J. Pharm. Pharmacol. 1995, 47: 131–137
Received March 28, 1994
Accepted September 7, 1994

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Novel 1,4 Substituted Piperidine Derivatives. Synthesis and Correlation of Antioxidant Activity with Structure and Lipophilicity

ALEXANDROS N. ALEXIDIS, ELENI A. REKKA, VASSILIS J. DEMOPOULOS AND PANOS N. KOUROUNAKIS

Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotelian University of Thessaloniki, Thessaloniki 54006, Greece

Abstract

A series of novel piperidine derivatives was prepared and their lipophilicity was determined (as R_M values). These compounds as well as two intermediate α-keto-esters were tested for antioxidant activity.

It was found that the cysteamine derivatives were efficient antioxidants, i.e. they could inhibit lipid peroxidation, act as hydroxyl radical scavengers and interact with 2,2-diphenyl-1-picrylhydrazyl radicals. This interaction could be attributed to the free SH group and this activity seemed to be favoured by increased lipophilicity.

Replacement of SH by NH_2 or OH resulted in a decreased antioxidant activity of the compounds. However, the described activities seem not to be connected with any $O_2^-\cdot$ scavenging ability, at least under the experimental conditions applied. Furthermore, cysteamine derivatives seem to induce $O_2^-\cdot$ generation, a phenomenon often observed with thiol compounds.

The antioxidant activity of the intermediate α-keto-esters varied and is probably mediated by different mechanisms.

In aerobic organisms, oxygen-derived free radicals may initiate a series of harmful biochemical events, or may be generated as a consequence of earlier tissue injury, thus aggravating the final damage. Under normal conditions, the physiological defence system can prevent oxidative damage, although these adaptive changes may themselves become detrimental if allowed to persist (Kehrer 1993). Uncontrolled free radical processes are involved in various pathological conditions. Furthermore, metabolic disruptions caused by diverse toxic agents, acting at various sites, may, in part, be expressed by abnormal levels of reactive oxygen species (Le Bel et al 1990). Although in such circumstances non-toxic drugs are required, very few are currently used for treating free radical-associated situations and new agents are desirable (Battioni et al 1991).

In this study, we report the synthesis and the evaluation of the antioxidant activity of some novel piperidine derivatives. These compounds have been designed to contain substituents present in many antioxidants as well as in chelating agents. Radical chain reactions are generally catalysed by transition metal ions, mainly iron and copper, and many antioxidants act by binding to metal ions. In addition, most known specific antidotes to heavy metal poisoning are metal chelators. Finally, the toxicity of metals such as mercury or cadmium (Manca et al 1991) has been connected with free radical processes. Thus, the investigated compounds are expected to offer protection, especially in conditions associated with metal overload and toxicity.

Correspondence: P. N. Kourounakis, Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotelian University of Thessaloniki, Thessaloniki 54006, Greece.

Materials and Methods

Melting points were taken on a Mel Temp II capillary apparatus and were uncorrected. The IR spectra were determined on an FTIR-8101M Shimadzu Fourier Transform infrared spectrophotometer. The ^1H NMR spectra were recorded on a Bruker AW-80 NMR spectrometer. The chemical shifts are reported in δ units downfield from tetramethylsilane. Elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyser. The UV measurements were carried out on a Perkin-Elmer 554 UV-vis spectrophotometer. All reagents were obtained from commercial sources.

Methyl 1-(2-(diethylamino)ethyl)-4-oxo-piperidine-3-carboxylate (1)

To an ethanolic solution of *N,N*-diethylethylenediamine (22·1 g, 0·19 mol), methyl acrylate (51·6 g, 0·6 mol) was added dropwise at room temperature (21°C). After 90 min, the mixture was distilled under reduced pressure. From the obtained dimethyl β,β'-(2-(diethylamino)ethylamino)dipropionate (43·3 g, 79%), 25·7 g (0·11 mol) was gradually added to an equivalent amount of sodium in dry toluene, following the Dieckmann condensation procedure (Schaefer & Bloomfield 1967). The crude **1** was converted to its hydrochloride salt and recrystallized from ethanol to yield 13·4 g (46%) of a white solid; mp, 176–178°C; ^1H NMR ($DMSOd_6$-CF_3COOD): δ 1·30 (t, 6H, $2CH_3$, J = 8 Hz), 2·63–3·00 (m, 3H, CH_2, CHCOO), 3·00–3·95 (m, 12H, $6CH_2N$), 4·00 (s, 3H, OCH_3); IR (nujol): cm^{-1} 1640 (C=O), 1690 ($COOCH_3$). Analysis: calculated for $C_{13}H_{26}Cl_2N_2O_3$: C, 47·42; N, 8·50; H, 7·95. Found: C, 47·27; N, 8·72; H, 7·91.

1-(2-(Diethylamino)ethyl)-4-(2-aminoethylamino)piperidine (3)

An aqueous solution of compound **1** (8·23 g, 0·025 mol) was refluxed with 10 mL conc. HCl until a negative ferric chloride test was given. The solution was then made alkaline with 50% NaOH and extracted with ether to yield crude 1-2-(diethylamino)ethyl-4-piperidone (**2**) (4·5 g, 89%). Compound **2** (2·24 g, 0·011 mol) and ethylenediamine (2 g, 0·033 mol) were added to dry benzene, and refluxed, using a Dean-Stark apparatus, for 8 h. The solvent was evaporated, $NaBH_4$ (0·63 g, 0·016 mol) and 50 mL isopropanol was added. The mixture was refluxed (18 h), the solvent was evaporated and the residue was stirred with 5 mL conc. HCl (30 min). The free base was obtained after basification (50% NaOH) and extraction with ether. The hydrochloride of **3** was precipitated from ethanol with the addition of an ethereal solution of HCl and recrystallized from methanol to yield 2 g (46%) of an off-white solid; mp, 264–265°C; ^1H NMR (CF_3COOD): δ 1·46 (t, 6H, $2CH_3$, J = 5·6 Hz), 2·50–2·88 (m, 4H, $2CH_2$), 3·30–4·22 (m, 17H, $8CH_2N$, CHN); the picrate salt (analytical sample) was obtained from ethanol with the addition of an ethanolic solution of picric acid (10%) and recrystallized from methanol; mp, 238–239°C. Analysis (including methanol of crystallization): calculated for $C_{37}H_{42}N_{16}O_{28}{\cdot}CH_3OH$: C, 38·32; N, 18·81; H, 3·89. Found: C, 37·95; N, 18·61; H, 3·76.

1-(2-(Diethylamino)ethyl)-4-(2-hydroxyethylamino) piperidine (4)

Compound **2** (1 g, 0·005 mol) was refluxed with ethanolamine (0·62 g, 0·01 mol), in 160 mL dry toluene, using a Dean-Stark apparatus for 2 h. The solvent was evaporated and $NaBH_4$ (0·38 g, 0·01 mol) and isopropanol (50 mL) were added. The mixture was refluxed (15 h), the solvent was evaporated and 5 mL conc. HCl was added with stirring. Basification with 50% NaOH and extraction with 200 mL $CHCl_3$ followed. The hydrochloride of **4** was obtained from ethanol and recrystallized from ethanol to yield 0·7 g (39·5%) of a white solid; mp, 243–245°C; ^1H NMR (CF_3COOD): δ 1·47 (t, 6H, $2CH_3$, J = 8·8 Hz), 2·5–2·88 (m, 4H, $2CH_2$), 3·26–4·37 (m, 17H, $7CH_2N$, CH_2OH, CHN); IR (nujol): cm^{-1} 3300 (–OH). Analysis: calculated for $C_{16}H_{32}Cl_3N_3O$: C, 44·26; N, 11·91; H, 9·14. Found: C, 43·75; N, 11·75; H, 9·05.

1-(2-(Diethylamino)ethyl)-4-(2-mercaptoethylamino)piperidine (5)

Compound **2** (1·25 g, 0·006 mol), cysteaminum chloride (2·17 g, 0·019 mol) and $NaBH_3CN$ (0·82 g, 0·013 mol), in 100 mL dry methanol, were stirred (24 h) while the pH was adjusted to 6 (CH_3COOH). The solvent was removed and 7 mL conc. HCl was added to the residue. After 1 h stirring, the water was removed azeotropically with ethanol. Hot ethanol was added to the dry residue, the inorganic precipitate was removed and after condensation, an off-white solid was obtained (35%) and recrystallized from methanol. ^1H NMR (CF_3COOD): δ 1·46 (t, 6H, $2CH_3$, J = 8·8 Hz), 2·52–3·12 (m, 6H, $2CH_2$, CH_2S), 3·30–4·10 (m, 15H, $7CH_2N$, CHN); the picrate (analytical sample) was obtained from the free base and recrystallized from methanol; mp, 229–231°C. Analysis (including methanol of crystallization): calculated for $C_{31}H_{38}N_{12}O_{21}S{\cdot}CH_3OH$: C, 39·26; N, 17·17; H, 4·32. Found: C, 39·47; N, 16·98; H, 4·07.

Methyl 1-(2-phenylethyl)-4-oxo-piperidine-3-carboxylate (6)

Phenylethylamine (14·5 g, 0·12 mol) and methyl acrylate (32·5 g, 0·38 mol) were reacted in the same manner as for compound **1** to yield dimethyl β,β'-(2-phenylethylamino)dipropionate (28·3 g, 81%), which was then added dropwise to a double molar quantity of sodium hydride suspended in benzene. The mixture was then heated (70°C, 90 min), and treated as described for **1** to give the hydrochloride salt of **6** (20·2 g, 71% yield); mp, 167–169°C; ^1H NMR (CF_3COOD): δ 2·72–4·02 (m, 14H, $PhCH_2$, $3CH_2N$, $COOCH_3$, CHCOO, CH_2CO), 7·31 (s, 5H, C_6H_5); IR (nujol): cm^{-1} 1630 (C=O), 1680 ($COOCH_3$). Analysis: calculated for $C_{15}H_{20}ClNO_3$: C, 60·50; N, 4·70; H, 6·76. Found: C, 60·45; N, 4·90; H, 6·76.

Compound **6**, as a free base, has been reported in the literature (Sheng-Hsi et al 1981).

1-(2-Phenylethyl)-4-(2-aminoethylamino)piperidine (8)

Compound **6** (10·2 g, 0·034 mol) was hydrolysed and decarboxylated to give 6·14 g (88%) of crude 1-(2-phenylethyl)-4-piperidone (**7**). Compound **8** hydrochloride was prepared from the reaction of 0·25 g (0·0012 mol) of compound **7** and 0·24 g (0·004 mol) ethylenediamine as described for compound **3**, to yield 0·2 g (46%) of product as white solid, which recrystallized from methanol; mp, 307–310°C; ^1H NMR (CF_3COOD): δ 2·39–2·85 (m, 4H, $2CH_2$), 3·10–4·25 (m, 16H, $PhCH_2$, $5CH_2N$, CHN, CH_3OH), 7·32 (s, 5H, C_6H_5); IR (nujol): cm^{-1} 3350 (CH_3OH). Analysis (including methanol of crystallization): calculated for $C_{15}H_{28}Cl_3N_3{\cdot}CH_3OH$: C, 49·42; N, 10·80; H, 8·29. Found: C, 49·48; N, 10·81; H, 7·98.

1-(2-Phenylethyl)-4-(2-hydroxyethylamino)piperidine (9)

Compound **9** hydrochloride was prepared following the method described for compound **4**, using 2·75 g (0·0085 mol) of compound **7**, yielding a white solid (37%, 1·6 g), recrystallized from methanol-acetone; mp, 237–239°C; ^1H NMR (CF_3COOD): δ 2·48–2·79 (m, 4H, $2CH_2$), 3·04–4·33 (m, 13H, $PhCH_2$, $4CH_2N$, CHN, CH_2OH), 7·13–7·41 (s, 5H, C_6H_5); IR (nujol): cm^{-1} 3250 (–OH); the picrate (analytical sample) recrystallized from methanol; mp, 243–246°C. Analysis: calculated for $C_{15}H_{26}Cl_2N_2O$: C, 45·89; N, 15·85; H, 4·27. Found: C, 45·96; N, 15·68; H, 4·12.

1-(2-Phenylethyl)-4-(2-mercaptoethylamino)piperidine (10)

Compound **10** hydrochloride was prepared as described for compound **5**, using compound **7** as the starting material, yielding (33·5%) a white substance, recrystallized from methanol; ^1H NMR (CF_3COOD): δ 2·49–2·82 (m, 2H, CH_2S), 2·89–4·19 (m, 15H, $PhCH_2$, $4CH_2N$, $2CH_2$, CHN), 7·32 (s, 5H, C_6H_5); the picrate (analytical sample) also recrystallized from methanol; mp, 230–231°C. Analysis (including methanol of crystallization): calculated for $C_{27}H_{30}N_8O_{14}S{\cdot}CH_3OH$: C, 44·56; N, 14·84; H, 4·54. Found: C, 44·83; N, 15·06; H, 4·31.

1-Isobutyl 4-(2-aminoethylamino)piperidine (11)
Isobutylamine (8·1 g, 0·11 mol) and methyl acrylate (22·9 g, 0·26 mol) were reacted as described above (compound **1**) to give 13·3 g (49%) dimethyl β,β'-isobutylaminodipropionate. Following the method described for the preparation of **3**, **11** hydrochloride was obtained and recrystallized from methanol (1·6 g, 42%); mp, 287–289°C; ^{1}H NMR (C_3FCOOD): δ 1·15 (d, 6H, $2CH_3$, J = 4 Hz), 2·07–2·31 (m, 1H, CH), 2·48–2·85 (m, 4H, $2CH_2$), 2·95–3·43 (m, 7H, $3CH_2N$, CHN), 3·73–4·21 (m, 7H, CH_3OH, NCH_2CH_2N); IR (nujol): cm^{-1} 3500 (CH_3OH). Analysis (including methanol of crystallization): calculated for $C_{11}H_{28}Cl_3N_3{\cdot}CH_3OH$: C, 42·29; N, 12·33; H, 9·46. Found: C, 41·93; N, 12·65; H, 9·66.

n-Propyl 4-(2-mercaptoethylamino)piperidine-1-acetate (13)
4-Piperidone monohydrate hydrochloride (3 g, 0·019 mol) and diisopropylamine (3·9 g, 0·039 mol) were stirred for 2 days, in 100 mL acetone, after the dropwise addition of propyl bromoacetate (3·5 g, 0·019 mol). The precipitated solid was filtered off and the solvent was evaporated. The residue was washed with a small amount of ether to remove propyl bromoacetate and to yield 3·1 g (79%) crude *n*-propyl 4-oxo-piperidine-1-acetate (**12**), a yellow oil, which was used in the next step without further purification. Compound **13** hydrochloride was synthesized as described for compound **5** in an overall yield of 45% after recrystallization from *n*-propanol; mp, 211–213°C; ^{1}H NMR (CF_3COOD): δ 1·00 (t, 3H, CH_3, J = 8 Hz), 1·64–2·00 (m, 2H, $MeCH_2$), 2·50–2·85 (m, 4H, $2CH_2$), 2·89–3·17 (m, 2H, CH_2S), 3·39–4·45 (m, 11H, $4CH_2N$, CHN, $COOCH_2$); IR (nujol): cm^{-1} 1750 (COOPr). Analysis: calculated for $C_{12}H_{26}Cl_2N_2O_2S$: C, 43·24; N, 8·40; H, 7·86. Found: C, 43·16; N, 8·22; H, 8·09.

Determination of R_M values from reversed-phase TLC and Σ_f values with Rekker's revised method
For the reverse phase thin layer chromatography (RPTLC), silica gel normal phase plates (Merck $60F_{254}$) impregnated with 5% (w/v) liquid paraffin in light petroleum ether were used as the stationary phase. A methanol/water/aqueous ammonia (27%) mixture (75/20/5, v/v) was used as the mobile phase. Spots were detected by iodine vapour, and R_f values were determined from at least ten individual measurements (Rekka et al 1989a). R_M values were calculated applying the equation $R_M = \log[(1/R_F) - 1]$. Lipophilicity calculations, expressed as Σ_f values, were performed using Rekker's revised *f*-system (Rekker & Mannhold 1992).

In-vitro lipid peroxidation
For the in-vitro lipid peroxidation, heat-inactivated hepatic microsomes, from untreated female Fischer 344 rats, were used. Lipid peroxidation was induced by the ascorbic acid/Fe^{2+} (0·2 mM/10 μM) system. The tested compounds were dissolved in Tris buffer (pH 7·4) and added at various concentrations (0·1–1 mM). The mixture was incubated at 37°C for 45 min and samples (0·3 mL) were taken at various time intervals. The lipid peroxidation was estimated by spectrophotometric determination (535 vs 600 nm) of the 2-thiobarbituric acid-reactive material (Rekka et al 1989b). Each experiment was performed at least in duplicate. All compounds have been tested and found not to interfere with the assay.

Hydroxyl radical (HO·) scavenging activity
For the hydroxyl radical scavenging activity, formaldehyde formed during the oxidation of dimethylsulphoxide by Fe^{3+}/ascorbic acid was determined. The incubation mixture contained 0·1 mM EDTA, 162 μM $FeCl_3$ (as 1:2 mixture with EDTA) and dimethylsulphoxide (3·3–66 mM), in phosphate buffer (50 mM, pH 7·4). The tested compounds, dissolved in buffer, were added at various concentrations (5–25 mM). The mixture was incubated for 30 min at 37°C, and the reaction was stopped by adding 250 μL CCl_3COOH (17·5% w/v). The HCHO formed was determined spectrophotometrically at 412 nm by the method of Nash (Nash 1953; Klein et al 1981). Each experiment was performed at least in duplicate.

Interaction of the synthesized compounds with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)
To an ethanolic solution of DPPH (final concentration 200 μM) an equal volume of the compounds, dissolved in ethanol, was added at various concentrations (50–200 μM). The mixture was kept at room temperature (21°C) for 90 min, and the absorbance (517 nm) was recorded at various time intervals (Blois 1958). Each experiment was performed at least in duplicate.

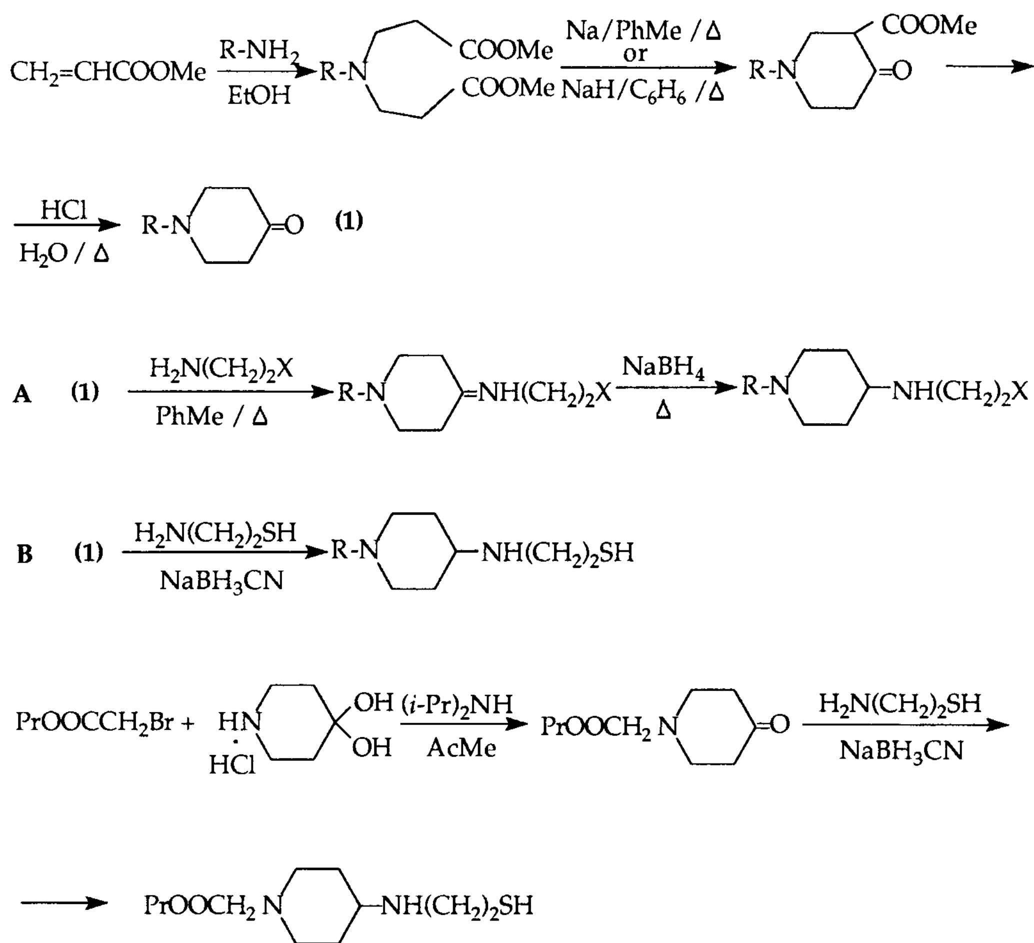
Superoxide (O_2^-·) scavenging activity
For the superoxide anion radical scavenging activity, the colour complex of nitro blue tetrazolium (NBT) with the superoxide anions, was measured at 560 nm. The mixture contained phosphate buffer (pH 7·4), NBT (600 μM), xanthine (200 μM), xanthine oxidase (0·07 units mL^{-1}) and the tested compounds, dissolved in buffer, at various concentrations (1–2 mM). The incubation time was 10 min and each experiment was performed at least in duplicate (Robak & Gryglewski 1988).

Toxicity of the examined compounds
Toxicity experiments were carried out using male Fischer 344 rats. The tested compounds were dissolved in 0·9% NaCl and administered by intraperitoneal injection, at various concentrations. Mortality was recorded after 24 h.

Results

Chemistry
In this study, we synthesized nine novel piperidine derivatives. The synthetic route followed is shown in Scheme 1. The structures of the synthesized compounds and their physicochemical properties are shown in Table 1. The structures of all compounds were supported by spectral data and confirmed by elemental analyses for the new compounds.

Evaluation of the antioxidant activity
Lipid peroxidation experiments demonstrated that of the substances tested, compounds **1** and **4** had no effect at 1 mM



$X = NH_2$ or OH $\quad$ $R = PhCH_2CH_2$-, $Et_2NCH_2CH_2$-, Me_2CHCH_2-

SCHEME 1. Synthetic pathway.

concentration. Compounds **3**, **8**, **9** and **11**, inhibited lipid peroxidation by 15, 20, 5 and 9%, respectively, at 1 mM after 45 min of incubation. All the other compounds (**5**, **6**, **10**, **13**) inhibited non-enzymatic lipid peroxidation by more than 90% at 1 mM. This inhibition was time- and concentration-dependent. The time course of lipid peroxidation, as affected by various concentrations of a representative compound **10**, is shown in Fig. 1. The IC50 values, after 45 min of

Table 1. Structures of the synthesized compounds, their physicochemical properties and acute toxicity.

Compound	R_1	R_2	R_3	$R_M \pm$ s.d.	Σ_f	Acute toxicity (mmol kg^{-1})
1	$Et_2NCH_2CH_2$–	–COOMe	=O	ND[b]	1·096	0·1–1
3	$Et_2NCH_2CH_2$–	–H	$-NH(CH_2)_2NH_2$	0·802 ± 0·07	0·512	0·05–0·1
4	$Et_2NCH_2CH_2$–	–H	$-NH(CH_2)_2OH$	0·260 ± 0·033	0·404	0·1–0·5
5	$Et_2NCH_2CH_2$–	–H	$-NH(CH_2)_2SH$	0·221 ± 0·026	1·806	0·1–0·2
6	$PhCH_2CH_2$–	–COOMe	=O	ND	2·151	> 1
8	$PhCH_2CH_2$–	–H	$-NH(CH_2)_2NH_2$	0·391 ± 0·029	1·567	> 0.1
9	$PhCH_2CH_2$–	–H	$-NH(CH_2)_2OH$	ND	1·459	< 1
10	$PhCH_2CH_2$–	–H	$-NH(CH_2)_2SH$	0·261 ± 0·04	2·861	> 0·1
11	$(Me)_2CHCH_2$–	–H	$-NH(CH_2)_2NH_2$	0·410 ± 0·039	0·908	> 1
13	$PrOOCCH_2$–	–H	$-NH(CH_2)_2SH$	–0·310 ± 0·029	1·659	> 0·1

ND: not determined.

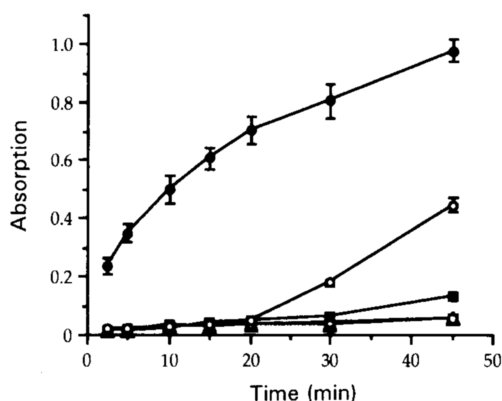


FIG. 1. Time course of lipid peroxidation, as affected by various concentrations of compound **10**, ● controls, ○ 0·1 mM, ■ 0·25 mM, □ 0·5 mM, ▲ 1 mM. Error bars indicate standard deviation of absorbance values; n = 3–5.

incubation, could be calculated (see discussion). The effect of the tested substances on lipid peroxidation could be placed in the following order, according to decreasing activity: **10** > **6** > **13** > **5** > **10** > **8** > **3** > **11** > **9**.

The competition between the tested compounds (at various concentrations) and dimethylsulphoxide (33 mM) for hydroxyl radicals, generated from the ascorbate/Fe^{3+} system, expressed as percent inhibition of formaldehyde production, was used for the estimation of the HO· scavenging activity. The order of decreasing activity (at 10 mM) was **1** > **6** > **13** > **5** > **10** > **8** > **9** > **4** > **3** > **11**. The HO· scavenging activity of the examined compounds is shown in Table 2. To calculate the second order rate constant (K_S) for the reaction of these compounds with HO·, we repeated this experiment varying the dimethylsulphoxide (DMSO) concentration (3·3, 33 and 66 mM) while the concentration of six of the tested compounds was fixed (**1**, 5; **5**, 5; **6**, 5; **8**, 25; **10**, 5; **13**, 5, all in mM). The K_S value of mannitol, used as a reference, was found to be $1{\cdot}7 \times 10^9\,M^{-1}\,s^{-1}$, which is in accordance with the values given in the literature ($1{\cdot}0$–$2{\cdot}0 \times 10^9\,M^{-1}\,s^{-1}$) (Goldstein & Czapski 1984; Halliwell et al 1987). The overall equation which enables the calculation of the rate constant, K_S, is:

$$K_S = \frac{A_o}{A} = 1 + \frac{k_s[S]}{k_{DMSO}[DMSO]}$$

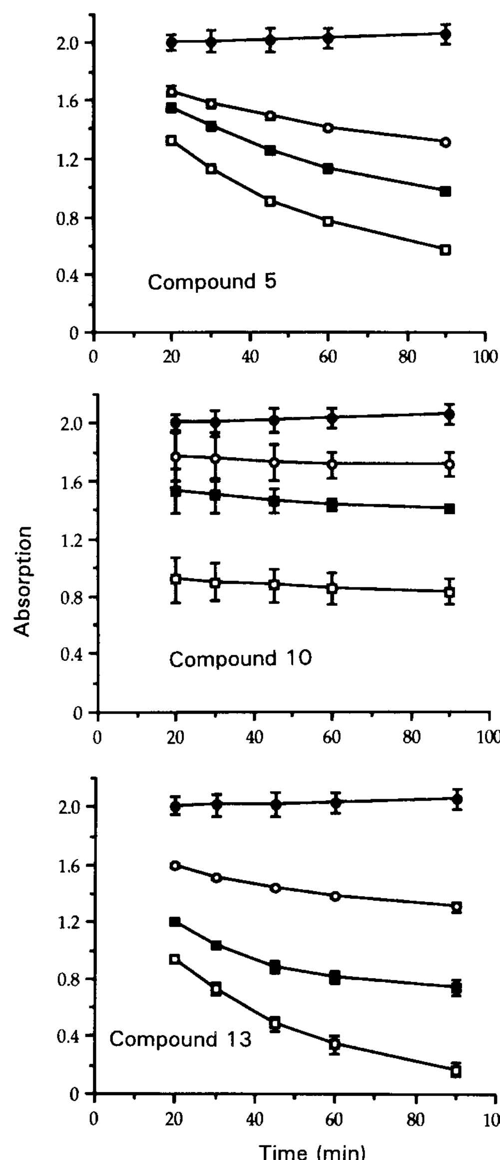


FIG. 2. Interaction of selected compounds with the stable free radical DPPH (200 μM), as a function of time. ● Controls, ○ 50 μM, ■ 100 μM, □ 200 μM. Error bars indicate standard deviation of absorbance values; n = 3–5.

Table 2. Effect of the examined compounds on the HO·-mediated oxidation of dimethylsulphoxide (33 mM), reaction rate constant (K_S) and interaction with DPPH (200 μM).

Compound	HO·-scavenging activity (Percent inhibition[b])			K_S	Interaction with DPPH Percent inhibition[b] (90 min)		
	25 mM	10 mM	5 mM	($M^{-1}\,s^{-1}$)	200 μM	100 μM	50 μM
1	100	100	61	12×10^{10}	28	13	9
3	37	25	0	ND	30	19	10
4	40	27	11	ND	25	16	8
5	100	81	55	$3{\cdot}3 \times 10^{10}$	72	53	36
6	100	98	51	$4{\cdot}5 \times 10^{10}$	0	–	–
8	51	40	37	$2{\cdot}01 \times 10^{10}$	29	24	15
9	51	43	24	ND	25	8·5	7
10	88	63	43	$4{\cdot}05 \times 10^{10}$	60	32	17
11	32	26	29	ND	33	24	15
13	100	88	44	$4{\cdot}5 \times 10^{10}$	92	64	36

Percent inhibition is based on absorbance values of samples with the tested compounds, against controls containing equal volume of the solvent. Standard deviation of absorbance values was less than ± 10%, n = 3–5. ND = not determined.

For these calculations we used the reported rate constant for this reaction of dimethylsulphoxide $0{\cdot}7 \times 10^{10}\,M^{-1}\,s^{-1}$ (Halliwell et al 1987).

The interaction of three of the examined compounds, at various concentrations, with DPPH, as a function of time, is shown in Fig. 2. Compound **6** was completely inactive at 200 mM after 90 min incubation. Reducing activity of compounds **1**, **3**, **4**, **8**, **9** and **11**, ranged from 25 to 40% at 200 mM (Table 2).

As superoxide anion scavengers, seven of the examined compounds had less than 12% activity at 2 mM. With sulphhydryl-containing compounds (**5**, **10**, **13**) an increase in the absorbance, compared with controls, was observed, ranging from 45 to 85%.

Discussion

Reactive oxygen species could be implicated in a number of human diseases including cancer and atherosclerosis, as well as the ageing process itself. Lipid peroxidation, the oxidative modification of polyunsaturated fatty acyl chains that can damage cell membranes, is considered to be a cause and effect of a range of conditions related to oxygen toxicity (Dix & Aikens 1993). Thus, agents such as antioxidants that can control states of oxidative stress represent a major line of defence regulating general health status (Cao et al 1993).

In this investigation, we synthesized novel piperidine derivatives, substituted at position 4 and also at position 1, with groups that are expected to offer protection against radical attack, by application of standard synthetic methods summarized in Scheme 1.

The lipophilicity of most of these compounds was determined from RPTLC and expressed as R_M values. This is considered to be a reliable, fast and convenient method for expressing lipophilicity (Andreadou et al 1991). The corresponding Σ_f values, the sum of the lipophilicity contribution, f, of a constituent part of a structure to the total lipophilicity (Rekker & Mannhold 1992), were also calculated. Apart from the important role of lipophilicity for the kinetics of biologically active compounds, antioxidants of hydrophilic or lipophilic character are both needed to act as radical scavengers in the aqueous phase or as chain-breaking antioxidants in biological membranes. From our results (Table 1), it can be concluded that the R_M values could not be used as a successful relative measure of the overall lipophilic/hydrophilic balance of these molecules, as this is indicated by the calculated Σ_f values, which express their theoretical log P in the standard octanol/water system. We could attribute this to the different nature of the hydrophilic and lipophilic phases in the two systems and, in addition, to the fairly high content of basic nitrogen atoms in the examined compounds, which could disturb the absorption/desorption process.

Compounds **5**, **10** and **13**, which are cysteamine derivatives, are potent antioxidants in almost all tests performed. They could inhibit non-enzymatic microsomal lipid peroxidation in a concentration-dependent manner. Their IC50 values, after 45 min incubation, were found to be 370 μM, < 100 μM and 330 μM, respectively. These variations in activity can be attributed to differences in lipophilicity which are due to the piperidine *N*-substituent, the most potent being the most lipophilic compound **10**. This can be further supported by the fact that cysteamine itself, with a Σ_f value of 0·088, could inhibit lipid peroxidation only by 38% at 1 mM after 45 min incubation under the same experimental conditions (Spyriounis et al 1993). These compounds presented a lag period in the time course of lipid peroxidation, followed by an acceleration of the reaction after about 30 min. This is often observed with chain-breaking antioxidants (Halliwell 1990), compounds usually possessing easily donatable hydrogen atoms (Wiseman et al 1991), which can scavenge intermediate radicals, such as peroxyl or alkoxyl radicals, and prevent continued allylic hydrogen abstraction from lipids. We could attribute the activity of cysteine derivatives to their free thiol group, which can easily donate the sulphhydryl hydrogen. A similar protection against non-enzymatic lipid peroxidation has been reported to occur with dihydrolipoate, a lipid soluble dithiol, and dithioerythritol, and to be mediated via direct chemical reactions (Murphy et al 1992). The synthesized thiol derivatives were also found to interact with the stable free radical DPPH (Table 2, Fig. 2), due to their reducing ability provided by the thiol group. Replacement of SH by NH_2 or OH groups resulted in a significant decrease of the ability of these compounds either to inhibit lipid peroxidation or to interact with DPPH.

The cysteamine derivatives could very effectively inhibit the oxidation of dimethylsulphoxide by HO· generated by ferric EDTA/ascorbate. This effect can be mainly attributed to their HO· scavenging activity. However, the apparent K_S values determined with 66 mM dimethylsulphoxide were higher than those appearing in Table 2, which were obtained using lower dimethylsulphoxide. This phenomenon may be due to a simultaneous effect on hydroxyl radical generation by these compounds, in addition to their scavenging effect. This activity was less pronounced with the ethanolamine and the ethylenediamine derivatives. However, piperidine *N*-substitution may also play a role in the development of the HO·-scavenging activity, possibly connected with the acquired lipophilicity.

Although HO· is considered to be the initiating species in most lipid peroxidation studies in-vitro, there is inadequate direct evidence to support its involvement. The generation of HO· in biological systems appears certain, but its major sites of attack are not the bis-allylic hydrogen atoms of lipids (Patterson 1981). It has been confirmed that the major reaction of HO· with lipids is reversible addition to effect stereochemistry of the lipid (Dix & Aikens 1993).

The antioxidant activity of these compounds could not be correlated with any ability to scavenge $O_2^-\cdot$. Although $O_2^-\cdot$ can be dangerous, since it results in H_2O_2 formation via superoxide dismutase and, being a strong reductant, can convert an Fe^{3+} complex to an Fe^{2+} complex, thus facilitating a Fenton reaction, it is ineffective in initiating lipid peroxidation. It is insufficiently reactive to abstract a hydrogen atom from lipids; furthermore, its ionic character would not permit its entrace to the hydrophobic interior of cell membranes. In this series of experiments, cysteamine derivatives were found to enhance NBT reduction, as indicated by the high absorbance values. It is known (Buettner 1993) that sulphhydryl compounds (RSH) can react with oxidizing species yielding thiyl radicals (RS·).

This radical, reacting with another RSH, gives the highly reducing species RSSR·, which can produce O_2^-· after reaction with oxygen. This series of events may explain the elevated O_2^- · generation observed with our compounds.

It is known that O, S and N can bind iron (Albert 1985). The antioxidant activity of these compounds cannot be totally attributed to their ability to bind iron due to their substituents at position 4 of piperidine, since cysteamine, ethylenediamine and ethanolamine were tested and found to have slight or no effect on lipid peroxidation at 1 mM. Furthermore, inhibition of lipid peroxidation and HO·-scavenging activities were correlated with the ability of these compounds to interact with DPPH ($r_1 = 0{\cdot}904$, $r_2 = 0{\cdot}923$, respectively), which is an iron-free system.

The intermediate α-keto-esters **1** and **6** (Table 1) were also tested for antioxidant activity, in an attempt to elucidate the structural characteristics responsible for this action. Compound **1** had no effect on lipid peroxidation and a weak ability to interact with DPPH, but it could very significantly inhibit the oxidation of dimethylsulphoxide by HO·. On the contrary, compound **6** acted as a potent inhibitor of lipid peroxidation (IC50 255 μM). It was incapable of interacting with DPPH, yet it could inhibit HO·-mediated oxidation of dimethylsulphoxide. A possible explanation for the similar effect of these compounds on dimethylsulphoxide oxidation could be their ability to interact with Fe^{3+} because of their enol-ester moiety, although the different rate constants of their reaction with HO· indicate that this may not be the only mechanism of action. In addition, the observation that compound **6** is an inhibitor of lipid peroxidation, while **1** is inactive, further indicates that the enol-ester-moiety is not entirely responsible for the antioxidant activity due to Fe^{3+} complexation. Similarly, the substituent at position 1 of the piperidine ring itself was found not to be decisive for the antioxidant activity of the other compounds tested. We could only explain this activity of compound **6** by the remarkable difference in lipophilicity found between compounds **1** and **6**. Compound **6**, the second most lipophilic in the whole series, was found to be a good inhibitor of lipid peroxidation.

In conclusion, of the novel compounds synthesized, cysteamine derivatives are potent antioxidants, most probably due to the easily oxidizable SH group. Replacement of the SH by NH_2 or OH groups caused a decrease in the antioxidant activity. The antioxidant activity of these compounds may be useful in the development of agents for the treatment of conditions involving free radicals.

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