SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME 2-SUBSTITUTED BENZIMIDAZOLES

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Summary

Benzimidazole nucleus is considered to be biologically most important, as its derivatives find wide pharmacological applications. The synthesis and evaluation of various biological activities of benzimidazoles with heterocyclic substitution at various positions were reported earlier[1,2]. Substituted Benzimidazoles are a major class of anti-infectives with significant potential for continuous development. In our present work 2-substituted benzimidazoles were synthesized and evaluated for their antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Candida albicans* and *Aspergillus niger*. Sulphadiazine and ketaconazole were used as standards for antibacterial and antifungal activity, respectively.

Key-words: Benzimidazole, antimicrobial activity, tube-dilution method

Introduction

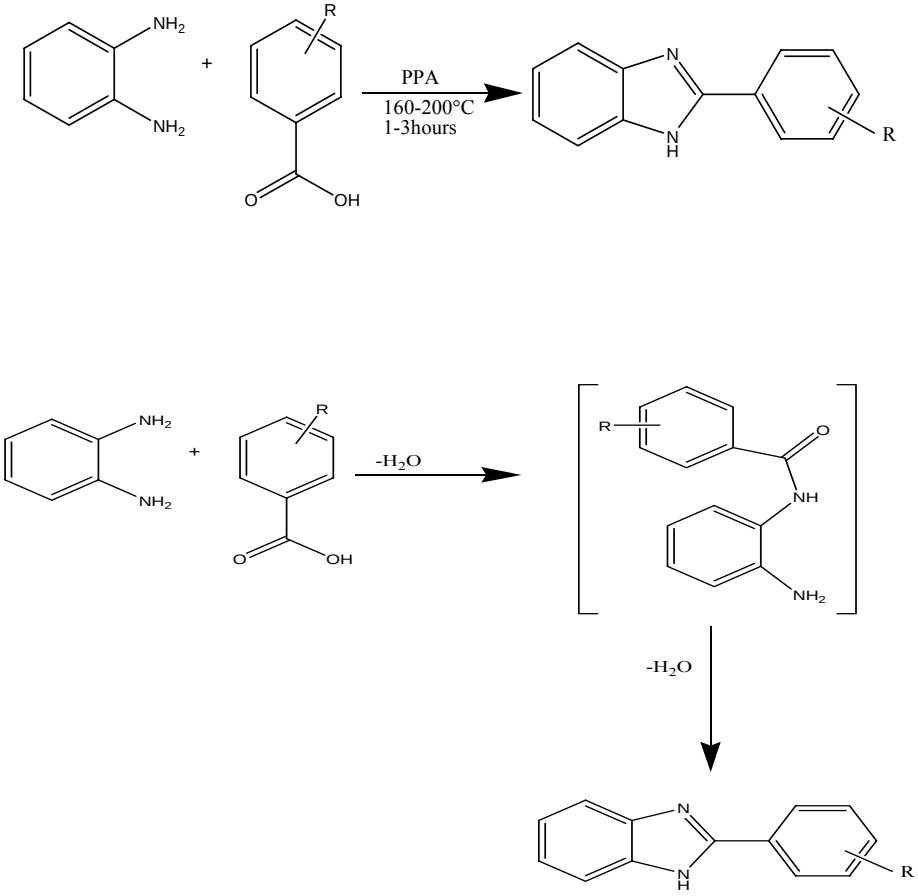
Diseases due to pathogenic bacteria and fungi represent a critical problem to human health and they are one of the main causes of morbidity and mortality world wide[3]. The evolution of multiple drug resistant human pathogenic microorganisms has driven the search for new sources of antimicrobial substances[4]. At the time of Hippocrates, wine and vinegar were used in wound dressings. Pasteur introduced the concept of sterilization of surgical instruments by heat and Hister used phenol to kill bacteria on instruments, dressings and other operating materials. Those led to the development of a number of non-antibiotic antimicrobial agents[5].

The versatility of Benzimidazole nucleus makes it a significant compound in the treatment of various diseases. Benzimidazole inhibits the growth of various bacteria, fungi, yeast, protozoa and helminthes. Determination of antimicrobial effectiveness against specific pathogen is essential for proper therapy. Bacterial genomic sequencing will greatly amplify the number of targets that can be screened for decades to come in the continuing and necessary search for new antibacterial agents[6]. The present work aims at, synthesis of various Benzimidazole derivatives, evaluation of antibacterial and antifungal activities.

Materials and Methods

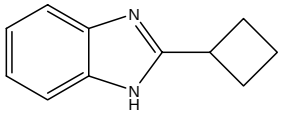
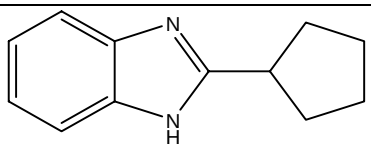
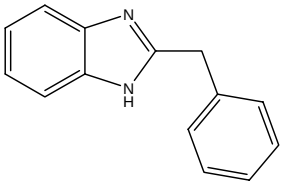
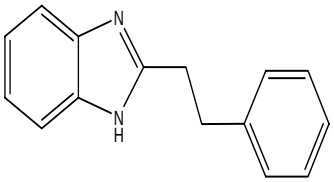
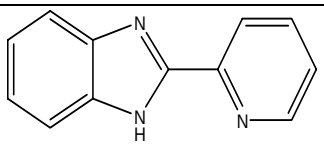
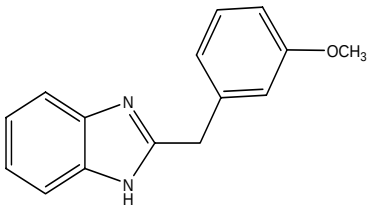
Synthesis of 2-substituted benzimidazoles

Compounds in which the aromatic or alicyclic ring is directly attached to second position of benzimidazole ring were synthesized from o-phenylenediamine by reaction with carboxylic acids in the presence of polyphosphoric acid. The reaction was carried out at 160-200^{o}C for 1-3 hours. In the first step, o-phenylenediamine reacts with aromatic or alicyclic acid to form the intermediate amide with the loss of a water molecule. In the next step, cyclization of the amide takesplace to form the corresponding benzimidazole, substituted at second position with aromatic or alicyclic ring.



PPA : Polyphosphoric acid, R: substitution

Table 1-Physical characteristics of synthesized compounds

Comp code	Empirical Formula	Structure	Yield	Melting Point	R_f Value	Mol.wt	Partition Coefficient
JP1	$C_{11}H_{12}N_2$		52%	138°C	0.86	172	3.837
JP2	$C_{12}H_{14}N_2$		39%	147°C	0.82	186	3.396
JP3	$C_{14}H_{12}N_2$		65%	181°C	0.74	208	3.632
JP4	$C_{15}H_{14}N_2$		53%	189°C	0.68	228	3.781
JP5	$C_{12}H_9N_3$		58%	214°C	0.78	195	2.5344
JP6	$C_{15}H_{14}N_2O$		48%	151°C	0.71	238	3.551

Physical Characteristics of Synthesized compounds:

The synthesized compounds were soluble in ethyl acetate and ethanol. The melting points were determined by open capillary method. The structures of the synthesized compounds were confirmed by NMR, MASS, and IR Spectroscopy. The physiochemical characteristics of the synthesised compounds were summarised in Table No.1. The NMR spectra were recorded on NMR-JEOL GSX-400 using $CDCl_3$ as solvent. The chemical shifts were reported in δ units relative to TMS. IR spectra were recorded on FT-IR Perkin Elmer 1000 using KBr pellets. The results are in confirmatory to the anticipated structures. The UV spectra were recorded on Shimadzu 160A spectrophotometer between 200-400 nm.

Evaluation of antimicrobial activity

Test organisms and drugs used

Ketoconazole obtained from Janssen-cilag pharmaceuticals, Bangalore. Dimethylsulphoxide was purchased from Qualigens Fine chemicals, Mumbai. Sulphadiazine was purchased from Ranbaxy Laboratories Ltd., Delhi. *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger* were used as test organisms.

The antimicrobial activity was evaluated by tube-dilution method. It depends upon the inhibition of growth of a microbial culture in a uniform solution of antibiotic in fluid media that is favourable to its rapid growth in the absence of the antibiotic[7]. In this method minimal inhibitory concentration (MIC) of the antimicrobial agent was determined. The MIC is the lowest concentration of an antimicrobial agent that inhibits the growth of the test organism[8].

Determination of antibacterial activity

Antibacterial activity of synthesized compounds was tested against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis.* The MIC of the synthesized compounds was determined by broth macrodilution using sterile glass test tubes containing MH broth (supplemented with magnesium and calcium cations). The inoculum contained $5X10^5$ c.f.u/ml. The concentration range tested was 0.078 to 2.5 µg/ml. Test solutions were prepared in DMSO and freshly diluted on the day of testing. Each test was performed in duplicate (CLSI, 1999). Sulphadiazine was used as standard.

Determination of Antifungal activity

Antifungal activity of synthesized compound was tested against *Candida albicans* and *Aspergillus niger*. The MIC of the synthesized compounds was determined by broth macrodilution using sterile glass test tubes containing Sabouraud's glucose broth[9]. The inoculum contained $5X10^5$ c.f.u/ml. The concentration range tested was 0.078 to 2.5 µg/ml. The test solutions were prepared in DMSO and freshly diluted on the day of testing. Each test was performed in duplicate (CLSI, 1999). Ketaconazole was used as standard.

Table 2: Microbial growth inhibition data (MIC)

Comp. Code	Compound Name	MIC (µg/ml)				
		S.a	B.s	E.c	C.a	A.n
JP1	2-(cyclobutyl) Benzimidazole	0.3120	0.6250	0.6250	0.6250	1.2500
JP2	2-(cyclopentyl) Benzimidazole	0.1560	0.6250	0.3120	0.3120	0.3120
JP3	2-(benzyl) Benzimidazole	0.3120	0.3120	0.6250	0.6250	0.3120
JP4	2-(2-phenyl ethyl) Benzimidazole	0.6250	0.3120	0.3120	0.1560	0.1560
JP5	2-(2-pyridyl) Benzimidazole	0.1560	0.3120	0.3120	0.3120	0.3120
JP6	2-(3-methoxy benzyl) Benzimidazole	0.3120	0.3120	0.1560	1.2500	0.6250
Std	Sulphadiazine	0.1560	0.1560	<0.1560	0.3120	0.1560
Std	Ketoconazole	0.3120	0.1560	0.3210	<0.1560	0.1560

S.a - *Staphylococcus aureus* B.s - *Bacillus subtilis* E.c - *Escherichia coli*
C.a - *Candida albicans* A.n - *Aspergillus niger*

Results and Discussion

Evaluation of Antimicrobial activity

The antimicrobial activity of the synthesized compounds was evaluated by tube-dilution method against bacterial species such as *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* and fungal species such as *Candida albicans* and *Aspergillus niger*. The *in vitro* antimicrobial activity was studied for synthesized compounds in comparison with clinical standards sulphadiazine for antibacterial activity and ketoconazole for antifungal activity. Table- 2 summarizes the biological data gathered for the synthesized compounds.

Minimum inhibitory concentration is defined as the lowest concentration of the compound that prevented the growth of test organism after incubation at 37^{o}C for 24 hours for bacteria and incubation at 30^{o}C for 48 hours for *Candida albicans* and 7 days for *Aspergillus niger*.

The results of the antibacterial activity revealed that, all the synthesized compounds showed activity against *Staphylococcus aureus*. Compounds JP2 and JP5 showed maximum activity. All the compounds showed activity against *Bacillus subtilis.* But none of the compounds exhibited activity equal to clinical standard. All the compounds showed activity against *Escherichia coli*. Out of the different compounds synthesised, compound JP6 showed maximum activity with MIC value of 0.1560 µg/ml, against *Escherichia coli*.

The results of the antifungal activity revealed that all the synthesized compounds showed activity against *Candida albicans* and *Aspergillus niger*. Compound JP4 showed the maximum activity with MIC value of 0.1560 µg/ml against *Candida albicans* and *Aspergillus niger*.

In conclusion, our study revealed the antimicrobial properties of 2-substituted benzimidazoles. Although the results of *in vitro* studies were encouraging, further *in vivo* studies has to be performed, to establish the safety and efficacy of the synthesised compounds.

References

1. Haugwitz, R. D.; Maurer, B. V.; Jacobs, G. A.; Narayanan, V. L.; Cruthers, L.; Szanto, Synthesis and anthelmintic activities of novel 2-pyridinyl-5-isothiocyanatobenzimidazoles. Journal of Medicinal Chemistry (1979), 22(9), 1113-18.

2. Dubey, Rashmi; Abuzar, Syed; Sharma, Satyavan; Chatterjee, R. K.; Katiyar, J. C.. Synthesis and anthelmintic activity of 5(6)-[(benzimidazol-2-yl)carboxamido]- and (4-substituted piperazin-1-yl)benzimidazoles. Journal of Medicinal Chemistry (1985), 28(11), 1748-50.

3. World Health Organization, The World Health Report. Life in the 21st Century: A vision for all 2. Measuring health. World Health Organization, Geneva,Switzerland 1998: 39-60.

4. Nostro A, Germano MP, Angelo V, Marino A, Cannatelli MA., Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lettent Applied Microbiology 2000; 30:379-384.

5. Kalant M.D, Rochlan, W.H.F., Principles of Medical Pharmacology, 5th Edn. B.C. Deckor Inc., Toronto, 1985; 595.

6. Chu, Daniel T. W.; Plattner, Jacob J.; Katz, Leonard. New Directions in Antibacterial Research. Journal of Medicinal Chemistry (1996), 39(20), 3853-3874.

7. Pharmacopoeia of India, Vol. II.Published by Ministry of Health Department , Govt. of India, A-88.

8. Cappucino. J and Sherman N., Microbiology, A laboratory manual, 4th Edn., Addison Wesley Longman, Inc., California, 1999; 263.

9. Cruckshank R, Duguid J.O., Marimon B.P. and Swain, R.H.A., Medical Microbiology, 12th Edn., Vol.II, Churchill Livingstone Publication, Newyork, 1975;136.