Carcinogenesis vol.20 no.5 pp.911–914, 1999

SHORT COMMUNICATION

Relation of structure of curcumin analogs to their potencies as inducers of Phase 2 detoxification enzymes

Albena T.Dinkova-Kostova and Paul Talalay[1]

Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

[1]To whom correspondence should be addressed
Email: ptalalay@welchlink.welch.jhu.edu

A series of naturally occurring as well as synthetic structural analogs of the dietary constituent curcumin were examined in order to elucidate which portions of the molecule are critical for the ability to induce Phase 2 detoxification enzymes in murine hepatoma cells, and hence to assess the chemoprotective potential of these compounds. Two groups of compounds were studied: classical Michael reaction acceptors such as curcumin and related β-diketones such as dibenzoylmethane which lack direct Michael reactivity. The presence of two structural elements was found to be required for high inducer potency: (i) hydroxyl groups at *ortho*-position on the aromatic rings and (ii) the β-diketone functionality. All curcuminoids elevate the specific activity of quinone reductase in both wild type and mutant cells defective in either the aryl hydrocarbon (*Ah*) receptor or cytochrome P4501A1 activity. This indicates that neither binding to this receptor, nor metabolic activation by P4501A1 are required for the signaling process originating from this family of electrophiles and ultimately resulting in Phase 2 enzyme induction.

Introduction

Curcumin **1** (diferuloylmethane) is a component of turmeric, a yellow spice extracted from the rhizome of the East Indian herb *Curcuma longa* L. (Zingiberaceae), that is widely used as a food flavoring and coloring agent (e.g. in curry). Extracts of the plant rich in curcumin usually also contain lesser quantities of demethoxycurcumin **2** and bisdemethoxycurcumin **3**. Curcumin has a long history of medicinal use in India and Southeast Asia for a wide variety of medical conditions (1) and has been shown in experimental studies to have anti-inflammatory properties, to prevent tumorigenesis and mutagenesis, to block carcinogen–DNA adduct formation, and to inhibit angiogenesis (2–5). Interest in curcumin and its promising cancer-preventive potential is growing, especially since it does not appear to be significantly toxic (1). Plans for its clinical development as an anticancer agent are in progress (6).

Several possibilities have been raised regarding the potential mechanisms of the observed antitumor effects of curcumin, and among these its antioxidant and anti-inflammatory properties have received major attention. Curcumin scavenges active oxygen species including superoxide, hydroxyl radical, and nitric oxide (7,8). It decreases the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced expression of *c-jun*, *c-fos* and *c-myc* proto-oncogenes (9), and suppresses activation of nuclear factor κB (NF-κB) (10). Curcumin represents a candidate for a natural chemoprotective agent, since it also elevates the activities of Phase 2 detoxification enzymes of xenobiotic metabolism, such as glutathione transferases (11) and NAD(P)H: quinone reductase (QR) (5), while inhibiting procarcinogen activating Phase 1 enzymes, such as cytochrome P4501A1 (12).

In the light of compelling evidence that the coordinate induction of Phase 2 enzymes [e.g. glutathione transferases, NAD(P)H:QR, glucuronosyltransferases, epoxide hydrolase] is a critical and sufficient condition for protection against toxicity and carcinogenicity (13,14), the present study was designed to define which structural features of the curcumin molecule contribute to its ability to serve as an inducer of Phase 2 enzymes. For this purpose, we employed the Prochaska test (15), which provides a highly quantitative bioassay that measures quinone reductase activity (a prototype Phase 2 enzyme) in Hepa1c1c7 murine hepatoma cells grown in 96-well microtiter plates. Potencies of inducers are customarily expressed as concentration required to double the quinone reductase specific activity (CD value). The response of this cell line to a variety of inducers mimics the response of many rodent tissues. Furthermore, the ability to predict very accurately the competence or incompetence of a given compound to raise tissue levels of Phase 2 enzymes and ultimately to protect against toxicity and carcinogenicity is a recognized merit of this assay system.

With use of animal models, as well as the microtiter plate assay, it was established unambiguously in this laboratory that Michael reaction acceptors (olefins or acetylenes conjugated to electron-withdrawing groups) constitute a major class of Phase 2 enzyme inducers, and their inducer potency was closely correlated with their reactivities with nucleophiles in the Michael reaction (16). Not surprisingly, curcumin **1** [1,7-bis-(3-methoxy-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione], which contains two Michael reaction acceptor functionalities in its molecule, induced quinone reductase with a CD value of 7.3 μM (Figures 1 and 2).

The initial goals of our experiments were to establish the contribution of individual portions of the curcumin molecule to its inducer potency. Thus, vanillin **7** (3-methoxy-4-hydroxybenzaldehyde) was chosen as the first model compound in order to determine whether an aromatic ring system possessing one methoxyl and one hydroxyl substituent, but no Michael reaction acceptor functionality, could induce quinone reductase. As expected, vanillin **7** was inactive (Figure 3). Next, ferulic acid **8**, which contains an olefin conjugated to a carboxyl group (weakly electron withdrawing) was tested. In this case as well, no inducer activity was found. However, its methyl ester **9**, a better Michael reaction acceptor, was a definite although weak inducer (CD = 83 μM). Removal of the methoxyl substituent, as in methyl *p*-coumarate **10** (CD = 83 μM) or methyl *m*-coumarate **11** (CD = 83 μM), did not affect the inducer potency. In sharp contrast, the methyl ester

Abbreviations: *Ah* receptor, aryl hydrocarbon receptor; DMBA, 7,12-dimethylbenz[*a*]anthracene; NF-κB, nuclear factor κB; QR, quinone reductase; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

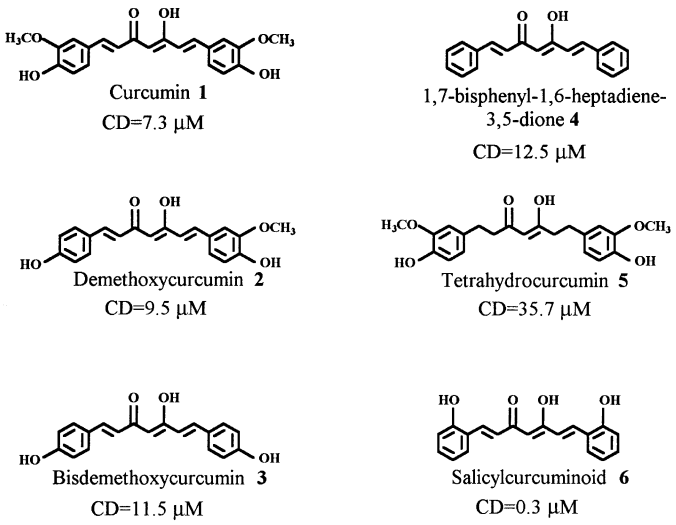

Fig. 1. Structures of curcuminoids tested in this study and their inducer potencies (CD values) in the QR assay in Hepa1c1c7 murine hepatoma cells.

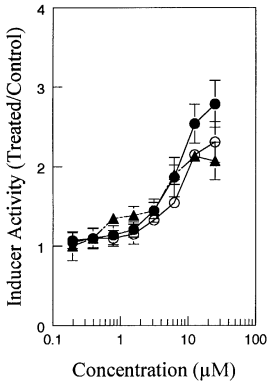

Fig. 2. Induction of QR as a function of concentration by curcumin in murine hepatoma cells Hepa 1c1c7 (●) and its mutants Bp^{r}c1 (○) and c1 (▲).

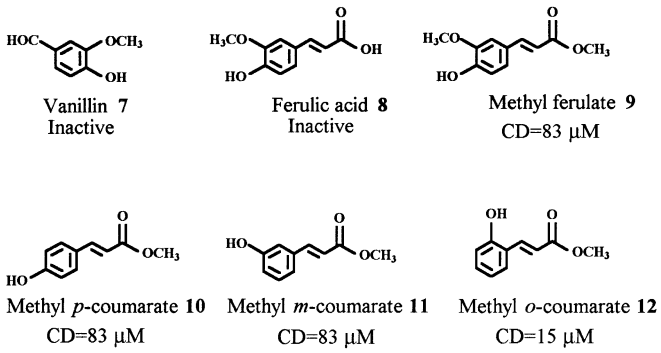

Fig. 3. Structures of vanillin and cinnamic acid derivatives used as model compounds and their inducer potencies (CD values) in the QR assay in Hepa1c1c7 murine hepatoma cells.

of *o*-coumaric acid **12** was a much better inducer (CD = 15 μM), indicating the critical contribution of an *o*-hydroxyl substituent to inducer potency.

As expected on the basis of the above observations, removal of one methoxyl group from the molecule of curcumin, as in demethoxycurcumin **2**, affected the inducer potency only very slightly (CD = 9.5 μM) (Figure 1). Removal of both methoxyl groups, as in bisdemethoxycurcumin **3** [1,7-bis-(4-hydroxy-

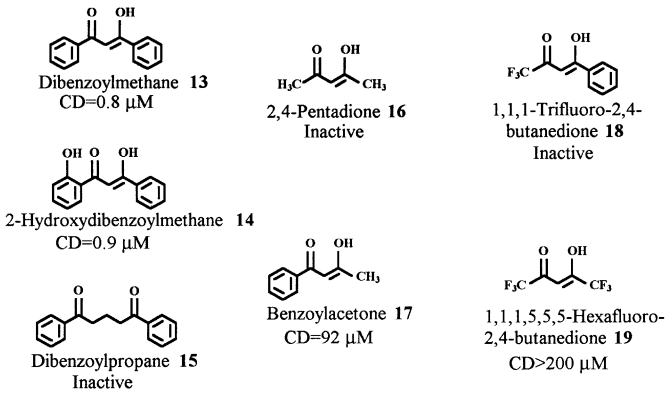

Fig. 4 Structures of diketone model compounds and their inducer potencies (CD values) in the QR assay in Hepa1c1c7 murine hepatoma cells.

phenyl)-1,6-heptadiene-3,5-dione] (CD = 11.5 μM) decreased the inducer potency negligibly. The unsubstituted derivative **4** [1,7-bis-phenyl-1,6-heptadiene-3,5-dione] was found to have essentially the same potency (CD = 12.5 μM). Surprisingly, tetrahydrocurcumin **5** (not a classical Michael reaction acceptor) was still found to elevate QR, although much more weakly, with a CD value of 35.7 μM (Figure 1). These findings are in agreement with the studies by Huang *et al.* (17) and Anto *et al.* (18) who showed that curcumin **1**, demethoxycurcumin **2** and bisdemethoxycurcumin **3** were all very potent inhibitors of TPA (phorbol ester)-induced mouse ear edema and skin carcinogenesis, respectively, whereas tetrahydrocurcumin **5** was less effective. However, when tested as a direct antioxidant *in vitro*, tetrahydrocurcumin **5** was very potent and a mechanism was proposed, which involves its β-diketone moiety (19). The possible significance of the same functionality in the process of QR induction was suggested by two facts: (i) β-diketones retain some features of a Michael reaction acceptor because of keto-enol tautomerism; furthermore the keto-enol form is stabilized by the formation of an intramolecular hydrogen bond (20); (ii) the 'parent' compound ferulic acid is inactive. Inducer activity is acquired when two ferulic acid molecules are linked together via a methylene bridge to give a β-diketone. To test this conclusion, simpler β-diketone compounds were examined. Thus, the structurally related dibenzoylmethane **13** was found to be 10 times more potent (CD = 0.8 μM) than was curcumin (Figure 4). The inducer potency of its *ortho*-hydroxylated derivative **14** (CD = 0.9 μM) was essentially the same. It has been shown that intramolecular hydrogen bonding is responsible for the dominance of the keto-enol tautomers of both dibenzoylmethane and 2-hydroxydibenzoylmethane in non-polar, as well as polar environments (21,22). That the observed QR inducer activity of these compounds is specifically due to their keto-enol tautomers was further supported by the finding that dibenzoylpropane **15** was inactive as an inducer.

Derivatives of dibenzoylmethane occur in several plant families, mainly bearing isoprenoid- or furano-substitutions on the aromatic rings, or more rarely an allyl group bonded to the central carbon of an aliphatic chain (23,24). Existing exclusively as keto-enolic tautomers, such molecules have been described as 'β-hydroxychalcones'. Some accumulate upon pathogen attack (25), others are potent antimutagens (26) and powerful sunscreens in the UVA region (27). Furthermore, 1% of dibenzoylmethane in the diet potently reduces the incidence of 7,12-dimethylbenz[*a*]anthracene (DMBA)-

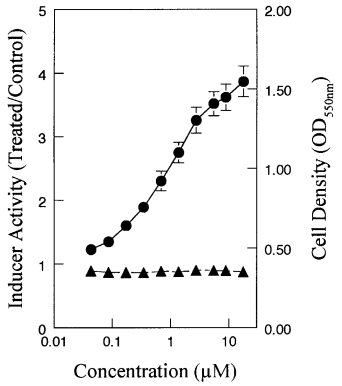

Fig. 5. Induction of QR by salicylcurcuminoid as a function of concentration in Hepa1c1c7 cells. ●, inducer activity; ▲, cell density.

induced mammary tumors in the rat (28) and the mouse (29). Importantly, in the same experimental models, 1% dietary curcumin **1** was without effect, implying large differences in their bioavailability and/or mechanism(s) of action.

The next question was whether other types of compounds containing the tautomeric keto-enol function would be inducers. To this end, four 2,4-pentanedione derivatives were tested, namely 2,4-pentanedione **16**; 1-benzoylacetone **17**; 1,1,1-trifloro-2,4-butanedione **18** and 1,1,1,5,5,5-hexafluoro-2,4-pentanedione **19**. All were either very weakly effective or inactive as inducers (Figure 4). Taken together, these findings established that neither the presence of a keto-enol functionality nor of the aromatic ring system is sufficient alone to provide inducer activity; both must be present to confer this biological activity.

Since it was concluded previously that the only substituent on the aromatic ring with significant impact on the inducer potency was a hydroxyl group at the *ortho*-position with respect to the Michael reaction acceptor portion of the molecule (30 and this study), it was important to examine the biological effect of such a substituent on a curcumin analog. For this purpose, salicylcurcuminoid **6** [1,7-bis-(2-hydroxyphenyl)-1,6-heptadiene-3,5-dione] was synthesized according to the method of Dinesh Babu and Rajasekharan (31). The crystalline product had the appropriate ^{1}H NMR spectrum (DMSO-d_6, 300 MHz) δ (p.p.m.): 6.16 (1H, s, C_4-H), 6.91 (2H, d, J=16 Hz, C_2-H + C_6-H), 6.95–7.00 (4H, m, Ar-H), 7.29 (2H, m, Ar-H), 7.68 (2H, d, Ar-H), 7.92 (2H, d, J=16 Hz, C_1-H + C_7-H), 10.3 (2H, s, Ar-OH). When tested as a QR inducer, it was shown to have a CD value of 0.3–0.4 μM (Figures 1 and 5), and consequently was the most potent inducer in the curcuminoid series identified by us. Importantly, no cytotoxicity was observed even at 50-fold higher concentrations (the highest tested) in this assay system (Figure 5). The reason for this >30-fold increase in inducer potency of salicylcurcuminoid compared with the parent compound **4** is not clear. However, the impact of the *o*-hydroxyl groups is critical, as shown here for the model compound methyl *o*-coumarate **12**, as well as in a study by Anto *et al*. (18), involving a two-stage mouse skin tumor-promotion model. These researchers demonstrated that salicylcurcuminoid **6** was the most effective tumor inhibitor among a series of curcuminoids, completely inhibiting the appearance of papillomas for 10 weeks, at which time point 90% of the control animals had developed tumors. This provides further support for the view that Phase 2 detoxification enzyme induction, as monitored by the elevation of the specific activity of QR, can be used as a highly reliable tool to predict the *in vivo* tumor-preventive properties of various natural and synthetic products.

Investigations directed towards unraveling the molecular mechanism(s) of induction of enzymes of xenobiotic metabolism have revealed that some planar aromatic hydrocarbons are capable of elevating the activities of both Phase 1 (cytochromes P450) and Phase 2 enzymes, and have been designated bifunctional inducers (32). These compounds are ligands for the aryl hydrocarbon (*Ah*) receptor. After binding, the ligand–receptor complexes are transported into the nucleus, and ultimately act as ligand-induced transcription factors. It has been suggested that such inducers undergo metabolism by cytochromes P450, and the resultant (usually electrophilic) products then act as inducers of Phase 2 enzymes via the antioxidant (electrophile) response element (ARE or EpRE). In contrast, a second family of inducers (designated monofunctional inducers) do not require a functional *Ah* receptor or the cytochromes P450 regulated by this receptor, and can selectively elevate the activities of Phase 2 enzymes without significant effects on Phase 1 enzymes. By replacing Hepa1c1c7 cells in the Prochaska test with mutant hepatoma cells, lacking either an intact *Ah* receptor (e.g. Bp^{r}c1) or a functional cytochrome P4501A1 gene (e.g. c1), it is possible to determine whether an inducer falls into the monofunctional or bifunctional category, and which of these systems (if not both) is involved in the mechanism of induction of QR by a particular stimulant.

When induction of QR by curcumin was compared in Hepa1c1c7 murine hepatoma cells and the two aforementioned mutants that lack a functional *Ah* receptor or cytochrome P4501A1 gene product, the inducer potencies of the curcuminoids tested here were similar (Figure 2) with the following CD values: curcumin, 8.9 and 10.9 μM; demethoxycurcumin, 12.5 and 14.3 μM; bisdemethoxycurcumin, 19.1 and 21.7 μM; and salicylcurcuminoid, 0.8 and 3.1 μM, respectively. In the same experiment, β-naphthoflavone, a very potent synthetic bifunctional inducer had a CD of 15 nM in the wild-type Hepa1c1c7 cells, but was inactive as an inducer in both mutants. Curcumin would accordingly be classified as a monofunctional inducer. However, a recent study has indicated that curcumin can also be a Phase 1 enzyme inducer. It binds to the *Ah* receptor, activates the transcription of the cytochrome P4501A1 gene, and elevates its enzyme activity (12). Curcumin is therefore a bifunctional inducer, but differs from 'conventional' bifunctional inducers (such as polycyclic aromatic hydrocarbons, β-naphthoflavone and azo dyes), in that induction of Phase 2 enzymes proceeds by transcription-signaling mechanisms that are independent of the *Ah* receptor or its gene targets. The existence of such mechanisms has been suggested earlier (32,33), and curcumin provides a clear-cut example.

In conclusion, curcumin and a number of naturally occurring and synthetic analogs are Phase 2 enzyme inducers, as demonstrated by their ability to elevate the enzyme activity of QR in murine hepatoma cells. It is reasonable to assume that Phase 2 enzyme induction plays a significant role in the chemoprotective and antioxidant activities of these curcuminoids. The introduction of aromatic *o*-hydroxyl groups into the curcuminoid skeleton raises the inducer potency >30-fold. The activities of these compounds are generally attributable to their Michael reaction acceptor centers. The finding that dibenzoylmethane, a β-diketone that is not a classical Michael reaction acceptor, is also a potent inducer, focuses attention

on the fact that the β-diketone moiety of curcuminoids may also play a significant role in their biological activities.

Acknowledgements

We thank Professor Gary H.Posner and M.Cristina White for advice on the synthesis of salicylcurcuminoid, and Jack Arbiser and Mou-Tuan Huang for the generous gift of pure samples of curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin. This work was supported by a program-project (PO1 CA 44530) from the National Cancer Institute, Department of Health and Human Services, and by a postdoctoral fellowship (to A.D.-K.) from the Cancer Research Foundation of America.

References

1. Ammon,H.P.T. and Wahl,M.A. (1991) Pharmacology of *Curcuma longa*. *Planta Med.*, **57**, 1–6.
2. Huang,M.-T., Lou,Y.-R., Ma,W., Newmark,H.L., Reuhl,K.R. and Conney,A.H. (1994) Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. *Cancer Res.*, **54**, 5841–5847.
3. Kim,J.M., Araki,S., Kim,D.J. *et al.* (1998) Chemopreventive effects of carotenoids and curcumins on mouse colon carcinogenesis after 1,2-dimethylhydrazine initiation. *Carcinogenesis*, **19**, 81–85.
4. Mukundan,M.A., Chacko,M.C., Annapurna,V.V. and Krishnaswamy,K. (1993) Effect of turmeric and curcumin on BP–DNA adducts. *Carcinogenesis*, **14**, 493–496.
5. Arbiser,J.L., Klauber,N., Rohan,R., van Leeuwen,R., Huang,M.-T., Fisher,C., Flynn,E. and Byers,H.R. (1998) Curcumin is an *in vivo* inhibitor of angiogenesis. *Mol. Med.*, **4**, 376–383.
6. Kelloff,G.J., Crowell,J.A., Hawk,E.T. *et al.* (1996) Strategy and planning for chemopreventive drug development: clinical development plans II. *J. Cell. Biochem. Suppl.*, **26**, 54–71.
7. Kunchandy,E. and Rao,M.N.A. (1990) Oxygen radical scavenging activity of curcumin. *Int. J. Pharmacol.*, **58**, 237–240.
8. Sreejayan and Rao,M.N.A. (1997) Nitric oxide scavenging by curcuminoids. *J. Pharm. Pharmacol.*, **49**, 105–107.
9. Kakar,S.S. and Roy,D. (1994) Curcumin inhibits TPA induced expression of c-fos, c-jun and c-myc proto-oncogenes messenger RNAs in mouse skin. *Cancer Lett.*, **87**, 85–89.
10. Singh,S. and Aggarwal,B.B. (1995) Activation of transcription factor NF-κB is suppressed by curcumin (diferuloylmethane). *J. Biol. Chem.*, **270**, 24995–25000.
11. Susan,M. and Rao,M.N.A. (1992) Induction of glutathione *S*-transferase activity by curcumin in mice. *Arzneim.-Forsch.*, **42**, 962–964.
12. Ciolino,H.P., Daschner,P.J., Wang,T.T.Y. and Yeh,G.C. (1998) Effect of curcumin on the aryl hydrocarbon receptor and cytochrome P450 1A1 in MCF-7 human breast carcinoma cells. *Biochem. Pharm.*, **56**, 197–206.
13. Talalay,P., Fahey,J.W., Holtzclaw,W.D., Prestera,T. and Zhang,Y. (1995) Chemoprotection against cancer by Phase 2 enzyme induction. *Toxicol. Lett.*, **82**/**83**, 173–179.
14. Kensler,T.W. (1997) Chemoprevention by inducers of carcinogen detoxication enzymes. *Environ. Health Perspect.*, **105** (Suppl.), 965–970.
15. Prochaska,H.J. and Santamaria,A.B. (1988) Direct measurement of NAD(P)H:quinone reductase from cells cultured in microtiter wells: a screening assay for anticarcinogenic enzyme inducers. *Anal. Biochem.*, **169**, 328–336.
16. Talalay,P., De Long,M.J. and Prochaska,H.J. (1988) Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis. *Proc. Natl Acad. Sci. USA*, **85**, 8261–8265.
17. Huang,M.-T., Ma,W., Lu,Y.-P., Chang,R.L., Fisher,C., Manchand,P.S., Newmark,H.L. and Conney,A.H. (1995) Effects of curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin on *12-O*-tetradecanoylphorbol-13-acetate-induced tumor promotion. *Carcinogenesis*, **16**, 2493–2497.
18. Anto,R.J., George,J., Dinesh Babu,K.V., Rajasekharan,K.N. and Kuttan,R. (1996) Antimutagenic and anticarcinogenic activity of natural and synthetic curcuminoids. *Mutat. Res.*, **370**, 127–131.
19. Sugiyama,Y., Kawakishi,S. and Osawa,T. (1996) Involvement of the β-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. *Biochem. Pharm.*, **52**, 519–525.
20. Pedersen,U., Rasmussen,P.B. and Lawesson,S.-O. (1985) Synthesis of naturally occurring curcuminoids and related compounds. *Liebigs Ann. Chem.*, 1557–1569.
21. Tobita,S., Ohba,J., Nakagawa,K. and Shizuka,H. (1995) Recovery mechanism of the reaction intermediate produced by photoinduced cleavage of the intramolecular hydrogen bond of dibenzoylmethane. *J. Photochem. Photobiol. A: Chemistry*, **92**, 61–67.
22. Hansen,P.E. (1994) Hydrogen bonding and tautomerism studied by isotope effects on chemical shifts. *J. Mol. Struct.*, **321**, 79–87.
23. Demizu,S., Kajiyama,K., Hiraga,Y., Kinoshita,K., Koyama,K., Takahashi,K., Tamura,Y., Okada,K. and Kinoshita,T. (1992) Prenylated dibenzoylmethane derivatives from the root of *Glycyrrhiza inflata (*Xinjiang Licorice). *Chem. Pharm. Bull.*, **40**, 382–395.
24. Magalhaes,A.F., Tozzi,A.M.A., Magalhaes,E.G., Blanco,I.S. and Nogueira, M.A. (1997) Three dibenzoylmethane derivatives from *Lonchocarpus* species. *Phytochemistry*, **46**, 1029–1033.
25. Ayabe,S., Iida,K. and Furuya,T. (1986) Stress-induced formation of echinatin and a metabolite, 5′-prenyl-licodione, in cultured *Glycyrrhiza echinata* cells. *Phytochemistry*, **25**, 2803–2806.
26. Wang,C.Y., Lee,M.-S. and Zukowski,K. (1991) Inhibition by diacylmethane derivatives of mutagenicity in *Salmonella typhimurium* and tRNA-binding of chemical carcinogens. *Mutat. Res.*, **262**, 189–193.
27. Roelandts,R. (1991) Which components in broad-spectrum sunscreens are most necessary for adequate UVA protection? *J. Am. Acad. Dermatol.*, **25**, 999–1004.
28. Singletary,K., MacDonald,C., Iovinelli,M., Fisher,C. and Wallig,M. (1998) Effect of the β-diketones diferuloylmethane (curcumin) and dibenzoyl-methane on rat mammary DNA adducts and tumors induced by 7,12-dimethylbenz[*a*]anthracene. *Carcinogenesis*, **19**, 1039–1043.
29. Huang,M.-T., Lou,Y.-R., Xie,J.G., Ma,W., Lu,Y.-P., Yen,P., Zhu,B.T., Newmark,H. and Ho,C.-T. (1998) Effect of dietary curcumin and dibenzoylmethane on formation of 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors and lymphomas/leukemias in Sencar mice. *Carcinogenesis*, **19**, 1697–1700.
30. Dinkova-Kostova,A.T., Abeygunawardana,C. and Talalay,P. (1998) Chemoprotective properties of phenylpropenoids, bis(benzylidene) cycloalkanones, and related Michael reaction acceptors: correlation of potencies as Phase 2 enzyme inducers and radical scavengers. *J. Med. Chem.*, **41**, 5287–5296.
31. Dinesh Babu,K.V. and Rajasekharan,K.N. (1994) Simplified conditions for the synthesis of curcumin I and other curcuminoids. *Org. Prep. Procedure Int.*, **26**, 674–677.
32. Prochaska,H.J. and Talalay,P. (1988) Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. *Cancer Res.*, **48**, 4776–4782.
33. Hayes,J.D. and Pulford,D.J. (1995) The glutathione *S*-transferase supergene family: regulation of GST and the contribution of the isozymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.*, **30**, 445–600.

Received November 2, 1998; revised January 22, 1999; accepted January 25, 1999