Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 2738–2744
Bioorganic & Medicinal Chemistry Letters

# Antioxidant and antiproliferative activity of curcumin semicarbazone

Sabari Dutta,[a,*,†] Subhash Padhye,[a,*] K. Indira Priyadarsini[b] and Chris Newton[c]

[a]*Department of Chemistry, University of Pune, Pune 411 007, India*
[b]*Radiation Chemistry and Chemical Division, Bhabha Atomic Research Center, Trombay, Mumbai 400 085, India*
[c]*Department of Medicine, University of Hull, Hull, HU6 7RX, UK*

Received 14 November 2004; revised 31 March 2005; accepted 1 April 2005
Available online 4 May 2005

**Abstract**—A new semicarbazone derivative of curcumin (CRSC) was synthesized and examined for its antioxidant, antiproliferative, and antiradical activity and compared with those of curcumin (CR). The antioxidant activity was tested by their ability to inhibit radiation induced lipid peroxidation in rat liver microsomes. The antiproliferative activity was tested by studying the in vitro activity of CRSC against estrogen dependant breast cancer cell line MCF-7. Kinetics of reaction of (2,2′-diphenyl-1-picrylhydrazide) DPPH, a stable hydrogen abstracting free radical was studied to measure the antiradical activity using stopped-flow spectrophotometer. Finally one-electron oxidized radicals of CRSC were generated and characterized by pulse radiolysis. The results suggest that the probable site of attack for CRSC is both the phenolic OH and the imine carbonyl position. CRSC shows efficient antioxidant and antiproliferative activity although its antiradical activity is less than that of CR.


## 1. Introduction

Curcumin, 1,7-bis(4-hydroxy-3-methoxy phenyl)-1,6-heptadione-3,5-dione, or diferuloyl methane (CR), is a yellow pigment obtainable from the rhizomes of the plant *Curcuma longa*,[1] which is one of the major spices used in the Indian culinary practices. It is considered to be a safe phytochemical without having any toxic, genotoxic, and teratogenic properties even at high doses.[2] There is compelling evidence that curcumin has cancer chemopreventive properties in a range of animal models of chemical carcinogenesis.[3] The compound has antioxidative and antiinflammatory properties, and several mechanisms have been proposed by which it might block initiation and progression of cancer. Loo and co-workers have proposed that $H_2O_2$ is the indirect DNA damaging agent in curcumin treated Jurkat T-lymphocytes. After the uptake of curcumin, $H_2O_2$ is generated intracellularly causing cell death due to its stressful and cytotoxic effects.[4] Curcumin has also been shown to induce mitochondrial swelling, calcium release, respiration impairment, and collapse of mitochondrial membrane potential, which are events related to pore opening. The formation of a pore, called the permeability transition pore (PTP) could lead to cell death by releasing apoptogenic factors from mitochondria.[5] Morin et al. have shown that curcumin is able to inhibit the $Ca^{2+}$ induction of PTP and this effect is related to its antioxidant properties, as it inhibits both superoxide anion production and lipid peroxidation.[6] Thus curcumin has a dual effect, inducing PTP despite its antioxidant properties.

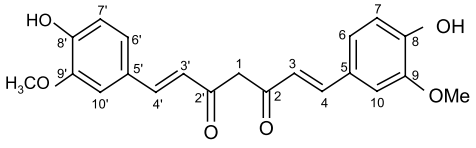
The remarkable antioxidant properties of curcumin[7] are thought to arise from the hydroxyl groups in the aromatic side chains or from the $CH_2$ group of the β-diketone moiety.[8] Jovanoic et al. have indicated that hydrogen abstraction from the methylene $CH_2$ group is responsible for the remarkable antioxidant activity of curcumin.[9] Recently, Priyadarsini et al. have confirmed that phenolic OH is mainly responsible for the activity of curcumin.[10] Attempts have been made wherein the phenolic OH is blocked on both the rings keeping the β-diketone moiety intact.[11] However, modifications

*Keywords*: Curcumin semicarbazone; Pulse radiolysis; Antioxidant activity; Lipid peroxidation; Antiproliferative activity.
* Corresponding authors. Tel.: +1 313 993 9113; fax: +1 313 577 2765 (S.D.); tel.: +91 20 5601227; fax: +91 20 5691728 (S.P.); e-mail addresses: sdutta@med.wayne.edu; sbpadhye@chem.unipune.ernet.in
† Present address: Department of Biochemistry and Molecular Biology, School of Medicine, Wayne State University, Detroit, MI 48201, USA.

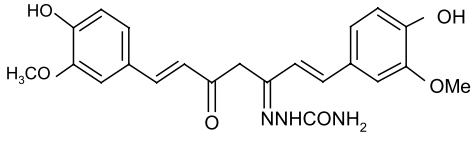
0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.04.001

in the β-diketone are less explored except where this moiety is modified with hydrazide[12] and cyclohexanone groups.[6] Preliminary studies in appending the β-carbonyl functionality in curcumin with known pharmacophore semicarbazone by us have shown to enhance its antioxidant potential.[13]

Morin et al.[6] had earlier proposed that during mitochondrial respiration curcumin interacts with $O_2^{\cdot -}$ to give rise to a radical that is toxic for the mitochondria. Thus in the present communication we have investigated the interactions of curcumin semicarbazone (CRSC) with specific one electron oxidants such as azide ($N_3^{\cdot}$), methyl and halocarbonperoxyl radicals employing the pulse radiolytic technique and the stopped-flow method involving DPPH radicals. The effect of the modified curcuminoid is also examined on the lipid peroxidation induced by γ-radiation in microsomes and the results are compared with those on curcumin. Thus we propose that the ability of a mimetic compound to interact with one electron oxidants, should also exhibit antiproliferative activity. The compounds CR and CRSC were therefore, tested against MCF-7 breast cancer cell lines wherein CRSC exhibits enhanced antiproliferative activity.




**Curcumin (CR)**




**Curcumin semicarbazone (CRSC)**

The synthesis and physical measurements have been published earlier.[13] The compositional analysis along with the $^1$H NMR and electrochemical data are summarized in Table 1. Our earlier studies on the superoxide scavenging abilities of CR and CRSC generated by $KO_2$–DMSO employing the nitroblue tetrazolium assay (NBT) assay had shown that the latter was 10-fold more effective in the radical scavenging activity.[13] Radiolysis studies generating specific radicals were therefore, undertaken to explore the molecular mechanism of such antioxidant action.

## 2. Inhibition of γ-radiation induced lipid peroxidation by CRSC

Lipid peroxidation (estimated as TBARS) in microsomes induced by γ-radiation at pH 7.4 saturated with nitrous oxide and air was studied both in absence and presence of CRSC and compared with the normal microsomes exposed to the same amount of radiation (Fig. 1). The formation of TBARS in the normal microsomes increases in a radiation dose dependant manner from 136 to 408 Gy. In the presence of CRSC the TBARS formation is inhibited significantly at almost all doses, indicating its value as a protector of lipid peroxidation through its interaction with radical species. At 10 μM concentration the extent of inhibition varied from 85% to 54%, while CR showed about 80–60% protection when the absorbed dose was varied from 136 to 408 Gy and is similar to that reported according to the reference.[10] This indicates that CRSC has the potential to act as an antioxidant and modification of the β-diketonic moiety does not alter its antioxidant activity. Since free radical scavenging is responsible for the antioxidant action of curcumin, even in CRSC it is worth examining the free radical reactions, which are presented below.[12,14]

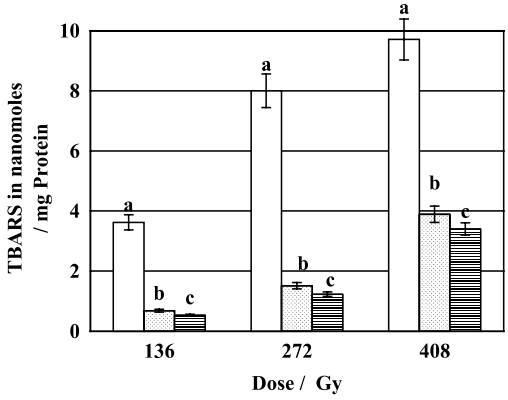



**Figure 1.** Effects of CUR and CURSC on ·OH induced lipid peroxidation. Microsomes in $N_2O$-purged buffer (pH 7.4) were exposed to different doses using $^{60}$Co γ-source (a) normal microsomes, (b) in presence of 10 μM of CR, (c) in presence of 10 μM of CRSC.

**Table 1.** Analytical and $^1$H NMR data for CR and CRSC

| Compound | % Analysis | | $^1$H NMR (ppm) | | | Potentials/V |
|---|---|---|---|---|---|---|
| | %C (Obsd) | %H (Obsd) | NH | $NH_2$ | 1-H | |
| CR | 68.47 | 5.47 | Ab* | Ab* | 5.8 | 0.25, 0.53, and −1.02 |
| $C_{21}H_{20}O_6$ | (67.98) | (5.21) | | | | |
| CRSC | 62.11 | 5.45 | 9.6 | 1.3 | 5.9 | 0.28, 0.57, and −1.14 |
| $C_{22}H_{23}N_3O_6$ | (62.66) | (5.33) | | | | |

Ab* = absent.

### 3. Pulse radiolysis studies

The nature of the interaction of CRSC with small radicals that can cause both electron transfer and H atom transfer processes was probed further using nanosecond pulse radiolysis technique. The radicals selected for the purpose were azide, methyl, and the halocarbon peroxyl radicals. While azide radicals react by specific electron transfer, methyl and halocarbon peroxyl radicals react by H atom transfer. The rate constants and the resultant transient spectra and kinetics on reaction of these radicals with CRSC were determined and compared with those for curcumin. The interaction of the azide radicals with CRSC produced a transient absorbing in the region 300–600 nm with a strong absorption maximum at 405 nm (Fig. 2) and another weak and broad absorption with maximum around 495–500 nm region. Curcumin reacts with $N_3^{\bullet}$ radicals to give a strong absorption at 500 nm and relatively weak absorption at 310–340 nm and a simultaneous bleaching of the ground state absorption at 420 nm. The transient absorbing at 500 nm is assigned to the phenoxyl radical of CR formed by one electron oxidation followed by proton loss from phenolic OH group.[15] Comparing the transient spectrum from CRSC with that for CR, the strong 405 nm absorption in CRSC is attributed to the formation of radicals produced by the one-electron oxidation of the imine carbonyl of the semicarbazone moiety. Earlier Bordwell and Bausch have shown that $NH_2CO$ though an acceptor group, has radical stabilizing properties.[16] The weak band around 495–500 nm is attributed to the phenoxyl radical from the phenolic OH group.[15]

The reactions of methyl and halocarbon peroxyl radicals produced similar transients as observed with azide radical reactions. The rate constants of the reactions of methyl and halocarbon peroxyl radicals are summarized in Table 2. On the whole the reactions of all the radicals examined with both the curcuminoid compounds are found to be comparable within experimental limits. However, in case of CR the phenoxyl radical is the predominant species,[17] while in the case of CRSC it is the imine carbonyl radical, which is a major species being formed in this reaction (Scheme 1).

### 4. Interaction with DPPH radicals

DPPH is a stable free radical that can abstract hydrogen from any molecule having capacity to donate such atom, leading to bleaching of the strong absorption at 517 nm. The reaction can, therefore, be conveniently used to examine the radical scavenging ability of the prospective molecule.

The kinetics of DPPH decay was thus examined in the presence of CR and CRSC compounds at different

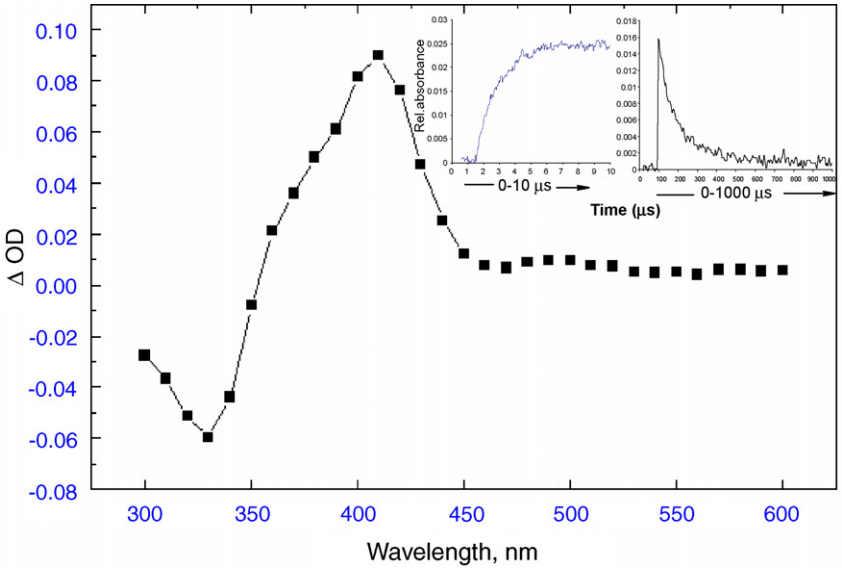
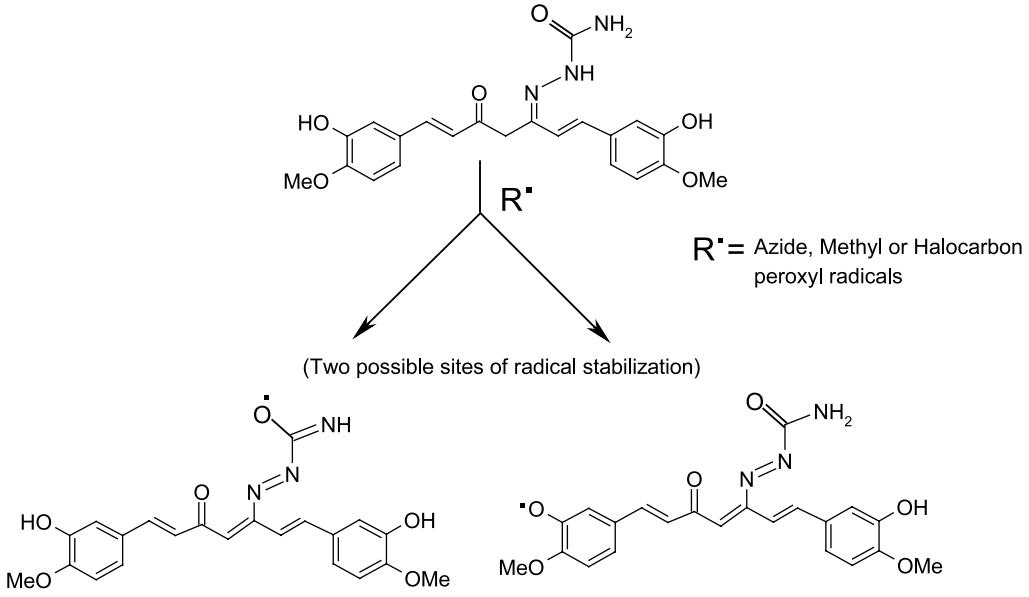



**Figure 2.** Transient spectra obtained by pulse radiolysis of $1.88 \times 10^{-4}$ M of CRSC in 10% acetonitrile–water saturated with $N_2O$, containing 0.01 mol $dm^{-3}$ sodium azide at pH 7. *Inset*: Time variation of absorption of CRSC derived phenoxyl radicals produced by azide radical reactions under similar conditions.

**Table 2.** Bimolecular rate constants ($M^{-1}$ $s^{-1}$) for the reactions of CR and CRSC with different radicals

| Reaction | CR | CRSC |
|---|---|---|
| Azide radical (10% acetonitrile, pH 7) | $9.4 \times 10^9$ | $3 \times 10^9$ |
| Methyl radical (40% DMSO, pH 5) | $3.5 \times 10^9$ | $2.4 \times 10^9$ |
| Halocarbon peroxyl radicals (48% isopropanol, 4% $CCl_4$, pH 7) | $1.0 \times 10^8$ | $1.6 \times 10^8$ |
| DPPH radicals (acetonitrile) | 1640 | 274 |




**Scheme 1.** The proposed reaction mechanism for radical formation in CRSC.

concentrations. In a typical kinetic run 25 μM of DPPH and 100 μM of CR or CRSC were mixed in a stopped-flow cell and the change in absorption at 517 nm was measured over a period of 200 s. Figure 3a and b shows the absorption–time plots for this reaction in absence and presence of CR and CRSC, respectively. It is observed that the reaction is much faster with CR as compared to CRSC compound. The rate constant for CR was found to be $1640 \pm 38\ M^{-1}\ s^{-1}$ and the reaction was completed within 10–20 s (Fig. 3a), while the reaction with CRSC was much slower under similar conditions (Fig. 3b) and took more than 200 s for completion of the reaction. The decay traces could be fitted to exponential function to obtain the observed rate constants ($k_{obs}$), which was followed as a function of CRSC concentration. From the linear dependency of $k_{obs}$ as a function of CRSC concentration (Fig. 3, inset) rate constant for the reaction of CRSC with DPPH was determined to be $274 \pm 7\ M^{-1}\ s^{-1}$. The difference in their rate constants is attributed to the steric hindrance by the bulky semicarbazide side chain and its higher reduction potential.

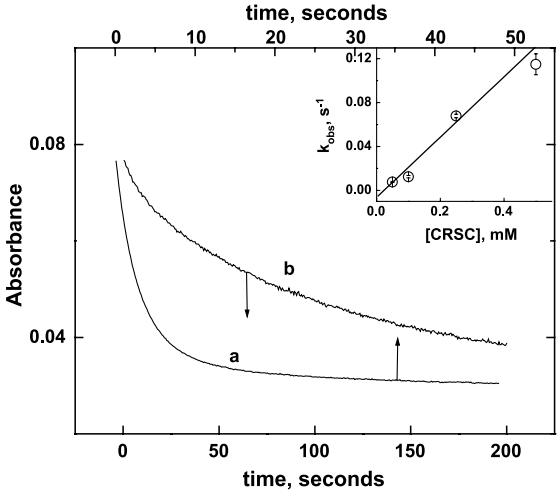



**Figure 3.** Absorption–time plot showing the decay of DPPH radical at 517 nm in presence (a) 100 μM curcumin and (b) in presence of 100 μM CRSC. Inset shows linear variation of observed rate constant as a function of CRSC concentration.

The semicarbazone is an electron withdrawing group and hence there is a slight increase in the oxidation potential of phenolic OH in CRSC as compared to CR. For CR the oxidation potential observed in DMSO solvent is +0.25 V, which increases to +0.28 V in case of CRSC. The peak at 0.53 V for CR and 0.57 V for CRSC correspond to the oxidation of $CH_2$ group of the β-diketo group. The peaks obtained at −1.02 V for CR is due to the reduction of the carbonyl oxygen, while the peak obtained at −1.14 V for CRSC correspond to the reduction of the amide group.[13]

The electrochemical data obtained on both the compounds (Table 1) indicates that CRSC is slightly more difficult to oxidize making H atom transfer to DPPH radical thermodynamically and kinetically unfavorable and thereby reducing the rate constant.

## 5. Anticancer activity

CRSC and CR were tested for their antiproliferative activities against estrogen dependant breast cancer cell line MCF-7. MCF-7 cells (5000/0/1 mL) were plated overnight removed from media and then exposed to 1 μg/mL concentration of the test compound for 72 h followed by determination of the cell viability by MTT method. Figure 4 represents the antiproliferative activities of both the compounds. The results indicate that CRSC is a potent antiproliferative compound with

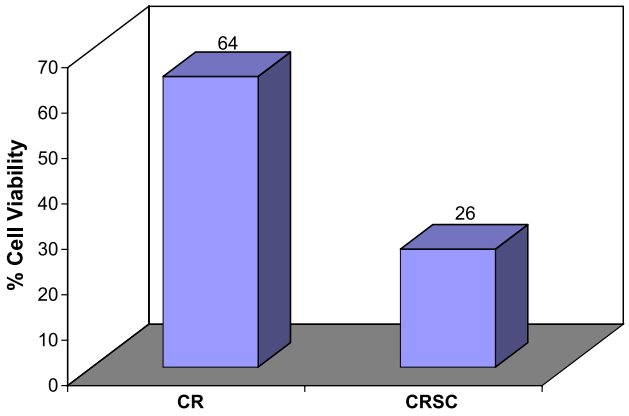



**Figure 4.** Effect of 1 μg/mL of CR and CRSC on breast cancer MCF-7 cells viability after 24 h of treatment. All determinations are expressed as percentage of the control (untreated cells).

26% cell viability as compared to CR (64%) (24 h). When compared to untreated cells, MCF-7 cells treated with curcumin for 24 h does not show any serious changes in their morphology. However, 48 h treatment with CR in general and 72 h treatment in particular, induce some morphological changes in the cell line. Holy[18] had also made a similar observation for CR in sulforhodamine B assay system on MCF-7 cells. However, no satisfactory explanation is presently available for this observation. The CRSC does not show such effects when compared to CR.

Present studies indicate that modification of the β-diketone functionality in CR serves to up-regulate its liposolubility and retain its antioxidant property and free radical scavenging ability as indicated by the results of lipid peroxidation and pulse radiolytic experiments. The pulse radiolysis studies obtained with $N_3$ radicals for these two systems produces significantly different optical spectra. CR produces a strong absorption peak at 500 nm attributed to the phenoxyl radical formed by one electron oxidation followed by proton loss from phenolic OH group. In case of CRSC a strong absorption at 405 nm is produced suggesting that the radical species formed is the imine carbonyl along with a less intense peak at 500 nm due to the formation of phenoxyl radicals. The stability of the imine carbonyl and phenoxyl radical imparts ability for CRSC to scavenge the oxidizing free radicals. The radical scavenging abilities of CR and CRSC are comparable, which is evident from their biomolecular rate constants obtained with azide, methyl, and halocarbon peroxyl radicals. However, the hydrogen donating ability of CRSC is five times slower as compared to CR. Here both steric and higher oxidation potentials are responsible for such changes in the reaction rate constants. CR has been studied for its antiproliferative activities by Mehta et al.[19] and they have also reported 48–72 h treatment to observe some morphological changes in MCF-7 cell lines. However, CRSC shows significant morphological changes within 24 h indicating different mode of action probably due to the different radical species and life time of such species being generated and is currently under investigation.

## 6. Experimental

*Materials*: Thiobarbituric acid (TBA) and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co., St. Louis, USA. All other reagents were of analytical grade. Nitrous oxide gas ($N_2O$) used was of Iolar grade. Curcumin semicarbazone was prepared according to the method described earlier.[13]

## 7. Cell viability assay

The number of viable cells remaining after appropriate treatment was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co.) assay.[20]

Briefly, cells were plated (4000 cells/well per 0.2 mL RPMI 1640 medium) in 96-well microtiter plates and incubated overnight. The test agent was then added at indicated concentrations to quadruplicate wells. After 24, 48, and 72 h, MTT was added to each well at a final volume of 0.5 mg/mL and the microplates were incubated at 37 °C for 3 h. After the supernatant was removed, the formazan salt resulting from the reduction of MTT was solubilized in dimethyl sulfoxide (DMSO, Sigma Chemical Co.) and the absorbance was read at 570 nm using an automatic plate reader (Molecular Devices Corporation, Sunnyvale, CA). The cell viability was extrapolated from optical density $(OD)_{570}$ values and expressed as percent survival using this formula and the graph was plotted.

$$\% \text{ Cell viability} = \frac{(OD)_{570} \text{ of drug treated sample}}{(OD)_{570} \text{ of drug Untreated sample}} \times 100$$

## 8. Isolation of microsomes

Male albino wistar strain rats (180–200 g) were killed by decapitation and livers were quickly removed and washed with isolation medium (0.25 M sucrose containing 10 mM Tris–HCl, pH 7.4). A 10% liver homogenate was made in isolation medium and microsomes were isolated by differential centrifugation as described elsewhere.[21] All operations were carried out at 0–4 °C.

## 9. Incorporation of CRSC into the microsomes

CRSC was incorporated into the microsomes by adding 100 μL solution from a stock concentration of $3 \times 10^{-3}$ M in ethanol to the microsomal pellet and diluting it to 10 mL with the buffer A (0.15 M Tris–HCl, buffer pH 7.4) followed by homogenization. The solution was then re-sedimented at 105,000*g* for 1 h to remove traces of ethanol yielding the microsome pellets with CRSC. They were re-suspended in the buffer A at a protein concentration of 6–8 mg protein per mL and stored at −20 °C for the maximum period of 2 weeks.

In the control experiments, 100 μL ethanol was added to the microsome pellet and the sample processed as above. The protein was estimated by the Lowry method[22] while the concentration of CRSC incorporated into the microsomes was measured by extracting it into a chloroform layer. The absorbance of the chloroform layer was measured at 425 nm and the concentration of the compound was calculated by using the extinction coefficient of $4.2 \times 10^3\ M^{-1}\ cm^{-1}$. During the microsomal experiments the solutions were diluted with phosphate buffer having pH 7.4.

## 10. γ-Radiolysis

Steady state γ-radiolysis of microsomal solution containing CRSC was carried out using $^{60}Co$ source with a dose rate of 7.4 Gy/min measured by Fricke dosimetry.[23] The antioxidant activity of CRSC against γ-ray induced lipid peroxidation was studied in $N_2O$ purged microsomal solution at physiological pH 7.4. The γ-radiolysis of aqueous solutions generates primary radicals as given in Eq. 1, which under $N_2O$-saturated conditions convert $e^-_{aq}$ to the hydroxyl radicals as shown in Eq. 2 leading exclusively to ·OH induced lipid peroxidation.

$$H_2O \xrightarrow{\gamma\ \text{ray}} e^-_{aq}, {}^{\cdot}OH, H^{\cdot} \quad (1)$$

$$N_2O + e^-_{aq} \rightarrow N_2 + {}^{\bullet}OH + OH^- \quad (2)$$

The microsome samples in the presence and absence of the test compounds were prepared as follows:

Two sets of sealed vials were prepared and vortexed one containing normal microsomes diluted to 2 mL (buffer pH 7.4) at a protein concentration of 0.4–0.6 mg/mL and the other containing microsomes with the test substances with identical dilution and protein concentration. Both sets were irradiated for different time intervals and purging the microsomal suspensions with $N_2O$ gas for 2 or 3 min in such a way that some dissolved oxygen still remained inside. For the blank correction, identical sets were prepared to see the extent of lipid peroxidation in the absence of irradiation.

The extent of lipid peroxidation was estimated in terms of thiobarbituric acid reactive substance (TBARS) as follows: At regular time intervals, 0.5 mL of the microsomal suspension from the respective vial of both the sets were removed and added to the TBA reagent (TBA reagent: 15% w/v trichloroacetic acid, 0.375% w/v TBA, 0.25 N hydrochloric acid, 0.05% w/v BHT) and were incubated for 20 min at 80 °C in a water bath. The precipitates were removed upon cooling by centrifugation and the absorbances of the supernatants were measured at 535 nm ($\varepsilon = 1.56 \times 10^5\ M^{-1}\ s^{-1}$) to calculate TBARS, which is a direct measure of the lipid damage.[24] The extent of inhibition of ·OH induced lipid peroxidation was measured at different dose rates of irradiation.

## 11. Pulse radiolysis

Pulse radiolysis experiments were done on a 7 MeV linear accelerator at Bhabha Atomic Research Centre producing 50 ns electron pulse with an absorbed dose of 10–12 Gy as described by Guha et al.[25] The absorbed dose was measured using thiocyanate dosimetry monitoring $(SCN)_2^{\cdot -}$ at 500 nm.[26]

Azide radicals ($N_3^{\cdot}$) were generated by irradiating $N_2O$ saturated aqueous solution containing sodium azide where all the ·OH radicals were exclusively converted to $N_3^{\cdot}$ by the reaction shown in 3.

$$ {}^{\cdot}OH + N_3^- \rightarrow OH^- + N_3^{\cdot} \quad (3)$$

Since both the test substances are insoluble in water, 10% acetonitrile was added to solubilize the required amounts of the compounds. In order to avoid direct reaction of the hydroxyl radical with acetonitrile and its preferential reaction with azide, atleast 0.05 M sodium azide is used. For the generation of methyl radicals in DMSO and halocarbon peroxyl radicals literature protocols were followed.[27]

## 12. Stopped-flow measurements

Stopped-flow experiments were carried out using SX-18 MV multimixing reaction analyzer from Applied Photo Physics Ltd, UK. It was used in single mixing CR/CRSC in methanol and syringe II contained the DPPH solution (μM) in methanol. The absorbance due to DPPH was measured at 517 nm as a function of time. All the experiments were carried out at room temperature (25 °C). At least three independent runs were used to determine the observed rates at any particular concentration. All the experiments were repeated twice.

## 13. Physico-chemical measurements

Elemental analyses were carried out on a Hosley C H N analyzer at the Microanalytical Laboratory, University of Pune, India. Proton NMR spectra were obtained on Perkin–Elmer instrument operating at 300 MHz in DMSO-$d_6$ and referenced to $\delta$ DMSO at 2.5 ppm. Cyclic voltammetric measurements were made in DMSO solvent on BAS CV-27 instrument with an X–Y recorder using Pt as working electrode against SCE and Pt wire as an auxiliary electrode with TEAP as the supporting electrolyte.

## Acknowledgements

S.D. would like to thank CSIR for the SRF Fellowship. Dr. Prasad Kulkarni is thanked for technical assistance.

## References and notes


1. (a) Ammon, H. P. T.; Wahl, M. A. *Planta Med.* **1991**, *57*, 1; (b) Sharma, O. P. *Biochem. Pharmacol.* **1976**, *25*, 1811.
2. Soni, K. B.; Kuttan, R. *Ind. J. Physiol. Pharmacol.* **1992**, *36*, 273.
3. (a) Singletary, K.; MacDonald, C.; Wallig, M.; Fischer, C. *Cancer Lett.* **1996**, *103*, 137; (b) Singh, S.; Agarwal, B. B. *J. Biol. Chem.* **1995**, *270*, 24995; (c) Chuang, S. E.; Kuo, M. L.; Hsu, C. H.; Chen, C. R.; Lin, J. K.; Lai, G. M.; Hseih, C. Y.; Cheng, A. L. *Carcinogenesis* **2000**, *21*, 331, and references cited therein.
4. (a) Kelly, M. R.; Xu, J.; Alexander, K. E.; Loo, G. *Mut. Res.* **2001**, *485*, 309; (b) Loo, G. *J. Nutr. Biochem.* **2003**, *14*, 64.
5. (a) Crompton, M. *Biochem. J.* **1999**, *341*, 233; (b) Morin, D.; Barthelemy, S.; Zini, R.; Labidalle, S.; Tillement, J. P. *FEBS Lett.* **2001**, *495*, 131.
6. (a) Ligeret, H.; Barthelemy, Zini R.; Tillement, J.-P.; Labidall, S.; Morin, D. *Free Rad. Biol. Med.* **2004**, *36*, 919; (b) Ligeret, H.; Barthélássy, S.; Doulakas, G. B.; Carrupt, P. A.; Tillement, J.-P.; Labidalle, S.; Morin, D. *FEBS Lett.* **2004**, *569*, 37.
7. Kapoor, S.; Priyadarsini, K. I. *Biophys. Chem.* **2001**, *92*, 119, and references cited therein.
8. Jovanoic, S. V.; Steenken, S.; Borne, C. W.; Simic, M. G. *J. Am. Chem. Soc.* **1999**, *121*, 9677.
9. Jovanoic, S. V.; Boone, C. W.; Steenken, S.; Trionga, M.; Kaskey, R. B. *J. Am. Chem. Soc.* **2001**, *123*, 3064.
10. Priyadarsini, K. I.; Maity, D. K.; Naik, G. H.; Sudheer Kumar, M.; Unnikrishnan, M. K.; Satav, J. G.; Hari Mohan *Free Rad. Biol. Med.* **2003**, *35*, 475.
11. Tonneson, H. H.; GreenHill, J. V. *Int. J. Pharm.* **1992**, *87*, 79.
12. Shim, J. S.; Kim, D. H.; Jung, H. J.; Kim, J. H.; Lim, D.; Lee, S. K.; Kim, K. W.; Ahn, J. W.; Yoo, J. S.; Rho, J. R.; Shim, J.; Kwon, H. *J. Bioorg. Med. Chem.* **2002**, *10*, 2439.
13. Dutta, S.; Murugkar, A.; Gandhe, N.; Padhye, S. *Met.-Based Drugs* **2001**, *8*, 183.
14. Nakayama, T.; Ono, K.; Hashimoto, K. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1005.
15. Khopde, S. M.; Priyadarsini, K. I.; Venkatesan, P.; Rao, M. N. A. *Biophys. Chem.* **1999**, *80*, 85.
16. Bordwell, F. G.; Bausch, M. J. *J. Am. Chem. Soc.* **1986**, *108*, 1979.
17. Gorman, A. A.; Hanblett, V. S.; Srinivasan, V. S.; Wood, P. D. *Photochem. Photobiol.* **1994**, *59*, 389.
18. Holy, J. M. *Mut. Res.* **2002**, *518*, 71.
19. Mehta, K.; Pantazis, P.; McQueen, T.; Aggarwal, B. *Anticancer Drugs* **1997**, *8*, 470.
20. Chen, J. S. K.; Konopleva, M.; Andreeff, M.; Multani, A.; Pathak, S.; Mehta, K. *J. Cell Physiology.* **2004**, *200*, 223.
21. Satav, J.; Katyare, S.; Fatterpekar, P.; Srinivasan, A. *Biochem. J.* **1976**, *156*, 215.
22. Lowry, O. H.; Rosebourgh, M. J.; Faer, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 275.
23. *An Introduction to Radiation Chemistry*; Spinks, J. W. T., Woods, R. J., Eds.; John Wiley and Sons: New York, 1990, pp 243–313.
24. Buege, J. A.; Aust, S. D. Microsomal Lipid Peroxidation. In *Methods In Enzymology*; Fleisher, S., Packer, L., Eds.; Academic: New York, 1978, pp 302–310.
25. Guha, S. N.; Moorthy, P. N.; Kishore, D.; Rao, K. N. *Proc. Indian Acad. Sci. (Chem. Sci).* **1987**, *99*, 261.
26. Feilden, E. M. In *The Study of Fast Processes and Transient Species by Electron Pulse Radiolysis*; Baxendale, J. H., Busi, R. D., Eds.; Reidel: London, 1984, p 59.
27. (a) Alfassi, Z. B.; Mosseri, S.; Neta, P. *J. Phys. Chem.* **1981**, *91*, 3383; (b) Alfassi, Z. B.; Harriman, A.; Huie, R. E.; Mosseri, S.; Neta, P. *J. Phys. Chem.* **1987**, *91*, 2120; (c) Russ, M. E.; Bors, W.; Saran, M. *Photochem. Photobiol.* **1994**, *59*, 389.