Food Chemistry 111 (2008) 573–579

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Antimutagenicity of curcumin and related compounds against genotoxic heterocyclic amines from cooked food: The structural requirement

Shishu *, Indu Pal Kaur [1]

*University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India*

ARTICLE INFO

*Article history:*
Received 7 December 2007
Received in revised form 5 January 2008
Accepted 4 April 2008

*Keywords:*
Curcumin
Heterocyclic amines
Antimutagenic
Ames test
Cooked food

ABSTRACT

Curcumin (a major constituent of widely-used spice and colouring agent, turmeric) was found to be very effective in antagonising the S9-mediated mutagenicity of several food-derived heterocyclic amines. In order to understand the chemical basis of antimutagenic properties of curcumin against these mutagens, we have studied the structure–activity relationship between curcumin and its naturally-occurring derivatives, namely demethoxycurcumin and bisdemethoxycurcumin, and other structurally-related natural and synthetic analogues of curcumin, namely tetrahydrocurcumin, dibenzoylmethane, dibenzoylpropane, vanillin, ferulic acid, isoferulic acid and caffeic acid, using Ames *Salmonella*/reversion assay, against different classes of cooked food mutagens. We conclude that unsaturation in the side chain, a methoxy group on the benzene ring and a central β-diketone moiety in the curcumin molecule are the important structural requirements responsible for high antimutagenic potential of curcumin against cooked food heterocyclic amines.



## 1. Introduction

Cooking procedures such as broiling, frying, barbecuing, heat processing and pyrolysis of protein-rich foods, such as beef, chicken and fish, induce the formation of potent mutagenic and carcinogenic compounds called heterocyclic amines (Felton & Knize, 1991). Several epidemiological studies published in recent years show a correlation between intake of fried meat and development of cancer (de Meester & Gerber, 1995; Steck et al., 2007). The carcinogenic risk imposed by these probable human carcinogens depends not only on the level of exposure, but is also modulated by other dietary factors. It has been well established that chemical mutagenesis and carcinogenesis can be inhibited by a large number of naturally-occurring compounds of plant origin (Steinmetz & Potter, 1996; Surh, 2003).

One dietary polyphenol which has been of focus of considerable attention is curcumin. It is the major constituent of spice turmeric, obtained from dried powdered rhizome of *Curcuma longa* (Aggarwal, Sundaram, Malani, & Ichikawa, 2007; Nadkarni, 1976). The turmeric or its major yellow pigment curcumin (C) has been reported to possess antioxidant, anti-inflammatory (Anto, George, DineshBabu, Rajasekharan, & Kuttan, 1996; Jayaprakasha, Jaganmohan Rao, & Sakariah, 2006; Menon & Sudheer, 2007) and inhibitory activity against chemically-induced carcinogenesis in several experimental models (Huang et al., 1995; Shishodia, Chaturvedi, & Aggarwal, 2007). Curcumin has also been shown to be a potent inhibitor of several environmental mutagens requiring metabolic activation (Nagabhushan, Amonkar, & Bhide, 1987). Besides natural C, other naturally-occurring and structurally-related curcuminoids, such as demethoxycurcumin (dmC) and bisdemethoxycurcumin (bdmC) and synthetic curcuminoids have been reported to be inhibitors of 2-acetamidofluorene-induced (2-AAF) mutagenesis in *Salmonella typhimurium* strain TA98.

It has been observed that curcuminoids containing free hydroxyl groups showed a marked anti-promoter activity against croton oil-induced tumours and a potent inhibitory activity against 2-AAF-induced mutagenesis (Anto et al., 1996). In addition to the presence of these hydroxyl groups on the benzene rings in curcuminoids, the presence of double bonds in the alkene portion of the molecule and a central β-diketone moiety are also responsible for their biological activity (Fig. 1). Inhibitory effects of topically-applied C and dmC on TPA-induced tumour promotion in 7,12-dimethylbenz[*a*]anthracene (DMBA)-initiated mouse skin cancer, were observed to be greater than that of bdmC (Huang et al., 1995). Recently, structure–activity relationship studies on the three naturally-occurring curcuminoids, in growth inhibition studies against various cell lines (Ramsewak, deWitt, & Nair, 2000; Sandur et al., 2007), and for the evaluation of antioxidant activities (Ahsan, Parveen, Khan, & Hadi, 1999; Jayaprakasha et al., 2006; Ramsewak et al., 2000) have demonstrated that curcumin (the most non-polar among these three natural curcuminoids) is the

* Corresponding author. Tel.: +91 172 2534281(O)/2782099(R); fax: +91 172 2541142.
*E-mail addresses:* shishugoindi@yahoo.co.in, goindi@satyam.net.in (Shishu), indupalkaur@yahoo.com (I.P. Kaur).
[1] Tel.: +91 172 2534113(O)/2701914(R); fax: +91 172 2541142.

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.04.022

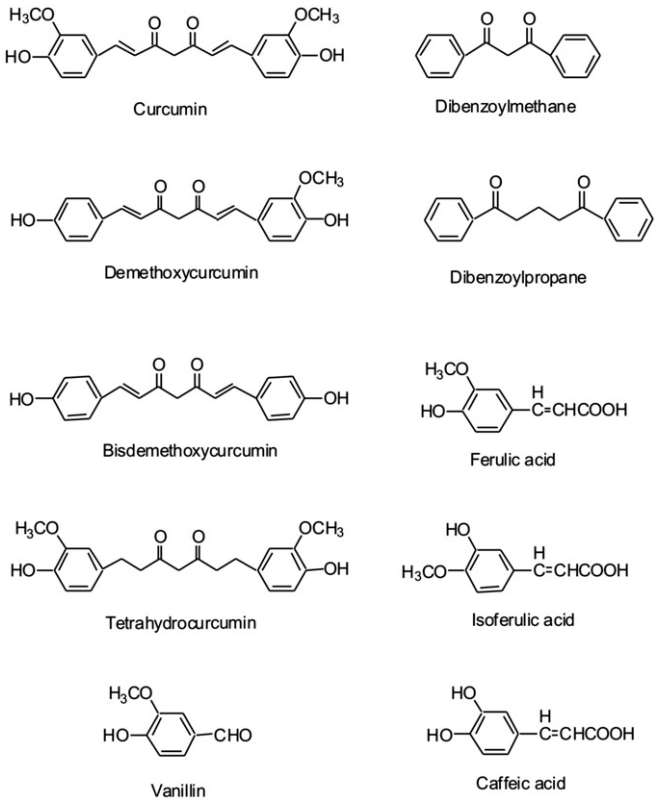


**Fig. 1.** Structures of curcumin and its analogues.

most active natural curcuminoid, followed by dmC, and bdmC. These studies suggest that the presence of a methoxy group on both or one of the phenyl rings is responsible for higher activities of C and dmC, compared to bdmC (having hydroxy groups on the aromatic rings). Very recently, it has been reported that the relative potency for suppression of tumour necrosis factor (TNF)-induced nuclear factor-kB (NF-kB) activation was C > dmC > bdmC, again suggesting the critical role of methoxy groups on the phenyl ring (Sandur et al., 2007.) However, in some of the anti-inflammatory studies, dmC was found to exhibit the highest activity (Anto, Kuttan, DineshBabu, Rajasekharan, & Kuttan, 1998; Ramsewak et al., 2000; Rao, Basu, & Siddique, 1982) and earlier reports on antimutagenicity studies of curcuminoids against S9-mediated mutagenicity of DMBA and 2-AAF, in TA98, proposed bdmC to be the most active among natural curcuminoids (Anto et al., 1996; Mulky, Amonkar, & Bhide, 1988).

So far, these curcuminoids have been studied for suppressing the mutagenicity of chilli extract, capsaicin, tobacco and cigarette smoke condensate, 2-AAF, benzo(*a*)pyrene, DMBA and aflatoxin $B_1$ (Anto et al., 1996; Nagabhushan et al., 1987; Soni, Lahiri, Chackradeo, Bhide, & Kuttan, 1997) in *S. typhimurium*. In our earlier studies, we have evaluated and reported the inhibitory effect of natural curcuminoids against various cooked food mutagens (Shishu & Kaur, 2002). It was observed that curcuminoids are very potent inhibitors of mutagenicity induced by different classes of cooked food-derived heterocyclic amines. Therefore, in view of these observations, present investigations were planned to identify the specific structural features in the curcumin molecule responsible for its potent inhibitory activity against these mutagens. In the present study, C and its natural analogues, dmC and bdmC from turmeric (Fig. 1), and other natural as well as synthetic structurally-related compounds were examined. These compounds included tetrahydrocurcumin (thC, a colourless, synthetic derivative and major metabolite of C, that differs from C in having a saturated alkyl chain connecting two aromatic rings), dibenzoylmethane (DBM, a minor constituent of licorice and a reported antimutagen; resembles C in that it possesses a β-diketone moiety linking two phenyl groups), and dibenzoylpropane (DBP, a synthetic structurally-related diketone compound, that lacks the β-diketone configuration): vanillin (V), ferulic acid (FA), isoferulic acid (isoFA) and caffeic acid (CA), degradation products of curcuminoids, possessing aromatic rings similar to those present in different natural curcuminoids, found in vegetables, fruits and certain beverages were also studied. These compounds were investigated for their potential antimutagenic properties against different classes of cooked food heterocyclic amine mutagens, e.g., imidazoazaarenes: IQ, MeIQ, MeIQx and PhIP; pyridoindole derivatives: Trp-P-1 and Trp-P-2; and dipyridoimidazole derivative: Glu-P-1. Based on results of these *in vitro* antimutagenicity studies, structural features essential for inhibitory effect of curcumin against cooked food mutagens are proposed herein.

## 2. Materials and methods

### 2.1. Bacterial strains

A histidine-requiring TA98 strain of *S. typhimurium* was obtained as a kind gift from Dr Bruce N. Ames (University of California, Berkley, USA).

### 2.2. Chemicals

2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) acetate and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) were purchased from Toronto Research Chemicals Inc., North York, Ontario, Canada. 2-Amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1) hydrochloride (monohydrate) was purchased from Wako Pure Chemicals, Osaka, Japan. 3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) acetate was kindly donated by Dr T. Nohmi, National Institute of Hygienic Sciences, Tokyo, Japan. Curcumin (C), demethoxycurcumin (dmC) and bisdemethoxycurcumin (bdmC) were received as a gift from Sami Labs Ltd. Bangalore, India. Dibenzoylmethane (DBM) and 1,3-dibenzoylpropane (DBP) were purchased from Aldrich Chemical Company, Inc. (St. Louis, MO). Albumin (bovine) and nicotinamide adenine dinucleotide phosphate (NADP) sodium salt, were purchased from Sisco Research Laboratories, Mumbai, India. D-Glucose-6-phosphate monosodium salt and d-biotin were purchased from Sigma Chemical Company, (St. Louis, MO). Oxoid nutrient broth No. 2 was purchased from Oxoid Ltd., Basingstoke, UK. Nutrient agar was purchased from Himedia Laboratories. Mumbai, India. All other chemicals and reagents used were of AR grade.

### 2.3. Preparation of liver homogenate S9 fraction

The S9 fraction was prepared from the pooled livers of four male Sprague-Dawley rats, previously induced with Aroclor 1254, by the method of Garner, Miller, and Miller (1972).

### 2.4. Determination of protein concentration of S9

Protein concentration of induced rat liver S9 was determined by biuret method (Gornall, Bardwill, & David, 1949) and was found to be 54 mg/ml.

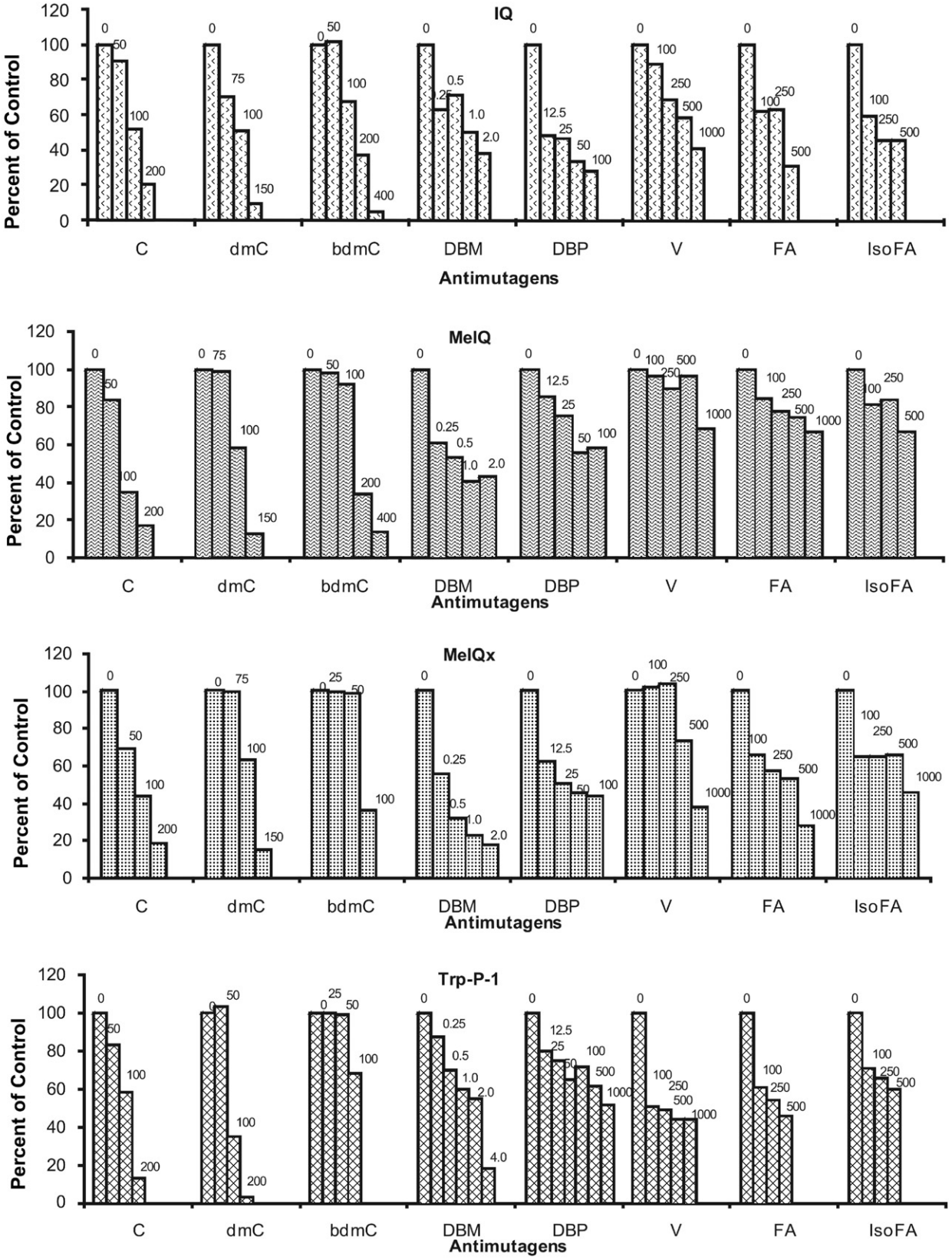


**Fig. 2.** Effect of varying concentrations of antimutagens on S9-mediated mutagenicity of various cooked food mutagens: IQ (0.010 μg/plate; 471 ± 40), MeIQ (0.005 μg/plate; 1000 ± 176), MeIQx (0.026 μg/plate; 819 ± 131), Trp-P-1 (0.225 μg/plate; 370 ± 50), Trp-P-2 (0.021 μg/plate; 511 ± 88), PhIP (0.897 μg/plate; 321 ± 67) and Glu-P-1 (0.050 μg/plate; 932 ± 70) towards a TA98 strain of *S. typhimurium*. With 2-AF (positive control) the revertant count is 5326 ± 187 ($n$ = 15). Dose of antimutagen (in mg/plate) is indicated on the top of each bar.

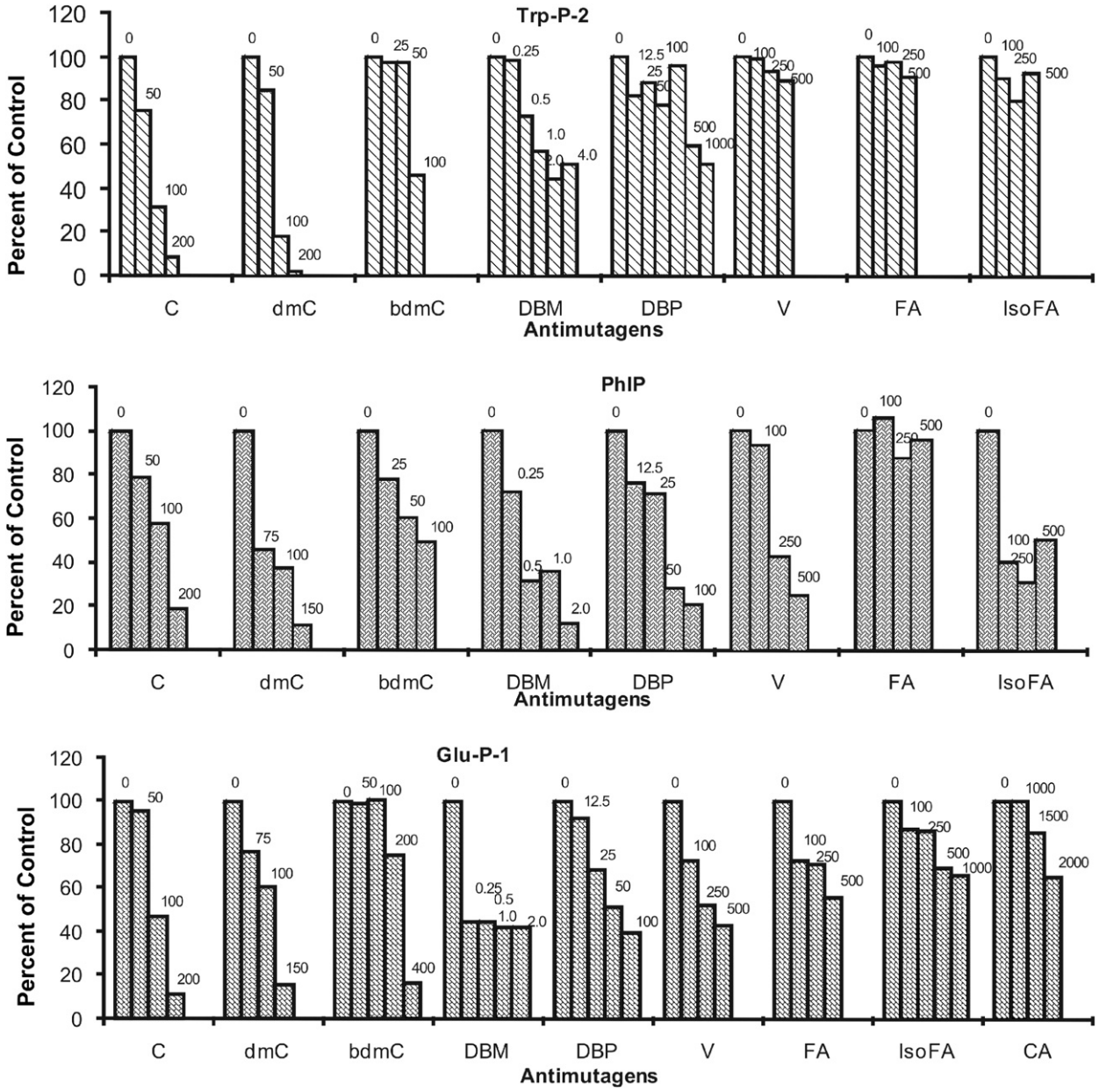


Fig. 2 (*continued*)

### 2.5. Antimutagenicity testing

The plate incorporation procedure of Maron and Ames (1983) was used for antimutagenicity testing, with the inclusion of a pre-incubation step (Yahagi et al., 1977), and with some minor modifications. Negative and positive controls were included in each assay (see legend of Fig. 2). Doses of antimutagens (shown on the top of each bar in Fig. 2) used in the study were also tested for any toxic or mutagenic effects toward strain TA98 and no change in spontaneous revertant count at the tested dose levels indicated absence of any mutagenic/toxic effects (see legend of Fig. 2 for revertant counts). A suitable dose of the test mutagens was selected from the linear portion of the dose–response curve of the respective mutagen. Further, mutagens were applied to the test in such doses which resulted in a maximum of about 2000 $His^+$ revertants/plate, so as to ensure accurate counting, since at this count overlapping of bacterial colonies is avoided and inhibition or enhancement by modulators can be detected with a minimum statistical variation (for dose of mutagens see legend of Fig. 2).

All assays were carried out in duplicate/triplicate on separate occasions. Results are expressed as mean ± SD ($n = 6$) of $His^+$ revertants per plate (uncorrected for spontaneous count 30 ± 4) for each dose. Number of $His^+$ revertants induced by the mutagen alone is expressed as 100% mutagenicity or as control (see legend of Fig. 2), and the reduction in mutagenicity at each dose level of the antimutagen is expressed as the percent of control:

$$\%\ \text{control} = \frac{\text{No. of His}^+\text{revertants induced by the mutagen per plate in the presence of antimutagen}}{\text{No. of His}^+\text{revertants induced per plate by the mutagen alone}} \times 100 \quad (1)$$

Percent of control values were plotted against varying concentrations of the antimutagen and $ID_{50}$ value, i.e., the dose corresponding to 50% mutagenicity was calculated from these dose–response curves. In those cases where 50% inhibition of mutagenicity was not achieved at the tested dose level, the minimum dose showing maximum inhibition is indicated.

### 2.6. Statistical analysis

All the data were statistically analysed by one way analysis of variance (ANOVA) followed by Student–Newman–Keuls method. The data which was not normal or where variations, were not equal, was subjected to Kruskal–Wallis ANOVA on ranks. Linear regression was used to test for linearity of dose–response relationship.

## 3. Results

Results of *in vitro* antimutagenicity assay indicate that C in the nontoxic dose range of 50–200 μg/plate, effectively inhibited mutagenicity induced by all the seven tested cooked food mutagens (Fig. 2). A linear dose–response relationship was observed for all the mutagens tested. Doses less than 50 μg/plate did not show any significant inhibition (only 10–25% inhibition) and a complete inhibition of mutagenicity was observed at 200 μg/plate dose. $ID_{50}$ value for IQ, MeIQ, MeIQx, Trp-P-1, Trp-P-2, PhIP and Glu-P-1 was found to be 137, 85, 86, 120, 80, 120, and 98 μg/plate, respectively. (Table 1 and Fig. 2).

DmC, a natural analogue of curcumin also exhibited strong inhibitory effects against all the tested cooked food mutagens. These inhibitory effects were also linearly related with the dose of dmC and the extent of inhibition was closely similar to that observed with curcumin. $ID_{50}$ values ranged between 77 and 113 μg/plate. Highest inhibitory activity was observed against PhIP. Almost complete inhibition was achieved at the dose level of 200 μg/plate (Table 1 and Fig. 2).

Like C and dmC, bdmC could also effectively suppress the mutagenicity induced by all the tested heterocyclic amines. However, bdmC appeared to be a relatively less potent antimutagen (Table 1). Linear dose-dependent inhibitory activity was observed in the range of 25–400 μg/plate. The antimutagenic potency, quantified as $ID_{50}$ value, ranged between 88 and 292 μg/plate and was lowest in the case of MeIQx.

Tetrahydrocurcumin failed to show any antimutagenic effect against the tested heterocyclic amine mutagens in the tested dose range (data not shown).

DBM, possessing beta-diketone configuration similar to C, was found to be the most potent of all the tested compounds (50–400 times more active than C; Table 1). It suppressed the mutagenicity induced by all the tested cooked food mutagens at very low doses ($ID_{50}$ values lie between 0.16 and 2.29 μg/plate). Maximum inhibition of approximately 60–90% was observed against imidazoazaarenes (IQ, MeIQ, MeIQx and PhIP), compared to 46–54% inhibition against pyridoindole derivatives (Trp-P-1 and Trp-P-2) and 58% inhibition against dipyridoimidazole derivative Glu-P-1 at the dose level of 2 μg/plate (Fig. 2). A linear dose–response relationship was observed against all of the tested mutagens except MeIQ and Glu-P-1.

Evaluation of antimutagenic properties, against various cooked food mutagens, revealed that DBP, although, lacking the diketone configuration indicated to be essential for biological activities of curcumin, was very effective in suppressing the mutagenicity induced by the tested mutagens, except Trp-P-2 (Table 1 and Fig. 2). $ID_{50}$ values of 12, 28, 33 and 61 μg/plate for IQ, MeIQx, PhIP and Glu-P-1, respectively, were less than the $ID_{50}$ values obtained with C against these mutagens, indicating that DBP is more active than curcumin. Highest inhibitory effect was observed against IQ. A linear dose–response relationship was observed against all of the tested mutagens except MeIQx and Trp-P-1.

Vanillin, also exhibited a dose-dependent inhibition but at a relatively higher dose range (100–1000 μg/plate) compared to the curcuminoids. $ID_{50}$ values obtained against various food-derived heterocyclic amines indicated that inhibitory effects of vanillin were more marked against Trp-P-1, PhIP and Glu-P-1, compared to IQ, MeIQ, MeIQx and Trp-P-2 (Table 1 and Fig. 2).

Ferulic acid dose-dependently inhibited the mutagenicity induced by the tested cooked food mutagens, except PhIP, in the concentration range of 100–1000 μg/plate (Fig. 2). It showed stronger inhibitory effects than V against IQ, MeIQ, MeIQx and was found to be most active against IQ and least active against Trp-P-2 and PhIP (Table 1).

Antimutagenic effects of isoFA against IQ, MeIQ and PhIP, were found to be more marked than those of FA. Stronger inhibitory activities were observed against IQ, MeIQx and PhIP. However, it showed weaker antimutagenic effects against Trp-P-2 and Glu-P-1. A linear dose–response relationship was observed for all the tested mutagens except for PhIP and Trp-P-2, in the concentration range of 100–1000 μg/plate (Table 1 and Fig. 2).

Caffeic acid did not show any inhibitory effect against the tested cooked food heterocyclic amines, except a moderate inhibitory activity against Glu-P-1 (maximum inhibition of 35% was observed at 2000 μg/plate). A linear dose–response effect was observed for Glu-P-1 in the concentration range of 500–2000 μg/plate (Table 1).

**Table 1**
Comparison of $ID_{50}$ values of curcumin and its analogues against Aroclor induced S9-mediated mutagenicity of heterocyclic amines in TA98 strain of *Salmonella typhimurium*

| Antimutagens | $ID_{50}$ (μg/plate) | | | | | | |
|---|---|---|---|---|---|---|---|
| | IQ (0.010) | MeIQ (0.005) | MeIQx (0.026) | Trp-P-1 (0.225) | Trp-P-2 (0.021) | PhIP (0.897) | Glu-P-1 (0.050) |
| Curcumin | 137 | 85 | 86 | 120 | 80 | 120 | 98 |
| Demethoxycurcumin | 101 | 112 | 113 | 90 | 77 | 68 | 112 |
| Bisdemethoxycurcumin | 157 | 172 | 88 | 131 | 97 | 97 | 292 |
| Tetrahydrocurcumin | – | – | – | – | – | – | – |
| Dibenzoylmethane | 1.07 | 0.67 | 0.31 | 2.29 | 1.60 | 0.38 | 0.21 |
| Dibenzoylpropane | 12 | 44% inhibition at 50 μg/plate | 28 | 48% inhibition at 1000 μg/plate | 49% inhibition at 1000 μg/plate | 33 | 61 |
| Vanillin | 735 | 31% inhibition at 1000 μg/plate | 829 | 168 | 11% inhibition at 500 μg/plate | 230 | 324 |
| Ferulic acid | 356 | 33% inhibition at 1000 μg/plate | 558 | 379 | 10% inhibition at 500 μg/plate | 13% inhibition at 250 μg/plate | 44% inhibition at 500 μg/plate |
| Isoferulic acid | 209 | 32% inhibition at 500 μg/plate | 838 | 40% inhibition at 500 μg/plate | 21% inhibition at 250 μg/plate | 84 | 34% inhibition at 1000 μg/plate |
| Caffeic acid | – | – | – | – | – | – | 35% inhibition at 2000 μg/plate |

## 4. Discussion

Analysis of the results of these studies as indicated in Table 1 and Fig. 2, clearly demonstrate that all three natural curcuminoids (C, dmC and bdmC) are highly effective in antagonising the S9-mediated mutagenic effects of all the seven tested heterocyclic amines in a dose-dependent manner. In general, C and dmC showed stronger inhibitory effects compared to bdmC (Table 1). More than 80% inhibition of mutagenicity was observed at 200 μg/plate in the cases of C and dmC, whereas bdmC showed 60–80% inhibition at this dose. Thus, suggesting the critical role of methoxy groups on the phenyl ring of the curcumin molecule.

Tetrahydrocurcumin, a colourless, synthetic derivative, prepared by hydrogenation of C, although reported to be a very efficient and potent antioxidant, was found to be ineffective against the cooked food mutagens at the tested dose levels. These findings indicate that probably the presence of unsaturation in the side chain of curcumin molecule is essential for antimutagenic activity against food-derived heterocyclic amines. Moreover, the stronger antioxidant activity of thC compared to natural curcuminoids has been attributed to the presence of benzylic hydrogens involved in the oxidation process of thC and not to the β-diketone moiety in the chain (Portes, Gardrat, & Castellan, 2007).

These screening studies also revealed that DBP, although lacking the central β-diketone configuration indicated to be essential for biological activities of C (Anto et al., 1996), was very effective in suppressing the mutagenicity induced by the tested mutagens, except for Trp-P-2.

Interestingly, DBM, possessing a central β-diketone moiety (as present in the C molecule) was found to be the most active amongst the compounds investigated in the present study (Table 1). Such a strong antimutagenic effect of DBM might be because of the relatively small size of the molecule, absence of hydroxyl groups on the aromatic rings and presence of the central β-diketone moiety (Shishu, Singla, & Kaur, 2003).

Vanillin, a natural flavouring agent (a hydroxy and a methoxy group on the aromatic ring as in C molecule), also showed a very good inhibitory activity against Trp-P-1, PhIP and Glu-P-1 (60–70% inhibition at a dose of 500 μg/plate). At a higher dose of 1000 μg/plate, vanillin was found to be effective against MeIQ and IQ as well (58–63% inhibition). However, only 11% inhibition was observed against Trp-P-2 at dose of 500 μg/plate. Overall, these findings suggest that vanillin, which is also a degradation product of C, is an effective inhibitor of S9-mediated mutagenicity of cooked food mutagens.

Both FA and its structural analogue, isoFA, like V, were observed to be effective in antagonising the mutagenicity of all the tested heterocyclic amines except for Trp-P-2. Inhibitory effects of isoFA were stronger against IQ and MeIQ, compared to V and FA. Caffeic acid, lacking a methoxy group on the benzene ring, unlike FA and isoFA, and possessing two hydroxy groups, did not exhibit any antimutagenic effect against the tested heterocyclic amines except Glu-P-1.

In order to understand the chemical basis of the antimutagenic properties of curcumin, the structure–activity relationship between C and its two naturally-occurring derivatives, namely dmC and bdmC, and other structurally-related natural and synthetic analogues of C (Fig. 1) was studied. For the various biological activities of curcumin, the presence of hydroxy groups on the benzene rings, the double bonds in the alkene portion of the molecule and the central β-diketone configuration are implicated as essential chemical structures (Anto et al., 1996). Recently, structure–activity relationship studies on the three naturally-occurring curcuminoids, for growth inhibition studies against various cell lines, and for evaluation of the antioxidant activities, have demonstrated that C is the most active natural curcuminoid, followed by dmC, then bdmC (Ramsewak et al., 2000; Sandur et al., 2007), indicating that the presence of a methoxy group on one or both of the phenyl rings is responsible for higher activities of curcumin and dmC, compared to bdmC.

In the current studies, the ineffectiveness of thC to suppress mutagenicity of food mutagens in the tested dose range reinforces the fact that double bonds in the alkene portion of the curcumin molecule are essential for antimutagenic effect of curcumin against cooked food mutagens. DBM, possessing a central β-diketone moiety similar to C, was found to be a much more active antimutagen against cooked food heterocyclic amines, when compared to DBP, which does not possess a central β-diketone configuration. Thus, it can be concluded that the central β-diketone moiety in the curcumin molecule is responsible for its higher antigenotoxic effect and the presence of double bonds in the alkene portion of the molecule is essential for the antimutagenic effect of C against cooked food mutagens.

Further, results of our investigations indicate that C (two ferulic acid moieties joined by a methylene bridge) and dmC (a ferulic acid and a caffeic acid moiety joined by a methylene bridge) were the most active inhibitors among the natural curcuminoids, while bdmC (two caffeic acid moieties joined by a methylene bridge) showed the least activity. Thus, it may be suggested that presence of methoxy group on the benzene rings were responsible for the high antigenotoxic effects of C and dmC, as compared with bdmC molecule, in which both the methoxy groups on the benzene rings, are replaced with hydroxy groups. This observation can be further supported by the results obtained from antimutagenicity studies of V, FA, isoFA and CA against various heterocyclic amines. Caffeic acid, carrying two hydroxy groups on the benzene ring was ineffective in antagonising the mutagenic effects of all the tested cooked food mutagens except for Glu-P-1, whereas V and FA, both possessing one hydroxy and a methoxy group on the benzene ring, exhibited significant antimutagenic effects against various tested heterocyclic amines. Also, V carrying an aldehyde group in place of the side chain on the benzene ring was less active than FA against IQ and MeIQ. IsoFA, which differs from FA in the position of its methoxy group, which is at the *para* position instead of at the *meta* position, showed higher antimutagenic effect against IQ, MeIQ and PhIP.

Summarising all the above findings, it may be suggested that unsaturation in the side chain, a methoxy group on the benzene ring and a central β-diketone moiety in the curcumin molecule are the important structural requirements responsible for the high antimutagenic potential of curcumin against cooked food heterocyclic amines. Results of these studies taken together suggest that these dietary compounds, which are present in spices, vegetables, fruits and certain beverages and consumed daily may contribute towards chemoprotection against cooked food-derived heterocyclic amines. These findings might merit further investigations to establish the chemoprotective role of these agents in human cancer. Moreover, the potent activities of DBM and DBP need strong attention from the scientific community to further explore their chemopreventive potential.

## References

Aggarwal, B. B., Sundaram, C., Malani, N., & Ichikawa, H. (2007). Curcumin: The Indian solid gold. *Advances in Experimental Medicine and Biology, 595*, 1–75.

Ahsan, H., Parveen, N., Khan, N. U., & Hadi, S. M. (1999). Pro-oxidant, anti-oxidant and cleavage activities on DNA of curcumin and its derivatives demethoxycurcumin and bisdemethoxycurcumin. *Chemico-Biological Interactions, 121*, 161–175.

Anto, R. J., George, J., DineshBabu, K. V., Rajasekharan, K. N., & Kuttan, R. (1996). Antimutagenic and anticarcinogenic activity of natural and synthetic curcuminoids. *Mutation Research, 370*, 127–131.

Anto, R. J., Kuttan, G., DineshBabu, K. V., Rajasekharan, K. N., & Kuttan, R. (1998). Anti-inflammatory activity of natural and synthetic curcuminoids. *Pharmacy and Pharmacology Communications, 4*, 103–106.

De Meester, C., & Gerber, G. B. (1995). The role of cooked food mutagens as possible etiological agents in human cancer. A critical appraisal of recent epidemiological investigations. *Epidemiology Reviews Sante Publique, 43*, 147–161.

Felton, J. S., & Knize, M. G. (1991). Occurrence, identification and bacterial mutagenicity of heterocyclic amines in cooked food. *Mutation Research, 259*, 205–218.

Garner, R. C., Miller, E. C., & Miller, J. A. (1972). Liver microsomal metabolism of aflatoxin $B_1$ to a reactive derivative toxic to *Salmonella typhimurium* TA1530. *Cancer Research, 32*, 2058–2066.

Gornall, A. G., Bardwill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry, 177*, 751–766.

Huang, M. T., Ma, W., Lu, Y. P., Chang, R. L., Fischer, C., Manchand, P. S., et al. (1995). Effect of curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin on TPA-induced tumor promotion. *Carcinogenesis, 16*, 2493–2497.

Jayaprakasha, G. K., Jaganmohan Rao, L., & Sakariah, K. K. (2006). Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin. *Food Chemistry, 98*, 720–724.

Maron, D. M., & Ames, B. N. (1983). Revised methods for Salmonella mutagenicity test. *Mutation Research, 113*, 173–215.

Menon, V. P., & Sudheer, A. R. (2007). Antioxidant and anti-inflammatory properties of curcumin. *Advances in Experimental Medicine and Biology, 595*, 105–125.

Mulky, N., Amonkar, A. J., & Bhide, S. V. (1988). Antimutagenicity of curcumin and related compounds: The Structural requirement for the antimutagenicity of curcumins. *Indian Drugs, 25*, 1–3.

Nadkarni, K. M. (1976). Curcuma longa. In K. M. Nadkarni (Ed.), *India materia medica* (pp. 414–418). Bombay, India: Popular Prakashan Publishing Company.

Nagabhushan, M., Amonkar, A. J., & Bhide, S. V. (1987). *In vitro* antimutagenicity of curcumin against environmental mutagens. *Food Chemical Toxicology, 25*, 545–547.

Portes, E., Gardrat, C., & Castellan, A. (2007). A comparative study of the antioxidant properties of tetrahydrocurcuminoids and curcuminoids. *Tetrahedron, 63*, 9092–9099.

Ramsewak, R. S., deWitt, D. L., & Nair, M. G. (2000). Cytotoxicity, antioxidant and anti-inflammatory activities of curcumins I–III from *Curcuma longa*. *Phytomedicine, 7*, 303–308.

Rao, T. S., Basu, N., & Siddique, H. H. (1982). Anti-inflammatory activity of curcumin analogues. *Indian Journal of Medical Research, 75*, 574–578.

Sandur, S. K., Pandey, M. K., Sung, B., Ahn, K. S., Murakami, A., Sethi, G., et al. (2007). Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. *Carcinogenesis, 28*, 1765–1773.

Shishodia, S., Chaturvedi, M. M., & Aggarwal, B. B. (2007). Role of curcumin in cancer therapy. *Current Problems in Cancer, 31*, 243–305.

Shishu & Kaur, I. P. (2002). Inhibitory effect of curcumin and its natural analogues on genotoxicity of heterocyclic amines from cooked food. *Indian Journal of Experimental Biology, 40*, 1365–1372.

Shishu, Singla, A. K., & Kaur, I. P. (2003). Inhibitory effect of dibenzoylmethane on mutagenicity of food-derived heterocyclic amine mutagens. *Phytomedicine, 10*, 575–582.

Soni, K. B., Lahiri, M., Chackradeo, P., Bhide, S., & Kuttan, R. (1997). Protective effect of food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity. *Cancer Letters, 115*, 129–133.

Steck, S. E., Gaudet, M. M., Eng, S. M., Britton, J. A., Teitelbaum, S. L., Neugut, A. I., et al. (2007). Cooked meat and risk of breast cancer – lifetime versus recent dietary intake. *Epidemiology, 18*, 373–382.

Steinmetz, K. A., & Potter, J. D. (1996). Vegetables, fruit and cancer prevention: A review. *Journal of American Dietetic Association, 96*, 1027–1039.

Surh, Y. J. (2003). Cancer chemoprevention with dietary phytochemicals. *Nature Reviews Cancer, 3*, 768–780.

Yahagi, T., Nagao, M., Seino, Y., Matsushima, T., Sugimura, T., & Okada, M. (1977). Mutagenicity of *N*-nitrosamines on Salmonella. *Mutation Research, 48*, 121–130.