Syntheses of Novel Indole Lipoic Acid Derivatives and Their Antioxidant Effects on Lipid Peroxidation

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The aim of the study was to examine antioxidant properties of conjugates based on indole and lipoic acid moieties. The design and syntheses of novel indole α-lipoic acid derivatives were performed. The antioxidant properties of target compounds were investigated using rat liver microsomal, NADPH-dependent lipid peroxidation inhibition. Some of the target compounds, especially those containing amide linker at position 5 of indole ring, proved to be highly effective in inhibiting lipid peroxidation as compared to α-lipoic acid.

Keywords: α-Lipoic acid; Indole; Lipid peroxidation; Antioxidant

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Introduction

Impaired antioxidant defense mechanisms are important features in the pathogenesis of various diseases including diabetes, cancer, and neurological disorders. Antioxidants are required to prevent cellular damage observed in many of these diseases. Impairment of these defense mechanisms may be due to increased oxidative stress. α-Lipoic acid (α-LA; 1,2-dithiolane-3-pentanoic acid; thioctic acid) is a natural multifunctional antioxidant [1] and acts as a co-enzyme in biological group transfer reactions [2−4]. Its main function is to increase production of glutathione, which eliminates toxic substances in the liver.

α-LA (α-Lipoic acid) is found endogenously as lipoamide in animals and plants [5]. Its carboxylic group is covalently bound by amide linkage to the ε-amino group of lysine residues. It functions as a cofactor of mitochondrial enzymes in catalyzing oxidative decarboxylation of α-keto acids such as pyruvate, α-ketoglutarate, and branched-chain α-keto acids [6]. α-LA is readily absorbed and rapidly converted to reduced form, dihydrolipoic acid (DHLA) in many tissues [7]. Usually, antioxidant substances exhibit antioxidant properties in their reduced form. α-LA is unique, because it retains protective functions in both its reduced and oxidized forms, although DHLA is the more effective than α-LA [8]. The antioxidant properties of α-lipoic acid can be related to four categories: its ability to scavenge free radicals; metal ion chelating activity; capacity to regenerate endogenous antioxidants for example glutathione and tocopherol; and the ability to repair oxidative damage in macromolecules [9, 10]. On the other hand, the indole nucleus is an important element in many pharmacologically active endogenous and exogenous compounds. Tryptophane is an indole amino acid found in the constitution of numerous proteins. Another well-known indole derivative is the neurotransmitter serotonin which displays a wide range of physiological actions including protection of biological tissues against radiation injury [11]. The mechanism for this protection is thought to include inhibition of excessive free radical formation. Melatonin is also a well-known indole compound having the properties of scavenging free radicals [11] that protects against DNA strand breaks and lipid peroxidation [12].

This study was designed to determine whether novel indole-lipoic acid derivatives will be able to provide certain antioxidant properties compared to α-lipoic acid which has been shown to have substantial multifunctional antioxidant properties.

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Results and discussion

Several novel α-lipoic acid-indole (α-LAI) derivatives were synthesized. The target compounds were evaluated for antioxidant properties using *in vitro* non-enzymatic lipid peroxi-

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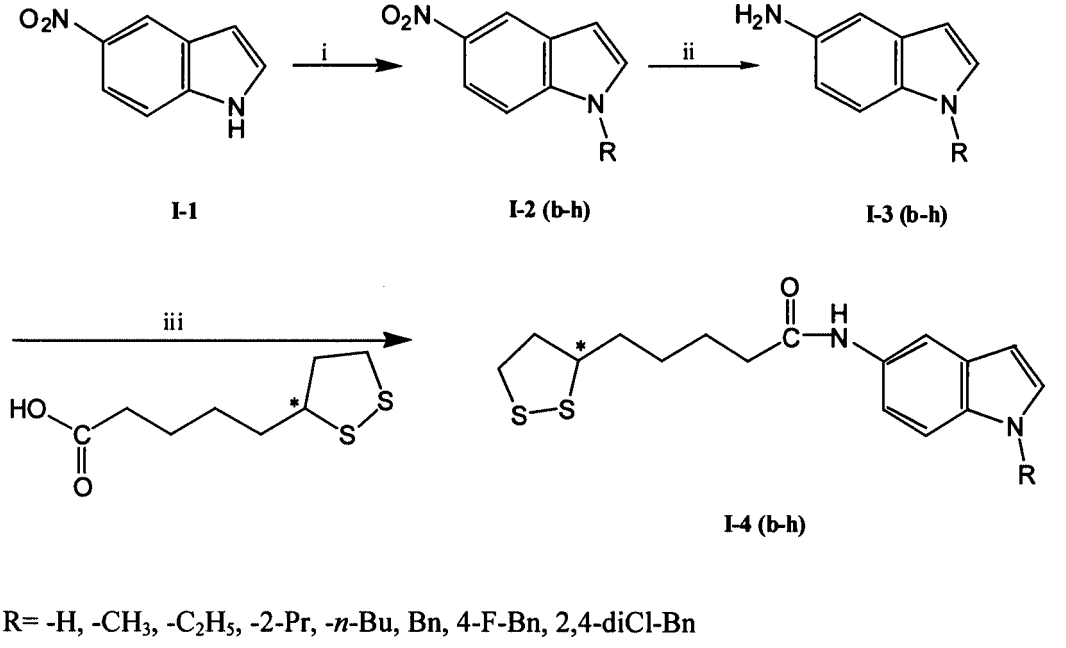
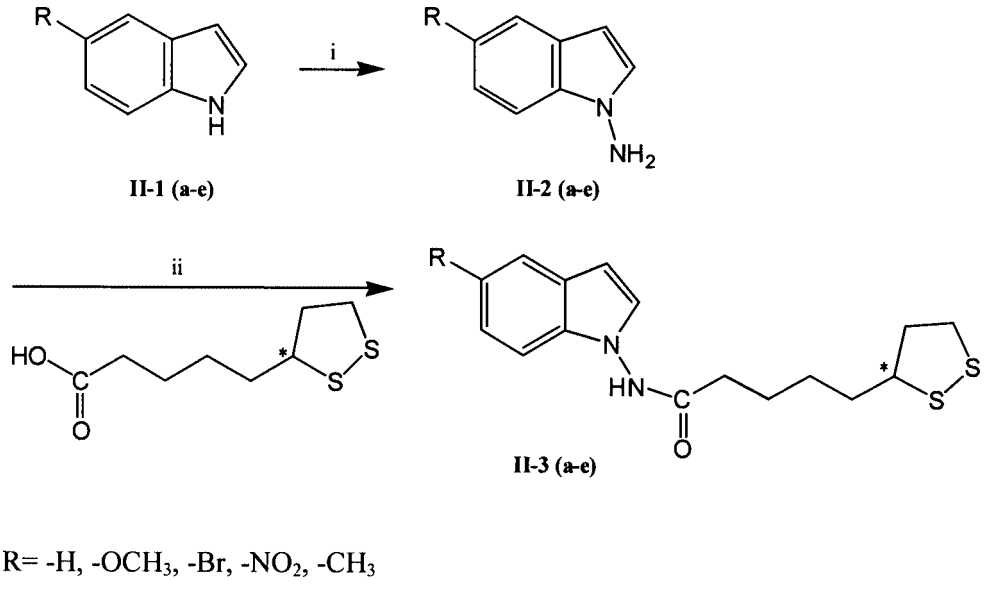
dation of rat hepatic microsomal membranes by measurement of the formation of 2-thiobarbituric acid (TBA) reactive substances. Lipid peroxidation consists of a radical-initiated reaction that serves for the evaluation of the antioxidant properties of a compound. The compounds exhibited significant lipid peroxidation inhibitory effects at the concentration of 1.0 mM with exception of compounds **I-4f** and **II-3d**, which showed 14.7% and 16.1% inhibition, respectively.

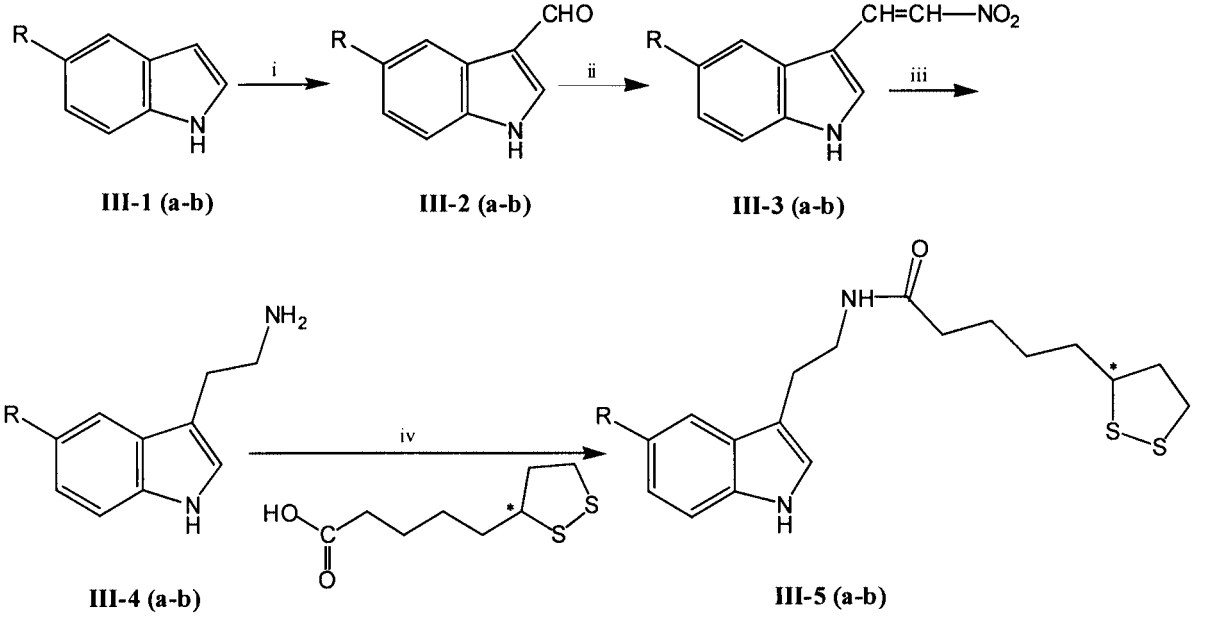
For the first series of compounds (Scheme 1), substitution at the amine of 5-nitroindole followed by reduction generated the amine intermediate. Conjugation of this with α-LA through amide bond formation was then conducted. The second series involved introduction of amine at position 1 followed by conjugation. The third series involved introduction of ethylamine at position 3 via formylation and methyl nitrite followed by conjugation to the nitrogen. The conjugation of α-LA to indole moiety resulted in substitution patterns which were classified in three groups. The first group involved amidation of 5-amino indoles with α-LA. The subtitution pattern for this group lies on the indole nitrogen where alkyl homologues and various benzyl substitents were employed. The most active compound within the first group was 4-fluoro benzyl derivative (compound **I-4g**) which exerted lipid peroxidation (LPO) inhibitory activity as 90.7% inhibition. However, inconsistency was found with compound **I-4f** which exhibited 14.7% inhibition. This compound has a bare benzyl substitution and the activity observed was significantly less than for the fluorobenzyl compound. This difference in terms of inhibitory levels for two similar substitution patterns generated some interest and assays were repeated several times. However, the difference seems to be due to the significance of the fluoro group being located at the *para* position of the benzyl ring. The 2,4-dichlorobenzyl derivative in this series also gave moderate inhibitory activity with confirmation of the important role of halogen substitution on the benzyl ring. A feasible approach to continuing studies in this area might be rationalized based on the difference between the halogens, including size and electronegativity, and the location. Removal of alkyl substitution (except **I-4d**) from the indole nitrogen resulted in a slight drop in the inhibitory activity. The *i*-propyl derivative (**I-4d**) might contain the optimal braching feature for the alkyl substitution. In order to perceive the activity of α-LA conjugation to indole moiety at the first (1) position, we synthesized α-LA derivatives of indole in which the indole nitrogen was maintained to establish the α-LA integration while at position 5 of indole moiety was further modified by substituting electron donating (OCH_3, Br, CH_3) and electron withdrawing (NO_2) groups in order to optimize the electronic and lipophilic characteristics. The amino linker at the position 1 of the indole ring was required for the conjugation of α-LA, due to the failure of direct bonding of α-LA to indole nitrogen.



Scheme 1. Reaction scheme of the first series of compounds. (i) alkyl / benzyl /substituted benzyl halogenid, NaH, DMF, r.t.; (ii) 10% Pd/C, H_2; (iii) *N,N′*-CDI, DMF, r.t.
R = -H, -CH_3, -C_2H_5, -2-Pr, -*n*-Bu, Bn, 4-F-Bn, 2,4-diCl-Bn



Scheme 2. Reaction scheme for the second series of compounds. (i) H-*O*-SA, KOH, DMF, 0 °C; (ii) *N,N′*-CDI, DMF, r.t.
R = -H, $-OCH_3$, -Br, $-NO_2$, $-CH_3$



Scheme 3. Reaction scheme for the third series of compounds. (i) $POCl_3$, DMF, 0 °C; (ii) CH_3NO_2, CH_3COONH_4, 70 °C, reflux; (iii) $LiAlH_4$, THF, reflux; (iv) *N,N′*-CDI, DMF, r.t.
R = -H, $-OCH_3$

The reaction depicted in Scheme 2 was used for preparation of the second series of α-LA-indole conjugates. All compounds showed good inhibitory activity except compound **II-3d** (16.1% LPO inhibition) which bears a nitro substituent at position 5. The non-substituted derivative, **II-3a** showed good activity (89.6%), higher than those with electron donating substituents. These data illustrate that the presence of substituents might not be necessary to obtain

good potency while electron-withdrawing substitution might be involved in significant alteration of the electron density of the indole ring to cause major loss in LPO inhibition activity. However, further studies are needed to elucidate the impact of such modifications or the influence of such substitution patterns for α-LA-indole compounds on lipid peroxidation inhibitory activity. The 5-substituted tryptamine conjugates synthesized as shown in Scheme 3 also exhibited greater than 75% inhibition.

In conclusion, this study designed and synthesized α-LA-indole conjugates. These compounds possess good inhibitory activities on lipid peroxidation. These findings become significant in that α-lipoic acid is less active than some of the synthesized compounds. This could indicate a new strategy in designing α-LA derivatives with improved antioxidant capacity which could have an indirect beneficial effect on the human antioxidant defence system.

Acknowledgments

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Experimental

General

The structures of all synthesized compounds were assigned on the basis of IR, ^{1}H-NMR, and Mass Spectra analyses. Silica gel 60 (Merck, 230-400 Mesh; Merck, Darmstadt, Germany) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 GF_{256}, 0.20 mm) were used for TLC. Melting points were determined with Büchi SMP-20 and Büchi 9100 melting point apparatus and are uncorrected (Büchi Labortechnik, Flawil, Switzerland). IR spectra were recorded on a Jasco FTIR-420 spectrophotometer (JASCO Research Ltd., Victoria, British Columbia, Canada), ^{1}H-NMR (400 MHz) spectra were obtained on a Varian Mercury-400 spectrometer (Varian Inc., Palo Alto, CA, USA), and MS were recorded on a Waters ZQ micromass LC-MS spectrometer by the method of ES^+ (Waters Corporation, Milford, MA, USA).

Chemistry

Commercially available α-lipoic acid (racemic mixture) and appropriate indole compounds served as starting materials. Fifteen α-LA derivatives were designed and synthesized, composing three series bearing substituents at positions 1, 3, and 5 of the indole ring. Conjugation of the two moieties was through amide bond which is between the carboxyl group of α-LA and the amine group on position 1, 3, or 5 of the indole ring (Tables 1 and 2). Fourteen out of fifteen conjugates are novel α-LA compounds. Designed compounds are shown as three groups in Schemes 1–3.

Table 1. 1-Substituted-5-nitro/5-amino-1*H*-indole and 5-substituted-1*H*-indole-1-amino derivatives.

Comp.	R	mp [°C]	yield [%]
I-2b	CH_3	164 (165–167, [14])	94
I-2c	C_2H_5	77 (78–81, [14])	61
I-2d†	***i*-C_3H_7**	**66–-67**	**21**
I-2e†	***n*-C_4H_9**	**46–47**	**77**
I-2f	Bn	102 (100–103, [14])	73
I-2g	4-F-Bn	120–121 (114–115, [15])	78
I-2h†	**2,4-diCl-Bn**	**149–151**	**82**
I-3b	CH_3	104 (105, [16])	
I-3c	C_2H_5	oil [17]	
I-3d	*i*-C_3H_7	oil	
I-3e	*n*-C_4H_9	oil	
I-3f	Bn	oil [17]	
I-3g	4-F-Bn	oil [no ref. in Sci Finder]	
I-3h	2,4-diCl-Bn	oil	
II-2a	H	40 (41–41.5, [19])	29
II-2b	OCH_3	115 (115–117, [20])	8
II-2c	Br	85 (oil, [20])	33
II-2d	NO_2	165 (166–167, [20])	8
II-2e†	**CH_3**	**84**	**34**

† Detailed properties of novel compounds are given in Experimental.

Table 2. Physicochemical properties of synthesized compounds as 1st, 2nd, 3rd group and their inhibition of lipid peroxidation (LPO).

Comp. (10^{-4}M)	R	†R_f	yield [%]	mp [°C]	‡LPO [% inh]
I-4a	H	0.62	62	96–98	82.1
I-4b	CH_3	0.68	36	116–117	60.4
I-4c	C_2H_5	0.76	9	93–95	77.4
I-4d	*i*-C_3H_7	0.82	52	130	88.7
I-4e	*n*-C_4H_9	0.89	69	65–66	78.1
I-4f	Bn	0.85	61	105	14.7
I-4g	4-F-Bn	0.86	51	111	90.7
I-4h	2,4-diCl-Bn	0.94	29	69–71	64.6
II-3a	H	0.88	32	105–107	89.6
II-3b	OCH_3	0.84	25	88–90	78.2
II-3c	Br	0.82	27	118	79.3
II-3d	NO_2	0.54	21	124–126	16.1
II-3e	CH_3	0.83	50	43–44	63.4
III-5a	H	0.16	54	58–60	83.3
III-5b	OCH_3	0.16	38	oil	75.7

† Ethylacetate/*n*-Hexane (1:1).
‡ α-LA; LPO inh.: 45%.

Syntheses of compounds I-4 (a–h) comprising the 1st group

Designed compounds bearing substituent at position 1 were synthesized in three steps (Scheme 1). Syntheses of 1-substituted-5-nitro-1*H*-indole derivatives **I-2(b–h)** (Table 1) were obtained according to the procedure described by Mor et al. [13], starting from **I-1** and using an alkylating agent (Scheme 1). Catalytic hydrogenation of

the aromatic nitro group in ethanol was conducted at room temperature and under a pressure of 35 psi with 10% Pd/C as catalyst. The reaction was filtered through celite to remove the Pd/C, and the solvent evaporated. The compounds (**I-3 (c–h)**) (Table 1) obtained as brown oils were used *in situ* and without purification in the next step. The amide compounds **I-4 (b–h)** (Table 2) were prepared from α-LA and appropriate amine as illustrated above after activation of *N*,*N*′-Carbonyldiimidazole (*N*,*N*′-CDI) according to the method described by Tomihisa [18]. The same method was used to synthesize the compound **I-4a** starting from α-LA and 5-amino-indole.

Syntheses of compounds II-3 (a–e) comprising the 2nd group

Designed compounds bearing substituent at position 5 were synthesized in two steps (Scheme 2). Syntheses of 5-substituted-1*H*-indole-1-amine derivatives **II-2 (a–e)** (Table 1) were achieved following the procedure described by Somei and Natsume [19], starting from 5-substituted-1*H*-indoles **II-1 (a–e)** and using Hydroxylamine-*O*-sulfonic acid (H-*O*-SA) as amination agent. The amide compounds **II-3 (a–e)** (Table 2) were prepared from α-LA and appropriate amine after activation of *N*,*N*′-CDI in accordance with the literature [18].

Syntheses of compounds III-5 (a–b) comprising the 3rd group

Designed compounds bearing substituent at position 5 were synthesized in four steps (Scheme 3). By treating **III-1 (a–b)** with DMF and $POCl_3$ [13], the corresponding aldehydes **III-2 (a–b)** were obtained. These aldehydes were condensed with nitromethane to give **III-3 (a–b)** as described by Mor et al. [13]. 3-2′-vinylindoles were reduced to the subsequent aminoethylindoles **III-4 (a–b)** by $LiAlH_4$ [21]. The amide compounds **III-5 (a–b)** (Table 2) were prepared from α-LA and aforementioned amine as described above after activation of *N,N*′-CDI.

Spectral analyses of intermediate compounds

1-Isopropyl-5-nitro-1H-indole ***(I-2d)***

^{1}H-NMR δ ppm ($CDCl_3$, 400 MHz): 1.57 (d, 6H, N-CH*(CH$_3$)$_2$*), 4.72 (m, 1H, N-*CH*(CH_3)$_2$), 6.70 (d, 1H, *J* = 3.6), 7.38 (d, 1H, *J* = 3.2), 7.40 (s, 1H), 8.11 (dd, 1H, J_m = 2.4, J_o = 9.2), 8.59 (d, 1H, *J* = 2); LC-MS *m*/*z* 204 $(M+H)^+$.

1-n-Butyl-5-nitro-1H-indole ***(I-2e)***

^{1}H-NMR δ ppm ($CDCl_3$, 400 MHz): 0.95 (t, 3H, -*CH$_3$*), 1.34 (m, 2H, -*CH$_2$*CH_3), 1.83 (m, 2H, N-CH_2*CH$_2$*-), 4.16 (t, 2H, N-*CH$_2$*-), 6.66 (d, 1H, *J* = 3.6), 7.24 (d, 1H, *J* = 3.2), 7.34 (d, 1H, *J*=8.8), 8.10 (dd, 1H, J_m = 2, J_o = 9.2), 8.57 (d, 1H, *J*=2); LC-MS *m*/*z* 218 $(M+H)^+$.

1-(2,4-dichloro benzyl)-5-nitro-1H-indole ***(I-2h)***

^{1}H-NMR δ ppm ($CDCl_3$, 400 MHz): 5.42 (s, 2H, N-*CH$_2$*-), 6.56 (d, 1H, *J* = 8), 6.77 (d, 1H, *J* = 3.2), 7.11 (dd, 1H, J_m = 2.4, J_o = 8.8), 7.27 (m, 2H), 7.46 (d, 1H, *J* = 2), 8.09 (dd, 1H, J_m = 2.4, J_o = 8), 8.61 (d, 1H, *J* = 2.4).

5-Methyl-1H-indole-1-amine ***(II-2e)***

^{1}H-NMR δ ppm ($CDCl_3$, 400 MHz): 2.44 (s, 3H, -*CH$_3$*), 4.74 (s, 2H, -*NH$_2$*), 6.29 (d, 1H, *J* = 2.8), 7.06 (dd, 1H, *J* = 8.4), 7.12 (d, 1H, *J* = 3.2), 7.29 (d, 1H, *J* = 8.4), 7.37 (s, 1H); LC-MS *m*/*z* 146 $(M+H)^+$.

Spectral analyses of synthesized compounds

5-[1,2]Dithiolane-3-yl-penthanoic acid (1H-indole-5-yl)-amide ***(I-4a)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.39–3.65 [1.42 (m, 2H), 1.57–1.72 (m, 4H), 1.87 (m, 1H), 2.29 (t, 2H), 2.44 (m, 1H), 3.15 (m, 2H), 3.64 (m, 1H); α-*LA-protons*], 6.35–7.86 [6.35 (m, 1H), 7.17 (m, 1H), 7.28 (m, 2H), 7.86 (d, 1H); Ar-*H*], 9.66 (s, 1H, indole-N*H*), 10.98 (s, 1H, N*H*-CO) ; LC-MS *m*/*z* 321 $(M+H)^+$; IR (KBr, cm^{-1}): 3271 (NH), 1653 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid (1-methyl-1H-indole-5-yl)-amide ***(I-4b)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.38–3.64 [1.41 (m, 2H), 1.56–1.69 (m, 4H), 1.86 (m, 1H), 2.27 (t, 2H), 2.38 (m, 1H), 3.13 (m, 2H), 3.61 (m, 1H); α-*LA-protons*], 3.74 (s, 3H, N-*CH$_3$*), 6.33–7.86 [6.33 (d, 1H), 7.24 (m, 2H), 7.32 (d, 1H), 7.86 (d, 1H); Ar-*H*], 9.69 (s, 1H, N*H*-CO); LC-MS *m*/*z* 335 $(M+H)^+$; IR (KBr, cm-1): 3254 (NH), 1637 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid (1-ethyl-1H-indole-5-yl)-amide ***(I-4c)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.33 (t, 3H, N-CH_2*CH$_3$*), 1.41–3.64 [1.42 (m, 2H), 1.67 (m, 4H), 1.87 (m, 1H), 2.29 (t, 2H), 2.42 (m, 1H), 3.15 (m, 2H), 3.63 (m, 1H); α-*LA-protons*], 4.15 (m, 2H, N-*CH$_2$*CH_3), 6.35–7.86 [6.35 (m, 1H), 7.22 (d, 1H), 7.35 (m, 2H), 7.86 (s, 1H); Ar-*H*], 9.69 (s, 1H, N*H*-CO); LC-MS *m*/*z* 349 $(M+H)^+$; IR (KBr, cm^{-1}): 3255 (NH), 1639 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid (1-isopropyl-1H-indole-5-yl)-amide ***(I-4d)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.41 (d, 6H, N-CH*(CH$_3$)$_2$*), 1.40–3.62 [1.40 (m, 2H), 1.65 (m, 4H), 1.86 (m, 1H), 2.28 (t, 2H), 2.40 (m, 1H), 3.14 (m, 2H), 3.62 (m, 1H); α-*LA-protons*], 4.67 (m, 1H, N-*CH*(CH_3)$_2$), 6.36–7.85 [6.36 (s, 1H), 7.19 (d, 1H), 7.39 (m, 2H), 7.85 (s, 1H); Ar-*H*], 9.67 (s, 1H, N*H*-CO); LC-MS *m*/*z* 363 $(M+H)^+$; IR (KBr, cm^{-1}): 3258 (NH), 1645 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid (1-n-butyl-1H-indole-5-yl)-amide ***(I-4e)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 0.87 (t, 3H, -*CH$_3$*), 1.21 (m, 2H, -*CH$_2$*CH_3), 1.39–3.65 [1.42 (m, 2H), 1.57–1.73 (m, 4H), 1.87 (m, 1H), 2.29 (t, 2H), 2.41 (m, 1H), 3.15 (m, 2H), 3.63 (m, 1H); α-*LA-protons*], 1.57–1.73 (m, 2H, N-CH_2*CH$_2$*-), 4.12 (t, 2H, N-*CH$_2$*-), 6.34–7.87 [6.34 (d, 1H), 7.22 (d, 1H), 7.31 (d, 1H), 7.36 (d, 1H), 7.87 (s, 1H); Ar-*H*], 9.70 (s, 1H, N*H*-CO); LC-MS *m*/*z* 377 $(M+H)^+$; IR (KBr, cm^{-1}): 3253 (NH), 1635 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid (1-benzyl-1H-indole-5-yl)-amide ***(I-4f)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.39–3.56 [1.40 (m, 2H), 1.65 (m, 4H), 1.86 (m, 1H), 2.28 (t, 2H), 2.40 (m, 1H), 3.12 (m, 2H), 3.56 (m, 1H); α-*LA-protons*], 5.36 (s, 2H, N-*CH$_2$*-), 6.41–7.88 [6.41 (s, 1H), 7.16–7.31 (m, 7H), 7.45 (s, 1H), 7.88 (s, 1H); Ar-*H*], 9.68 (s, 1H, N*H*-CO); LC-MS *m*/*z* 411 $(M+H)^+$; IR (KBr, cm^{-1}): 3248 (NH), 1644 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid [1-(4-fluorobenzyl)-1H-indole-5-yl]-amide ***(I-4g)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.39–3.61 [1.40 (m, 2H), 1.65 (m, 4H), 1.86 (m, 1H), 2.27 (t, 2H), 2.39 (m, 1H), 3.12 (m,

2H), 3.61 (m, 1H); α-*LA-protons*], 5.35 (s, 2H, N-CH_2-), 6.40−7.88 [6.40 (s, 1H), 7.11−7.21 (m, 5H), 7.34 (d, 1H), 7.45 (s, 1H), 7.88 (s, 1H); Ar-*H*], 9.69 (s, 1H, N*H*-CO); LC-MS *m/z* 429 $(M+H)^+$; IR (KBr, cm^{-1}): 3315 (NH), 1686 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid [1-(2,4-dichlorobenzyl)-1H-indole-5-yl]-amide ***(I-4h)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.39−3.65 [1.42 (m, 2H), 1.63 (m, 4H), 1.87 (m, 1H), 2.29 (t, 2H), 2.42 (m, 1H), 3.15 (m, 2H), 3.63 (m, 1H); α-*LA-protons*], 5.47 (s, 2H, N-CH_2-), 6.47−7.93 [6.47 (d, 1H), 6.55 (d, 1H), 7.17−7.32 (m, 3H), 7.41 (d, 1H), 7.68 (d, 1H), 7.93 (d, 1H); Ar-*H*], 9.73 (s, 1H, N*H*-CO); LC-MS *m/z* 479 $(M+H)^+$; IR (KBr, cm^{-1}): 3254 (NH), 1639 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid indole-1-yl-amide ***(II-3a)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.44−3.66 [1.47 (m, 2H), 1.60−1.71 (m, 4H), 1.89 (m, 1H), 2.34 (t, 2H), 2.42 (m, 1H), 3.11−3.19 (m, 2H), 3.66 (m, 1H); α-*LA-protons*], 6.44−7.54 [6.44 (m, 1H), 7.05 (t, 1H), 7.15 (m, 2H), 7.25 (d, 1H), 7.54 (d, 1H); Ar-*H*], 11.10 (s, 1H, N*H*-CO); LC-MS *m/z* 321 $(M+H)^+$; IR (KBr, cm^{-1}): 3195 (NH), 1667 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid (5-methoxy-indole-1-yl)-amide ***(II-3b)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.45−3.67 [1.46 (m, 2H), 1.63 (m, 4H), 1.90 (m, 1H), 2.33 (t, 2H), 2.45 (m, 1H), 3.14 (m, 2H), 3.67 (m, 1H); α-*LA-protons*], 3.74 (s, 3H, O-CH_3), 6.35−7.20 [6.35 (d, 1H), 6.78 (m, 1H), 7.06 (m, 2H), 7.20 (m, 1H); Ar-*H*], 11.06 (s, 1H, N*H*-CO); LC-MS *m/z* 351 $(M+H)^+$; IR (KBr, cm^{-1}): 3180 (NH), 1668 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid (5-bromo-indole-1-yl)-amide ***(II-3c)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.45−3.69 [1.47 (m, 2H), 1.67 (m, 4H), 1.91 (m, 1H), 2.36 (t, 2H), 2.44 (m, 1H), 3.15−3.31 (m, 2H), 3.68 (m, 1H); α-*LA-protons*], 6.47−7.78 [6.47 (d, 1H), 7.18 (d, 1H), 7.28 (d, 1H), 7.35 (s, 1H), 7.78 (s, 1H); Ar-*H*], 11.22 (s, 1H, N*H*-CO); LC-MS *m/z* 400 $(M+H)^+$, 401 (M+2); IR (KBr, cm^{-1}): 3187 (NH), 1674 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid (5-nitro-indole-1-yl)-amide ***(II-3d)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.46−3.71 [1.49 (m, 2H), 1.66 (m, 4H), 1.91 (m, 1H), 2.40 (t, 2H), 2.46 (m, 1H), 3.17 (m, 2H), 3.69 (m, 1H); α-*LA-protons*], 6.79-8.61 [6.79 (d, 1H), 7.41 (d, 1H), 7.58 (d, 1H), 8.07 (d, 1H), 8.61 (d, 1H); Ar-*H*], 11.43 (s, 1H, N*H*-CO); LC-MS *m/z* 366 $(M+H)^+$; IR (KBr, cm^{-1}): 3254 (NH), 1669 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid (5-methyl-indole-1-yl)-amide ***(II-3e)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.35−3.63 [1.37 (m, 2H), 1.49−1.66 (m, 4H), 1.88 (m, 1H), 2.21 (t, 2H), 2.39 (m, 1H), 3.15 (m, 2H), 3.61 (m, 1H); α-*LA-protons*], 2.37 (s, 3H, -CH_3), 6.35−7.34 [6.35 (d, 1H), 6.99 (d, 1H), 7.07 (d, 1H), 7.20 (d, 1H), 7.34 (d, 1H); Ar-*H*], 11.07 (s, 1H, N*H*-CO); LC-MS *m/z* 335 $(M+H)^+$; IR (KBr, cm^{-1}): 3248 (NH), 1671 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid [2-(1H-indole-3-yl)ethyl]-amide ***(III-5a)***

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.30−3.55 [1.30 (m, 2H), 1.51 (m, 3H), 1.61 (m, 1H), 1.82 (m, 1H), 2.06 (t, 2H), 2.36 (m, 1H), 2.82 (m, 2H), 3.12 (m, 2H), 3.34 (m, 2H), 3.55 (m, 1H); α-*LA-protons* and −CH_2CH_2NH], 6.66−7.53 [6.66 (t, 1H), 7.06 (t, 1H), 7.14 (s, 1H), 7.33 (d, 1H), 7.53 (d, 1H); Ar-*H*], 7.92 (s, 1H, indole-N*H*), 10.82 (s, 1H, N*H*-CO); LC-MS *m/z* 349 $(M+H)^+$; IR (KBr, cm^{-1}): 3291 (NH), 1648 (C=O).

5-[1,2]Dithiolane-3-yl-penthanoic acid [2-(5-methoxy-1H-indole-3-yl)ethyl]-amide ***(III-5b)*** [18]

^{1}H-NMR δ ppm (DMSO-d_6, 400 MHz): 1.30−3.56 [1.30 (m, 2H), 1.49 (m, 3H), 1.62 (m, 1H), 1.83 (m, 1H), 2.05 (m, 2H), 2.36 (m, 1H), 2.76 (m, 2H), 3.13 (m, 2H), 3.30 (m, 2H), 3.56 (m, 1H); α-*LA-protons* and -CH_2CH_2NH], 3.74 (s, 3H, O-CH_3), 6.69−7.20 [6.69 (d, 1H), 6.99 (s, 1H), 7.08 (s, 1H), 7.20 (d, 1H); Ar-*H*], 7.89 (s, 1H, indole-N*H*), 10.63 (s, 1H, N*H*-CO); LC-MS *m/z* 379 $(M+H)^+$; IR (KBr, cm^{-1}): 3298 (NH), 1647 (C=O).

Assay of lipid peroxidation

NADPH-dependent lipid peroxidation was determined under the optimum conditions as earlier described [22]. Male Wistar rats (200−225 g) 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. Livers were removed immediately and washed in ice-cold distilled water, and microsomes were prepared using literature methods [23]. NADPH-dependent lipid peroxidation was measured spectrophotometrically by estimation of thiobarbituric acid reactive substances (TBARS). Amounts of TBARS were expressed in terms of nmol malondialdehyde (MDA) per mg protein. A typical optimized assay mixture contained 0.2 mM Fe^{++}, 90 mM KCl, 62.5 mM potassium phosphate buffer, pH 7.4, a NADPH generating system consisting of 0.25 mM $NADP^+$, 2.5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate buffer, pH 7.8, and 0.2 mg microsomal protein in a final volume of 1.0 mL. Protein was measured by the method of Lowry et al., using bovine serum albumin as standard [24].

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