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Novel *N*-acyl dehydroalanine derivatives as antioxidants: Studies on rat liver lipid peroxidation levels and DPPH free radical scavenging activity

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Abstract
Oxidative stress has been implicated in the development of many neurodegenerative diseases such as Parkinson and Alzhemier's disease and is also responsible for aging, artherosclerosis, rheumatoid arthritis and carcinogenesis. Olefins such as dehydroalanines have been shown to inactivate free radicals by forming stabilized free radical adducts. Among these molecules N-acyl dehydroalanines react with and scavenge oxygen and hydroxyl radicals. This study describes the synthesis, characterization and *in vitro* effects on rat liver lipid peroxidation levels, and DPPH free radical scavenging activities of some *N*-acyl dehydroalanine derivatives. Compounds **c**, **f** and **j** slightly scavenged the level of DPPH radical at 10^{-3} M concentration by about 27, 46, and 56%, respectively while compounds **a, d, e, f, g, h** showed a strong inhibitory effect on lipid peroxidation at 10^{-3}M and 10^{-4}M concentrations and inhibition was in the range of 76–90%. The possible antioxidant mechanism of the compounds was discussed.

Keywords: *Dehydroalanine, lipid peroxidation, radical scavenging, synthesis, free radical*

Introduction

In disease states, the immune system produces an excess of superoxide which overwhelms the native superoxidesismutase (SOD) enzymes' ability to remove superoxide free radicals so leading to free radical-induced damage to cells and tissues. Free radical damage has been associated with a growing number of diseases and conditions, such as auto-immune diseases like rheumatoid arthritis, neurode-generative disorders like Parkinson's disease, multiple types of cancer, complications of diabetes mellitus, stroke, heart attacks, reperfusion injury during transplants of organs, pain and inflammation [1]. Free radicals are chemically reactive species that can attack and damage several biomolecules such as DNA and enzymes. Oxidative damage to DNA by reactive oxygen species (ROS) is a continuous problem that cells must guard against to survive [2]. All amino acid residues of proteins are susceptible to oxidative modification by one or more forms of ROS [3,4].

Dehydroamino acids represent an important class of compound as they are key intermediates in amino acid and peptide synthesis and are constituents of a variety of naturally occurring antibiotic and phytotoxic peptides including several fungal metabolites which posses antibiotic activity [5–9]. They also play an important role at the active site of some enzymes and show free radical scavenging activity [4]. It has been postulated that dehydroamino acids play an important role in giving the definite peptide conformation that is required for expression of biological activities [10]. Recently, much attention has been given to the oxidative stress theory to explain increased risks to life-threatening and chronic or degenerative diseases [11–13]. Singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radicals often cause deleterious effects on cells and macromolecules and cause

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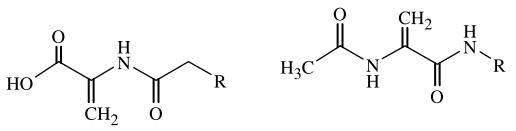

AD Derivatives
R: acyl, aryl

N-acetyl dehydroalanine derivatives
R: aliphatic, aryl

Figure 1. General structure of AD[23,25] and *N*-acetyl DHA derivatives.

oxidative stress-induced neuronal cell death in a number of neurological disorders.

Antioxidants and antioxidant enzymes function as major protective systems in organisms against free radical attack [14–17]. Dehydro amino acids and corresponding peptides can function as radical scavengers [18–21]. Oxygen radicals are involved in tumor promotion and they are considered as the principle causative agents of the deleterious effects of ionising radiations. Wagner *et al* [22] showed that the dehydroalanyl residue can capture the 5′-deoxyadenosyl radical generated from S-adenosylmethionine by pyruvate formate-lyase-activating enzyme. N-(acylaryl)-dehydroalanine derivatives have shown inhibitory activity towards oxygen radicals [23–24]. The *o*-methoxyphenylacetyl dehydroalanine derivative, refund to as AD-20 has free radical scavenging activity and protects against the effects of ionising radiation [25] (Figure 1). N-substituted dehydroalanines, a class of compounds with both acceptor and donor substituents react with and scavenge oxygen radicals. The reactivity of these compounds with hydroxyl radical is evident from their inhibition of hydroxyl radical adduct formation. ESR spin trapping studies of the species formed by reaction of the dehydroalanine derivatives with the hydroxyl radical was reported by Sipe *et al* [26]. Therefore it was of interest to investigate antioxidant ability of N-substituted dehydro amino acid derivatives. Eleven *N*-acetyl dehydroalanine (*N*-acetyl DHA) derivatives were synthesised to evaluate their antioxidant properties on rat liver lipid peroxidation (LP) levels and DPPH free radical scavenging activity. R groups were chosen aliphatic and cyclyc chains in order to identify the possible structure antioxidant activity relationships of the compounds and to guide perspectively the design of new analogues (Figure 2).

Materials and methods

Uncorrected melting points were determined with a Büchi SMP-20 apparatus. The ^{1}H NMR spectra were measured with a Varian mercury 400 MHz using TMS as internal standard and DMSO-d_6 or $CDCl_3$. All chemical schifts were reported as δ (ppm) values. ESI Mass spectra were determined on a Waters micromass ZQ. Chromatography was carried out using Merck silica gel 60 (230–400 mesh). Cytochrome c, 2,2,diphenyl-1-picrylhydrazyl (DPPH), α-tocopherol and thiobarbituric acid (TBA) were purchased from

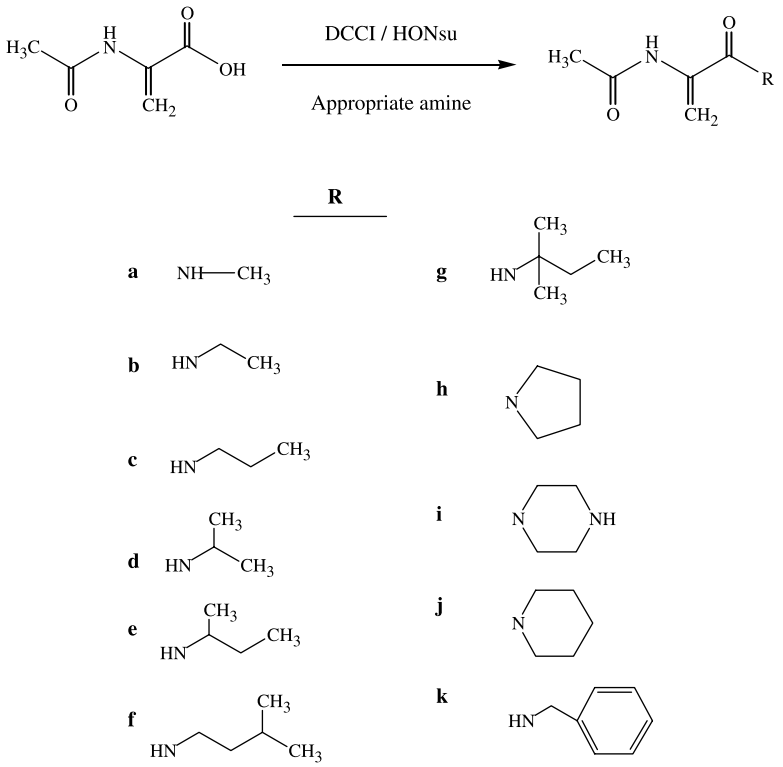

Figure 2. Synthesised *N*-Acetyl dehydroalanine derivatives.

Sigma Chemical Co. (St Louis, MO, USA). The chemical reagents used in synthesis were purchased from Sigma (Germany) and Aldrich (USA). The abbreviations used for chemicals are as follows: DCCI (1,3-dicyclohexyl carbodiimide), HONSu (N-hydroxysuccinimide). Male Wistar rats weighing 200–220 g were used for experiments. They received a standard diet. All the antioxidant experiments were performed in triplicate. Procedures involving the animals and their care conformed to Institutional guidelines, in compliance with National and International laws and guidelines for the use of animals in biomedical research.

Chemistry

For the synthesis of N-acetyl-DHA derivatives (**a**–**k**) the method described by Harada and Tagasaki described [31] was performed. DCCI and HONSu were used for the coupling of acetamidoacrylic acid and the appropriate amine. Synthesis and characterization of compounds **c**[27], **d**[28], and **k**[28] were published previously by our research group. Compounds **a** and **b** were characterized by Palmer *et al* [29] and Gulzar *et al*[30] respectively. The physical and spectral data of the synthesized compounds are given in Table I.

General procedure for the preparation of N-*acetyl dehydroalanines*

Acetamidoacrylic acid (1 mol), DCCI (1.2 mol), HONSu (1.1 mol), and appropriate amine (1 mol) in ethyl acetate were cooled at −10°C. The heterogeneous reaction mixture was then stirred at r.t for 24 h (48 h for compound **h**, **i** and **j**). At the end of the reaction the precipitated dicyclohexylurea was filtered and the filtrate was evaporated to dryness. The crude product was purified by column chromatography (ethyl acetate/petrolum spirit 60°–80°). For compounds **g** and **h** column chromatography was applied twice for purification.

Antioxidant activity studies

DPPH free radical scavenging activity. The free radical scavenging activity of *N*-acetyl DHA derivatives was tested by their ability to bleach the free radical 2,2,diphenyl-1-picrylhydrazyl (DPPH) [32]. Because of its odd electron, DPPH gives a strong absorption bound at 517 nm in visible spectroscopy. The reaction mixture contained 100μM DPPH in methanol and different concentrations of synthesized compounds. Absorbance at 517 nm was determined after 30 min at 37°C and the scavenging activity were calculated as a percentage of the radical reduction. Each experiment was performed in triplicate. α-Tocopherol was used as a reference compound. The radical scavenging activity was obtained from the equation: Radical scavenging activity % : $\{(OD_{control} - OD_{sample})/OD_{control}\} \times 100$

Assay of lipid peroxidation. The effect of the *N*-acetyl DHA derivatives on rat liver homogenate induced with $FeCl_2$-ascorbic acid was determined. LP was examined by the method of Mihara [33]. Animals were starved for 24 h. prior to sacrifice and then sacrificed by decapitation. The livers were immediately removed and washed in ice-cold distilled water, then immediately homogenized with a Teflon homogenizer. LP was measured spectrophotometrically by estimation of thiobarbituric acid reactants (TBARS). A typical optimized assay mixture contained 0.5 ml of liver homogenate, 0.1 ml of Tris-HCl buffer (pH 7.2), 0.05 ml of 0.1 mM ascorbic acid, 0.05 ml of 4 mM $FeCl_2$ and 0.05 ml of various concentrations of *N*-acetyl DHA derivatives or α-tocopherol, and was incubated for 1 h at 37°C. After incubation, 3.0 ml of H_3PO_4 and 1 ml of 0.6% TBA were added, the mixture shaken vigorously and then boiled for 30 min. After cooling the mixture to room temperature the absorbance of the supernatant was read at 532 nm against a blank, which contained all reagents except liver the homogenate.

Results

Eleven *N*-acetyl-DHA derivatives were synthesized, characterized and their *in vitro* antioxidant activity tested on rat liver lipid peroxidation levels and DPPH free radical scavenging activity. The scavenging effects of the compounds are shown in Table II. Compounds **c**, **f** and **j** slightly scavenged the level of DPPH radical at 10^{-3} M concentration by about 27, 46, and 56%, respectively. However compounds **a, d, e, f, g, h** showed a strong inhibitory effect on LP at 10^{-3}M and 10^{-4}M concentrations and the inhibition was in the range 76–90%. Compounds **b, c** and **i** also inhibited LP by about 54%, 58% and 66%, respectively but compounds **j** and **k** had no inhibitory LP activity at the same concentrations.

The superoxide anion radical scavenging activity was measured by the inhibition of cytochrome c reduction according to the modified method of Mc Cord *et al*[34]. The assay was also adapted to assess the capacity of antioxidants to react with superoxide anion radical. However none of the compounds at final concentrations of 10^{-3} and 10^{-4}M showed any significant ability to scavenge $O_2^{\cdot -}$ (data not shown).

Discussion

The synthesized *N*-acetyl DHA derivatives had strong effects on LP while having no significant activity on

Table I. Physical and spectral data of synthesised compounds.

No	Formula	Yield (%)	m.p°C	^{1}H NMR Data (δ ppm)	Mass Data
c	$C_8H_{14}N_2O_2$	48	75–78	$CDCl_3$: 0.95 (t, 3H, CH_2-*CH_3*), 1.57 (m, 2H, CH_2), 2.14 (s, 3H, $COCH_3$), 3.31 (q, 2H, NH*CH_2*), 5.18 (s, 1H, = CH), 6.26 (brs, NH), 6.43 (s, 1H, = CH), 8.18 (brs, NH)	209 (M + K, 18.20), 194 (M + 1 + Na, 16.15), 193 (M + Na, 100),150 (12.26), 129 (39.64)
e	$C_9H_{16}N_2O_2$	27	92–94	$CDCl_3$: 0.94 (t, 3H, CH_2-*CH_3*), 1.19 (d, 3H, CH*CH_3*), 1.59 (m, 2H, CH_2), 2.14 (s, 3H, $COCH_3$), 3.37 (m, 1H, CH), 5.14 (s, 1H, = CH), 5.86 (brs, NH), 6.43 (s, 1H, = CH), 8.01 (brs, NH)	223 (M + K, 17.32), 208 (M + 1 + Na, 19.80), 207 (M + Na, 100), 185 (M + 1, 97.01), 186 (M + 2, 12.17), 169 (25, 63), 147 (22.58)
f	$C_{10}H_{18}N_2O_2$	25	82–85	d_6-DMSO: 0.89 (d, 6H, $(CH_3)_2$) 1.35 (m, 2H, *CH_2*-CH), 1.57 (m, 1H, CH), 2.00 (s, 3H, $COCH_3$), 3.15 (t, 2H, NH*CH_2*), 5.32 (s, 1H, = CH), 6.01 (s, 1H, = CH), 8.27 (brs, NH), 9.02 (brs, NH)	237 (M + K, 12.10), 221 (M + Na, 100), 199 (M + 1, 28.76), 157 (59.05), 155 (11.18), 147 (14.14)
g	$C_{10}H_{18}N_2O_2$	18	Oily	$CDCl_3$: 0.81 (t, 3H, CH_2*CH_3*) 1.18-1.28 (m, 8H, $(CH_3)_2CH_2$), 2.04 (s, 3H, $COCH_3$), 5.02 (s, 1H, = CH), 5.85 (brs, NH), 6.33 (s, 1H, = CH), 8.02 (brs, NH)	237 (M + K, 13.29), 225 (100) (M + Na, 35.67), 199 (M + 1, 17.52), 169 (18.95), 147 (13.35)
h	$C_9H_{14}N_2O_2$	15	Oily	d_6-DMSO: 1.79 (m, 4H, N(CH_2-*CH_2*)$_2$) 1.89 (s, 3H, $COCH_3$), 3.25 (m, 4H, N(*CH_2*-CH_2)$_2$), 4.65 (s, 1H, = CH), 5.28 (s, 1H, = CH), 9.56 (brs, NH), second N*H* not observed	205 (M + Na, 7.38), 183 (M + 1, 9.55), 169 (29.85), 147 (49.59) 137 (24.36)
i	$C_9H_{15}N_3O_2$	20	Decomp. at 40	d_6-DMSO: 1.73 (m, 4H, *CH_2*)$_2$NH), 1.96 (s, 3H, $COCH_3$), 3.10 (m, 4H, N(*CH_2*)$_2$), 5.27 (s, 1H, = CH), 6.01 (s, 1H, = CH), 8.18 (brs, NH), 9.05 (brs, NH)	236 (M + K, 11.02), 225 (100), 221 (M + 1 + Na, 19.89), 174 (33.56), 166 (21.16), 143 (62.75), 139 (14.25), 125 (28.61)
j	$C_{10}H_{16}N_2O_2$	31	80–83	d_6-DMSO: 1.33-1.58 (m, 10 H, cyclic-H), 1.89 (s, 3H, $COCH_3$), 5.15 (s, 1H, = CH), 5.90 (s, 1H, = CH), 8.80 (brs, NH), 9.78 (brs, NH)	235 (M + K, 23.27), 225 (100), 219 (M + Na, 58.61), 165 (19.37), 141 (47.32), 112 (28.15)

Table II. Effects of the compounds **a-k,** on LP levels and scavenging activity of DPPH radical[a].

Compound No	Concentration in incubation medium (M)	LP (Percent of control)	DPPH free radical scavenging activity (percent of control)
Control[b] **DMSO-EtOH**		100	100
a	10^{-3}	10 ± 1	103 ± 1
	10^{-4}	17 ± 2	101 ± 1
b	10^{-3}	46 ± 3	102 ± 2
	10^{-4}	93 ± 1	100 ± 1
c	10^{-3}	42 ± 1	73 ± 1
	10^{-4}	102 ± 2	99 ± 2
d	10^{-3}	24 ± 2	98 ± 1
	10^{-4}	40 ± 1	102 ± 2
e	10^{-3}	15 ± 3	95 ± 3
	10^{-4}	88 ± 2	97 ± 1
f	10^{-3}	10 ± 1	54 ± 2
	10^{-4}	25 ± 1	101 ± 2
g	10^{-3}	11 ± 1	91 ± 1
	10^{-4}	40 ± 2	100 ± 3
h	10^{-3}	18 ± 2	103 ± 2
	10^{-4}	43 ± 1	104 ± 1
i	10^{-3}	34 ± 2	90 ± 1
	10^{-4}	91 ± 1	100 ± 2
j	10^{-3}	202 ± 3	44 ± 2
	10^{-4}	240 ± 4	99 ± 2
k	10^{-3}	203 ± 2	90 ± 2
	10^{-4}	168 ± 4	99 ± 2
α-tocopherol	10^{-3}	5 ± 1	10 ± 2
	10^{-4}	10 ± 1	13 ± 2

[a] Each value represents the mean ± SE of 2–4 independent experiments
[b] DMSO, control for compounds and α\-tocopherol

the DPPH radical. The scavenging effects of the compounds on the DPPH radical and inhibitory effect on LP were not similar. Since the mechanisms of these methods are different it is possible to observe different effects [35–38].

Non-proteinogenic amino acids constitute an important group of compounds in the field of peptide chemistry. These compounds have several applications, either as biologically active substrates or as individual structural components. Among these amino acids are α,β-dehydroamino acids and β-substituted alanines [39]. There is still speculation as to the role of the dehydro units in biologically active compounds. Certainly, they have an influence as a conformational constraint due to their sp^2 hybridized carbon structure, however, they could well have the ability to add on nucleophiles via Michael type conjugative addition, as recently proven in a set of model experiments [39,40]. Wagner *et al*[22] stated that the free radical scavenging mechanism of the dehydroalanyl residue appeared to involve addition of the nucleophilic radical to the electrophilic carbon-carbon double bond. DHA type compounds behave as highly effective trapping agents of free radicals which add covalently to the olefinic β-carbon. Also another finding was established by Garrison [41] that the oxidation of protein by ROS can possible lead to the cleavage of peptide chains, called the α-amidation pathway. Since the *N*-acyl DHA derivatives contain a carbon-carbon double bond and a peptide bond, an addition to the double bond [42] and/or cleavage reactions may happen during their antioxidant process (Figure 3). N-acetyl DHA derivatives most probably follow route(b) depending on our studies on dehydrothreonine derivatives [42].

N-Acyl dehydroalanines have shown free radical scavenging activity [23,25]. They react by scavenging mainly hydroxyl radical. Olefins such as DHA derivatives by offering a reactive site may inactive free radicals of different polarities. In DHA derivatives, free radicals are most probably attacted to the carbon-carbon double bond, and the resulting radical adduct is formed easily by the effect of both electron donating groups. According to our results in the LP assay the most active compounds have electron donating aliphatic side chains. An interesting result was obtained from compound **k** which had no LP inhibitory activity, the aromatic ring did not scavenge hydroxyl radical to give the hydroxylated aromatic derivative. Buc-Calderon et al [23,25] have stated that *N*-acyl and aryl dehydroalanines were effected superoxide anion scavengers. Contrary to some of their findings we found no scavenging effect on superoxide anion with the synthesized DHA derivatives, (data was not shown). This may be due to the differences between the substituents of the compounds.

In conclusion, this study has shown that *N*-acetyl DHA derivatives which are substituted with aliphatic

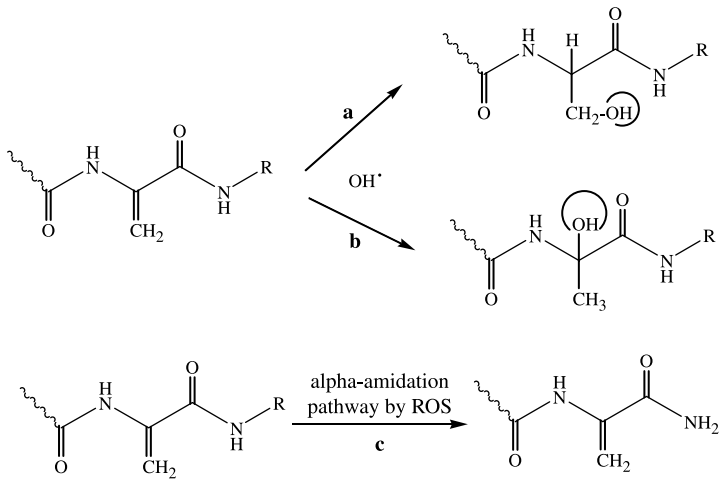

Figure 3. Possible antioxidant mechanisms of *N*-acetyl DHA derivatives.

(up to 3 or 4 carbons) and cyclic side chains (5 membered) have significant hydroxyl radical scavenging activity.

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References

[1] Ateş-Alagöz Z, Çoban T, Suzen S. Evaluation of ınvitro antioxidant activity of melatonin analogue some new ındole derivatives. Med Chem Res 2006; In press.

[2] Suzen S, Ates-Alagöz Z, Püsküllü O. Antioxidant Activities of Indole and Benzimidazole Derivatives. FABAD J Pharm Sci 2000;25(3):113–119.

[3] Stadtman ER, Van Remmen H, Richardson A, Wehr NB, Levine RL. Methionine oxidation and aging. Biochim Biophys Acta 2005;1703(2):135–140.

[4] Suzen S. The role of Dehydroalanines in Enzyme and Peptide Chemistry. J Fac Pharm Ankara Univ 1999;28(2):129–141.

[5] Bierbaum G, Gotz F, Peschel A, Kupke T, VandeKamp M, Sahl HG. The biosynthesis of the lantibiotics epidermin, gallidermin, Pep5 and epilancin K7. Antonie Van Leeuwenhoek 1996;69:119–127.

[6] Boeck LD, Berry DM, Mertz FP, Wetzel RW. A10255, a complex of novel growth-promoting thiopeptide antibiotics produced by a strain of Streptomyces gardneri. Taxonomy and fermentation studies. J Antibiot (Tokyo) 1992;45: 1222–1230.

[7] Dunn AD, Corsi CM, Myers HE, Dunn JT. Tyrosine 130 is an important outer ring donor for thyroxine formation in thyroglobulin. J Biol Chem 1998;273:25223–25229.

[8] Paik SH, Chakicherla A, Hansen JN. Identification and characterization of the structural and transporter genes for, and the chemical and biological properties of, sublancin 168, a novel lantibiotic produced by Bacillus subtilis 168. J Biol Chem 1998;273:23134–23142.

[9] Rohrkasten A, Meyer HE, Nastainczyk W, Sieber M, Hofmann F. cAMP-dependent protein kinase rapidly phosphorylates serine- 687 of the skeletal muscle receptor for calcium channel blockers. J Biol Chem 1988;263:15325–15329.

[10] Nomoto S, Sano A, Shiba T. A new synthetic method for dehydroalanine peptides through Hofmann degradation of diaminopropionyl residue. Tetrahedron Lett 1979;20: 521–522.

[11] Dekkers JC, van Doornen LJP, Kemper HCG. The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. Sports Med 1996;21: 213–238.

[12] Gutteridge JM. Antioxidants, nutritional supplements and life-threatening diseases. Br. J Biomed Sci 1994;51:288–295.

[13] Toyokuni S. Iron and carcinogenesis: from Fenton reaction to target genes. Redox Rep. 2002;7(4):189–197.

[14] Buettner GR, Jurkiewicz BA. Catalytic metals, ascorbate and free radicals: combinations to avoid. Radiation Res 1996; 145:532–541.

[15] Marx JJ, van Asbeck BS. Use of iron chelators in preventing hydroxyl radical damage: adult respiratory distress syndrome as an experimental model for the pathophysiology and treatment of oxygen-radical-mediated tissue damage. Acta Haematolog. 1996;95:49–62.

[16] McCord JM. Effects of positive iron status at a cellular level. Nutr Rev 1996;54(3):85–88.

[17] Toyokuni S. Iron-induced carcinogenesis: the role of redox regulation. Free Rad. Biol. Med. 1996;20(4):553–566.

[18] Stadtman ER, Levine RL. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 2003;25:207–218.

[19] Ghoshal AK, Rushmore TH, Buc-Calderon P, Roberfroid M, Farber E. Prevention by free radical scavenger AD5 of prooxidant effects of choline deficiency. Free Rad Biol Med 1990;8(1):3–7.

[20] Buc-Calderon P, Roberfroid M. AD 5, a dehydroalanine derivative, decreases the amount of reactive oxygen species formed during nitrofurantion microsomal metabolism. Life Sci. 1990;46(3):207–215.

[21] Buc-Calderon P, Sipe Jr, HJ, Flitter W, Mason RP, Roberfroid M. N-acyl dehydroalanines scavenge oxygen radicals and inhibit in vitro free radical mediated processes. Chem Biol Interact 1990;73(1):77–88.

[22] Wagner AF, Demand J, Schilling G, Pils T, Knappe J. A dehydroalanyl residue can capture the 5′-deoxyadenosyl radical generated from S-adenosylmethionine by pyruvate formate-lyase-activating enzyme. Biochem Biophys Res Commun 1999;54(2):306–310.

[23] Buc-Calderon P, Defrense MP, Barvais C, Roberfroid M. N-acyl dehydroalanines protect from radiation toxicity and

inhibit radiation carcinogenesis in mice. Carcinogenesis 1989; 10:1641–1644.
[24] Vo TK, Fischer SM, Slaga TJ. Effects of N-acyl dehydroalanines on phorbol ester-elicited tumor development and other events in mouse skin. Cancer Lett. 1991;60(1):25–32.
[25] Buc-Calderon P, Roberfroid M. Increase in the survival time of mice exposed to ionizing radiation by a new class of free radical scavengers. Experienta 1990;46:708–710.
[26] Sipe HJ, Buc-Calderon P, Roberfroid M, Mason RP. Identification of the free radical formed by addition of hydroxyl radical to dehydroalanine compounds. Chem Biol Interact. 1993;86:93–102.
[27] Suzen S, Williams JM. Behaviour of dehydroalanine derivatives under hydrazinolysis conditions. Possible relevance to glycoprotein hydrazinolysis. J Pep Sci 1999;5:283–286.
[28] Suzen S, Williams JM. Investigation of the synthesis of some dehydroalanine derivatives. Turkish J Chem 2000;24: 361–370.
[29] Palmer ED, Pattaroni C, Nunami K, Goodman M. Effects of dehydroalanine on peptide conformations. J Am Chem Soc 1992;114:5634–5642.
[30] Gulzar MS, Morris KB, Gani D. Control of the regioselectivity of N-nucleophile addition to N-carbonyl protected dehydroalanines. Chem Soc Chem Commun 1995;10:1061–1062.
[31] Harada K, Takasaki M. Asymmetric synthesis of alanine by catalytic hydrogenation of chiral N-acetyldehydroalanine. Bull Chem Soc Jap 1984;57:1427–1428.
[32] Blois MS. Antioxidant determinations by the use of a free free radical. Nature 1958;181:1199–1200.
[33] Mihara M, Uchiyama M, Fukuzawa K. Thiobarbituric acid value on fresh homogenate of rat as a parameter of lipid peroxidation in aging, CCl_4 intoxication, and vitamin E deficiency. Biochem Med 1980;23:303–311.
[34] McCord J, Fridowich I. An enzymic function for erythrocuprein. J Biol Chem 1969;243:6049–6055.
[35] Aikens J, Dix TA. Effect of solution ionic strength on lipid peroxidation initiation by the perhydroxyl (xanthine oxidase-derived) and peroxyl radicals. Chem Res Toxicol 1992; 5(2):263–267.
[36] Dix TA, Aikens J. Mechanisms and biological relevance of lipid peroxidation initiation. Chem Res Toxicol. 1993;6(1):2–18.
[37] Litwinienko G, Ingold KU. Abnormal solvent effects on hydrogen atom abstractions. 1. The reactions of phenols with 2,2-diphenyl-1-picrylhydrazyl (dpph*) in alcohols. J Org Chem 2003;68(9):3433–3438.
[38] Parke DV, Ioannides C, Lewis DFV. The 1990 Pharmaceutical Manufacturers Association of Canada keynote lecture. The role of the cytochromes P450 in the detoxication and activation of drugs and other chemicals. Can J Physiol Pharmacol 1991;69:537–549.
[39] Abreu AS, Silva NO, Ferreira PMT, Queiroz MJRP. Synthesis of novel amino acids and dehydroamino acids containing benzothiophene moiety. Eur J Org Chem 2003;:1537–1545.
[40] Monteiro LS, Ferreira PM, Maia HLS, Sacramento J, Peptides. The Wave of the Future, Proc. 2nd Int and 17th American Peptide Symposium. San Diego 2001. p 44
[41] Garrison WM. Reaction mechanism in radiolysis of peptides, polypeptides and proteins. Chem. Rev. 1987;87:381–398.
[42] Suzen S, Davies JS. In vitro Antioxidative properties of N-Benzyloxycarbonyl dehydrothreonine methyl ester 2nd Joınt Italian-Swiss Meeting on Medicinal Chemistry, 2005 September 12–16; Modena, Italy, Abstract P61 2005. p 137