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Important chemical structural features of curcumin and its derivatives: How do they influence their anticancer activity?

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Curcumin is the active component of the Indian spice turmeric, known since ancient times for medicinal properties. Extensive research in the last two to three decades has confirmed its promising pharmacological properties such as anti-cancer, anti-oxidant, anti-inflammatory *etc*., leading to several ongoing/completed clinical trials. Curcumin has three reactive functional groups: one diketone moiety, and two phenolic groups. Curcumin interacts with several biomolecules through non-covalent and covalent binding. However, the properties limiting its potential are low bioavailability and fast degradation. The metabolites as well as degradation products of curcumin show biological activities but not as much as curcumin. To overcome these limitations, new analogues with modifications on both o-methoxy group and the diketo structures of curcumin have been developed. Of several analogues, dimethyl curcumin, where the phenolic OH is absent showed better anti-tumor activity. Also, the isoxazole and pyrazole derivatives of curcumin, derivatized at the diketo moiety have been investigated in our group. Hispolon, which is a half curcumin analogue also showed interesting cellular activity. Here in the present manuscript, the comparative cytotoxic effect of curcumin and some of these derivatives in cancer cells is presented. The results indicated that specific structural modifications on curcumin can be adopted to fine–tune its desired anticancer activity.

Keywords: Curcumin derivatives, Pro-oxidant, Structure-activity correlation

Curcumin, the active principle of *Curcuma* species has been one of the highly researched molecules[1-5]. There are at least 200 known varieties of curcuma species all over the world. Even before curcumin was investigated, turmeric, or *Curcuma longa*, was known to Indians and Chinese as a medicinal herb as mentioned in Indian scriptures, Ayurveda and other related documents[6]. To the Europeans and Americans, turmeric is not much known, the latest, one can trace back is the mention of it by Marcopolo in his travel logs around 13th century[6]. India is the major producer of turmeric[6]. Apart from *Curcuma longa*, other known Curcuma species are *Curcuma zedoaria*, *Curcuma aromatic etc*. Curcumin content in curcuma species varies with the soil, location, climatic conditions *etc*.

Although curcumin was isolated from turmeric nearly two centuries ago, there were only a few reports till the 1970s on its chemical structure, synthesis, and biological activity[4]. However, after reports on its potential anticancer effect were known in 90s, the pace of curcumin research has grown rapidly[1-5] with more than 18000 citations to date. While the majority of researchers have been pursuing the medicinal aspects, a few others were reporting chemistry, development of new analogues and recently its formulations. Curcumin research has become one of the most favorite subjects for all the branches of chemistry, including organic, inorganic, physical and analytical chemists[1-5]. Important findings are extraction methodologies, synthesis of curcumin derivatives, preparation of metal chelates with modified biochemical activities, understanding molecular mechanisms on its interactions with biomolecules and use of its unique spectroscopic properties to identify and quantitatively

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Abbreviations: BDMC, Bisdemethoxycurcumin; CI, Curcumin isoxazole; CP, Curcumin pyrazole; DCFDA, 2′,7′-Dichlorofluorescin diacetate; DHC, Dihydrocurcumin; DIMC, Dimethyl curcumin; DMC, Demethoxycurcumin; GSH, Glutathione; HHC, Hexahydrocurcumin; HME, Hispolonmonomethyl ether; HMEP, Hispolonmonomethyl ether pyrazole; HP, Hispolonpyrazole; HS, Hispolon; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium; OHC, Octahydrocurcumin; ROS, Reactive oxygen species; THC, Tetrahydrocurcumin; TrxR, Thioredoxin reductase

estimate trace elements[1-7]. Other important areas of chemistry research are its reactivity with reactive oxygen species (ROS), degradation and formation of nanoconjugates and formulations[4,8].

The extensive biological research on curcumin has confirmed its anti-inflammatory, chemopreventive and therapeutic potential against varieties of cancers[1-3,5]. Free radicals formed from reactive oxygen and nitrogen species act as key players in the initiation and progression of tumor cells and enhance their metastatic potential[9]. Some of the factors leading to anti-cancer effects of curcumin are inhibition of different types of enzyme kinases or signal transducers, transcription factors and cytokines and induction of apoptosis in cancer cells[1-3,5]. Currently, curcumin is being examined in the clinic against several diseases such as multiple myeloma, pancreatic cancer, colon cancer, head and neck cancers, liver and skin diseases, metabolic disorders, neurological diseases *etc*[10].

Curcumin supplementation is recommended against many chronic diseases. Curcumin has been reported to be safe for humans even in gram quantities[11]. This has led to a big market for curcumin all over the world and curcumin nutraceuticals, curcumin based food products and cosmetics are being sold across the counters[5].

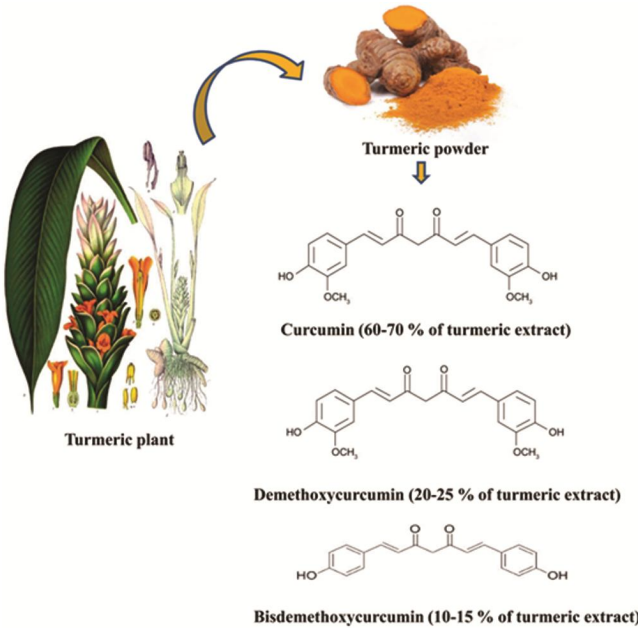
Demand for Ayurvedic medicinal formulations has been gaining momentum in several developed countries as well. As a result of all these, the global curcumin market size has reached USD 23552.9 thousand in 2016 and is projected to see a yearly growth rate of 13.3%[12]. Some of the side effects reported with curcumin administration are its influence in inhibiting blood clotting, aggravating gallstone problems, iron metabolism, liver function *etc*[13].

The major limitation restricting the therapeutic usage of curcumin is its stability and low bioavailability[3,7,14,15]. Even after administration in gram quantities, only a nanogram of curcumin is found in the plasma. Due to its hydrophobic nature, curcumin is poorly soluble in neutral water. It undergoes fast degradation in solution[3,4,7]. The stability of curcumin is crucial to maintain its physiological activities. The decomposition of curcumin is pH-dependent and it degrades more rapidly at neutral-basic conditions[4,7]. The presence of 10% fetal calf serum in cell culture medium and human blood improves the hydrolytic stability of curcumin, and the addition of anti-oxidants such as ascorbic acid, N-acetylcysteine and glutathione (GSH) slows the degradation of curcumin[3,4,7]. Curcumin is also sensitive to light and is rapidly decolorized upon exposure to UV light[7].

Curcumin also participates in a variety of chemical reactions in biological systems. Important among these are the hydrogen donation reactions with ROS leading to oxidation of curcumin, reversible and irreversible nucleophilic addition reactions, hydrolysis, degradation, and enzymatic reactions[4,16-18]. The hydrogen bonding and hydrophobicity of curcumin, arising from the aromatic and tautomeric structures along with the flexibility of the linker group are responsible for the non-covalent interactions with proteins and other biomolecules[4]. The α, β unsaturated β-diketone moiety covalently interacts with protein thiols, through Michael reaction[4,7]. The β-diketo group forms chelates with transition metals, thereby reducing the metal– induced toxicity and some of the metal complexes exhibit improved antioxidant activity as enzyme mimics[19,20].

Curcumin metabolites, degradation products, and synthetic analogues

Turmeric contains three curcumin analogues, curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC), collectively known as curcuminoids (Scheme 1)[1-7]. The three compounds differ in substitution on the aromatic ring, while curcumin has two symmetric o-methoxy phenols



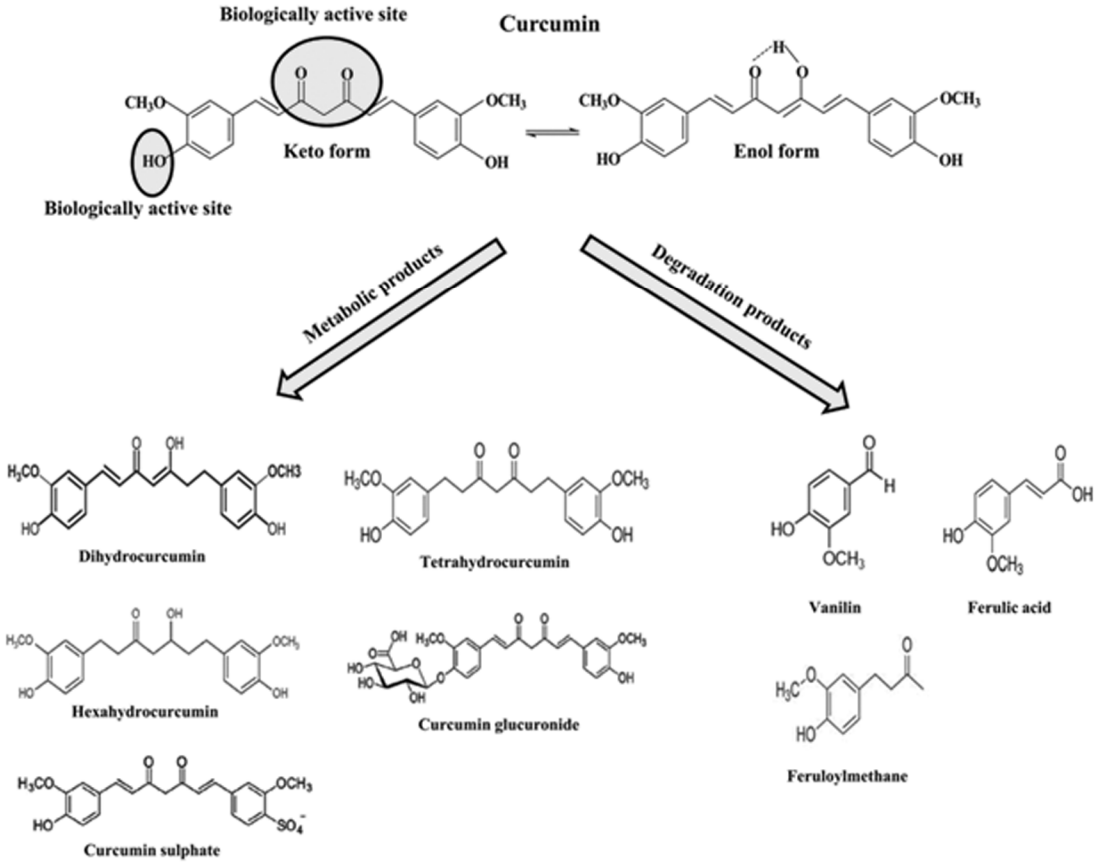
Scheme 1 — Important chemical constituents of turmeric extract

linked through the α, β-unsaturated β-diketone moiety, BDMC, also symmetric but lacks in two o-methoxy substitutions, and DMC has an asymmetric structure with only one o-methoxy substitution. Of the three curcuminoids, curcumin is the most abundant in turmeric, followed by DMC and BDMC. A lesser known curcuminoid from turmeric is cyclocurcumin. Both DMC and BDMC have also been reported to exhibit biological activities.

Metabolism of curcumin produced partially and fully reduced and conjugated derivatives, which include, dihydrocurcumin (DHC), tetrahydrocurcumin (THC), hexahydrocurcumin (HHC), octahydrocurcumin (OHC), curcumin glucuronide, and curcumin sulfate (Scheme 2)[4,7,21]. THC, a partially reduced derivative of curcumin, is one of the major metabolites of curcumin and has also been studied extensively. THC shows excellent antioxidant activity. Other reduced forms of curcumin, HHC and OHC, have not been examined as extensively as THC. The degradation products of curcumin are trans-6-(4′-hydroxy-3′-methoxyphenyl)-2,4,-dioxo-5-hexenal (major products), vanillin, ferulic acid and feruloylmethane (Scheme 2). All these products have been reported to exhibit biological activities[4,22].

Structurally, curcumin has three important functionalities, which can be synthetically modified. These are an aromatic o-methoxy phenolic group, α, β unsaturated diketo moiety and a seven carbon linker. The *o*-methoxyphenol group and methylenic hydrogen are responsible for the antioxidant activity of curcumin, and curcumin donates an electron/hydrogen atom to ROS[4,7]. While the phenolic OH is essential for ROS scavenging, the diketone moiety is necessary for its biological activity. Additionally diketone moiety is involved in its degradation and hydrolysis and also for chelation reaction with many metal ions[4,7,15,19,22].

To overcome the problem of fast degradation and to improve the anti-tumor activity of curcumin, new synthetic derivatives have been made and studied extensively in the literature[4,23-26]. Essentially three main modifications were reported. In the first category, the basic structural features of curcumin, are retained and a slight modification in the three above mentioned functionalities were carried out. In the second group, these analogues have structural

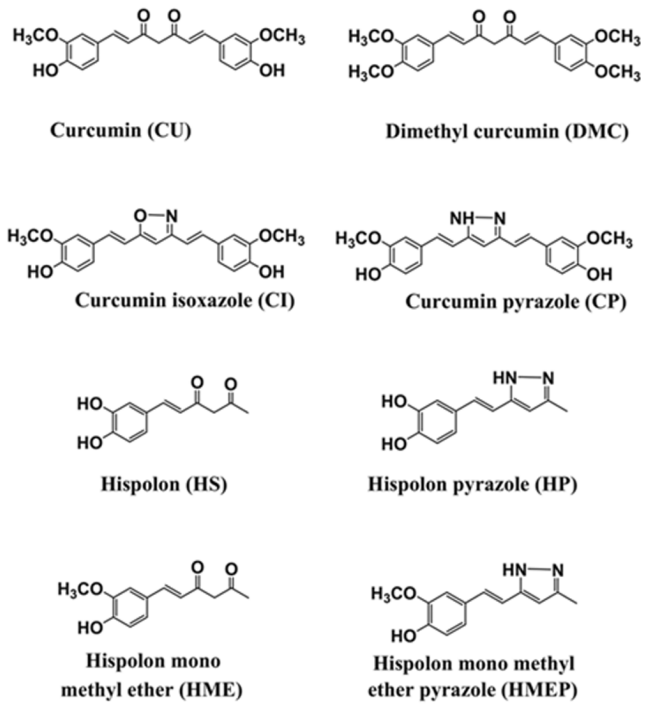


Scheme 2 — Important metabolic and degradation products of curcumin

similarity but do not have basic curcumin skeleton. The third category of compounds is metal complexes of curcumin and its analogues. The number of reports on the second and third groups is vast and outnumber the first group and is not included in this article. In the first category, our group has undertaken research on methylated curcumin analogue, known as dimethyl curcumin (DIMC), where the phenolic OH groups are converted to methoxy groups. DIMC did not show ROS scavenging activity but was found to exhibit better anti-tumor activity than curcumin[27,28]. We have also undertaken studies on pyrazole and isoxazole derivatives of curcumin, where the diketo group converted to more electron rich groups. These compounds showed less degradation and exhibited interesting physico-chemical properties[17]. Finally hispolon (HS), which is also a natural curcumin analogue, having similarity to half curcumin is also investigated for antioxidant and anticancer activities[29]. Results from these three different types of analogues (as shown in Scheme 3) were compared with those from curcumin and included in this manuscript for discussion.

Comparative cytotoxicity studies of curcumin and DIMC in cancer cells

DIMC is the metabolically stable derivative of curcumin[25]. The phenolic hydroxyl moiety of the curcumin is susceptible to the glucuronidation and sulphonation reactions during hepatic metabolism in the body[4,14,21,22]. Since DIMC has phenolic hydroxyl moiety replaced with methoxy group, it shows remarkable resistance against hepatic metabolism. There are only a few reports in the literature on the comparison of the biological activity of DIMC and curcumin[26,27,30]. On similar lines, our group has previously compared the cytotoxicity, cellular uptake and prooxidant activities of curcumin and DIMC in human breast carcinoma (MCF-7) cells[27,31] (Table 1). In brief, human breast carcinoma cells (MCF-7) were treated with equimolar concentration (10 μM, 48 h) of curcumin and DIMC and the viability was determined by MTT assay. Notably, both curcumin and DIMC displayed comparable cytotoxicity (~30%). Subsequently to understand the mechanism of cytotoxicity, the cellular uptake and pro-oxidant activities of curcumin and DIMC were studied in MCF-7 cells[28,31]. The pro-oxidant activity of curcumin and DIMC was measured in terms of their abilities to modulate cellular levels of ROS as well as GSH. The ROS and GSH estimation was done by using standard fluorometric assays (2′,7′-Dichlorofluoresc in diacetate (DCFDA) and o-phthalaldehyde, respectively)[32,33]. This analysis indicated that treatment with either curcumin or DIMC led to a concentration dependant increase in the production of ROS and a concurrent decrease in GSH. At equimolar concentration (10 μM for 2 h) both curcumin and DIMC showed ~3 fold induction in basal ROS level[28,31]. Further, cellular uptake of curcumin and DIMC in cells was estimated by following their absorbance at ~420 nm in cellular lysate as described in our previous reports[34,35]. The cellular uptake of curcumin or DIMC was normalized



Scheme 3 — Chemical structure of curcumin and hispolon derivatives studied in our laboratory

Table 1 — Comparative cytotoxicity and pro-oxidant activity of curcumin and hispolon derivatives in human breast carcinoma (MCF-7) cells[24,27]. Values are mean ± SEM (n=3)

Compounds	Cytotoxicity (MTT assay)	ROS generation (DCFDA assay)
	Treatment condition (10 μM for 48 h)	Treatment condition (10 μM for 2 h)
Curcumin	28±4%	~ 3 folds
DIMC	35 ±3%	~3 folds
CI	42±5%	~1.5 folds
CP	38±2%	~1.5 folds
HS	30±3%	No significant increase
HME	33 ± 5%	No significant increase
HP	2.0 ±0.12%	No significant increase
HMEP	29 ±3%	No significant increase

to cell number or the protein content. From these studies, it was found that cellular uptake of curcumin (44.2 ± 7.2 pmoles/million) in MCF-7 cells was marginally higher as compared to that of DIMC (37.6 ± 5.6 pmoles/million cells)[25,28,31,32]. Subsequently, similar parameters of curcumin and DIMC were determined in human lung carcinoma (A549) cells. The results indicated that DIMC exhibited ~ 3 folds higher (52%) cytotoxicity as compared to that of curcumin (16 %) at an equimolar concentration (10 μM for 48 h) of treatment (Fig. 1A). On the contrary DIMC exhibited ~1.5 folds lesser cellular uptake (~ 220 ng of DIMC/mg of cellular protein) as compared to that of curcumin (~390 ng of curcumin/mg of cellular protein) in this cell line[36,37]. The ROS generation by curcumin and DIMC in A549 cells was comparable to each other (Fig. 1B). From these studies, it appears that the phenolic hydroxyl or methoxy group does not play any role in the pro-oxidant activity of these compounds in the tumor cells as both of these compounds behaved similarly in terms of ROS generation. A second important finding

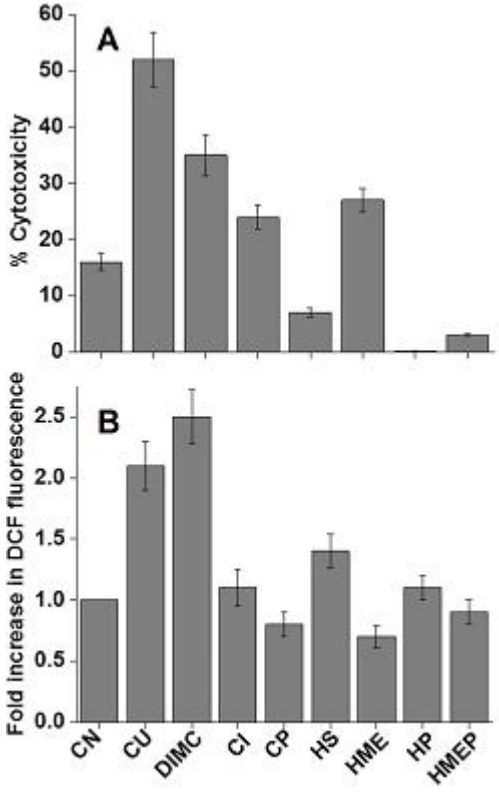


Fig. 1 — The cytotoxic effect of curcumin and hispolon derivatives in human lung carcinoma (A549) cells at equimolar concentration (10 μM) after (A) 48 h of treatment by MTT assay; and (B) 2 h of treatment by DCFDA assay. Values are mean ± SEM (n = 3)

of the above observations is that despite having lower uptake by cells, DIMC exhibits either comparable or higher toxicity than curcumin. One of the explanations for this could be the presence of a methyl group in the ring structure of DIMC increasing its chemical and metabolic stability of DIMC over curcumin. It is also likely that curcumin and DIMC may be differentially regulating signalling proteins like NF-κB, thioredoxin reductase (TrxR) and DNA repair pathways that need to be investigated[30]. The lower cellular uptake of DIMC could be due to its higher hydrophobicity as compared to curcumin. Further to establish the selective cytotoxicity of curcumin derivatives towards cancer cells, our group has also reported the cytotoxicity of curcumin and DIMC in normal cells such as murine splenic lymphocytes by MTT assay[28]. The results of this study indicated that both curcumin and DIMC caused concentration dependant cytotoxicity in spleen lymphocytes. However, when compared at equimolar treatment concentration (10 μM), the percent cytotoxicity caused by curcumin and DIMC in splenic lymphocytes was significantly lower as compared to those observed in MCF-7 and A549 cells[28]. This suggested the differential cytotoxicity of curcumin and DIMC in normal versus tumor cells.

Comparative cytotoxicity studies of isoxazole and pyrazole derivatives of curcumin in cancer cells

As discussed in the previous sections, the α, β-unsaturated diketone group present in the structure of curcumin has been linked with its anticancer activity as well as instability under physiological conditions[4,7]. For example, several reports have established that ketone group of curcumin acts as a Michael acceptor and therefore can form adducts (covalent modification) with the thiol (-SH), groups containing bio-molecules such as GSH and signalling proteins[4,21,22,38]. During this process, curcumin generates ROS as well as affects the functionality of several signalling proteins leading to apoptosis. Based on these studies, several researchers have synthesized curcumin derivatives by a conjugating β-diketo group with pyrazole (CP) or isoxazole (CI) group and have demonstrated the change in its anticancer activities[26,39,40]. These reports together indicated that CP and CI derivatives exhibited higher cytotoxicity compared to curcumin in cancer cell lines. In continuation of these reports, here we have compared the cytotoxicity of curcumin with its CP and CI derivatives in A549 cells under identical treatment

conditions (10 µM for 48 h) by MTT assay. Notably, the cytotoxicity of curcumin derivatives followed the order CI> CP > curcumin (Fig. 1A). A similar trend of cytotoxicity has been reported in MCF-7 cells (Table 1). About pro-oxidant activity, both CI and CP did not induce any significant ROS generation in A549 cells under equimolar treatment condition (10 µM for 2 h) by DCFDA assay (Fig. 1B). From these results, it is clear that the enhanced toxicity of CI and CP is not due to their pro-oxidant activity. Previously researchers have reported that the diketo moiety of curcumin interacts with cellular proteins not only through Michael addition reaction but also by involving non covalent interactions[41]. The conjugation of diketo group of curcumin with electron– rich moieties like pyrazole and/or isoxazole is expected to enhance its interaction with cellular proteins and thus can enhance its cytotoxicity in tumor cells; however, this hypothesis needs to be validated in future[38]. The decrease in the pro-oxidant activity of CI and CP as compared to curcumin is attributed to the unavailability of free ketone group which is responsible for their reactivity towards cellular thiol[4,38]. Finally, there are no reports on the cellular uptake of CP and CP derivatives and therefore it would be interesting in the future to correlate their cytotoxic effects in tumor cells with cellular uptake.

Comparative cytotoxcity studies of HS derivatives with curcumin in cancer cells

As discussed in the introduction section, HS is another important natural product which is structurally similar to curcumin[29]. This compound per se has been reported for various pharmacological activities like anti-inflammatory, anticancer, antioxidant and anti-bacterial among others[42-45]. Encouraged by these reports, our group has recently established the structure– activity correlation of various HS derivatives (Scheme 3)[29,46]. In this study, four derivatives namely HS, hispolonpyrazole (HP), hispolonmonomethyl ether (HME), and hispolonmonomethyl ether pyrazole (HMEP) were evaluated in detail for chemical stability and cytotoxic effects in tumor cells[29]. The chemical stability of HS derivatives was found in the order of HS<HP~HME<HMEP[29]. The instability of HS is attributed to the auto-oxidation of phenolic hydroxyl group as well as hydrolysis of the conjugated diene structure. Accordingly substituting phenolic hydroxyl group with methoxy group in the ring structure of HME and blocking the diketo group with pyrazole group in HMEP increased the stability of hispolon derivatives. Similar observations have been reported previously with curcumin justifying our results[4]. Further, cytotoxicity of HS derivatives was evaluated in cancer cells including A549 and MCF-7 and in murine splenic lymphocytes representing normal cell type (Fig. 1 & Table 1). Notably, all four HS derivatives exhibited lower cytotoxicity as compared to curcumin in tumor cells. Among HS derivatives, HME exhibited higher toxicity followed by HS in tumor cells at equimolar treatment conditions (10 µM for 48 h) (Fig. 1 & Table 1). The compounds, HP and HMEP wherein the diketo moiety is conjugated with pyrazole group showed lesser toxicity compared to respective parent compounds HS and HME under similar treatment conditions (Fig. 1 & Table 1). Comparing the cytotoxic effect of HS derivatives in splenic lymphocytes with those in MCF-7 and A549 cells, it was observed that only HS exhibited selective cytotoxicity towards tumor cells[29]. The mechanistic investigations suggested that modulation of intracellular redox status coupled with the inhibition of intracellular TrxR activity is one of the factors responsible for the cytotoxic effect of HS derivatives in tumor cells[29]. For example, HS per se induced mild oxidative stress in MCF-7 cells by increasing ROS generation and decreasing GSH level (Fig. 1B). On the other hand, HME, HP and MHEP showed induction of reductive environment (lower ROS level) within cells by affecting the utilization-recycling pathway of GSH (Fig. 1B). Further TrxR is a very important intracellular redox enzyme responsible for DNA synthesis and maintaining reductive environment in reduced state[29]. The inhibition or decrease in the activity of TrxR is reported to cause anti-proliferative effects and/or cytotoxicity. The enzyme kinetics and *in silico* analysis indicated that HME was the strongest inhibitor of TrxR followed by HS and respective pyrazole derivatives such as HMEP and HP[29]. Together these results indicated that replacing phenolic hydroxyl with methoxy group and /or conjugating pyrazole moiety with the diketo group in HS changes its behaviour from pro-oxidant to antioxidant nature in cells[29,46]. Additionally, diketo moiety of HS plays an important role in its interaction with cellular protein like TrxR[29]. It would be interesting in the future to understand the interaction of HS derivatives with other important cellular proteins.

Conclusions

The studies reported on various structural modifications of curcumin indicated that its diketo

moiety regulates the redox moldulatory activity. Additionally, diketo moiety is also responsible for the interaction of curcumin with cellular proteins. Since the anticancer activity of curcumin derivatives has mainly been attributed to their redox modulatory behaviours as well as the ability to interact with various signalling proteins, the structural modification of diketo moiety is expected to increase its activity. Indeed isoxazole and pyrazole substitutions at the diketo moiety of curcumin have shown an increase in its anticancer potential. Further, methoxy substitution in the phenolic moiety of curcumin has also shown a very significant increase in its anticancer activity. However, most of these results have come from *in vitro* studies and need to be validated in animal models. Taken together, it is concluded that specific structural modifications on curcumin can be adopted as a tool to fine– tune its anticancer activity.

Conflict of Interest

All authors declare no conflict of interest.

References

1 Prasad S, Gupta SC, Tyagi AK & Aggarwal BB, Curcumin, a component of golden spice: from bedside to bench and back. *Biotechnol Adv*, 32 (2014) 1053.

2 Gupta SC, Patchva S, Wonil K & Aggarwal BB, Discovery of curcumin, a component of the golden spice, and its miraculous biological activities. *Clin Exp Pharmacol Physiol*, 39 (2012) 283.

3 Sanidad KZ, Sukamtoh E, Xiao H, McClements DJ, Zhang G. Curcumin: recent advances in the development of strategies to improve oral bioavailability. *Annu Rev Food Sci Technol*, 10 (2019) 597.

4 Priyadarsini KI, The chemistry of curcumin: from extraction to therapeutic agent. *Molecules*, 19 (2014) 20091.

5 Kunwar A & Priyadarsini KI, Curcumin and its role in chronic diseases. *Adv Exp Med Biol*, 928 (2016) 1.

6 Sasikumar B, Turmeric. In: *Handbook of Herbs and Spices*, (Ed. by KV Peter; Woodhead Publishing Limited, New Delhi), 2012, 526.

7 Priyadarsini KI, Photophysics, photochemistry and photobiology of curcumin: Studies from organic solutions, bio-mimetics and living cells. *J Photochem Photobiol C: Photochem Rev*, 10 (2009) 81.

8 Mukherjee A, Sarkar S, Jana S, Swarnakar S, Das N. Neuro-protective role of nanocapsulated curcumin against cerebral ischemia-reperfusion induced oxidative injury. *Brain Res*, 1704 (2019) 164.

9 Shrivastava A, Aggarwal LM, Mishra SP, Khanna HD, Shahi UP & Pradhan S, Free radicals and antioxidants in normal versus cancerous cells — An overview. *Indian J Biochem Biophys*, 56 (2019) 7.

10 Salehi B, Stojanović-Radić Z, Matejić J, Sharifi-Rad M, Anil Kumar NV, Martins N & Sharifi-Rad J, The therapeutic potential of curcumin: A review of clinical trials. *Eur J Med Chem*, 163 (2019) 527.

11 Hewlings SJ & Kalman DS, Curcumin: A review of its' effects on human health. *Foods*, 6 (2017) 92.

12 https://www.transparencymarketresearch.com/curcumin-market.html.

13 Burgos-Morón E, Calderón-Montaño JM, Salvador J, Robles A & López-Lázaro M, The dark side of curcumin. *Int J Cancer*, 126 (2010) 1771.

14 Anand P, Kunnumakkara AB, Newman RA & Aggarwal BB, Bioavailability of curcumin: problems and promises. *Mol Pharm*, 4 (2007) 807.

15 Kunnumakkara AB, Harsha C, Banik K, Vikkurthi R, Sailo BL, Bordoloi D, Gupta SC & Aggarwal BB, Is curcumin bioavailability a problem in humans: lessons from clinical trials. *Expert Opin Drug Metab Toxicol*, 15 (2019) 705.

16 Priyadarsini KI, Maity DK, Naik GH, Kumar MS, Unnikrishnan MK, Satav JG & Mohan H. Role of phenolic O-H and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. *Free Radic Biol Med*, 35 (2003) 475.

17 Singh U, Barik A, Singh BG & Priyadarsini KI, Reactions of reactive oxygen species (ROS) with curcumin analogues: Structure-activity relationship. *Free Radic Res*, 45 (2011) 317.

18 Shaikh SAM, Singh BG, Barik A, Balaji NV, Subbaraju GV, Naika DB & Priyadarsini KI, Unravelling the effect of β-diketo group modification on the antioxidant mechanism of curcumin derivatives: A combined experimental and DFT approach. *J Mol Struct*, 1193 (2019) 166.

19 Agrawal ND, Nirala SK, Bhadauria M, Srivastava S & Shukla S, Protective potential of *Moringa oleifera* Lam. along with curcumin and piperine against beryllium-induced alterations in hepatorenal biochemistry and ultramorphology in rats. *Indian J Biochem Biophys*, 56 (2019) 70.

20 Barik A, Mishra B, Shen L, Mohan H, Kadam RM, Dutta S, Zhang HY & Priyadarsini KI, Evaluation of a new copper(II)-curcumin complex as superoxide dismutase mimic and its free radical reactions. *Free Radic Biol Med*, 39 (2005) 811.

21 Metzler M, Pfeiffer E, Schulz SI & Dempe JS, Curcumin uptake and metabolism. *Biofactors*, 39 (2013) 14.

22 Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF & Walters MA, The essential medicinal chemistry of curcumin. *J Med Chem*, 60 (2017) 1620.

23 Zhong H, Zhang Z & Jin Y, Research progress in the synthesis of curcumin derivatives. *Pharm Care Res*, 14 (2014) 350.

24 Rao EV & Sudheer P, Revisiting curcumin chemistry Part I: A new strategy for the synthesis of curcuminoids. *Indian J Pharm Sci*, 73 (2011) 262.

25 Wanninger S, Lorenz V, Subhan A & Edelmann FT, Metal complexes of curcumin – synthetic strategies, structures and medicinal applications. *Chem Soc Rev*, 44 (2015) 4986.

26 Bairwa K, Grover J, Kania M & Jachak SM, Recent developments in chemistry and biology of curcumin analogues. *RSC Adv*, 4 (2014) 13946.

27 Tamvakopoulos C, Dimas K, Sofianos ZD, Hatziantoniou S, Han Z, Liu ZL, Wyche JH & Pantazis P, Metabolism and anticancer activity of the curcumin analogue, dimethoxycurcumin. *Clin Cancer Res*, 13 (2007) 1269.

28 Kunwar A, Jayakumar S, Srivastava AK & Priyadarsini KI, Dimethoxycurcumin-induced cell death in human breast carcinoma MCF-7 cells: evidence for pro-oxidant activity, mitochondrial dysfunction, and apoptosis, *Arch Toxicol*, 86 (2012) 603.

29 Chethna P, Iyer SS, Gandhi VV, Kunwar A, Singh BG, Barik A, Balaji NV, Ramani MV, Subbaraju GV & Priyadarsini KI, Toxicity and antigenotoxic effect of hispolon derivatives: Role of structure in modulating cellular redox state and thioredoxin reductase. *ACS Omega*, 3 (2018), 5958.

30 Jayakumar S, Patwardhan RS, Pal D, Sharma D & Sandur SK, Dimethoxycurcumin, a metabolically stable analogue of curcumin enhances the radiosensitivity of cancer cells: Possible involvement of ROS and thioredoxin reductase. *Biochem Biophys Res Commun*, 478 (2016) 446.

31 Kunwar A, Barik A, Sandur SK & Priyadarsini KI, Differential antioxidant/pro-oxidant activity of dimethoxycurcumin, a synthetic analogue of curcumin. *Free Radic Res*, 45 (2011) 959.

32 Eruslanov E & Kusmartsev S, Identification of ROS using oxidized DCFDA and flow-cytometry. *Methods Mol Biol*, 594 (2010) 57.

33 Hissin PJ & Hilf R, A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Anal Biochem*, 74 (1976) 214.

34 Kunwar A, Barik A, Pandey R & Priyadarsini KI, Transport of liposomal and albumin loaded curcumin to living cells: an absorption and fluorescence spectroscopic study. *Biochim Biophys Acta*, 1760 (2006) 1513.

35 Kunwar A, Barik A, Mishra B, Rathinasamy K, Pandey R & Priyadarsini KI, Quantitative cellular uptake, localization and cytotoxicity of curcumin in normal and tumor cells. *Biochim Biophys Acta*, 1780 (2008) 673.

36 Das RP, Singh BG, Kunwar A, Ramani MV, Subbaraju GV, Hassan PA & Priyadarsini KI, Tuning the binding, release and cytotoxicity of hydrophobic drug by Bovine Serum Albumin nanoparticles: Influence of particle size. *Colloids Surf B Biointerfaces*, 158 (2017) 682.

37 Das RP, Gandhi VV, Singh BG, Kunwar A, Kumar N & Priyadarsini KI, Preparation of albumin nanoparticles: optimum size for cellular uptake of entrapped drug (Curcumin). *Colloid Surface A*, 567 (2019) 86.

38 Awasthi S, Pandya U, Singhal SS, Lin JT, Thiviyanathan V, Seifert WE Jr, Awasthi YC & Ansari GA, Curcumin-glutathione interactions and the role of human glutathione S-transferase P1–1. *Chem Biol Interact*, 128 (2000) 19.

39 Chakraborti S, Dhar G, Dwivedi V, Das A, Poddar A, Chakraborti G, Basu G, Chakrabarti P, Surolia A & Bhattacharyya B, Stable and potent analogues derived from the modification of the dicarbonyl moiety of curcumin. *Biochemistry*, 52 (2013)7449.

40 Ishida J, Ohtsu H, Tachibana Y, Nakanishi Y, Bastow KF, Nagai M, Wang HK, Itokawa H & Lee KH, Antitumor agents. Part 214: synthesis and evaluation of curcumin analogues as cytotoxic agents. *Bioorg Med Chem*, 10 (2002) 3481.

41 Shaikh SAM, Singh BG, Barik A, Ramani MV, Balaji NV, Subbaraju GV, Naik DB & Priyadarsini KI, Diketo modification of curcumin affects its interaction with human serum albumin. *Spectrochim Acta A Mol Biomol Spectrosc*, 199 (2018) 394.

42 Ali NAA, Jansen R, Pilgrim H, Liberra K & Lindequist U, Hispolon, a yellow pigment from *inonotus hispidus*. *Phytochemistry*, 41 (1996**)** 927.

43 Wu Q, Kang Y, Zhang H, Wang H, Liu Y & Wang J, The anticancer effects of hispolon on lung cancer cells. *Biochem Biophys Res Commun*, 453 (2014**)** 385.

44 Chen W, Zhao Z, Li L, Wu B, Chen SF, Zhou H, Wang Y & Li YQ, Hispolon induces apoptosis in human gastric cancer cells through a ROS-mediated mitochondrial pathway. *Free Radic Biol Med*, 45 (2008) 60.

45 Balaji NV, Ramani MV, Viana AG, Sanglard LP, White J, Mulabagal V, Lee C, Gana TJ, Egiebor NO, Subbaraju G & Tiwari AK, Design, synthesis and *in vitro* cell-based evaluation of the anti-cancer activities of hispolon analogs. *Bioorg Med Chem*, 23 (2015) 2148.

46 Shaikh SA, Barik A, Singh BG, Modukuri RV, Balaji NV, Subbaraju GV, Naik DB & Priyadarsini KI, Free radical reactions of isoxazole and pyrazole derivatives of hispolon: kinetics correlated with molecular descriptors. *Free Radic Res*, 50 (2016) 1361.