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Scavenging of Free-radicals and Inhibition of Lipid Peroxidation by 3-Phenylsydnone

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Abstract

The antioxidant properties of 3-phenylsydnone were studied in various models in-vitro.

3-Phenylsydnone scavenged the stable free radical, 1,1-diphenyl-2-picrylhydrazyl, and inhibited the degradation of deoxyribose mediated by hydroxyl radicals, although, to a lesser extent than trolox, a water soluble analogue of vitamin E. Many antioxidants possess pro-oxidant properties due to their ability to reduce ferric ions; however, 3-phenylsydnone was free from pro-oxidant properties, and also inhibited the lipid peroxidation induced by iron, in rat brain homogenates.

Recently we reported sydnonylchalcones (Fig. 1) as a novel class of antitumour agents with antioxidant properties (Ruby et al 1994). These compounds were found to inhibit lipid peroxidation and were able to scavenge free radicals. To understand the mechanism of action, we were interested to know whether the sydnone moiety or the chalcone moiety was responsible for the antioxidant activities. Although a number of compounds containing chalcone or related styryl ketone moieties are reported to be free-radical scavengers (Lovina et al 1990; Rajkumar & Rao 1993), we have not come across any report on the antioxidant properties of sydnones. Sydnones are a novel class of mesionic compounds containing the 1,2,3-oxadiazole ring system. We were prompted to investigate the antioxidant properties of a model compound 3-phenylsydnone (Fig. 1). The present study shows that this compound has appreciable free-radical scavenging activity and is also able to inhibit iron-induced lipid peroxidation.

Materials and Methods

Reduction of the stable free radical, 1,1-diphenyl-2-picryl-hydrazyl (DPPH)

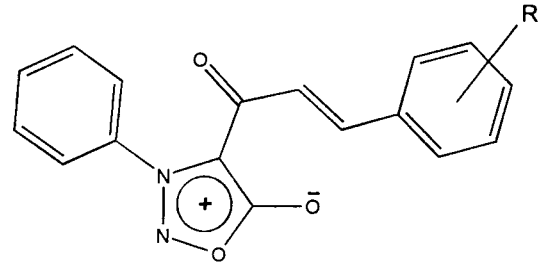
Solutions of various concentrations of 3-phenylsydnone in 95% ethanol were added to DPPH (0·1 mM) in ethanol. After 20 min, the absorbance at 517 nm was measured (Kato et al 1988). The difference in the absorbance between test and the control was taken and expressed as percent reduction of the DPPH radical. Experiments were performed in triplicate.

Effect on deoxyribose degradation by iron-dependent hydroxyl radicals

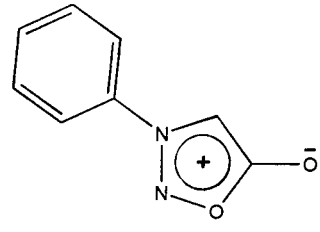
Reaction mixtures contained, in a final volume of 1 mL, the following reagents at the final concentrations stated: deoxyribose (2·8 mM), KH_2PO_4-KOH buffer, pH 7·4 (20 mM), $FeCl_3$ (0·1 mM), EDTA (0·1 mM), H_2O_2 (1 mM), ascorbate (0·1 mM) and test compound. The reaction mixture was incubated for 1 h at 37°C. The degradation of deoxyribose was estimated as thiobarbituric acid-reactive substances (TBARS) by measuring the absorbance at 532 nm as described by us earlier (Rajkumar & Rao 1993). Experiments were performed in triplicate.

Effect on lipid peroxidation induced by iron in rat brain homogenates

Rat brain homogenate 10% (w/v) was prepared in 0·15 M KCl and centrifuged at 800 *g* for 10 min. The supernatant was used for the study of lipid peroxidation induced by iron as described by us earlier (Sreejayan & Rao 1994). Briefly, lipid peroxidation was initiated by adding ferrous sulphate



Sydnonylchalcones



3-Phenylsydnone

FIG. 1. Structures of compounds used.

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Table 1. Reduction of stable free radical, 1,1-diphenyl-2-picrylhydrazyl by 3-phenylsydnone.

3-Phenylsydnone (μM)	Reduction (% ± s.d.)[a]
5	14·3 ± 0·3
25	39·5 ± 0·7
50	48·8 ± 0·5
250	90·7 ± 0·9

[a] Reduction of 1,1-diphenyl-2-picrylhydrazyl (100 μM) by 3-phenylsydnone was estimated in ethanolic solution at 517 nm. Percent reduction was calculated by comparing test with control and expressed as mean ± s.d. (n = 3).

(100 mM) or ferric chloride (100 μM) to the reaction mixture containing 0·5 mL brain homogenate, KCl (0·15 M) and ethanol (10 μL) or test compound dissolved in ethanol, in a final volume of 1·5 mL. The reaction mixture was incubated for 20 min at 37°C. The amount of TBARS formed was estimated as described earlier. A correction was made in both test and control for spontaneous peroxidation, by conducting experiments without the inducing agents (iron). Percent inhibition was calculated by comparing control with test experiments and expressed as means ± s.d. of triplicate experiments.

Results and Discussion

Table 1 gives the effect of 3-phenylsydnone on the reduction of DPPH. The reduction was found to be concentration-dependent. At 250 μM, the reduction was 90%. Thus 3-phenylsydnone scavenges DPPH to a significant extent.

The effect of 3-phenylsydnone on scavenging hydroxyl radicals was measured by studying the competition between 3-phenylsydnone and deoxyribose for the hydroxyl radical generated from the ferric-ascorbate-EDTA-H_2O_2 system. The hydroxyl radical attacks deoxyribose and sets off a series of reactions that eventually result in TBARS formation. When a molecule scavenges a hydroxyl radical, it decreases TBARS formation. Table 2 shows the inhibition of TBARS formed in the presence of test compounds. 3-Phenylsydnone showed concentration-dependent inhibition, but was less active than trolox, a water-soluble derivative of vitamin E, used as a standard drug for comparison. The reaction mixture containing ferric-ascorbate-EDTA-H_2O_2, generates hydroxyl radicals at a rapid rate, but, in the absence of ascorbate, generation is very slow. Such a system is useful in identifying compounds capable of accelerating hydroxyl radical formation (Laughton et al 1989). 3-Phenylsydnone was tested in such a system (Table 3). The formation of hydroxyl radical was not accelerated when 3-phenylsydnone was added to the reaction mixture which did not contain ascorbate, but 3-phenylsydnone further reduced the hydroxyl radical generated. Many phenolic antioxidants are known to act as pro-oxidants in such a system (Laughton et al 1989). Thus 3-phenylsydnone differs from the classical phenolic antioxidants, since it is free from a pro-oxidant effect. In the absence of EDTA, deoxyribose undergoes site-specific degradation, when the hydroxyl radical is generated from the ferric-ascorbate-H_2O_2 system (Halliwell 1990). Iron binds directly to deoxyribose in the absence of chelator (EDTA) to cause site-specific degradation. Hence, the molecules that can inhibit deoxyribose degradation in the absence of EDTA are those that are capable of chelating iron, thus rendering it inactive or poorly active in the Fenton reaction. The effect of 3-phenylsydnone on the degradation of deoxyribose was tested in such a system (Table 3); 3-phenylsydnone was inactive in such a system indicating that it is a poor complexing agent.

Both ferrous and ferric ions induce lipid peroxidation through various mechanisms involving reactive oxygen species (Braughler et al 1986). Most of the antioxidants inhibit iron-induced lipid peroxidation (Rajkumar & Rao 1993; Sreejayan & Rao 1994). Hence, 3-phenylsydnone was

Table 2. Effect of 3-phenylsydnone and trolox on the degradation of deoxyribose mediated by hydroxyl radicals.

Drug	Inhibition of TBARS % (± s.d.)[a]			
	5 μM	10 μM	50 μM	100 μM
3-Phenylsydnone	0	24·2 ± 0·8	42·3 ± 1·0	64·9 ± 1·1
Trolox	25·4 ± 0·6	65·1 ± 1·0	71·3 ± 1·4	79·8 ± 1·7

[a] Reaction mixture containing deoxyribose (2·8 mM), KH_2PO_4-KOH buffer, pH 7·4 (20 mM), $FeCl_3$ (0·1 mM), EDTA (0·1 mM), H_2O_2 (1 mM), ascorbate (0·1 mM) and test compound, in a final volume of 1 mL was incubated at 37°C for 1 h. The degradation of deoxyribose was estimated as TBARS by measuring absorbance at 532 nm. Percent inhibition was calculated by comparing the amount of TBARS formed in test with control and expressed as mean ± s.d. (n = 3).

Table 3. Effect of 3-phenylsydnone on the hydroxyl radical-induced degradation of deoxyribose in the presence and absence of EDTA and ascorbic acid.

Reaction	A 532
Fe^{3+} + H_2O_2 + EDTA + ascorbate	0·131
+ phenylsydnone	0·046
Fe^{3+} + H_2O_2 + EDTA	0·070
+ phenylsydnone	0·025
Fe^{3+} + H_2O_2 + ascorbate	0·044
+ phenylsydnone	0·040

Table 4. Effect of 3-phenylsydnone and trolox on the lipid peroxidation induced by ferrous ions in rat brain homogenates[a].

Drug	Inhibition % (± s.d.)		
	5 μM	10 μM	25 μM
3-Phenylsydnone	48·1 ± 1·1	81·8 ± 0·8	87·5 ± 1·0
Trolox	51·3 ± 0·9	82·7 ± 0·8	84·9 ± 0·7

[a] The lipid peroxidation was stimulated by the addition of ferrous sulphate (100 μM) to a reaction mixture containing test compound, rat brain homogenate (0·5 mL, 10% w/v) and KCl (0·15 M) in a final volume of 1·5 mL. The reaction was stopped after 20 min. The amount of lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS). Percent inhibition was calculated by comparison with control experiments without test compounds and expressed as mean ± s.d. (n = 3).

Table 5. Effect of 3-phenylsydnone and trolox on the lipid peroxidation induced by ferrous ions in rat brain homogenate[a].

Drug	Inhibition % (± s.d.)		
	10 μM	25 μM	100 μM
Phenylsydnone	28·4 ± 0·2	33·2 ± 0·4	92·6 ± 1·1
Trolox	22·6 ± 0·2	71·5 ± 0·7	87·8 ± 1·0

[a] The lipid peroxidation was stimulated by the addition of ferric chloride (100 μM). Inhibition was measured as in Table 4.

tested for its effect on the lipid peroxidation induced by ferrous ions (Table 4) and ferric ions (Table 5) in rat brain homogenates. For the experiment with ferrous ion (Table 4), 3-phenylsydnone was very potent and showed concentration-dependent inhibition of peroxidation. Its potency was similar to the standard antioxidant, trolox. For the ferric ion 3-phenylsydnone was marginally more active than trolox at 10 and 100 μM, but at 25 μM, trolox was more active.

The present study demonstrates that 3-phenylsydnone is an efficient scavenger of free radicals and an inhibitor of iron-induced lipid peroxidation. It is capable of scavenging both nitrogen-centred free radicals such as DPPH and oxygen-centred free radicals such as hydroxyl radicals. Many antioxidants similar to ascorbate and phenolic compounds possess pro-oxidant properties since they enable redox cycling of ferric to ferrous ions so as to maintain a supply of electrons for the Fenton reaction (Laughton et al 1989). Lactic acid is also reported to accelerate lipid peroxidation due to its ability to reduce ferric to ferrous ions (Fauconneau et al 1993). However, 3-phenylsydnone is free from such pro-oxidant properties, since it is unable to reduce ferric ions.

Thus, 3-phenylsydnone is considered to be a novel, non-phenolic antioxidant and the antioxidant properties shown by the antitumour agents, sydnonylchalcones, may also be due to the sydnone nucleus.

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