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Synthesis and Pharmacochemical Investigation of Some Novel 1,2,4-4*H*-triazoles with Potential Antiviral Activity

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Abstract

We report the synthesis of some mercaptotriazole derivatives in an effort to discover underlying structural requirements for antiviral activity. A preliminary antiviral study was performed and the contribution of the compounds to free radical processes was investigated. Because lipophilicity influences both biological activity and antioxidant potential we calculated lipophilicity and attempted to correlate this with antioxidant activity.

Treatment of the *N*-(aryl)piperazineacetohydrazides (compounds **1**) with 2,4-dichlorophenylisothiocyanate gave the *N*-(aryl)piperazinethiosemicarbazides (compounds **2**) in good yield. Cyclization of these compounds after treatment with NaOH solution provided the corresponding 5-(4-aryl-1-piperazinylmethyl)-4-(2,4-dichlorophenyl)-4*H*-1,2,4-triazole-3-thiols (compounds **3**) in good yield. Reaction of compounds **3** with 2,4-dichloro- or 4-fluorobenzyl chloride in acetone in the presence of potassium carbonate gave the target compounds (compounds **4**) in about 70% yield. The antioxidant effect of the compounds on non-enzymatic lipid peroxidation of rat hepatic microsomal membranes was studied. Most of the examined compounds were active at concentration of 0·1 mM and most were found to prevent dimethylsulphoxide oxidation moderately (20–50%) at a concentration tenfold less than that of dimethylsulphoxide. The interaction of the synthesized compounds with 1,1-diphenyl-2-picrylhydrazyl stable free radical was also studied. Correlation was found between the two expressions of calculated lipophilicity, antioxidant activity and the lipophilicity of the synthesized compounds, and a correlation was derived between antioxidant activity and ΔlogP, which expresses the compounds' hydrogen-bonding capacity.

Derivatives of 1,2,4-4*H*-triazoles and their open-chain counterparts are a potentially important class of antiviral drugs (Wood et al 1985; Gabrielsen et al 1992; Todoulou et al 1994). Although their exact mechanism of action has not yet been fully elucidated it seems that open-chain thiosemicarbazones are metabolized to the cyclic substituted, triazoles or thiadiazoles (Jones et al 1965). Because we wanted to investigate the stereo-electronic requirements for optimum effectiveness of these triazole derivatives, we have already studied the synthesis of some new *N,N'*-disubstituted thioureas (Todoulou et al 1994).

In this paper we report the synthesis of some mercaptotriazole derivatives in an effort to determine the underlying structural requirements for antiviral activity. A preliminary antiviral study was performed and the involvement of these compounds in free-radical processes was investigated. It has been suggested that for some natural products, such as quercetin and rosemary extracts, antioxidant and antiviral effects might be related (Aruoma et al 1996a, b) and that hydrogen peroxide, a hydroxyl-radical precursor, can cause amplification of viral and endogenous gene sequences in mammalian cells. This might be yet another potential mechanism by which active oxygen species contribute to viral activation (Weitzman et al 1990). Finally, because lipophilicity is a physicochemical property that influences both the biological activity and the antioxidant

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potential of compounds (Ohkawa et al 1991), we calculated this property for the synthesized compounds and related their lipophilicity to the determined antioxidant activity. Furthermore, because ΔlogP values [$\Delta\log P = \log P_{SK(octanol)} - \log P_{SK(cyclohexane)}$] are indicative of the hydrogen-bonding capacity of a compound and the likelihood of penetration of the blood-brain barrier, we attempted to estimate these values and to investigate the possibility of correlation between ΔlogP and antioxidant activity for most of the synthesized compounds, in addition to determining their antioxidant properties.

Materials and Methods

Synthesis

Melting points were determined by use of a Buchi capillary apparatus and are uncorrected. Microanalysis was performed by Service Central de Microanalyse (CNRS), France; the results obtained were within 0·4% of the theoretical values. Fourier-transform proton magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AC 200 MHz spectrometer in DMSO-d_6 or $CDCl_3$ and are reported as chemical shift, δ, relative to tetramethylsilane as internal standard.

*4-(2,4-Dichlorophenyl)-1-[4-(4-nitrophenyl)piperazineacetyl]thiosemicarbazide (**2a**).* A solution of *N*-(4-nitrophenyl)piperazineacetohydrazide (27·9 g, 0·1 mol) in absolute EtOH (660 mL) was treated with 2,4-dichlorophenylisothiocynate (24 g, 0·1 mol). The reaction mixture was heated under reflux for 30 min and then concentrated in-vacuo to give **2a** (43·8 g, 0·09 mol). Yield 91%; mp 190–192°C (EtOH); Anal. C, H, N ($C_{19}H_{20}Cl_2N_6O_3S$).

^{1}H NMR (200 MHz) DMSO-d_6 δ: 2·10–2·45 and 3·05–3·25 (2m, 10H, piperazine H and N-CH_2), 6·95 (d, 2H, m– to NO_2, J = 9·3), 7·50–7·75 (m, 2H, ArH), 7·90 (d, 1H, *o*– to Cls, J = 2·1), 8·01 (d, 2H, *o*– to NO_2, J = 9·3), 9·41, 9·79, 10·05 (3 Br s, 3H, 3 × NH).

The same method was followed in preparing compounds **2b–2d**.

*4-(2,4-Dichlorophenyl)-1-[4-(4-methoxyphenyl)-piperazineacetyl]semicarbazide (**2b**).* Yield 90%; mp 181–182°C (EtOH); Anal. C, H, N ($C_{19}H_{23}Cl_2N_5O_2S$).

^{1}H NMR (200 MHz) DMSO-d_6 δ: 2·55–2·80 and 2·90–3·10 (m, 8H, piperazine H), 3·13 (s, 2H, NCH_2), 3·66 (s, 3H, OCH_3), 6·70–6·94 (m, 4H, ArH), 7·30–7·50 (m, 2H, ArH), 7·60–7·71 (m, 1H, ArH), 9·41, 9·79, 10·05 (3 br s, 3H, 3 × NH).

*4-(2,4-Dichlorophenyl)-1-[4-(2-methoxyphenyl)-piperazineacetyl]thiosemicarbazide (**2c**).* Yield 87%; mp 178–180°C (EtOH); Anal. C, H, N ($C_{19}H_{23}Cl_2N_5O_2S$).

^{1}H NMR (200 MHz) DMSO-d_6 δ: 2·55–2·80 and 2·87–3·07 (m, 8H, piperazine H), 3·13 (s, 2H, NCH_2), 3·75 (s, 3H, OCH_3), 6·80–7·00 (m, 4H, ArH), 7·30–7·52 (m, 2H, ArH), 7·60–7·70 (m, 1H, ArH), 9·41, 9·80, 10·05 (3 br s, 3H, 3 × NH).

*4-(2,4-Dichlorophenyl)-1-(4-phenylpiperazine-acetyl)thiosemicarbazide (**2d**).* Yield 85%; mp 184–186°C (EtOH); Anal. C, H, N ($C_{19}H_{21}Cl_2N_5OS$).

^{1}H NMR (200 MHz) DMSO-d_6 δ: 2·55–2·75 and 2·95–3·20 (m, 10H, piperazine H and NCH_2), 6·65–6·80 (m, 1H, ArH), 6·85–6·95 (m, 2H, ArH), 7·10–7·25 (m, 2H, ArH), 7·31–7·47 (m, 2H, ArH), 7·62–7·72 (m, 1H, ArH), 9·41, 9·81, 10·05 (3 br s, 3H, 3 × NH).

*4-(2,4-Dichlorophenyl)-5-[[4-(4-nitrophenyl-1-piperazinyl]methyl]-4H-1,2,4-triazole-3-thiol (**3a**).* 4-(2,4-Dichlorophenyl)-1-[4-(4-nitrophenyl)piperazine-acetyl]thiosemicarbazide (**2a**) (43·63 g, 0·1 mol) was heated under reflux with NaOH (1 N; 25 mL) for 8 h. The cooled reaction mixture was neutralized with 10% CH_3COOH and the precipitated solid was filtered to give **3a** (42 g, 0·09 mol). Yield 90%; mp 246–248°C (EtOH); Anal. C, H, N ($C_{19}H_{18}Cl_2N_6O_2S$).

^{1}H NMR (200 MHz) DMSO-d_6 δ: 2·10–2·45 and 3·05–3·25 (2m, 8H, piperazine H), 3·27, 3·35, 3·44, 3·51 (AB quartet, N-CH_2, J = 14·3), 6·95 (d, 2H, *m*– to NO_2, J = 9·3), 7·50–7·75 (m, 2H, ArH), 7·90 (d, 1H, *o*– to Cls, J = 2·1), 8·01 (d, 2H, *o*– to NO_2, J = 9·3).

The same method was followed in preparing **3b–3d**.

*4-(2,4-Dichlorophenyl)-5-[[4-(4-methoxyphenyl-1-piperazinyl]methyl]-4H-1,2,4-triazole-3-thiol (**3b**).* Yield, 92%; mp 210–212°C (EtOH); Anal. C, H, N $C_{20}H_{21}Cl_2N_5OS$).

^{1}H NMR (200 MHz) DMSO-d_6 δ: 2·20–2·45 and 2·60–2·90 (2m, 8H, piperazine H), 3·26, 3·33, 3·43, 3·50 (AB quartet, N-CH_2, J = 14·4), 3·65 (s, 3H, OCH_3), 6·70–6·83 (m, 4H, ArH), 7·55–7·70 (m, 2H, ArH), 7·89 (d, 1H, *o*– to Cls, J = 2·1).

*4-(2,4-Dichlorophenyl)-5-[[4-(2-methoxyphenyl)-1-piperazinyl]methyl]-4H-1,2,4-triazole-3-thiol (**3c**).* Yield, 90%; mp 239–240°C (EtOH); Anal. C, H, N ($C_{20}H_{21}Cl_2N_5OS$).

^{1}H NMR (200 MHz) DMSO-d_6 δ: 2·20–2·45 and 2·60–2·90 (2m, 8H, piperazine H), 3·30, 3·35, 3·45, 3·51 (AB quartet, N-CH_2, J = 14·3), 3·75 (s, 3H,

OCH_3), 6·70–7·00 (m, 4H, ArH), 7·55–7·70 (m, 2H, ArH), 7·91 (d, 1H, *o*– to Cls, J = 2·1), 13·9 (s, 1H, SH).

*4-(2,4-Dichlorophenyl)-5-[[(4-phenyl)-1-piperazinyl]methyl]-4H-1,2,4-triazole-3-thiol (**3d**).* Yield, 86%; mp 181–182°C (EtOH); Anal. C, H, N ($C_{19}H_{19}Cl_2N_5S$).

^{1}H NMR (200 MHz) DMSO-d_6 δ: 2·20–2·47 and 2·75–3·10 (2m, 8H, piperazine H), 3·50, 3·43, 3·33, 3·26 (AB quartet, N-CH_2, J = 14·3), 6·65–7·00 (m, 3H, ArH), 7·05–7·30 (m, 2H, ArH), 7·50–7·75 (m, 2H, ArH), 7·88 (d, 1H, *o*– to Cls, J = 2·1).

*3-(2,4-Dichlorobenzyl)thio-4-(2,4-dichlorophenyl)-5-[[4-(4-nitrophenyl)-1-piperazinyl]methyl]-4H-1,2,4-triazole (**4a**).* A mixture of 4-(2,4-dichlorophenyl)-5-[[4-(4-nitrophenyl-1-piperazinyl] methyl]-4*H*-1,2,4-triazole-3-thiol (**3a**) (4·65 g, 0·001 mol), K_2CO_3 (0·69 g, 0·005 mol) and 2,4-dichlorobenzylchloride (0·23 g, 0·001 mol) in anhydrous acetone (100 mL) was heated under reflux for 5 h. The cooled reaction mixture was filtered and the filtrates were evaporated in-vacuo. Water was added to the residue and extracted with ether. The organic layer was washed with water, dried with Na_2SO_4 and concentrated in-vacuo to give **4a** (4·49 g, 0·007 mol). Yield 72%; mp 172–173°C (EtOH); Anal. C, H, N, ($C_{26}H_{22}Cl_4N_6O_2S$).

^{1}H NMR (200 MHz) $CDCl_3$ δ: 2·40–2·58 and 3·10–3·40 (2m, 8H, piperazine H), 3·51, 3·58, 3·59, 3·66 (AB quartet, N-CH_2, J = 14·1), 4·30, 4·36, 4·42, 4·47 (AB quartet, S-CH_2, J = 15·8), 6·74 (d, 2H, *m*– to NO_2, J = 9·4), 6·82–7·10 (m, 3H, ArH), 7·15–7·40 (m, 2H, ArH), 7·51 (d, 1H, *o*– to Cls, J = 2·3). 8·06 (d, 2H, *o*– to NO_2, J = 9·4).

The same method was followed in preparing **4b–4h**.

*4-(2,4-Dichlorophenyl)-3-(4-fluorobenzyl)thio-5-[[4-(4-nitrophenyl)-1-piperazinyl]methyl]-4H-1,2,4-triazole (**4b**).* Yield 65%; mp 148–149°C (Et_2O-hexane); Anal. C, H, N ($C_{26}H_{23}Cl_2N_6O_2S$).

^{1}H NMR (200 MHz) $CDCl_3$ δ: 2·38–2·55 and 3·05–3·30 (2m, 8H, piperazine H), 3·44, 3·51, 3·52, 3·59 (AB quartet, N-CH_2, J = 14·0), 4·29, 4·35, 4·39, 4·46 (AB quartet, S-CH_2, J = 12·9), 6·75 (d, 2H, *m*– to NO_2, J = 9·4), 6·85–7·00 (m, 3H, ArH), 7·18–7·40 (m, 3H, ArH), 7·53 (d, 1H, *o*– to Cls, J = 2·3), 8·07 (d, 2H, *o*– to NO_2, J = 9·4).

*3-(2,4-Dichlorobenzyl)thio-4-(2,4-dichlorophenyl)-5-[[4-(4-methoxyphenyl)-1-piperazinyl]methyl]-4H-1,2,4-triazole (**4c**).* Yield 75%; mp 115–117°C (EtOH); Anal. C, H, N ($C_{27}H_{25}Cl_4N_5OS$).

^{1}H NMR (200 MHz) $CDCl_3$ δ: 2·35–2·52 and 2·67–2·95 (2m, 8H, piperazine H), 3·42, 3·49, 3·53, 3·60 (AB quartet, N-CH_2, J = 14·0), 3·73 (s, 3H, OCH_3), 4·37, 4·44, 4·47, 4·54 (AB quartet, S-CH_2, J = 13·5), 6·72–7·36 (m, 7H, ArH), 7·37–7·48 (m, 2H, ArH), 7·51 (d, 1H, *o*– to Cls, J = 2·2).

*4-(2,4-Dichlorophenyl)-3-(4-fluorobenzyl)thio-5-[[4-(4-methoxyphenyl)-1-piperazinyl]methyl]-4H-1,2,4-triazole (**4d**).* Yield 70%; mp 94–96°C (Et_2O-hexane); Anal. C, H, N ($C_{27}H_{26}Cl_2FN_5OS$).

^{1}H NMR (200 MHz) $CDCl_3$ δ: 2·35–2·52 and 2·70–2·95 (2m, 8H, piperazine H), 3·46, 3·49, 3·52, 3·59 (AB quartet, N-CH_2, J = 14·0), 3·72 (s, 3H, OCH_3), 4·33, 4·35, 4·38, 4·44 (AB quartet, S-CH_2, J = 13·0), 6·71–7·03 (m, 7H, ArH), 7·17–7·37 (m, 3H, ArH), 7·51 (d, 1H, *o*– to Cls, J = 2·2).

*3-(2,4-Dichlorobenzyl)thio-4-(2,4-dichlorophenyl)-5-[[4-(2-methoxyphenyl)-1-piperazinyl]methyl]-4H-1,2,4-triazole (**4e**).* Yield 78%; mp 142–144°C (EtOH); Anal. C, H, N ($C_{27}H_{25}Cl_4N_5OS$).

^{1}H NMR (200 MHz) $CDCl_3$ δ: 2·38–2·55 and 2·65–3·00 (2m, 8H, piperazine H), 3·42, 3·49, 3·54, 3·61 (AB quartet, N-CH_2, J = 14·1), 3·72 (s, 3H, OCH_3), 4·37, 4·44, 4·47, 4·53 (AB quartet, S-CH_2, J = 13·2), 6·76–7·05 (m, 5H, ArH), 7·12 (dd, 1H, ArH, J = 8·3, 2·1),), 7·20–7·45 (m, 3H, ArH), 7·52 (d, 1H, *o*– to Cls, J = 2·2).

*4-(2,4-Dichlorophenyl)-3-(4-fluorobenzyl)thio-5-[[4-(2-methoxyphenyl)-piperazinyl]methyl]-4H-1,2,4-triazole (**4f**).* Yield 72%; mp 96–98°C (Et_2O-hexane); Anal. C, H, N ($C_{27}H_{26}Cl_2FN_5OS$).

^{1}H NMR (200 MHz) $CDCl_3$ δ: 2·40–2·55 and 2·68–3·00 (2m, 8H, piperazine H), 3·42, 3·49, 3·53, 3·60 (AB quartet, N-CH_2, J = 14·0), 3·79 (s, 3H, OCH_3), 4·26, 4·33, 4·37, 4·44 (AB quartet, S-CH_2, J = 12·9), 6·72–7·00 (m, 7H, ArH), 7·15–7·35 (m, 3H, ArH), 7·51, (d, 1H, o-to Cl_2, J = 2·3).

*3-(2,4-Dichlorobenzyl)thio-4-(2,4-dichlorophenyl)-5-[(4-phenyl-piperazinyl)methyl]-4H-1,2,4-triazole (**4g**).* Yield 77%; mp 128–130°C (Et_2O-hexane); Anal. C, H, N ($C_{26}H_{23}Cl_4N_5S$).

^{1}H NMR (200 MHz) $CDCl_3$ δ: 2·35–2·55 and 2·85–3·10 (2m, 8H, piperazine H), 3·42, 3·50, 3·53, 3·60 (AB quartet, N-CH_2, J = 14·0), 4·38, 4·45, 4·48, 4·54 (AB quartet, S-CH_2, J = 13·3), 6·75–6·90 (m, 3H, ArH), 6·92–7·02 (m, 1H, ArH), 7·09–7·38 (m, 5H, ArH), 7·40–7·47 (m, 1H, ArH), 7·52 (d, 1H, *o*– to Cls, J = 2·2).

*4-(2,4-Dichlorophenyl)-3-(4-fluorobenzyl)thio-5-[(4-phenyl-piperazinyl)methyl]-4H-1,2,4-triazole (**4h**).* Yield 72%; mp 93–95°C (Et_2O-hexane); Anal. C, H, N ($C_{26}H_{24}Cl_2FN_5S$).

^{1}H NMR (200 MHz) $CDCl_3$ δ: 2·35–2·55 and 2·80–3·05 (2m, 8H, piperazine H), 3·45, 3·51, 3·53, 3·60 (AB quartet, N-CH_2, J = 14·4), 4·26, 4·33, 4·43, 4·50 (AB quartet, S-CH_2, J = 14·3), 6·75–7·00 (m, 6H, ArH), 7·15–7·44 (m, 5H, ArH), 7·52 (d, 1H, *o*– to Cls, J = 2·3).

Antiviral activity assays

Briefly, confluent cell cultures, MDCK, HeLa cell cultures were seeded in microtitre trays and inoculated with virus at a multiplicity of 20 CCID50 per well (where 1 CCID50 is the cell culture infective dose) in the presence of different concentrations of the test compounds. After incubation for five days, virus-induced cytopathicity was recorded. The concentration of compound required to inhibit the appearance of virus-induced cytopathicity by 50% was determined as the 50% effective concentration (EC50). Cytotoxicity of the test compounds was based on a microscopically detectable alteration of normal cell morphology, as described previously (De Clercq 1985).

Calculation of lipophilicity

The lipophilicity of the synthesized compounds was calculated according to the method based on the Leo-Hansch fragmental constant (ClogP; Leo (1993)) and the Suzuki/Kudo ($logP_{SK}$) atom-based procedure (Suzuki 1991).

In-vitro lipid peroxidation

Heat inactivated hepatic microsomal fraction from untreated male Fischer-344 rats, corresponding to 0·125 g liver mL^{-1} was used. Lipid peroxidation was induced by the Fe^{2+} (10 μM)-ascorbic acid (0·2 mM) system. The test compounds were dissolved in dimethylsulphoxide (DMSO) and added at various concentrations (100 μL) to the incubation mixture (final volume 4 mL in Tris-HCl-KCl 50 mM–150 mM buffer solution, pH 7·4). The mixture was incubated at 37°C for 45 min. Samples (0·3 mL) were taken at various time intervals and the extent of lipid peroxidation determined spectrophotometrically (535 nm against 600 nm) as the 2-thiobarbituric acid-reactive material (Rekka et al 1989). Antioxidants inhibit the production of malondialdehyde and, therefore, the colour produced after addition of 2-thiobarbituric acid is less intense. None of the compounds interfered with the assay, neither with the conjugation of 2-thiobarbituric acid-reactive material with 2-thiobarbituric acid or with the absorption at 535–600 nm. Each experiment was performed in triplicate.

Competition of the synthesized compounds with DMSO for hydroxyl radicals

The hydroxyl radicals generated by the Fe^{3+}-ascorbic acid system were detected by determination of formaldehyde produced on oxidation of DMSO (Klein et al 1981). The reaction mixture contained EDTA (0·1 mL), Fe^{3+} (167 μM, as a 1 : 2 mixture with EDTA), DMSO (33 mM), in phosphate buffer (50 mM, pH 7·4). The compounds tested were dissolved in 0·5% dimethylformamide (DMF) in phosphate buffer and added at a concentration of 2·5 mM (200 μL) to the reaction mixture (final volume 750 μL). The mixture was incubated at 37°C for 30 min. The reaction was stopped by addition of 250 μL trichloroacetic acid (17·5% w/v) and the formaldehyde formed was determined spectrophotometrically by the method of Nash (1953). DMF, at the final concentration used (0·13% v/v) was found not to interfere with the assay. Each experiment was performed at least in triplicate and the spread of the highest and lowest values was within 10%.

Interaction of the synthesized compounds with 1,1-diphenyl-2-picrylhydrazyl stable free radical (DPPH)

To a solution of DPPH (final concentration 200 μM) in absolute ethanol, an equal volume of the compounds dissolved in ethanol was added at a concentration of 200 μM. Ethanol was added to the control solution. After 20 min at room temperature absorbance was recorded at 517 nm (Kato et al 1988). Each experiment was performed in triplicate and the spread of the highest and lowest values was within 10%.

Results

The yields and analyses of the synthesized compounds are shown in Table 1.

In this study a correlation was derived between the two expressions used to calculate lipophilicity (Table 2) and between the antioxidant activity and the lipophilicity of the synthesized compounds. Furthermore, a correlation was derived between antioxidant activity and the ΔlogP values, derived from the subtraction logP(octanol) – logP(cyclohexane), which express the hydrogen-bonding capacity of the compounds (El Tayar et al 1991).

The in-vitro effect of the compounds on the non-enzymatic lipid peroxidation of rat hepatic microsomal membranes is shown in Figures 1 and 2. Most of the compounds examined were very active at a concentration of 0·1 mM and also at a concentration of 0·01 mM for 30 min. At a concentration of 0·01 mM for 45 min most of the compounds

Table 1. Yields, melting points and elemental analyses of the synthesized compounds.

Compound*	Yield (%)	Melting point (°C)	Elemental analysis					
			Calculated (%)			Found (%)		
			C	H	N	C	H	N
2a	91	190–192	47·21	4·17	17·39	46·90	4·53	17·01
2b	90	181–182	51·28	4·95	14·95	51·56	5·00	15·12
2c	87	178–180	51·28	4·95	14·95	50·88	5·15	15·16
2d	85	184–186	52·05	4·79	15·97	52·19	4·79	15·63
3a	90	246–248	49·04	3·90	18·06	48·82	3·95	17·66
3b	92	210–212	53·34	4·70	15·55	53·14	4·64	15·48
3c	90	239–240	53·34	4·70	15·55	53·19	4·66	15·15
3d	86	181–182	54·29	4·56	16·66	54·20	4·65	16·50
4a	72	172–173	50·02	3·55	13·46	49·78	3·31	13·32
4b	65	148–149	54·45	4·04	14·65	54·51	4·12	14·73
4c	75	115–117	53·22	4·14	11·49	53·23	4·11	11·55
4d	70	94–96	58·07	4·69	12·54	58·00	4·77	12·39
4e	78	142–144	53·22	4·44	11·49	53·15	3·95	11·44
4f	72	96–98	58·07	4·69	12·54	58·19	4·68	12·18
4g	77	128–130	53·90	4·00	12·09	53·75	3·84	11·93
4h	72	93–95	59·09	4·58	13·25	59·21	4·73	13·12

Table 2. ClogP, $logP_{SK}$, ΔlogP and logInh.LP (0·01 mM) values of the examined compounds.

Compound	ClogP	$logP_{SK\ (oct)}$	$logP_{SK(cyclohex)}$	ΔlogP	logInh.LP (0·01 mM)
3a	5·70	5·46	5·07	0·39	0·95
3b	5·61	6·54	6·22	0·32	1·62
3c	5·59	5·74	5·36	0·37	1·03
4a	9·21	8·74	8·56	0·18	1·50
4b	9·12	8·82	8·65	0·17	1·33
4c	9·10	9·02	8·85	0·16	1·80
4d	7·93	7·70	7·45	0·24	1·46
4e	7·84	7·78	7·45	0·32	1·09
4f	7·82	7·97	7·74	0·23	1·25

inhibited lipid peroxidation by approximately 70% compared with controls. The competition of the compounds with DMSO for hydroxyl radicals and their interaction with the stable free radical DPPH was only moderate. The results are shown in Table 3.

Discussion

Treatment of the *N*-(aryl)piperazineacetohydrazides, compounds **1**, with 2,4-dichlorophenylisothiocyanate gave the *N*-(aryl)piperazinethiosemicarbazides, compounds **2**, in good yield (Kiritsy et al 1978). The cyclization of these compounds after treatment with 1 M NaOH solution furnished the corresponding 5-(4-aryl-1-piperazinylmethyl)-4*H*-1,2,4-triazole-3-thiols, compounds **3**, in good yield (Habib et al 1989). Reaction of compounds **3** with 2,4-dichloro- or 4-fluorobenzyl chloride in acetone in the presence of potassium carbonate (Papadaki-Valiraki et al 1993) gave the final products, compounds **4**, in ca. 70% yield (Table 1). The reactions followed for the synthesis of the compounds are shown in Fig. 3.

In a preliminary test the antiviral activity of most of the synthesized compounds was very poor. Only for compound **3d** was the EC50 5-fold lower than the MTC. Compound **3c** was weakly active ($EC50 = 24\ \mu g\ mL^{-1}$) but the compound was selectively active ($MTC = 250\ \mu g\ mL^{-1}$) against influenza A_2 (H_2N_2).

The antioxidant potential of compounds **3a–3d** and **4a–4h** was studied using a non-enzymatic lipid peroxidation assay and rat liver microsomal membranes as substrate. This membrane is known to be readily peroxidized in this type of experiment (Rekka et al 1990). Compounds **3a–3d** afforded significant protection against lipid peroxidation at all concentrations used (1–0·1 mM). This property

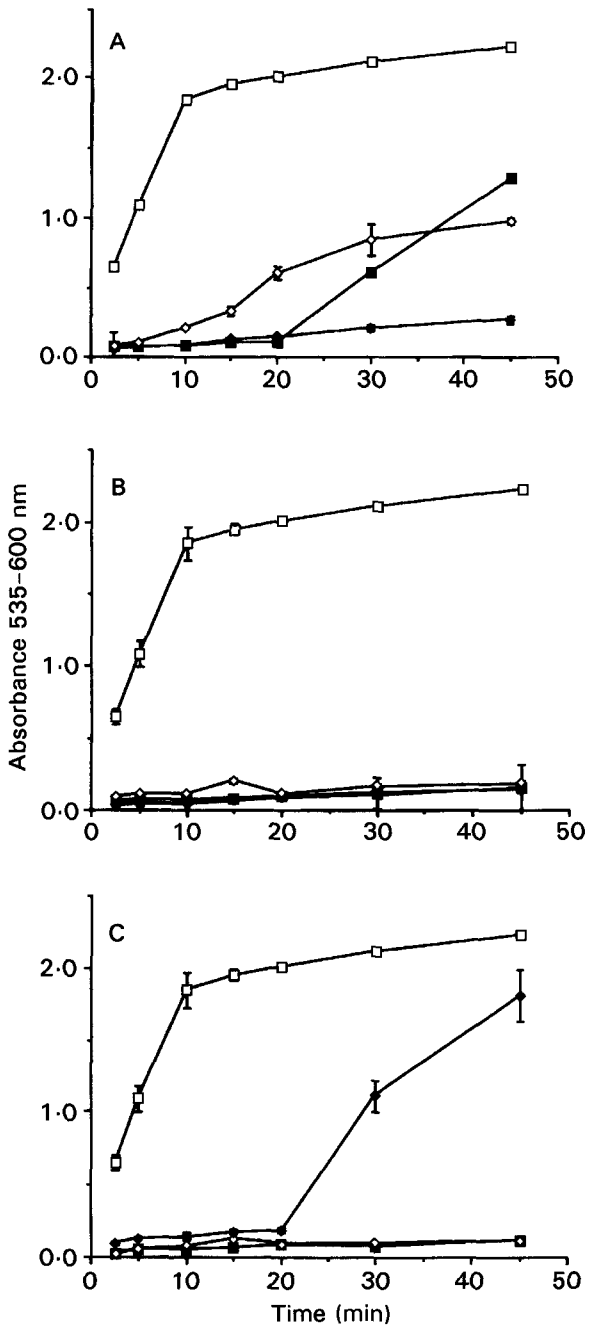

Figure 1. Effect of the compounds (0·1 mM) on lipid peroxidation as a function of time. A. □, control; ◆, compound **3a**; ■, compound **3b**; ◇, compound **3c**. B. □, control; ◆, compound **4a**; ■, compound **4b**; ◇, compound **4c**. C. □, control; ◆, compound **4d**; ■, compound **4e**; ◇, compound **4f**.

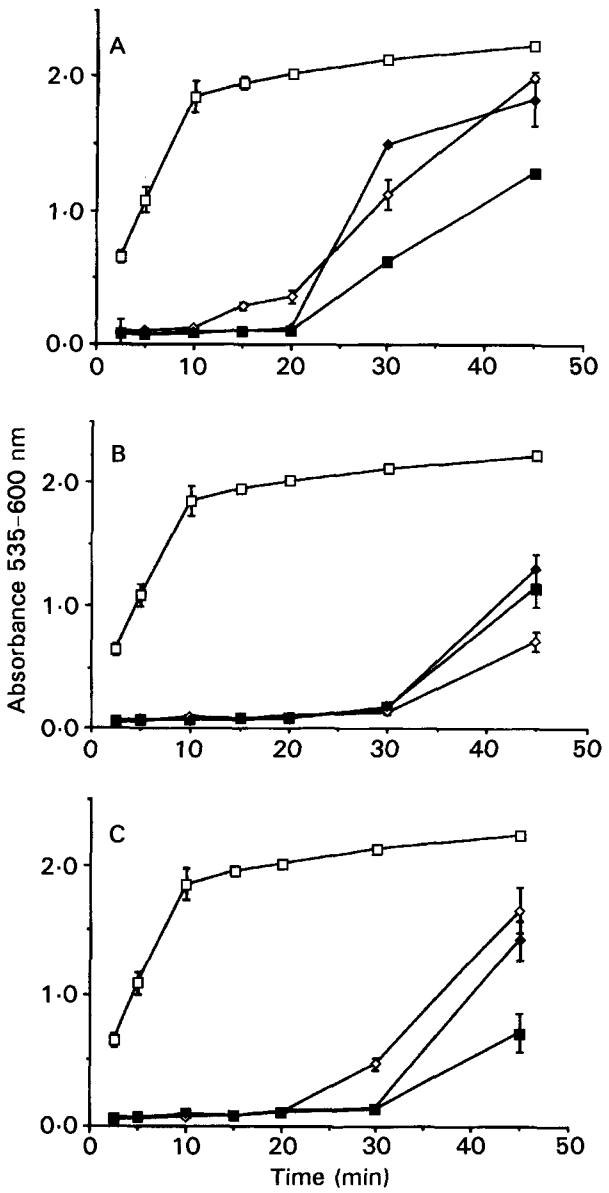

Figure 2. Effect of the compounds (0·01 mM) on lipid peroxidation as a function of time. A. □, control; ◆, compound **3a**; ■, compound **3b**; ◇, compound **3c**. B. □, control; ◆, compound **4a**; ■, compound **4b**; ◇, compound **4c**. C. □, control; ◆, compound **4d**; ■, compound **4e**; ◇, compound **4f**.

Table 3. Effect of the examined compounds on lipid peroxidation after 45-min incubation, $HO^{\bullet}$-mediated oxidation of dimethylsulphoxide (33 mM) and their interaction with 1,1-diphenyl-2-picrylhydrazyl stable free radical (200 μM).

Compound	**3a**	**3b**	**3c**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
Inhibition of lipid peroxidation by 1 mM compound (%)	90	96	96	89	97	94	93	93	95
Inhibition of lipid peroxidation by 0·5 mM compound (%)	88	96	85	95	95	90	26	95	95
Inhibition of $HO^{\bullet}$ scavenging activity by 2·5 mM compound (%)	45	39	44	41	27	56	28	22	48
Interaction with 1,1-diphenyl-2-picrylhydrazyl radical (% inhibition)	58	59	55	1	0	2	1	3	0

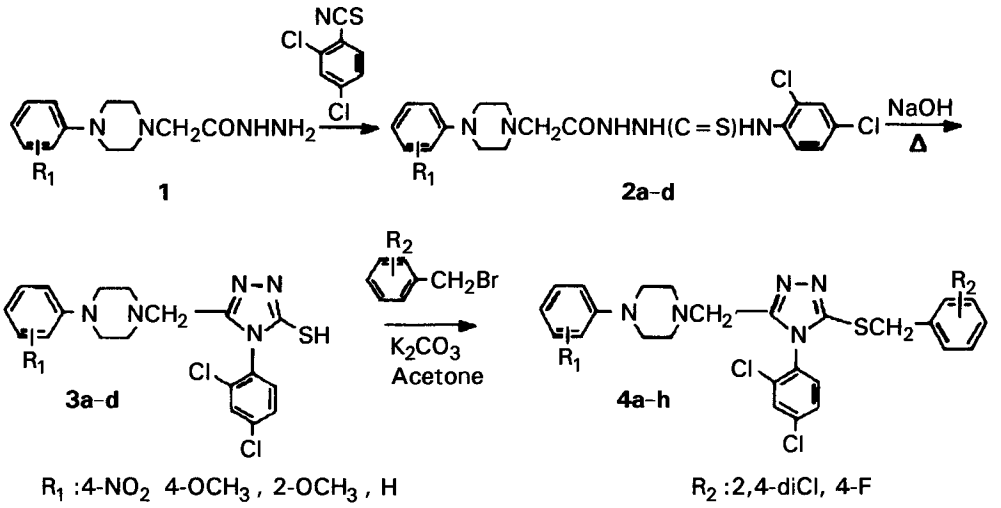

FIG. 3. The synthetic reactions and conditions used for the syntheses.

is attributed to the free thiol group of the triazole ring. Sulphydryl- containing compounds interfere with lipid peroxidation by more than one mechanism, e.g. by scavenging radicals or by elevating GSH levels (Wardman & von Sonntag 1995). Small differences between the protection against lipid peroxidation afforded by compounds **3** might be because of differences in lipophilicity. It is known that the lipophilic character of a compound can influence its antioxidant activity (Ohkawa et al 1991). Compounds **4** also had potent antioxidant activity, their activity at a concentration of 0·1 mM tending to be higher than that of compounds **3**. These compounds therefore afford effective protection of biological membranes.

From Figure 2 it is apparent that for all the compounds tested there was a lag period during lipid peroxidation, which accelerated after ca. 30 min. This is often observed with chain-breaking antioxidants which have easily-donated hydrogen atoms which can scavenge intermediate radicals, such as peroxy or alkoxy radicals (Halliwell 1990). In both series of compounds **3** and **4**, the higher the lipophilicity the greater the protective activity against lipid peroxidation. Because $HO^{\bullet}$ is one of the most potent oxidizing agents, which under certain conditions might be implicated in lipid peroxidation, and because hydrogen peroxide as a source of $HO^{\bullet}$ has been implicated in viral activation (Weitzman et al 1990), we attempted to investigate the ability of the synthesized compound to compete with DMSO for $HO^{\bullet}$. Most of the compounds were found to prevent DMSO oxidation (20 to 50%) when present at a concentration more than one-tenth that of the DMSO.

The interaction of the examined compounds with the stable free radical DPPH was also studied. This interaction is an indication of the reducing potency of compounds and indicates their ability to scavenge free radicals (Ratty et al 1988). All compounds **4** were inactive, which is not surprising because of the two groups of compounds only **3a–3c** have a free −SH group, a well known reducing group. Although it has been reported that the capacity to scavenge DPPH radicals can give an indication of inhibitory effect on lipid peroxidation (Rekka & Kourounakis 1991), such a correlation was obvious only for compounds **3a–3c** and not for compounds **4**.

Because there is often a linear correlation between lipophilicity and the antioxidant activity of some series of compounds (Ohkawa et al 1991), we calculated the lipophilicity of these compounds by two different theoretical methods using the fragmental-constant-based approach of Hansch and the Suzuki-Kudo atom-based procedure (Suzuki 1991; Leo 1993). As a measure of the lipophilicity of the compounds, we have also determined ΔlogP values, from $\log P_{SK(oct)}$ and $\log P_{SK(n\text{-}hex)}$.

The correlation equations obtained were:

$$\text{ClogP} = 1{\cdot}006(\pm 0{\cdot}019) + \log P_{SK} \quad (1)$$
$$n = 9,\ r = 0{\cdot}964,\ s = 0{\cdot}435,\ F = 93{\cdot}91$$

$$\log I_{(0{\cdot}01\ \text{mmol L}^{-1})} = 0{\cdot}167(\pm 0{\cdot}008)\text{ClogP} \quad (2)$$
$$n = 8,\ r = 0{\cdot}805,\ s = 0{\cdot}18,\ F = 11{\cdot}080$$

$$\log I_{(0{\cdot}01\ \text{mmol L}^{-1})} = -2{\cdot}711(\pm 0{\cdot}609)\Delta\text{logP} + 1{\cdot}999(\pm 0{\cdot}165) \quad (3)$$
$$n = 8,\ r = 0{\cdot}876,\ s = 0{\cdot}147,\ F = 19{\cdot}83$$

From equation 1 it is apparent that there is good correlation between values obtained from two different theoretical methods. For all three equations correlations were derived without data for compound **3b**, which was an outlier.

Our results have shown that all the compounds were very lipophilic; equation 2 demonstrates a trend of correlation between lipophilicity and antioxidant activity. However, the hydrogen-bonding capacity of a compound, expressed as ΔlogP values, has a greater influence on the antioxidant activity of the test compounds. The negative sign of ΔlogP in equation 3 demonstrates the inverse relationship between inhibition of lipid peroxidation and hydrogen-bond development, further supporting earlier reports of the relationship between inhibition of lipid peroxidation and lipophilicity, because hydrogen-bond formation is a measure of the polarity of molecules. It has been reported that small ΔlogP values are indicative of the capacity of compounds to cross the blood–brain barrier (El Tayar et al 1991). Because viral infections of the CNS are frequent, this information is valuable for prospective antiviral agents. The calculation of a parameter that expresses the hydrogen-bonding capacity of prospective antiviral compounds might be proved useful in determining the ability of such compounds to pass the blood–brain barrier effectively and thus prove useful at combating viral infections of the CNS.

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