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List of Papers Published/Communicated

- **D.Kumar**, Manish Kumar, Chinnadurai Saravanan, Sushil Kumar Singh Curcumin: A potential candidate for MMPIs in Expert Opinion on Therapeutic Targets, 16(10):959-72 (2012).

- **D.Kumar**, Manish Kumar, Ashoka kumar, Sushil k singh. Chalcone and curcumin derivatives: A way ahead for the antimalarial treatment in Mini-Reviews in Medicinal Chemistry, 13, 2116-2133 2013.

- **D. Kumar**, B.G Harish , Mayank Gangwar, Manish Kumar, Dharmendra Kumar, Ragini Tilak, Gopal Nath, Ashok kumar and Sushil Kumar Singh Synthesis, molecular docking and in vitro antimicrobial studies of new hexahydroindazole derivatives of curcumin. Letters in Drug Design & Discovery Volume 10, Number 2, February 2013, pp. 119-128(10)

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- **D.Kumar**, Renata C Paula, Sushil K Singh, Synthesis and *In silico* study of Pyrazoline analogues of curcumin as novel *Plasmodium falciparum* inhibitors Bioorg. Med. Chem. Lett.2015 (communicated)

EXPERT OPINION

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

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Article highlights.

- Curcumin and its analogs exhibit MMPI activity by inhibiting different cell signaling pathways including MAPK/NF-κB/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 IC_{50} values of 27.8 and 85.8 μg 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].

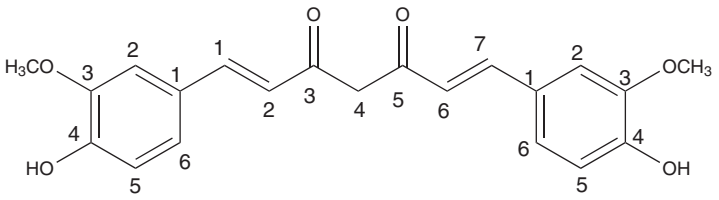
As illustrated in **Figure 2**, curcumin affects the level of MMPs in extracellular sites by modulating the inducers, such as growth factors and cytokines (IL-1α, IL-1β, TNF-α, TNF-β, HGF, etc.), occurring primarily at the transcriptional level and are initiated by the binding of the stimulating factor to its cell surface receptor. **JAK/STAT signaling pathway** is mainly activated by cytokines such as interferon α/β/γ. 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-κB signaling pathway**: NF-κB, a transcription factor, is activated by external stimuli such as TNF-α and IL-1. Upon activation, NF-κB translocates from cytoplasm to the nucleus and binds to the promoter region of the target gene. NF-κB 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 μg/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-κB-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.

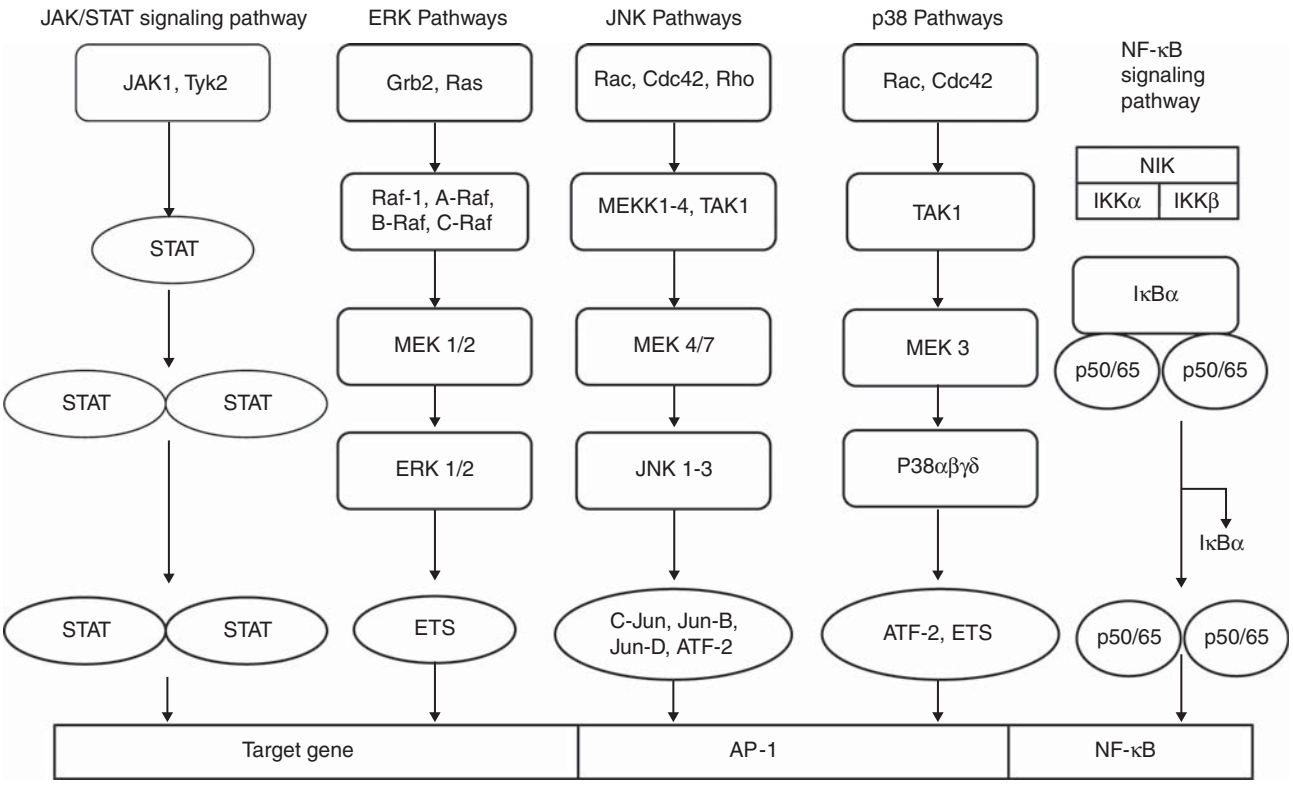


Figure 2. Signaling pathways that contribute to MMPs gene transcription.

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 κB (IκBα) by enhancing the activity of IκBα kinase (IKK). Then, translocation of NF-κB 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-κB-mediated pro-MMP-2 activation by blocking the IκBα/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 nonsmall 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-κB. CSC-induced NF-κB activation and NF-κB-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 IκBα kinase. This owes to the decreased expression of CSC-induced NF-κB-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-κB 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-κB signaling pathways [30]. It suppresses PMA-induced MMP-1, -3, -9, and -14 expressions in human astroglioma cells by inhibiting PKC. MMP-9 expression is mediated via NF-κB 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-κB. Curcumin inhibits cell proliferation and migration by down-regulation of bcl-2 and bclxl through NF-κB 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-κB-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-κB pathways through a cross talk between phosphatidylinositol 3-kinase/Akt/IκBκ 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-κB 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-κB 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-κB. Proliferation of Caski cells has been inhibited in a dose- and time-dependent manner. The expression of MMP-2, MT1-MMP, and NF-κB has been decreased when treated with 50 μmol/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-κB, 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 μM. 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-κB–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-1β, EMMPRIN, MMP-2, and MMP-9 expressions by inhibiting NF-κB 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].

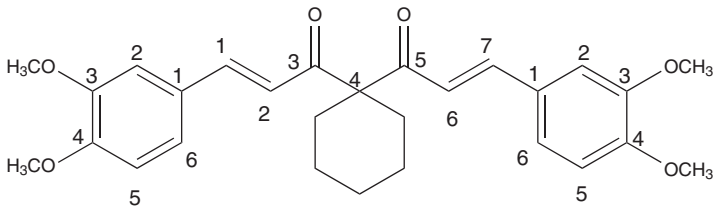
Detailed RNA interference study of human mesenchymal stem cells (hMSCs) revealed that gene knockdown of MMP-2, MT1-MMP, or TIMP-2 substantially impaired hMSC invasion, whereas silencing of TIMP-1 enhanced cell migration, indicating opposing roles of TIMP-1 and TIMP-2 in the process of hMSC mobilization and homing. The role of inflammatory cytokines such as TGF-β1, IL-1β, 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-κB 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-1β-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-κB, 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 (PKCδ) 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-κB activation induced by TNF-α/IL-1β [69]. Catabolic process in RA is mediated by pro-inflammatory cytokines like IL-1β, IL-6, TNF-α, IFN-γ, and NF-κB, resulting in induction of MMPs on human cartilage, which causes extracellular matrix degradation [70].



FLLL32

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-κB, 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 profibrotic 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

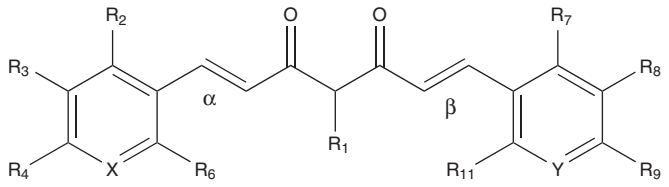


Figure 4. New analog of curcumin.

inhibition of TNF-α [88]. Recently, it has been shown that curcumin also downregulates MMP-3 by inhibiting NF-κB translocation in endometriosis [89]. The expressions of NF-κB, 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.

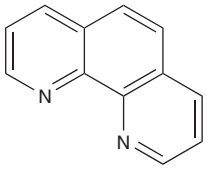
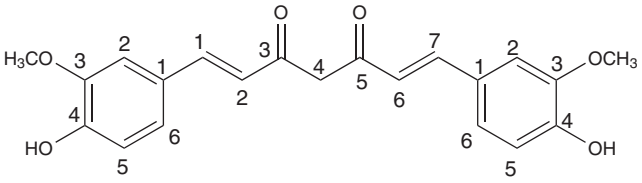
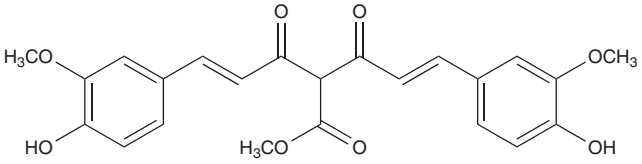
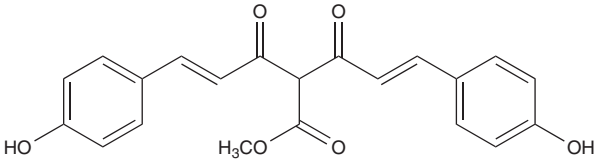
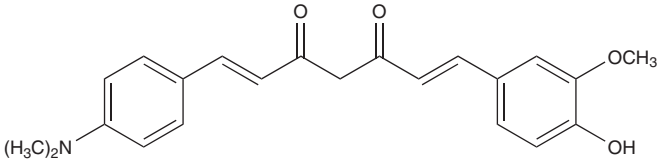
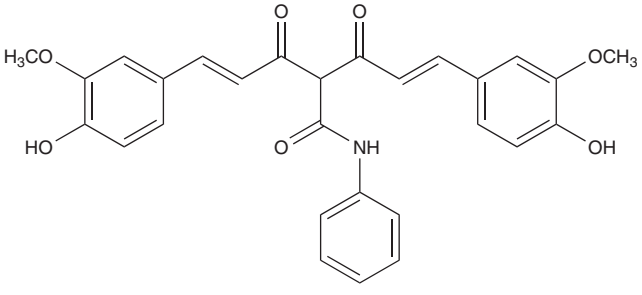
Introduction of electron-donating group on aryl rings affects enhancement of water solubility by increasing electron polarization within the molecule, for instance intermolecular dipole–dipole interaction with surrounding water molecules is enhanced. Electron-donating group may also increase water solubility 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 IC_{50} value of 10 μM, 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 IC_{50} ~ 6 μM.

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 μM), compound **1**, **2** and **3** have shown 72, 68 and 100% inhibition, respectively at 100 μM 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 IC_{50} 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 (IC_{50} = 6 μM) as an MMP inhibitor against MMP-9. All three chemically modified curcumin analogs 1, 4 and 3 showed lower IC_{50} values ~ 17 μM than natural curcumin (IC_{50} = 29 μM). 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.

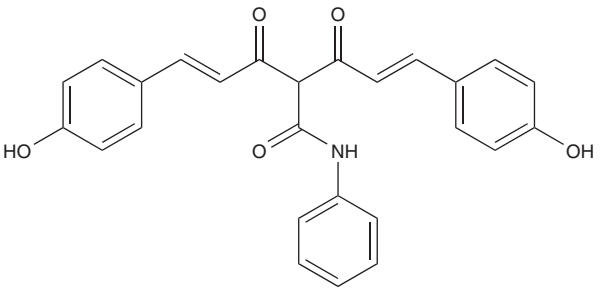
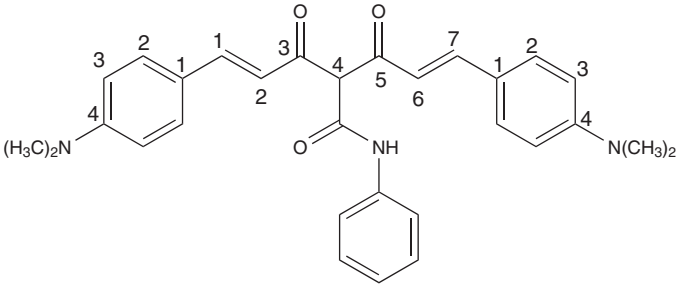
A recent study on curcumin and its analogs (dibenzoyl methane, dibenzoylpropane, and dibenzylideneacetone (**Figure 5**) has revealed that α,ω-bisaryl alkanone moiety is a minimal structural requirement for suppression of cell proliferation and inflammation. Furthermore, the activity can be improved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

Table 1. IC_{50} values of curcumin analogs against MMPs.

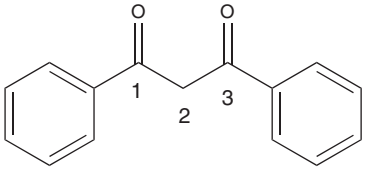
Compounds	IC50 values (μM)			
	MMP-8	MMP-9	MMP-2	MMP-13
 1,10-O-Phenanthroline	10 – 35	9	70	4
 Curcumin	14	29	85	110
 Compound **1**	10	16	48	15
 Compound **2**	≤ 5	6	70	8
 Compound **3**	ND	17	> 250	250
 Compound **4**	ND	ND	85	< 1

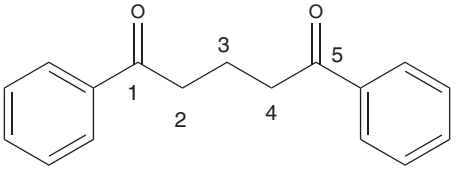
ND: Not determined.

Table 1. IC_{50} values of curcumin analogs against MMPs (continued).

Compounds	IC50 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

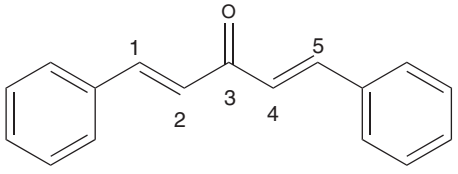

Dibenzylideneacetone

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-κB activation and translocation of p65. Further, it also downregulates NF-κB-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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Chalcone and Curcumin Derivatives: A Way Ahead for Malarial Treatment

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Abstract: Malaria has been a major cause of morbidity and mortality in developing countries, particularly in Sub-Saharan Africa and South Asia. The global malaria situation is increasingly being challenging owing to lack of credible malaria vaccine and the emergence of drug resistance to most of the available antimalarials. They demand search for novel generation of drugs. Versatility and flexibility for structural modification of natural and synthetic analogues of curcumin and chalcone have been explored extensively for designing new antimalarial agent. Recent advances to our knowledge of parasite biology as well as the availability of the genome sequence, have opened up new vista in the firmament of antimalarial drug designing for identifying novel molecular targets. Curcumin and chalcones has been reported to exert anti-malarial effect by binding directly to numerous signaling molecules, such as histone acetyltransferase, histone deacetylase, sarco (*endo*) plasmic reticulum Ca^{2+}-ATPase, cysteine proteases etc. This review highlights insights the more recent antimalarial activities of these compounds, their mechanisms of action, molecular targets and relevant structure-activity relationship studies. Natural lead compounds like chalcone and curcumin have shown good and optimal binding to many enzymes present in parasite and can be explored as molecular targets for *in silico* studies to develop new, affordable and effective antimalarial drugs. With no credible malaria vaccine in sight, there is an imperative need to develop new drugs with different mechanisms of action to help preclude issues of cross-resistance.

Keywords: Antimalarial, Bioavailability, Chalcone, Curcumin, Molecular Targets, Histone acetyltransferase.

INTRODUCTION

Malaria is one of the most fatal diseases in the world. According to a WHO estimate malaria infects nearly 300 million people every year and more than one million people die from the disease. Over, 90% of malaria related deaths occur in Sub-Saharan Africa [1, 2]. In absence of effective vaccines, chemotherapy is the mainstay of malarial control. The treatment with drugs that targets malaria parasite *Plasmodium* has become increasingly a challenge, since parasites turn to acquire resistance against the existing drugs. The emergence of multidrug-resistant parasites is a major concern of malaria control and development of novel drugs poses challenge to researchers. The frequency, virulence, and drug resistance have made malaria, the most serious and widespread parasitic disease. Human malaria transmitted by female *Anopheles* mosquitoes is caused by four species of *Plasmodium*, viz. *P. falciparum, P. vivax, P. ovale and P. malariae* [3].

Chalcones are a group of natural products characterised by the presence of 1,3-diphenylprop-2-en-1-one skeleton. The radical quenching property of phenolic groups present in many of the naturally occurring chalcones has raised interest for using these compounds or chalcone rich plant extracts as drugs or food preservatives. Chalcones are of considerable interest in drug discovery because of the diverse biological activities displayed by derivatives and also the ease of their synthesis. Naturally occurring and synthetic chalcones have shown promising biological activities, safety profiles and have the potential to be developed as lead compounds for the discovery of antimalarial agents. As antimalarials, they may exert their effect via inhibition of cysteine proteases or through new permeability pathways [4, 5].

Curcumin has been used in traditional medicines as household remedy for the treatment of various diseases, including biliary disorders, anorexia, cough, hepatic disorders, rheumatism and sinusitis. Turmeric is reported as a component of traditional remedies for malaria and fever in India. Curcumin, a polyphenol extracted from the roots of *Curcuma longa* L., is reported to exhibit multiple biological activities and pharmacological actions [6]. Recent studies have indicated that, curcumin inhibits chloroquine-sensitive (CQ-S) and chloroquine-resistant (CQ-R) *P. falciparum* growth. Many of the synthetic analogues of curcumin have expanded its antimalarial activity as they have shown more effective inhibition of *P. falciparum* growth than curcumin. Similar to artemesinin, ART, PfATP6, the parasite orthologue of mammalian sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase (SERCA), could be possible target for curcumin action [7]. New alternative of ART-based combination therapies (ACTs) demonstrate a better overall efficacy and delay the emergence of resistance. However, their cost, adverse drug reaction and pharmacokinetic mismatch of each drug of the combination present gloomy picture of alternatives of ACTs. Studies with animal models have indicated that a

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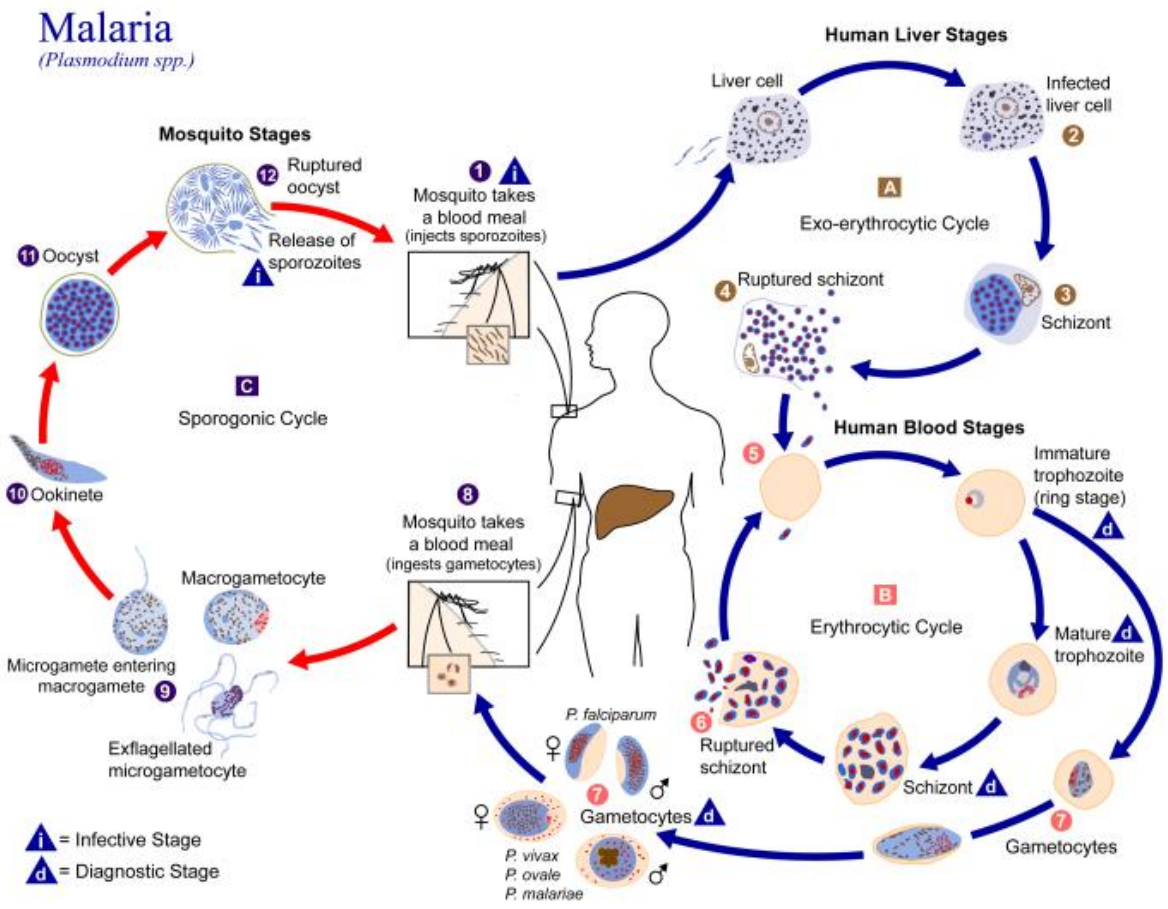


Fig. (1). Life cycle of malarial parasite.

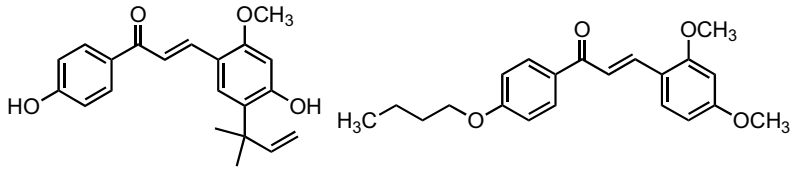
combination therapy with ART and curcumin can add a new dimension to malaria therapy in terms of its potential to prevent parasite recrudescence and relapse in *falciparum* and *vivax* malaria as well as protecting against cerebral malaria. Both these partner drugs have short half-lives and no resistance is known to curcumin [8]. This review highlights the recent advances and developments which have taken place to develop chalcones and curcumin as antimalarial.

The life cycle of malarial parasite is illustrated in Figure. (Fig. **1**)

ANTIMALARIAL ACTIVITY OF CHALCONES

Licochalcone A **1** (Fig. **2**), isolated from Chinese Licorice roots has been reported to inhibit *in vitro* growth of both chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) *P. falciparum* strains in [^{3}H] hypoxanthine uptake assay. Its *in vivo* activity was demonstrated by protecting the mice from lethal *Plasmodium yoelii* infection in mouse model after either intraperitoneal or oral administration for 3-6 days [9]. An analogue of licochalcone A, 2,4-dimethoxy-4'-butoxychalcone (2,4mbc) **2** (Fig. **2**) exhibited a concentration-dependent inhibitory effect on both chloroquine-susceptible and chloroquine-resistant strains of *Plasmodium falciparum* in a [^{3}H] hypoxanthine uptake assay. It also showed protective effect *in vivo* study in mice infected with *Plasmodium berghei* or *Plasmodium yoeli*. It fulfils all the promising criteria for potent antimalarial candidate viz. activity and selectivity against drug-resistant parasites, having no toxicity, and easy administration [10].

The antiparasitic potency of α,β-double bond modified chalcones differs only marginally from that of parent chalcones indicating that the propenone residue functions as

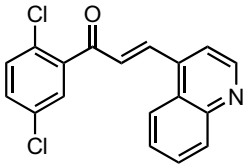


1. Licochalcone A

2. 2,4-dimethoxy-4' -butoxychalcone

Fig. (2). Licochalcone A and its derivative.

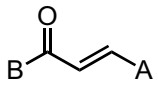
only spacer between the two benzene rings. Further, the difference in biological activity, observed in a series of chalcones, is because of the different substitution patterns at the two benzene rings [11]. Previously, it was suggested that the antibacterial effect was due to inactivation of essential enzymes by a conjugated addition of the chalcones to thiolo groups or other nucleophilic centres [12]. Among the novel series of chalcone and its derivatives, l-(2,5-dichlorophenyl)-3-(4-quinolinyl)-2-propen-l-one **3** (Fig. **3**) had an IC_{50} value of 200 nM against both a chloroquine-resistant strain (W2) and a chloroquine-sensitive strain (D6).



3.

Fig. (3). Chemical structure of l-(2,5-dichlorophenyl)-3-(4-quinolinyl)-2-propen-l-one.

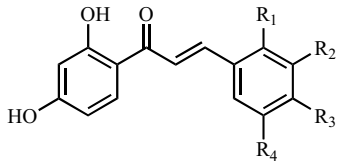
SAR study of chalcone derivatives **4** (Fig. **4**) revealed that chloro or fluoro substitution on A ring and electron-donating substitution (e.g. methoxy, imidazole, etc.) on B ring exhibited better antimalarial activity. The α,β-unsaturated ketone bridge is essential but steric constriants due to substituents in the bridge portion, reduce its activity. Substantially lower observed resistant indexes of these derivatives than that of chloroquine, suggest different mechanism of action of chalcones than that of chloroquine. Malarial cysteine protease, an enzyme used by the parasite *P. falciparum* for the degradation of host haemoglobin within the acidic food vacuole of the intra-erythrocytic parasite, is a promising target enzyme for action of these chalcones on chloroquine resistant malaria [13,14]. Experiential inferences are supported by computational structural analyses suggesting that malarial cysteine protease is the most likely target enzyme of chalcones [15].



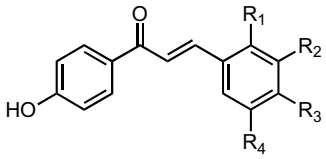
4.

Fig. 4. General structure of chalcone derivatives.

In vitro study of chalcone analogues against *P. falciparum* (K1) in a [^{3}H] hypoxanthine uptake assay has indicated that, good antimalarial activity is associated with structural features of ring B while selection of substituents on ring A determines the critical parameters viz. size, lipophilicity and electronic characteristics. It was found that hydroxylated chalcones were less active than the corresponding alkoxylated analogues as had been demonstrated in SAR study of hydroxylated and alkoxylated chalcones. Among the promising antimalarial hydroxylated chalcones (Fig. **5**), 4-chloro-2′,4′-dihydroxychalcone **6** with an IC_{50} of 12.3 μM, was found to be most active against chloroquine resistant *P. falciparum* (K_1) in [^{3}H] hypoxanthine uptake assay [16].



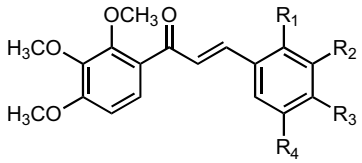
5. $R_1R_3R_4$=H R_2= naphthalenyl, IC_{50}=20.0 μM
6. $R_1R_2R_4$=H R_3= chloro, IC_{50}=12.3 μM



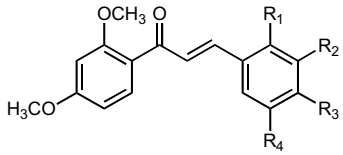
7. $R_1R_3R_4$=H R_2= methyl, IC_{50}=25.8 μM
8. $R_1R_2R_4$=H R_3= dimethylamino, IC_{50}=17.7 μM
9. R_1R_4=H R_2R_3= chloro, IC_{50}=18.4 μM
10. $R_2R_3R_4$=H R_1= pyridinyl, IC_{50}=16.3 μM

Fig. (5). Chemical structure of hydroxylated chalcone analogues.

While among the alkoxylated chalcones, 1-(2′,3′,4′ trimethoxyphenyl)-3-(3-quinolinyl)-2-propen-1-one **13** (Fig. **6**) was most active with an IC_{50} of 2 μM. Rest of the compounds also showed IC_{50} values below 10 μM [16].



11. R_2R_4=H R_1R_3= chloro, IC_{50}=5.4μM
12. $R_1R_2R_4$=H R_3= trifluoromethyl, IC_{50}=3.0 μM
13. $R_1R_3R_4$=H R_2= quinolinyl, IC_{50}=2.0 μM
14. $R_1R_2R_4$=H R_3= fluoro, IC_{50}=9.5 μM



15. R_2R_4=H R_1R_3= dimethoxy, IC_{50}=2.1 μM
16. R_2R_4=H R_1R_3= difluoro, IC_{50}=6.2 μM
17. $R_1R_2R_4$=H R_3= trifluoromethyl, IC_{50}=5.9 μM
18. $R_1R_2R_4$=H R_3= ethyl, IC_{50}=2.4 μM
19. $R_1R_3R_4$=H R_2= quinolinyl, IC_{50}=2.2μM

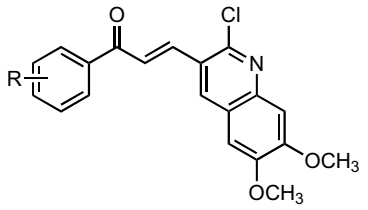
Fig. (6). Chemical structure of alkoxylated chalcone analogues.

Studies on series of oxygenated chalcone analogues suggested that good antimalarial activity is found among alkoxylated chalcones with polar A rings, in particular, those substituted with electron withdrawing groups or replaced by quinoline rings. Visualisation of the steric and electrostatic fields generated from comparative molecular field analysis (CoMFA) indicate that the size characteristics of ring B

(large, alkoxylated) and the electronic characteristics of ring A (electron deficient) are important [17]. The 3D QSAR analyses of antimalarial alkoxylated and hydroxylated chalcones by Comparative molecular field analysis (CoMFA), Comparative similarity indices analysis (CoMSIA) provided tools for predicting affinity of related compounds, and guided the design and synthesis of novel and potent antimalarial agents. The study indicated a preference for sterically large (alkoxylated) ring B and a hydrophobic ring A in chalcones, which was also in conformity with experimental results [18].

The alkoxylated and hydroxylated chalcones were found to inhibit sorbitol-induced hemolysis of parasitized erythrocytes to a significant extent (40% of control values) at a concentration of 10 μM by inhibiting the new permeation pathways induced by the parasite in host erythrocyte membrane. Chalcones with good sorbitol-induced haemolytic inhibitory activity were concurrently identified as active antiplasmodial agents but not all active antiplasmodial chalcones inhibit sorbitol-induced hemolysis. This observation suggested that structurally different chalcones might exert their antiplasmodial activity by different routes or target additional pathways in the parasitized cell [19].

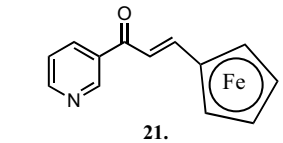
Evaluation of inhibitory action of quinolinyl chalcones against cultured *P. falciparum* parasites and *P. falciparum* cysteine protease, suggested moderate activity but probable mechanism was different from the inhibition of falcipain. 1-(2,4-Dichlorophenyl)-3-[3-(2-chloro-6,7-dimethoxiquinolinyl)]-2-propen-1-one **20** (Fig. **7**) was found to be the most promising compound among reported compounds (IC_{50} ~ 19.0 μM) and assessment of series of reported compounds of quinolinyl chalcones has also manifested the significance of substituted group in the benzoyl ring, in determining the antimalarial activity [20].



20. R = 2,4-Dichloro

Fig. (7). Chemical structure of most promising quinolinyl chalcone.

Similar to synthesis of various ferrocenyl analogues of known antimalarial agents (viz. artemisinin, mefloquine, chloroquine and quinine) with variable outcome of curative effects, several ferrocenyl chalcones have also been synthesised and evaluated for *in vitro* antimalarial activity against a chloroquine-resistant strain of *Plasmodium falciparum*. 1-(3-pyridyl)-3-ferrocenyl-2-propen-1-one **21** (Fig. **8**) and 1-ferrocenyl-3-(4-nitrophenyl)-2-propen-1-one **22** (Fig. **8**) were found to be most active compounds with IC_{50} of 4.5 and 5.1 μM, respectively. However, no significant structure–activity relationships could be drawn among the two series of analogues. Preliminary QSAR study implied that size, lipophilicity and electronic factors have a limited role in activity [21]. Evaluation of a series of ferrocenyl chalcones had shown that the location of ferrocene influenced the ease of oxidation of Fe^{2+} in ferrocene and polarity of the carbonyl linkage, which in turn influenced antiplasmodial activity. Compounds with ferrocene adjacent to the carbonyl linkage had polarised carbonyl linkages, lower lipophilicities and less readily oxidized ferrocene rings were associated with more selective and potent antiplasmodial activity. Among the most active compounds **21** and **22** (Fig. **8**)**,** with IC_{50} values in the low micro-molar range (4-5 μM), Compound **22** had shown selective activity for plasmodia compared to mammalian cell lines MDCK (SI = 14) and KB3-1 (SI = 37). The incorporation of ferrocene in the chalcone template enhanced its role in the processes that involved quenching and generation of free radicals. Participation of ferrocene in redox cycling may contribute to the antiplasmodial activity of ferrocenyl chalcones [22].



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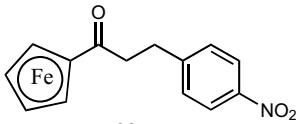
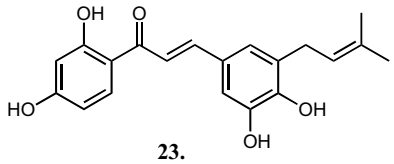


22.

Fig. (8). Chemical structure of ferrocenyl chalcones.

The ethyl acetate extract of stem bark of *E. abyssinica* has shown antiplasmodial activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *P. falciparum* with IC_{50} values of 7.9 ± 1.1 and 5.3 ± 0.7 μg/ml, respectively. A new chalcone**,** 2',3,4,4'-tetrahydroxy-5-prenylchalcone **23** (Fig. **9**) (trivial name, 5-prenylbutein) and a new flavanone, 7,40-dihydroxy-30-methoxy-50-prenylflavanone **24** (Fig. **9**) (trivial name, 5-deoxyabyssinin II) along with known flavonoids, isolated from the plant by chromatographic separations, have significant antiplasmodial activity [23].



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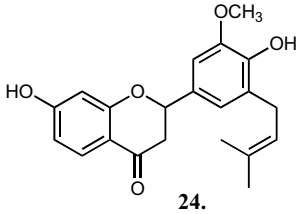


24.

Fig. (9). Chemical structure of 5-prenylbutein and 5-deoxyabyssinin II.

Chalcones can exist as Z-or E-isomers and undergo isomerisation on exposure to light. Previously, little difference in the activity of the isolated isomers was observed, leading to the conclusion that the isomers have equipotent antiplasmodial activity [24]. However, on synthesising and evaluating antiplasmodial activity of conformationally restricted Z- and E-analogues of different known antiplasmodial chalcones, it was found that the E-locked analogues were equipotent to the parent chalcones, indicating that the E-isomer was the active conformation and Z-isomer was nearly inactive [25].

Prenylated chalcone derivatives (Fig. **10**) from hops plant, *Humulus lupulus* were evaluated against chloroquine-sensitive strain poW and the multiresistant clone Dd2 using a [^{3}H] hypoxanthine-incorporation assay. The main hop chalcone, xanthohumol **25**, was most active with IC_{50} values of 8.2 ± 0.3 (poW) and 24.0± 0.8mM (Dd2). Interference in haemin-degradation process of *P. falciparum* may contribute to their antiplasmodial activity. Nevertheless, antiplasmodial activity of one derivative without being able to interact with GSH-dependent haemin degradation, suggested that other modes of action must add to its antiparasitic activity [26]. Prenylated chalcone, crotaorixin **26** has been isolated from the aerial parts of the *Crotalaria orixensis* and exhibited 100% inhibition of maturation of malaria parasite *P. falciparum* (Strain NF-54) from ring stage to schizont stage both at 50 and 10 µg/ml concentrations. The diprenylated compound, medicagenin **27**, isolated from the roots of *Crotalaria medicagenia,* inhibited 100% parasites at 2 µg/ml concentration while the chromenodihydro-chalcones (Fig. **10**) (viz. crotaramosmin **28**, crotaramin **29** and crotin **30**) isolated from the aerial parts of *Crotalaria ramosissima* showed lower order of activity. Substitution at the 4-hydroxyl group in ring-B in compounds crotaramosmin, crotaramin and crotin, decreases the activity. The 3, 4-disubstituted benzene system (ring-A) in crotaorixin has exhibited lower order of activity (10 µg/ml). However, prenylation with free 4,4-dihydroxy system (medicagenin) leads to improved activity [27].

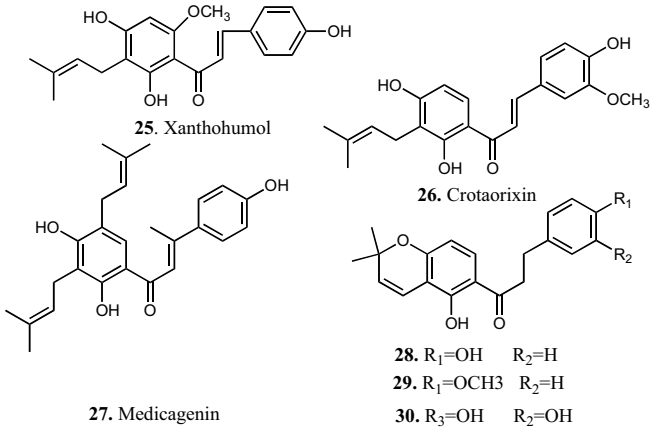


Fig. (10). Chemical structure of Prenylated and chromenodihydro chalcones.

Various analogues of sulfonamide chalcone (Fig. **11**) have been tested as inhibitors of β-hematin formation and also their activity against cultured *P. falciparum* parasites. Inhibition of β-hematin formation was highest with compounds **31** (IC_{50} ~ 0.48 µM) and **32** (IC_{50} ~ 0.50 µM) with substitution of 3,4,5-trimethoxyl and 3-pyridinyl, respectively (better than chloroquine, IC_{50} ~ 1.33µM). However, most active antimalarial compound, by the inhibition of cultured *P. falciparum* parasites, was found to be compound **33**. Electron withdrawing groups on the substituted aromatic ring should favour the Michael addition to an available nucleophilic side chain on the enzyme and thus showed excellent activity in inhibiting β-hematin formation. Basicity of compounds is essential for their accumulation in the malarial parasite acidic food vacuoles, in which hemozoin formation takes place. Mono Cl, F, Me, or OMe substituents in the aromatic ring did not improve the activity of the analogues compared to that of the corresponding disubstituted, trisubstituted analogues. Potent inhibitory activity of compound **33** on malaria parasite has not exhibited any correlation with inhibition of heme formation, suggesting that chalcones exert their antimalarial activity via multiple mechanisms [28].

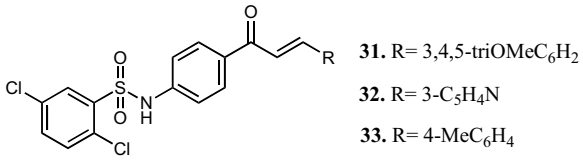
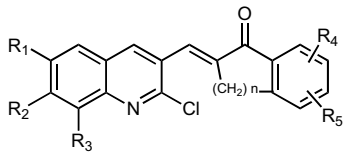


Fig. (11). Chemical structure of sulfonamide chalcones.

A series of E-2-quinolinylbenzocycloalcanones were prepared and evaluated for their *in vitro* inhibitory activity of β-hematin formation and the hydrolysis of hamoglobin. Among the promising compounds **34**, **35**, **36** and **37** (Fig. **12**), the most active analogue, 2-chloro-8-methyl-3-[(4′-methoxy-1′-indanoyl)-2′-methyliden]-quinoline **37** has shown efficacy as antimalarial agent. It targets β-hematin formation and the inhibition of hydrolysis of hamoglobin *in vitro* together with a better survival in murine malaria model, which should help in delaying the rapid onset of resistance to drugs acting at only single site. Inhibition of β-hematin formation was maximum with compounds (52%) and (90%) with a substitution of methoxy on position 6 and 7 or methyl on position 8 of the quinoline nucleus and methoxy or methyl groups on position 4′ of the indanone respectively [29].

Phenylurenyl chalcone derivatives have been synthesised and evaluated as inhibitors of *in vitro* development of chloroquine-resistant strain of *Plasmodium falciparum*, and murine *Plasmodium berghei* malaria. Among the other analogues having significant activity **38-44** (Fig. **13**), 1-[3′-N-(N′-phenylurenyl)phenyl]- (3,4,5-trimethoxyphenyl)-2-propen-1-one **38** was found to be most active antimalarial compound with an IC_{50} of 1.76 µM for inhibition of *Plasmodium falciparum.* The activity in most cases to a large extent was found to be governed by groups attached to the substituted aromatic ring A (difluoro, dichloro, trimethoxy). 3'-phenylurenyl chalcone derivatives were much better active than corresponding 4'-phenylurenyl chalcone derivatives. In the series of 4'-phenylurenyl chalcone derivatives, the

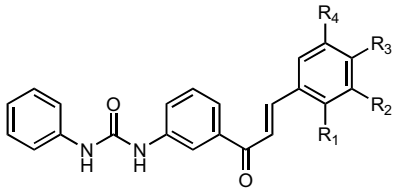


Compounds	R_1	R_2	R_3	R_4	R_5	%IHP	IGH	Day of survival over control
34	OCH_3	OCH_3	H	4'-CH_3	H	52	+	1.66 ± 0.42
35	OCH_3	OCH_3	H	5'-OCH_3	H	61	+	6.5 ± 0.5
36	H	H	CH_3	4'-CH_3	H	90	-	9.66 ± 1.31
37	H	H	CH_3	4'-OCH_3	H	79	-	12.16 ± 0.31

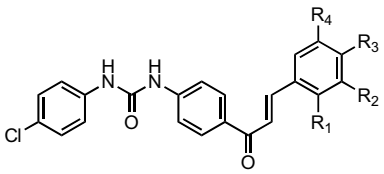
IGH: Inhibition of globin hydrolysis.

%IHP: percentage of inhibition of haem polymerization.

Fig. (12). Chemical structure of E-2-quinolinylbenzocycloalcanones.



38. $R_2R_3R_4=OCH_3$ R_1= H
39. R_1R_3=Cl R_2R_4=H
40. $R_1R_2R_4$= H $R_3=CH_3$
41. R_1R_4= H $R_2R_3=OCH_3$

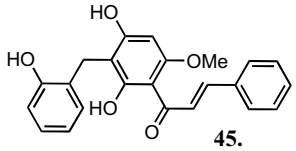


42. R_1R_3=Cl R_2R_4=H
43. R_1R3=F R_2R_4= H
44. R_1=H $R_2R_3R_4=OCH_3$

Fig. (13). Chemical structure of Phenylurenyl chalcones.

para-position in the urenyl ring plays an important role in their antimalarial activity [30].

A natural C-benzylated chalcone, 2',4'-dihydroxy-3'-(2-hydroxybenzyl)-6'-methoxychalcone **45** (Fig. **14**) was isolated from the aerial parts of *Ellipeiopsis cherrevensis* (Annonaceae) along with other flavanoids and alkaloids. It exhibited cytotoxic activity against malarial parasites *P. falciparum* (IC_{50} ~ 7.1 µg/mL) and against various cancer cell lines [31].



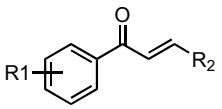
45.

Fig. (14). Chemical structure of C-benzylated chalcone.

In vitro investigation of a series of 1-phenyl-3-aryl-2-propen-1-ones **46-51** (Fig. **15**) resulted in identification of inhibitors with submicromolar efficacy against drug-resistant strains of *Plasmodium falciparum*. The attachment of R_2-substituents (quinolines and naphthalenes) at 3 position of 2-propen-1-one has greater influence on its antiplasmodial activity. Tabular representation of following analogues study suggests that nitrogen containing 3-quinolines as R_2 -substituent, appears to be more active than their corresponding carbocyclic analogues, the 2-naphthalenes. However, this is not the case with corresponding 4-quinoline R_2-substituent. In both series, di- and tri-methoxy substituted compounds at 1-phenyl ring were found to be more active than the corresponding mono-methoxy analogues. Failure of *in vivo* activity of these analogues in a *Plasmodium berghei* infected mouse model suggested its metabolic instability [32].

A series of 'retinoid-like chalcones' were synthesized from a new enaminone synthon which occurred via a new aromatic annelation. 4-hydroxy-chalcone-like (derived from β-ionone) **52** (Fig. **16**), exhibited good and reproducible inhibitory effect in *in vitro* culture of *Plasmodium falciparum*, with an IC_{50} value lower than 10 µM in [^{3}H] by hypoxanthine uptake assay (respectively IC_{50} values 4.93 and 8.47 µM for strains K1 and Thaï) [33].

Both enantiomers of hinokiresinol **53** (Fig. **17**) possessed almost same antiplasmodial activity, and were used as templates for developing new antimalarial drugs. Synthetic procedure was based on the method using chalcone as starting material [34]. The potencies of the hinokiresinol analogues were successfully improved up to 10 times from an IC_{50} value of 13µg/mL (hinokiresinol) to an IC_{50} value of 1.5 µg/ mL (10h) by changing the substitution pattern on the

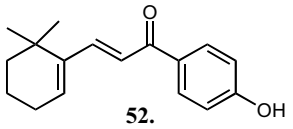


No.	R_1	R_2	P.f.W_2 IC_{50} (μM)	P.f.D_6 IC50(μM)	*In vivo* activity
46	4-MeO–	3-Quinolinyl-	3.3	1.8	-
47	4-MeO–	2-Naphthyl-	>17	>17	-
48	3,4-DiMeO–	4-Quinolinyl-	0.50	0.88	inactive
49	3,4-DiMeO–	2-Naphthyl-	7.7	9.8	-
50	4-MeO–	4-Quinolinyl-	10	5.4	-
51	4-MeO–	1-Naphthyl-	10	9.1	-

pfW2: *Plasmodium falciparum W2,* pfD6: *Plasmodium falciparum D6.*

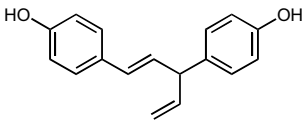
Fig. (15). Chemical structure of 1-phenyl-3-aryl-2-propen-1-one derivatives.

rings [35]. The bioassay-guided purification of n-hexane extract from the leaves of *Piper hostmannianum* var. *berbicense* led to isolation of various monoterpene or prenyl-substituted dihydrochalcones along with other known compounds. 2',6'-dihydroxy-4'-methoxydihydrochalcone **54**, linderatone **55** and (-)-Methyllinderatin **56** (Fig. **17**) exhibited potent antiplasmodial activities with IC_{50} values of 12.69, 10.33 and 5.64 μM respectively against both chloroquine-sensitive and resistant strains of *P. falciparum* (F32, FcB1) [36].

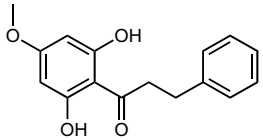


52.

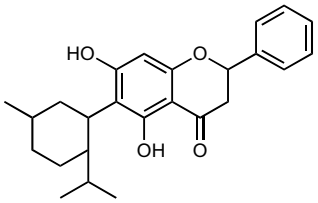
Fig. (16). Chemical structure of retinoid-like 4-hydroxy-chalcone.



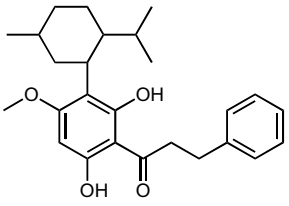
53. Hinokiresinol



54. 2',6'-Dihydroxy-4'-methoxy dihydrochalcone



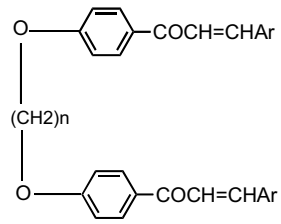
55. linderatone



56. (-)-Methyllinderatin

Fig. (17). Chemical structure of hinokiresinol and prenyl-substituted dihydrochalcones.

Compounds belonging to the bischalcone **57** (Fig. **18**) and chalcone series significantly inhibited DNA topoisomerase (topo) II of chloroquine-sensitive and chloroquine-resistant strains of the rodent malaria parasite *Plasmodium berghei*. *In vitro* topo II inhibition can be correlated with their *in vivo* antimalarial activity, as some of the compounds inhibited both *in vitro* activity of topo II and *in vivo* parasitaemia of the chloroquine-sensitive strain of *Plasmodium berghei*. Methylene chain length in bischalcones seems to be crucial for exhibiting topoisomerase inhibitory activity and methoxy substitution at position 2 and 4 of the phenyl ring produced more active compounds than the compounds having methoxy substitution at positions 3 and 4 of the phenyl ring [37].



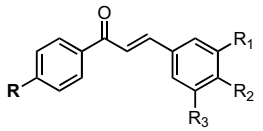
Ar = 3,4-$(CH_3O)_2C_6H_3$ or 2,4-$(CH_3O)_2C_6H_3$

57.

Fig. (18). Chemical structure of bischalcone series.

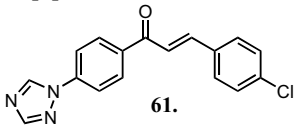
The *in vitro* antiplasmodial activity of 4-chloro **58**, 4-methoxy **59** and 3,4,5-trimethoxy **60** (Fig. **19**) in the series suggested that small or medium sized highly lipophilic groups containing multiple nitrogen or amine in acetophenone moiety impart antiplasmodial activities. Compound **61** (Fig. **19**), a triazole substituted chalcone was found to be the most effective (IC_{50} = 1.52±0.04). However, pyrrole and benzotriazole showed comparably less activities, suggesting that small lipophilic groups containing single or multiple nitrogen can enhance antimalarial activity *in vitro*. In triazole substituted chalcones, spacing of nitrogen and orientation of the molecule on active site of enzyme, are good enough to provide stronger and effective hydrogen bonding with histidine 67 of cysteine protease. This study concluded that

chalcones containing small, highly polar, lipophilic cyclic amines (chalcones with azoles on acetophenone ring) are showing antimalarial potential [38].



58. R_1=H, R_2=Cl, R_3=H
59. R_1=H, R_2=OCH_3, R_3=H
60. R_1= R_2= R_3= OCH3

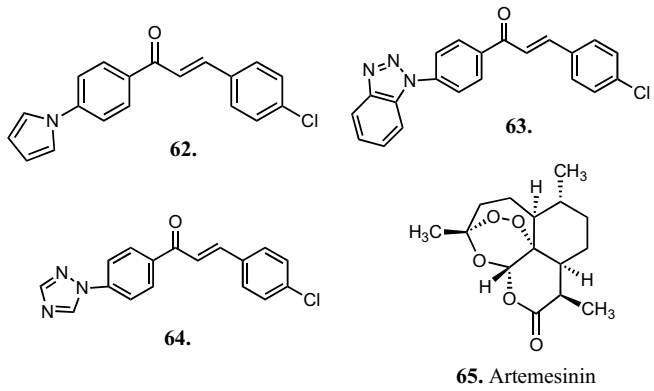
R can be substituted with pyrrole, imidazole, benzotriazole, pyrazole, pyrrolidine, morpholine and piperidine.



61.

Fig. (19). Chemical structure of heterocyclic ring substituted chalcones.

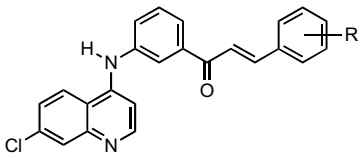
Antiplasmodial activity of chalcone derivatives is exerted at all stages of asexual cycle, predominantly acting on trophozoite stages. Artemisinin **65** (Fig. **20**) in combination with three different synthesized novel chalcone azole derivatives **62-64** (Fig. **20**) showed synergistic or additive interactions and decreased hemozoin formation in parasitized erythrocytes [39].



62. **63.** **64.** **65.** Artemesinin

Fig. (20). Chemical structure of artemisinin and chalcone azole derivatives.

The [(7-Chloroquinolin-4-yl)amino]chalcone derivatives (Fig. **21**), synthesized from corresponding 3- or 4-[(7-chloroquinolin-4-yl)amino]acetophenone, have been evaluated for *in vitro* antimalarial activity evaluating their potential to inhibit heme crystallization and globin proteolysis. The 4-amino-7-chloroquinoline subunit is an antimalarial pharmacophore that inhibits heme dimerization into the non-toxic hemozoin, while appropriate substitution on phenyl ring increased its lipophilic property. The most active compounds **66-70** from the 3-substituted series displayed inhibitory effects against heme crystallization in the range of 93.14±1.74 - 94.93±1.50 % [40].

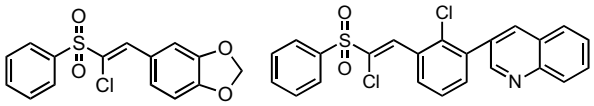


Compounds	R	%IβHS	IC_{50}(μg/ml)
66	4-N(CH3)2	94.55 ± 0.25	23.66 ± 1.24
67	4-Cl	94.34 ± 0.99	7.93 ± 2.05
68	2-Cl	94.42 ± 1.89	10.09 ± 2.29
69	3-F	94.93 ± 1.50	7.11 ± 2.06
70	H	93.14 ± 1.74	6.95 ± 1.62
71. Chloroquine	-	96.61 ± 0.26	-

IβHS: inhibition of β-hematin synthesis.

Fig. (21). Chemical structure of [(7-Chloroquinolin-4-yl)amino] chalcone derivatives.

Chlorovinyl sulfone-like chalcone derivatives (Fig. **22**) were prepared via Claisen-Schmidt condensation from available chloromethylphenyl sulfones with substituted aldehydes. [2(3',4'-methylendioxyphenyl)-1-chloro]ethenylphenyl sulfone **72** and [2(2'-chloroquinolin-3'-yl)-1-chloro]ethenylphenyl sulfone **73** have shown most potent antimalarial effects against *P. falciparum*, with IC_{50} values of 0.053 and 0.025 μM respectively. 3',4'-methylendioxy substituted derivative located in the aromatic ring of the α,β-unsaturated chlorophenyl sulfone, as well as the 2'-chloroquinolinyl group that forms part of the aromatic system, play an important role in mediating activity against *P. falciparum.* The inhibitory potency of compounds against the cultured *P. falciparum* parasites were found to be in good correlation with their inhibitory potency against hemoglobin hydrolysis. However, the same against hemozoin formation was found to be very weak and suggested involvement of various mechanisms [41].



72. **73.**

Fig. (22). Chemical structure of Chlorovinyl sulfone-like chalcones.

Out of the various chalcone analogues, compound 3-(4-methoxyphenyl)-1-(4-pyrrol-1-yl-phenyl)prop-2-en-1-one **74** (Fig. **23**) was found to be the most active with 50% inhibition concentration (IC_{50}) of 1.61μg/ml, and was comparable to prototype chalcone, licochalcone A (IC_{50} of 1.43 μg/ml). The excellent potency of the compound suggests that small or medium-sized, saturated or unsaturated, but highly lipophilic nitrogen containing heterocyclic substituents can be introduced into ring B. Whereas small, hydrophobic functional groups are most suitable for the ring A. Additional hydrogen bonding or hydrophobic interaction between active site amino acid (histidine 67) of cysteine protease enzyme

and substituted chalcones, p–p interactions between imidazole of histidine and substituted acetophenone along with the aromatic ring might be responsible for enhanced antimalarial activity [42]. The cyclin dependent protein kinases, Pfmrk and PfPK5, play an essential role in cell cycle control and differentiation in *P. falciparum* and are thus attractive targets for the development of antimalarial drugs. Series of chalcone analogues have been reported to inhibit Pfmrk (selectivity over PfPK5) in the low micromolar range with good correlation between Pfmrk inhibition and *in vitro* activity against the parasite. Molecular modelling study shows a pair of amino acid residues within the Pfmrk active site appear to confer this selectivity. Compound **75** (Fig. **23**), found to be most potent Pfmrk inhibitor with an IC_{50} of 1.3 µM, has been explained by the number of polar interactions between Lys-39, the glycine rich region, and a particular substituted chalcone moiety [43].

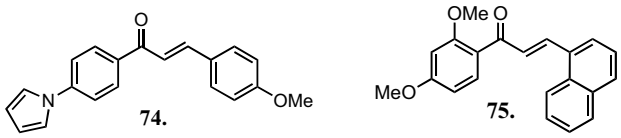
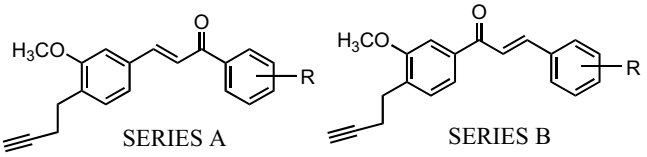


Fig. (23). Chemical structure of chalcone analogues.

On evaluation of series of synthetic acetylenic chalcones (Fig. **24**) for antimalarial activity, data suggested that growth inhibition of the W2 strain of *P. falciparum* can be imparted by the introduction of a methoxy group ortho to the acetylenic moiety. However, the series which lacked this ortho methoxy group showed activity against the chloroquine-sensitive (D10) strain. The most active compound **76** had IC_{50} of 3.4 µM against the D10 and 3.8 µM against the W2 strain, respectively. Mechanism of action was not clearly defined as none of the chalcones in this series showed activity against the *P. falciparum* cysteine protease falcipain-2. Expansion of the series A and B will provide further evidence regarding correlation of activity and the degree of methoxylation, position of attachment of the acetylenic group and the aromatic ring preference for substituents [44].



76. R= 2,4-dimethoxy(series A)

Fig. (24). Chemical structure of acetylenic chalcones.

Various α-pyranochalcones and pyrazoline analogs were synthesized to obtain chemically diverse antimalarial leads. The (E)-3-(3-(2,3,4-trimethoxyphenyl)-acryloyl)-2H-chromen-2-one **77** (Fig. **25**), turned out to be the most potent analogue of the series, showing IC_{50} of 3.1 µg/ml against chloroquine-sensitive (3D7) strain and IC_{50} of 1.1 µg/ml against chloroquine-resistant field isolate (RKL9) of *Plasmodium falciparum*. The antimalarial activity, evaluated using the microtiter plate based SYBR-Green-I assay, had shown high therapeutic indices, suggesting its selectivity in action against the malaria parasite. Further, docking of most active compound into active site of falcipain enzyme revealed the predicted interactions with active site residues [45].

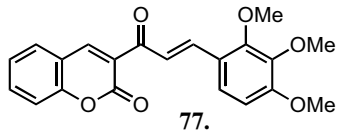
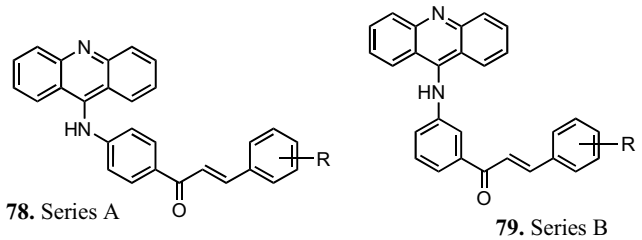


Fig. (25). Chemical structure of pyranochalcone.

A series of novel chalcones bearing acridine moiety attached to the amino group in their ring B had been synthesized through noncatalyzed nucleophilic aromatic substitution reaction between various 3'-aminochalcone or 4'-aminochalcones and 9-chloroacridine. *In vitro* antimalarial activity of chalcones **78 & 79** (Fig. **26**) have displayed complete inhibition at concentration of 10 µg/mL and above, while three compounds showed significant inhibition at concentration of 2 µg/mL against *P. falciparum* NF-54. Location and nature of the substituent in ring A of the chalcone derivatives were crucial in determining antimalarial potency as they played a vital role in interaction with the enzyme [46].



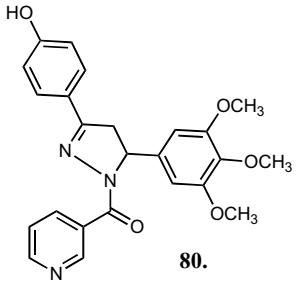
R is 4-OCH_3, 3,4,5-Trimethoxy, 4-Cl, H, 3-NO_2, 3-CH_3 and 4-CH_3

Fig. (26). Chemical structure of acridine bearing amino chalcones.

A series of 1,3,5-trisubstituted pyrazolines were synthesized using recrystallized substituted chalcones by the well-known Claisen-Schmidt reaction. 1,3,5-trisubstituted pyrazolines analogues showed *in vitro* antimalarial efficacy against chloroquine sensitive (MRC-02) as well as chloroquine resistant (RKL9) strains of *P. falciparum*. Compound **80** (Fig. **27**) was found to be most active against parasite. It showed activity at nano molar concentration and good correlation was observed between normalized antimalarial activity and β-hematin formation inhibition (BHIA50) against both sensitive and resistant strains [47].

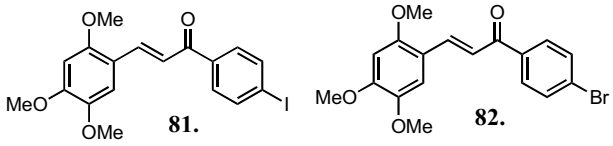
Structure activity relationship of a series of methoxylated chalcones against *P. falciparum* (3D7 strain) using fluorescence-based SYBR Green assay revealed that basic chalcone scaffold (having 2,4,5-trimethoxy substitution on aryl ring A) was crucial for antimalarial activity. Electron releasing methoxy groups on ring A and electron withdrawing groups on ring B increased antimalarial potency while the positional interchange of these groups decreased it. Natural β-asarone, obtained from *Acorus calamus* oil, is rich source for synthesis of 2,4,5-trimethoxy substituent (ring A), which

constitute potent analogues. Most active analogue **81** (Fig. **28**) (IC_{50}=1.8 μM) and **82** (Fig. **28**) (IC_{50}=2 μM) were also relatively non-toxic [48].



80.

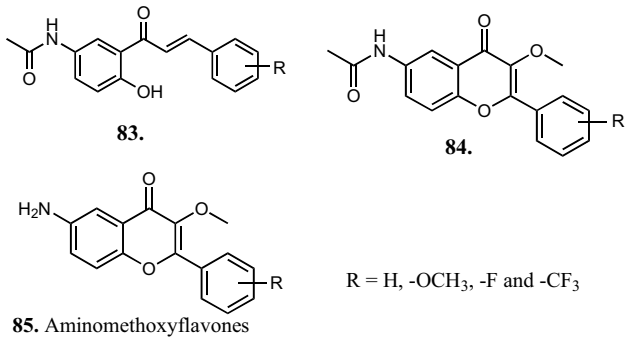
Fig. (27). Chemical structure of 1,3,5-trisubstituted pyrazoline.



81. **82.**

Fig. (28). Chemical structure of methoxylated chalcones.

Novel series of flavonoid derivatives and their chalcone intermediates **83** (Fig. **29**) have been synthesized and evaluated for antiproliferative activity on *P. falciparum* (W_2) parasites. Chalcones exhibited more selective antiplasmodial activity than flavonoids. Methoxyflavone **84** and aminomethoxyflavone **85** derivatives (Fig. **29**) exhibited highest specific activity against *P. falciparum* strain W2 and different substitutions on the phenyl ring A, e.g. fluoro at position 2, promoted its specific antiplasmodial activity. Para substitutions on A ring of amino derivatives of methoxyflavones were needed to promote antiplasmodial activity [49]. Another novel series of β-amino alcohol hybrids which included thiolactone-chalcone hybrids **86- 87** (Fig. **30**) and isatin-chalcone hybrids **88-89** (Fig. **30**) displayed promising antimalarial activity against chloroquine-resistant W2 strain *P. falciparum*. Envisaged Hybrid design included β-amino alcohol moiety, a known antimalarial pharmacophore and the solubility enhancing 1,2,3-triazole ring system which functions as linker. The β-amino alcohol thiolactone-chalcone hybrids had shown promising antiplasmodial activity with IC_{50}s < 6.08 μM against W2 strain but did not show falcipain-2 inhibitory activity at the maximum concentration tested, suggesting that different pathways or target inhibitions are responsible for antimalarial activity. Antiplasmodial activity increases with increase in the number of methoxy substituents on the chalcone moiety. The preference for methoxy substituent on phenyl ring and activity of compounds show penchant for *m*-substitution as far as the attachment of the triazole moiety to the chalcone scaffold is concerned. The isatin-chalcone hybrids generally displayed less activity than thiolactone-chalcone hybrids (with IC_{50} of 14.9μM or less). This study has confirmed the interaction capabilities of isatin scaffold with thiols of cysteine proteases and falcipain-2 inhibitory activity when meta substitution is preferred [50].

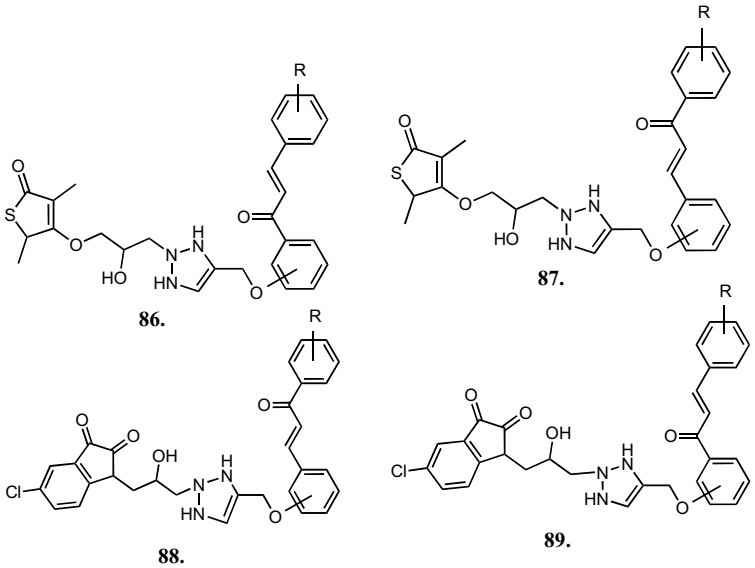


83. **84.**

85. Aminomethoxyflavones

R = H, -OCH_3, -F and -CF_3

Fig. (29). Chemical structure of amino substituted chalcone and methoxyflavones.



86. **87.**

88. **89.**

Where, R = 4-methoxy; 2,4-dimethoxy; 2,3,4-trimethoxy

Fig. (30). Chemical structure of β-Amino alcohol hybrids.

The Cu (I)-catalyzed cycloaddition of azides and terminal alkynes have been applied as the hybridization strategy for the synthesis of a targeted series of chalcone and dienone hybrid compounds containing aminoquinoline and nucleoside templates. The AZT hybrid compounds did not exhibit notable higher *in vitro* antimalarial activity in comparison to their acetylenic precursors while their solubility and possibly the oral bioavailability has enhanced. The chloroquinoline hybridization strategy led to enhanced antimalarial activity, with most active compound **90** (Fig. **31**) exhibited submicromolar IC_{50} values against the D10, Dd2 and W2 strains of *Plasmodium falciparum.* This study also pointed out the importance of α,β-unsaturated carbonyl system in hybrid compounds for antimalarial activity. Inhibitory activity of these compounds against β- hematin formation accounted for the primary mechanism of action, though other mechanisms of action are also quite possible. Enone-chloroquinoline triazole intermediate **91** (Fig. **31**) showed notably high *in vitro* antimalarial activity with submicromolar IC_{50} values against D10 and Dd2 *P. falciparum* and modifications on its α-carbon is likely to be crucial for the retention of activity [51].

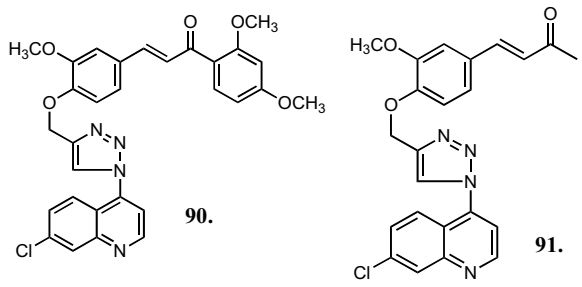


Fig. (31). Chemical structure of chloroquinoline triazole hybrid.

Various analogues of hybrid compounds **90** and **91** (Fig. **31**) were proposed with the aim of identifying compounds with improved solubility and antimalarial potency, which were supported by their *in silico* characterization. They retained acceptable predicted permeability properties but were predicted to be susceptible to hepatic metabolism, principally N-dealkylation by hepatic cytochromes. Though, none were as active as **90**, compounds **92** and **93** (Fig. **32**) were found to be most active among various hybrid analogues. To certain extent the mechanism of action of these analogues is inhibition of hemozoin formation and inhibition of falcipain-2 [52].

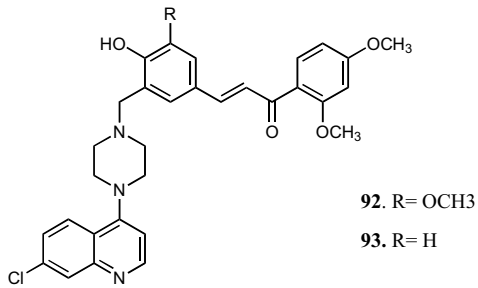


Fig. (32). Chemical structure of Enone and chalcone chloroquinoline hybrid analogues.

Chalcone, intermediate in synthesis of 7-Methoxyflavenes and 5,7,8-trimethoxyflavenes, were found to undergo stereoselective acid-catalyzed rearrangement to generate the benzopyrano [4,3-b]benzopyran ring system present in the natural product, dependensin 1 **94** (Fig. **33**). Antimalarial growth inhibition assays of dependensin and its analogue against *P. falciparum* were found to have IC_{50} values ranging between 1.9 and 3.9 μM [53].

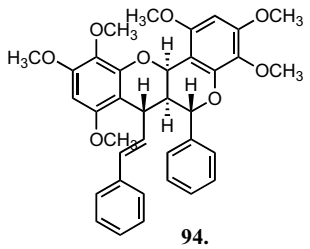


Fig. (33). Chemical structure of Dependensin 1.

Evaluation of a series of synthetic dibenzylideneacetones (Fig. **34**) and some of their pyrazolines in *in vitro* blood stage *P. falciparum* culture using SYBR- green-I fluorescence assay has shown that the compound (1E, 4E)-1,5-bis(3,4-dimethoxyphenyl)penta-1,4-dien-3-one **95** was most active with IC_{50} of 1.97μM against chloroquine-sensitive strain (3D7) and 1.69 μM against chloroquine-resistant field isolate (RKL9). The MTT based cytotoxicity assay on HeLa cell line confirmed its selective action against malaria parasite with a therapeutic index of 166. This initial study concluded that symmetrical dibenzylideneacetones requires electron donating group (methoxy) at the meta and para positions and electron withdrawing (chloro) group at the ortho position **96** to achieve desirable improvement of antimalarial activity. Thus, low molecular weight dibenzylideneacetones represent a novel antimalarial scaffold, and a potential starting point for the development of new, safe, effective, cheap and potent inhibitors/drugs against malaria [54].

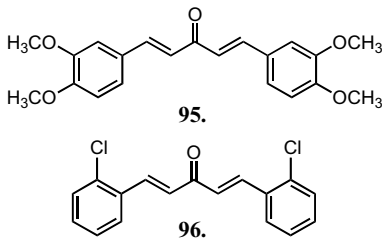


Fig. (34). Chemical structure of synthetic dibenzylideneacetones.

ANTIMALARIAL ACTIVITY OF CURCUMIN

Development of simple and efficient method for the separation of three phenolic diketones (Fig. **35**), curcumin **97**, demethoxycurcumin **98**, and bis-demethoxycurcumin **99** from the rhizomes of *Curcuma longa* provided unique opportunity to explore their therapeutic dimensions. These molecules show activity against *P. falciparum* with IC_{50}

value of 3.5, 4.2 and 3.0 μg/ml respectively [55]. Further, polyphenolic organic molecule, curcumin was found to be potent against both chloroquine-sensitive (IC_{50} of ~ 3.25 μM, MIC = 13.2 μM) and chloroquine-resistant (IC_{50} of ~ 4.21 μM, MIC = 14.4 μM) *P. falciparum* strains [56].

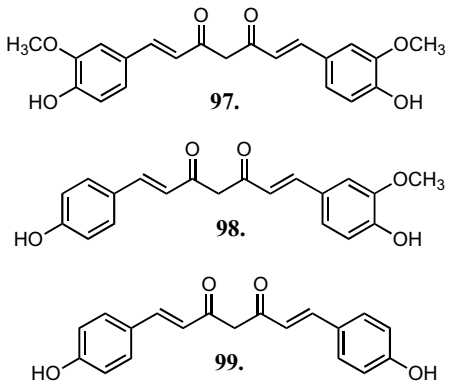


Fig. (35). Chemical structure of curcuminoids.

Curcumin inhibited chloroquine-resistant *P. falciparum* growth in culture in a dose dependent manner with an IC_{50} of 5 μM in a [^{3}H] hypoxanthine uptake assay. Oral administration of curcumin to mice infected with malaria parasite (*Plasmodium berghei*) reduced blood parasitemia by 80–90% and enhanced their survival significantly. PfATP6, the parasite orthologue of mammalian sarcoplasmic–endoplasmic reticulum Ca^{2+}-ATPase (SERCA) could be possible target for curcumin action [57].

In view of its abundance, non-toxic nature, and therapeutic effects, curcumin can be an ideal antimalarial molecule especially for use in combination with antimalarials such as artemisinin to overcome the problems of high cost, recrudescence, and drug resistance. Artemisinin and curcumin showed an additive effect in inhibiting *P. falciparum* in culture. In *in vivo* study, three oral doses of curcumin followed by a single injection of α,β-arteether to *Plasmodium berghei*-infected mice, were able to prevent recrudescence due to α,β-arteether monotherapy and ensured almost 100% survival of animals [58].

Curcumin showed competitive inhibition towards both CYP2B6 and CYP3A4, which may partly explain the fact that, in combination with artemisinin, it completely prevented recrudescence of malarial parasites and death in animal models [58, 59]. The efficacy of curcumin as a partner drug of artemisinin against an artemisinin-resistant clone of *Plasmodium chabaudi* and efficacy of piperine in increasing the bioavailability of curcumin was evaluated. it was evident that curcumin, alone and in combination with piperine has only a modest antimalarial effect and was unable to reverse the artemisinin-resistant phenotype when used in combination with artemisinin [60].

Cerebral malaria (CM) is severe complication of *P. falciparum* infections. CM is a result of dysregulated immune response *i.e.* results of an immunopathological process. Curcumin, having antimalarial [56] and immunomodulatory activities [61], can be envisaged as ultimate treatment of CM. Among the various compounds tested, only fasudil and curcumin had significant effects on the progression of the disease in a murine model of cerebral malaria. Neither of the drugs caused a reduction in parasitemia but survival of the treated mice was significantly increased and the development of cerebral malaria was either delayed or prevented. This supports the hypothesis that immunomodulators efficient in preventing CM should be administered together with antiplasmodial drugs to prevent severe malaria disease [62].

Adjunctive therapy of CM patients with an appropriate immunomodulatory compound possessing even moderate anti-malarial activity with the capacity to down regulate excess production of pro-inflammatory cytokines and expression of adhesion molecules, could potentially reverse cytoadherence, improve survival and prevent neurological sequelae. Well tolerated curcumin might exert its therapeutic effects by inhibiting NF-κB activation, followed by downregulation of pro-inflammatory cytokine production and expression of cytoadhesion molecules on endothelial cells. Cytoadherence of the malaria parasite continues long after parasites have been killed by antimalarial drugs, supports the development of adjunctive therapies to reverse the pathophysiological consequences of cytoadherence. Thus, Drug discovery efforts focused on molecules with dual, immunomodulatory and anti-parasitic action, may pave the way for their use as an adjunctive therapy for the management of uncomplicated and severe malaria [63].

The Pro-oxidant activity of curcumin promotes production of reactive oxygen species (ROS), resulting in damage of both mitochondrial and nuclear DNA (probably due to its intracellular elevation). This cytotoxic effect can be antagonized by co-incubation with antioxidants and ROS scavengers. Curcumin inhibits the histone acetyltransferase (HAT) activity of the recombinant *Plasmodium falciparum,* thus leading to down-regulation of PfGCN5 HAT activity (specific inhibition) and these two activities account for the parasiticidal effect of curcumin [64].

Now, the design of synthetic strategy has been focused on the development of curcumin analogues with high anti-malarial activity especially against CQ-R strains. Possibilities of synthesizing a number of derivatives around 'curcumin' scaffold open up new opportunities for anti-malarial therapy. Among the various analogues, compounds **100**, **101**, and **102** (Fig. **36**) were found to be most potent and have shown inhibitory activity for CQ-S *P. falciparum* with IC_{50} of 0.48, 0.87, 0.92 μM and for CQ-R *P. falciparum* at IC_{50} of 0.45 μM, 0.89, 0.75 μM, respectively. Pyrazole analogues of curcumin exhibited seven fold higher antimalarial potency against CQ-S strains and nine fold higher antimalarial potency against CQ-R strains [65]. Recent study on monocarbonyl analogues of curcumin indicated their safety towards mammalian cells. Considering the selectivity index for cytotoxicity (Vero cell) to antimalarial activity, it was demonstrated that all the monocarbonyl analogues of curcumin are non toxic [66].

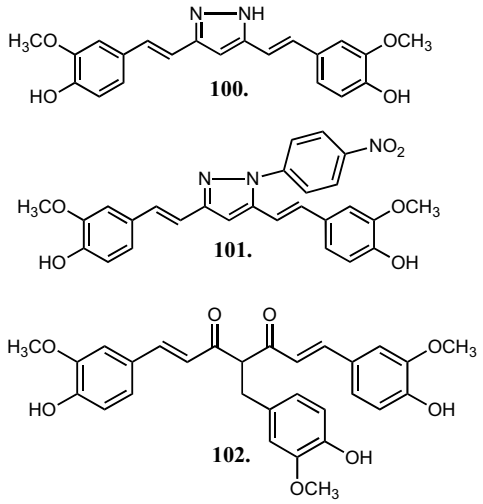


Fig. (36). Chemical structure of potential curcumin analogues.

Curcumin revealed potential antimalarial activity in synergistic manner with indigenous plants *Andrographis paniculata* and *Hedyotis corymbosa*. Both extracts inhibited *P. falciparum* culture at the ring stage of parasite. Increased *in vivo* potency was observed with the combination of plant extracts over the individual extracts and curcumin. Thus, this combination could be an effective, alternative source of herbal anti-malarial drugs [67]. Interactions of curcumin with PfATP6, an important antimalarial target, were investigated by molecular docking studies and its effective inhibition of PfATP6 as characterized by the theoretical binding ability, provided some new clues to the antimalarial mechanisms of curcumin. The phenolic hydroxyls are important for the binding of curcumin to PfATP6 and their chemical modifications may affect the binding ability of curcumin. On performing parallel calculations on the methylcurcumin, The Ludi scores (tool for calculate binding affinity) decreased to 528 and 504 from 594 and 561 for complexes of PfATP6 with the keto **103** and enol **104** forms (Fig. **37**) respectively, [68].

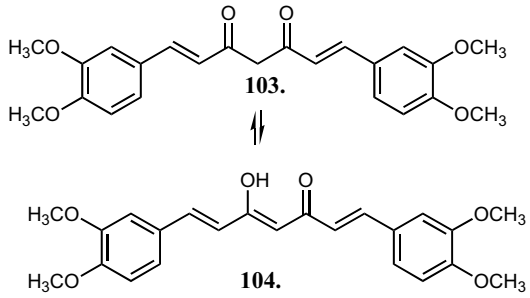


Fig. (37). Chemical structure of keto and enol forms of methylcurcumin.

Therapeutic intervention accelerating suicidal death of infected erythrocytes (Premature eryptosis) has the potential to foster elimination of infected erythrocytes, delay the development of parasitemia and favourably influence the course of malaria. Curcumin is one of responsible factors for triggering stimulation of suicidal erythrocyte death. Most importantly, counteracting plasmodia by inducing eryptosis is not expected to generate resistance of the pathogen as the proteins involved in suicidal death of the host cells are not encoded by the pathogen and thus cannot be modified by mutations of its genes [69,70]. In ultrafiltration, liquid chromatography and mass spectrometry (UF and LC/MS) based binding assays, curcuminoids (bis-demethoxycurcumin, demethoxycurcumin, and curcumin) were used to study the binding affinity for PfTrxR and PfGR enzymes, which are highly interesting drug targets to develop new antimalarial drugs. The developed method was specific, fast and needs very low amounts of test compounds due to the low detection limits of the LC/MS and thus, have a potential for automated high-throughput screening to discover potential ligands for PfTrxR and PfGR enzymes [71]. Clinical trials of combinations of pure compounds (such as artemisinin + curcumin + piperine) and of combinations of herbal remedies (such as *Artemisia annua* leaves + *Curcuma longa* roots + *Piper nigrum* seeds) conclude that former may enhance the activity of existing pharmaceutical preparations, and the latter may improve the effectiveness of existing herbal remedies for use in remote areas where modern drugs are unavailable [72].

Chloroquine phosphate (CQ), on account of its rapid action on blood schizontocide of all strains of malarial parasites, has become the most widely prescribed drug for prophylaxis and treatment of malaria in most endemic areas. This led to commonly encountered toxicity of CQ at therapeutic and higher doses of treatment, which brought about significant decrease in Protein content with a decline in SDH, ATPase and ALKase activities, whereas ACPase activity was found to be significantly increased. Antioxidant enzyme, SOD registered a significant reduction as opposed to TBARS, which was found to be elevated in a significant manner in the CQ treated groups as compared to control. Administration of curcumin led to significant reversal of CQ induced toxicity in hepatic tissues as protein contents, SDH, ATPase, ALKase, ACPase, SOD, TBARS were found to be comparable to that of control group after curcumin administration [73].

Curcuminoids-loaded lipid nanoparticles for parenteral administration were successfully prepared by nanoemulsion technique employing high-speed homogenizer and ultrasonic probe. For the production of nanoparticles, trimyristin, tristerin and glycerylmonostearate were selected as solid lipids and medium chain triglyceride (MCT) as liquid lipid, which influenced the entrapment efficiency (EE) and drug loading capacity (LC). The *in vivo* pharmacodynamic activity revealed 2-fold increase in antimalarial activity of curcuminoids entrapped in lipid nanoparticles when compared to free curcuminoids at the tested dosage level as controlled release characteristics and parentral nature of formulation (by passing the gastro-intestinal route) may improve bioavailability of the drug in the active, native form. Furthermore, lipid nanoparticles may increase drug concentrations at the site of action and will help to treat cerebral malaria [74]. Formulation of hydrogel nanoparticles of curcumin using a combination of hydroxyl propyl methyl cellulose and polyvinyl pyrrolidone enhanced absorption and prolonged the rapid clearance of curcumin due to possible evasion of the reticulo-endothelial system. In addition to its

in vivo anti-malarial studies which revealed its significance as an adjunct anti-malarial therapy along with the standard therapy, acute and subacute toxicity studies have confirmed the oral safety of the formulation [75].

Poor bioavailability and chemical instability of curcumin hindered its development as drug. This could be improved by binding curcumin to chitosan nanoparticles, which not only increased its bioavailability from 0.04% to 0.4%, but also enhanced circulation hour from 30 minute to 6 hours. Oral delivery of these particles to normal mice has shown that they can cross the mucosal barrier intact, and confocal microscopy detected the curcumin bound chitosan nanoparticles in the blood. Further, it has improved uptake of curcumin by mouse RBC along with delayed *in vitro* degradation. Oral administration of curcumin bound to chitosan nanoparticles cured mice from *Plasmodium yoelii* (N67) infection. Curcumin inhibited parasite induced β-hematin synthesis *in vitro* in a dose dependent manner and has demonstrated lower IC_{50} value (122 μM±2.7) than chloroquin (198 μM±3.7). Thus, by enhancing bioavailability and chemical stability, curcumin can inhibit hemozoin synthesis which is lethal for the malaria parasite [76]. The above mentioned combinations would be good candidates to take them forward into clinical trials.

PROTEIN TARGETS OF CURCUMIN AND CHALCONES

Extensive research during the past couple of decades has shown the ability of curcumin and chalcone derivatives to modulate multiple cellular targets and hence posses preventative and therapeutic values against malaria. Examples of such targets includes Histone acetyl transferase, Ca(2+)–ATPase (PfATP6), and Cysteine proteases.

Curcumin inhibits the histone acetyltransferase (HAT) activity of the recombinant *P. falciparum* general control nonderepressed 5 (PfGCN5) *in vitro* and reduces nuclear HAT activity of the parasite in culture. It induced hypoacetylation of histone H3 at K9 and K14, but not H4 at K5, K8, K12, and K16, suggested that curcumin causes specific inhibition of PfGCN5HAT. These observations indicated that generation of ROS and down-regulation of PfGCN5HAT activity accounted for the cytotoxicity of curcumin. The importance of epigenetics in development of malarial parasite suggests that the pathways involved in parasite chromatin modifications may be practical drug targets [77].

Another target of curcumin is PfATP6 (*P. falciparum* ATP6), the parasite analogue of SERCA (Sarcoplasmic/ endoplasmic reticulum Ca^{2+}-ATPase). PfATP6 is the solitary SERCA-type Ca^{2+}-ATPase in *P. falciparum* which is accountable for the maintenance of calcium ion concentrations for the generation of calcium-mediated signalling, correct folding and post-translational processing of the proteins. Ca^{2+}-ATPase (PfATP6) of *P. falciparum* is the target of many antimalarial drugs. However, the mechanism of inhibition of Ca^{2+}-ATPase (PfATP6) is not known [78]. It was suggested that, curcumin interacts with PfATP6 mainly through hydrophobic interactions and hydrogen bonds. Moreover, the theoretically predicted binding affinity implies that curcumin can efficiently inhibit PfATP6, which gains some deeper insights of the antimalarial mechanism of curcumin [79].

C-Jun N terminal kinases (JNK) belong to the family of mitogen activated kinases called MAP Kinases, which are activated in response to inflammatory cytokines and environmental stress conditions. Their activation induces the transcription-dependent apoptotic signalling pathway resulting in neuronal cell death during experimental cerebral malaria. Curcumin suppresses JNK activation and thus protects the neurons. It may also affect the JNK pathway by interfering with the signalling molecule(s) at the same level or proximally upstream of the MAPKKK level. Taken together, the inhibition of the MEKK1-JNK pathway reveals a possible mechanism of suppression of AP-1 and NF-κB signalling by curcumin [80]. Curcumin activates PPAR-γ (peroxisome proliferator activated receptor γ), which is a transcription factor that regulates inflammatory responses. Its activation inhibits translocation of NF-κB and also microglia from producing proinflammatory cytokines. In a recent *in vivo* study, curcumin significantly reduced the parasitemia and development of cerebral malaria and was also found to activate PPARs. PPARs have been shown to exhibit anti-inflammatory and immunomodulatory properties. PPARs agonists can be considered as a therapeutic option for the treatment of cerebral malaria, where inflammation sustains as a basic pathology. Clinical trials for assessing the efficacy and safety of these agonists in humans, should confirm their promising perspectives. Thus, targeting these receptors might be a breakthrough in treatment of cerebral malaria [81].

Recent studies have shown that curcumin was able to interfere with ubiquitin proteasome pathway. Inhibitor studies in *Plasmodium* certified the essential role of proteasome in the liver, blood and transmission stage. Thus, suggesting the proteasome may be a promising multi stage target in malaria therapy. *In silico* study has shown that genome of *Plasmodium* spp contains several gene encoding proteins predicted to be involved in the Ubiquitin Proteasome System. Ubiquitylation is a regulated post translational modifications of proteins in which ubiquitin molecule is attached to a lysine amino acid in the target protein. The removal of ubiquitin is carried out by deubiquitylating enzymes (DUBs). DUBs have also been implicated in antimalarial drug resistance as confirmed by mutations found in a gene encoding a de-ubiquitylating enzyme UBP-1 in *Plasmodium chabaudi* parasites resistant to artemisin and artesunate. *In vivo* efficacy of curcumin was studied in BALB/c mice infected with *Plasmodium chabaudi* clones resistant to chloroquine and artemisinin, and drug interactions were analyzed. Treatment of mice with subtherapeutic dose of the drug resulted in transient increase in gene encoding DUBs signifying UPS intervention [82, 83]. The *Plasmodium falciparum* S-adenosyl-L-homocysteine hydrolase (pfSAHH) enzyme, a regulator of biological methylations has been considered as a potential chemotherapeutic target against malaria due to the amino acid differences found on binding sites of pfSAHH related to human SAHH. Curcumin has shown a strong interaction with hydrophobic amino acid residues of pfSAHH. Molecular Docking and ADMET predictions suggest that curcumin can be a potent inhibitor of pfSAHH with ability to

modulate the target in comparatively smaller dose. Therefore, curcumin is likely to become a good lead molecule for the development of effective drug against malaria [84].

Cysteine proteases of the malarial parasite are of particular interest as therapeutic targets since they play major roles in parasite development. *P. falciparum* parasite expresses four cysteine proteases from the papain family known as falcipains, required for degradation of hemoglobin by erythrocytic malaria parasites, of which falcipain-2 and falcipain-3 are the most obvious drug targets. QSAR and Docking studies revealed that the mechanism of action of chalcone derivatives appears to be based on the competitive inhibition of malarial cysteine protease (falcipain). chalcone–cysteine protease interaction may be dependent on a nucleophilic interaction with one or more amino acid residues (Cys 39 and Gln 36) of the active site of the parasitic enzyme [85]. Thus, curcumin and chalcones appear to have several targets in the parasite that singly or collectively may be responsible for their observed antiparasitic action.

CONCLUSIONS

The development of novel agents is essential and has given impetus to researcher across the globe to search molecules from natural sources owing to drug resistance threatening the effectiveness of currently available malaria therapies. Several studies pointed out that chalcone and curcumin possess potential to be developed as lead compounds for the discovery of antimalarial agents. An important limitation in the clinical advancement of curcumin is its poor aqueous solubility and ADME properties. Attempts are being made to improve the bioavilability of curcumin by curcumin-PVP solid dispersion, solid-lipid curcumin nanoparticles, synthetic analogues of curcumin, polymeric nanoparticles, implants, cyclodextrin based preparation, liposomal prearation, curcumin phytosomes, adjuvants (piperine) [86] etc. Determination of genome sequence of *Plasmodium falciparum* and advancement in malaria genetics offer a multitude of potential drug targets. Natural lead compounds like chalcone and curcumin have shown good and optimal binding to many enzymes present in parasite and can be explored as molecular targets for *in silico* studies to develop new, affordable and effective antimalarial drugs.

A recent rational approach of antimalarial drug design characterized as "covalent bitherapy" involves linking two molecules with individual intrinsic activity into a single agent, thus packaging dual-activity into a single hybrid molecule [87]. In view of this background and reported antimalarial synergism between artemisinin and curcumin, we need to delve deep into computer-assisted docking to predict molecular interaction and binding affinity of Artemisinin-curcumin hybrid and its derivatives with *Plasmodium falciparum* Ca2+-ATPase (PfATP6) to endorse hybrid molecules as the next-generation antimalarial drugs. With no credible malaria vaccine in sight, there is an urgent need to develop new drugs with different mechanisms of action to help preclude issues of cross-resistance.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

ACPase	=	Acid phosphatase
ACTs	=	ART combination therapies
ADMET	=	Absorption, Distribution, Metabolism and Excretion
ALKase	=	Alkaline phosphatase
ART	=	Artemisinin
ATPase	=	Adenosine triphosphatase.
AZT	=	Zidovudine
BHIA50	=	β-hematin formation inhibition
CM	=	Cerebral malaria
CoMFA	=	Comparative molecular field analysis
CoMSIA	=	Comparative similarity indices analysis
CQ-R	=	Chloroquine-resistant
CQ-S	=	Chloroquine-sensitive
DUBs	=	Deubiquitylating enzymes
EE	=	Entrapment efficiency
HAT	=	Histone acetyltransferase
IC_{50}	=	Half maximal inhibitory concentration
JNK	=	C-Jun N terminal kinases
LC	=	Loading capacity
LC/MS	=	Liquid chromatography-mass spectrometry
MAP	=	Mitogen activated kinases
MAPK	=	Mitogen-activated protein kinase
MCT	=	Median chain triglyceride
MDCK	=	Madin-Darby canine kidney
MEKK1-JNK	=	Mitogen and extracellular kinase kinase
MIC	=	Minimum inhibitory concentration
MTT	=	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide.
NF-κB	=	Nuclear factor kappa beta.
P. falciparum	=	*Plasmodium falciparum*
PfATP6	=	*Plasmodium falciparum* ATPase 6
PfGCN5	=	*Plasmodium falciparum* GCN5 homologue
PfGR	=	*Plasmodium falciparum* glutathione reductase
Pfmrk	=	*Plasmodium falciparum* MO15-related protein kinase

PfPK5 = *Plasmodium falciparum*

pfSAHH = *Plasmodium falciparum* S-adenosyl-L-homocysteine hydrolase

PfTrxR = *Plasmodium falciparum* thioredoxin reductase

PPAR = Peroxisome proliferator activated receptor

PPAR-γ = Peroxisome proliferator activated receptor-γ

QSAR = Quantitative structure activity relationship

ROS = Reactive oxygen species.

SAHH = S-adenosyl-L-homocysteine hydrolase

SAR = Structure activity relationship

SDH = Succinate dehydrogenase

SERCA = Sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase

SOD = Superoxide dismutase

TBARS = Thiobarbituric acid reactive substances

UBP-1 = Upstream-binding protein 1

UPS = Ubiquitin proteasome system

WHO = World Health Organisation

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Letters in Drug Design & Discovery, **2013,** *10,* **00-00** **1**

Synthesis, Molecular Docking and *In Vitro* Antimicrobial Studies of New Hexahydroindazole Derivatives of Curcumin

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Abstract: A series of hexahydroindazole analogues of curcumin were synthesized and investigated for *in vitro* and *in silico* antimicrobial activity. The structures of synthesized compounds were identified on the basis of satisfactory analytical and spectral data (^{1}H NMR, ^{13}C NMR, EI-MASS techniques and elemental analysis). Synthesized compounds showed moderate to high activity against both bacterial and fungal strain. All compounds were docked computationally to the active site of enzyme L-glutamine: D-fructose-6-phosphate amido-transferase [GlcN-6-P] (EC 2.6.1.16). The autodock programme 4.0 was employed to perform automated molecular docking. *(E)-1-(7-(3-methoxybenzylidene)-3-(3-methoxyphenyl)-3,3a,4,5,6,7-hexahydro-2H-indazol-2-yl)ethanone* (**A7**) turned out to be the most potent analogue of the series, showing best activity against bacterial and fungal strain. Compound **A7** showed minimum binding and docking energy and may be considered as good inhibitor of GlcN-6-P synthase. Further investigation and optimization of this new lead could provide new antimicrobial molecules.

Keywords: Autodock 4.0; Bacterial strain; Fungal strain; GlcN-6-P synthase; Hexahydroindazole; *In silico.*

INTRODUCTION

In recent years, a number of antibiotics have lost their effectiveness due to the development of resistant strains. Extremely resistant bacteria such as methicillin-resistant *Styphlococoous aureus* (MRSA) and vancomycin-resistant Enterococci accounts for a soaring percentage of hospital acquired infections [1]. Multidrug resistance (MDR) to antibiotics presents a serious therapeutic problem in the treatment of bacterial infections. The importance of mechanism of resistance in clinical settings is reflected in increasing number of reports of multidrug resistant isolates [2]. In addition to this problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, immune-suppression allergic reactions etc. Therefore, there is a need to develop novel antimicrobial drugs for the treatment of infectious diseases. The search for not only the improved versions of existing drugs but also new drug targets have become an urgent need. Large number of marketed drugs has enzymes or membrane receptors as molecular targets [3]. In most cases, elucidating the intermolecular interactions in these drug-target complexes was a pre-requisite of the success [4].

The enzyme, L-glutamine:D-fructose-6-phosphateamidotransferase, known under the trivial name of glucosamine-6-phosphatesynthase (EC 2.6.1.16) is a new target for antimicrobial studies. The enzyme catalyses uridine 5'diphospho-N-acetyl-D-glucosamine, the first and practically irreversible step in the pathway of hexosamine metabolism end-product, and serves as an activated form of *N*-acetylglucosamine. This amino sugar is incorporated into several macromolecules viz. peptidoglycan, lipopolysaccharides and teichoic acids in bacteria, chitin in fungi, insects and crustaceans, and glycoproteins, glycosaminoglycans and mucopolysaccharides in mammals. All these molecules are essential for the assembly of the cell wall [5]. Several GlcN-6-P inhibitors, exhibiting antimicrobial activity have been reported, from both natural and synthetic origin.

Curcumin, a naturally occurring polyphenol is extracted from the rhizomes of *Curcuma longa*. It has been used as an important dietary component for long time. It exhibits various biological activities including antiproliferative activity against various cancer cells, antioxidant activity, wound healing ability and antimicrobial activity. Curcumin possesses antibacterial property against a number of Gram-positive and Gram-negative bacteria. It has been shown to kill several pathogenic Gram-positive bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis* and *Enterococcus* that cause skin infections, pneumonia, meningitis and urinary tract infections in human being. Understanding the mechanism of the antibacterial activity of curcumin will greatly assist in designing potent analogues of curcumin, a natural remedy for several bacterial diseases [6-10].

Compounds containing hexahydroindazole are biologically active and have been reported as potent antimicrobial, anti-inflammatory, depressant of central

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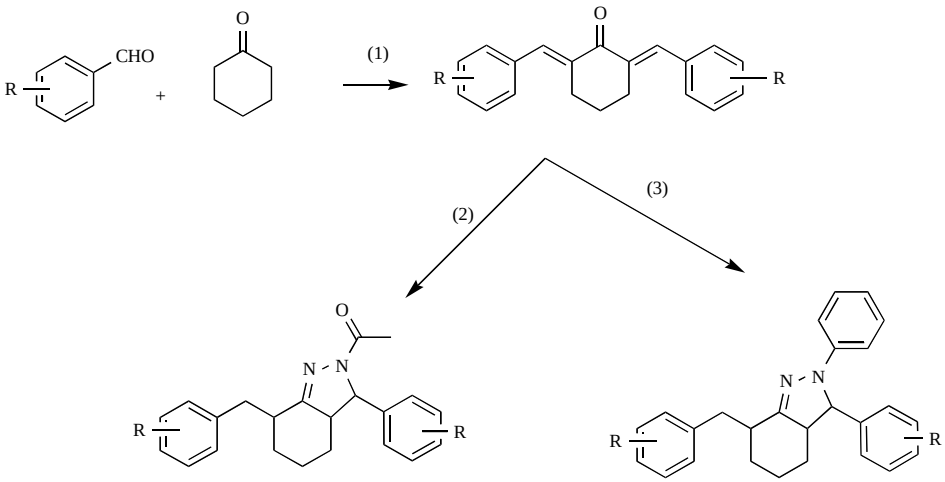


Scheme 1. Synthesis of hexahydroindazole derivatives of Curcumin (**A1-A9**) and (**B1-B5**). Reagents and conditions: (1) 10 % NaOH, EtOH, H_2O; (2) Hydrazine hydrate, Glacial acetic acid, 12 h reflux; (3) Phenyl hydrazine, Glacial acetic acid, 12 h reflux.

nervous system and additionally some are also found to be monoamine oxidase inhibitors [11-12].

Minu *et al* has reported synthesis and antimicrobial activity of 2,3-disubstituted-3,3a,4,5,6,7-hexahydro-2H-indazole derivatives. They observed that the compounds with electron withdrawing groups were generally more active than other derivatives. Among the electron withdrawing halogen groups, presence of P-flurophenyl group at third position of hexahydroindazole improved the antibacterial activity. Whereas, the presence of P-chlorophenyl group at third position of hexahydroindazole improved the antifungal activity [13].

Synthesis of furfurylidene containing hexahydroindazoles with different pharmacophore fragments (furan and pyrazoline cycles, nitro-, azomethine, and other groups) was reported by Golikov *et al*. They observed that nitrofuran cycle linked to the pyrazoline ring containing azomethine group via avinilydene unit was most potent against gram positive bacteria. While, other substitutions (replacement of nitro group in furan by a methyl group or hydrogen) to hexahydroindazole resulted in diminished activity against all the tested organisms [14].

Present study includes the synthesis, molecular docking and antimicrobial studies of some novel hexahydroindazole derivatives of curcumin and attempt has been made to ascertain the role of various substituents on aryl ring with respect to the antibacterial activity.

MATERIALS AND METHODS

All reagents were obtained from commercial suppliers and were used without further purification. Reaction progress was monitored by thin layer chromatography (TLC) on pre-coated Merck alufoil plates (silica gel 60F-254, 0.25 mm thickness). Melting points were determined on a Veego capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrophotometer. All NMR spectra were obtained in deuterated chloroform ($CDCl_3$); chemical shifts are reported in parts per million, and coupling constants in hertz (Hz). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), m (multiplet). Mass spectra were recorded on a LC-MS-2010A spectrometer and mass values are reported in m/z. Elemental analysis of synthesized compounds were recorded on EXETER-CE-440 elemental analyzer.

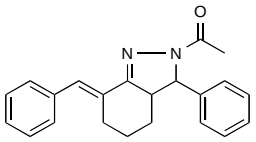
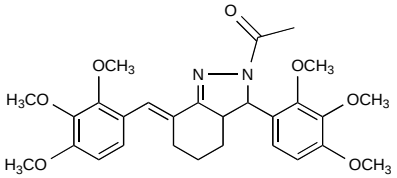
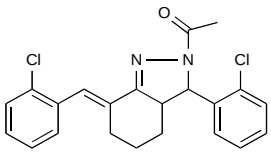
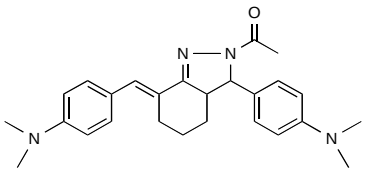
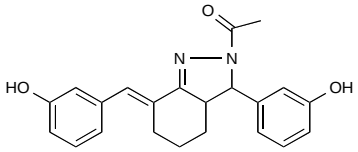
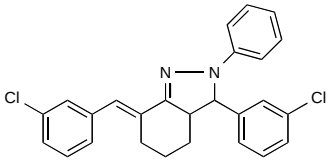
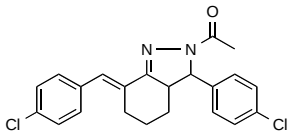
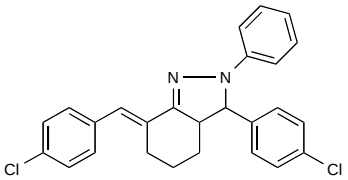
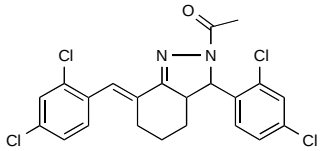
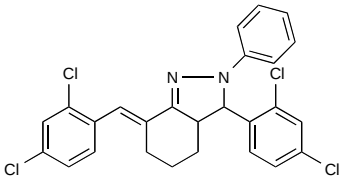
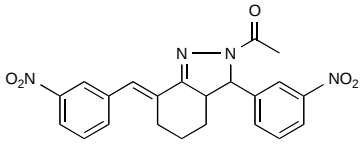
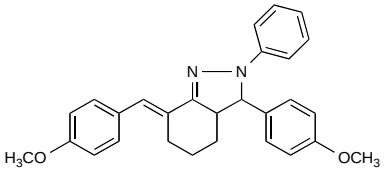
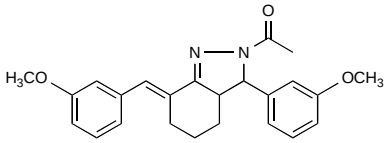
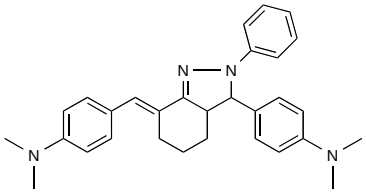
Molecular Docking Studies

Automated docking was used to determine the orientation of inhibitors bound to the active site of GlcN-6-P synthase. A genetic algorithm method, implemented in the program AutoDock 4.0, was employed [15]. The 3D structure file of all the curcumin analogues and fluconazole molecule were loaded on to PRODRG server [16] and PreADMET server for energy minimization and drug likeliness prediction respectively. The protein structure file 1Jxa was downloaded from Protein Data Bank (www.rcsb.org/pdb) was edited by removing the hetero atoms, adding C-terminal oxygen [17]. For docking calculations, Gasteigere-Marsili partial charges [18] were assigned to the ligands and non-polar hydrogen atoms were merged. All torsions were allowed to rotate during docking. The grid map was centered at the residues of the protein predicted from the CASTp server [19]. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization using default parameters. The number of docking runs was 50, the population in the genetic algorithm was 250, the number of energy evaluations was 100,000, and the maximum number of iterations was 10,000. The docking results for ligand molecules against glucosamine-6-phosphate synthase [PDB Id: 1jka], showed minimum docking energy, binding energy, inhibition constant, intermolecular energy with 0.0 RMS as documented. Computer with Microsoft Windows XP operating system, Intel Pentium 3.40 GHz processor, 1 GB RAM and Hard disk: 500 GB, Python: 2.4 was used.

Synthesis

The compounds were prepared by coupling substituted benzaldehydes with cyclohexanone (2:1) in a base catalyzed Claisen-Schmidt condensation followed by reflux with

Table 1. Different substitutions on aryl ring of synthesized compounds.

H	A1		2,3,4 triOCH$_3$	A8	
2-Cl	A2		4-N(CH$_3$)$_2$	A9	
3-OH	A3		3-Cl	B1	
4-Cl	A4		4-Cl	B2	
2,4-diCl	A5		2,4-diCl	B3	
3-NO$_2$	A6		4-OCH3	B4	
3-OCH$_3$	A7		4-N(CH$_3$)$_2$	B5	

hydrazines (hydrazine hydrate, phenyl-hydrazine) and acetic acid (scheme **1**, Table **1**).

Synthesis of E-1-(7-(substituted bezylidene)-3-(substituted phenyl)-3,3a,4,5,6,7-hexahydro 2 H indazole-2yl) Ethanones (A1-A9)

These were prepared in two steps. In First step, respective dibenzylidiene cyclohexanones were synthesized. A mixture of ethanol (20ml) and sodium hydroxide solution (10%, 10 ml) was taken in a beaker, maintained at temperature between 15 to 25ºC. The solution was vigorously stirred and one half of previously prepared mixture of appropriate aromatic aldehyde and cyclohexanone (2:1) was added to it. After 20 min, the remaining of the aromatic aldehyde-cyclohexanone mixture was added. The reaction mixture was further stirred for 45 minutes. The solid separated was filtered off, washed with distilled water, and subjected to further purification by recrystallization with ethyl alcohol.

In second step, synthesized substituted dibenzylidene cycloheaxanone (1.2 mmol) from first step was dissolved in glacial acetic acid (5 mL), and hydrazine hydrate (1.5 mmol) was added to the solution. The solution was refluxed for 12 hours andreaction was monitored monitored by TLC .The solvent was removed in vacuum and the residue was

recrystallized with ethyl alchol. All the derivatives were also prepared by the same procedure.

(E)-1-(7-benzylidene-3-phenyl-3,3a,4,5,6,7-hexahydro-2H-indazol-2-yl)ethanone (A1):

Yellow powder, mp 90-92^{0}C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.61-7.25 (m, 10H), 6.31 (s, 1H, =CH), 4.72 (d, 1H), 2.67 (m, 1H), 2.12 (s, 3H, CH_3), 1.81-1.42 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 166.9, 154.4, 141.7, 136.0, 134.9, 131.9, 128.5, 127.6, 127.1, 127.9, 126.3, 70.2, 41.8, 27.5, 24.8, 23.7. ESI-MS: m/z: (M^+, %) 330.14 (M^+ 6%), 329(97%), 200(100%). Elemental analysis found (Calc.) for $C_{22}H_{22}N_2O$: C, 79.97 (79.65); H, 6.71 (6.73); N, 8.48(8.45).

(E)-1-(7-(2-chlorobenzylidene)-3-(2-chlorophenyl)-3,3a,4,-5,6,7-hexahydro-2H-indazol-2-yl)ethanone (A2):

Yellow powder, mp 102-104^{0}C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.72-7.20 (m, 8H), 6.69 (s, 1H, =CH), 4.5 (d, 1H), 2.67 (m, 1H), 2.02 (s, 3H, CH_3), 1.85-1.43 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 166.5, 154.6, 141.5, 136.5 134.7, 133.5, 133.1, 130.7, 129.5, 129.3, 129.3, 128.2, 127.1, 127.0, 126.6, 126.4, 65.9, 42.4, 23.3. ESI-MS: m/z: (M^+, %) 398.07. (M^+ 9%), 397(22%), 220(100%). Elemental analysis found (Calc.) for $C_{22}H_{20}Cl_2N_2O$: C, 66.17(66.43); H, 5.05(5.03); N, 7.02(7.30).

(E)-1-(7-(3-hydroxybenzylidene)-3-(3-hydroxyphenyl)-3,-3a,4,5,6,7-hexahydro-2H-indazol-2-yl)ethanone (A3):

Yellow powder, mp 166-168^0 C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.52-6.51 (m, 8 H), 6.31 (s, 1H, =CH), 5.30 (s, 2H), 4.6 (d, 1H), 2.55 (m, 1H), 2.32 (s, 3H, CH_3), 1.75-1.37 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 167.2, 158.9, 156.6, 155.5, 140.7, 135.6, 135.1, 131.7, 130.4, 129.7, 121.8, 120.4, 115.6, 113.4, 113.2, 22.2. ESI-MS: m/z: (M^+, %) 362 (M^+ 7%),361(96%), 221(100%). Elemental analysis found (Calc.) for $C_{22}H_{22}N_2O_3$: C, 72.91(72.71); H,6.12 (6.09); N, 7.73(7.76).

(E)-1-(7-(4-chlorobenzylidene)-3-(4-chlorophenyl)-3,3a,4,-5,6,7-hexahydro-2H-indazol-2-yl)ethanone (A4):

Yellow powder, mp 126-128^0 C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.66-7.58 (m,8 H), 6.27 (s, 1H, =CH), 4.2 (d, 1H), 2.65 (m, 1H), 2.01 (s, 3H, CH_3), 1.77-1.45 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 168.5, 155.6, 139.7, 136.0, 133.3, 131.5, 129.0, 128.6, 128.4, 70.9, 42.8, 27.8, 24.5, 21.4. ESI-MS: m/z: (M^+,%) 398 (M^+ 12%), 397(47%),229(100%). Elemental analysis found (Calc.) for $C_{22}H_{20}$ $Cl_2N_2O_2$: C, 63.62(63.36); H,4.85 (4.86); N,6.75 (6.77).

(E)-1-(7-(2,4-dichlorobenzylidene)-3-(2,4-dichlorophenyl)-3,3a,4,5,6,7-hexahydro-2H-indazol-2-yl)ethanone (A5):

Yellow powder, mp 152-154^0 C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.71-7.02 (m, 6H), 6.56 (s, 1H, =CH), 4.7 (d, 1H), 2.66 (m, 1H), 2.02 (s, 3H, CH_3), 1.85-1.23 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 163.5, 152.6, 150.1, 137.6, 136.8, 132.4, 132.1, 131.4, 131.0, 130.3, 127.9, 126.8, 126.5, 125.2, 120.8, 75.8, 42.1, 27.9, 24.3, 23.1. ESI-MS: m/z: (M^+, %) 468(M^+ 12%), 467(22%), 221(100%). Elemental analysis found (Calc.) for $C_{22}H_{18}$ Cl_4N_2O : C, 56.44(56.66); H,3.88 (3.83); N, 5.98 (5.96).

(E)-1-(7-(3-nitrobenzylidene)-3-(3-nitrophenyl)-3,3a,4,5,6-,7-hexahydro-2H-indazol-2-yl) ethanone (A6):

Yellow powder, mp 130-132^0 C. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 8.29-7.54 (m, 8H), 6.42 (s, 1H, =CH), 4.4 (d, 1H), 2.63 (m, 1H), 2.02 (s, 3H, CH_3), 1.81-1.43 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 167.5, 154.6, 146.8, 146.2, 140.4, 136.1, 135.3, 134.6, 134.2, 129.5, 129.3, 128.7, 123.8, 122.7, 121.7, 120.7, 69.7, 42.8, 27.8, 24.5, 23.4. ESI-MS: m/z: 420(M^+ 12%),.418(100%). Elemental analysis found (Calc.) for $C_{22}H_{20}$ N_4O_5 : C, 62.85(62.60); H,4.79 (4.80); N, 13.33(13.28)

(E)-1-(7-(3-methoxybenzylidene)-3-(3-methoxyphenyl)-3,3-a,4,5,6,7-hexahydro-2H-indazol-2-yl)ethanone (A7):

Yellow powder, mp 110-112^0 C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.49-6.21 (m,8 H), 6.11 (s, 1H, =CH), 4.1 (d, 1H), 3.75 (s, 6H), 2.68 (m, 1H), 2.04 (s, 3H, CH_3), 1.78 -1.32 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 169.5, 161.5, 160.4, 155.6, 142.5, 136.0, 134.8, 128.6, 126.5, 120.8, 120.4, 112.5, 112.0, 111.5, 71.2, 55.8, 42.8, 27.1, 24.5, 23.4. ESI-MS: m/z: 390((M^+ 8%), 389(26%), 212(100%). Elemental analysis found (Calc.) for $C_{24}H_{26}N_2O_3$: C, 73.82(73.63); H, 6.71(6.72); N, 7.17(7.16)

(E)-1-(7-(2,3,4-trimethoxybenzylidene)-3-(2,3,4-trimethoxy-phenyl)-3,3a,4,5,6,7-hexahydro-2H-indazol-2-yl)ethanone (A8):

Yellow powder, mp 210-212^0 C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.47 -6.25 (m,4 H), 6.59 (s, 1H, =CH), 4.4 (d, 1H), 3.75 (s, 9H), 2.68 (m, 1H), 2.04 (s, 3H, CH_3), 1.80-1.35 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 168.5, 156.8, 155.9, 152.9, 151.1, 149.1, 142.3, 141.6, 136.0, 135.7, 132.7, 122.7, 121.4, 119.6, 108.2, 106.8, 104.7, 65.3, 61.7, 60.8, 60.3, 56.1, 43.1, 27.8, 24.5, 23.4. ESI-MS: m/z: 510(M^+ 12%), 507(20%), 215(100%). Elemental analysis found (Calc.) for $C_{28}H_{34}N_2O_7$: C, 65.87(65.92); H, 6.71(6.70); N, 5.49(5.48).

(E)-1-(7-(4-(dimethylamino)benzylidene)-3-(4-(dimethyla-mino)phenyl)-3,3a,4,5,6,7-hexahydro-2H-indazol-2-yl)ethanone (A9) :

Yellow powder, mp 150-152^0 C.^{1}H NMR (CDCl3, 300 MHz, 25 °C): 7.64 -6.56 (m,10 H), 6.25 (s, 1H, =CH), 4.7 (d, 1H), 3.78 (s, 12 H), 2.68 (m, 1H), 2.54 (s, 3H, CH_3), 1.81-1.36 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 167.5, 154.6, 151.3, 149.3, 133.7, 132.1, 131.4, 128.7, 124.7, 112.7, 111.7, 72.9, 41.3, 26.8, 24.5, 23.4. ESI-MS: m/z: 416(M^+ 9 %), 415(21%), 221(100%). Elemental analysis found (Calc.) for $C_{26}H_{32}N_4O$: C, 74.97(74.96); H, 7.74(7.72); N, 13.45(13.43).

Synthesis of E-7-(substituted benzylidene)-2-phenyl-3-(substituted phenyl)-3, 3a, 4,5,6,7 hexahydro 2H indazoles (B1-B5)

In the first step, the dibenzylidene cycloheaxanones were synthesized as reported. The synthesized dibenzylidene cycloheaxanon(1.2 mmol) from first step was dissolved in glacial acetic acid (5 mL) and phenyl hydrazine (1.5mml) was added to the solution. The solution was refluxed for 12 hours and monitored by TLC. Solvent was removed in vacuum and recrystallized with ethlyl alcohol. The

Table 2. Antimicrobial activity of new hexahydroindazole derivatives of curcumin.

Comp no.	Bacterial Strains Zone of inhibition (in mm)					Fungal Strains Zone of inhibition (in mm)		
	E.coli ATCC 35218	*S. typhi* MTCC 3216	*K. pneumonia*	*S. aureus* ATCC 25323	*E. faecalis*	*C. albicans* ATCC 90028	*C. tropicalis* ATCC 750	*C. krusie*
A1	NA	NA	NA	10.37±0.38	9.70±0.36	NA	NA	NA
A2	15.60±0.26	14.37±0.12	14.87±0.15	13.20±0.17	15.83±0.19	14.80±0.25	12.53±0.18	12.83±0.15
A3	13.30±0.25	14.93±0.18	17.63±0.09	16.77±0.03	14.57±0.15	16.27±0.23	11.30±0.15	11.20±0.15
A4	9.40±0.12	NA	NA	11.73±0.38	10.50±0.21	13.37±0.09	NA	NA
A5	8.93±0.09	NA	10.27±0.09	11.70±0.12	NA	12.67±0.09	NA	NA
A6	8.07±0.09	NA	NA	10.23±0.15	9.03±0.12	NA	NA	NA
A7	21.43±0.18	17.20±0.17	20.40±0.11	22.53±0.09	20.33±0.15	23.0±0.12	21.50±0.12	19.77±0.15
A8	19.67±0.11	15.10±0.15	16.80±0.17	17.0±0.16	15.23±0.20	20.33±0.12	18.20±0.17	17.87±0.19
A9	10.90±0.06	NA	NA	10.03±0.18	12.10±0.20	11.93±0.09	NA	12.47±0.07
B1	13.93±0.09	NA	8.87±0.13	7.10±0.17	6.63±0.09	7.63±0.05	5.73±0.12	NA
B2	8.07±0.06	NA	NA	6.77±0.15	4.07±0.18	NA	NA	NA
B3	7.87±0.10	NA	NA	NA	NA	6.70±0.13	NA	5.93±0.09
B4	9.33±0.07	8.33±0.09	NA	5.57±0.12	4.87±0.09	NA	NA	NA
B5	8.90±0.08	NA	NA	NA	4.17±0.15	NA	NA	3.30±0.17
Cip	26.23±0.16	19.98±0.06	26.71±0.08	27.44±0.15	22.50±0.09	NA	NA	NA
Fluc	NA	NA	NA	NA	NA	25.33±0.05	24.65±0.05	24.06±0.04

The value of each compound consisted of Mean ± SE of 03 replicates.
Level of significance $p < 0.05$

remaining derivatives were also prepared by the same procedure.

(E)-7-(3-chlorobenzylidene)-3-(3-chlorophenyl)-2-phenyl-3,3a,4,5,6,7-hexahydro-2H-indazole (B1):

Yellow powder, mp 58-60^0 C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.34-6.75 (m, 13 H), 6.24 (s, 1H, =CH), 3.7 (d, 1H), 2.25 (m, 1H), 1.78-1.43 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 145.6, 143.8, 140.9, 136.0, 134.9, 134.2, 134.1, 130.0, 129.9, 129.5, 128.0, 126.9, 126.2, 119.7, 62.6, 40.5, 26.8, 24.8, 23.5. ESI-MS: m/z: 432(M^+ 7%), 430(100%). Elemental analysis found (Calc.) for $C_{26}H_{22}Cl_2N_2$: C, 72.06(71.97); H, 5.12(5.11); N, 6.46(6.52).

(E)-7-(4-chlorobenzylidene)-3-(4-chlorophenyl)-2-phenyl-3,3a,4,5,6,7-hexahydro-2H-indazole (B2):

Yellow powder, mp 78-80^0 C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.78-6.73 (m, 13 H), 6.23 (s, 1H, =CH), 3.5 (d, 1H), 2.26 (m, 1H), 1.82-1.41 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 154.6, 143.8, 138.6, 136.0, 133.5, 133.3, 131.7, 131.5, 129.0, 129.5, 128.6, 128.4, 116.7, 63.1, 42.9, 28.6, 27.8, 24.8, 24.5. ESI-MS: m/z: 432(M^+ 6%), 430(100%). Elemental analysis found (Calc.) for $C_{28}H_{24}Cl_2N_2$: C, 73.20(73.37); H, 5.27(5.28); N, 6.10(6.11).

(E)-7-(2,4-dichlorobenzylidene)-3-(2,4-dichlorophenyl)-2-phenyl-3,3a,4,5,6,7-hexahydro-2H-indazole (B3):

Yellow powder, mp 70-72^0 C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.58-6.75 (m, 13H), 6.59 (s, 1H, =CH), 3.8 (d, 1H), 2.27 (m, 1H), 1.87-1.30 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 154.6, 150.5, 141.8, 138.6, 136.0, 133.8, 133.1, 131.7, 131.1, 130.3, 129.4, 128.9, 128.1, 126.7, 120.8, 116.7, 58.0, 41.4, 28.7, 24.5. ESI-MS: m/z: 502(M^+ 3%), 501(15%), 225(100%). Elemental analysis found (Calc.) for $C_{26}H_{20}Cl_4N_2$: C, 62.17(62.31); H, 4.01(4.02); N, 5.58(5.56).

(E)-7-(4-methoxybenzylidene)-3-(4-methoxyphenyl)-2-phenyl-3,3a,4,5,6,7-hexahydro-2H-indazole (B4):

Yellow powder, mp 80-82^0 C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.52-6.84 (m,8 H), 6.32 (s, 1H, =CH), 3.5 (d, 1H), 3.73 (S, 6H), 2.25 (m, 1H), 1.84-1.42 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 158.8, 156.8, 144.8, 134.0, 131.8, 131.2, 130.2, 129.5, 127.8, 127.5, 121.8, 116.7, 113.2, 112.1, 63.1, 55.8, 42.9, 27.8, 24.8, 24.5. ESI-MS: m/z: 424(M^+ 4%), 215(100%). Elemental analysis found (Calc.) for $C_{28}H_{28}N_2O_2$: C, 79.22(79.43); H, 6.65(6.66); N, 6.60(6.62).

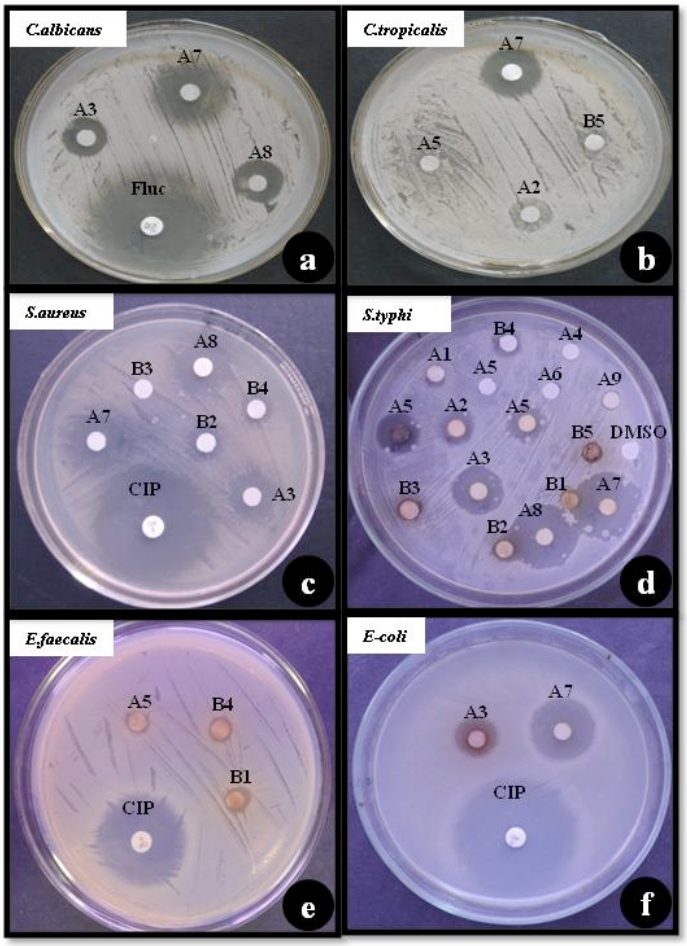


Fig. (1). Antimicrobial activity of hexahydroindazole derivatives of Curcumin **(a)** antifungal activity against *candida albicans* with refrence to standard drug Fluconazole, A7 showing the strongest inhibition compare to A3 and A8 **(b)** antifungal activity against *C.tropicalis*, A7 showing clear zone of inhibition as compare to A5, B5 and A2 **(c)** Antibacterial against *S.aureus* with reference to standard drug Ciprofloxacin, A3, A8 and A7 showing inhibition much better than B3, B2,and B4 **(d)** antibacterial against *S.typhi*, A3, A5, A8, A7, A2, A5 and B4 showing inhibition zone **(e)** antibacterial against *E.faecalis* with reference to Ciprofloxacin as standard, A5, B4, and B1 showing less inhition compare to standard **(f)** antibacterial activity against *E-coli*, A7 and A3 showing good inhibition as compared to standard Ciprofloxacin.

(E)-4-(7-(4-(dimethylamino) benzylidene)-2-phenyl-3, 3a, 4, 5, 6, 7-hexahydro-2H-indazol-3-yl)-N,N-dimethylaniline (B5):

Yellow powder, mp 178- 179^0 C.^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.62-7.01 (m, 13 H), 6.24 (s, 1H, =CH), 3.1 (d, 1H), 3.07 (s, 12 H) 2.31 (m, 1H), 1.78 -1.20 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 154.2, 150.1, 146.3, 143.8, 136.7, 130.7, 130.4, 130.0, 129.7, 129.5, 128.9, 128.4, 127.1, 126.9, 116.7, 112.7, 111.7, 63.1, 42.9, 40.3, 27.8, 24.8, 24.5. ESI-MS: m/z: 450(M^+ 3%), 135(100%). Elemental analysis found (Calc.) for $C_{30}H_{34}N_4$: C, 79.96(80.02); H, 7.61(7.62); N, 12.43(12.41).

Antimicrobial Studies

Antimicrobial activities of newly synthesized compounds were first screened by disc diffusion method [20] against various Gram positive and Gram negative human pathogenic bacteria viz. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27893, *S. typhi* MTCC 3216, *E. faecalis* and *Staphylococcus aureus* ATCC 25323 and different fungal strains of *Candida* according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1997).

Freshly grown microbial strains were mixed in sterile saline (0.85%) and the turbidity was matched with McFarland No. 2 system to achieve concentration of 107 CFU/ml. Sterile petri plates containing 20 mL of Mueller Hinton agar (MHA, Hi-Media) were used for all bacterial culture and Sabouraud's dextrose agar (SDA)/Potato dextrose agar (PDA) (Hi-Media) were used for all fungal culture. The bacterial inoculums' suspension was spread on the surface of agar plates. Sterile disc (5mm) of Whatman paper no. 1 was then placed on the surface of the media and the test compounds (25µl/ml) was put and allowed to diffuse and plates were incubated for 24 h at 37ºC for bacterial cultures and 72 h at 25 ºC for fungal culture. Ciprofloxacin (5µg/disc, Hi-Media) was used as positive control for bacteria and Fluconazole (10µg/disc, Hi-Media), was used as a positive control for fungi. Zone of inhibition was measured

Table 3. Theoretical prediction of different properties of new hexahydroindazole derivatives of curcumin using PreADMET Server.

Entry	Log_p	Human intestinal Absorption (%)	*In vitro* skin permeability (logKp, cm/hour)	*In vitro* plasma protein binding (%)	Water solubility in buffer system Mg/L
A1	4.62	99.54	-2.45	93.96	403.4
A2	5.70	99.68	-2.42	94.26	45.21
A3	3.61	94.30	-2.95	90.87	207.1
A4	5.98	99.68	-2.45	100	144.0
A5	7.01	99.59	-2.27	100	5.57
A6	3.69	93.90	-2.42	93.48	5.53
A7	4.69	97.37	-2.82	89.91	128.1
A8	4.10	98.91	-3.40	87.51	15.57
A9	4.82	98.31	-2.52	92.61	55.88
B1	8.23	100	-1.77	100	$5.37x10^{-6}$
B2	8.27	100	-1.76	100	$1.50x10^{-5}$
B3	8.85	100	-1.69	100	$5.77x10^{-7}$
B4	7.07	99.32	-2.05	96.34	$3.77x10^{-5}$
B5	7.10	100	-1.78	93.07	$5.83x10^{-6}$

Table 4. Molecular docking of new hexahydroindazole derivatives of curcumin with glucosamine-6-phosphate synthase.

Entry	Binding Energy (Kcal/mol)	Docking Energy (Kcal/mol)	Inhibition constant (M)	Amino acid residue involved in H-bond	Bond Length (Å)	RMSD
A1	-9.25	-10.03	$1.67x10^{-7}$	Arg 73:HH1::Dp1:NA	1.67	0.81
				His 77:HE2::Dp1:NA	2.18	
A2	-9.99	-10.78	$4.79x10^{-8}$	Arg 73:HE::Dp2:OA	2.05	0.92
				Arg 73:HH4::Dp2:NA	2.08	
A3	-9.57	-10.28	$9.63x10^{-8}$	Gly 99:HN::Dp3:OA	1.90	0.99
A4	-8.19	-8.54	$9.97x10^{-7}$	No H- Bonds	--	1.12
A5	-9.56	-9.64	$9.80x10^{-8}$	No H- Bonds	--	1.20
A6	--	--	--	--	--	
A7	-10.02	-10.96	$4.50x10^{-8}$	Gly 99:HN::Dp7:OA	2.00	0.56
A8	-6.93	-8.48	$8.57x10^{-6}$	Arg 73:HH21::Dp8:OA	1.68	1.02
				His 77:HE2::Dp8:OA	2.13	
A9	-5.40	-4.86	$1.10x10^{-4}$	His 71:HE2::Dp9:NB	1.88	1.11
B1	-7.64	-8.35	$2.49x10^{-6}$	His 77:HE2::Dp10:NA	2.01	0.96
B2	-7.51	-8.40	$3.14x10^{-6}$	No H- Bonds	--	1.23
B3	-8.69	-9.07	$4.29x10^{-7}$	No H- Bonds	--	1.20
B4	-8.53	-8.40	$5.59x10^{-7}$	Cys 1:SG::Dp13:OA	2.93	0.95
B5	-7.12	-7.55	$6.05x10^{-6}$	No H- Bonds	--	1.23
Fluconazole	-5.81	-5.36	$5.53x10^{-5}$	Arg 73:HH21::DF:OA	2.20	1.97
				Arg 73:HE::DF:OA	2.09	
				His 77:HE2::Dp10:NA	1.87	

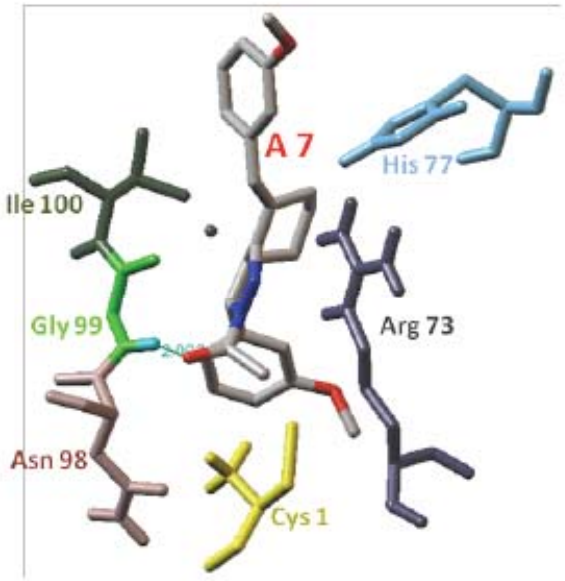


Fig. (2). Interaction of compound A7 with GlcN-6-P.

in millimeters after 24 h. All tests were performed in triplicate.

RESULT AND DISCUSSION

The compounds were prepared by coupling substituted benzaldehydes with cyclohexanone in (ratio of 2:1) in a base catalyzed Claisen-Schmidt condensation followed by reflux with hydrazines (hydrazine hydrate, phenyl-hydrazine) and acetic acid. The results of antimicrobial screening of synthesized hexahydroindazole derivatives of curcumin are presented in table (Table **2**).

The results of antibacterial screening revealed that among the compounds screened compounds **A2**, **A3**, **B1** and **B2** showed moderate antibacterial activity while compound **A7** & **A8** displayed good antibacterial activity when compared with ciprofloxacin used as standard. Particularly, compound **A7** which is carrying methoxy group on aryl ring appears to exhibit maximum antibacterial activity (zone of inhibition up to 21 mm) against *E. coli* and *S. aureus*. Interestingly, triple substitution of methoxy group at ortho, meta and para position leads to a slight decrease in the potency (compound A8 zone of inhibition = 19.67mm), this may be due to the steric hindrance by these groups. Electron donating group (methoxy) significantly increases antibacterial activity.

Derivatization of **B1** with chloro substitution on phenyl rings enhanced the potency (Compound **B1** zone of inhibition = 13.93mm) indicating that substitutions may result in restoration of potency. Whereas, methoxy (Compound **B4** zone of inhibition = 9.33mm) and N, N-dimethylamino (B5, zone of inhibition = 8.90mm) substitutions exhibited moderate potency. Phenyl pyrazoline analogues (**B1-B5**) did not significantly increase the antibacterial potency.

The results of antifungal screening indicated that compounds, **A2**, **A3**, **A4**, **A7** and **A8** showed moderate to excellent antifungal activity. Compounds **A2**, **A3** and **A4** showed moderate activity against *C. albicans*. Interestingly, compound **A3** also showed moderate activity against three fungal strains viz. *C. albicans*, *C. tropicalis*, *C. krusie*. Among the halogenated derivatives chloro substitution at ortho or para position increased antifungal potency appreciably (compound **A2** zone of inhibition = 14.80mm), (compound **A4** zone of inhibition = 13.37mm) Fig. (**1**).

Chloro substitution at both ortho and para positions resulted in marginal decrease in potency (compound **A5** zone of inhibition = 12.67mm). This may be attributed to successful interactions of the compound with target protein(s) when chloro substitution occurred either at ortho or para position. Out of 14 compounds synthesized the majority of them showed considerable antimicrobial activity against tested strains.

The combinatorial chemistry and virtual screening have rapidly increased in drug discovery. Need for minimizing extremely time-consuming steps of synthesis, biological screening and good tools for predicting drug-likeness is very much desired. The molecular descriptors have been employed to predict various human ADMET processes and other pharmacokinetic parameters such as oral absorption, bioavailability, skin penetration, clearance, volume of distribution, and metabolism [21-22]. Predicting ADME properties at an early stage of drug discovery and development process is very important to remove compounds with poor pharmacokinetic properties and minimize extremely expensive and time-consuming steps. Different properties viz. drug-likeliness, Human intestinal absorption, *In vitro* skin permeability, *in vitro* plasma protein binding and water solubility in buffer system of all the 14 synthesized hexahydroindazole analogues of curcumin were determined using PreADMET server. The results are summarized in table (Table **3)**.

Comparative docking of glucosamine-6-phosphate synthase (GlcN-6-P synthase) protein with the curcumin

analogues and the standard drug fluconazole and amphotericin B yielded best possible conformations with parameters including the binding energy, docked energy, inhibition constant and RMSD (Table **4**). Theoretically, molecules showed very good binding energy and docking energy ranging from -5.40 Kcal/mol to -10.02 Kcal/mol and -4.86 Kcal/mol to -10.96 Kcal/mol respectively Fig. (**2**). The minimum docked energy was found in the analogue A7 (-10.96 kcal/mol) with an estimated inhibition constant of $4.50x10^{-8}$ and RMSD 0.56. Whereas, docked energy of the standard drugs fluconazole was -5.67 kcal/mol with an inhibition constant of $5.53x10^{-5}$ and RMSD 1.97.

The incorporation of halogen atoms increase membrane permeability, improve the oral absorption and skin penetration etc [26]. It is generally accepted that halogen atoms are not capable of significant hydrogen bonding. Six analogues containing chlorine as halogen atom (**A2**, **A4**, **A5**, **B2**, **B3** and **B5**) have not formed any hydrogen bond with the active site amino acids of GlcN-6-P synthase, but showed very good binding and docking energy, since the halogens are endowed with the ability to establish intermolecular bonds in a fashion that resembles the H-bonds.

In the present study, the molecular docking results of compound **A7** showed very good binding energy and RMSD, even in *in vitro* studies also **A7** emerged s active against all tested microorganisms.So it can be predicted that the activity may be due to the inhibition of enzyme GlcN-6-Psynthase, which catalyses a complex reaction involving ammonia transfer from L-glutamine to Fru-6-P followed by isomerisation of the formed fructosamine-6-phosphate to glucosamine-6-phosphate.

CONCLUSION

This study allows us to conclude that enviable improvement of antimicrobial activities in synthesized compounds require electron donating group and methoxy substitution may be the reason for highest activity of Compound **A7** among tested compounds. It is juvenile to arrive at the conclusion on structure activity aspect of these compounds and further assessment is desirable to use them for clinical study. Molecular docking studies also revealed that compound **A7** has minimum binding and docking energy and may be considered as good inhibitor of GlcN-6-P. The study may exalt the scope of developing these hexahydroindazole derivatives of curcumin as promising antibacterial and antifungal agents.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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The accuracy of dockings was evaluated by the root-mean square deviation (RMSD) of docked ligand from original crystal structure. RMSD less than 1.0 Å was considered as excellent.

A significant number of drugs and drug candidates in clinical development are halogenated structures. The formation of halogen bonds in ligand-target complexes is recognized as a kind of intermolecular interaction that favorably contributes to the stability of protein-ligand complexes. The insertion of halogen atoms has been used in innumerous cases of hit-to-lead or lead-to-drug conversions [23-25].

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

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Synthesis, Molecular Docking and *In Vitro* Antimicrobial Studies of Novel Pyrazole Analogues of Curcumin

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Abstract: A series of pyrazole analogues of curcumin were synthesized and investigated for *in vitro* and *in silico* antimicrobial activity. The structures of newly synthesized compounds were ascertained on the basis of their analytical and spectral profiles. The compounds were subjected to molecular docking studies for the inhibition of the enzyme glucosamine-6-phosphate synthase [GlcN-6-P]. The autodock program 4.2 was employed to perform automated molecular docking. The docking study was performed on two different active sites of the enzyme reside with the amino acid series Cys1, Arg73, Thr76, His77, Asn98, Gly99, Ile100 and Gly301, Thr302, Ser303, Ser347, Gln348, Ser349, Thr352, Lys485, Ala602, Val605 respectively. Among the thirteen molecules taken for docking studies, compounds **cp10, cp11 and cp12** showed minimum docking energy and inhibition constant and may be considered as good inhibitor of GlcN-6-P synthase.

Keywords: Autodock 4.2, Active sites, Antimicrobial activity, GlcN-6-P synthase, Pyrazole, *In silico*.

INTRODUCTION

The rapid evolution of antibiotic resistance poses a grave threat to the therapy of many bacterial infections. It necessiciates imperative and scrupulous efforts to develop next generation of antibiotics. The availability of complete microbial genome sequence has led to devise concerted strategy to look at novel antibacterials. Nevertheless, in spite of the identification of many new potential drug targets, novel antimicrobial agents have been sluggish to emerge from these efforts [1]. Multidrug resistance (MDR) to antibiotics is a problem that has long plagued public health [2]. Extremely resistant bacteria such as methicillin- resistant *Stayphlococoous aureus* (MRSA) and vancomycin resistant *Enterococci* account for a soaring percentage of hospital acquired infections [3]. However, the significant technological advances in the past decade, in the fields of genomics, molecular biology, high throughput screening and structural biochemistry have led to essentially new standards in the quest for novel antimicrobial agents [4]. In the past forty years, only two structural types i.e. Daptomycin and Linezolid have been introduced to the clinical use following their discovery using empirical screening methods [5]. Therefore, the discovery of drugs with novel mode of action will be imperative to meet the threats by the emergence of resistance.

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One important qualitative difference between the microbial and mammalian cells is the presence of a cell wall in the former. In consequence, enzymatic pathways leading to the formation of the cell wall are potential targets for antimicrobial drugs. Ubiquitous enzymes, namely L-glutamine: D-fructose-6-phosphate amidotransferase and GlcN-6-P synthase have been emerging as a new target for antimicrobialand antifungal agent [6, 7]. Curcumin possesses multifunctional pharmacological applications in a variety of disorders such as liver fibrosis, inflammation, malaria, cardiovascular disease and cancer. Curcumin and its analogues have been shown to exterminate several pathogenic Gram positive and Gram negative bacteria. Understanding the nuances of mechanism of antibacterial activity of curcumin will give new insight into design and development of new lead compounds as antibacterial agents and extrapolated agents for bacteria-infecteddiseases [8].

Curcumin possesses good pharmacological properties however, its utility as a potential therapeutic drug is limited because of its poor bioavailability, fast metabolism and its instability above pH 6.5 [9]. Pyrazoles are a significant class of compounds for new drug development that engrossed much attention. Several pyrazole analogues have been synthesized as target structures and evaluated for their biological activities. The improvement in potency and selectivity of the pyrazole series has suggested that, several microbial enzymes may be useful targets for discovering other series of inhibitors with potency and selectivity [10]. Pyrazole analogues of curcumin have also been reported to possess antioxidant, antitumor, anti-inflammatory, antimalarial, neuro-

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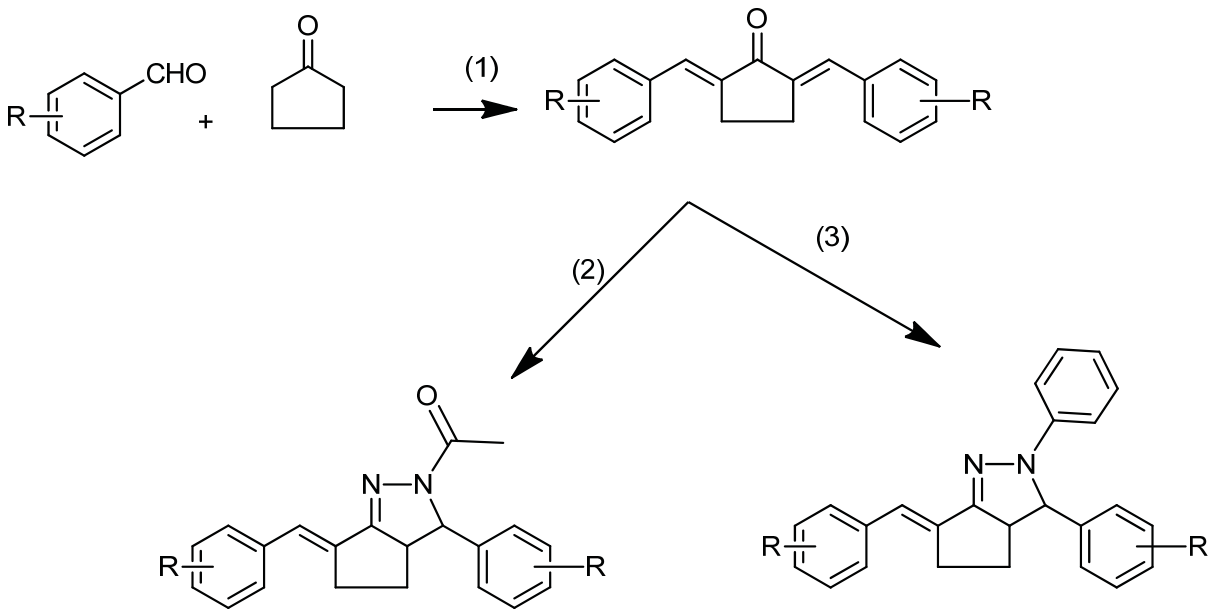

Scheme (1). Synthesis of pyrazole analogues of curcumin(**cp1-cp14);** Reagents and conditions: (1) 10% NaOH, EtOH, H_2O; (2) Hydrazine hydrate, Glacial acetic acid, 14 h reflux; (3) Phenyl hydrazine, Glacial acetic acid, 20 h reflux.

protective, anti-Alzheimer's and memory enhancer activities [11-16].

In our earlier study, we reported synthesis, molecular docking and *in vitro* antimicrobial activity of a series of fused pyrazole (Hexahydroindazole) analogues of curcumin. It was observed that, electron releasing group and methoxy substitution were responsible for optimum activity. Molecular docking study also revealed that methoxy substitution had minimum binding and docking energy and was good inhibitor of GlcN-6-P, while other substitutions (Nitro and Hydroxyl group replacement on aryl rings) resulted in diminished activity against all the tested organisms [17].

MATERIALS AND METHODS

All reagents were obtained from commercial suppliers and used without further purification. Reaction progress was monitored by thin layer chromatography (TLC) on precoated Merck alu-foil plate (silica gel 60F-254, 0.25 mm thickness). Melting points were determined on a Veego capillary melting point apparatus and are uncorrected. FTIR spectra were recorded on KBr pellets on Shimadzu FTIR spectrophotometer (model no 8400S). ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrophotometer. All NMR spectra were obtained in deuterated chloroform ($CDCl_3$); chemical shifts are reported in parts per million, and coupling constants in Hertz (Hz). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), m (multiplet). Mass spectra were recorded on a LC-MS-2010A spectrometer on ESI positive mode and molecular ion peaks are reported as m/z ratio. Elemental analysis of synthesized compounds was recorded on EXETER-CE-440 elemental analyzer.

MOLECULAR DOCKING STUDIES

Automated docking was used to determine the orientation of inhibitors bound to the active site of GlcN-6-P synthase. A genetic algorithm method, implemented in the program AutoDock4.2, was employed [18]. The 3D structure file of all fourteen curcumin analogues and fluconazole molecule was loaded on to PRODRG server [19] and PreADMET server for energy minimization and drug likeliness prediction respectively. The protein structure file (PDB ID: 1Jxa) was downloaded from Protein Data Bank (www.rcsb.org/pdb) and was edited by removing the heteroatoms, adding C-terminal oxygen [20]. For docking calculations, Gasteigere-Marsili partial charges [21] were assigned to the ligands and non-polar hydrogen atoms were merged. All torsions were allowed to rotate during docking. The grid map was centered at the residues of the protein predicted from the CASTp server [22]. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization using default parameters. The number of docking runs was 50, the population in the genetic algorithm was 250, the number of energy evaluations was 100,000, and the maximum number of iterations was 10,000. The docking results for ligand molecules against glucosamine-6-phosphate synthase [PDB Id: 1Jxa], showed minimum docking energy, binding energy, inhibition constant, intermolecular energy with 0.0 RMS as documented. Specification of the computer used was, Operating system: Microsoft Windows XP, Processor: Intel Pentium 3.40 GHz, RAM: 1 GB, Hard disk: 500 GB, Python: 2.4.

SYNTHESIS

The compounds were prepared by coupling of substituted benzaldehydes with cyclopentanone (In ratio of 2: 1) in a base catalyzed Claisen-Schmidt condensation followed by reflux with hydrazines (hydrazine hydrate, phenyl-hydrazine) and acetic acid (Scheme **1**). Structures of all synthesized compounds are given in table (Table **1**).

Table 1. Different substitutions on aryl ring of synthesized compounds.

SN	cp1	cp2	cp3	cp4	cp5	cp6	cp7	cp8	cp9	cp10	Cp11	Cp12	Cp13	Cp14
R	H	2-Cl	3-OH	4-Cl	2, 4-diCl	3-NO_2	3-OCH_3	2, 3, 4-triOCH_3	4-$N(CH_3)_2$	3-Cl	4-Cl	2, 4-diCl	4-OCH_3	4-$N(CH_3)_2$

General procedure for the synthesis of *(E)*-1-(6-(substituted-benzylidene)-3-(substituted phenyl)-3a,4,5,6-tetrahydrocyclopenta[c]pyrazol-2(3*H*)-yl) ethanone(cp1-cp9):

The compounds were prepared in two steps. In first step, respective dibenzylidiene cyclopentanone was synthesized. A mixture of ethanol (20 ml) and sodium hydroxide solution (10%, 10 ml) was taken in a beaker and was maintained at temperature between 15 to 25ºC. The solution was vigorously stirred and one half of previously prepared mixture of appropriate aromatic aldehyde and cyclopentanone (in molar ratio of 2: 1) was added to it. After 20 min, the remaining aromatic aldehyde-cyclopentanone mixture was added. The reaction mixture was further stirred for 45 minutes. The solid obtained was filtered off, washed with distilled water, and subjected to further purification by crystallization with ethyl alcohol.

In second step, synthesized substituted dibenzylidene cyclopentanone (1.2 mmol) from first step was dissolved in glacial acetic acid (5 ml), and hydrazine hydrate (1.5 mmol) was added to the solution. The solution was refluxed for 14 hours and monitored by TLC. The solvent was removed in vacuum and the residue was crystallized with ethyl alcohol. The remaining derivatives were also prepared by the same procedure.

The specified stereoselectivity (E) of pyrazole analogues of curcumin might be a reason for biological activity which has been previously reported in literature [9].

*(E)-1-(6-benzylidene-3-phenyl-3a,4,5,6-tetrahydrocyclopenta[c]pyrazol-2(3H)-yl)ethanone (**cp1**):*

Yellow powder; mp 88–90 °C; FTIR (KBr, ν_{max}/cm^{-1}): 1567 (C=N), 1493 (C=C), 1232 (C–N). ^1H NMR ($CDCl_3$, 300 MHz, 25°C): 7.30 - 7.08 (m, 10H), 6.21 (s, 1H, =CH), 4.72 (d, 1H), 2.67 (m, 1H), 2.02 (s, 3H, -CH_3), 1.75 - 1.42 (m, 4H).MS (ESI^+) m/z: $(M^+ H)^+$ 317. Elemental analysis Calc. (found) for $C_{21}H_{20}N_2O$: C, 79.72 (79.75); H, 6.37 (6.39); N, 8.85 (8.82).

(E)-1-(6(2-chlorobenzylidene-3-(2-chlorophenyl)-3a,4,5,-6-tetrahydrocyclopenta[c]pyrazol-2(3H)-yl)ethanone (**cp2**):

Yellow powder, mp 107 - 109°C; FTIR (KBr, ν_{max}/cm^{-1}): 1586 (C=N), 1494 (C=C), 1234 (C–N). ^1H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.22 - 7.05 (m, 8H), 6.69 (s, 1H, =CH), 4.50 (d, 1H), 2.67 (m, 1H), 2.02 (s, 3H, CH_3), 1.91-1.51 (m, 4H).MS (ESI^+) m/z: $(M^+ H)^+$ 386. Elemental analysis Calc. (found) for $C_{21}H_{18}Cl_2N_2O$: C, 65.46 (65.48); H, 4.71 (4.72); N, 7.27 (7.29).

*(E)-1-(6(3-hydroxybenzylidene-3-(3-hydroxyphenyl)-3a,4,5,6-tetrahydrocyclopenta[c]pyrazol-2(3H)-yl)ethanone (**cp3**):*

Yellow powder, mp 170 – 172 °C; FTIR (KBr, ν_{max}/cm^{-1}): 3502 (O-H), 1582 (C=N), 1467 (C=C), 1239 (C–N). ^1H NMR ($CDCl_3$, 300 MHz, 25°C): 7.04-6.51 (m, 8 H), 6.25 (s, 1H, =CH), 5.30 (s, 2H, OH), 4.60 (d, 1H), 2.45 (m, 1H), 2.12 (s, 3H, CH_3), 2.01-1.2 (m, 4H).MS(ESI^+) m/z: $(M^+ H)^+$ 349. Elemental analysis Calc. (found) for $C_{21}H_{20}N_2O_3$: C, 72.40 (72.40); H, 5.79 (5.81); N, 8.04 (8.06).

*(E)-1-(6(4-chlorobenzylidene-3-(4-chloroyphenyl)-3a,4,5,6-tetrahydrocyclopenta[c]pyrazol-2(3H)-yl)ethanone (**cp4**):*

Yellow powder, mp 120 – 122 °C; FTIR (KBr, ν_{max}/cm^{-1}): 1596 (C=N), 1474 (C=C), 1244 (C–N). ^1H NMR ($CDCl_3$, 300 MHz, 25°C): 7.21 - 7.06 (m, 8 H), 6.27 (s, 1H, =CH), 4.50 (d, 1H), 2.65 (m, 1H), 2.01 (s, 3H, CH_3), 1.77-1.45 (m, 4H).MS (ESI^+) m/z: $(M^+ H)^+$ 386. Elemental analysis Calc. (found) for $C_{21}H_{18}Cl_2N_2O$: C, 65.46 (65.20); H, 4.71 (4.72); N, 7.27 (7.25).

*(E)-1-(6(2,4-dichlorobenzylidene-3-(2,4-dichlorophenyl)-3a,4,5,6-tetrahydrocyclopenta[c]pyrazol-2(3H)-yl)ethanone (**cp5**):*

Yellow powder, mp 150 – 152 °C; FTIR (KBr, ν_{max}/cm^{-1}): 1576 (C=N), 1458 (C=C), 1249 (C–N). ^1H NMR ($CDCl_3$, 300 MHz, 25°C): 7.23 - 7.02 (m, 6H), 6.46 (s, 1H, =CH), 4.80 (d, 1H), 2.40 (m, 1H), 2.07 (s, 3H, CH_3), 2.01-1.24 (m, 4H).MS (ESI^+) m/z: $(M^+ H)^+$ 455. Elemental analysis Calc. (found) for $C_{21}H_{16}Cl_4N_2O$: C, 55.53 (55.31); H, 3.55 (3.56); N, 6.17 (6.19).

*(E)-1-(6(3-nitrobenzylidene-3-(3-nitrophenyl)-3a,4,5,6-tetrahydrocyclopenta[c]pyrazol-2(3H)-yl)ethanone (**cp6**):*

Yellow powder, mp 126 – 128 °C;FTIR (KBr, ν_{max}/cm^{-1}): 1596 (C=N), 1477 (C=C), 1215 (C–N). ^1H NMR ($CDCl_3$, 300 MHz, 25 °C): 8.22 - 7.44 (m, 8H), 6.32 (s, 1H, =CH), 4.6 (d, 1H), 2.63 (m, 1H), 2.02 (s, 3H, CH_3), 2.04 - 1.23 (m, 4H). MS (ESI^+) m/z: $(M^+ H)^+$ 407. Elemental analysis Calc. (found) for $C_{21}H_{18}N_4O_5$: C, 62.06 (62.10); H, 4.46 (4.47); N, 13.79 (13.84).

*(E)-1-(6(3-methoxybenzylidene-3-(3-methoxyphenyl)-3a,4,5,6-tetrahydrocyclopenta[c]pyrazol-2(3H)-yl)ethanone (**cp7**):*

Yellow powder, mp 116–118 °C; FTIR (KBr, ν_{max}/cm^{-1}): 1584 (C=N), 1487 (C=C), 1265 (C–N). ^1H NMR ($CDCl_3$, 300 MHz, 25°C): 7.15–6.52 (m, 8H), 6.11 (s, 1H, =CH), 4.10 (d, 1H), 2.48 (m, 1H), 2.05 (s, 3H, CH_3), 3.70; 3.74 (s, 6H, $2OCH_3$), 1.75-1.22(m, 4H).MS (ESI^+) m/z: $(M^+ H)^+$ 377. Elemental analysis Calc. (found) for $C_{23}H_{24}N_2O_3$: C, 73.38 (73.40); H, 6.43 (6.41); N, 7.44 (7.45).

*(E)-1-(6(2,3,4-trimethoxybenzylidene-3-(2,3,4-trimethoxyphenyl)-3a,4,5,6-tetrahydrocyclopenta[c]pyrazol-2(3H)-yl)ethanone (**cp8**):*

Yellow powder, mp 208 - 210 °C; FTIR (KBr, ν_{max}/cm^{-1}): 1577 (C=N), 1469 (C=C),1281 (C–N). ^1H NMR ($CDCl_3$, 300 MHz, 25°C): 7.21 - 6.20 (m, 4H), 6.49 (s, 1H, =CH), 4.20

(d, 1H), 2.68 (m, 1H), 2.01 (s, 3H, CH_3), 3.65; 3.70; 3.75 (s, 18H, 6OCH_3), 1.70 - 1.15 (m, 4H). MS (ESI^+) m/z: $(M^+ H)^+$ 497. Elemental analysis Calc. (found) for $C_{27}H_{32}N_2O_7$: C, 65.31 (65.57); H, 6.50 (6.52); N, 5.64 (5.60).

*(E)-1-(6-(4-(dimethylamino)benzylidene)-3-(4-dimethylaminophenyl)-3a,4,5,6-tetrahydrocyclopenta[c]pyrazol-2(3-H)-yl)ethanone (**cp9**):*

Yellow powder, mp 146 – 148 °C; FTIR (KBr, ν_{max}/cm^{-1}): 1596 (C=N), 1477 (C=C), 1258(C–N). ^1H NMR ($CDCl_3$, 300 MHz, 25°C): 7.14 - 6.46 (m, 8H), 6.15 (s, 1H, =CH), 4.70 (d, 1H),2.68 (m, 1H), 2.03 (s, 3H,CH_3), 2.78; 2.80(s, 12 H,4N-CH_3),1.75 - 1.26 (m, 4H).MS (ESI^+) m/z: $(M^+ H)^+$ 403. Elemental analysis Calc. (found) for $C_{25}H_{30}N_4O$: C, 74.59 (74.61); H, 7.51 (7.49); N, 13.92 (13.90).

General Procedure for the synthesis of (*E*)-6-(substitutedbenzylidene)-2-phenyl-3-(substitutedphenyl)-2,3,3a,4,5,6-hexahydrocyclopenta[c]pyrazole (cp10-cp14):

The compounds were also prepared in two steps. In first step, respective dibenzylidiene cyclopentanone was synthesized. A mixture of ethanol (20 ml) and sodium hydroxide solution (10%, 10 ml) was taken in a beaker and was maintained at temperature between 15 to 25 ºC. The solution was vigorously stirred and one half of previously prepared mixture of appropriate aromatic aldehyde and cyclopentanone (in molar ratio of 2: 1) was added to it. After 20 min, the remaining aromatic aldehyde-cyclopentanone mixture was added. The reaction mixture was further stirred for 45 minutes. The solid was filtered off, washed with distilled water, and subjected to further purification by crystallization with ethyl alcohol.

In second step, the synthesized dibenzylidiene cyclopentanone (1.2 mmol) from first step was dissolved in glacial acetic acid (5 ml) and phenyl hydrazine (1.5 mml) and was added to the solution. The solution produced was refluxed for 20 hours and monitored by TLC. Solvent was removed in vacuum and recrystallized with ethyl alcohol. The remaining derivatives were prepared by the same procedure.

*(E)-6-(3-chlorobenzylidene)-3-(3-chlorophenyl)-2-phenyl-2,3,3a,4,5,6-hexahydrocyclopenta[c]pyrazole (**cp10**):*

Yellow powder, mp 70 – 72 °C; FTIR (KBr, ν_{max}/cm^{-1}): 1566 (C=N), 1483 (C=C). ^1H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.24-6.45 (m, 13 H), 6.20 (s, 1H, =CH), 3.50 (d, 1H), 2.21 (m, 1H), 1.75 - 1.23 (m, 4H).MS (ESI^+) m/z: $(M^+ H)^+$418. Elemental analysis Calc. (found) for $C_{25}H_{20}Cl_2N_2$: C, 71.60 (71.66); H, 4.81 (4.82); N, 6.68 (6.70).

*(E)-6-(4-chlorobenzylidene)-3-(4-chlorophenyl)-2-phenyl-2,3,3a,4,5,6-hexahydrocyclopenta[c]pyrazole (**cp11**):*

Yellow powder, mp 76 – 78 °C; FTIR (KBr, ν_{max}/cm^{-1}): 1574 (C=N), 1484 (C=C). ^1H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.28 - 6.13 (m, 13H), 6.03 (s, 1H, =CH), 3.50 (d, 1H), 2.26 (m, 1H), 1.62 - 1.21 (m, 4H). MS (ESI^+) m/z: $(M^+ H)^+$ 420. Elemental analysis Calc. (found) for $C_{25}H_{20}Cl_2N_2$: C, 71.60 (71.75); H, 4.81 (4.84); N, 6.68 (6.70).

*(E)-6-(2,4dichlorobenzylidene)-3-(2,4-dichlorophenyl)-2-phenyl-2,3,3a,4,5,6-hexahydrocyclopenta[c]pyrazole (**cp12**):*

Yellow powder, mp 86 – 88 °C; FTIR (KBr, ν_{max}/cm^{-1}): 1579 (C=N), 1450 (C=C). ^1H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.28 - 6.05 (m, 11H), 6.02 (s, 1H, =CH), 3.50 (d, 1H), 2.22 (m, 1H), 1.77-1.20 (m, 4H).MS (ESI^+) m/z: $(M^+ H)^+$ 489. Elemental analysis Calc. (found) for $C_{25}H_{18}Cl_4N_2$: C, 61.50 (61.47); H, 3.72 (3.78); N, 5.74 (5.72).

*(E)-6-(4-methoxybenzylidene)-3-(4-methoxyphenyl)-2-phenyl-2,3,3a,4,5,6-hexahydrocyclopenta[c]pyrazole (**cp13**):*

Yellow powder, mp 92 – 94 °C; FTIR (KBr, ν_{max}/cm^{-1}): 1584 (C=N), 1496 (C=C). ^1H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.22 - 6.14 (m, 13H), 6.11 (s, 1H, =CH), 3.5 (d, 1H), 2.15 (m, 1H), 3.68; 3.71 (s, 6H, 2OCH_3), 1.84 - 1.4 (m, 4H).MS (ESI^+) m/z: $(M^+ H)^+$410. Elemental analysis Calc. (found) for $C_{27}H_{26}N_2O_2$: C, 79.00 (79.04); H, 6.38 (6.40); N, 6.82 (6.80).

*(E)-4-(3-(4-(dimethylamino)phenyl)-2-phenyl-3,3a,4,5-tetrahydrocyclopenta[c]pyrazol-6(2H)-ylidene) methyl)-N,N-dimethylaniline (**cp14**):*

Yellow powder, mp 94 – 96 °C; FTIR (KBr, ν_{max}/cm^{-1}): 3406 (=NH), 1596 (C=N), 1477 (C=C). ^1H NMR ($CDCl_3$, 300 MHz, 25 °C): 7.22-6.51 (m, 13H), 6.24 (s, 1H, =CH), 3.1 (d, 1H), 2.31 (m, 1H), 2.82; 2.86 (s, 12 H, 4N-CH_3), 1.78-1.20(m, 4H).MS (ESI^+) m/z: $(M^+ H)^+$ 437. Elemental analysis found (Calc.) for $C_{29}H_{32}N_4$: C, 79.78 (79.80); H, 7.39 (7.41); N, 12.83 (12.80).

ANTIMICROBIAL STUDIES

Antimicrobial activity of newly synthesized compounds was first screened by disc diffusion method [23] against various Gram positive and Gram negative human pathogenic bacteria viz. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27893, *S. typhi* MTCC 3216, *E. faecalis* and *Staphylococcus aureus* ATCC 25323 and different fungal strains of *Candida* according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1997). Fresh grown bacteria were mixed in sterile saline (0.85%) and the turbidity was matched with McFarland No. 2 system to achieve concentration of 107 CFU/ml. Sterile petri plates containing 20 ml of Mueller Hinton agar (MHA, Hi-Media) were used for all bacterial culture and Sabouraud's dextrose agar (SDA)/Potato dextrose agar (PDA) (Hi-Media) were used for all fungal culture. The bacterial inoculums' suspension was spread on the surface of agar plates. Sterile disc (5mm) of Whatman paper no. 1 was then placed on the surface of the media and the test compounds (25µl/ml) was put and allowed to diffuse and plates were incubated for 24 h at 37 °C for bacterial cultures and 72 h at 25 °C for fungal culture. Ciprofloxacin (5 µg/disc, Hi-Media) was used as positive control for bacteria and Fluconazole (10 µg/disc, Hi-Media) was used as a positive control for fungi. Zone of inhibition was measured in millimeters after 24 h. All tests were performed in triplicate.

RESULTS AND DISCUSSION

The compounds were prepared by coupling of substituted benzaldehydes with cyclopentanone in (molar ratio of 2:1) in a base catalyzed Claisen-Schmidt condensation followed by reaction with hydrazines (hydrazine hydrate, phenyl-

Table 2. Antimicrobial activity of pyrazole analogues of curcumin.

Comp.	Microbial Species							
	Bacteria					Fungi		
	E. coli ATCC 35218	*S. typhi*MTCC 3216	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25323	*E. faecalis* (Clinical isolate)	*C. albicans* ATCC 90028	*C. Tropicalis* ATCC 750	*C. Krusie* ATCC 6258
cp1	12.73 ± 0.20	14.23 ± 0.70	10.66 ± 0.40	11.43 ± 0.90	-	8.73 ± 0.75	11.66 ± 0.17	-
cp2	10.45 ± 0.47	-	-	-	10.17 ± 0.47	11.49 ± 1.03	-	12.06 ± 0.15
cp3	12.35 ± 0.40	13.26 ± 0.15	12.06 ± 0.90	14.63 ± 0.85	12.26 ± 0.33	-	11.66 ± 0.47	10.70 ± 0.51
cp4	11.35 ± 1.07	-	-	11.30 ± 0.34		10.80 ± 0.51	10.30 ± 1.15	-
cp5	09.45 ± 1.15	-	9.60 ± 0.50	10.33 ± 0.61	11.26 ± 0.94	10.87 ± 0.80	-	09.24 ± 0.23
cp6	-	-	-	-	-	-	-	-
cp7	11.50 ± 0.37	-	13.60 ± 0.98	14.13 ± 0.21	-	12.65 ± 0.86		9.76 ± 0.45
cp8	13.40 ± 0.02	-	-	10.56 ± 0.45	-	10.15 ± 0.17	-	-
cp9	-	10.87 ± 0.79	10.53 ± 0.44	11.53 ± 0.25	-	-	10.13 ± 0.34	
cp10	21.50 ± 0.72	17.26 ± 0.15	15.76 ± 0.19	20.13 ± 0.22	14. 26 ±1.27	11.53 ± 0.32	-	10.51 ± 0.16
cp11	18.40 ± 1.10	-	17.33 ± 1.06	14.01 ± 0.15	-	13.86 ± 0.65	-	11.20 ± 0.86
cp12	19.64 ± 0.93	15.30 ± 0.91	16.06 ± 0.52	19.13 ± 1.65	15.40 ± 1.17	10.10 ± 0.53	-	10.53 ± 0.39
cp13		-	-	11.26 ± 0.25	10.90 ± 0.62	11.66 ± 0.40	-	16.13 ± 0.70
cp14	11.33 ± 0.35	10.53 ± 0.57	-	10.76 ± 0.56	-	09.16 ± 0.06	-	12.50 ± 0.66
Ciprofloxacin	28.06 ± 1.30	22.17 ± 0.45	29.76 ± 0.26	30.63 ± 0.49	26.40 ± 0.19	-	-	-
Fluconazole	-	-	-	-	-	21.35 ± 0.55	16.80 ± 0.15	20.93 ± 0.18
DMSO	-	-	-	-	-	-	-	-

The values of each compound consisted of Mean ± SE of 03 replicates.

hydrazine) and acetic acid. Antimicrobial activity of the synthesized pyrazole analogues of curcumin compounds is given in the table (Table **2**).

The results of antibacterial screening revealed that among the screened compounds **cp1**, **cp2** and **cp8** showed moderate antibacterial activity. However, compounds **cp10**, **cp11** and **cp12** showed good antibacterial activity when compared with ciprofloxacin used as standard. Particularly, compound **cp10** showed maximum antibacterial activity (Zone of inhibition up to 21mm) against *E.coli* and *S aureus* (Fig. **1**). Double substitution with chloro group at *ortho* and *para* position led to slender decrease in potency (compound **cp12** zone of inhibition =19mm).

Level of significance $p < 0.05$

The introduction of methoxy group at *meta* position in order to increase electron density resulted in diminished antibacterial activity (Compound **cp7** zone of inhibition = 11mm). While substitution of methoxy group at *ortho*, *meta* and *para* positions led to slight increase in potency (compound **cp8** zone of inhibition=13 mm). This may be due to the favorable steric interaction of these groups. The results of antifungal screening indicated that compounds **cp2**, **cp7**, **cp11**, **cp13** showed moderate to excellent antifungal activity. Compounds **cp2**, **cp7** and **cp11** showed moderate activity against *C. albicans*. While, compound **cp13** showed good activity against two fungal strains viz. *C. albicans* and *C. krusie*. Methoxysubstituition (compound **cp13** zone of inhibition=16mm) increased antifungal potency significantly. Among the halogenated analogues chloro substitution at *para* position increased antifungal activity appreciably (compound **cp11** zone of inhibition=13mm). The increase in potency by *para* substitution may be attributed to appropriate orientation of chloro to fit in binding site. Out of fourteen compounds synthesized, majority of them showed substantial antimicrobial activity against tested strains.

A significant bottleneck in the drug discovery procedures, especially in the later stages of lead discovery, is analysis of the ADME and overt toxicity properties of drug candidates. Nearly half of the drugs fail in clinical trials because of poor ADME and toxicity properties. Consequently, there is increasing interest in the early prediction of ADME properties, with the objective of escalating the success rate of compounds reaching development [24]. Predicting ADME

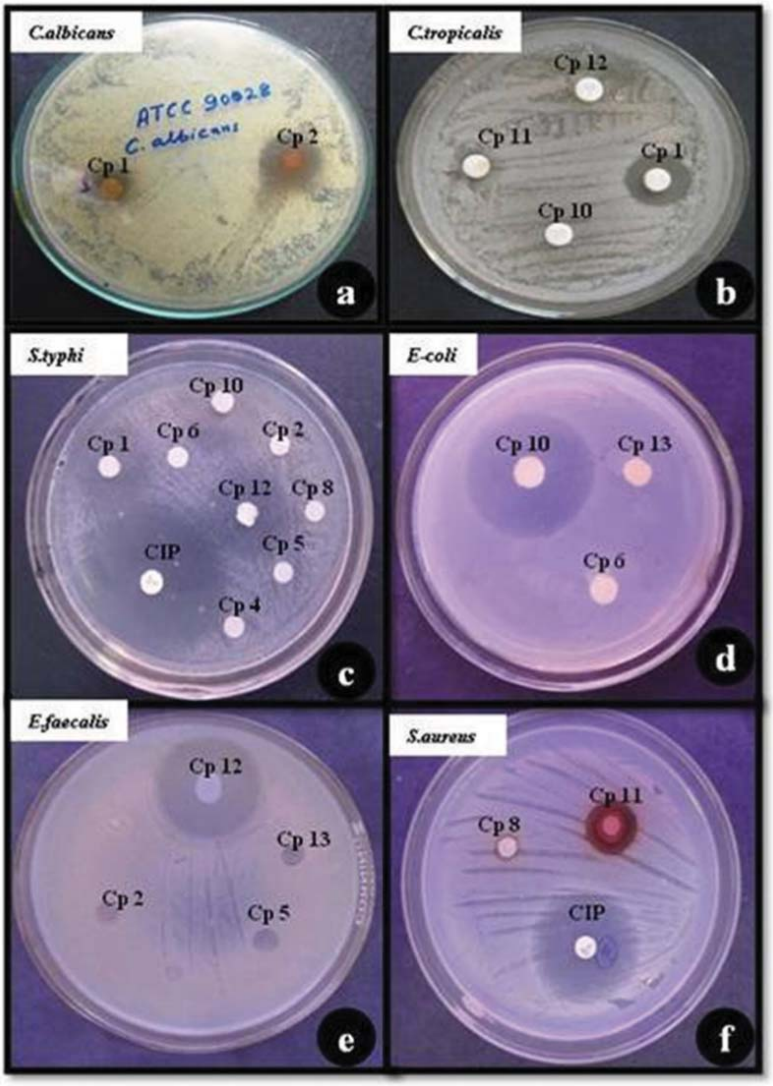

Fig. (1). Bioassay plate showing antimicrobial effect of pyrazole analogues of Curcumin by agar well diffusion methods **(a)cp1**&**cp2** showing effective antifungal activity against *C. albicans* with refrence to standard drug Fluconazole **(b)cp1** showing effective inhibition zone against *C.tropicalis*, as compare to **cp10**, **cp11**, **cp12(c)** Antibacterial activity of standard drug Ciprofloxacin (CIP), **cp1**, **cp10** and **cp12** against *S.typhi* showing inhibition in comparision to **cp2**, **cp4**, **cp5**, **cp6** and **cp8(d)** cp10 showing excellent inhibition against *E. coli*, in comparision to **cp6** and **cp13(e)cp12** showing effective antibacterial activity against *E.faecalis*in comparision to other derivatives **(f)cp11**and Ciprofloxacin showing zone inhibition against *S. aureus*.

properties at an initial stage of drug discovery and development process is imperative to discard problematic candidates, to reduce the amount of wasted time and resources, and streamline the overall development process. In the present study, pyrazole analogues of curcumin were predicted for different properties like Log P, Human intestinal absorption, *in vitro* skin permeability, *in vitro* plasma protein binding and *in vivo* blood-brain barrier penetration using PreADMET server. The results are summarized in Table **3**.

Molecular docking of two different active sites (Active site 1, amino acid series: Cys1, Arg73, Thr76, His77, Asn98, Gly99, Ile100 and active site 2, amino acid series: Gly301, Thr302, Ser303, Ser347, Gln348, Ser349, Thr352, Lys485, Ala602 and Val605) of glucosamine-6-phosphate synthase (GlcN-6-P synthase) protein with the pyrazole analogues of curcumin and the standard drug fluconazole yielded best possible conformations with parameters including the docked energy, inhibition constant and RMSD as presented in the table (Table **4 & 5**).

Comparative docking of GlcN-6-P synthase with the pyrazole analogues of curcumin and the standard drug fluconazole revealed that the docked energy for the compound **cp10**, **cp11**, **cp12** was -10.85, -10.58, -11.12 kcal/mol at active site 1 and -12.18, -12.32, -11.35 kcal/mol at active site 2 with an estimated inhibition constant of $1.08x10^{-7}$, $1.44\ x10^{-7}$, $5.23\ x10^{-8}$ and $1.44\ x\ 10^{-8}$, $8.31x10^{-9}$,$5.35x10^{-8}$ respectively (Fig. **2**). The docking energy of the fluconazole was only -5.36 at active site 1 and -6.57 kcal/mol at active site 2 with an inhibition constant of $5.53x10^{-5}$ and $4.7\ x\ 10^{-5}$respectively. In the first active site the geometry of compounds **cp10**, **cp11** and **cp12** are "frozen" in the binding pocket due to strong and stable hydrogen bonds formed between the amido moiety of the inhibitor and the His77 amino acid residue present in the binding site and well conserved in GlcN-6-P

Table 3. Theoretical ADME prediction of new pyrazole analogues of curcumin using PreADMET Server.

Entry	Log_p	Human Intestinal Absorption (%)	*In vitro* Skin Permeability (logKp, cm/hour)	*In vitro* Plasma Protein Binding (%)	*In vivo* Blood-Brain Barrier Penetration
CP-1	4.07	99.52	-2.62	94.33	1.16
CP-2	5.39	99.67	-2.58	93.29	0.66
CP-3	3.30	94.13	-3.18	89.01	0.63
CP-4	5.41	99.67	-2.61	99.50	0.52
CP-5	5.80	99.61	-2.40	100	1.91
CP-6	4.16	92.93	-2.59	94.58	0.07
CP-7	4.06	97.37	-2.99	89.06	1.11
CP-8	3.61	99.06	-3.55	85.14	0.58
CP-9	4.41	98.27	-2.67	92.19	0.13
CP-10	6.42	100	-1.86	82.52	8.14
CP-11	6.79	100	-1.86	82.31	8.91
CP-12	7.69	100	-1.77	81.43	11.92
CP-13	5.92	99.31	-2.16	95.36	0.63
CP-14	6.07	100	-1.87	93.15	3.29

Table 4. Molecular docking of new pyrazole analogues of curcumin with glucosamine-6-phosphate synthase at Active Site1.

Entry	Docking Energy (Kcal/mol)	Inhibition Constant (M)	Amino Acid Residue Involved in H-bond	Bond Length (Å)	RMSD
CP-1	-9.77	2.16×10^{-7}	Thr76	2.17	1.05
			His77	1.84	
CP-2	-9.34	4.08×10^{-7}	NB	NB	1.09
CP-3	-10.22	6.87×10^{-8}	Trp74	2.04	0.85
			Thr76	1.69	
			Gly99	1.73	
CP-4	-9.14	7.70×10^{-7}	Thr76	1.97	1.10
CP-5	-8.51	4.76×10^{-7}	His86	1.8	1.19
CP-6	NA	NA	NA	NA	NA
CP-7	-10.33	1.16×10^{-7}	His77	1.89	0.72
CP-8	-9.51	3.35×10^{-7}	Gly99	2.13	1.01
CP-9	-8.55	5.41×10^{-6}	Arg73	1.96	1.20
CP-10	-10.85	1.08×10^{-7}	His77	1.90	0.82
CP-11	-10.58	1.44×10^{-7}	His77	1.81	0.81
CP-12	-11.12	5.23×10^{-8}	His77	1.91	0.74
CP-13	-10.51	3.43×10^{-7}	His77	2.10	0.85

Table 4. contd…

Entry	Docking Energy (Kcal/mol)	Inhibition Constant (M)	Amino Acid Residue Involved in H-bond	Bond Length (Å)	RMSD
CP-14	-9.74	1.61×10^{-6}	Arg73	1.89	1.00
			His104	2.11	
Flucanozole	-5.36	5.53×10^{-5}	Arg 73	2.20	1.34
			Arg 73	2.09	
			His 77:	1.87	

Table 5. Molecular docking of new pyrazole analogues of curcumin with glucosamine-6-phosphate synthase at Active Site 2.

Entry	Docking Energy (Kcal/mol)	Inhibition Constant (M)	Amino Acid Residue Involved in H-Bond	Bond Length (Å)	RMSD
CP-1	-9.34	1.36×10^{-6}	Ala602	2.15	1.18
CP-2	-9.67	3.3×10^{-7}	Gln348	1.95	1.11
			Ser349	2.22	
CP-3	-9.80	2.80×10^{-7}	Gln348	1.81	1.08
			Ser349	1.90	
CP-4	-10.57	3.0×10^{-7}	Gln348	2.07	0.86
			Ser349	2.12	
CP-5	-9.48	4.12×10^{-7}	Gln348	2.10	1.16
CP-6	NA	NA	NA	NA	NA
CP-7	-10.75	2.59×10^{-7}	Gln348	2.07	0.83
			Ala602	2.13	
CP-8	-10.60	6.91×10^{-7}	Val605	1.83	0.85
CP-9	-10.64	1.18×10^{-7}	Thr302	1.92	0.85
CP-10	-12.18	1.44×10^{-8}	NB	NB	0.68
CP-11	-12.32	8.31×10^{-9}	NB	NB	0.61
CP-12	-11.35	5.35×10^{-8}	NB	NB	0.71
CP-13	-10.51	1.03×10^{-6}	Gly301	2.04	0.86
CP-14	-11.04	1.20×10^{-7}	NB	NB	0.73
Fluconazole	-6.57	4.7×10^{-5}	Gln348	2.35	1.28
			Ser349	2.02	

synthase sequences from various organisms [25]. Whereas, in the second active site there is no hydrogen bond formed with any of the three compounds but the docking energy and inhibition constant was minimum.

This effect is caused by specific physical supramolecular interactions with a set of amino acid side-chains, may be ion-charge or dipole interactions, charge transfers, and hydrophobic or hydrophilic interactions. The electronegative nucleus of the chlorine connected to a carbon atom withdraws electrons from other parts of the molecule, thus strongly polarizing that bond causing a dipole moment. A significant number of drugs and drug candidates in clinical development are halogenated structures. The formation of halogen bonds

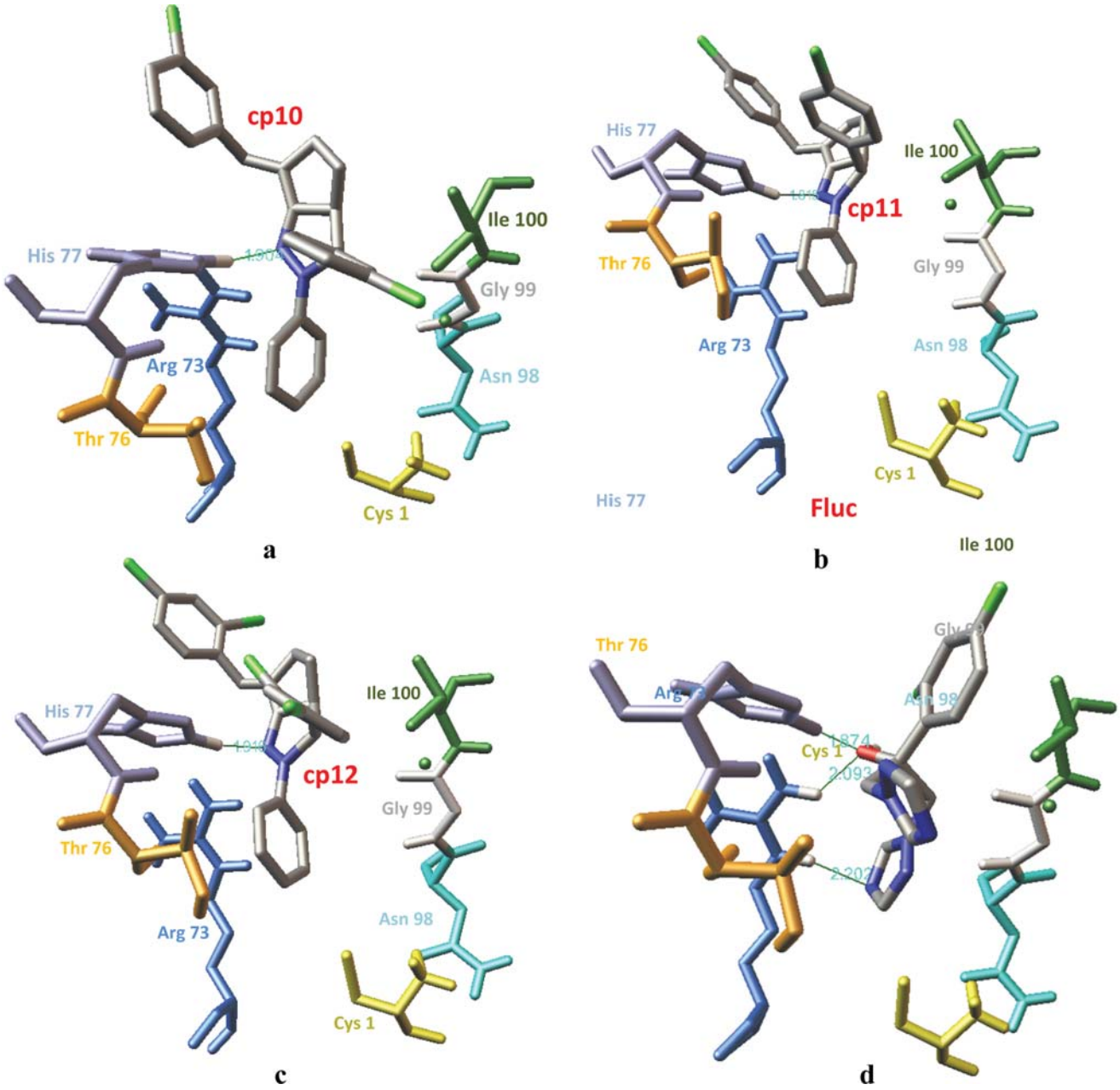

Fig. (2). Comparative docking of pyrazole analogues of curcumin and fluconazole with (Ball and Stick model) glucosamine-6-phosphate synthase showing hydrogenbond formation within the active site 1 (**a**) compound **cp10**, (**b**) compound **cp11**, (**c**) compound **cp12**, (**d**) fluconazole (in active site 2 no hydrogen bonds are formed).

in ligand-target complexes is recognized as a kind of intermolecular interaction that favorably contributes to the stability of protein-ligand complexes. The insertion of halogen atoms has been used in innumerous cases of hit-to-lead or lead-to-drug conversions [26-28]. The incorporation of halogen atoms increases membrane permeability, improves the oral absorption and skin penetration [29].

In the present study, the molecular docking results of compound **cp10, cp11 and cp12** showed very good binding energy and RMSD, even in *in vitro* studies these analogues showed promising activity against all tested microorganisms. So, it may be concluded that the activity is due to the inhibition of enzyme GlcN-6-P synthase, which catalyses a complex reaction involving ammonia transfer from L-glutamine to Fru-6-P followed by isomerisation of the formed fructosamine-6-phosphate to glucosamine-6-phosphate.

CONCLUSION

The desirable improvement in the antimicrobial activity of the pyrazole analogues of curcumin requires electron releasing groups and chloro substitution and may be the reason for higher activity of compounds cp10, cp11andcp12. It is infantile to draw conclusions on structure activity aspect of these compounds and additional evaluation is enviable to use them for clinical study. Docking studies were carried out to rationalize the screening results. These pyrazole analogues of curcumin could embody fruitful matrix for the development

of a new class of antibacterial and antifungal agents which can be of interest for further exploration and derivatizations.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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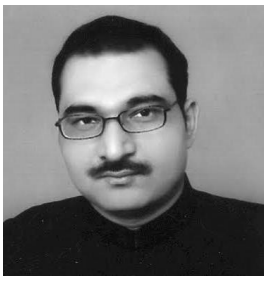
Candidate's Personal Profile



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