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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTI-MICROBIAL ACTIVITY OF 2-SUBSTITUTED BENZIMIDAZOLES

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ABSTRACT

Four new 2-substituted-benzimidazole derivatives (ADMRY1-4**)** were synthesized by simple synthesis of 2-methylchloride benzimidazole (I) from *o*-phenylenediamine. It is a versatile synthetic intermediate because removal of hydrochloric acid when treated with substituted aniline. The equimolar concentration compound (I) (0.01mol) is treated with different substituted aniline (0.01mol) in presence of ethanol as medium and catalytic amount of hydrochloric acid, refluxed it for 1-2 hrs resulted in to compound (II) to build a linear 2- substituted benzimidazole derivatives by a simple route. The newly synthesized compounds were characterized by its melting point and I.R, ^{1}HNMR, MASS spectroscopic methods. Further the synthesized compounds were screened for antimicrobial activity. Few compounds have shown moderate to mild antimicrobial activity when compared to standard ampicilin.

KEYWORDS

Benzimidazole, 2-substitututed benzimidazole, Antibacterial and Antifungal activity.

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INTRODUCTION

Benzimidazole (Figure No.1) is a heterocyclic aromatic organic compound. It is an important pharmacophore and a privileged structure in medicinal chemistry. This compound is bicyclic in nature which consists of the fusion of benzene and imidazole. Nowadays is a moiety of choice which possesses many pharmacological properties. The most prominent benzimidazole compound in nature

is N-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin B12[1].

Study on Structural modifications and their pharmacological actions

The use of Benzimidazole dates many years back[2]. In 1990 various benzimidazole derivatives were synthesized with substitution of fluorine, propylene, tetrahydroquinoline and cyclised compound which resulted in compounds with increased stability, bioavailability and significant biological activity[3, 4]. It was also showed that substitution on pyridine by electron donating group increases activity. In 1991 benzimidazole derivatives were synthesized by derivatization at N-H of benzimidazole by electron donating group and substitution with long chain of propyl, acetamido, thio, thiazole-amino, tetramethyl piperidine on pyridine resulting in good antiulcer activity[5, 6]. Nowadays Infectious microbial diseases are causing problems world-wide, because of resistance to number of antimicrobial agents (p-lactam antibiotics, macrolides, quinolones, and vancomycin). A variety of clinically significant species of microorganisms has become an important health problem globally[7]. One way to fight with this challenge is the appropriate usage of the available marketed antibiotics the other is the development of novel anti-microbial agents[8]. Hence, there will always be a vital need to discover new chemotherapeutic agents to overcome the emergence of resistance and ideally shorten the duration of therapy. Due to the structural similarity to purine, antibacterial ability of benzimidazoles are explained by their competition with purines resulting in inhibition of the synthesis of bacterial nucleic acids and proteins[9, 10]. Benzimidazole having some important activities such as anti-microbial and anti-bacterial effects, HIV Inhibitors, anti-parasitic effectuates, viral effect, anti-hypertensive agents, anti-ulcer activity, anti-microbial and anti-fungal activity, antiproliferative, anti-inflammatory, anti-protozoa, anti-inflammatory, anti-protozoal, androgen receptor antagonist, anti-oxidant activity. The benzimidazole ring is an important pharmacophore in modern drug discovery. Attention has been increasingly given to the synthesis of benzimidazole derivatives as a source of new antimicrobial agents. The benzimidazole derivatives are a resource for medicinal research. The knowledge gained by various researches has suggested that substituted benzimidazoles and heterocycles, which are the structural isosteres of nucleotides allow them to interact easily with the biopolymers, possess pharmacological activity with lower toxicities. Since now, researchers have been attracted toward designing more potent benzimidazole derivatives having wide diverse of biological activity.

MATERIAL AND METHODS

All chemicals used were of analytical grade from, SD Fine. Melting points of all the synthesized compounds were determined by open capillary tube method. These are uncorrected. The purity of all compounds was checked by TLC was run on Silica Gel G plates using Chloroform: alcohol (9:1). Spots were visualized using iodine vapour chamber. IR spectra were recorded on Shimadzu IR spectrophotometer by using KBr pellets technique (Figure No.2). ^{1}H-NMR was recorded on Bruker AMX 60 MHz spectrophotometer by using DMSO as solvent (Figure No.3) (Table No.1).

Procedure for synthesis of substituted benzimidazoles

Take 54gms of *O*-phenyl diamine in 250 mL round bottom flask and add 39 mL of acetyl chloride. Heat the mixture on water both at 100^{o}C for 4hrs, cool, add 10% NaOH solution, slowly constant rotation of the flask, until the mixture is just alkaline to litmus. Then the filter off the crude Benzimidazole by using filter paper and the compound is dried and kept in desiccator.

Procedure for recrystallisation

Dissolve the crude product in 400 mL boiling water add 2gms of decolorizing carbon (activated charcoal) filter rapidly at the pump through a pre-heated buccner funnel and flask. Cool the filtrate to about 10^{0}C, filter off the benzimidazole, wash with 25ml of cold water and dry it finally to get pure benzimidazole.

Synthesis of 2 substituted benzimidazoles

Take equimolar concentration substited benzimidazole (0.01 Mole) and (0.01Mole) different substituted aniline in 100ml beaker and add 10-15ml of acetone and 2-3 drops of Hcl then stirring for 1 hr. After stirring the mixture will be poured in beaker containing ice water, in this add sodium sulphate to remove the neutralize excess acid liberated. Then that mixture washed with petroleum ether for preventing sticky nature and form precipitate. Finally filter the mixture and drying (Scheme 1).

Biological Activity

Evaluation of the Anti-bacterial activity of synthesized compounds by minimal inhibitory concentration (MIC) method

Method

In vitro antibacterial activity of all synthesized compounds was evaluated against four strains of microorganisms namely *S. aureus* (MTCC 96, Gm+ve), *P. aeruginosa* (MTCC 1688, Gm–ve), *S. pyogenus* (MTCC 442, Gm+ve) *and E. coli* (MTCC 443, Gm–ve\) by MIC method (Broth dilution method).Mueller Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test bacteria. Ampiciln is used as a reference standard.

Evaluation of the Anti-fungal activity of synthesized compounds by Agar cup method

All those compounds screened for antibacterial activity were also tested for their antifungal activity by using Agar cup method. The fungi employed for screenings were; *Aspergillus niger* (MTCC 282), *Aspergillus clavatus* (MTCC 1323) and *Candida albicans* (MTCC 227). Potato dextrose agar-agar was used as a medium for the growth of fungi and Grieseofulvin as reference standard drug.

RESULTS AND DISCUSSION

Anti bacterial activity

Among the synthesized compounds, ADMRY-1 and ADMRY-2 exhibited highest antibacterial activity against both Gm+ve and Gm-ve pathogens compared to other tested compounds. These compounds contain *m*-chloro, and *o*-methyl, some of the above mentioned compounds bear electron withdrawing groups and some other compounds contain electron donating groups. This does not indicate any relationship between the structure and the anti-bacterial activity of the compounds. Further research is required to get the clear idea about relationship between anti-bacterial activity and structure of the compounds (Table No.2).

Anti-fungal activity

Compounds ADMRY-1 and ADMRY-2 exhibited potent antifungal activity against tested fungal strain than any other tested compounds. These compounds contain, both electron donating and electron withdrawing groups like, m-chloro, *o*-methyl respectively. This fails to give any conclusive idea about the relationship between structure and antifungal activity as among these compounds some contain electron withdrawing groups and some other contain electron donating groups. However, further studies are required to come to a conclusion about establishing a correlation between antifungal activity and structure of the synthesized compounds (Table No.3).

Table No.1: Characterization of new compounds

S.No	Compd. Code	C-Cl cm^{-1}	C=C cm^{-1}	C=N cm^{-1}	Ali-CH cm^{-1}	Ar-CH cm^{-1}	Imidazole-NH	^{1}HNMR (in δ ppm) and Mass spectral data
1	ADRMY-1	705	1470	1630	2954	3288	3398	1.339 (S, 1H, -CH); 2.95 (S, 1H, imi-NH); 2.36 (S, 1H, ali-NH); 6.96-7.265 (M,8H,Ar-H); EIMS m/z = 258
2	ADRMY-2	-	1468	1629	2955	3056	3291	-
3	ADRMY-3	-	1437	1630	2954	3100	3453	-
4	ADRMY-4	710	1445	1610	2940	3150	3462	-

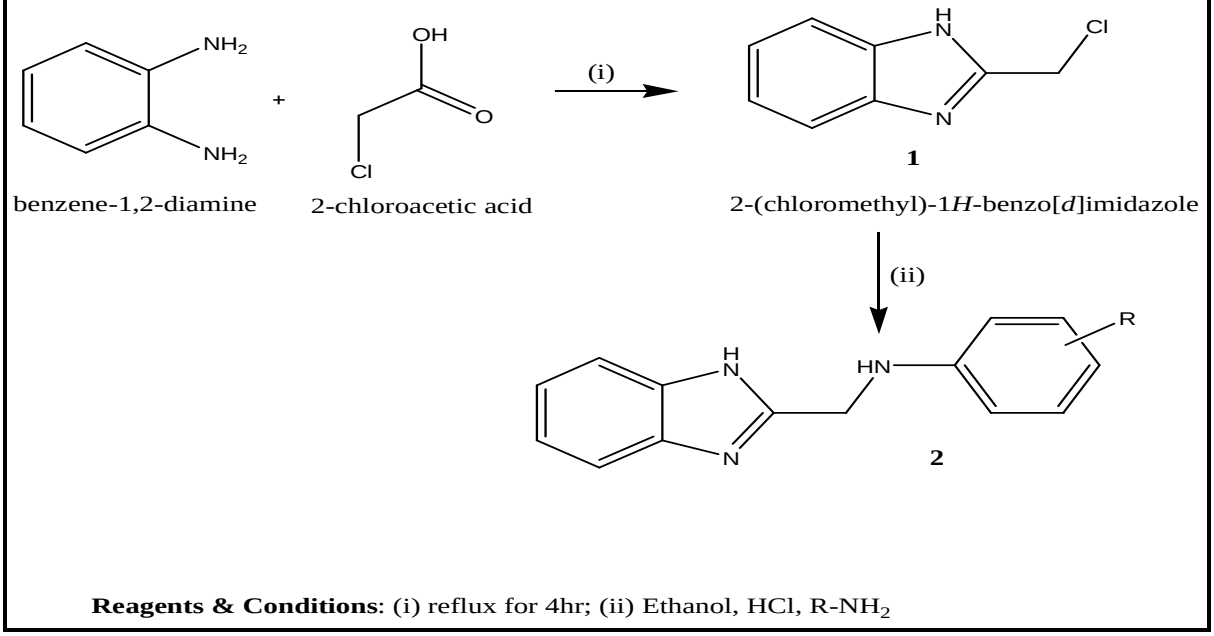
Table No.2: Anti bacterial activity by MIC method for the synthesized compounds (MIC in μg/ml)

S.No	Compound Code	*E. coli* [MTCC 443] (Gm-ve)	*P.aeruginosa* [MTCC 1688] (Gm-ve)	*S.aureus* [MTCC 96] (Gm +ve)	*S.pyogenus* [MTCC 442] (Gm +ve)
1	Ampicillin (Std)	100	100	250	100
2	ADMRY-1	100	50	200	100
3	ADMRY-2	100	100	50	100
4	ADMRY-3	100	500	250	500
5	ADMRY-4	200	250	200	150

Table No.3: Antifungal activity by Micro broth dilution method for the synthesized compounds (MIC in μg/ml)

S.No	Compound code	*C. albicans* (MTCC227)	*A. niger* (MTCC 282)	*A. clavatus* (MTCC 1323)
1	Greseofulvin (std)	500	100	100
2	ADMRY-1	200	1000	>1000
3	ADMRY-2	200	>1000	>1000
4	ADMRY -3	250	1000	1000
5	ADMRY -4	500	500	500

Scheme 1



Reagents & Conditions: (i) reflux for 4hr; (ii) Ethanol, HCl, R-NH_2

Scheme 1: Synthesis of 2 substituted benzimidazoles

S.No	Name of the compound (code)	R
1	ADMRY-1	m-Chloro-aniline
2	ADMRY-2	m-toludine
3	ADMRY-3	N-methyl-aniline
4	ADMRY-4	4-chloro-aniline

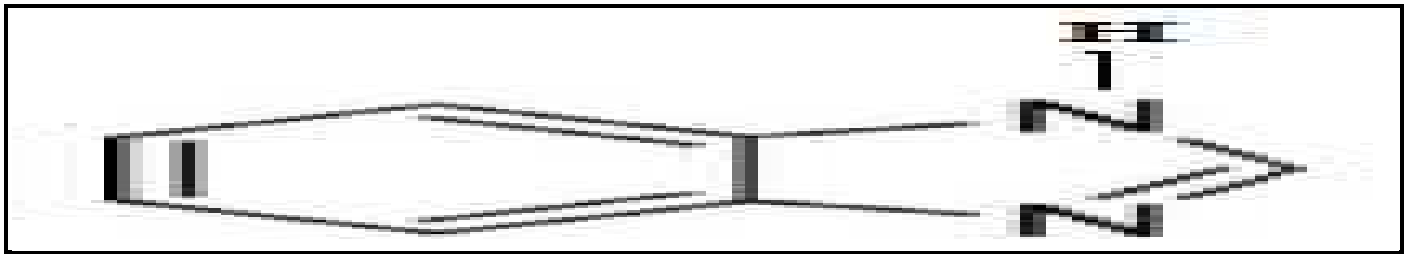


Figure No.1: Structure of 1H-benzimidazole

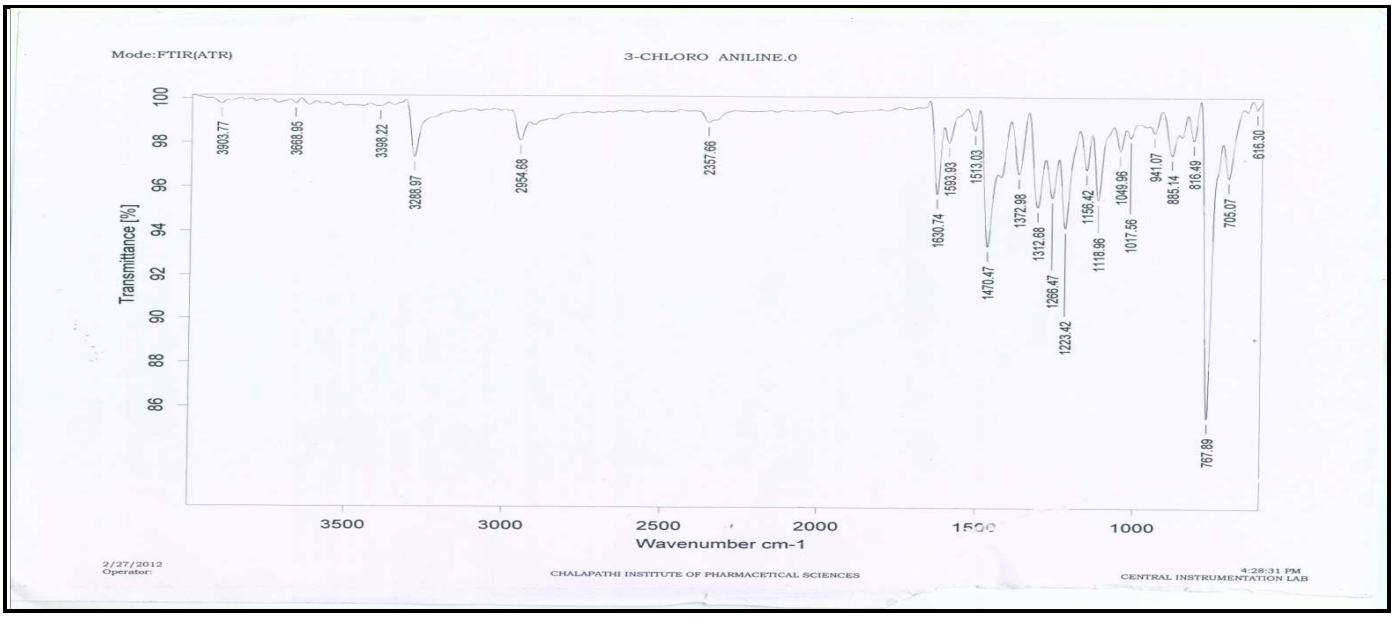


Figure No.2: IR Spectrum of 3-Chloro Aniline

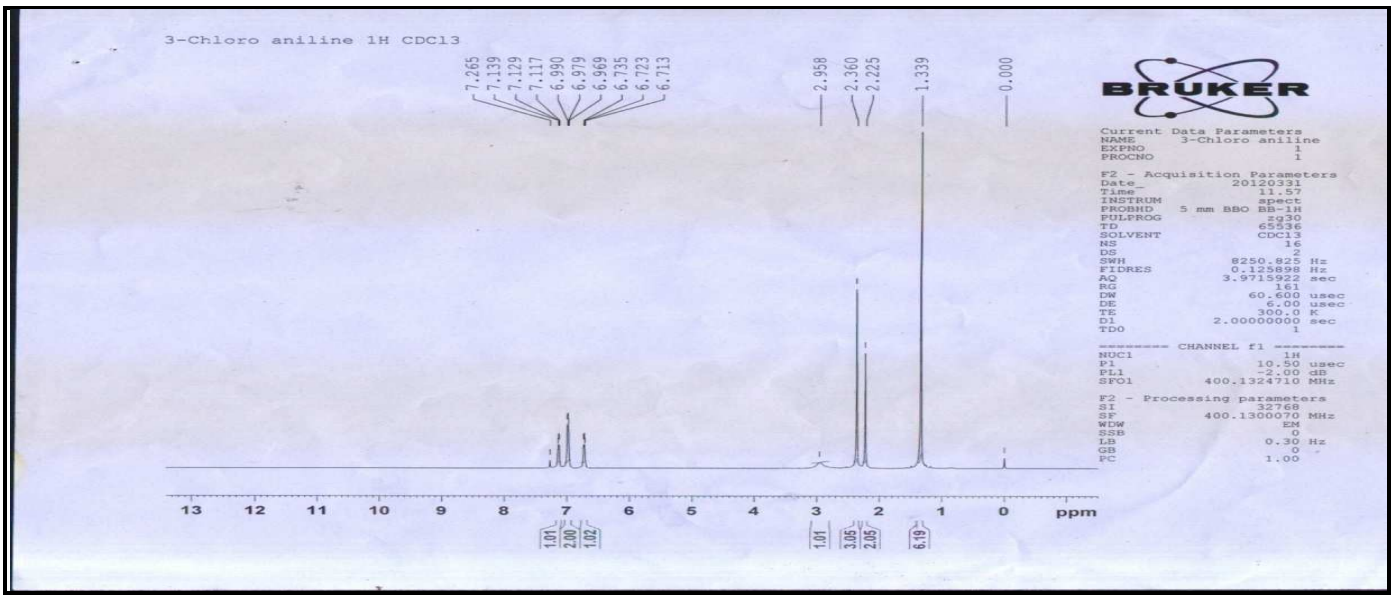


Figure No.3: NMR Spectrum of 3-Chloro Aniline

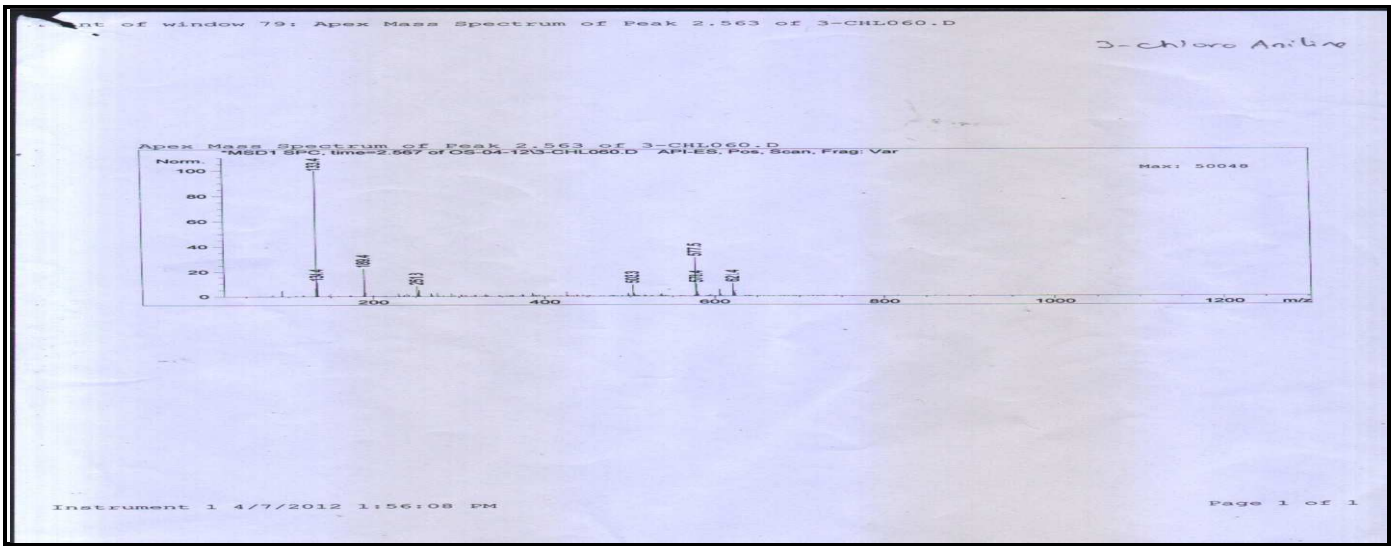


Figure No.4: Mass Spectrum of 3-Chloro Aniline

CONCLUSION

Four new 2-substituted-benzimidazoles derivatives ADMRY (1-4) were synthesized. Analytical and spectral data were used to characterize synthesized compounds (Figure No.2-4). All synthesized compounds were screened for antibacterial and anti-fungal activity. Some of the tested compounds exhibited potent to moderate antibacterial activity against both (Gm+ve) *S.aureus*, *S.pyogenus* and (Gm-ve) *E.coli*, *p.auregensa* when compared to standard Ampicilline. Few of the tested compounds exhibited significant and potent antifungal against *C. albicans* but none of synthesized compounds shown significant antifungal activity against *A. Niger* and *A. clavatus* when compared to standard griseofulvin.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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