Chapter 2
Literature Review

2. LITERATURE REVIEW

2.1 Microbial Infections

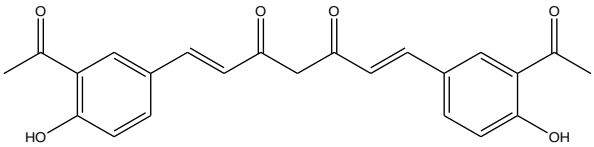
Bacterial infection is an omnipresent health peril. There are a number of clinically efficient antibiotics in the market, but the development of bacterial resistance has rendered almost all of them less effective. This decisive circumstance necessitates the design of newer antibacterial agents. The agents must target essential bacterial pathways, which may have new modes of action or even interfere with novel bacterial targets.

The availability of complete bacterial genome sequences (with the first, *Haemophilus influenza*, genome in 1995) spurred a renewed interest in novel antibiotic research. Genomic information fostered the target-based approach of finding new classes of drugs with a novel mode of action. Several unconventional targets have been selected based on genomics, isolated, characterized and screened in pursuit of novel drug candidates.

However, the success rate has been rather disappointing. Genomic information is now being used to find genes that encode the production of natural products. Since these are usually clustered in the genome, analysis is facilitated and with the help of bioinformatics tools, prediction of biosynthetic pathways and structures has become possible. This has recently resulted in the discovery of many novel natural compounds. Another approach to find new antibiotics is to target previously unexploited essential enzymes amongst others, those involved in the fatty acid metabolic pathway, cell division, DNA replication and others.

The importance and value of antibiotics cannot be overestimated; we are totally dependent on them for the treatment of infectious disease. It is vital that there should be absolutely no letup in the search for new antimicrobial agents. We are in the midst of an emerging crisis of antibiotic resistance for microbial pathogens throughout the world 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 therefore, the discovery of drugs with novel mode of action will be imperative to meet the threats by the emergence of resistance.

2.2 Curcumin as antimicrobial agent



11.

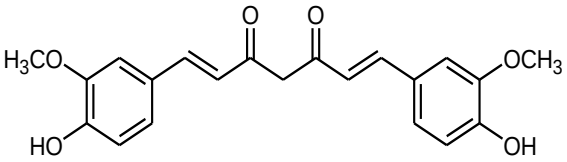
Structure of Curcumin

Curcumin, a natural yellow pigment, chemically known as 1, 7-bis (4 hydroxy-3-methoxyphenyl)-1, 6-heptane-3, 5-dione is derived from the rhizome of *Curcuma longa* linn. belongs to the family Zingiberaceae. It is the principal curcuminoid of the popular Indian spice turmeric. Curcumin incorporates several functional groups. The aromatic ring systems, which are phenols, are connected by two α, β-unsaturated carbonyl groups. The diketones form stable enols and are readily deprotonated to form enolates; the α, β-unsaturated carbonyl group is a good Michael acceptor and undergoes nucleophilic addition reactions. Curcumin has wide range of activities against inflammation, ulcer, cancer, diabetes, depression, contraception, viral diseases etc. 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. A single blind, randomized, and placebo controlled study on 20 tropical pancreatitis patients has revealed that oral administration of capsule containing 500mg of pure extract of Curcumin (95%) with 5mg of piperine, three times a day, can significantly reverse the erythrocyte malonyl dialdehyde level.

The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. Both curcumin and the oil fraction suppress growth of several bacteria like *Streptococcus, Staphylococcus, Lactobacillus,* etc. along with long history of therapeutic use, antibacterial and antifungal activity of curcumin has been reported [Chattopadhyay *et al.* 2004; Di Mario *et al*. 2007; and Rai *et al*. 2008]. Curcumin is highly safe and its intake at as high as 8 g every day for 3 months by humans causes no ill effects [Chattopadhyay *et al*. 2004].

Singh *et al.* (2002) has reported Antibacterial activity of *Curcuma longa* rhizome extract on pathogenic strains of Gram-positive (*S. aureus*, *S. epidermidis*) and Gram-negative (*E. coli*, *P. aeruginosa*, *S. typhimurium*) bacteria. The study suggests that essential oil fraction from turmeric possesses significant ($P < 0.001$) antibacterial activity at very low concentration (20 µg/disc) on pathogenic Gram-positive *S. aureus* bacteria, which were resistant to the standard antibiotics [Singh *et al.* 2002].

Han *et al.* (2005) has reported Curcumin as a common non-toxic natural dye used in textile and food, has antimicrobial ability on wool. The antimicrobial ability of curcumin finished wool is semi-durable, more durable to home laundering than to light exposure [Han *et al.* 2005].



Curcumin (Diferuloyl methane)

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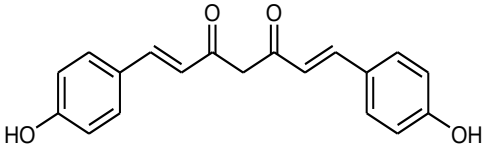

Bisdemetohxy curcumin

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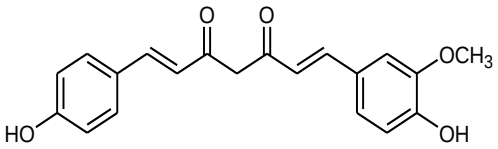

Demethoxy curcumin

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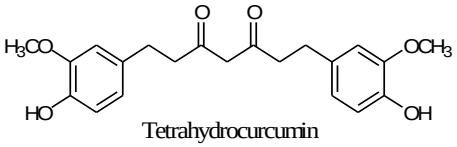


Tetrahydrocurcumin

19.

Chemical structure of curcuminoid

Park *et al.* (2005) has demonstrated that curcuminoid has inhibitory activity against sortase A, a bacterial surface protein anchoring transpeptidase, from *S. aureus* ATCC 6538p. Curcumin was a potent inhibitor of sortase A, with an IC_{50} value of 13.8±0.7 µg/mL. It also exhibited potent inhibitory activity against *S. aureus* cell adhesion to fibronectin. The suppression of fibronectin-binding activity by curcumin highlights its potential for the treatment of *S. aureus* infections via inhibition of sortase activity [Park *et al*. 2005].

Tajbakhsh *et al.* (2008) has investigated antibacterial activity of new analogues of curcumin-indium complex against *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 14990), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922) and reported to have more antibacterial effect than curcumin [Tajbakhsh *et al*. 2008].

Rai *et al.* (2008) has shown the inhibitory action of curcumin on bacterial cell division, apparently by perturbing the cytokinetic Z-ring through a direct interaction with assembly

dynamics of **F**ilamenting **t**emperature-**s**ensitive mutant **Z** (FtsZ). It is a prokaryotic homologue of eukaryotic cytoskeletal protein tubulin, polymerizes to form a Z-ring at the mid cell that orchestrates bacterial cell division [Rai *et al.* 2008].

Di Mario *et al.* (2007) reported Curcumin in combination with lactoferrin, N-acetylcysteine and pantoprazole has recently been shown to significantly reduce the symptoms caused by *Helicobacter pylori* infection in humans [Di Mario *et al.* 2007].

Dahl *et al.* (1989) and **Oda *et al*. (1995)** has further reported, curcumin have a potent phototoxic effect against several bacteria such as Salmonella serotype *Typhimurium* and *Escherichia coli*. It also inhibits repair system used by the cell when DNA damage could interfere significantly with DNA replication (SOS) induction in them [Dahl *et al.* 1989 and Oda *et al*. 1995].

Bhawana *et al.* (2011) reported that administration of curcumin to patients has a serious practical problem despite having multiple medicinal benefits and extremely superior safety profile, Its low aqueous solubility, poor bioavailability and rapid metabolism led to pharmacologically insignificant therapeutic effects. The *in vitro* biological assays against various microbes clearly demonstrated that transformation to the nano form greatly improves the water solubility and efficacy of curcumin as an antimicrobial agent [Bhawana *et al.*2011].

Naz *et al.* (2010) has studied antibacterial activity of different variety of *Curcuma longa*. Among all the three turmeric varieties, Kasur variety had most inhibitory effect on the growth of all bacterial strains tested as compared to Faisalabad and Bannu varieties [Naz *et al.* 2010].

Moghaddam *et al.* (2009) demonstrated enhancement effect of curcumin on the antibacterial activity of different antibiotics (Cefixime, Cephotaxime, Vancomycin and Tetracycline) against *S. aureus* strain. Therefore, this safe natural compound or its future derivatives have a good potential for combination therapy [Moghaddam *et al.* 2009].

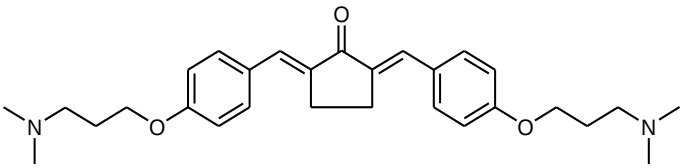
Wang *et al.* (2009) has improved the stability and solubility problem of curcumin through microencapsulation method. The microcapsule curcumin exhibited broad spectrum inhibitory effect on studying their antibacterial and antifungal activities against some foodborne pathogens and spoilage microbe such as *Escherichia coli*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, etc. [Wang *et al.* 2009].

De *et al.* (2009) reported that Curcumin arrested *H. pylori* growth irrespective of the genetic makeup of the strains, although its minimum inhibitory concentration (MIC) is

relatively high; this may be due to the poor bioavailability of curcumin. Moreover, the gastric damage induced by *H. pylori* infection was almost completely restored by curcumin, thus highlighting its potential as an alternative therapy against *H. pylori* infection [De *et al.* 2009].

Singh *et al*. (2009) evaluated the antitubercular, antifungal and antibacterial activities of curcumin analogues out of several compound synthesized α, α′-(EE)-bis(benzylidene)-cycloalkanones displayed moderate antitubercular activity against *M. tuberculosis* H37Rv with MIC value 12.5-1.56 µg/mL. However, none of the compounds displayed any significant antifungal activity [Singh *et al*. 2009].

Liang *et al*. (2008) has reported the effect of curcumin on the therapy of bacteria infected diseases through inhibiting the bacterial endotoxin-induced cytokines secretion, pathways activation, and directly suppressing pathogen cell growth.



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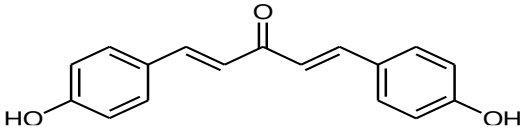

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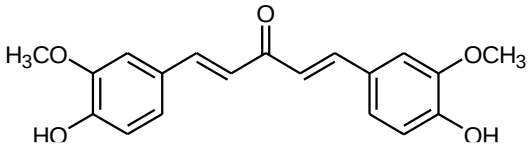

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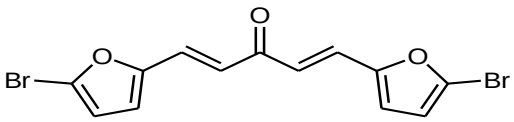

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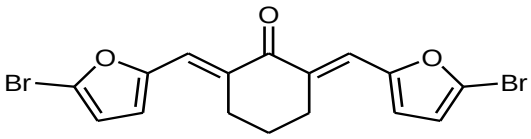


24.

Various mono-carbonyl analogues of curcumin

In vitro antibacterial activities of three series of curcumin analogues with mono-carbonyl showed that heterocyclic or long-chain substituents may enhance the activity of curcumin analogues. Compounds 20, 21, 22, 23 and 24 have shown remarkable *in vitro* antibacterial activity against the Ampicillin-resisted *E. cloacae* [Liang *et al.* 2008].

Talwar *et al.* (2008) has also reported that curcumin has antimicrobial activity against the many pathogens. It inhibits the growth of *N. gonorrhoeae* and *C. albicans* [Talwar *et al*. 2008].

Rudrappa *et al.* (2008) have shown that the curcumin inhibits *Pseudomonas aeruginosa* (PAO1) virulence factors such as biofilm formation, pyocyanin biosynthesis, elastase/protease activity, and acyl homoserine lactone (HSL) production. In addition, transcriptome analysis of curcumin-treated PAO1 revealed down regulation of 31 quorum sensing (QS) genes. Thus, the effect of curcumin on multiple targets such as virulence, QS, and biofilm initiation makes curcumin a potential supplemental molecule for the treatment of *P. aeruginosa* infections [Rudrappa *et al.* 2008].

2.3 Curcumin as anti-malarial agent

As per the *National Guidelines for treatment of malaria* in the Drug Policy 2013, it has been advised that *P. vivax* cases should be treated with chloroquine for three days and Primaquine for 14 days. Primaquine is used to prevent relapse but is contraindicated in pregnant women, infants and individuals with glucose -6-phosphate dehydrogenase deficiency (G6PD). *P. falciparum* cases should be treated with Artemisinin combination therapy (ACT) (Artesunate 3 days + Sulphadoxine Pyrimethamine 1 day). This is to be accompanied by single dose Primaquine preferably on day 2. However, considering the reports of resistance to partner drug Sulphadoxine Pyrimethamine (SP) in North Eastern States, the Technical Advisory Committee has recommended to use Coformulated tablet of Artemether (20 mg) - Lumefantrine (120 mg) as per the age-specific dose schedule for the treatment of Pf cases in North Eastern States (Not recommended during the first trimester of pregnancy and for children weighing < 5 kg). In case of pregnant woman uncomplicated *P. falciparum*, Primaquine is contra indicated so the 1st trimester can be treated with Quinine and 2nd& 3rd trimester with ACT. Presumptive treatment with Chloroquine is no more recommended. Resistance should be suspected if in spite of full treatment with no history of vomiting, diarrhoea, patient does not respond within 72 hours, clinically and parasitologically. Such cases not responding to ACT, should be treated with oral quinine with Tetracycline / Doxycycline [The National Drug Policy on Malaria, 2013].

Rasmussen *et al*. (1998) reported the development of simple and efficient method for the separation of three phenolic diketones, curcumin **11**, bis-demethoxycurcumin **17** and demethoxycurcumin **18**, 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 [Rasmussen *et al.* 1998].

Mishra *et al.* (2008) reported the 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 [Mishra *et al.* 2008].

Reddy *et al.* (2005) has reported that Curcumin inhibited Chloroquine-resistant *P. falciparum* growth in culture in a dose dependent manner with an IC_{50} of 5 µM by using 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 [Reddy *et al.* 2005].

Nandakumar *et al*. (2006) reported, curcumin can be an ideal antimalarial molecule due to its abundance, non-toxic nature, and therapeutic effects, 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 [Nandakumar *et al*. 2006].

Opong *et al.* (2007) & **Martinelli *et al.* (2008)** showed Curcumin has 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 [Opong *et al*. 2007 and Martinelli *et al*. 2008].

Yadav *et al.* (2005) has reported 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 [Yadav *et al*. 2005].

Grinberg *et al.* (2010) has reported that the 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 and immunomodulatory activities, 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 immune modulators efficient in preventing CM that should be administered together with antiplasmodial drugs to prevent severe malaria disease [Grinberg *et al.* 2010].

Mimche *et al.* (2011) has reported 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 nuclear transcription factor -κB (NF-Ķb) 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 [Mimche *et al.* 2011].

Cui *et al*. (2007) has reported 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 acetyl transferase (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 [Cui *et al.* 2007].

Mishra *et al.* (2008) has reported that the design of synthetic strategy has been focused on the development of curcumin analogues with high anti-malarial activity especially against

Chloroquine resistant (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 **28**, **29**, and **30** were found to be most potent and have shown inhibitory activity for Chloroquine susceptible (CQ-S) *P. falciparum* with IC_{50} of 0.48µM, 0.87µM, 0.92µM and for CQ-R *P. falciparum* at IC_{50} of 0.45µM, 0.89µM , 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 [Mishra *et al.* 2008].

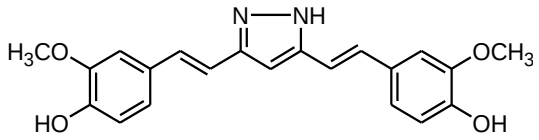
Manohar *et al.* (2013) has reported the study on monocarbonyl analogues of curcumin and indicated their safety towards mammalian cells. Considering the selectivity index for cytotoxicity (Vero cell) to antimalarial activity, it was demonstrated that all the monocarbonyl analogues of curcumin are non toxic [Manohar *et al.* 2013].

Mishra *et al.* (2009) has reported that 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 [Mishra *et al*. 2009].

Ji *et al.* (2009) has reported the interactions of curcumin with calcium ATPase from *Plasmodium falciparum* (PfATP6), an important antimalarial target, investigated by molecular docking studies and its effective inhibition of PfATP6 as characterized by the theoretical binding ability. The study 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 methyl curcumin, the Ludi scores (tool for calculate binding affinity) decreased to 528 and 504 from 594 and 561 for complexes of PfATP6 with the keto **31** and enol **32** forms respectively [Ji *et al.* 2009].

Foller *et al* (2009) and **Bentzen *et al*. (2007)** has repoted the 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 [Foller *et al*. 2009 and Bentzen *et al*. 2007].



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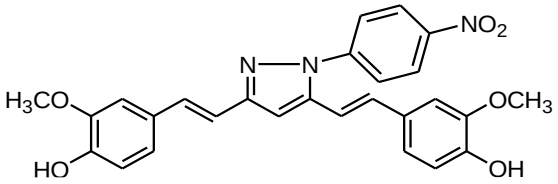

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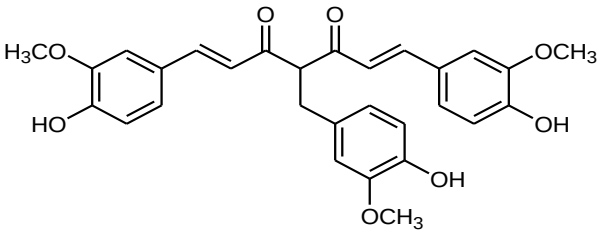
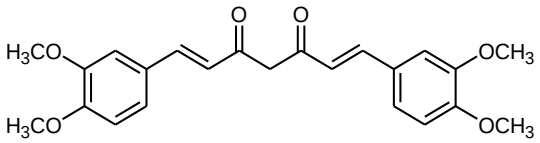


30.

Chemical structures of potential curcumin analogues.



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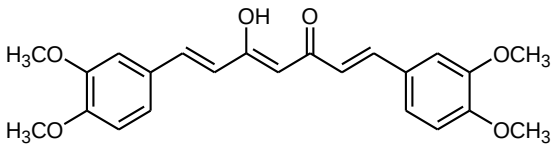


32.

Chemical structures of keto and enol forms of methyl curcumin.

Mulabagal *et al.* (2010) has repoted that in ultrafiltration, liquid chromatography and mass spectrometry (UF and LC/MS) based binding assays, curcuminoids (bis-demethoxycurcumin, demethoxycurcumin, and curcumin), used to study the binding affinity for thioredoxin Reductase from *Plasmodium falciparum* (PfTrxR) and *Plasmodium falciparum* Glutathione Reductase enzymes (PfGR), which are highly interesting drug targets to develop new antimalarial drugs. The developed method was specific and fast, needed 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 [Mulabagal *et al.* 2010].

Rasoanaivo *et al.* (2011) reported 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) and concluded 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 [Rasoanaivo *et al.* 2011].

Dattani *et al.* (2010) has reported that 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 succinic dehydrogenase (SDH), ATPase and alkaline phosphatase (ALKase) activities, whereas acid phosphatase (ACPase) activity was found to be significantly increased. Antioxidant enzyme, superoxide dismutase (SOD) registered a significant reduction as opposed to Thiobarbituric acid reactant substances (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 [Dattani *et al.* 2010].

Nayak *et al.* (2010) has reported that 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 parenteral 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 [Nayak *et al*. 2010].

Dandekar *et al.* (2010) has reported that 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 [Dandekar *et al.* 2010].

Akhtar *et al.* (2012) has reported that the 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 the 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 chloroquine (198 μM ± 3.7). Thus, by enhancing bioavailability and chemical stability, curcumin can inhibit hemozoin synthesis which is lethal for the malaria parasite [Akhtar *et al*. 2012]. The above mentioned combinations would be good candidates to take them forward into clinical trials.

2.4 Pyrazole and hexahydra indazole as antimicrobial agent

Gomez *et al.* (2007) & **Bradbury *et al.* (2008)** reported that the Emergence of bacterial resistance against almost all major classes of antibiotics viz. β-lactum, Macrolides, Quinolones and Vancomycin, has posed a challenge to world-wide health. Intense efforts in antimicrobial drug discovery are still needed to develop more promising and effective antibacterial and antifungal agents for use in the clinical arena. In pursuit of new antibacterial molecules, recent study revealed that pyrazole derivatives could be promising

candidates for the development of antibacterial agents. Pyrazoles have been reported to possess antibacterial activity as they inhibited bacterial DNA gyrase and topoisomerase IV at their respective ATP-binding sites [Gomez *et al.* 2007; Bradbury *et al.*2008].

Bondock *et al.* (2011) in quest of finding new antimicrobial compounds, synthesized a series of 4-hetaryl pyrazoles and furo [2, 3-c] pyrazoles. Among the synthesized compounds, 1-(5-(5-hydroxy-3-methyl-1-phenyl-1H-pyrazole-4-yl)-2-methylfuran-3 -yl) ethanone **33** showed equal activity with chloramphenicol against *B. subtilis* (MIC 3.125 mg/mL), while its activity was 50% lower than of chloramphenicol against *B. thuringiensis*. In addition, N-[(4Z)-3-Methyl-1-phenyl-1H-furo[2,3-c] pyrazol-4(5H)-ylidene]- 1H-benzimidazol -2-amine **34** and 2-(5-hydroxy -3-methyl -1-phenyl-1H-pyrazol-4-yl)-4H-furo[3,2-c]chromen-4-one **35** were found to exhibit potent *in-vitro* antifungal activity with MIC (6.25 mg/mL) against *B. fabae* and *F. oxysporum* respectively [Bondock *et al*. 2011].

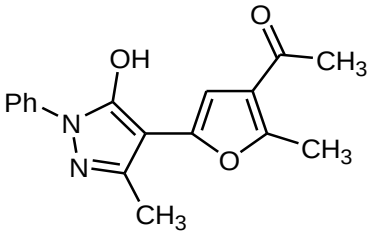
Rai *et al.* (2008) synthesized a series of novel pyrazoles namely 1-aryl-3-(5-nitro-2-thienyl)-4-aroyl-pyrazoles **36-38** and screened for their antibacterial and antifungal activities against a variety of microorganisms. 1, 3-Dipolar cycloaddition reaction of sydnones with 5-nitrothiophene containing acetylenic ketones has resulted in the formation of biologically active pyrazoles. Compounds **36**, **37** and **38** have exhibited higher activity against all the tested microorganisms (*S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *C. albicans*) compared to the standard. The higher potency of compounds may be attributed to the presence of chlorine in the aryl moiety [Rai *et al.* 2008].

Gouda *et al.* (2010) reported synthesis and antimicrobial activity of pyrazole derivative based on 4, 5, 6, 7-tetrahydrobenzothiophene moiety. Results of their antibacterial (against *Klebsiella pneumoniae* and *Bacillus theringiensis*) and antifungal (against *B. fabe* and *F. oxysporum*) activities signify the rational of incorporation of benzo-thiophene nucleus to pyrazole moieties. Compounds **39** (pyrazolo[5,1-c] [1,2,4] triazine), **40** (pyrolo pyrazole derivative) and **41** (pyrazole derivative) exhibited good antibacterial and antifungal activities as compared to reference chemotherapeutics [Gouda *et al*. 2010].

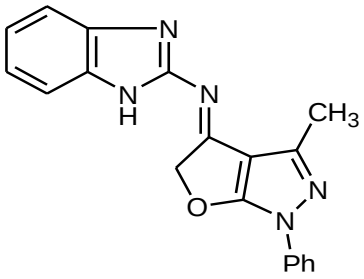
Gadakh *et al.* (2010) designed and synthesized a series of fluorine containing 4-(substituted-2-hydroxybenzoyl) pyrazoles and pyrazolyl benzoxazoles which exhibited promising antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus subtilis*. The compounds 1-(3,4-difluorophenyl)-4-(5-fluoro-2-hydroxybenzoyl)- 1H-pyrazole **42**, oxime derivatives such as1-(3,4-difluoro phenyl)-1H-pyrazol-4-yl) (2-hydroxy- 4-methyl phenyl) methanone oxime **43** and (5-

chloro-2-hydroxyphenyl) (1-(3,4-difluorophenyl) -1H-pyrazol-4-yl)methanone oxime **44** exhibited potential activities against tested bacterial strains [Gadakh *et al.* 2010].

Abdel *et al.* (2009) reported a new series of benzofuran derivatives, 1-(benzofuran-2-yl)-4-nitro-3-arylbutan-1-ones and 3-(benzofuran-2-yl)-4,5-dihydro-5-aryl-1-[4-(aryl) -1,3-thiazol-2-yl] -1H-pyrazoles **45-48** and screened for their antibacterial and antifungal activities at 100 µg concentration. In general, the inhibitory activity against the Gram-negative bacteria was higher than that of the Gram-positive bacteria.



33.



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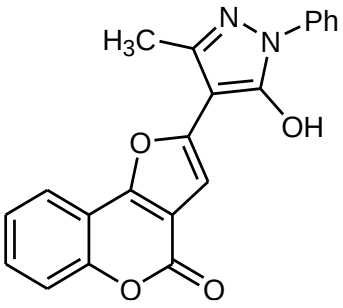

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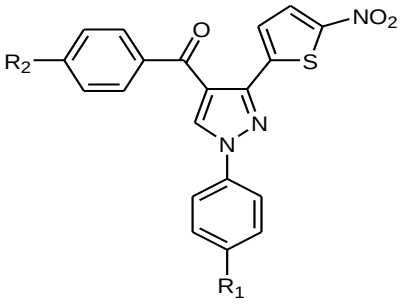
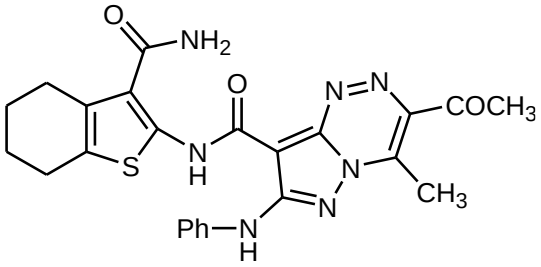


36. R_1=OCH_3 R_2=OCH_3

37. R_1=H R_2=Cl

38. R_1=OCH_3 R_2=Cl



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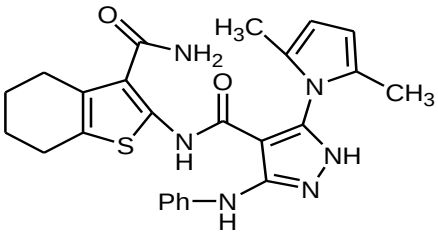

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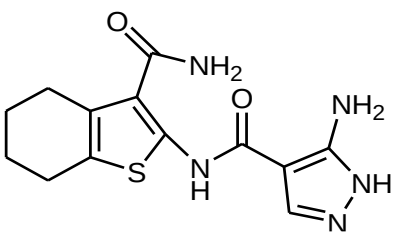
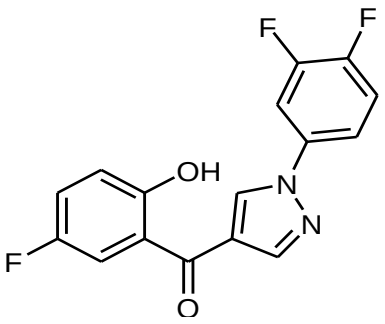


41.

Pyrazoles having antimicrobial action

The 1-(thiazol-2-yl) pyrazoline **45** showed excellent activity against Gram-negative bacteria (inhibitory zone 25 mm) and against Gram-positive bacteria (inhibitory zone 20 mm). Compounds **46**, **47** and **48** showed antifungal activities against *C. albicans* which was comparably better than the reference sample Flucanazol [Abdel *et al.* 2009].



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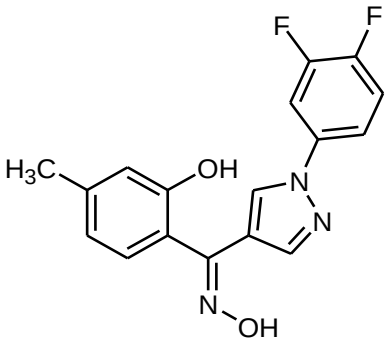

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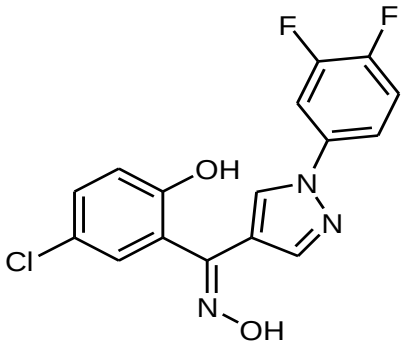

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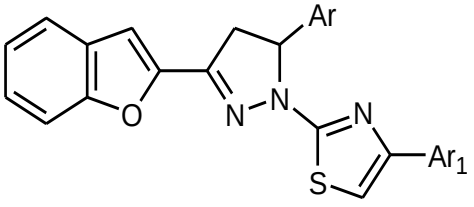
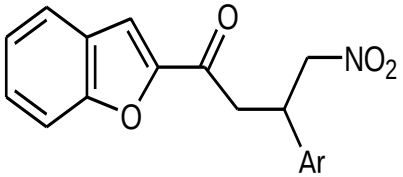


45. Ar = -C_6H_5 Ar_1 = -C_6H_5

46. Ar = 4-Cl-C_6H_4 Ar_1 = 4-Br-C_6H_4



47. Ar = -C_6H_5

48. Ar = 4-MeO-C_6H_4

Pyrazoles having antimicrobial action

Thumar *et al.* (2011) synthesized a new series of substituted 4-pyrazolyl-N-arylquinoline-2, 5-dione derivatives via multi component reaction approach. This synthetic strategy allows the construction of therapeutically active moieties, pyrazole and quinolin-2-one.

Compound **49** were found to have more efficient (MIC<100 µg/mL) antibacterial activity as compared with standard drug ampicillin. However, its antifungal activity were found to be equipotent to the standard griseofulvin (MIC = 500 µg/mL). Structural activity relationship (SAR) study revealed that compounds with *para*-fluorophenyl at 1-position of quinoline nucleus and with methyl substituted phenyl ring in pyrazole gave better results against *C. tetani*, *B. subtilis* and *E. coli*. Also, compound with *para*-methoxyphenyl substituent at the 1-position of the quinoline nucleus were found to be highly active against *C. Tetani* [Thumar e*t al.* 2011].

Boschi *et al.* (2011) synthesized new (cyano-NNO-azoxy) pyrazole derivatives by linking 1, 5-dimethyl-4-(cyano-NNO-azoxy)pyrazol-3-yl and 1,3-dimethyl-4-(cyano-NNO-azoxy)pyrazol-5-yl moieties to various heterocyclic ring viz. pyridine, pyrazole, isoxazole, thiophene and the furan. All compounds displayed interesting antifungal activity. The most active compound of the series being the thiophene derivative **50**, has shown remarkable activity against *Candida krusei* and *Candida glabrata* (MIC = 0.25 and 0.5 µg/mL, respectively), two fungal species resistant to azoles. The presence of the azoxycyano function in these products appeared to be essential to their activity [Boschi *et al.* 2011].

Vijesh *et al.* (2011) synthesized and characterized two series of novel imidazole derivatives containing substituted pyrazole moiety. Among the screened samples, compound **51** has shown excellent antimicrobial activity at 1 and 0.5 mg/mL concentrations against tested microbial strains as compared to the standard drug streptomycin. Imidazole and pyrazole nucleus which is present in both the series are responsible for biological activity. Thioanisyl moiety of this compound is accounted for enhanced antibacterial activity. The acute oral toxicity study for the compound **52** revealed that it is safe up to 3000 mg/kg and no death of animals were recorded [Vijesh *et al.* 2011].

Chandrakantha et al. (2011) synthesized a novel series of ethyl-1-(N-substituted) 5-phenyl-1H-pyrazole-4 carboxylate derivatives and screened for their antibacterial activity by serial dilution (MIC) method. After screened for their antibacterial properties against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, compounds, **53**, **54**, **55** and **56** were found to exhibit excellent antibacterial activity against all the tested bacterial strains as compared to the standard drug Ceftriaxone. Relationship between structure of the heterocyclic scaffold and the observed antibacterial properties showed that the presence of halogen substituent on compounds lead to enhance

antibacterial activity [Chandrakantha *et al.* 2011].

Sharma *et al.* (2011) synthesized some novel 1,3-diaryl-4-functionalized pyrazoles bearing benzene sulfonamide moiety at position-1 and an aldehyde, carboxylic acid, cyano and carbothioamide functionality at position-4 and evaluated for their *in vitro* antibacterial activity against four pathogenic bacterial strains namely, *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative), and *in vitro* antifungal activity against two pathogenic fungal strains namely, *Aspergillus niger* and *Aspergillus flavus*. Out of the tested compounds, carboxylic acid and carbothioamide containing **57**, **59** and **60** exhibited moderate antibacterial activity against Gram-positive bacteria and **58** showed moderate antifungal activity against the tested fungi [Sharma *et al*. 2011].

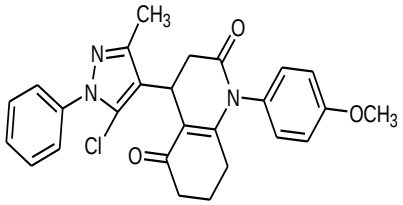
Kanagarajan *et al.* (2011) reported synthesis of novel 1,1'-(5,5'-(1,4-phenylene) bis (3-aryl-1H-pyrazole-5,1-(4H,5H)-diyl)) diethanones by clean, efficient and economical method using ultrasound irradiations. *In vitro* antibacterial and antifungal activities profile revealed that halogen substituted (X = F, Cl, Br) aromatics are more active than non-substituted aromatic ring system. Among all the tested compounds, electron withdrawing group substituted compounds **61**, **62** and **63** exerted moderate antimicrobial activities and the range of MIC values of **61-63** are 200-6.25 μg/mL. Moreover, all the tested compounds revealed promising antitubercular activity against *Mycobacterium tuberculosis* H37Rv and INH-resistant *M. tuberculosis*. Among them, compound 61 against *M. tuberculosis* and compound 63 against INH-resistant *M. tuberculosis* exhibited the percentage of reduction in relative light units (RLU) at 89 and 85%, respectively [Kanagarajan *et al*. 2011].

Bugaev *et al* (2005) & Gokhan-Kelekci *et al* (2009), reported that compounds containing hexahydroindazole are biologically active and have been used as potent antimicrobial, anti-inflammatory, depressant of central nervous system and additionally some are also found to be monoamine oxidase inhibitors [Bugaev *et al.* 2005 & Gokhan-Kelekci *et al*. 2009].

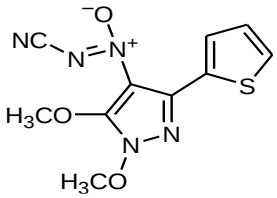
With regard to their antibacterial potency **Golikov *et al* (2005)** reported synthesis of furfurlidene containing hexahydroindazoles with different pharmacophore fragments (furan and pyrazoline cycles, nitro-, azomethine, and other groups). They observed that nitrofuran cycle linked via avinilydene unit to the pyrazoline ring containing azomethine group was most potent active against gram positive bacteria. While other substituents on hexahydroindazole (replacement of nitro group in furan cycle by a methyl group or

hydrogen) resulted in diminished activity against the entire tested organism [Golikov *et al*. 2005].

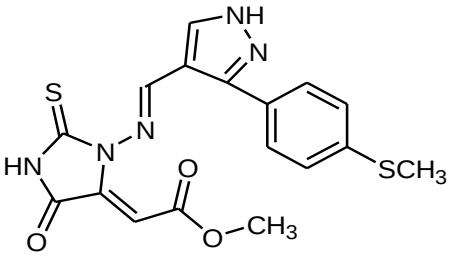
Minu *et al* (2009) demonstrated synthesis and antimicrobial activity of 2, 3-disubstituted-3,3a,4,5,6,7-hexahydro-2H-indazole derivatives. They observed that electron withdrawing group was generally more active than other derivatives.



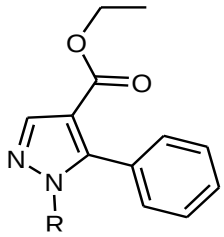
49.



50.



51.

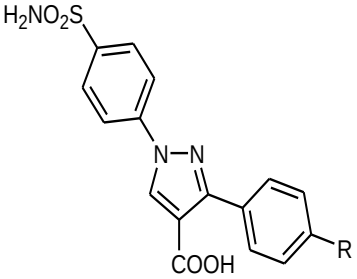


52. R= 4-CF_3-C_6H_5

53. R= 2-Br-C_6H_5

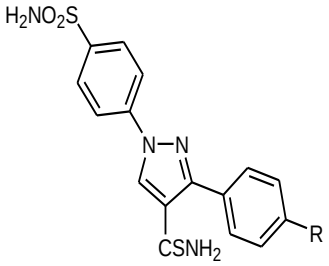
54. R= 2, 4-diCl-C_6H_5

55. R= 4-$C_5H_{10}N$



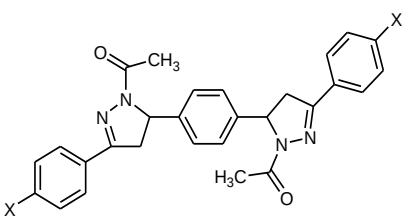
56. R = Cl

57. R = NO_2



58. R = CH_3

59. R = Br



60. X=F **61.** X = Cl **62.** X = Br **63.** X = CH_3

Pyrazoles having antimicrobial action

And among the electron withdrawing halo group, presence of *para*-flurophenyl group at 3rd position of hexahydroindazole improved the antibacterial activity. Whereas the presence of *para*-chlorophenyl group at 3rd position of hexahydroindazole improved the antifungal activity [Minu *et al.* 2009].

2.5 Glucosamine-6-phosphate synthase: a novel target for antimicrobial agents

Wojciechowski *et al*. (2005) has reported L-Glutamine: D-fructose-6-phosphate amidotransferase, known under trivial name of glucosamine-6-phosphate synthase, as the only member of the amidotransferase subfamily of enzymes, does not display any ammonia-dependent activity. This enzyme, catalysing the first committed step in a pathway leading to the eventual formation of uridine 5'-diphospho-N-acetyl-D-glucosamine (UDP-GlcNAc), is an important point of metabolic control in biosynthesis of amino sugar-containing macromolecules. The molecular mechanism of reaction catalysed by GlcN-6-P synthase is complex and involves both amino transfer and sugar isomerisation. Substantial alterations to the enzyme structure and properties have been detected in different neoplastic tissues. GlcN-6-P synthase is inflicted in phenomenon of hexosamine-induced insulin resistance in diabetes. Finally, this enzyme has been proposed as a promising target in antifungal chemotherapy. Glucosamine-6-phosphate synthase (GlmS) catalyzes the formation of D-glucosamine 6-phosphate from D-fructose 6-phosphate using L-glutamine as the ammonia source. Because N-acetylglucosamine is an essential building block of both bacterial cell walls and fungal cell wall chitin, the enzyme is a potential target for antibacterial and antifungal agents. The most potent carbohydrate-based inhibitor of GlmS reported to date is 2-amino-2-deoxy-D-glucitol 6-phosphate, an analogue of the putative cis-enolamine intermediate formed during catalysis [Wojciechowski *et al*. 2005]

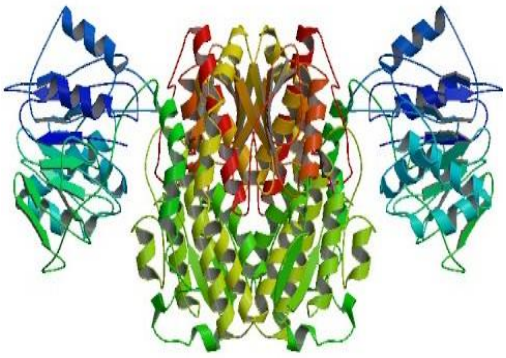


Fig 2.1. Biological Assembly Image for Glucosamine 6-phosphate synthase with Glucose 6-phosphate (1JXA)

2.6 Cucrumin and MMP:

Curcumin the natural yellow pigment in turmeric has become focus of interest with regard to its role in regulation of MMPs. MMPs are metal-dependant endopeptidases capable of degrading any one of the components of the extracellular matrix. In normal physiological conditions it is regulated by tissue inhibitors of metalloproteinases and any alteration in this regulatory process leads to pathological conditions. Curcumin controls regulation and production of MMP-2, MMP-9 and MMP-1 in various types of cancer, arthiritis, ulcer and other diseases.

Shin *et al.* (2010) reported that curcumin inhibits the TPA-induced MMP-9 expression and cell invasion through the suppression of the PKCα/MAPK/NF-kB/AP-1 pathway in MCF-7 breast cancer cells. Accordingly, curcumin may have the therapeutic potential in restricting breast cancer metastasis [Shin *et al.* 2010].

Swarnakar *et al.* (2009) demonstrated that curcumin reduced the MMP-9 activity and increased the total MMP-2 (both pro and active forms) activities, dose dependently, in indomethacin induced gastric model. Intriguingly, only a 1.5 times higher dose (60mg/kg) was required to treat ulcer through oral route when compared to the intraperitoneal route (40mg/kg) thus concluding that antiulcer activity of curcumin is primarily attributed to MMP-9 inhibition, one of the major pathways of ulcer healing [Swarnakar *et al.* 2009].

Kundu *et al.* (2011) reported that Curcumin dose dependently suppressed MMP-3 and -9 expression in *Helicobacter pylori* (Hp) infected human gastric epithelial (AGS) cells. MMP-3 and -9 are inflammatory molecules associated to the pathogenesis of Hp-infection and this action was linked to decreased pro-inflammatory molecules and activator protein-1 activation in Hp-infected gastric tissues. Conventional triple therapy of Hp-infected gastric tissues was found to be less efficient than curcumin in restoring the altered balance between MMPs and TIMPs in gastric mucosa during protection of Hp-infection [Kundu *et al.* 2011].

Zhao *et al*. (2010) reported that curcumin by Inhibiting expression and activity of MMP-9 and MMP-2 which may be one of its protective mechanisms of cerebral ischemic/reperfusion injury in rats through inhibiting the inflammatory reaction and improving BBB integrity, which might be associated with inhibition of TNF-α and MMP-9 [Zhao *et al.* 2010].

Claramunt *et al.* (2009) evaluated N-unsubstituted curcuminoid pyrazoles by using human intestinal epithelial cells *in vitro* for regulating the activity of MMPs, showed significant down-regulation of MMP-9 activity on inflammation-induced intestinal

epithelial cells, making them probable candidate for the treatment of inflammatory bowel disease [Claramunt *et al.* 2009].

Zhao *et al.* (2010) reported that curcumin has anti-inflammatory properties and may prevent the migration of human aortic smooth muscle cells HASMCs by suppressing MMP-9 expression through suppression of production of ROS and the nuclear translocation of NF-κB p50 and p65 induced by TNF-α , thus can restrict the development of atherosclerosis [Zhao *et al.* 2010].