International Journal of Engineering Research & Management Technology ISSN: 2348-4039
September-2016 Volume 3, Issue-5
Email: editor@ijermt.org www.ijermt.org

SPECTRAL AND BIOLOGICAL ANALYSIS OF 2-PHENYL-1H-BENZIMIDAZOLE

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ABSTRACT: 2-Phenyl-1H-benzimidazole is synthesized by benzene-1, 2-diamine and benzoic acid and its structure was also established using FTIR, UV-Vis and 1H-NMR spectroscopic method. The synthesized compound was also tested for antimicrobial activity against Escherichia coli, Bacillus subtitles and Staphylococcus aureus and fungus Candida albican, Aspergillus Niger and Candida krusei.

KEYWORDS: FTIR, NMR, Raman spectroscopy, UV-Visible spectroscopy, pyrimidin-4-one, antimicrobial activity.

INTRODUCTION

Bacterial infection is a ubiquitous health hazard. There are a number of very good clinically efficacious antibiotics in use today; however, the development of bacterial resistance has rendered almost all of them less effective. This critical situation necessitates the design of novel antibacterial agents. These agents must target essential bacterial pathways, but may have new modes of action or even interfere with novel bacterial targets. Many essential bacterial proteins have been identified as potential drug targets. However, an ideal target is recognized as that different from existing targets, essential for microbial cell survival, highly conserved in a clinically relevant spectrum of species, absent or radically different in man, easy to assay, and has an understood biochemistry.

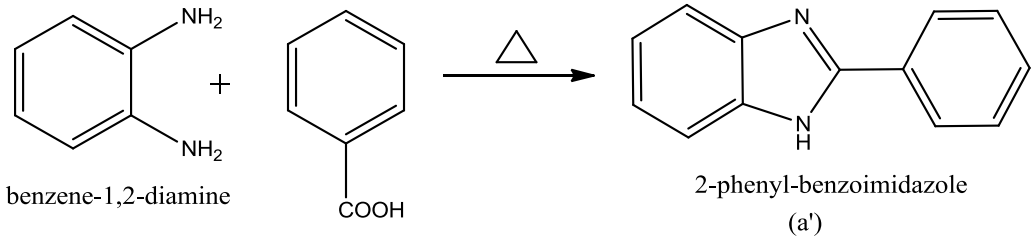
Antibacterial agents present an extremely heterogeneous class of compounds with a large and continuously growing number of commercially available drugs. This extraordinary diversity can be structured by the chemistry of the agents, their mode of action or by their main indication, thus many substances of these major drug classes are also used in the treatment of infectious diseases like respiratory tract infections, sexually transmitted diseases, gastrointestinal infections etc. As various pathogenic bacteria continuously produce mechanism of resistance to currently used antibacterial drugs, so the discovery of novel and efficacious antibiotics is the best way to overcome bacterial resistance.

In this direction, various scientists have been engaged to synthesize new compounds related to various chemical groups. Literature survey revealed that benzimidazole derivatives possessed broad spectrum of biological activities *viz* anthelmintic [1], anticancer [2], antiprotozol [3], anticonvulsant [4-5], anti-inflammatory [6], analgesic [7], antifungal [8], antibacterial [9] and many more.

The vibrational analysis of the title compound has been studied [10-11].The complete vibrational analysis of the polyatomic molecules is possible only when both the IR and Raman spectral data are available [12].The 1H-NMR analysis [13] and UV-Vis analysis [14] of the title compound also has been studied.

METHODS AND MATERIALS

A solution of benzoic acid (0.01 mol) and benzene-1,2-diamine (0.01mol) in 20ml of glacial acetic acid was stirred in 15 min with heating at 150 to 200 ^{0}C the precipitate is obtained after addition of 10%NaOH in ice bath. Then the product is filtered, dried in hot air oven and recrystallized from ethanol [15]. The yield of 2-phenyl benzoimidazole is 95 % (**scheme1**).



benzene-1,2-diamine

benzoic acid

2-phenyl-benzoimidazole

(a')

SCHEME 1

All the reagents and solvents were generally received form commercial supplier. Reactions were done in dried glassware. Melting points were taken in open capillaries by thermionic melting point apparatus, (Campbell Electronic Mumbai, India) and are uncorrected. The purity of the newly synthesized compounds was checked by thin layer chromatography (TLC) on silica gel-G coated plates by using different solvent systems. Infrared (IR) spectra were determined on Bruker IFS-66 FTIR (Bruker Bioscience, USA) using KBr pallets and wave number (ν) was reported in cm^{-1}. The ^{1}H-NMR spectra were taken on Jeol GSX -300 FT NMR (Jeol, Tokyo, Japan) in $CDCl_3$ or DMSO-d_6, and chemical shifts (δ) are given in ppm. Tetramethylsilane (TMS) was used as internal reference standard. Mass spectra were recorded on Spec Finnigan Mat 8230 MS. The carbon, hydrogen and nitrogen analysis were performed on Carlo Erba-1108 (Carlo Erba, Milan, Italy), and the results were found within ± 0.4% of the theoretical values. The electronic spectra (UV-Vis) were recorded on a Perkin-Elmer Lambda 15 UV-Vis spectrophotometer, using 10^{-3} mol·dm^{-3} solutions in DMF.

ANTIMICROBIAL ACTIVITY

The antimicrobial activity was assayed *in vitro* by the twofold broth dilution [16] against bacteria *Escherichia coli, Bacillus subtitles* and *Staphylococcus aureus* and fungus *Candida albican, Aspergillus Niger* and *Candida krusei*. The minimal inhibitory concentrations (MIC, µg/ml) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain. All compounds, dissolved in dimethylsulfoxide, were added to culture media .Mueller Hinton Broth for bacteria and Sabouraud Liquid Medium for fungi to obtain final concentrations ranging from 125 µg/ml to 1.592 µg/ml. The amount of dimethylsulfoxide never exceeded 1% v/v. Inocula consisted of 5.0 x10^4 bacteria/ml and 1.0 x10^3 fungi/ml. The MICs were read after incubation at 37 °C for 24 h (bacteria) and at 30°C for 48 h (fungi). Media and media with 1% v/v dimethylsulfoxide were employed as growth controls. Chloroamphenicol and fluconazole were used as reference antibacterial and antifungal drugs, respectively. To detect the type of antimicrobial activity, subcultures were performed by transferring 100 µl of each mixture remaining clear in 1 ml of fresh medium. The minimal bactericidal concentrations (MBC, µg/ml) and the minimal fungicidal concentrations (MFC, µg/ml) were read after incubation at 37 °C for 24 h and at 30 °C for 48 h, respectively.

RESULT AND DISCUSSION

Spectral analysis

The heteroaromatic structure shows the presence of C-H stretching, in-plane bending vibrations in the regions 3200-3000 cm^{-1} and 900- 1200 cm^{-1} respectively. In this region the bands are not affected appreciably by the nature of the substituents. The FTIR bands at 3150, 3108, 2908, 2852, and 2603 cm^{-1} and FT-Raman bands at 3068, 2892, 2852 and 2601 cm^{-1} in benzoimidazole is assigned to C-H stretching modes. The bands at 1138, 1076, 1016 cm^{-1} have been assigned to C-H in-plane bending vibrational modes. The IR

and Raman bands identified at 3443 cm^{-1} are assigned to N-H stretching mode. The N-H in-plane bending vibration is found at 1311 and 1231 cm^{-1}. The C=N stretching frequencies in the Raman spectrum of crystalline 2-phenyl benzoimidazole occur in the range 1625-1480 cm^{-1}. In the present investigation, the Raman bands observed at 1551, 1480 cm^{-1} have been assigned to C=N stretching vibrations. The very strong IR peak and the strong Raman peak observed at 1625 cm^{-1} is assigned to C-N stretching mode. The carbon-carbon stretching vibrations of the title compound have been observed at 1510, 1495 and 1460 cm^{-1}. The medium Raman bands identified at 872 and 752 cm^{-1} have been assigned to C-C in-plane bending (**Table1)**.

In the ^{1}H-NMR spectra, the singlet signal at δ 12.96 ppm is assigned to NH based on the position of this peak in the spectrum of the parent benzoimidazole molecule. The assignment of the peak at δ 8.21-8.20 ppm of two proton of CH of benzoimidazole molecule is obtained. doublet signal of H (1) and H (2) of benzoimidazole are found. Multiplet signal of CH of phenyl group are found (**Table2**).

UV-Vis absorption spectra of 2-phenyl benzoimidazole after the continuous prolonged irradiation (0, 5, 15, 30, 45 and 60 min) with UV-A light. Both the absorption maxima (λ_{max}= 303 nm and λ_{max}= 315 nm) decrease, and a slight bathochromic shift have been detected, at the end of any particular UV-irradiating period. The log values of the absorbance maxima plotted against irradiation time yielded a linear plot, suggesting the involved kinetics to be probably of pseudo-first order, depending on the 2-phenyl benzoimidazole concentration only (**Table3**).

Antimicrobial activity (Minimal inhibitory concentration)

Antibacterial activity of 2-phenyl benzoimidazole (a') and standard drug, chloroamphenicol, was carried out at a concentration 250 µg/ml against *E. coli ATCC 25922*, *B. subtitles ATCC 1633* and *S. aureus ATCC 25923*. Results show the varying degree of antibacterial activity of all the compounds tested (T**able 4**). From the results obtained, it is clear that 2-phenyl benzoimidazole exhibited less activity against *E. coli ATCC 25922*, *B. subtilis ATCC 1633* than chloroamphenicol but *S. aureus ATCC 25923* displayed antibacterial property moderate to the reference drug.

The compound 2-phenyl benzoimidazole (a') along with reference drug, fluconazole, were also tested for antifungal activity at a concentration of 250 µg/ml against *C. albicans ATCC 2091*, *A. Niger ATCC 9029* and *C. krusei ATCC 6518*, and it is found that synthesized is showed very weak or moderate active as compared to standard drug.

CONCLUSION

2-phenyl benzoimidazole established using FTIR, UV-Vis and ^{1}H-NMR spectroscopic method. Vibrational and electronic spectra confirmed the synthesized compound, 2-phenyl benzoimidazole. The compound was tested for its *in vitro* antimicrobial activity and its activity against bacteria *Escherichia coli*, *Bacillus subtitles* and *Staphylococcus aureus* and fungus *Candida albican*, *Aspergillus Niger* and *Candida krusei* compared to chloramphenicol and fluconazole, respectively.

ACKNOWLEDGMENT

The authors are thankful to Sophisticated Analytical Instrument Facility, Indian Institute of Technology Madras, Chennai, India for spectral and elemental analysis and Head, Department of Microbiology, L. L. R. M. Medical College, Meerut, India for antifungal and antibacterial activities. This paper is the part of Ph.D. thesis of Rovin.

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Table: 1 Vibrational assignment of fundamental frequencies (cm-1) of 2-Phenyl-1H-benzimidazole.

Species	Observed Frequencies (cm-1)		Calculate Frequencies (cm^{-1})	Assignment
	FTIR	Raman		
a'	3443(ms)	-	3458	N-H stretching
a'	3150(s)	-	3215	C-H stretching
a'	-	3068(s)		C-H stretching
a'	2908(s)	-	-	C-H stretching
a'	2852(s)	-	-	C-H stretching
a'	-	2803(s)	-	C-H stretching
a'	2603(s)	-	-	C-H stretching
a'	2503(s)	-		C-H stretching
a'	1625(s)	-	1635	C=N stretching
a'	1602(w)	-	-	C=N stretching
a'	-	1551(s)	-	C=N stretching
a'	1510(ms)	-	1565	C=C stretching
a'	1460(ms)	-	-	C=C stretching
a'	1404(w)	-	1465	C-N stretching
a'	-	1375(w)	-	C-N stretching
a'	1345(w)		-	C-N stretching
a'	1311(s)	-	1365	N-H in plane bending
a'	1231(w)	-	1275	N-H in plane bending
a'	1138(s)	-	1138	C-H in plane bending
a'	1076(s)	-	-	C-H in plane bending
a'	1016(s)	-	-	C-H in plane bending
a'	872(w)	-	968	C-C in plane bending
a'	752(w)	-	664	C-N-C in plane bending
a'		698 (s)	-	C-N-C in plane bending
a'	622(w)	-	564	C-C-H in plane bending
a'	527(s)			C-C-H in plane bending

Table 2: 1H-NMR data of 2-Phenyl-1H-benzimidazole.

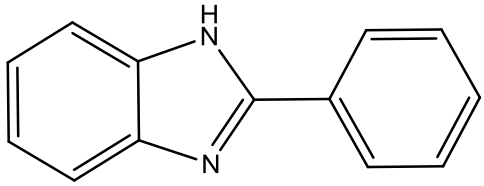
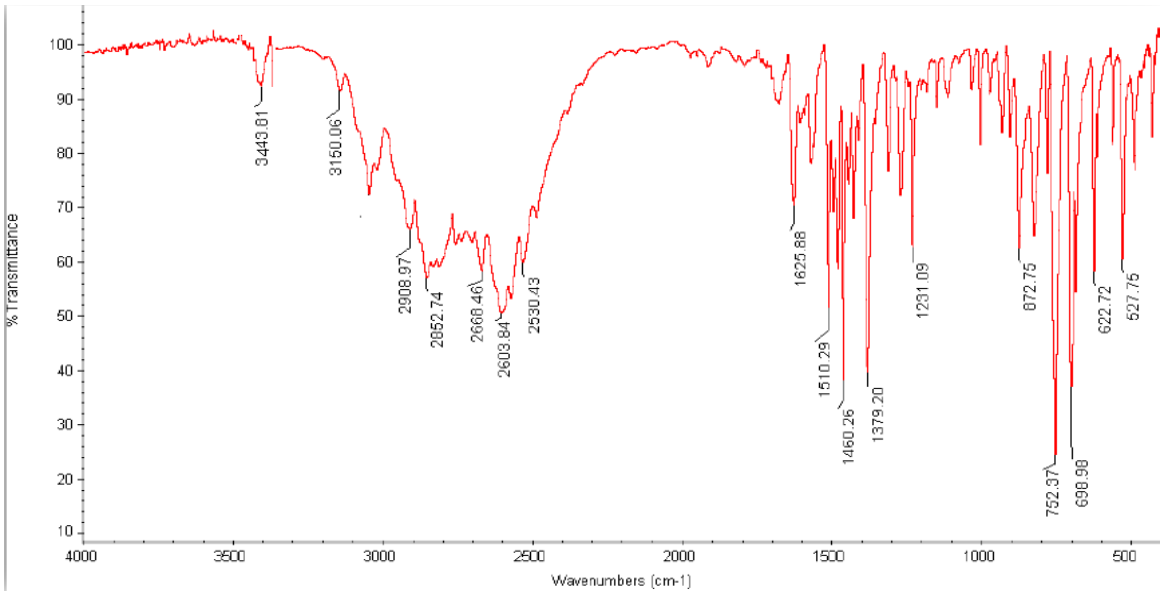
Compound	δ/ ppm	Assignments
	7.26-7.20 7.62-7.49 8.21-8.20 12.96	m, 2H of benzimidazole m, 5H of CH of phenyl t, (*J=9.1Hz*) 2H of benzimidazole s, H of NH

Table 3: Electronic spectral data in 95% ethanol and DMF, λmax (nm) / εmax (10^3 $mol^1.dm^3.cm$)

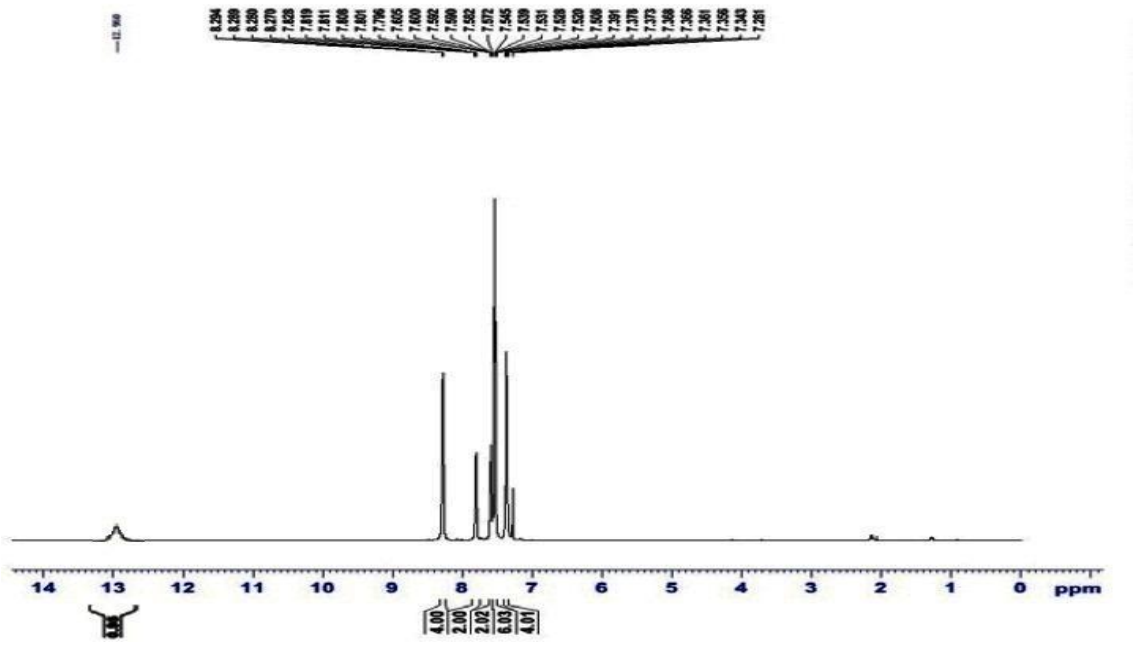
Solvent	2-Phenyl-1H-benzimidazole			
	I	II	III	IV
Ethanol	202.5/0.82	222.5/0.34	306.5/0.47	250.00/0.42
DMF	-	225.6/0.61	-	315.2/0.52

Table 4: Minimal inhibitory concentration (MIC) µg/ml of 2-Phenyl-1H-benzimidazole against tested bacterial and fungal strains

Compound No.	Minimal inhibitory concentration (MIC) µg/ml					
	E. coli	B. subtitles	S .aureus	C. albicans	A. Niger	C. krusei
a’	1.592	3.125	6.25	1.592	3.125	1.592
Chloroamphenicol	12.5	6.25	12.5	-	-	-
Fluconazole	-	-	-	6.25	12.5	3.125



FTIR-Raman Spectroscopy of 2-Phenyl-1H-benzimidazole



H-NMR Spectroscopy of 2-Phenyl-1H-benzimidazole