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Investigation of antioxidant activity of some bis-benzimidazole in *Tetrahymena thermophila* stressed cells

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Abstract

In this paper, we present experimental findings on the protective effect of some bis-benzimidazole molecules against oxidative (H_2O_2) and nitrosative (SNP) stress using the protozoan *T. thermophila* as a cellular model. The results of this article show that the molecules 2,2'-Benzendiyl-1,4-Bis-(5-amino-1H-benzimidazole) and 2,2'-Octandiyl-1,8-Bis-(quinolin-8-ol-5-azo-1H-benzimidazole) have a protective effect against stress important than the molecule 2,2'-Benzendiyl-1,4-Bis-(1H-benzimidazole).

Keywords: *Bis-benzimidazole; Antioxidant activity; Toxicity; Cell; Tetrahymena; Stress.*

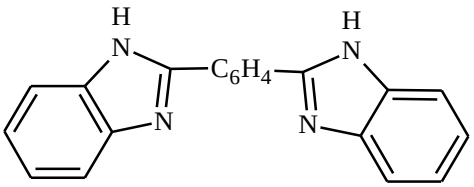
1. Introduction

In our research on the synthesis of substituted and unsubstituted bis-benzimidazoles [1], our research group is interested in studying the biological activity of these molecules [2]. Benzimidazole and its derivatives are one of the most bioactive heterocyclic compounds, owing to an important position in different research fields such as medicinal chemistry. They are well known as pharmacophores due to their privileged structure. Benzimidazole nuclei have been commonly used as preferred scaffolds for the development of therapeutic molecules of pharmaceutical or biological interest [3], several of the studies show that this type of molecule has antihemic activities [4], anti-HIV [5], anticonvulsant [6], anti-inflammatory [7], antihepatic [8], antineoplastic [9], antiulcer [10], and antioxidants [11]. Nowadays the antioxidants value of these aromatic compounds attracted the interest of the scientific community due to their ability to reduce the risk of chronic diseases such as cancer and heart disease [12]. There are several methods of evaluating antioxidant activity among these methods 1,1-diphenyl-2-picrylhydrazyl, superoxide scavenging, reducing power, nitric oxide scavenging, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonate), hydroxyl radical, and ferric reducing antioxidant power [13]. In this study, we have chosen the protozoan *T. thermophila* as a cell model for the *in-vivo* antioxidant activity. *T. thermophila* is one of the well-studied phylum of the eukaryotic kingdom, due to its diversity and biological parameters including its hormonal system similar to the human system, it can be easily cultured in different axenic medium and in small volumes. Indeed that *Tetrahymena* is considered as an ideal tool to discuss many cellular processes such as sexual reproduction, oxidative stress, and toxicity of many xenobiotic [14,15].
In this article, we have evaluated for the first time the protective effect (*in-vivo*) of molecules A, B, and C (Figure 1) on the protozoan *T. thermophila*, against both types of oxidative stress by hydrogen peroxide (H_2O_2) and nitrosative by sodium nitroprusside (SNP) according to the method reported previously in literature of Addoum et al. [16]. The measurement of the antiradical activity has been tested for these molecules [2], and we have shown that the two molecules B and C have a significant antioxidant activity which exceeds 46% as regards the percentage of DPPH inhibition, whereas the A molecule has a very low DPPH antioxidant effect that does not exceed 7%.

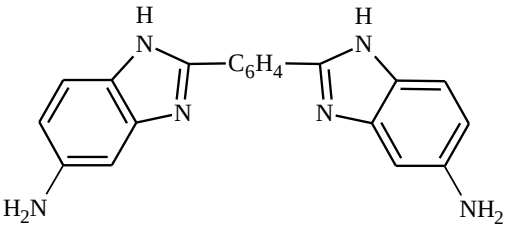
2. Materials and Methods

2.1. General synthesis procedure

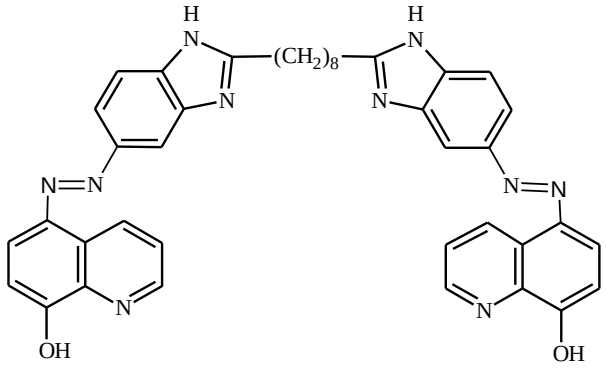
The molecules A, B, and C mentioned in the following Figure 1 were carried out according to the experimental methods described previously [1].



A: 2,2'-Benzendiyl-1,4-Bis-(1H-benzimidazole)



B: 2,2'-Benzendiyl-1,4-Bis-(5-amino-1H-benzimidazole)



C : 2,2'-Octandiyl-1,8-Bis-(quinolin-8-ol-5-azo-1H-benzimidazol)

Figure 1: Semi-developed formula of molecules A, B, and C

2.2. Protozoa and culture conditions

To evaluate the *in-vivo* antioxidant activity of our bis-benzimidazole derivatives, we used protozoa *T. thermophila* grown in PPYE at 32°C for 72h. This liquid medium (PPYE) is composed by 1.5% (w/v) of the Trypton USP and 0.25% (w/v) of yeast extract [16], after sterilization of the culture medium we transplanted the protozoan and we incubated it as shown in Figure 2.

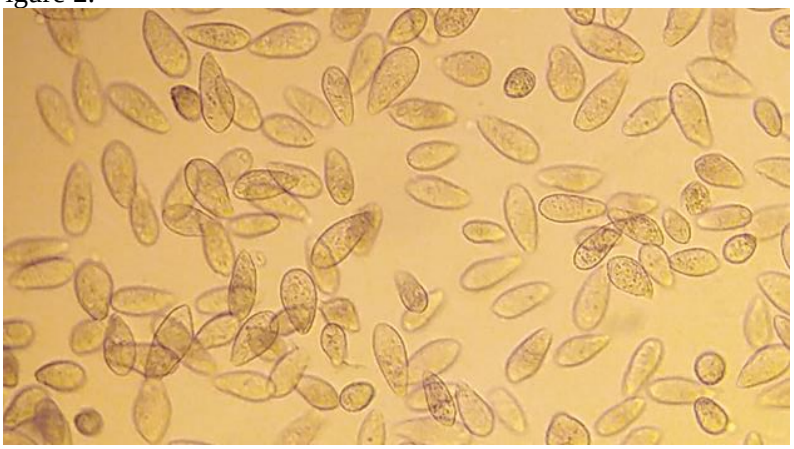


Figure 2: Microscopy images of T. thermophile (objective × 10)

2.3. Kinetics of Tetrahymena growth

To monitor the growth of *T. thermophila*, we measured absorbance at 600 nm using the UV-Visible spectrophotometer.

2.4. Microscopic observation

In order to study the morphology and mobility of *T. thermophila*, a 1mL aliquot was removed immediately, and the samples were fixed with buffered neutral formol, prepared at 2% in phosphate-buffered saline: PBS [16], then 6µL of suspensions were taken to perform the visualization under the light microscope: A. KRÜSS Optronic optical microscope (objective × 10). All experiments presented were performed at least in duplicate or more.

2.5. Stress conditions

To evaluate the effects of SNP and H_2O_2 on the growth of *T. thermophila*, Erlenmeyer flasks containing PPYE medium are inoculated with 1% (v/v) of protozoal *T. thermophila*, while cultures were supplemented with IC_{50} (the half maximal inhibitory concentration) stressors after 24 hours of cell growth. Protozoan growth was then monitored by sterile sampling every 24h and *T. thermophila* absorbance was measured at 600nm for 120h. Simultaneously, a negative control was prepared in the same stress-free state in order to follow the normal growth of *T. thermophila*. The IC_{50} used in this experiment are determined in Table 1.

Table 1: The concentration of H_2O_2 and SNP used to induce stress [17]

IC_{50}	
H_2O_2	SNP
0,4 mM	1,8mM

3. Results

3.1. Toxicity of molecules and determination of the MIC

From the stock solution (2mg/mL), dilutions were made to obtain solutions of concentrations 400, 300, 200 and 100µg/mL. The toxicity evaluation of our molecules (A, B and C) during 120h on *T. thermophila*, show that the form, the mobility and the number of protozoa remain normal like the control (0µg / mL) by using the concentrations 100, 200 and 300µg/mL, while above 400µg/mL the number of protozoa decreased and high mobility with different forms (pear shapes and elongated forms) was observed. The MIC (minimum inhibitory concentration) was the lowest concentration showing no turbidity after 72 hours incubation of our protozoan [18]. The non-toxic (non-lethal) concentration of our bis-benzimidazole was the concentration that did not affect the growth and shape and mobility of the cells. Therefore, the three molecules have no negative effects on the protozoa (no toxicity) as long as the concentration is less than 300µg/mL (Table 2). In order to work with optimal conditions, the test was carried out using a concentration of 100µg/mL.

Table 2: Toxicity evaluation of molecules on *T. thermophila*

Compounds	Concentrations used					
	2mg/mL (Mother Solution)	400µg /mL	300µg/ mL	200µg/ mL	100µg/ mL	Control (0µg/mL)
A	+	+	++	++	+++	
B	+	+	++	++	+++	+++
C	+	+	++	++	+++	

2.2. Kinetics growth of T. thermophila

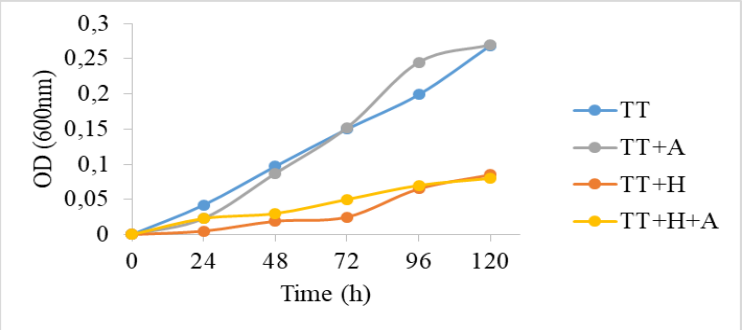


Figure 3: Growth curve of *T. thermophile* (TT) in the presence of molecule A and H_2O_2 (H)

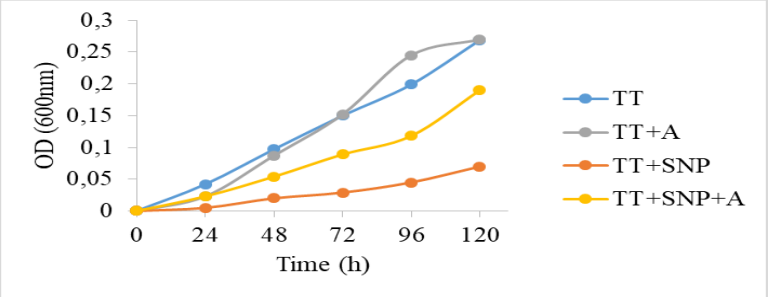


Figure 4: Growth curve of *T. thermophila* (TT) in the presence of molecule A and SNP

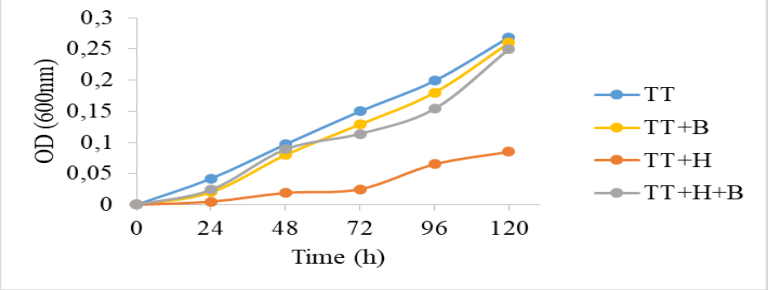


Figure 5: Growth curve of *T. thermophila* (TT) in the presence of molecule B and H_2O_2 (H)

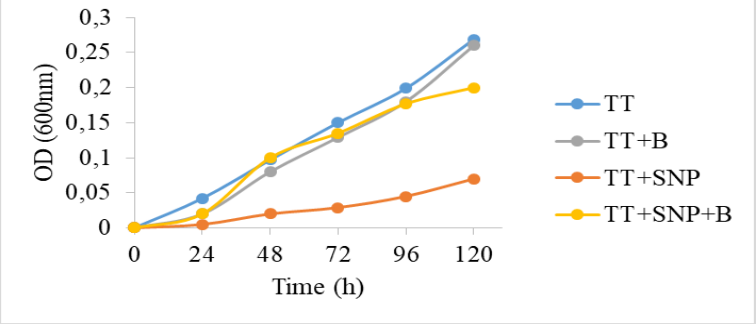


Figure 6: Growth curve of *T. thermophila* (TT) in the presence of molecule B and SNP

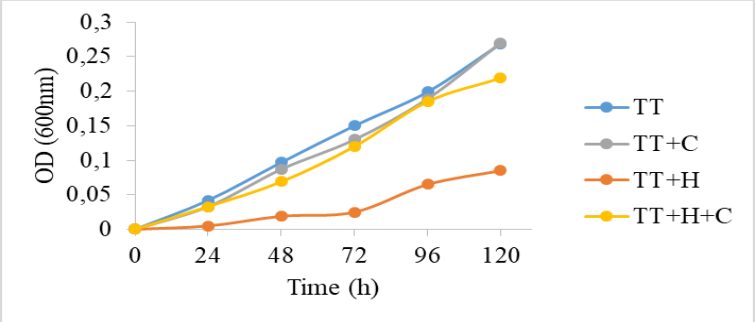


Figure 7: Growth curve of *T. thermophila* (TT) in the presence of molecule C and H_2O_2 (H)

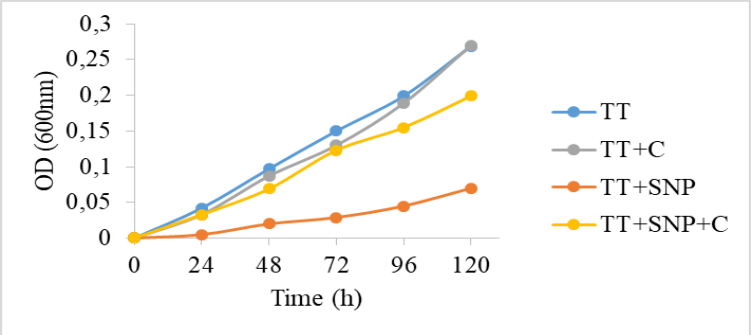


Figure 8: Growth curve of *T. thermophila* (TT) in the presence of molecule C and SNP

According to the figures Figure 3Figure 8, we notice that oxidative stress with hydrogen peroxide (H_2O_2) and nitrosative with sodium nitroprusside (SNP) negative influence on the growth of *T. themophila* (TT and TT+H and TT+SNP), which corroborate with several works including the studies of Errafiy et al. [19]. The result shows clearly that both stressors (H_2O_2 and SNP) completely inhibited the growth of *T. thermophila*. The presence of molecules B (Figure 5 and Figure 6) and C (Figure 7 and Figure 8) in the culture media exposed to two types of stress protects the protozoan *T. themophila* against the two types of stress because we found that the growth of *T. thermophila* is comparable to control (TT). By screening the protective effect of molecule A against stress, we noticed from the growth curves of the protozoan *T. thermophila* that this molecule (A) is unable to protect the protozoan against oxidative stress by H_2O_2 (Figure 3) and we can notice that the curves TT+H and TT+H+A are superimposed after a time of 96h. While the same molecule protects the protozoan against nitrosative stress by SNP (Figure 4) but remains low compared to other molecules B and C.

2.3. Cell enumeration of T. Thermophila

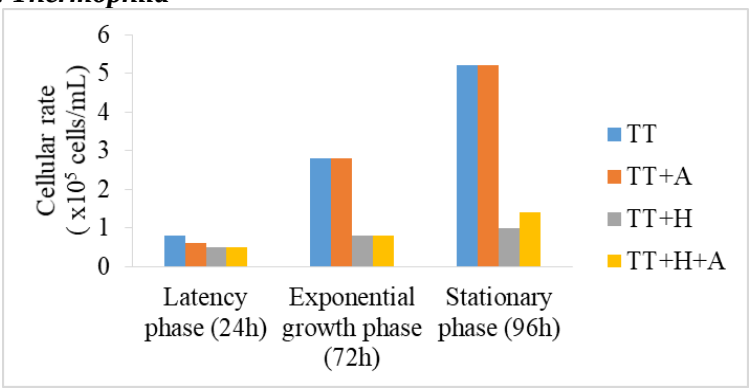


Figure 9: Cell number de *T. thermophila* (TT) in the presence of the molecule A and H_2O_2 (H)

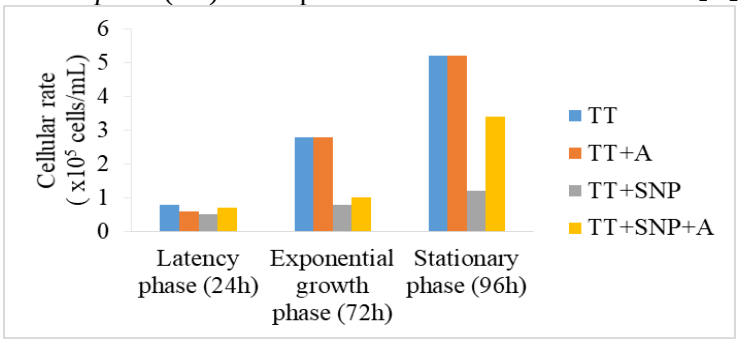


Figure 10: Cell number de *T. thermophila* (TT) in the presence of the molecule A and SNP

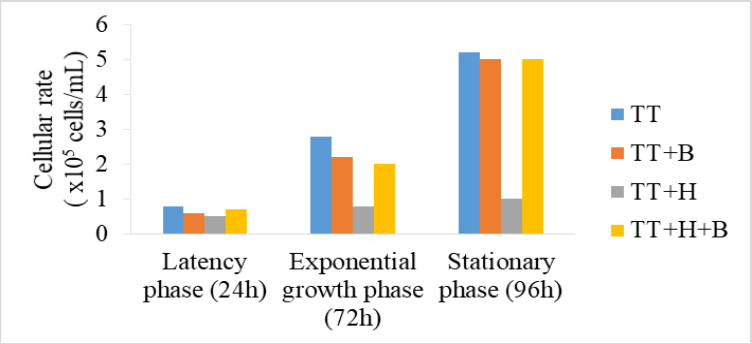


Figure 11: Cell number de *T. thermophila* (TT) in the presence of the molecule B and H_2O_2 (H)

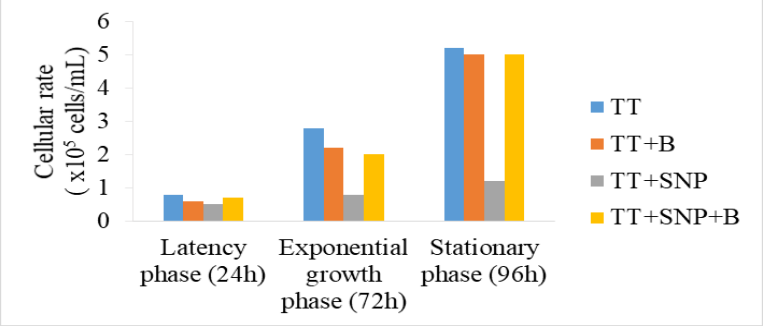


Figure 12: Cell number de *T. thermophila* (TT) in the presence of the molecule B and SNP

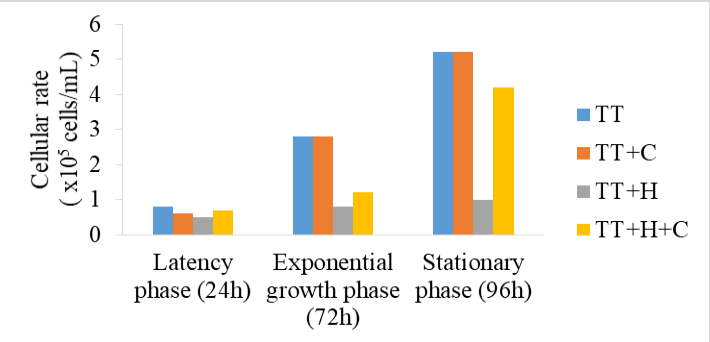


Figure 13: Cell number de *T. thermophila* (TT) in the presence of the molecule C and H_2O_2 (H)

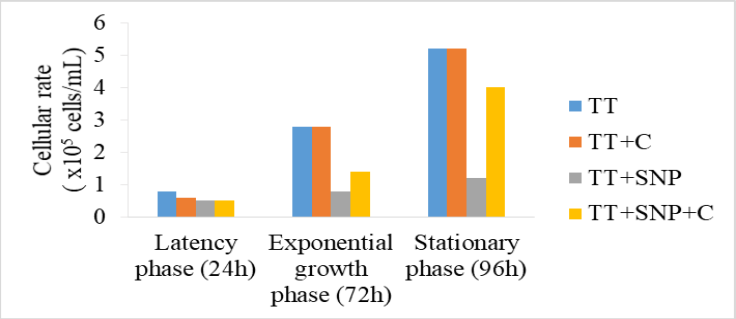


Figure 14: Cell number de *T. thermophila* (TT) in the presence of the molecule C and SNP

The counting of cells number (Figure Figure 9-Figure 14) confirmed the growth results of *T. thermophila* reported previously in Figure Figure 3-Figure 8. The analysis of different growth phase of *Tetrahymena* especially from the second phase 72h (exponential phase), we find that the B and C molecules have a protective effect against both types of stress: oxidative and nitrosative, more important than the molecule A, and we can clearly see this result in the stationary phase (96h).

2.4. Morphology and mobility of T. thermophile

The results of the morphological studies summarized in Table 3 show that the mobility and form of this ciliate -*T. thermophila*- exposed to different stressors are affected, we noticed the presence of elongated forms and conjugations with cellular debris. The mobility is very low in this experimental condition. Accordingly, the same observation is recorded when the PPYE medium is supplemented with the derivative A which demonstrated once again that this molecule hasn't a protective effect especially against oxidative stress.

Table 3: Morphology and mobility of *T. thermophila*

	Mobility	Morphology
TT	Fast	Normal
TT+H	Slow	Rounded shape and form of conjugations
TT+SNP	Slow	Shape shrinks and cellular debris
TT+H+B	Fast	Normal
TT+H+A	Slow	Rounded shape and form of conjugations
TT+H+C	Fast	Normal
TT+SNP+B	Fast	Normal
TT+SNP+A	Fast	Normal
TT+SNP+C	Fast	Normal
TT+B	Fast	Normal
TT+A	Fast	Normal
TT+C	Fast	Normal

4. Discussion

Nowadays the medicinal and pharmacological studies classified the benzimidazole molecules as bioactive molecules with a wide range of biological activities [4–11] related to the presence of a bioactive nucleus which is a constituent of vitamin B12. As continuity to our previous study on bis-benzimidazole [2], the purpose of the current study is the evaluation of the protective effect of the bis-benzimidazole molecules. The results of the toxicity show that our bis-benzimidazole don't have a toxic effect on the cells according to the concentration used (Table 2), for that we chose a minimum inhibitory concentration (MIC) of 100 µg/ml which does not influence our protozoan in terms of mobility, growth, and form. The ongoing results confirmed once again that stressors (H_2O_2 and SNP) induce changes in physiological parameters in our *T. thermophila*, such as growth, shape, and cell mobility. These results confirm the results found by Errafiy et al. [19], Mar et al. [18] and Addoum et al. [16], they demonstrated that stressors SNP and H_2O_2 have negatively influenced the growth of *T. thermophila* cells. In the framework of classifying the molecules according to their protective behavior, the molecule C takes the first position in the protection level against both types of stress, then the molecule B is the second one, whereas the molecule A has a weak effect for the protective effect especially against oxidative stress. The biological potential of these molecules can be explained by the semi-developed forms (chemical formulas) of molecule A, B, and C (Figure 1). As the semi-developed formula of the molecule A shows that this bis-benzimidazole molecule has no substitution at the benzimidazole group level. For the second bis-benzimidazole molecule B is substituted by the amine group in the 5(6) position benzimidazole while the molecule C which is electron-rich compound is substituted in the 5(6) position of benzimidazole by the quinoline molecule, this is a well-known bioactive molecule (quinoline) among the N-heterocycles and displayed wide roles in the pharmaceutical and agrochemical industries. Accordingly, compounds B and C contain a large number of nitrogen atoms in their formula, since the presence of nitrogen in an organic molecule, in general, confers a significant

biological activity such as metronidazole [20], nitrazepam [21], chloramphenicol [22], etc. The *in-vivo* results reported herein are in harmony with the results in-vitro published previously, such as the molecules that have antioxidant activity in vitro also have a protective effect *in-vivo*.

5. Conclusion

The purpose of this study is to assess and compare the effectiveness of the protective effect in benzimidazoles molecules against oxidative and nitrosative stresses. The results presented in this article showed that the synthesized benzimidazole derivatives have remarkable antioxidant activity. Especially the derivative substituted with quinoline whereas their effects on various antioxidants enzymes should be studied and discussed to underlying their cellular mechanism of action.

References

[1] Ouaket A, Hamdouch S, Moughaoui F, Anbaoui Z, Berrada M, Bennamara A, et al. Synthesis and Characterization of 2, 2'-Alkyl/Aryl-Bis(Quinoline-8-Ol-5-Azobenzimidazole). Ijppr Hum 2018;11:231–8.

[2] Ouaket A, Moughaoui F, Laaraibi A, Hamdouch S, Berrada M, Knouzi N. DPPH scavenging activity of some Bis-benzimidazole derivatives. Mediterr J Chem 2019;8:103–7.

[3] Saber A, Kheira Sebbar N, Essassi EM. Synthese, reactivite et activites biologiques des derives du benzimidazole synthesis, reactivities and biolobical properties of benzimidazole derivatives. Moroccan J Heterocycl Chem 2019;18:1–50.

[4] Rajiv M.H. RM. Synthesis of 6-nitrobenzimidazol-1-acetyl amino acids and peptides as potent anthelmintic agents. INDIAN J Heterocycl Chem 2002;12:121–4.

[5] Gardiner JM, Loyns CR, Burke A, Khan A, Mahmood N. Synthesis and HIV-1 inhibition of novel benzimidazole derivatives. Bioorganic Med Chem Lett 1995;5:1251–4. doi:10.1016/0960-894X(95)00203-6.

[6] Shingalapur R V, Hosamani KM, Keri RS, Hugar MH. Derivatives of benzimidazole pharmacophore: synthesis, anticonvulsant, antidiabetic and DNA cleavage studies. Eur J Med Chem 2010;45:1753–9. doi:10.1016/j.ejmech.2010.01.007.

[7] Achar KCS, Hosamani KM, Seetharamareddy HR. In-vivo analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives. Eur J Med Chem 2010;45:2048–54. doi:10.1016/j.ejmech.2010.01.029.

[8] Luo Y, Yao J-P, Yang L, Feng C-L, Tang W, Wang G-F, et al. Design and synthesis of novel benzimidazole derivatives as inhibitors of hepatitis B virus. Bioorg Med Chem 2010;18:5048–55. doi:10.1016/j.bmc.2010.05.076.

[9] Murugan V, Prasad KR, Sarma GV, Ramanathan M SB. Synthesis of triazole, thiadiazole and oxadiazole bearing 2-thiomethyl benzimidazole and their biological evaluation. INDIAN J Heterocycl Chem 2001;11:169–70.

[10] Lombardy RL, Tanious FA, Ramachandran K, Tidwell RR, Wilson WD. Synthesis and DNA Interactions of Benzimidazole Dications Which Have Activity against Opportunistic Infections. J Med Chem 1996;39:1452–62. doi:10.1021/jm9507946.

[11] Ateş-Alagöz Z, Kuş C, Çoban T. Synthesis and antioxidant properties of novel benzimidazoles containing substituted indole or 1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene fragments. J Enzyme Inhib Med Chem 2005;20:325–31. doi:10.1080/14756360500131706.

[12] Chandra Shekhar T, Anju G. Antioxidant Activity by DPPH Radical Scavenging Method of Ageratum conyzoides Linn. Leaves. vol. 1. 2014.

[13] Venkatachalam U, Muthukrishnan S. Free radical scavenging activity of ethanolic extract of Desmodium gangeticum. J Acute Med 2012;2:36–42. doi:10.1016/j.jacme.2012.04.002.

[14] Ye Q, Feng Y, Wang Z, Jiang W, Qu Y, Zhang C, et al. Effects of gelsemine on oxidative stress and DNA damage responses of Tetrahymena thermophila. PeerJ 2018;6:e6093. doi:10.7717/peerj.6093.

[15] Ruehle MD, Orias E, Pearson CG. Tetrahymena as a Unicellular Model Eukaryote: Genetic and Genomic Tools. Genetics 2016;203:649–65. doi:10.1534/genetics.114.169748.

[16] Addoum Boutaina, El Kkhalfi Bouchra, Papa Daouda Mar, Najoie Filali Ansari, Elmakssoudi Abdelhakim SA. Synthesis and Antioxidant Activity of some A-Aminophosphonate Compounds on Tetrahymena Protozoan. Der Pharma Chem 2018;10:58–67.

[17] Mar PD, Otto H, ElKhalfi B, Soukri A. Biochemical Study of the Protective Effect of Salvia officinalis Essential Oil against the Oxidative Stress Induced by Hydrogen Peroxide in Tetrahymena thermophila. Der Pharma Chem 2019;11:8–12.

[18] Mar PD, El Khalfi B, Soukri A. Protective effect of oregano and sage essentials oils against the effect of extracellular H2O2and SNP in Tetrahymena thermophila and Tetrahymena pyriformis. J King Saud Univ - Sci 2018. doi:10.1016/j.jksus.2018.05.005.

[19] Errafiy N, Ammar E, Soukri A. Protective effect of some essential oils against oxidative and nitrosative stress on Tetrahymena thermophila growth. J Essent Oil Res 2013;25:339–47. doi:10.1080/10412905.2013.775681.

[20] Al-Masoudi NA, Abbas ZAA. Synthesis and biological activity of new metronidazole derivatives. Monatshefte Fur Chemie 2016;147:383–90. doi:10.1007/s00706-015-1612-7.

[21] Gilli G, Bertolasi V, Sacerdoti M, Borea PA. 7-Nitro-1,3-dihydro-5-phenyl-2 H -1,4-benzodiazepin-2-one (nitrazepam). Acta Crystallogr Sect B Struct Crystallogr Cryst Chem 1977;33:2664–7. doi:10.1107/S0567740877009157.

[22] Neu HC, Fu KP. In vitro activity of chloramphenicol and thiamphenicol analogs. Antimicrob Agents Chemother 1980;18:311–6. doi:10.1128/aac.18.2.311.