

EXPERT OPINION

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Curcumin: a potential candidate for matrix metalloproteinase inhibitors

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Introduction: Curcumin, a natural yellow pigment of turmeric, has become focus of interest with regard to its role in regulation of matrix metalloproteinases (MMPs). MMPs are metal-dependent endopeptidases capable of degrading components of the extracellular matrix. MMPs are involved in chronic diseases such as arthritis, Alzheimer's disease, psoriasis, chronic obstructive pulmonary disease, asthma, cancer, neuropathic pain, and atherosclerosis.

Areas covered: Curcumin regulates the expression and secretion of various MMPs. This review documents the matrix metalloproteinase inhibitory activity of curcumin on various diseases viz., cancer, arthritis, and ulcer. Finally, the steps to be taken for getting potent curcuminoids have also been discussed in the structure–activity relationship (SAR) section. From this review, readers can get answer to the question: Is curcumin a potential MMPI candidate?

Expert opinion: Numerous approaches have been taken to beget a molecule with specificity restricted to a particular MMP as well as good oral bioavailability; however, nearly all the molecules lack these criteria. Using quantitative structure–activity relationship (QSAR) modeling and virtual screening, new analogs of curcumin can be designed which will be selectively inhibiting different MMPs.

Keywords: arthritis, cancer, curcumin, MMP, MMPI, structure–activity relationship, ulcer

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1. Introduction

Curcumin, a natural yellow pigment, chemically known as 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptane-3,5-dione (Figure 1), is derived from the rhizome of *Curcuma longa* Linn. belonging to the family Zingiberaceae. It is the principal curcuminoid of the popular Indian spice turmeric. Curcumin has wide range of activities against inflammation, ulcer, cancer, Dejerine-Sottas disease, cholangiocarcinoma, diabetes, depression, contraception, viral diseases, etc. [1–5]. Bioavailability of curcumin is poor because it is rapidly metabolized in liver and intestinal wall by glucuronidation. Oral bioavailability of curcumin can be increased when co-administered with piperine. Piperine, an active constituent of black pepper, is a strong hepatic and intestinal aryl hydrocarbon hydroxylation and glucuronidation inhibitor, thus increases the pharmacokinetic property of curcumin [6,7]. A single-blind, randomized, and placebo-controlled study on 20 tropical pancreatitis patients has revealed that oral administration of capsule containing 500 mg of pure extract of curcumin (95%) with 5 mg of piperine, three times a day, can significantly reverse the erythrocyte malonyldialdehyde level [8]. Readers can find more information related to bioavailability and pharmacokinetics of curcumin in a recent review by Belkacemi *et al.* [9]. *Curcuma longa* and *Curcuma aromatica* have shown significant

Article highlights.

- Curcumin and its analogs exhibit MMPI activity by inhibiting different cell signaling pathways including MAPK/NF- κ B/PI3-K and JAK/STAT signaling pathways.
- MMP inhibitory activity of curcumin can be useful for the treatment of cancer, ulcer and arthritis.
- Oral bioavailability and MMP inhibitory potency of curcumin can be altered by structural modifications.

This box summarizes key points contained in the article.

matrix metalloproteinases-13 (MMP-13) inhibitory activity with IC₅₀ values of 27.8 and 85.8 μ g mL⁻¹, respectively [10].

Matrix metalloproteinases (MMPs), a group of extracellular degrading endopeptidases, are known to be involved in most of the physiological and pathological conditions where an extracellular membrane plays its role. Recently, it has been reported for its intracellular activities [11,12]. Thanks to Gross and Lapiere who discovered the involvement of collagenases in resorption of tail in tadpoles, which is an origin of the MMPs research [13]. The term “matrix metalloproteinases” and its numbering system were introduced during an international MMP conference held in Destin, Florida, on September 11 – 15, 1989 [14]. MMPs are classified into different subgroups based on their substrate selectivity and domain structure: collagenases (MMP-1, -8, -13, and -18), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, -10, and -11), matrilysins (MMP-7 and -26), membrane-bound MMPs (MT-MMPs; MMP-14, -15, -16, -17, -24, and -25), and others. MMPs are involved in normal physiological processes such as embryogenesis, reproduction, uterine involution, general maintenance of joints, organ development, angiogenesis, apoptosis, and wound healing. Proteolytic activities of MMPs are inhibited by the specific tissue inhibitors of the metalloproteinases (TIMPs). The net balance of matrix metalloproteinases (MMP) and tissue inhibitor of metalloproteinases (TIMP) system has been known to be a key factor in maintaining ECM homeostasis. Tissue inhibitors of metalloproteinases (TIMPs) are specific inhibitors of matrixins that bind to the highly conserved zinc binding site of active MMPs in 1:1 stoichiometry and participate in controlling the local activities of MMPs in tissues. Any deviation from stoichiometry can lead to diseased condition. Hitherto, four TIMPs were identified: TIMP-1, TIMP-2, TIMP-3, and TIMP-4 [15]. Molecular structure has shown interaction between catalytic domain of MT1-MMP and TIMP-2 which is a big step toward understanding how proMMP-2 assembles with TIMP-2 and MT1-MMP on the cell surface. TIMPs differ in their specificity toward MMPs and in their expression pattern [16]. Under pathological condition, changes of TIMP levels directly affect the level of MMPs that may result in diseases such as arthritis, cancer, atherosclerosis, aneurysms, nephritis, tissue ulcers, and fibrosis [17].

As illustrated in Figure 2, curcumin affects the level of MMPs in extracellular sites by modulating the inducers, such as growth factors and cytokines (IL-1 α , IL-1 β , TNF- α ,

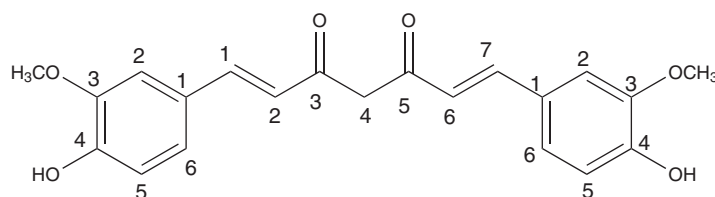
TNF- β , HGF, etc.), occurring primarily at the transcriptional level and are initiated by the binding of the stimulating factor to its cell surface receptor. **JAK/STAT signaling pathway** is mainly activated by cytokines such as interferon $\alpha/\beta/\gamma$. Receptor tyrosine kinases (EGFR, PDGFR), nonreceptor tyrosine kinases, and GPCR also utilize this pathway to transfer signals. Cell-surface receptors act by phosphorylating the STATs. The phosphorylated STATs leave the membrane and then dimerize before migrating into the nucleus where they activate the transcription of a number of target genes. **MAPK signaling** is used to control gene transcription, cell proliferation, apoptosis, motility, synaptic plasticity, etc. It comprises of three major pathways viz. ERK, JNK, and p38 pathways. **NF- κ B signaling pathway:** NF- κ B, a transcription factor, is activated by external stimuli such as TNF- α and IL-1. Upon activation, NF- κ B translocates from cytoplasm to the nucleus and binds to the promoter region of the target gene. NF- κ B is responsible for inflammation, cell proliferation, and apoptosis.

This review documents the cellular, molecular, and biochemical mechanism of action of curcumin in regulation of MMPs in multiple diseases such as cancer, arthritis, and ulcer. Finally, the steps to be taken for getting potent curcuminoids have been discussed in the SAR section. From this review, readers can get answer to the question: Is curcumin a potential MMPI candidate?

2. Cancer

Curcumin has been shown to suppress invasion of tumors through downregulation of MMPs and cell surface adhesion molecules. Demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) show higher antimetastasis potency than curcumin. In addition, active-MMP-2, MMP-9 and uPA levels were reduced by curcumin, DMC, and BDMC in a dose-dependent manner [18]. MMP-2 mRNA and activity of MMP-2 were decreased significantly in curcumin (10 μ g/mL)-treated ECV304 cells and the effect was dose- and time-dependent. Thus, curcumin could inhibit the growth of the vessel lining endothelial cells and decrease the expression of MMP-2 [19]. Curcuminoids inhibit the MMP-9 and angiogenesis induced by fibroblast growth factor in stromal cells of rabbit cornea [20]. Various studies have concluded that curcumin downregulates the expression of a range of NF- κ B-regulated genes including bcl-2, COX-2, MMP-9, TNF, cyclin D1, and the adhesion molecules. Good correlation was observed in binding affinity of tetrahydrocurcumin and bisdemethoxy curcumin against few well-validated targets of anticancer therapy including MMPs indicating that these derivatives were potent procarcinogen activating enzyme inhibitors [21]. MMPs have been considered prognostic factors in various types of cancer as well as promising targets for cancer therapy [22].

It is interesting to underline that western blot analysis showed evidence of presence of TIMP-like proteins within



Curcumin

Figure 1. Structure of curcumin.

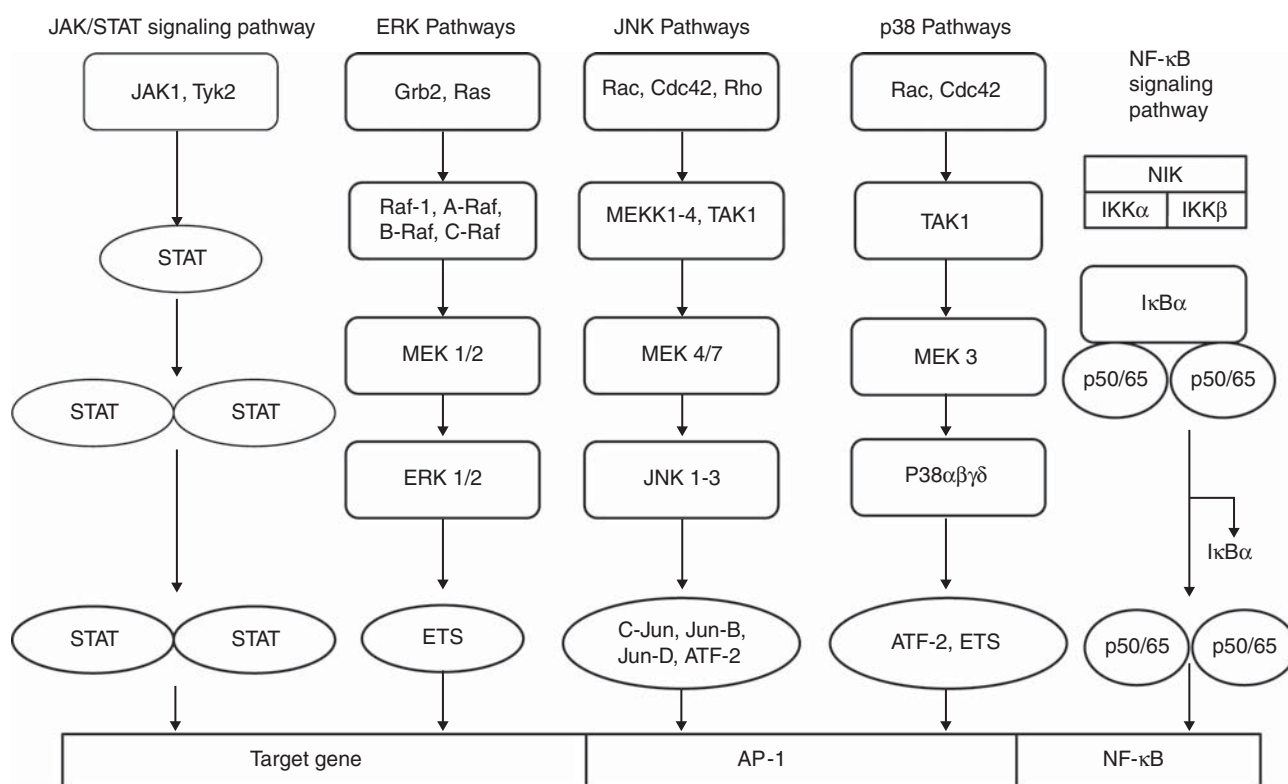


Figure 2. Signaling pathways that contribute to MMPs gene transcription.

AE-941, investigational drugs from standardized extracts derived from shark cartilage, could be responsible for its specific MMP inhibitory property, which are strongly linked to its anti-angiogenic and anti-metastatic effects [23]. There is direct evidence that suggests role of MMPs in tumor invasion and progression and increased expression of TIMPs by either host or tumor cells result in reduced invasion and metastatic capacity of transformed cells. Kwak *et al.* have suggested that TGF-β1 modulates the net balance of the MMPs/TIMPs, the systems in HT1080 cells for anti-invasion and anti-migration by augmenting TIMP-1 through ERK1/2 pathway and Sp1 transcription factor. Using quantitative

real-time PCR method, he demonstrated that TGF-β1 significantly augmented MMP-2 and TIMP-1 mRNA levels in HT1080 cells [24]. Curcumin has also been demonstrated to affect a number of cellular adhesion molecules involved in the processes of tumor growth and metastasis. A study of curcumin in metastatic melanoma demonstrated a dose-dependant reduction in binding to extracellular matrix proteins, decreased expression of alpha5beta1 and alpha5beta3 integrin receptors and increased expression of various anti-metastatic proteins including tissue inhibitor metalloproteinase (TIMP-2), nonmetastatic gene 23(Nm23) and E-cadherin [25].

When osteopontin (OPN) – a member of the ECM protein – binds to its integrin receptor, it induces phosphorylation and degradation of inhibitor of nuclear factor κ B ($\text{I}\kappa\text{B}\alpha$) by enhancing the activity of $\text{I}\kappa\text{B}\alpha$ kinase (IKK). Then, translocation of NF- κ B from cytoplasm into nucleus takes place, and raises the levels of MT1-MMP in the nucleus. Elevated levels of MT1-MMP on the cell surface assist the activation of pro-MMP-2 into active MMP-2. Curcumin inhibits OPN-induced NF- κ B-mediated pro-MMP-2 activation by blocking the $\text{I}\kappa\text{B}\alpha$ /IKK signaling pathways. Thus, curcumin inhibits OPN-induced cell proliferation, cell migration, and extracellular matrix invasion [26].

2.1 Lung cancer

Curcumin suppresses migration and invasion of human non-small cell lung cancer cells (A549) by inhibition of MMP-2 and -9 expressions through MEKK3, p-ERK signaling pathways which suggest that curcumin has anti-metastatic potential by decreasing invasiveness of cancer cells [27]. Further, Li *et al.* revealed a dose-dependent proliferation-inhibitory effect of curcumin on A549 cells mediated by ERK1/2 MAPK signal pathway with decreased expression of p-ERK1/2 and MMP-9, and increased expression of TIMP-1 protein [28]. Cigarette smoke condensate (CSC) – benzo[*a*]pyrene and free radicals such as superoxide radicals, hydroxyl radicals and hydrogen peroxide – are known to activate NF- κ B. CSC-induced NF- κ B activation and NF- κ B-regulated gene expressions were suppressed in human bronchial epithelial cells (BEAS-2B), human nonsmall cell lung carcinoma (H1299), and human lung epithelial cell carcinoma (A549) by curcumin through inhibition of $\text{I}\kappa\text{B}\alpha$ kinase. This owes to the decreased expression of CSC-induced NF- κ B-dependent MMP-9 level as well as cyclin D1 and cyclooxygenase-2 [29].

2.2 Brain cancer

Curcumin inhibits GRB2, Ras, PKC, MKK7, FAK, Rho A, ROCK1, MMP-2, MMP-9, iNOS, NF- κ B p65, COX-2, JNK1/2, and ERK1/2 expressions in mouse–rat hybrid retina ganglion cells (N18) in a time-dependent manner via MEKK, ERK, and NF- κ B signaling pathways [30]. It suppresses PMA-induced MMP-1, -3, -9, and -14 expressions in human astrogloma cells by inhibiting PKC. MMP-9 expression is mediated via NF- κ B and AP-1, and dependent on ERK, JNK, and p38 MAPK signaling pathways. Therefore, it might have therapeutic potential for controlling the growth and invasiveness of brain tumor [31,32]. Bcl-2 and MMP-9 promote the pathogenesis and progression of medulloblastoma which are regulated by the transcription factor NF- κ B. Curcumin inhibits cell proliferation and migration by down-regulation of bcl-2 and bclxl through NF- κ B suppression leading to caspase-mediated cell death [33].

2.3 Breast cancer

Curcumin formulated with phosphatidyl-choline exhibited fivefold increased oral bioavailability in rodents and human, and significantly reduced the expression of MMP-9 and

lung metastasis of mammary gland tumor cell line (ENU1564), thus claimed to be more effective anticancer agent [34]. Western blot analysis using specific antibodies showed that curcumin suppresses the paclitaxel-induced expression of MMP-9. Dietary administration of curcumin in a human breast cancer xenograft model has significantly decreased the incidence of breast cancer metastasis to the lung through decreased expression of MMP-9 and other NF- κ B-regulated gene products [35]. Recently, it has been found that curcumin suppresses MMP-2 and -9 expressions by the inhibition of both mammalian target of rapamycin (mTOR) and NF- κ B pathways through a cross talk between phosphatidylinositol 3-kinase/Akt/ $\text{I}\kappa\text{B}\kappa$ kinase signaling axis [36].

Curcumin significantly inhibits adhesion, motility, invasion, and MMP-9 activity of breast cancer cell lines, MCF-7 and MDA-MB-231 [37,38]. Like curcumin, hydrazinocurcumin also inhibits invasion and migration of the human breast cancer cells MDA-MB-231 by inhibiting STAT3 activity, and thereof reduces MMP-2 and MMP-9 expressions [39]. Dramatic upregulation of MMP-9 secretion by splenic and tumor-infiltrating T lymphocytes from D1-DMBA-3 mammary tumor-bearing mice has been found compared to T cells from normal animals. Thus, MMP-9 promotes neoplastic proliferation, angiogenesis, and invasion in tumor-infiltrating lymphocytes [40]. On investigating the anti-metastatic properties of curcumin at various levels (*viz.* DNA, messenger, and enzyme), curcumin shows anti-invasive effects in human breast cancer cells which depends on the decrease in MMP-2 and on the increase in TIMP-1 levels [41,42].

2.4 Miscellaneous cancers

The antiproliferative effects of curcumin on progression of head and neck squamous cell carcinoma (HNSCC) through inhibiting cancer cell migration and invasion have been evidenced, where downregulation of pS6 (mTOR's downstream target) was associated with a significant decrease in MMP-9. Curcumin inhibits the adverse effects of nicotine by blocking nicotine-induced activation of the Akt/mTOR pathway in HNSCC, thus it may be useful as an oral chemopreventive agent [43]. In highly invasive human YD-10B oral squamous carcinomas cell lines, curcumin inhibited activation of ERK/MAP kinase and NF- κ B that consequently downregulated uPA and MMP-2/9 expression [44].

A significant clinical correlation has been established between increased MMP levels and poor prognosis of prostate cancer [45]. Decreased expressions of MMP-2 and -9 were noticed in curcumin-treated prostate cancer cells (DU-145). Curcumin has also significantly reduced the metastatic nodules in a xenocraft model [46]. TNF- α -related apoptosis-inducing ligand (TRAIL) can be sensitized with curcumin to TRAIL-induced apoptosis of prostate cancer cells by inhibition of NF- κ B and its gene products such as cyclin D1, VEGF, uPA, MMP-2, MMP-9, Bcl-2, and Bcl-XL [47]. Combination of curcumin with paclitaxel can be an effective

therapy for hormone-refractory prostate cancer (HRPC). This combination significantly reduced the MMP-2 gene expression and proliferating cell nuclear antigen (PCNA) in human prostate cancer cells (PC3) xenografted tumor model, and decreased the side effect of paclitaxol (reduced dose) [48].

Probable molecular mechanism of curcumin, on invasion and metastasis of the human cervical cancer cells (Caski), could be the inhibition of expression of MMP-2, MT1-MMP, and NF- κ B. Proliferation of Caski cells has been inhibited in a dose- and time-dependent manner. The expression of MMP-2, MT1-MMP, and NF- κ B has been decreased when treated with 50 μ mol/L curcumin for 72 h [49].

Curcumin targets several cell signaling pathways including cell cycle regulatory protein and has shown distinct apoptotic role in different types of colon cancer cell lines. Overexpressions of MMP-1, -2, -3, -7, -9, -13, and MT1-MMP have been demonstrated in human colorectal cancers and are correlated with different stages of disease and/or prognosis [50]. Curcumin downregulates the expression of NF- κ B, COX-2, MMP-2, MMP-9, Ca^{2+} mobilization and inhibits the neurotensin-mediated AP1 activation in colon cancer [51].

Antimetastatic effect of curcumin has been demonstrated in highly invasive SK-Hep-1 cell line of human hepatocellular carcinoma (HCC). Curcumin inhibited 17.4% and 70.6% of cellular migration and invasion of SK-Hep-1, respectively, at the dose of 10 μ M. It also inhibits MMP-9 secretion in SK-Hep-1 in a dose-dependent fashion [52]. Treatment of human laryngeal cancer cells (HEp2) with curcumin has resulted in decreased expression of MMP-2, FAK, MT1-MMP, and integrin receptors. Downregulation of integrin receptors and low levels of FAK may hinder integrin-mediated signal transduction and prevents upregulation of MMP-2 activity. MMP-2 mRNA expression has been abolished on curcumin treatment, indicates specific inhibition of MMP-2 [53]. Curcumin potentiates the anticancer effect of gemcitabine in pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of NF- κ B-regulated gene products including MMP-9 [54].

A novel curcumin analog, FLLL32 (Figure 3), inhibited STAT3 DNA binding and induced proteasome-mediated degradation of STAT3, resulting in a subsequent loss of VEGF, MMP-2, and survivin expression, thus downregulates proliferation and promotes apoptosis of human osteosarcoma cells [55]. Intraperitoneal injection of curcumin (50 mg/kg) to melanoma cell line B16-bearing mice has decreased the level of MMP-2 and MT1-MMP mRNA by about 50.94% and 52.85%, respectively [56].

All together, curcumin can be used as a broad-spectrum modulator offering clinical benefits as an anticancer agent.

3. Inflammation

Curcumin significantly downregulates TNF- α , IL-1 β , EMM-PRIN, MMP-2, and MMP-9 expressions by inhibiting

NF- κ B in inflammatory conditions [57-59]. MMPs play role in inflammation directly by tissue destruction or indirectly by generation of an inflammatory signal or recruitment of inflammatory cells which leads to a pathological state [60]. Elevated level of MMPs in acute and chronic wound fluid suggests that nonhealing ulcers develop an environment containing high levels of activated metalloproteinases, which may result in chronic tissue turnover and failure of wound closure [61].

Detailed RNA interference study of human mesenchymal stem cells (hMSCs) revealed that gene knockdown of MMP-2, MT1-MMP, or TIMP-2 substantially impaired hMSC invasion, whereas silencing of TIMP-1 enhanced cell migration, indicating opposing roles of TIMP-1 and TIMP-2 in the process of hMSC mobilization and homing. The role of inflammatory cytokines such as TGF- β 1, IL-1 β , and TNF- α but not SDF-1 was also identified to strongly promote chemotactic invasion of hMSCs by upregulation of MMP activity [62].

3.1 Arthritis

A short-term, double-blind, crossover study on 18 rheumatoid arthritis patients revealed that oral administration of 1200 mg/day curcumin (three divided doses) for 2 weeks significantly improved the morning stiffness and walking time [63]. Pharmacokinetics and pharmacodynamics of curcumin including the involvement of AP-1/NF- κ B signaling in chondrocytes, osteoblasts, and synovial fibroblasts have well been described in a recent review article [64]. Among the matrix metalloproteinases, MMP-1 and -13 levels were found to be significantly elevated in the RA joints. Curcumin blocks IL-1 β -induced upregulation of MMP-3 and downregulation of type II collagen syntheses that are known contributors in the pathogenesis of RA [65].

Curcumin inhibits ERK, JNK and p38 MAPK pathways resulting in inhibition of AP-1 and NF- κ B, which leads to downregulation of IL-1-mediated MMP-3 and -13 in articular chondrocytes [66]. Interestingly, Mun *et al.* have shown that oral administration of curcumin to CIA mouse model suppresses MMP-1 and -3 expressions in fibroblast-like synoviocytes (FLS) and chondrocytes by inhibiting PKC isoforms (PKC δ) and JNK pathway, but not by inhibiting ERK and p38 pathways [67]. Macrophage migration inhibitory factor (MIF)-induced expressions of MMP-1 and -3 in synovial fibroblasts of rheumatoid arthritis and osteoarthritis can be reversed by curcumin. This effect is due to the inhibition of multiple pathways including AP-1, PKC, and tyrosine kinase-dependent pathways [68].

Curcumin dose dependently downregulates the expressions of MMP-1, -3, and -13 in arthritis model by inhibition of NF- κ B activation induced by TNF- α /IL-1 β [69]. Catabolic process in RA is mediated by pro-inflammatory cytokines like IL-1 β , IL-6, TNF- α , IFN- γ , and NF- κ B, resulting in induction of MMPs on human cartilage, which causes extracellular matrix degradation [70].

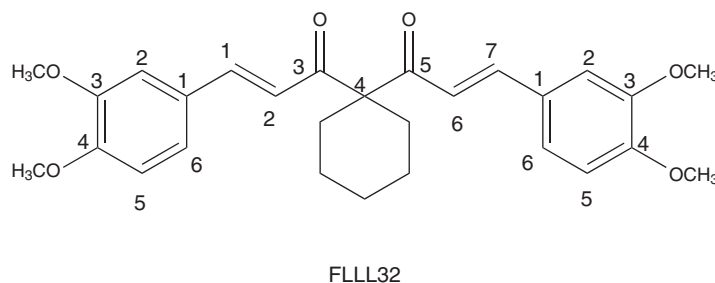


Figure 3. Structure of FLLL32.

3.2 Ulcer

Indomethacin-ulcerated stomach extracts exhibit significant upregulation of pro-MMP-9 (92 kDa) and moderate reduction of MMP-2 activity that strongly correlate with indomethacin dose and severity of ulcer. Curcumin accelerates healing process and protects gastric ulcer through attenuation of MMP-9 activity and amelioration of MMP-2 activity [71]. Curcumin dose dependently suppresses MMP-3 and -9 expression in *Helicobacter pylori* (Hp)-infected human gastric epithelial (AGS) cells. MMP-3 and -9 are inflammatory molecules associated with the pathogenesis of Hp infection and this action is linked to decreased pro-inflammatory molecules and activator protein-1 activation in Hp-infected gastric tissues. Conventional triple therapy of Hp-infected gastric tissues is found to be less efficient than curcumin in restoring the altered balance between MMPs and TIMPs in gastric mucosa during protection of Hp infection [72].

Abundance and activation of MMPs significantly increased ulcerative colitis and Crohn's mucosa, thus inhibitors of these proteolytic enzymes may, therefore, be of therapeutic value in the treatment of inflammatory bowel disease [73]. Evaluation of N-unsubstituted curcuminoid pyrazoles by using human intestinal epithelial cells *in vitro* for regulation of the activity of MMPs shows significant downregulation of MMP-9 [74]. Curcumin suppresses MMP-3 production in *ex vivo* colonic myofibroblasts culture from IBD patient and the effect is due to inhibition of p300 acetyl transferase [75].

4. Miscellaneous diseases

Oral administration of curcumin capsule (100 mg, three times a day) in addition to standard fundamental treatment to unstable angina pectoris patients has significantly reduced the MMP-2 and -9 expressions [76]. Interestingly, it has been revealed that curcumin upregulates MMP-2 and -9 expressions in experimental heart failure model. Migration and proliferation of vascular smooth muscle cells can contribute to the pathogenesis of atherosclerosis [77]. Curcumin suppresses the activation and expression of MMP-9 in TNF- α -treated human aortic smooth muscle cells (HASMCs) through the inhibition of nuclear translocation of NF- κ B, p50 and p65, which leads to decreased HASMCs migration [78]. Curcumin

protects kidney of experimental diabetic rats by upregulating the expression of MMP-2 and downregulating the expression of TIMP-2 [79,80].

Fibrosis is a hallmark feature of scleroderma (SSc), which is a result of perturbations in gene expression of TIMP-1, MMP-1, and HGF. Excess TIMP-1 relative to the MMPs under the influence of TGF- β is thought to promote a pro-fibrotic state. However, hepatocyte growth factor (HGF) is a potent epithelial cell mitogen and has anti-fibrotic properties [81,82]. In multiple sclerosis, MMPs play crucial role in different pathogenic stages viz. blood-brain barrier breakdown, invasion of brain parenchyma by immune cells and demyelination. On quantifying the transcriptional expression of different MMPs and TIMPs, Lindberg *et al.* have concluded that imbalance between MMP and TIMP expression may cause a persistent proteolytic over activity in multiple sclerosis. The mRNA expression of MMP-7 and -9, but not other MMPs were equally upregulated throughout all stage of inflammation. However, none of the TIMPs showed significant induction over baseline expression of control [83].

Dietary supplement of curcumin (1%, w/w) for 6 months to *Opisthorchis viverrini*-infected hamsters significantly reduced periductal fibrosis via inhibition of TIMPs expression and enhancement of MMPs expression mediated by cytokines [84]. Curcumin has shown protective function on liver tissues in hepatic fibrosis, induced by *Schistosoma japonicum* in mice, which may be related to the upregulation of MMP-1 mRNA and downregulation of TIMP-1 mRNA, leading to decrease in the content of hydroxyproline (Hyp) and collagen in liver [85]. Administration of bis-desmethoxy curcumin analog has significantly decreased the levels of collagen and TIMPs and positively modulated the expression of MMPs; and thus curcumin acts as an efficient anti-fibrotic agent [86]. Aplin *et al.* have indicated the critical role of MT1-MMP and TIMPs in both neovessel sprouting and vascular regression during angiogenesis in the aortic ring model. This may help in understanding complex nature of MMPs role and designing improved anti-angiogenic molecules [87].

Intraperitoneal injection of curcumin (48 mg/kg) to peritoneal endometriosis mice model has revealed that curcumin protects endometriosis in a time-dependent manner by decreasing MMP-9 activity and elevating TIMP-1 through

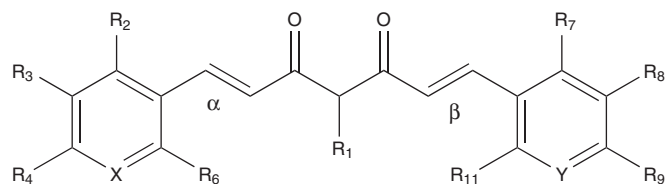


Figure 4. New analog of curcumin.

inhibition of TNF- α [88]. Recently, it has been shown that curcumin also downregulates MMP-3 by inhibiting NF- κ B translocation in endometriosis [89]. The expressions of NF- κ B, p65, and MMP-9 mRNA in the placenta of premature birth mouse are downregulated by the administration of curcumin [90]. It has been demonstrated with human dermal fibroblasts and human skin in organ culture that botanical composition comprising curcumin and gingerol act synergistically for enhanced repair of damaged skin and prevention of developing wound. It also suppresses elaboration of MMP-1 [91]. Anti-lymphangiogenic effect of curcumin in lymphatic endothelial TR-LE cells is dependent on Akt (serine/threonine protein kinase) and MMP-2, and not on IKK and EGFR tyrosine kinase [92]. It has been reported that curcumin can improve BBB integrity in cerebral ischemic/reperfusion injury via inhibition of TNF- α , MMP-2 and -9 [93,94].

5. Structure-activity relationship

Structure-activity relationship of various analogs of curcumin has revealed that they show variable inhibitory activities against different MMPs. MMPs inhibitory potency of curcumin can be enhanced by increasing its solubility. Poor oral absorption of curcumin in both humans and animals has raised quite a lot of concerns about its clinical efficacy. A new analog was invented by altering some functional group on aromatic moiety of curcumin, which provided a method of increasing water solubility, metal binding activity, and MMP inhibition activity.

In this invention (Figure 4) the improved biological activity of curcumin and its analogs is attributed in part to their ability to access and bind zinc ions and an enhanced solubility through addition of electron withdrawing and electron releasing group at C-4 carbon and on aryl skeleton of curcumin, resulting in increased inhibition of MMP activity. Incorporation of groups at C-4 carbon has led to stabilization of enol form as well as enolate formed from deprotonation of C-4 carbon and thereby, facilitating their water solubility and metal binding.

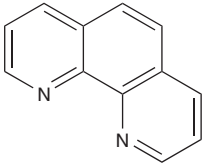
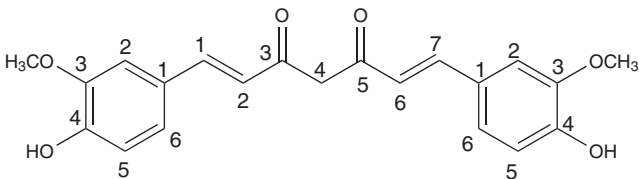
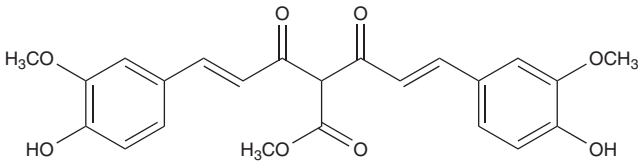
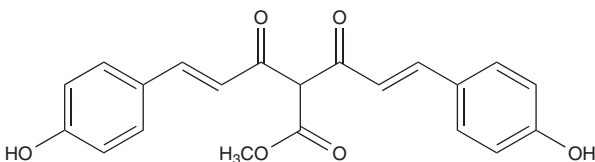
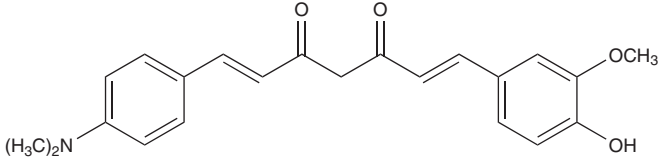
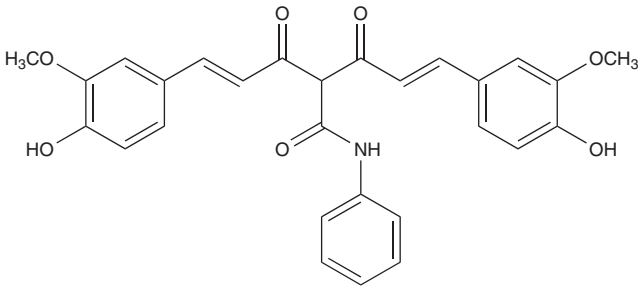
Introduction of electron-donating group on aryl rings affects enhancement of water solubility by increasing electron polarization within the molecule, for instance intermolecular dipole-dipole interaction with surrounding water molecules is enhanced. Electron-donating group may also increase water solubility by enhancing hydrogen bonding interactions with surrounding water molecules.

Compounds 1 and 2 (Table 1) were synthesized with improved solubility and tested against MMP-8. Compound 1 showed excellent MMPI with an IC₅₀ value of 10 μ M, which is equivalent to that of 1,10-phenanthroline, a standard zinc binding MMPI; whereas compound 2 which lacked substituents on aryl moieties did not show a dose response. Compounds 3 showed more solubility and potency against *in vitro* inhibition of MMP-8 in comparison to compound 1 (a methoxy carbonyl curcumin), which in turn more soluble and potent than curcumin. When compound 1, 3 and 4 were compared for inhibiting MMP-9, it was found that compound 3 showed lowest IC₅₀ ~ 6 μ M.

In Table 1, analogs of curcumin have been tested against MMP-8, -9, -2, and -13. Like 1,10-*O*-Phenanthroline (100% inhibition at 100 μ M), compound 1, 2 and 3 have shown 72, 68 and 100% inhibition, respectively at 100 μ M analogs concentration, and are better than curcumin which shows only 58% inhibition at the same concentration. Thus, compounds 2 & 3 show excellent activity as MMP-9 inhibitor. Curcumin has been found to be less potent as MMP-2 inhibitor than 1,10-*O*-Phenanthroline. However, compound 1 has shown maximum potency as MMP-2 inhibitor, having IC₅₀ ratio of 0.7 compared to standard 1,10-*O*-Phenanthroline. Amide-containing derivatives, compounds 4, 5 & 6, are much more potent inhibitor of MMP-13 than curcumin and other analogs. While comparing MMP-2 inhibitory potency, compound 4 was found to be showing similar efficacy than curcumin and amide-containing compounds are much more soluble than curcumin [95]. Compound 3 showed lowest (IC₅₀ = 6 μ M) as an MMP inhibitor against MMP-9. All three chemically modified curcumin analogs 1, 4 and 3 showed lower IC₅₀ values ~ 17 μ M than natural curcumin (IC₅₀ = 29 μ M). It can be inferred that change on aryl moiety was responsible for different inhibiting activities. Though substitution with amide group did not bring too much changes, substitution with methoxy group was able to show its inhibitory activity against MMP-9. However, chemically modified curcumin analogs having a carbonyl amide phenol group at carbon -4 exhibited greater activity against MMP-13 in comparison to curcumin.

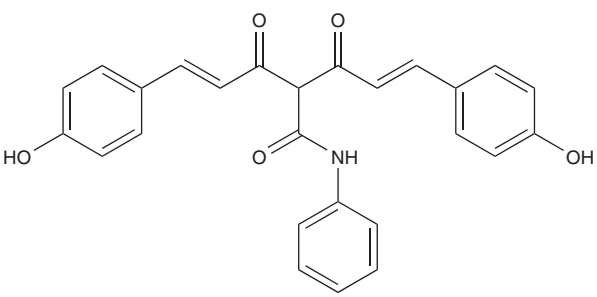
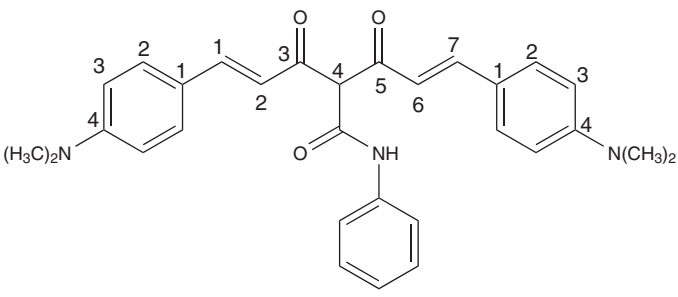
A recent study on curcumin and its analogs (dibenzoyl methane, dibenzoylpropane, and dibenzylideneacetone (Figure 5) has revealed that α,ω -bisaryl alkanone moiety is a minimal structural requirement for suppression of cell proliferation and inflammation. Furthermore, the activity can be improved by the presence of a Michael acceptor enone moiety [96].

Table 1. IC₅₀ values of curcumin analogs against MMPs.

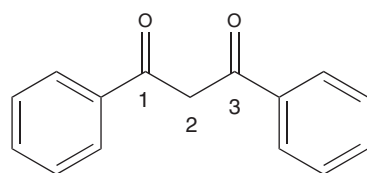
Compounds	IC ₅₀ values (μM)			
	MMP-8	MMP-9	MMP-2	MMP-13
 1,10-O-Phenanthroline	10 – 35	9	70	4
 Curcumin	14	29	85	110
 Compound 1	10	16	48	15
 Compound 2	≤ 5	6	70	8
 Compound 3	ND	17	> 250	250
 Compound 4	ND	ND	85	< 1

ND: Not determined.

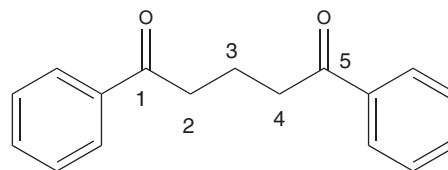
Table 1. IC₅₀ values of curcumin analogs against MMPs (continued).

Compounds	IC ₅₀ values (μM)			
	MMP-8	MMP-9	MMP-2	MMP-13
 Compound 5	ND	ND	125	< 1
 Compound 6	ND	ND	–	45

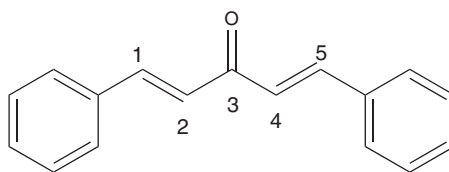
ND: Not determined.



Dibenzoylmethane



1,3-Dibenzoylpropane



Dibenzylideneacetone

Figure 5. Structures of curcumin analogs.

Antioxidant activity of 22 structurally related curcumin derivatives has revealed that compounds with low hardness and dipole values, and high softness and HOMO-LUMO_{gap} along with number of hydroxyl groups show good antioxidant activity [97]. Curcumin can exist in two tautomeric forms

namely, enol and β-diketone forms. Enolic form of curcumin predominantly exists in solution, and enolic proton is the most easily dissociable proton during the proton transfer/dissociation-associated radical-scavenging mechanisms [98,99].

Table 2. Effect of curcumin on various isoforms of MMPs involving different diseases.

SN.	Diseases	Effect of curcumin
1	Cancer	↓MMP-1, ↓MMP-2, ↓MMP-3, ↓MMP-9, ↓MMP-14, ↓COX-2, ↓ cyclin D1, ↓uPA, ↑MT1-MMP, ↑TIMP-1
2	Arthritis	↓MMP-1, ↓MMP-2, ↓MMP-3, ↓MMP-9, ↓MMP-13
3	Ulcer	↓MMP-2, ↓MMP-3, ↓MMP-9

6. Expert opinion

The need for alternative and less toxic therapy for various types of cancer and other diseases has given impetus to researchers across the globe to search molecules from natural products. Several data pointed out that curcumin's role in expression and downregulation of various MMPs, a group of proteolytic enzymes, is pivotal. Lack of systemic toxicity and broad-reaching mechanism of curcumin may make it best suited. Use of curcumin in diet or in treatment may be an ideal way to put an end to menace of cancers and other diseases. Curcumin has not only shown to be fairly effective in human trials, but also proven to be safe at high doses with no side effects.

Phase I clinical trial has shown safety of curcumin at high dose, 12 g/day, in human but due to poor bioavailability its efficacy is marred. Numerous approaches such as curcumin nanoparticles [100], solid-lipid nanoparticles [101], magnetic microspheres [102], metal complexes [103], synthetic analogs of curcumin and adjuvants (piperine) have been taken to improve its bioavailability. Bioavailability of curcumin can be enhanced by 32 – 155 times through the formulation of solid-lipid nanoparticles and this can be effectively delivered to the brain [101]. Endocytosis of curcumin by nasopharyngeal cells can be enhanced effectively through curcumin nanoparticles [100]. Synergistic effect was noted in the gastroprotective and antidepressant activity of curcumin when curcumin complexes with zinc (zinc (II)-curcumin complex) [103]. Enhanced

bioavailability of curcumin will pave new way to cure multiple human diseases. Recently, it has been found that novel cyclodextrin complex of curcumin is more active than free curcumin. Cyclodextrin complex of curcumin significantly suppresses the TNF-induced NF- κ B activation and translocation of p65. Further, it also downregulates NF- κ B-regulated gene products associated with proliferation (cyclin D1), invasion (MMP-9), and angiogenesis (VEGF) [104]. Table 2 summarizes the inhibitory role of curcumin on various isoform of MMPs which got elevated in various diseased conditions.

Numerous approaches have been taken to beget a molecule with specificity restricted to a particular MMP as well as good oral bioavailability; however, nearly all the molecules lack these criteria. Oral bioavailability is a major concern, as the MMPs are involved in chronic diseases such as arthritis, Alzheimer's disease, psoriasis, COPD, asthma, cancer, neuropathic pain and atherosclerosis to name a few, where one is supposed to take the drug throughout their lifetime. By using QSAR modeling and virtual screening, new analogs of curcumin can be designed which will be selectively inhibiting different MMPs.

Extensive literature search conducted on MMPs has brought light to the fact that we should be sanguine of healing role of curcumin in expression of different diseases such as cancer, arthritis, ulcer, and inflammation. However, it will take a long and passionate research to find curcumin analogs that inhibit specific MMPs.

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D Kumar, M Kumar and C Saravanan contributed equally to this work.

Declaration of interest

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