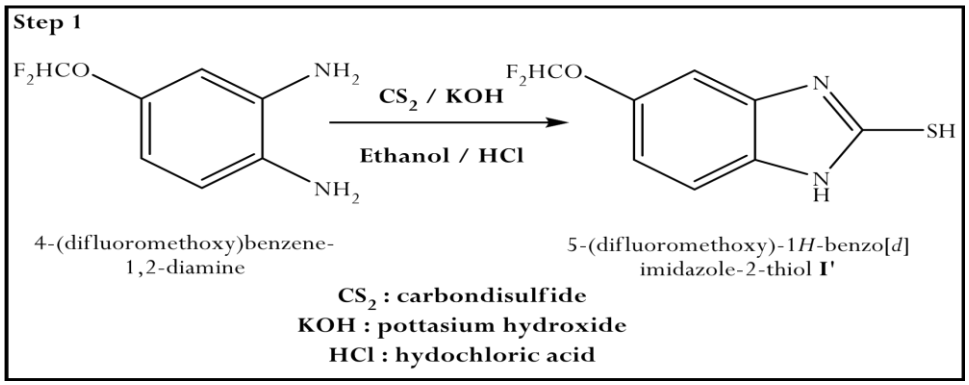

Fig.2 Synthesis of 5-(difluoromethoxy)-1H-benzo[d]imidazole-2-thiol I'

C. Synthesis of 2-chloro-N-(aryl) acetamide derivatives $II_{1\text{-}13}$ and $II_{a\text{-}j}$ (step 2)

Various substituted amines (0.01 mol) were added to a solution of DMF (35ml) containing TEA (3-4 drops). The mixture was stirred for 10 minutes at room temperature. CAC (0.015 mol, 113gm/mol, 1.19 ml) was added to the above mixture, maintaining the temperature between 0 to 5°C. The obtained solution was then stirred at room temperature for 4-6h.

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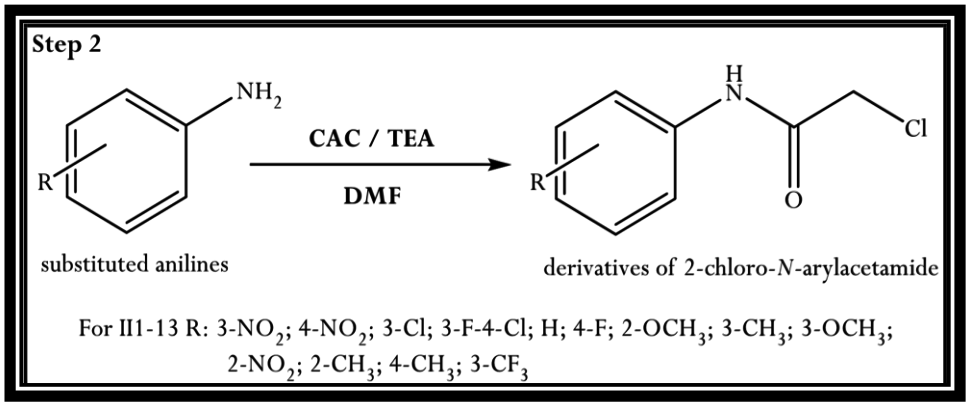

Fig.3 Synthesis of 2-chloro-N-(aryl)acetamide derivatives II_{1-13}

D. Synthesis of 2-(5-methoxy-1H-benzo[d]imidazol-2-ylthio) N(aryl) acetamide derivatives III_{1-13} (step 3)

5-methoxy-1H-benzo[d]imidazole-2-thiol **I** (0.01 mol, 180 gm/mol, 1.8 gm) was made soluble in acetone. To this well stirred solution different 2-chloro-Narylacetamide derivatives $\mathbf{II_{1-13}}$ (0.01 mol) were added to the above solution. K_2CO_3 (0.02 mol, 138 gm/mol, 2.76 gm) was added to the solution containing mixture of 5methoxy-1H-benzo[d]imidazole-2-thiol **I** and different acetamide derivatives. The mixture was allowed to stir for 4h at room temperature.

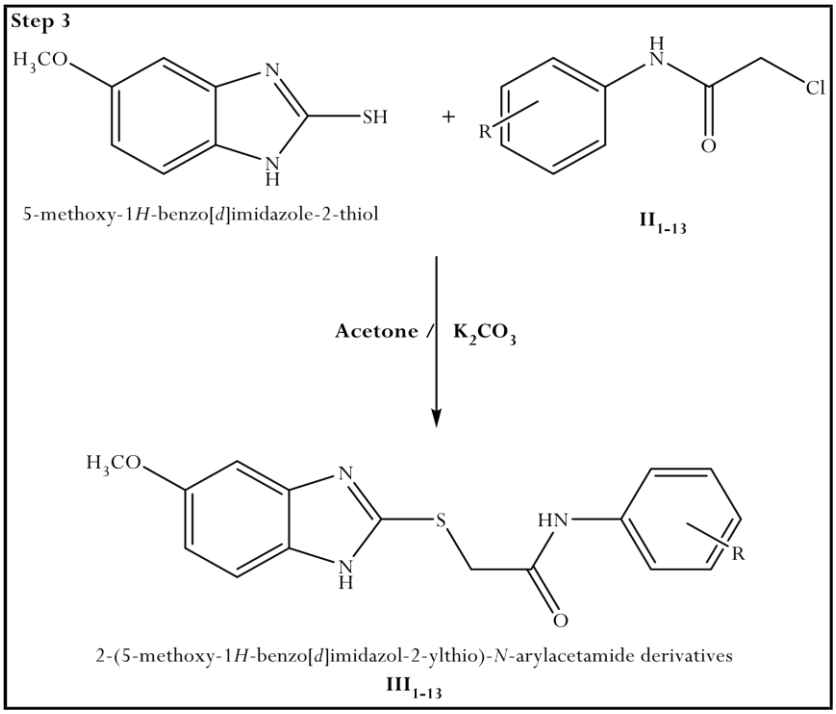

Fig.4 Synthesis of 2-(5-methoxy-1H-benzo[d]imidazol-2-ylthio)N(aryl) acetamide derivatives III_{1-13}

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SPECTROSCOPIC EXPERIMENTS

A. Melting point

Melting point of drug sample was determined by using melting point apparatus. The drug sample was taken and placed in a thin walled capillary tube; the tube was approximately 10-12 cm in length with 1mm in diameter and closed at one end. The capillary was placed in melting point apparatus and heated and when drug sample was melted the melting point of synthesized compound was recorded. The melting point of drug sample was determined by using melting point apparatus. The melting point was found to be in the range of 118-175^{0}C. The melting point of synthesized compounds are shown in the table:

Table no.1 melting Point of compound III_{1-13}

Derivatives	Substituent	Molecular formula	Molecular weight gm/mol	m.p. °C	% Yield
III_1	-3-NO_2	C16H14N4O4S	358	118°C	50%
III_2	-4-NO_2	C16H14N4O4S	358	125°C	59%
III_3	-3-Cl	C16H14N3O2SCl	347	121°C	63%
III_4	-3-F-4-Cl	C16H13N3O2SClF	365	202°C	59%
III_5	-H	C16H15N3O2S	313	118°C	55%
III_6	-4-F	C16H14N3O2SF	331	158°C	58%
III_7	-2-OCH_3	C17H17N3O3S	343	156°C	44%
III_8	-3-CH_3	C17H17N3O2S	327	105°C	54%
III_9	-3-OCH_3	C17H17N3O3S	343	195°C	62%
III_{10}	-2-NO_2	C16H14N4O4S	358	105°C	60%
III_{11}	-2-CH_3	C17H17N3O2S	327	156°C	53%
III_{12}	-4-CH_3	C17H17N3O2S	327	175°C	76%
III_{13}	-3-CF_3	C17H14N3O2SF	381	109°C	66%

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B. Determination of Rf value by Thin layer chromatography:

Rf value of synthesized compounds and the intermediates were determined by Thin Layer Chromatography on glass plates using silica gel-G as absorbent and acetate: benzene (6:4 v/v%), Toluone : Acetone (8:2 v/v %) and Ethyl Acetate: n- Hexane (6:4 v/v %) as solvent system and spots were detected in iodine chamber. The differences in Rf value between starting compound and product were indicative of the conversation of starting compound into the product. Rf values are mentioned in table no. 8.2.1.

Table no. 2: Molecular Formula, Rf value, solvent system of compound III_{1-13}

Derivative	Molecular formula	Rf	Solvent system
III_1	C16H14N4O4S	0.71	Ethyl acetate: Benzene (6:4)
III_2	C16H14N4O4S	0.69	Ethyl acetate: Benzene (6:4)
III_3	C16H14N3O2SCl	0.72	Ethyl acetate: Benzene (6:4)
III_4	C16H13N3O2SClF	0.65	Ethyl acetate: Benzene (6:4)
III_5	C16H15N3O2S	.067	Ethyl acetate: Benzene (6:4)
III_6	C16H14N3O2SF	0.63	Ethyl acetate: Benzene (6:4)
III_7	C17H17N3O3S	0.62	Ethyl acetate: Benzene (6:4)
III_8	C17H17N3O2S	0.73	Ethyl acetate: Benzene (6:4)
III_9	C17H17N3O3S	0.64	Ethyl acetate: Benzene (6:4)
III_{10}	C16H14N4O4S	0.74	Ethyl acetate: Benzene (6:4)
III_{11}	C17H17N3O2S	0.65	Ethyl acetate: Benzene (6:4)
III_{12}	C17H17N3O2S	0.67	Ethyl acetate: Benzene (6:4)
III_{13}	C17H14N3O2SF	0.75	Ethyl acetate: Benzene (6:4)

C. Infra-red spectral Analysis

Spectral interpretation for compound III_{13}

If we observe the data for 2-(5-methoxy-1H-benzo[d]imidazol-2-ylthio)-N (3(trifluoromethyl) phenyl) acetamide **III_{13}**, An absorption peak obtained at 3393 cm^{-1} has helped in confirming the presence of -NH in vicinity to the carbonyl group. The presence of aromatic C-H linkage is confirmed by the peak obtained at 3095 cm^{-1}. A sharp peak at 2921 cm^{-1} helped to assign the presence of -C-H bond in $-OCH_3$ group. A stretching band at 2853 cm^{-1} concluded the presence of methylene group in the final motif. The presence of carbonyl (-C=O) in the structure is proved due to the presence of a sharp peak at 1698 cm^{-1}. The presence of -C=N in the Benz imidazole nucleus was also confirmed by the presence of a sharp absorption band at 1580 cm^{-1}.

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Table no 3: IR spectral data for compound III_{13}

Functional group	Frequency (Cm^{-1})	Functional group	Frequency (Cm^{-1})
-N-H sec. amine (str.)	3393	-C=O carbonyl group (str.)	1698
-C-H aromatic ring (str.)	3095	-C=N sec. amine (str.)	1580
-C-H methoxy group (str.)	2921	-C-F (str.)	1161
$-CH_2$ methylene group (str.)	2853	-Nil-	-Nil-

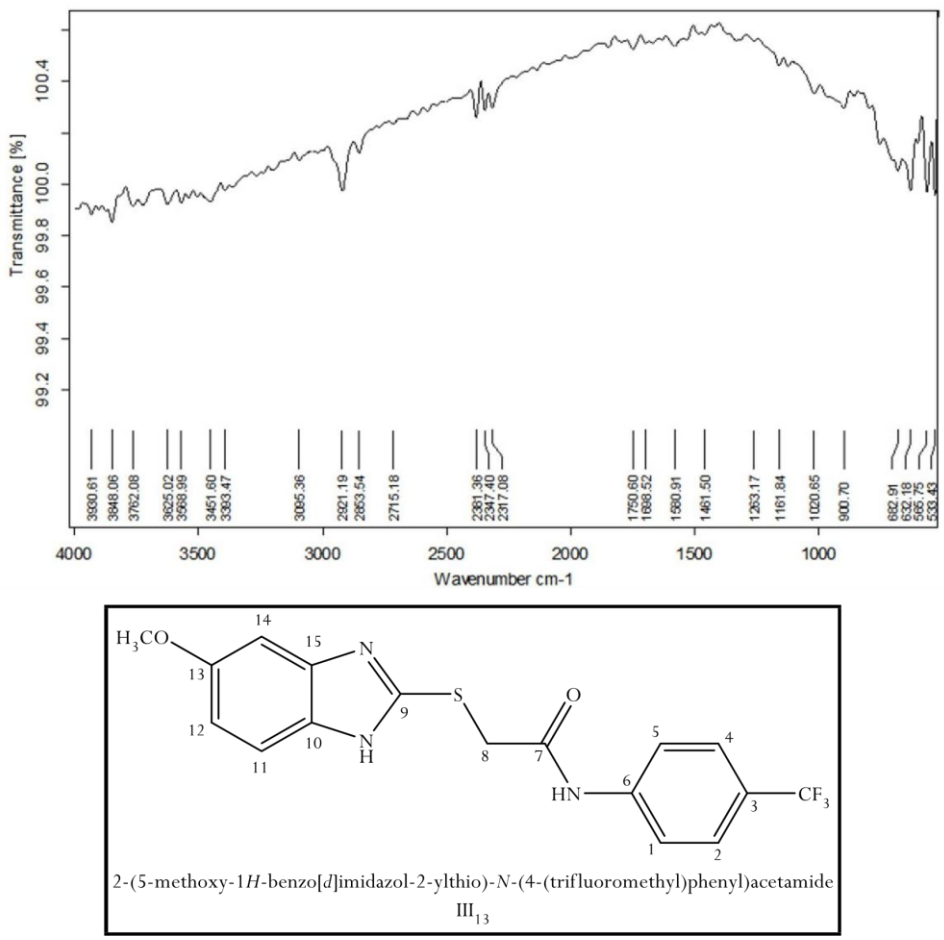

Fig. 2 IR spectra for compound III_{13}

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BIOLOGICAL EVALUATION OF SYNTHESIZED COMPOUND

Therapeutic studies – antimicrobial studies

The results obtained were depicted in the form of Minimum Inhibitory Concentration (MIC) values for the synthesized derivatives. The samples were tested by standard protocols like micro dilution/broth titer method. The antibacterial and antifungal details for each compound synthesized are discussed below.

Antibacterial Properties for compounds III_{1-13}

Gram-positive bacteria *S. aureus* (ATCC No. 25923) and *E. faecalis* (ATCC No. 27853) were introduced for testing the antibacterial potential of the synthesized molecules **III_{1-13}**. When tested against *S. aureus*, it was found that from the complete series synthesized, compounds **III_9** (p-OCH_3) and **III_{10}** (o-NO_2) exhibited activity equivalent to that of the standard ciprofloxacin (62.5 µg/ml). Other derivatives from the series exhibited higher MIC value than the standard resulting in poor results against gram-positive bacteria *S. aureus*. Similarly the synthesized series was tested against another gram-positive bacterial strain *E. faecalis*, where it was observed that the derivatives **III_3** (m-Cl), **III_4** (3-F-4-Cl), **III_5** (H), **III_9** (p-OCH_3), **III_{10}** (o-NO_2) and **III_{11}** (o-CH_3) exhibited equivalent (125 µg/ml) activity as compared to the standard. Overall half of the derivatives showed equivalent activity against gram-positive bacteria *S. aureus* and *E. faecalis* when compared with the standard drug ciprofloxacin. The final derivatives **III_{1-13}** were also tested against two gram-negative bacteria *E. coli* (ATCC No. 25922) and *P. aeruginosa* (27853) and compared with the same standard ciprofloxacin. The results of most of the compounds as antibacterial were excellent against both the gram-negative bacterial strains. The compounds **III_4** (3-F-4-Cl), **III_5** (H), **III_6** (p-F), **III_{10}** (o-NO_2), **III_{11}** (o-CH_3), **III_{12}** (p-CH_3) and **III_{13}** (m-CF_3) showed MIC value (62.5 µg/ml) even better than that of the standard drug ciprofloxacin proving excellent potency as antibacterial. The remaining derivatives from the same series which included **III_1** (m-NO_2), **III_2** (p-NO_2), **III_3** (m-Cl), **III_8** (m-CH_3), **III_9** (p-OCH_3) exhibited activity equivalent to the standard (125 µg/ml). These derivatives when tested against *P. aeruginosa* also resulted in very good antibacterial activity. Compounds **III_1** (m-NO_2), **III_2** (p-NO_2), **III_3** (m-Cl), **III_4** (3-F-4-Cl) and **III_9** (p-OCH_3) showed excellent activity (62.5 µg/ml), even better than ciprofloxacin. There were other compounds exhibiting equivalent activity (125 µg/ml) as compared to the standard viz. **III_5** (H), **III_6** (p-F), **III_{10}** (o-NO_2), **III_{11}** (o-CH_3), **III_{12}** (p-CH_3) and **III_{13}** (m-CF_3). Thus from the series of synthesized derivatives **III_{1-13}** more than half of the compounds showed equivalent activity.

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Table 4: Antimicrobial screening for compounds III_{1-13}

Compounds	Minimum Inhibitory Concentration (MIC) in µg/ml					
	Gram-positive bacteria		Gram-negative bacteria		Fun	Gi
	S. aureus ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 10231	*A. niger* ATCC 1015
III_1	250	250	125	62.5	250	250
III_2	125	250	125	62.5	125	250
III_3	125	125	125	62.5	125	250
III_4	125	125	62.5	62.5	125	250
III_5	125	125	62.5	125	125	125
III_6	125	250	62.5	125	125	125
III_7	31.25	125	62.5	125	250	125
III_8	250	250	125	250	250	250
III_9	62.5	125	125	62.5	250	250
III_{10}	62.5	125	62.5	125	250	250
III_{11}	125	125	62.5	125	125	125
III_{12}	250	250	62.5	125	250	250
III_{13}	250	250	62.5	125	250	125
Ciprofloxacin	62.5	125	125	125	-	-

CONCLUSION

From the antimicrobial results carried above, it can be concluded that the derivatives possessing electron with-drawing substituent were found to exhibit excellent antimicrobial property in **III_{1-13}**. On the basis of above results, attempts are made to optimize the lead structure to obtain more potent antimicrobial molecules. The series of synthesized derivatives **III_{1-13}** more than half of the compounds showed equivalent activity. Antimicrobial screening of the compounds III_{1-13} up to a great extent have proved to be potent antibacterial agents. In this study we have synthesized a novel series of 1, 2-benzimidazole derivatives starting from easily available starting materials. Using this method we have prepared series of 1,2-disubstituted benzimidazoles containing pyrimidine and other functional groups, which provides advantages such as, easy workup and high yield.

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