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A STUDY ON THE ANTIOXIDANT ACTIVITIES OF SOME NEW BENZAZOLE DERIVATIVES

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The *in vitro* antioxidant properties of some new benzazole derivatives (**1–10**) such as benzoxazoles, benzimidazoles, and benzothiazoles were determined by their effects on the rat liver microsomal NADPH-dependent lipid peroxidation (LP) level, the scavenging of superoxide anion and the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Compounds **1**, **2**, **4** and **6**, showed potent scavenging effect on superoxide radical at 10^{-3} M. Compound **8**, 5-nitro-2-(phenoxymethyl)benzimidazole, strongly inhibited lipid peroxidation at 10^{-3} M concentration.

Keywords: Antioxidant activity – benzazole derivatives – superoxide dismutase – lipid peroxidation – DPPH

INTRODUCTION

In vivo molecular oxygen easily converted to reactive free radicals which include the superoxide anion ($O_2^{-\cdot}$), hydroxyl radical ($HO\cdot$) are highly reactive substances that react with lipids, proteins and DNA, provoking irreversible changes of their biomolecular structure. Reactive oxygen species (ROS) are formed by the transfer of one electron to oxygen molecule during various physiological processes such as respiration chain, oxygenase and cellular immunization reactions. They play an essential role in the control of cell functions. They are intermediate metabolites in several enzymatic reactions, involved in the post-translational protein turnover and play a role in the control of signal transduction. Many components of the vascular system, such as leukocytes, monocytes and endothelial cells are able to release ROS upon

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appropriate stimulation. Thus, ROS are associated with incidence of various diseases such as heart diseases, thrombosis, hypertension, Alzheimer's disease, Parkinson's disease and amyotropic lateral sclerosis [1–5]. Besides oxidative stress due to reactive oxygene species is associated with the induction of DNA single- and double-strand breaks and is considered to be a first step in several human degenerative diseases, cancer and aging [6–8].

Antioxidants such as glutathione and antioxidant enzyme systems including superoxide dismutase and catalase are the major protective systems of the organisms [9].

In addition, nutriotional antioxidants such as flavanoids and vitamins (e.g. A, C and E) the minerals, coenzyme Q10, lipoic acid [10, 11] and synthetic antioxidant compounds can protect oxidative stress, so that they prevent various diseases which are directly related to lack of the antioxidant capacity of organisms [12–15].

The biological activities of benzazole derivatives such as benzoxazole, benzimidazole, and benzothiazole are the structural isosters of natural nucleotides and interact easily with the biopolymers. Hence, benzazoles possess potential antitumor, antiviral and antibiotic activities as the new topoisomerase I poisons, HIV-1 reverse transcriptase inhibitors and/or potent DNA gyrase inhibitors with low toxicities [16–21].

In this study, the antioxidant properties of some synthesized compounds [21–24] were investigated by examining their effects on lipid peroxidation and scavenging capacity of superoxide and DPPH stable radical.

MATERIALS AND METHODS

Chemistry

The compounds **1–6** were synthesized 5-amino-2-*p*-substitutedphenylbenzoxazoles by treatment with the appropriate substitutedbenzoylchlorid, 2-furoilchlorid, *p*-chlorophenoxyacetylchlorid obtained from substituted benzoic acids, furoic acid, *p*-chlorophenoxyacetic acid. The compounds **7–10** were preaperad by heating 2-aminothiophenol and/or 5-substituted-2-phenylendiamines with appropriate phenoxyacetic acid for benzotiazole derivative in PPSE and for benzimidazole derivatives in 6N HCl. All reagents were purchased from Sigma and Aldrich.

Antioxidant activity studies

In these experiments used chemicals: Xanthine, xanthine oxidase, cytochrome c, 2,2,diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene and α-tocopherol were purchased from Sigma Chemical Co. Nicotine amide adenine dinücleotide phosphate sodium salt ($NADP^+$), D-glucose-6-phosphate monosodium salt, thiobarbitüric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Crystalline bovine serum albumin was obtained from BDH Chemicals Ltd. (Poole, UK). All the other chemicals were used were of analytical grade.

Superoxide radical scavenging activity

The capacity of some benzimidazole compounds to scavenge superoxide anion formation was determined spectrophotometrically on the basis of inhibition of cytochrome c reduction according to the modified method of McCord et al. [25]. Superoxide anion was generated in the xanthine/xanthine oxidase system. The reaction mixture contained in a final volume of 1 ml, 0.05 M phosphate buffer pH 7.8, 0.32 U xanthine oxidase, 50 μM xanthine, 60 mM ctytochrome c and different concentration of synthesized compounds at 100 μl. The absorbance was measured spectrophotometrically at 550 nm for cytochrome c reduction. Each experiment was performed in triplicate and the result are expressed as the percent of the control.

DPPH radical scavenging activity

The free radical scavenging activities of these compounds were tested by their ability to bleach the stable radical 2,2,diphenyl-1-picrylhydrazyl (DPPH) [26]. This assay has often been used to estimate the antiradical activity of the synthesized compounds. Because of its odd electron DPPH gives a strong absorption bound at 517 nm in visible spectroscopy. Reaction mixture contained 100 μM DPPH in methanol and different concentrations of synthesized compounds. Absorbance at 517 nm was determined after 30 min at room temperature and the scavenging activity were calculated as a percentage of the radical reduction. Each experiment was performed in triplicate. BHT was used as a reference compound.

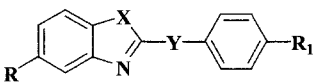
Assay of lipid peroxidation

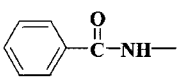
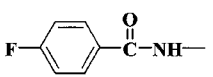
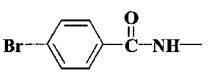
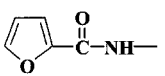
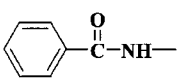
Male albino Wistar rats (200–225 g) were used in the experiments. Animals were fed with standard laboratory rat chow and tap water *ad libitum.* The animals were starved for 24 h prior to sacrifice and then killed by decapitation under anaesthesia. The livers were removed immediately and washed in ice-cold water and the microsomes were prepared as described previously [27].

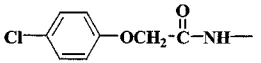
NADPH-dependent LP was determined using the optimum conditions determined and described previously [27]. NADPH-dependent was measured spectrophotometrically by estimation of thiobarbituric acid reactive substances (TBARS). Amounts of TBARS were expressed in terms of nmol malondialdehyde (MDA)/mg protein. The assay was essentially derived from the methods of Wills [28, 29] as modified by Bishayee [30]. A typical optimized assay mixture contained 0.2 mM Fe^{++}, 90 mM KCl, 62.5 mM potassiumphosphate buffer, pH 7.4, NADPH generating system con-

Table 1

Effects of the compounds **1–10**, on LP levels and scavenging activity of superoxide and DPPH radical[a]



Com. No.	R	R_1	X	Y	Concentration in incubation medium (M)	Superoxide anion ($O_2^{\cdot-}$) scavenging activity (percent of control)	LP (percent of control)	DPPH free radical scavenging activity (percent of control)
Control[b] DMSO-EtOH						100	100	100
1		H	O	CH_2	10^{-3} 10^{-4}	2±1 101±1	72	15±2 95±1
2		H	O	CH_2	10^{-3} 10^{-4}	4±2 119±9	125	105±4 102±6
3		Cl	O	CH_2	10^{-3} 10^{-4}	13±1 138±11	103	103±1 99±3
4		H	O	–	10^{-3} 10^{-4}	1±2 36±2	722	113±1 102±5
5		H	O	–	10^{-3} 10^{-4}	110±5 105±7	125	118±6 97±2

Com. No.	R	R_1	X	Y	Concentration in incubation medium (M)	Superoxide anion ($O2^{\cdot-}$) scavenging activity (percent of control)	LP (percent of control)	DPPH free radical scavenging activity (percent of control)
Control[b] DMSO-EtOH						100	100	100
6		H	O	–	10^{-3} 10^{-4}	1±2 3.0±0	89	119±7 111±4
7	H	Cl	S	CH_2O	10^{-3} 10^{-4}	18±2 108±4	77	101±2 100±6
8	NO_2	H	NH	CH_2O	10^{-3} 10^{-4}	49±5 101±4	9	103±3 106±4
9	H	H	NH	CH_2O	10^{-3} 10^{-4}	49±6 69±1	194	104±2 96±4
10	Cl	H	NH	CH_2O	10^{-3} 10^{-4}	401±38 116±6	102	102±7 99±4
BHT					10^{-3} 10^{-4}		12	7±4 25±4
SOD					30 IU 45 IU	24±2 11±1		

[a] Each value represents the mean ±S.D. of 2–4 independent experiments.
[b] DMSO-EtOH, control for compounds and BHT.

sisting of 0.25 mM $NADP^+$, 2.5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate, 1.0 U glucose-6-phosphate dehydrogenase and 14.2 mM potassium phosphate buffer pH 7.8 and 0.2 mg microsomal protein in a final volume of 1.0 ml.

RESULTS AND DISCUSSION

In the present study, we have investigated the antioxidant capacity of the synthesized compounds in three different *in vitro* assays, superoxide anion ($O_2^{\cdot-}$), DPPH stable radical scavenging activity and lipid peroxidation levels (Table 1).

The inhibitory effects of the compounds on the NADPH-dependent lipid peroxidation levels were determined using rat liver microsomes by measuring the formation of 2-thiobarbituric acid reactive substances. As seen in Table 1, compound **8**, 5-nitro-2-(phenoxymethyl)benzimidazole, significantly inhibited (91%) lipid peroxidation at 10^{-3} M concentration. This value was similar to that observed with BHT (88%) a well-known antioxidant at 10^{-3} M concentration. Additionally, compounds **1**, **6**, and **7** showed slight inhibition by about 28%, 11% and 23% at the same concentrations, respectively. However, the rest of the compounds had no effects on lipid peroxidation. The anthracycline antibiotic doxorubicin and its congener, daunorubicin, have been in use for more than 30 years for the treatment of a variety of malignancies. Therefore, a number of different mechanisms have been proposed for the cytostatic and cytotoxic actions of these agents [31]. These include intercalation into DNA with consequent inhibition of macromolecular biosynthesis, free radical formation with consequent induction of DNA damage or lipid peroxidation, DNA binding and alkylation, DNA cross-linking, interference with DNA unwinding or DNA strand separation and helicase activity, direct membrane effects, and the initiation of DNA damage via the inhibition of topoisomerase II. Even if investigators have failed to detect any relationship between lipid peroxidation and topoisomerase II inhibition, we were surprised with compound **8**. Compound **8** not only inhibited lipid peroxidation, but it was also found to be significantly active as topoisomerase II inhibitor having IC_{50} value 28.4 μM that was pretty close to IC_{50} value of standard drug, etoposide (21.8 μM) [19]. We could consider that compound 8 could be a further target structure for developing new antitumor agents via both inhibition of topoisomerase II and lipid peroxidation.

The superoxide anion radical scavenging activities of the synthesized compounds at different concentration were investigated and results are presented in Table 1. The results showed that almost all synthesized compound at the 10^{-3} M concentrations showed superoxide anion scavenging activity, and the scavenging rates were in the range of 51–99%. Compounds **1**, **2**, **3**, **4**, **6** and **7** have strong scavenger effect on superoxide anion at 10^{-3} M concentration (82–99%). Additionally, compounds **1**, **2**, **4** and **6** had comparable scavenger effects on superoxide anion as that of SOD (89% inhibitor at 45 IU). Compounds **8** and **9** have also decreased the level of superoxide anion by about 51%, at 10^{-3} M concentration. However, the level of superoxide anion radical at 10^{-3} M concentration increased if a chlorine atom (compound 10) attached

at 5th position of 2-(phenoxymethyl) benzimidazole instead of hydrogen (compound 9) or nitro group (compound 8). Morover, compound **5** had no scavenging effect on superoxide anion at 10^{-3} M and 10^{-4} M concentrations. Compounds **4**, **6**, and **9** had scavenging effect on superoxide anion at 10^{-4} M concentration by about 64%, 97% and 31%, respectively. It could be pointed out that benzoxazole ring posseses more antioxidant capacity as compared to with benzimidazole and benzothiazole with respect to their superoxide radical scavenging activities. Moreover, substitutions with benzamido and/or phenylacetamido and/or 2-furylcarbonylamino groups on the 5 position of benzoxazole system were significantly important for superoxide radical scavenging activity.

Compounds **1**, **2**, **4**, **6** have promising antioxidant activity by scavenging of superoxide radical. Furthermore, compounds increased and decreased the superoxide anion level at concentrations of 10^{-4} and 10^{-3} M, respectively. The distinct pattern of effects of chemicals can be seen in biological assays, where increases may occur at lower concentrations, whereas inhibition follows at higher concentrations [32, 33]. Biphasic effects of other chemicals such as thiazolidinedione [12], hydroxychalgones [25, 34, 35] on the level of superoxide anions have been well established in various *in vitro* systems.

The scavenging effect of the synthesized compounds **1–10** on the DPPH radical was examined as well. As seen in Table 1, compound **1**, which have a benzamido moiety, as an R substituent of 2-benzylbenzoxazole, was found to have the highest DPPH scavenger activity at 10^{-3} M concentration (85%). This compound was found to be as effective as the BHT, a well-known antioxidant utilized as positive control. When the same substitutent came on the R position of 2-phenylbenzoxazole refers to compound 5 did not show DPPH scavenger activity. Compound **1** scavenged superoxide and DPPH radical at 10^{-3} M concentration. The other compounds showed different patterns of effect on these parameters. The inhibitory effects of compounds were noted on the superoxide radical but not on DPPH radical. Such contradictory results have also been found in previous studies [12, 15, 34]. Therefore, the observation of different effects of synthetic compounds on superoxide anion and DPPH radical scavenger activity was not surprising since the mechanism of production of oxidative stress in these methods was different [25, 35–37].

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