INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

BIOACTIVITY OF 1, 3-BENZIMIDAZOLYL BENZENE AND ITS COPPER COMPLEX

Sakuntala Samuelson and Shubharani Ramnath*

Department of Biological Sciences, Surana College,
South End Road, Bangalore-560 004, Karnataka, India.

ABSTRACT

Bioactivity of benzimidazoles and their metal complexes have been reported extensively since they exhibit a wide range of biological properties. In the present study, the evaluation of the antioxidant effect of 1, 3- bis (benzimidazolyl) benzene and its copper complex was studied by DPPH method. The percentage of inhibition at 200-1000µg/ml concentration of 1, 3- benzimidazolyl benzene and its Cu 2+ complex was compared with a standard phenol. The tested compounds were found to have less antioxidant activity compared to the standard. The antibacterial effect of these compounds on gram-positive bacteria, Staphylococcus aureus, Strptococus mutans and Enterococcus faecalis was studied using Resazurin microtitre plate method. These bacteria are frequently found in dental caries, which is one of the most prevalent and chronic dental diseases. The studied compounds showed an effective antibacterial activity against all three bacteria. The highest activity was observed against Streptococcus mutans and lowest activity was observed against Enterococcus faecalis.

Keywords: 1, 3- benzimidazolyl benzene, copper complex, dental bacteria and bioactivity.

INTRODUCTION

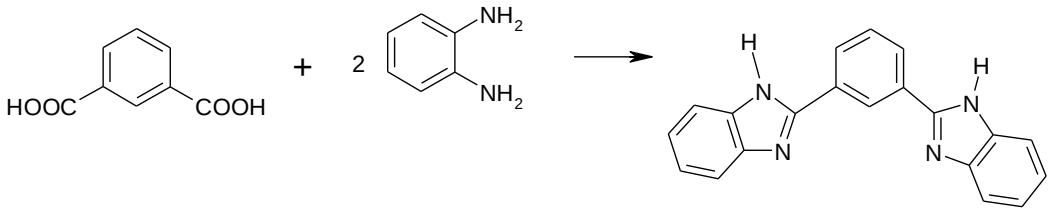
Benzimidazoles are a group of heterocyclic, aromatic and bioactive organic compounds which exhibit a wide range of biological properties. They are sometimes used as pharmaceutical targets[1]. Benzimidazoles are synthesized by the condensation of o-phenylenediamine and benzoic acids. Benzimidazoles and their derivatives have a broad spectrum of therapeutic uses specifically as antitumor, antifungal, antiparasitic, analgesics, anti-viral,anti-histamine,anti-ulcerative, anti-inflammatory, antiproliferative, anti-allergic, anti- hypersensitivity, anthelmentic, anti-convulsant, anti-diabetic and anti-inflammatory compounds. They are also used in treating cardiovascular diseases and in neurology, endocrinology and ophthalmology[2-6]. Since benzimidazoles and their metal complexes are associated with clinical medicine and development of new drugs, several scientists have synthesized and reported various derivatives of benzimadazoles for the pharmacological applications[7,8,9]. Benzamidazole derivatives are structural isomers of naturally occurring nucleotides, which allow them to interact freely with biopolymers of living systems[10]. Hence, benzimidazoles, their derivatives and metal complexes have been a subject of interest concerning their therapeutic potential. Based on this, the present study was conducted to evaluate the antioxidant effect of 1, 3-bis (benzimidazolyl) benzene and its copper complex by DDPH method. Their antibacterial effect on gram-positive bacteria which are frequently found in dental caries is also studied by Resazurin micro titer plate method and reported. Resazurin is a redox indicator used to assess viability for antimicrobial activity[11, 12]. It is blue in colour and on reduction turns pink. A change in color from blue to pink indicated the growth of bacteria. In the presence of an antibacterial compound the wells of the plate remain blue. The Minimum Inhibitory Concentration (MIC) was estimated by using various concentrations of the compounds [1,3-bis(benzimidazolyl)benzene and its copper complex] to record the lowest concentration that inhibited the growth of bacteria after incubation for 24 h at 35-37 °C.

MATERIALS AND METHODS

Preparation of Ligand and complex

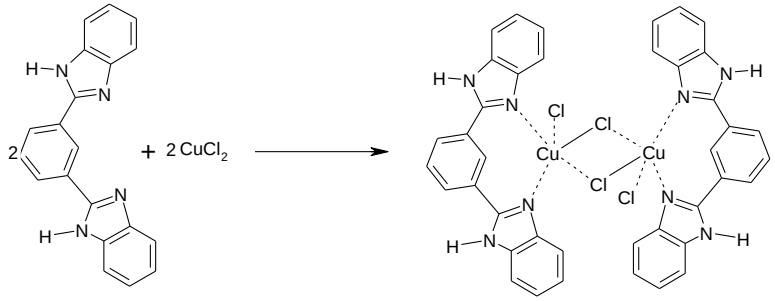
Preparation of 1, 3-bis (benzimidazolyl) benzene

Isophthalic acid (0.05mM) was added to a mixture of o-phenylene diamine (0.1mM) in phosphoric acid syrup and stirred for 4 hours in a sand bath at 240^{o}C. The greenish blue melt obtained was poured into cold water and subsequently neutralized with 10% sodium carbonate solution. The pink colour solid separated was recrystallised from ethanol. The resultant white needles were checked for melting point at 185^{o}C[13].



Preparation of Dichloro bis[1,3-bis (benzimidazolyl) benzene] dicopper (II)

To a solution of 1, 3-bis (benzimidazolyl) benzene (1mM) in ethanol, 1mM $CuCl_2$ was added. The brown coloured compound instantaneously precipitated was allowed to remain for 1 hour at room temperature and then washed with ethanol and dried in vacuum over P_2O_5 [14].



Determination of antioxidant activity by DPPH method

The antioxidant activity of 1,3- bis (benzimidazolyl) benzene and its copper complex was assessed on the basis of their radical scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to the standard method[15]. DPPH free radical accepts an electron from the compounds to form a stable diamagnetic molecule. This ability of DPPH to undergo reduction by an antioxidant is measured in terms of decrease in its absorbance at 517 nm. As DPPH radical reacts with a suitable reducing agent, the odd electron becomes paired and the solution loses color from purple to yellow stoichiometrically depending on the number of DPPH radicals that have undergone reduction. The degree of discoloration indicates the scavenging potential of antioxidant compounds. The lower absorbance of reaction mixture indicates higher free radical scavenging activity. Two milliliters of various dilutions of the compounds were mixed with 5mL of 0.1mM methanolic DPPH solution and incubated at 37^{o}C for 30 min. The wavelength of maximum absorbance of DPPH was measured at 517 nm using the spectrophotometer. The percentage of free radical scavenging activity of each concentration was calculated according to the formula:

$$\text{DPPH scavenged (\%)} = \frac{(A_{control} - A_{test})}{A_{control}} \times 100$$

Assays were performed in triplicate for each sample and at each concentration.

Determination of antibacterial activity by Resazurin microtiter plate method

The Gram-positive pathogens such as *Staphylococcus aureus, Stryptococus mutans* and *Enterococcus faecalis* were obtained from Azyme Biosciences, Bangalore. The antibacterial activity of 1, 3-bis (benzimidazolyl) benzene and its copper complex against these pathogens was studied by Resazurin microtiter plate method.

Resazurin microtitre plate preparation

Resazurin solution was prepared using 10g of Resazurin in one liter of deionized water as a stock solution. It was saved and frozen at -20°C and diluted 1:10 in deionized water just before the experiment. Bacterial culture was prepared with *Staphylococcus aureus, Strptococus mutans,* and *Enterococcus faecalis.* The samples 1, 3 bis (benzimidazolyl) benzene and its copper complex were prepared in serially diluted samples (1000, 500, 250, 125, 62.5, 31.25, 15.25, 7.81µg/ml). Lysogeny broth (LB), a nutrient-rich growth media primarily used for the bacterial growth. The control wells were prepared with LB broth, Resazurin dye, and deionized water, the wells turned blue in color. Blank wells were prepared with broth and water only which remained colorless.

To all the wells of microtiter plate, 200µl of deionised water (to prevent the samples from drying), 100µL of sterilized LB broth, 30µL of 0.1% Resazurin solution and 100µl each of serially diluted samples (1000, 500, 250, 125, 62.5, 31.25, 15.25, 7.81µg/ml) was added. Finally, the 100µl bacterial culture was inoculated into different wells. The plates were sealed and incubated for 24 hr at 37°C. After incubation, the activity of the samples was noted to see which samples inhibit the dye reduction. The changing color was then assessed visually. When there was no growth of organisms the wells remained blue. Whereas, when there was the positive growth of organisms, the color changed to pink and orange. The Minimum inhibitory concentration (MIC) was estimated by using various concentrations of the compounds 1, 3-bis (benzimidazolyl) benzene and its copper complex to record the lowest concentration that inhibited the growth of bacteria after incubation for 24 h at 35-37 °C.

RESULTS AND DISCUSSIONS

Antioxidant activity of 1, 3- bis (benzimidazolyl) benzene and its copper complex

The free radical scavenging properties of benzimidazole and its complexes were measured using DPPH, which is frequently used for the evaluation of the ant oxidation potential of various samples. DPPH undergo reduction by an antioxidant, measured in terms of decrease in its absorbance at 517nm. As DPPH radicals react with a suitable reducing agent, the electron becomes paired and color of the solution changes from purple to yellow. Table 1 shows the scavenging ability expressed as a percentage of inhibition of DPPH radical. The antioxidant activity of samples investigation showed a fall in absorbance indicating significant antioxidant activity in 1,3- bis (benzimidazolyl) benzene, the ligand, and its Cu^{2+} complex. The highest percentage of DPPH radical scavenging ability was 66% in Cu^{2+} complex of 1, 3 -benzimidazole benzene. The results clearly indicate that the ligand has less free radical scavenging potential of 46.6%. The percentage of inhibition at different concentrations (200-1000µg/ml) of 1, 3-bis (benzimidazolyl) benzene and its Cu^{2+} complex were compared with the standards Catechol (phenol), whereas, the tested samples were found to have less antioxidant activity compared to standard which ranged from 34.06 to 92.0% in different concentrations (Fig 1).

Table 1: Antioxidant activity of 1,3-bis (benzimidazolyl) benzene, its Cu^{2+} complex and standard

Sl.No	Samples	Percentage of inhibition (%)				
		200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml
1.	1,3bis(benzimidazolyl) benzene	1.9	16.8	20.2	41.6	46.6
2.	Cu^{2+} complex of the ligand	7.5	28.8	31.8	58.4	66.0
3.	Standard (Catechol)	34.06	61.86	87.32	90.41	92.0

A possible mechanism behind the increased rate of radical scavenging of DPPH with the copper (II) complex is suggested. Copper (II) can be reduced to copper (I) by the ligand.

$$Cu^{++} + L \rightarrow CuL^{++} \rightarrow Cu^{+} + L^{+}$$

followed by a reaction of the copper(I) with DPPH. Cu^{+} + DPPH → diamagnetic complex.

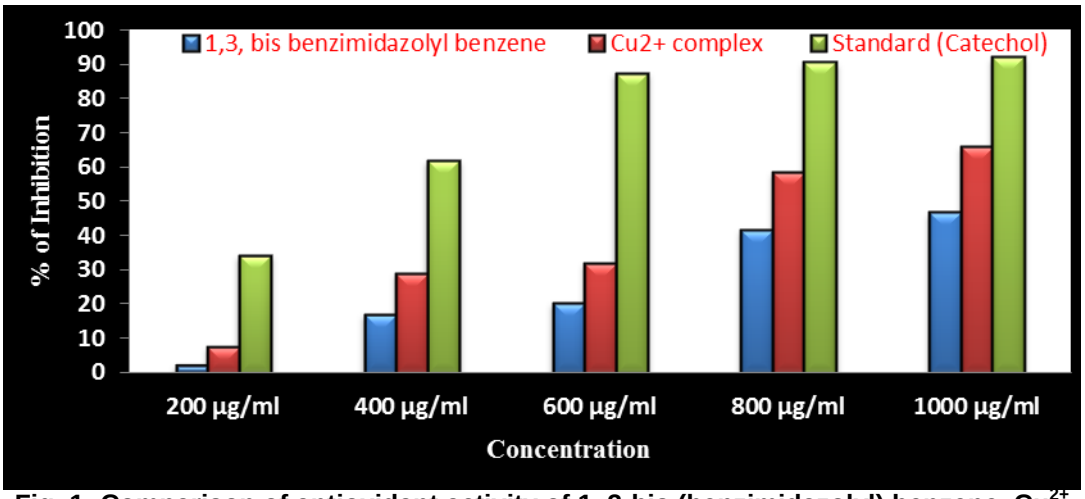


Fig. 1: Comparison of antioxidant activity of 1, 3-bis (benzimidazolyl) benzene, Cu^{2+} complex and standard in different concentrations

Antibacterial assay

The antibacterial activity was determined by Resazurin titer plate method and the results are summarized in Table 2. Resazurin titer plate method has provided a potentially useful technique for determining Maximum Inhibitory Concentration (MIC) of large numbers of test samples. MIC in microbiology is the lowest concentration of antimicrobials that will inhibit the visible growth of microorganisms after required incubation. MIC is important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agent[16].

Resazurin is an active blue compound, has been potentially used for a novel antibacterial therapy where the active bacterial cells reduce the non-fluorescent resazurin (blue) to the fluorescent resorufin (pink) which can be further reduced to hydroresorufin[17,18].

The antibacterial potential of both 1, 3 -bis (benzimidazolyl) benzene and its copper complex were evaluated according to their color change against *Staphylococcus aureus, Strptococus mutans,* and *Enterococcus faecalis*. The results revealed that the studied compounds showed an effective antibacterial activity against all three bacterial strains. The sample, 1,3-bis (benzimidazolyl) benzene was found to inhibit *Enterococcus faecalis* at a concentration of 1000 µg/ml and *Streptococcus mutans* was inhibited at the concentration of 500 µg/ml. Whereas, 1,3bis(benzimidazolyl) benzene did not inhibit the growth of *Staphylococcus aureus* up to the concentration of 7.81µg/ml. Similarly, the effect of Cu^{2+} complex of the benzimidazole was found to inhibit the growth of *Staphylococcus aureus* and *Enterococcus faecalis* at the concentration of 1000µg/ml and *Streptococcus mutans* at the concentration of 250µg/ml (Fig 2). Among the two compounds studied, the copper complex of the legend showed the highest degree of inhibition.

Table 2: Minimum Inhibition Concentration of 1, 3-bis (benzimidazolyl) benzene and its Cu^{2+} complex

Test Organism	Minimum Inhibition Concentration	
	Ligand	Cu^{2+} complex
Staphylococcus aureus	-	1000
Streptococcus mutans	500	250
Enterococcus faecalis	1000	1000

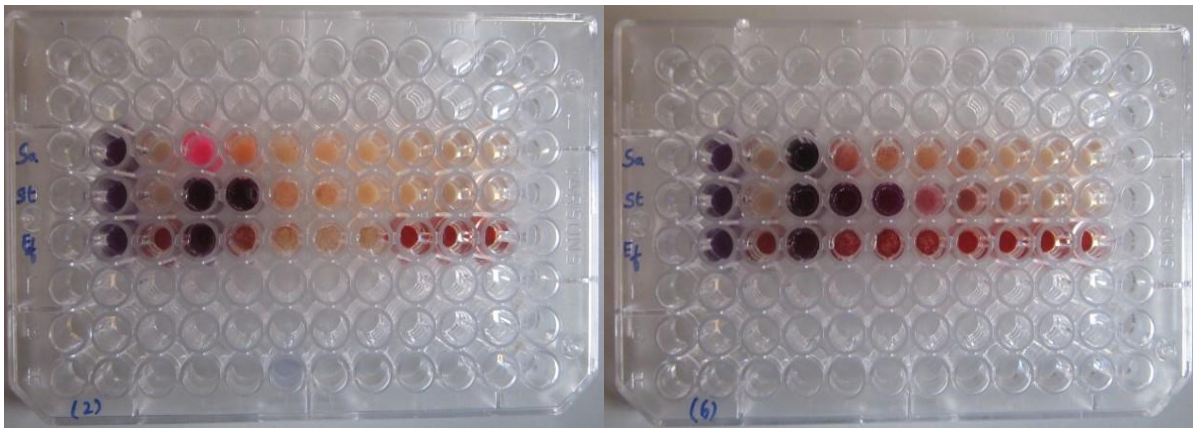


Fig. 2: Resazurin micro titer plate with 1, 3-benzimidazolyl benzene and its copper complex

CONCLUSION

Benzimidazole ring is an important pharmacophore in modern drug discovery and their derivatives are a resource for medicinal research. According to the present study,

1, 3-bis (benzimidazolyl) benzene, the ligand and its Cu^{2+} complex were found to have significant free radical scavenging potential. The results of the antibacterial assay of these compounds against gram-positive pathogens of oral cavity have proved their potential as antibacterial agents. The biological properties of benzimidazole derivatives may be because they are similar to naturally occurring nucleotides, which allow them to interact freely with biopolymers of living systems. Nevertheless, this study attempted a comparison of antioxidant and antibacterial activity among the ligand and its Cu^{2+} complex. The Cu^{2+} complex seems to be a more promising antioxidant and antimicrobial agent than 1, 3-bis (benzimidazolyl) benzene, the ligand. The results of the study may support the pharmaceuticals with the development of new antimicrobial and antioxidant drugs from both the compounds.

ACKNOWLEDGMENT

Authors are thankful to Azyme Biosciences, Bangalore, for providing the laboratory facilities and special thanks to Dr. Archana Surana, Managing Trustee, Surana College, South End Road, Bangalore, for her encouragement and financial support.

REFERENCES

1. Fatmah AS, Alasmary, Anna M, Snelling, Mohammed E, Zain Ahmed M, Alafeefy, Amani S , Awaad and Nazira Karodia. Synthesis and Evaluation of Selected Benzimidazole Derivatives as Potential Antimicrobial Agents. Molecules. 2015;20:15206-15223.
2. Sreerama Rajasekhar, Barnali Maiti, Balamurali and Kaushik Chanda. Synthesis and Medicinal Applications of Benzimidazoles. An Overview, Current Organic Synthesis, 2017:14(1):DOI**:** 10.2174/1570179413666160818151932.
3. Pavel Kopel, Dorota Wawrzak, Vratislav Langer, Kristyna Cihalova, Dagmar Chudobova, Radek Vesely, Vojtech Adam and Rene Kizek. Biological Activity and Molecular Structures of Bis(benzimidazole) and Trithiocyanurate Complexes. Molecules. 2015;20:10360-10376.
4. Babita Goswami and Arvind Kumar Singh. Pharmacological activities of benzimidazole derivatives Overview. International Journal of Science Innovations and Discoveries. 2012; 2(1):121-136.
5. Sivakumar R, Pradeepchandran R, Jayaveera KN, Kumarnallasivan P, Vijaianand PR and Venkatnarayanan R. Benzimidazole: An Attractive Pharmacophore in Medicinal Chemistry. International Journal of Pharmaceutical Research. 2011;3(3):19-31.
6. Gaba M, Sing D, Singh S, Sharma V and Gaba P. Synthesis and pharmacological evaluation of novel 5-substituted-1-(phenylsulfonyl)-2-methylbenzimidazole derivatives as anti-inflammatory and analgesic agents. European Journal of Medical Chemistry. 2010;45 2245.
7. Singh Gurvinder, Kaur Manninderjith and Chandu Mohan. Benzimidazoles. The latest information on Biological Activities. International Research Journal of Pharmacy. 2013;4(1): 82-87.

8. Ansari KF and Lal C. Synthesis and evaluation of some new benzimidazole derivatives as potential antimicrobial agents. European Journal of Medicinal Chemistry. 2009;44:2294-2299.
9. Mavrova AT, Anichina KK, Vuchev DI , Tsenov JA, Denkova PS, Magdalena SK and Micheva MK. Antihelminthic activity of some newly synthesized 5(6)-(un)substituted-1H-benzimidazol-2-ylthioacetylpiperazine derivatives. European Journal of Medicinal Chemistry. 2006;41(12):1412-1420.
10. Shobhit Srivastava, Pandeya SN, Meena K. Yadav and Singh BK. Synthesis and Analgesic Activity of Novel Derivatives of 1,2-Substituted Benzimidazoles. Journal of Chemistry. 2012;2013.
11. Palomino JC, Martin A, Camacho M , Guerra H, Swings J and Portaels F. American Society for Microbiology. 2002;46:2720-2722.
12. Karuppusamy S and Rajasekaran KM. High Throughput Antibacterial Screening of Plant Extracts by Resazurin Redox with Special Reference to Medicinal Plants of Western Ghats. Global Journal of Pharmacology. 2009;3(2):63-68.
13. Phillips MA. The formation of 2-substituted benziminazoles. Journal of Chemical Society (Resumed). 1928 :doi:10.1039/JR9280002393.
14. Shivakumaraiah N and Nanjegowda NM. Reactions of zinc salts with bis (benzimidazolyl) derivatives. Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry. 2003;33(7): 1207-1220.
15. Goyal AK, Mishra T and Sen A. Antioxidant profiling of Latkan (Baccaurea ramiflora Lour.) Wine. Indian Journal of Biotechnology. 2013;12:137-139.
16. Das K, Tiwari RKS and Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research. 2010;4(2):104-111.
17. Bauer J, Siala W, Tulkens PM and Van Bambeke F. A combined pharmacodynamic quantitative and qualitative model reveals the potent activity of daptomycin and delafloxacin against *Staphylococcus aureus* biofilms. Antimicrobial Agents of Chemotherapy. 2013;57: 2726-2737.
18. O'Brien J, Wilson I, Orton T and Pognan F. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. European Journal of Biochemistry. 2000;267(17):5421-5426.