DEVELOPMENT OF A CURCUMIN RESOURCE DATABASE AND IN-SILICO INTERACTION STUDIES WITH SELECTED TARGETS

by

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DECLARATION

It is to declare that the matter embodied in this thesis entitled "***Development of a Curcumin Resource Database and In-Silico Interaction Studies with selected Targets***" is the result of investigations carried out by me under the supervision of **Dr. Utpal Bora**, and is submitted to the Indian Institute of Technology Guwahati (Guwahati, India) for the award of degree of *Doctor of Philosophy in Biotechnology*. This work has not been submitted elsewhere for any degree or diploma of any institute or university to the best of my knowledge and belief.

In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work of other investigators are referred, and copyright licenses have been taken from respective publisher.

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Utpal Bora

June 2013 (Supervisor)

DEDICATION

Dedicated to
My Parents, Brother, Sister
and Teachers

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June, 2013 *Anil Kumar*

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SYNOPSIS

Turmeric has been used for thousands of years as a traditional medicine, coloring agent and spice in Asian countries. The major curcuminoids found in turmeric are curcumin (77%), demethoxycurcumin (17%) and bisdemethoxycurcumin (3%). The first chemical characterization of curcumin was done in 1910. It exists in keto and enol forms and regarded as the most active constituent of turmeric. Curcumin has been described in hundreds of published papers over the past few decades, studying its antioxidant, anti-inflammatory, cancer chemo-preventive and chemotherapeutic properties. It has been reported to interact with huge number of molecular targets like signaling molecules, growth factors, transcription factors, receptors, pro-inflammatory enzymes, protein kinases and adhesion molecules. In the pursuance of enhanced pharmacological properties, researchers have developed many synthetic curcumin derivatives by adding various functional groups in aromatic rings and linker region of the curcumin molecule to improve the binding of these derivatives to targets through polar interactions.

In the recent years it has been found in the *in-vitro* studies that curcumin can directly interfere in the binding of some transcription factors (NF-κB, Stat3 and AP1) to the DNA. It was also found to have DNA topoisomerase I and II inhibition activity in *in-vitro* studies. To understand the interaction of curcumin derivatives with these selected targets we fixed the following as the objectives of the present research work:

- Development of Curcumin Resource Database (CRDB): a database of curcumin analogs, their molecular targets and patents.
- Computational studies on interaction of curcumin derivatives with transcription factors [NF-κB, Stat3 and AP1 (Jun-Fos)].

- ❖ Computational studies on interaction of curcumin derivatives with topoisomerase I and II-DNA complexes.

Overall the thesis is divided into seven chapters as described below. The results obtained are presented in five chapters (2-6). These chapters are preceded by **Chapter One** which gives a brief introduction of curcumin, its disease and molecular targets. A review of curcumin analogs and their interactions to various molecular targets have been discussed with a focus on therapeutic applications.

Chapter 2 describes development of CRDB a database of curcumin analogs, their molecular targets and patents. Curcumin and its analogs exhibit wide range of pharmacological activities including antimicrobial, antioxidant, anti-inflammatory and anti-cancer activities. However, no database is available with comprehensive information of curcumin analogs and their molecular targets and patents till date. To address this issue, we developed CRDB an integrated and curated repository of curcumin analogs their molecular targets and patents. Currently, the database has 1186 curcumin analogs with their 196 molecular targets and information of 490 international and national patent documents curated from public domain databases and published literature in peer reviewed journals. Database can be either browsed through lists of curcumin analogs, molecular targets and patents or it can be searched by keywords. Provisions have been made for regular updation of the database. Freely available CRDB web portal contains user-friendly interfaces and is expected to be highly useful to the researchers working on structure/ligand based molecular design of curcumin analogs for therapeutic applications. The database is available on the web at http://www.iitg.ac.in/ubora/crdb.

Chapter 3 describes *in-silico* inhibition studies of NF-κB p50 subunit by curcumin and its natural derivatives. Nuclear factor-kappa B (NF-κB) is an important transcription factor, involved in many immune, inflammatory and apoptotic responses. Preventing its binding to DNA is a rational strategy that could be translated to potential therapeutic applications. In the present work the interference of curcumin and its derivatives in the binding of NF-κB to DNA has been explored by *in-silico* studies. Curcumin and its derivatives were docked on the p50 subunit of NF-κB and it was found that most of the compounds formed polar interactions with Lys144 and Lys241 which are the key residues involved in binding of NF-κB with DNA at the consensus sequence (κB site) through hydrogen bonding. Molecular docking studies and ADME predictions showed that curcumin sulphate can be a potent inhibitor of NF-κB p50 subunit amongst the natural curcumin derivatives and known inhibitors (aurine tricarboxylic acid, gallic acid and ellagic acid) docked.

Chapter 4 describes molecular docking studies on inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids. Stat3 is a mammalian transcription factor which regulates various genes involved in cell growth, proliferation, cell survival and other biological processes. Its constitutive activation promotes dysregulated growth, survival and immune responses which contribute to tumor progression and carcinogenesis. Inhibition of Stat3 dimerization which prevents its binding to DNA is a rational strategy that could be translated to potential therapeutic applications. The present computational study provides insights into the inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids. The involvement of key residues like Lys591, Arg609, Ser611, Glu612, Ser613, Ser636 and Val637 seems to play an important role in binding of curcumin natural derivatives and its amino acids conjugates with Src Homology (SH2) domain of Stat3 monomer.

Demethoxycurcumin, hexahydrocurcuminol followed by hexahydrocurcumin were predicted to be the most potent inhibitors amongst all the curcumin natural derivatives and known inhibitors (FLLL32, Sta21 and Stattic). Curcumin-proline conjugate (1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) was predicted to be the most potent inhibitor of Stat3 dimerization amongst the curcumin-amino acid conjugates and known peptide based inhibitor (Phpr-pTYR-LEU-cis-3,4-methanoPRO-GLN-NHBn).

Chapter 5 describes *in-silico* inhibition studies of Jun-Fos-DNA complex formation by curcumin derivatives. Activator protein-1 (AP1) is a transcription factor consists of the Jun and Fos family proteins. It regulates gene expression in response to a variety of stimuli and controls cellular processes including proliferation, transformation, inflammation and innate immune responses. AP1 binds specifically to 12-O-tetradecanoylphorbol-13-acetate (TPA) responsive element 5'-TGAG/CTCA-3' (AP1 site). It has been found constitutively active in breast, ovarian, cervical and lung cancers. Numerous studies have shown that inhibition of AP1 could be a promising strategy for cancer therapeutic applications. The present *in-silico* study provides insights into the inhibition of Jun-Fos-DNA complex formation by curcumin derivatives. These derivatives interact with the amino acid residues like Arg155 and Arg158 which play a key role in binding of Jun-Fos complex to DNA (AP1 site). Ala151, Ala275, Leu283 and Ile286 were the residues present at binding site which could contribute to hydrophobic contacts with inhibitor molecules. Curcumin sulphate was predicted to be the most potent inhibitor amongst all the natural curcumin derivatives docked.

Chapter 6 describes molecular docking studies of curcumin natural derivatives with DNA topoisomerase I and II-DNA complexes. DNA topoisomerase I (topo I) and II (topo

II) are essential enzymes that solve the topological problems of DNA by allowing DNA strands or double helices to pass through each other during cellular processes such as replication, transcription, recombination, and chromatin remodeling. Their critical roles make topoisomerases an attractive drug target against cancer. The present molecular docking study provides insights into the inhibition of topo I and II by curcumin natural derivatives. The binding modes suggested that curcumin natural derivatives docked at the site of DNA cleavage parallel to the axis of DNA base pairing. Cyclocurcumin and curcumin sulphate were predicted to be the most potent inhibitors amongst all the curcumin natural derivatives docked. The binding modes of cyclocurcumin and curcumin sulphate were similar to known inhibitors of topo I and II. Residues like Arg364, Asn722 and base A113 (when docked to topo I-DNA complex) and residues Asp479, Gln778 and base T9 (when docked to topo II-DNA complex) seem to play important role in the binding of curcumin natural derivatives at the site of DNA cleavage.

Chapter Seven presents the overall summary of the investigations, and the scope for further studies.

The work presented in the thesis has been peer reviewed and resulted in the following international journal publications:

Kumar A and Bora U (2013) Molecular docking studies of curcumin natural derivatives with DNA topoisomerase I and II-DNA complexes. *Interdisciplinary Sciences: Computational Life Sciences* ***(Accepted).*** - *Research Article*

Kumar A and Bora U (2013) Interactions of curcumin derivatives and its metal complexes with nucleic acids and their implications. *Mini Reviews in Medicinal Chemistry* 13(2):256-64. - *Review Article*

Kumar A and Bora U (2012) In silico inhibition studies of Jun-Fos-DNA complex formation by curcumin derivatives. *International Journal of Medicinal Chemistry* vol. 2012, Article ID 316972, 8 pages, 2012. doi:10.1155/2012/316972. - *Research Article*

Kumar A and Bora U (2012) Molecular docking studies on inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids. *Bioinformation* 8(20):988-993. - *Research Article*

Kumar A and Bora U (2012) In silico inhibition studies of NF-κB p50 subunit by curcumin and its natural derivatives. *Medicinal Chemistry Research* 21(10):3281-3287. - *Research Article*

Manuscripts in communication

Kumar A and Bora U (2013) CRDB: a database of curcumin derivatives their molecular targets and patents (Communicated) - *Research Article*

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ABBREVIATIONS

AGP	:	α1-acid glycoprotein
ALR2	:	Aldose reductase 2
AP-1	:	Activator protein 1
Bcl-2	:	B-cell lymphoma-2
Bcl-xl	:	B-cell lymphoma-extra-large
b-LG	:	b-lactoglobulin
BSA	:	Bovine serum albumin
CBP	:	CREB-binding protein
CCDS	:	Consensus CDS
CHIP	:	Carboxyl terminus of Hsc70- interacting protein
CMs	:	Casein micelles
COX	:	Cyclooxygenase
dGMP	:	Deoxyguanosine monophosphate
DNMT1	:	DNA methyltransferase1
EGFR	:	Epidermal growth factor receptor
EGFR	:	Epidermal growth factor receptor
EGR-1	:	Early growth response protein-1
ELAM-1	:	endothelial-leukocyte adhesion molecule 1
G6PDH	:	Glucose-6-phosphate dehydrogenase
GLOI	:	Glyoxalase I
GPx	:	Glutathione peroxidase
GR	:	Glutathione reductase
GSK-3β	:	Glycogen synthase kinase-3β
GST	:	Glutathione S-transferase
HAT	:	Histone acetyltransferases
HDAC	:	Histone deacetylases
HER2	:	Human Epidermal Growth Factor Receptor 2 kinase
HER-2	:	Human Epidermal Growth Factor Receptor-2

HGNC	:	HUGO Gene Nomenclature Committee
HIV	:	Human immunodeficiency virus
HPRD	:	Human Protein Reference Database
HSA	:	Human serum albumin
ICAM-1	:	Intercellular Adhesion Molecule 1
Ig	:	Immunoglobulin
IKK	:	IκB kinase
IL	:	Interleukin
JAK2	:	Janus kinase-2
JNK	:	c-Jun N-terminal kinase
MD-2	:	Myeloid differentiation protein 2
MDR	:	Multiple drug resistance
MMPs	:	Matrix metalloproteinases
NF-κB	:	Nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf-2	:	Nuclearfactor-E2 p45-related factor-2
OMIM	:	Online Mendelian Inheritance in Man
P-12-LOX	:	Platelet-type 12-lipoxygenase
PDB	:	Protein Data Bank
PKA	:	Protein kinase A
PKC	:	Protein kinase C
PPAR-γ	:	Peroxisome proliferator-activated receptor gamma
QR	:	Quinone reductase
STAT-1	:	Signal Transducers and Activators of Transcription-1
STAT-3	:	Signal Transducers and Activators of Transcription-3
TF	:	Tissue factor
TNF	:	Tumor necrosis factor
Topo I	:	Topoisomerase I
Topo II	:	Topoisomerase II
TrxR	:	Thioredoxin reductase
TYK2	:	Tyrosine kinase 2
uPA	:	Urokinase-type Plasminogen Activator

VCAM-1	:	Vascular cell adhesion protein 1
v-Src	:	Viral sarcoma
XO	:	Xanthine oxidase
α-GST	:	α-glutathione s-transferase

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CHAPTER 1

Introduction & Review of Literature

1.1 INTRODUCTION

Curcumin (diferuloylmethane) is a hydrophobic polyphenol derived from the spice turmeric (*Curcuma longa*). It has been used for thousands of years as a traditional medicine, coloring agent and spice in Asian countries (Goel *et al.*, 2008; Anand *et al.*, 2008a). Curcumin molecule has been shown to exhibit a wide range of pharmacological activities including antimicrobial, antioxidant, free radical-scavenging, anti-inflammatory, and anti-cancer activities (Srimal and Dhawan, 1973; Sharma, 1976; Satoskar *et al.*, 1986; Ammon and Wahl, 1991; Rao *et al.*, 1995; Jagetia and Aggarwal, 2007; Shishodia *et al.*, 2007; Anand *et al.*, 2008b; Barzegar and Moosavi-Movahedi, 2011). It has been found safe even at very high doses in various animal models and human (Goel *et al.*, 2008).

The turmeric plant is a perennial herb which belongs to the ginger family (Zingiberaceae), which is native to tropical South Asia. The rhizome is the most useful part of the plant which contains important pigments. The medicinal properties of turmeric have been known to ancient Indians as well as Chinese people. Since the time of Ayurveda (1900 BC) numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions (Aggarwal *et al.*, 2007). It has been used in poultices applied locally to relieve pain. It has also been used in the treatment of conditions such as jaundice, hemorrhage, toothache, flatulence, etc (Aggarwal *et al.*, 2007).

The rhizome is rich in curcuminoids. The major curcuminoids found in turmeric are curcumin (77%), demethoxycurcumin (17%) and bisdemethoxycurcumin (3%) (Huang *et al.*, 1995). Curcumin is the most biologically active curcuminoid of turmeric and makes up 2% to 5% of the spice. The characteristic yellow color of turmeric is due to curcumin. It was first isolated in 1815 by Vogel and Pelletier, obtained in crystalline form

in 1870 (Aggarwal *et al.,* 2007; Zhou *et al.*, 2011). The first chemical characterization of curcumin was done by Lampe and Milobedezka in 1910 (Sharma *et al.*, 2005; Shehzad and Lee, 2010). It exists in keto (**1**) and enol (**2**) forms (**Figure 1.1**) and regarded as the most active constituent of turmeric (Maheshwari *et al.*, 2006). Curcumin has been described in hundreds of published papers over the past few decades, studying its biological activities (Jayaprakasha *et al.*, 2005).

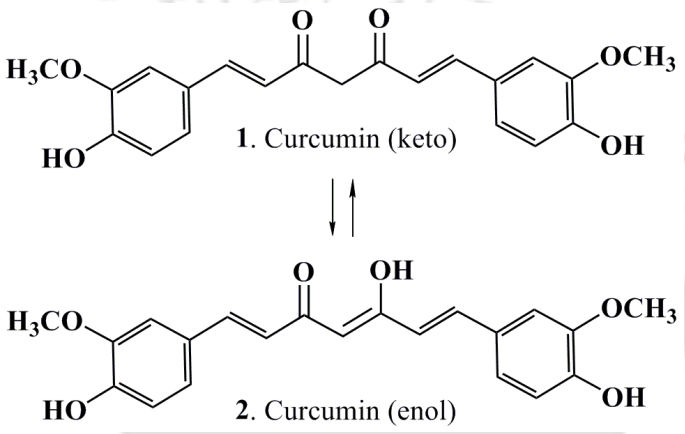

Figure 1.1 *Chemical structures of keto and enol forms of curcumin.*

It is only in recent years that scientists have recognized the medicinal value of turmeric. Curcumin, turmeric's yellow pigment, has shown significant anti-inflammatory activity in several experiments. Recent studies have demonstrated that curcumin has antioxidant properties and bolsters the human immune system. Turmeric is also effective in reducing joint inflammation in arthritis and rheumatism. Curcumin-based creams are commercially available for treating psoriasis. Currently, research is focused on the ability of this molecule to slow down the spread of cancer, inhibition of tumor growth, prevention of heart disease and to combat with degenerative neurological diseases.

It is important to develop our understanding of the mechanisms of action of curcumin at cellular and genetic levels, its targeted delivery to target cells and tissues, the enzymes involved in its degradation and metabolism, and the safety of using it in larger doses so that we can identify its wider therapeutic applications.

1.2 CURCUMIN ANALOGS

Curcumin analogs can be broadly divided into two categories natural analogs and metabolites made by Mother Nature and synthetic analogs made by man using different modification schemes. Turmeric itself contains three important analogs curcumin, demethoxycurcumin and bisdemethoxycurcumin, amongst which curcumin is the most abundant (**Figure 1.2**). These three compounds differ in methoxy substitution on the aromatic ring. While curcumin has two symmetric o-methoxy phenols linked through the α,β-unsaturated β-diketone moiety, bisdemethoxycurcumin is also symmetric but deficient in two o-methoxy substitutions, and demethoxycurcumin has an asymmetric structure with one of the phenyl rings having o-methoxy substitution. Commercially available curcumin mixture contains 77% curcumin, 17% demethoxycurcumin, and 3% bisdemethoxycurcumin. Some lesser known curcuminoids from turmeric are α-turmerone and β-turmerone and cyclocurcumin. Structurally, cyclocurcumin differs from curcumin in the β - diketone link. In this molecule, the α,β -unsaturated β-diketone moiety of curcumin is replaced by an α,β-unsaturated dihydropyranone moiety. Metabolites of curcumin reported are tetrahydrocurcumin, hexahydrocurcumin, curcumin glucuronide and curcumin sulfate.

The basic skeleton of curcuminoids is 1,7-Diarylheptane which have two phenolic groups (at 4,4'-positions on aromatic rings), two methoxy groups (at 3,3′-positions on

aromatic rings), Two double bonds in the 7-C chain (linker), β–Diketone and active methylene group at C-4 position. The double bond and β–Diketone together account for yellow color of curcumin.

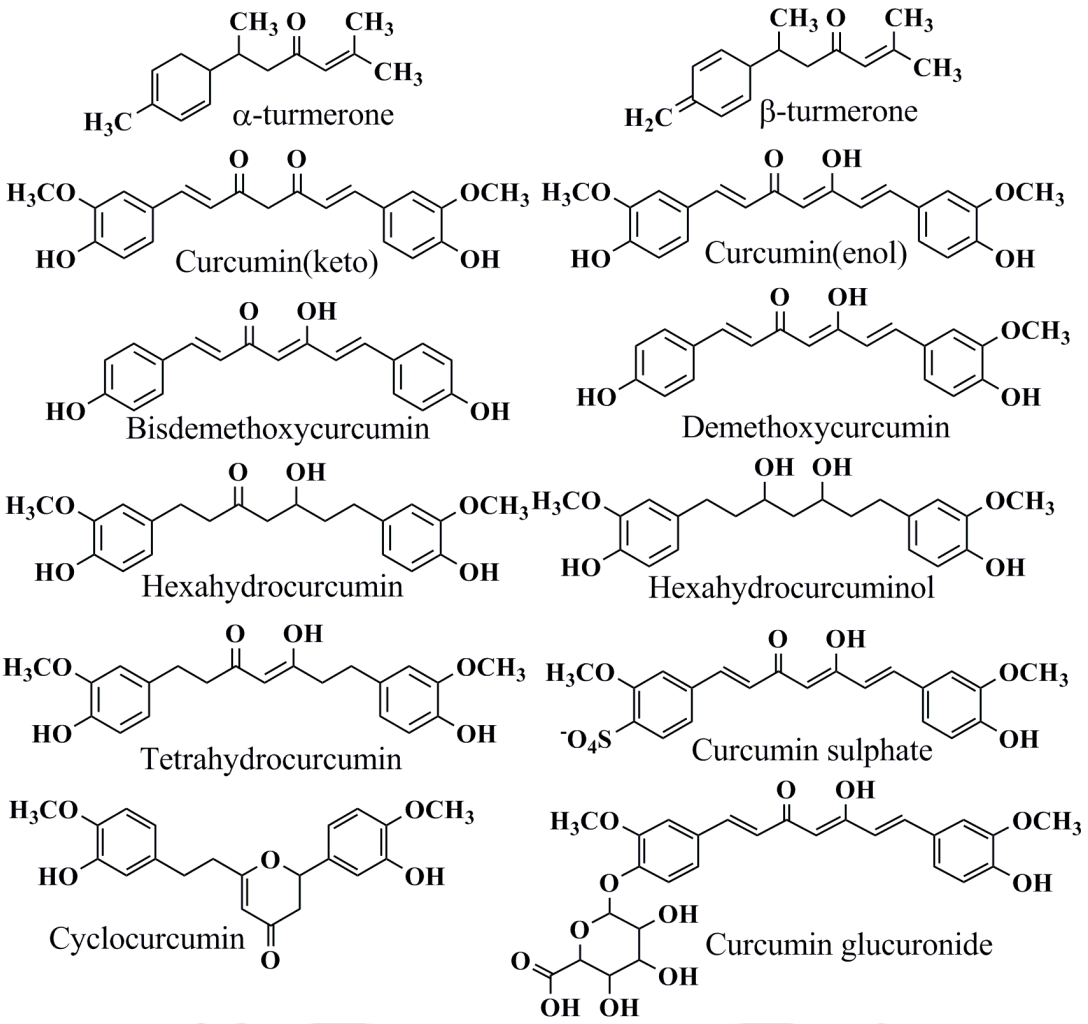

Figure 1.2 *Curcumin natural analogs*

In the pursuance to improve the pharmacological properties, curcumin was conjugated with various functional groups (Anand *et al.*, 2008b). Several researchers synthesized curcumin-amino acids conjugates using different substitution schemes which were tested for various biological activities including antioxidant, antimicrobial, antiviral,

antiproliferative and proteasome inhibition activities (Singh *et al.,* 2010; Dubey *et al.,* 2008; Parvathy *et al.,* 2010; Rai *et al.,* 2008; Wan *et al.,* 2010).

1.3 DISEASE TARGETS OF CURCUMIN

Ayurveda describe the use of curcumin in various inflammatory diseases including sprains and swellings caused by injury, wound healing, and abdominal problems. Texts on traditional chinease medicine also describe the use of curcumin for the treatment of abdominal pain and inflammatory conditions. Perhaps most of the effects associated with curcumin are based on its ability to suppress inflammation. Curcumin has been shown to be effective in a number of diseases. It has been taken as a dietary spice at doses up to 100 mg/day for centuries. Extensive investigations over the last few decades has indicated that curcumin reduces blood cholesterol (Rao *et al.,* 1970); prevents low-density lipoprotein (LDL) oxidation (Naidu and Thippeswami, 2002); inhibits platelet aggregation (Srivastava *et al.,* 1986); suppresses thrombosis (Srivastava *et al.,* 1985); suppresses symptoms associated with type 2 diabetes (Arun and Nalini, 2002), rheumatoid arthritis (Deodhar *et al.,* 1980), multiple sclerosis (Natarajan and Bright, 2002) and Alzheimer's disease (Lim *et al.,* 2001); inhibits human immunodeficiency virus (HIV) replication (Barthelemy *et al.,* 1998); enhances wound healing (Phan *et al.,* 2001); protects from liver injury (Morikawa *et al.,* 2002); protects from fibrosis (Punithavathi *et al.,* 2000); has anti-leishmaniasis (Saleheen *et al.,* 2002), anti-atherosclerotic (Chen and Huang, 1998) and anti-carcinogenic activities. Literature also suggests that curcumin has potential in the prevention and treatment of a variety of other diseases which are shown in **Figure 1.3**.

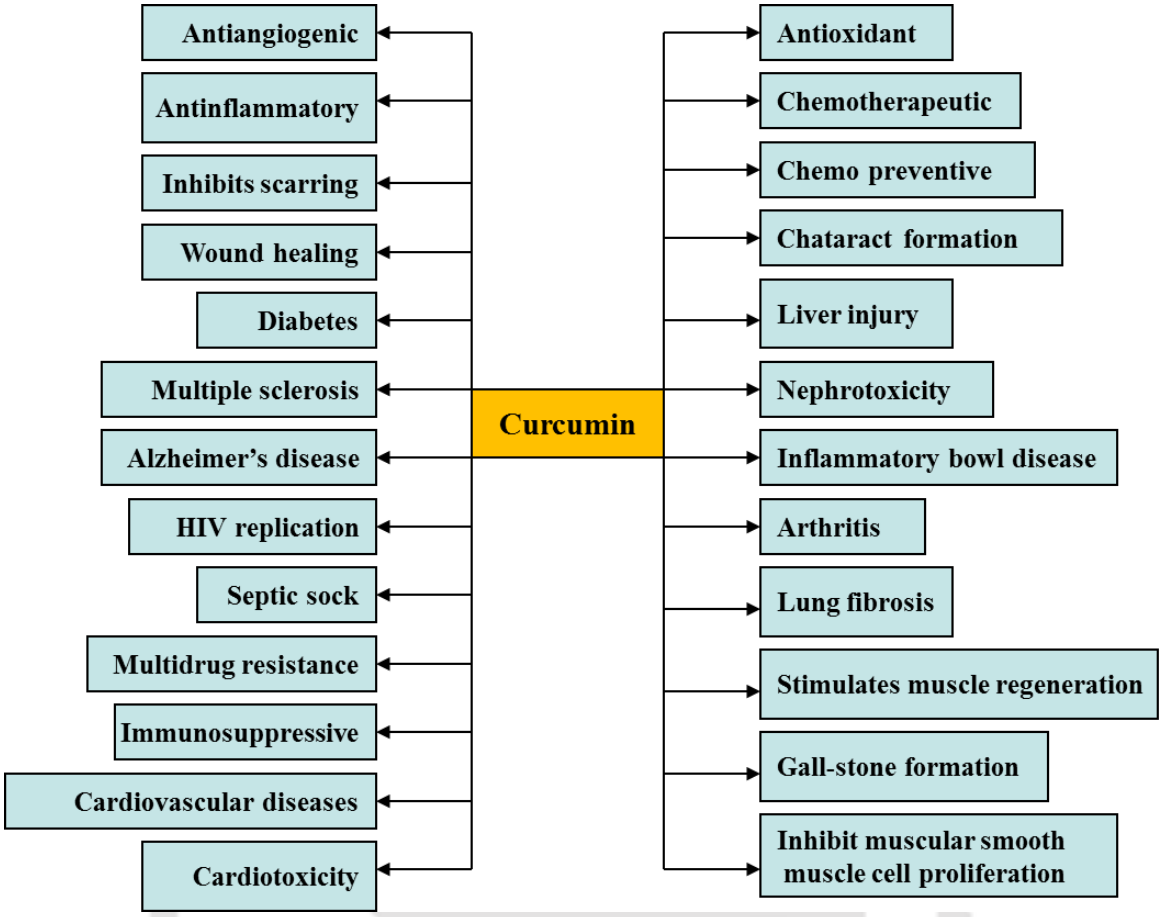

Figure 1.3 *Disease targets of curcumin (Aggarwal et al., 2007)*

1.4 MOLECULAR TARGETS OF CURCUMIN

Curcumin has been found to modulate various molecular targets including the growth factors, growth factor receptors, transcription factors, cytokines, enzymes, and genes regulating apoptosis as shown in **Figure 1.4**. Recent studies have revealed the direct interaction of curcumin with several molecular targets which are discussed in the following sections as well.

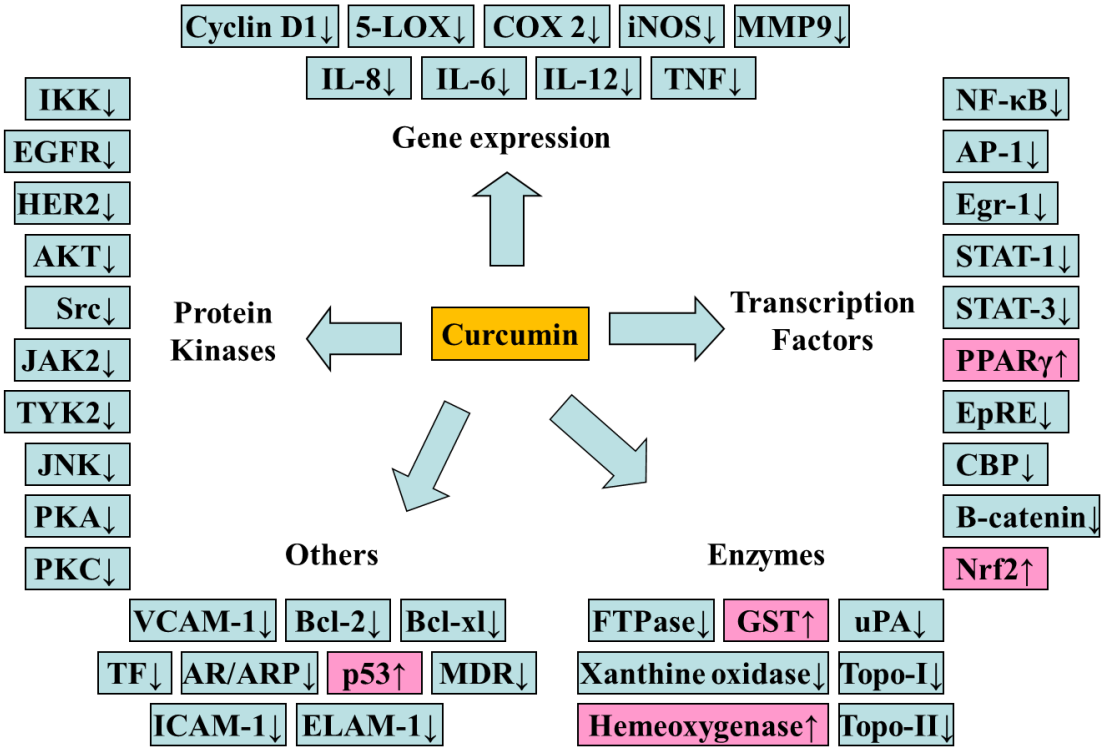

Figure 1.4 *Molecular targets of curcumin (Abbreviations: nuclear factor kappa-light-chain-enhancer of activated B cells (**NF-κB**), activator protein 1 (**AP-1**), early growth response protein-1 (**EGR-1**), signal Transducers and Activators of Transcription-1 (**STAT-1**), signal Transducers and Activators of Transcription-3 (**STAT-3**), peroxisome proliferator-activated receptor gamma (**PPAR-γ**), CREB-binding protein (**CBP**) nuclearfactor-E2 p45-related factor-2 (**Nrf-2**), IκB kinase (**IKK**), epidermal growth factor receptor (**EGFR**), human Epidermal Growth Factor Receptor-2 (**HER-2**), c-Jun N-terminal kinase (**JNK**), protein kinase A (**PKA**), protein kinase C (**PKC**), janus kinase-2 (**JAK2**), tyrosine kinase 2 (**TYK2**), vascular cell adhesion protein 1 (**VCAM-1**), tissue factor (**TF**) protein, multiple drug resistance (**MDR**), intercellular Adhesion Molecule 1 (**ICAM-1**), endothelial-leukocyte adhesion molecule 1 (**ELAM-1**), B-cell lymphoma-2 (**Bcl-2**), B-cell lymphoma-extra-large (**Bcl-xl**), glutathione S-transferase (**GST**), urokinase-type Plasminogen Activator (**uPA**), topoisomerase I (**topo-I**), topoisomerase II (**topo-II**)) (Aggarwal et al., 2007; Ravindram et al., 2007; Lin, 2007).*

1.4.1 Interaction with growth factors and cytokines

Various growth factors have been implicated in variety of human diseases, including Alzheimer's disease, cancer, major depression, inflammatory bowel disease, psoriasis, rheumatoid arthritis, systemic sclerosis, multiple sclerosis, and diabetes (Sethi *et al.,* 2009; Swardfager *et al.,* 2010; Locksley *et al.,* 2001; Dowlati *et al.,* 2010; Brynskov *et al.,* 2002). Curcumin has been reported to down-regulate expression of several cytokines, including tumor necrosis factor (TNF)-α, interleukin 6 (IL-6), IL-8, IL-12, and fibroblast growth factor 2 (Aggarwal *et al.,* 2005).

TNF-α, an essential component of the immune system, is produced by several type of cells including macrophages, CD4+ lymphocytes, NK cells and neurons. Wua *et al.* found that curcumin directly bind at the receptor binding sites of TNF-α through hydrogen bonds and hydrophobic interactions. Amino acid residues, such as Leu89, Asn90, Asp105, Asn106, and Cys129 play an important role in binding of curcumin to TNF-α. These studies suggested that curcumin may directly interrupt in the binding of TNF-α to its receptor which may suppress inflammation (Wua *et al.,* 2010).

1.4.2 Interaction with receptors

HER2/neu and epidermal growth factor receptor (EGFR) stimulate proliferation of breast cancer cells. Overexpression of these two proteins correlates with progression of human breast cancer. Curcumin has been shown to down-regulate the activity of EGFR and HER2/neu (Korutla *et al.,* 1995; Hong *et al.,* 1999). It has been also reported to have therapeutic potential against prostate cancer cells through down-regulation of androgen receptor (AR) and cAMP response element-binding protein (CBP)–binding protein (Nakamura *et al.,* 2002).

1.4.3 Interaction with transcription factors

Transcription factors play a critical role in all the cellular processes including cell proliferation, transformation, cell invasion, metastasis and angiogenesis. However, constitutively active transcription factors lead to cancers and several other human diseases (Garg and Aggarawal, 2002). Curcumin has been found to suppress constitutive expression of various transcription factors, including NF-κB, Stat3, AP-1 and beta-catenin which were implicated in cancers and other inflammatory diseases (Angel and Karin, 1991; Beg and Baldwin, 1993; Baeuerle and Baltimore, 1996; Bowman *et al.*, 2000; Shaulian and Karin, 2002; Darnell, 2002; Bharti *et al.*, 2003a; Alexandrow *et al.*, 2011). Hahm *et al.* reported that curcumin directly interrupt in AP1 binding to DNA (Hahm *et al.*, 2002). Bill *et al.* developed a curcumin analog (FLLL32) which induces apoptosis in melanoma cells via Stat3 inhibition (Bill *et al.*, 2010). Curcumin was dramatically reported to induce the expression of peroxisome proliferators–associated receptor gamma *(PPAR-γ)* gene and activated *PPAR-γ,* which inhibits the proliferation of nonadipocytes (Xu *et al.*, 2003).

1.4.4 Interaction with enzymes

Enzymes are the biological molecules responsible for the numerous chemical inter-conversions that sustain life. Curcumin has been reported to interact with several enzymes which are discussed in the following sections (Gupta *et al.*, 20011).

1.4.4.1 Histone acetyltransferases (HATs)

HATs such as p300/CBP, play an essential role in the epigenetic regulation of gene expression. It was found that curcumin can inhibit activity of HAT proteins of p300/CBP family by direct binding. Further immunoprecipitation and radiolabeling studies showed

that curcumin formed a covalent association with p300 and abolish histone hyperacetylation in both peripheral blood lymphocytes and PC3-M prostate cancer cells. These observations suggested that curcumin acts as a novel HAT inhibitor (Marcu *et al.*, 2006).

1.4.4.2 Histone deacetylases (HDAC)

HDAC counterbalance the acetyltransferase activity of HAT by deacetylating histone proteins. HDAC together with HAT plays a crucial role in epigenetic regulation of gene expression. In a study curcumin was found to be a potent HDAC inhibitor with free energy of binding of -8.55 kcal/mol and the Ki value of 539 nM. Molecular docking study revealed that the interaction of curcumin with HDAC8 was supported by hydrophobic contacts with the active site residues including Ile34, Pro35, Arg37 and Phe152. In addition, two hydrogen bonds (with Asp29 and Tyr100) contributed to low binding energy (Bora-Tatar *et al.*, 2009; Gupta *et al.*, 2011).

1.4.4.3 Glyoxalase I (GLOI)

GLOI is a key metalloenzyme involved in detoxification of reactive α-ketoaldehydes, such as methylglyoxal (Padmanabhan *et al.*, 2006). One study revealed that curcumin and its derivatives inhibit human GLOI, with a Ki value in the range of 2.6–4.6 mM. When docked to GLOI, curcumin (enol form) coordinated with Zn^{2+} in the active site of GLOI through oxygen atoms of the carbonyl group and formed strong hydrogen bonds with the Arg37, Arg122 and Lys156 residues (Yuan *et al.*, 2011). In another study, keto and enol

forms of curcumin bound to the active site of GLOI and enol form interacted with amino acid residues including Glu172 and Met179 (Liu *et al.,* 2010).

1.4.4.4 Cyclooxygenase (COX)

COX is an enzyme responsible for formation of important biological mediators including prostaglandins, prostacyclin and thromboxane. The COX isozymes, COX-1 and COX-2 are involved in the conversion of arachidonic acid into inflammatory prostaglandins. COX-1 expresses constitutively in all tissues, whereas COX-2 highly express under inflammatory conditions. Its inhibition could have therapeutic implications (Vane and Botting, 1998; Ryn *et al.,* 2000, Chun *et al.,* 2003). It has been found that curcumin molecule inhibits COX-1 and COX-2 by direct binding. *In-silico* studies showed that methoxy group of curcumin form a hydrogen bond with Ser530 while binding to COX-1 whereas the phenyl rings of the molecule were surrounded by the amino acid residues His90, Arg120, Tyr355, Leu357, Leu384, Tyr385, Phe518, Met522, Glu524 and Ser530. Curcumin also showed interactions with COX-2 through Val116, Tyr355, Ala516, and Val523 (Selvam *et al.,* 2005).

1.4.4.5 Xanthine oxidase (XO)

Molecular docking studies have revealed direct binding of curcumin to XO. Interestingly, the results suggested that degradation products of curcumin, such as trans-6-(40-hydroxy-30-methoxyphenyl)-2, 4-dioxo-5-hexenal, ferulic aldehyde, ferulic acid, feruloyl methane and vanillin, bind XO more efficiently than parent curcumin molecule. Phe914, Phe1009 and Thr1010 were identified as a key residue at the binding pocket for interaction. It was

suggested that the degradation products of curcumin exhibit better biological activities than curcumin molecule under physiological conditions (Shen and Ji, 2009).

1.4.4.6 DNA methyltransferase1 (DNMT1)

DNMTs are involved in the methylation of promoter CpG of tumor-suppressor genes (TSGs), resulting in transcriptional silencing of these genes in various solid and blood cancers (Herman and Baylin, 2003). Therefore, modulation of DNMT activity has therapeutic potential. Recently, curcumin was found to inhibit M.SssI, an analog of DNMT1 by covalently blocking the catalytic Cys1226 residue with an IC50 value of 30 nM. Tetrahydrocurcumin was also found to exhibit similar activity. Similar results were also observed with leukemia cell line where curcumin induced global DNA hypomethylation (Liu *et al.*, 2009). Results also indicated that the α,β-unsaturated group of curcumin covalently binds to the catalytic cysteine residue of DNMT which need further studies are necessary to confirm these claims.

1.4.4.7 Platelet-type 12-lipoxygenase (P-12-LOX)

P-12-LOX is another enzyme which was found overexpressed in different types of cancers, especially in prostate cancer. In a study, homology model of human P-12-LOX was used for computational docking studies with synthetic curcumin derivatives; it was observed that most of the derivative docked with similar binding modes into the active site. The amino acid residues Trp143, Phe351, Glu355, Leu360, His364, Leu365, Ile398, Ala402, Leu406, Leu407, Phe413 and Ile592 were found to be critical for hydrophobic

interactions. The derivatives inhibiting P-12-LOX reduced sprout formation of endothelial cells (Jankun *et al.,* 2006).

1.4.4.8 Matrix metalloproteinases (MMPs)

MMPs are zinc-dependent endopeptidases with capability of degrading extracellular matrix proteins. They play an important role in cell proliferation, differentiation, apoptosis, angiogenesis and host defense (Gupta *et al.,* 2011). These were implicated in inflammatory, vascular and auto-immune disorders (Page-McCaw *et al.,* 2007; Parks *et al.,* 2004; Egeblad and Werb, 2002). In a molecular docking study of tetrahydrocurcumin and bisdemethoxycurcumin with MMPs, bisdemethoxycurcumin docked with higher affinity than tetrahydrocurcumin and formed hydrogen bonds with Pro421 and Arg424 residues (Girija *et al.,* 2010).

1.4.4.9 Human immunodeficiency virus type 1 (HIV1) integrase and HIV1 protease

HIV-1 uses an integrase and a protease to propagate its life cycle which makes these enzymes potential therapeutic targets. As indicated by molecular docking studies, curcumin was shown to have a potential inhibitory effect on these enzymes by direct binding. When docked to integrase, curcumin formed contacts with residues Asp64, Thr66, His67, Glu92, Asp116, Ser119 and Lys159 and divalent metal Mg^{2+} at the binding site where as docking with protease revealed contacts with residues Asp25, Asp29, Asp30, Gly270, Asp290, and Asp300. These results suggested that the *o*-hydroxyl and/or keto-enol structures are important for inhibition of integrase and protease both (Vajragupta *et al.,* 2005). In another study by Mazumder *et al*. it was found that the anti-

integrase activity of curcumin was due to an intra-molecular stacking of two phenyl rings that brought the hydroxyl groups into close proximity (Mazumder *et al.*, 1995).

1.4.4.10 DNA topoisomerase I and II (topo I & II)

Topo I & II are essential ubiquitous nuclear enzymes that manage the DNA topology during cellular processes such as replication, transcription, recombination, and chromatin remodeling (Gellert, 1981; Champoux, 2001; Wang, 1985). Their critical roles during cellular processes make topoisomerases an attractive therapeutic target against cancer (Wang, 2002). Recent studies revealed that curcumin induce high levels of ternary complexes (curcumin-topo I/ II-DNA) in various human cancer cell lines such as TK-10, MCF-7, UACC-62, HL-60 and K562 cells, which results into permanent DNA strand breaks and triggers apoptosis (Martín-Cordero *et al.* 2003; López-Lázaro *et al.* 2007).

1.4.5 Interaction with protein kinases

Protein kinases are the integral part of the signal transduction pathway, curcumin has been reported to suppress a number of protein kinases including protein kinase A (PKA), protein kinase C (PKC), MAPK, c-Jun N-terminal kinase, src tyrosine kinase, phosphorylase kinase, protamine kinase, autophosphorylation-activated protein kinase, IκB kinase and the growth factor receptor protein tyrosine kinases (Reddy and Aggarwal, 1994). It was found that curcumin directly interact with some protein kinases including protein kinase C (PKC), v-Src, GSK-3b, and ErbB2 (HER2/neu). These direct interactions of curcumin with protein kinases are discussed in the following sections.

1.4.5.1 Protein kinase C (PKC)

PKC family of serine/threonine kinases plays a central role in cellular signaling. Members of this family control a range of physiological processes such as proliferation, differentiation, membrane transport, and the organization of cytoskeletal and extracellular matrix proteins (Battaini and Mochly-Rosen, 2007; Griner and Kazanietz, 2007; Alkon *et al.*, 2007). Previous studies identified the curcumin as a protein kinase C (PKC) inhibitor (Hasmeda and Polya, 1996; Liu *et al.*, 1993). In recent study, curcumin was found to inhibit PKC in the absence of membranes whereas stimulation was seen in the presence of membranes. Results suggested that curcumin decreased PKC activity by competing with Ca^{2+} at lower Ca^{2+} concentrations and stimulated PKC at higher Ca^{2+} concentrations. It was suggested that membrane facilitates Ca^{2+}-binding to the kinase, relieving the inhibition of PKC at low Ca^{2+} concentrations by curcumin (Mahmmoud, 2007).

To develop curcumin derivatives as effective PKC activators, Majhi *et al.* synthesized several long chain curcumin derivatives. Interaction of these derivatives with the activator-binding second cysteine-rich C1B subdomain of PKCδ, PKCε, and PKCθ was studied. Curcumin and its derivatives bound to PKC in the presence of activators. Molecular docking study revealed that curcumin and its derivatives formed hydrogen bonds with the tyrosine and tryptophan residues of PKC (Majhi *et al.*, 2010).

1.4.5.2 Glycogen synthase kinase-3β (GSK-3β)

GSK-3β is a multi-tasking serine/threonine kinase widely expressed in all tissues, with abundance in the brain (Lau *et al.*, 1999, Embi *et al.*, 1980; Rylatt *et al.*, 1980; Woodgett, 1990). It is found to involve in several diseases such as cancer, Alzheimer's disease, type

II diabetes, bipolar disorders, and stroke (Martinez *et al.*, 2002; Alonso and Martinez, 2004; Mohammad *et al.*, 2008; Taha *et al.*, 2008). Molecular docking studies revealed that the keto-enol groups of curcumin forms hydrogen bonds with the amidic carbonyl of Val135. Other amino acid residues involved in hydrogen bonding were Ile62, Lys85 and Arg141. *In vitro* studies suggested that curcumin inhibit GSK-3β with the IC50 value of 66.3 nM (Bustanji *et al.*, 2009).

1.4.5.3 Human Epidermal Growth Factor Receptor 2 (HER2) kinase

HER2, also known as ErbB2 and neu, is a transmembrane tyrosine kinase whose overexpression has been observed in approximately 30% of breast cancers (Anderson and Ahmad, 2002; Tan and Yu, 2007). Its over-expression is also found in ovarian, stomach, and aggressive forms of uterine cancer (Santin *et al.*, 2008). Its downregulation have potential therapeutic applications, particularly in breast cancer. Recently, curcumin was found to increase the association of a chaperone-dependent ubiquitin ligase [carboxyl terminus of Hsc70- interacting protein (CHIP)] with HER2, and subsequent ubiquitination and depletion of HER2. Site-directed mutagenesis and molecular docking studies revealed that curcumin inhibited HER2 by binding to its kinase domain. It was suggested that curcumin's Michael acceptor functionality was important for its covalent association with HER2 and curcumin mediated HER2 depletion (Jung *et al.*, 2007).

1.4.5.4 Phosphorylase kinase

Phosphorylase kinase phosphorylates glycogen phosphorylase which in turn metabolizes glycogen to supply energy for muscle contraction (Johnson, 2007). In a study with six

different protein kinases (PKA, PKC, protamine kinase, phosphorylase kinase, autophosphorylation-activated protein kinase, and a tyrosine kinase), it was found curcumin completely inhibited phosphorylase kinase at relatively lower concentrations. Results indicated that curcumin is a non-competitive inhibitor of phosphorylase kinase with a Ki value of 75 mM (Reddy and Aggarwal, 1994).

1.4.5.5 Viral sarcoma (v-Src)

Cellular sarcoma (c-Src), a cytoplasmic tyrosine kinase, is a homologue of viral sarcoma (v-Src). C-Src was found overexpressed in human tumors and regarded as a potential drug target (Biscardi *et al.*, 1999). In a study curcumin inhibited the kinase activity of v-Src, as a result of which a decrease in tyrosyl substrate phosphorylation of Shc, cortactin, and focal adhesion kinase (FAK) was observed. *In vitro* studies suggested that interaction of curcumin to v-Src was direct (Leu *et al.*, 2003).

1.4.6 Interaction with protein reductases

Protein reductases catalyze the reduction of other proteins. Curcumin was reported to inhibit protein reductases such as thioredoxin reductase (TrxR) and aldose reductase (ALR2) by direct binding. ALR2 is a member of the aldo–ketoreductase (AKR) super family which reduces glucose to sorbitol using cofactor NADPH. Subsequently, sorbitol is metabolized to fructose by sorbitol dehydrogenase (Kinoshita, 1990). Increased ALR2 activity leads to intracellular sorbitol accumulation which has been implicated in various complications of diabetes. In a study curcumin inhibited ALR2 activity in a non-competitive manner with IC50 value of 10 mM, it was also observed that curcumin failed

to inhibit ALR1 under similar experimental conditions. Curcumin interacted with active site residues Thr19, Lys21, Tyr48, Trp111, Gln183 and Leu300 as predicted by molecular docking (Muthenna *et al.,* 2009).

TrxR isoenzymes, TrxR1 and TrxR2, are essential mammalian selenocysteine (Sec)-containing flavoenzymes with a -Gly-Cys-Sec-Gly active site. TrxR catalyzes NADPH-dependent reduction of thioredoxin (Trx). Activity of this enzyme is essential for cell survival and growth which makes it a potential target for anti-cancer therapy (Arner and Holmgren, 2000; Fang *et al.,* 2005). It was found that curcumin has potential to inhibit TrxR1 in the presence of NADPH which persist after removal of curcumin. Mass spectrometry and blotting analysis revealed that inhibition was irreversible as curcumin bound covalently to Cys496/ Sec497 residue in the catalytic active site. It was found that modified TrxR sifted from an anti-oxidant to a pro-oxidant which provides a possible explanation for cancer preventive activity of curcumin (Fang *et al.,* 2005).

1.4.7 Interaction with cyclin-dependent kinase inhibitors

Curcumin induces G_0/G_1 and/or G_2/M phase cell cycle arrest. It up-regulates cyclin-dependent kinase inhibitors $p21^{WAF1/CIP1}$, $p27^{KIP1}$, and p53, and slightly down-regulated cyclin B1. It was also found to down-regulate cyclin D1 expression at the transcriptional and posttranscriptional levels (Reddy and Aggarwal, 1994; Hasmeda and Polya, 1996).

1.4.8 Interaction with adhesion molecules

The expression of various cell surface adhesion molecules is absolutely critical for tumor metastasis. Curcumin down-regulates expression of adhesion molecules (ICAM-1, VCAM-1 and ELAM-1) which are mediated through down-regulation of NF-κB activation (Kumar *et al.,* 1998). Curcumin has been also reported to inhibit TNF-induced

expression of adhesion molecules on human umbilical vein endothelial cells (Gupta and Ghosh, 1999).

1.4.9 Interaction with anti-apoptotic proteins

Curcumin suppresses the constitutive expression of apoptosis suppressor proteins like Bcl-2 and Bcl-X_L in many cancer cell lines. Curcumin induces apoptosis through a mitochondrial pathway involving caspase-8, Bid cleavage, cytochrome c release, and caspase-3 activation. It also activates caspase-7 and caspase-9 and induces polyadenosine-5'-diphosphate-ribose polymerase cleavage (Anto *et al.*, 2002; Bharti *et al.*, 2003b).

1.4.10 Interaction with inflammatory molecules

Curcumin was reported to interact with inflammatory molecules such as myeloid differentiation protein 2 (MD-2) and α1-acid glycoprotein (AGP). MD-2 was recognized as a key molecule for LPS signaling. In a study, it was found that curcumin binds to MD-2 at sub-micromolar affinity, which is the LPS-binding component of the endotoxin surface receptor complex MD-2/TLR4. *In-silico* studies suggested that curcumin binds to hydrophobic pocket of MD-2. Results suggested that interaction of curcumin with MD-2 may have therapeutic applications against chronic inflammatory diseases caused by bacterial infection (Gradisar *et al.*, 2007).

AGP or orosomucoid, has been suggested to have immunomodulatory and anti-inflammatory effects (Fournier *et al.*, 2000; Hochepied *et al.*, 2003). This glycoprotein remains involved in transport of a number of endogenous and exogenous compounds including drugs (Israili and Dayton; 2001; Kremer *et al.*, 1988). High level of AGP was observed during inflammatory and immunological processes. Molecular docking study of

curcumin with AGP indicated two possible binding sites for curcumin through hydrogen bonding with the phenol and enol moieties and pi–pi interactions with the aromatic rings of curcumin (Zsila *et al.,* 2004a).

1.4.11 Interaction with carrier proteins

Curcumin molecule has low solubility in aqueous solution and poor bioavailability (Letchford *et al.,* 2008; Anand *et al.,* 2007). Several attempts have been made by researchers to overcome this problem through encapsulation of curcumin in polymeric micelles, polymeric nanoparticles, liposomes, lipid-based nanoparticles, and hydrogels (Ma *et al.,* 2008; Li *et al.,* 2007; Bisht *et al.,* 2007; Sou *et al.,* 2008, Vemula *et al.,* 2006). Various proteins have been reported to act as carrier for curcumin molecule including milk casein, human serum albumin (HSA), bovine serum albumin (BSA), b-lactoglobulin (b-LG) and immunoglobulin (Ig) which are discussed in the following sections (Gupta *et al.,* 2011).

1.4.11.1 Casein

Caseins are the major milk proteins with good emulsification, gelation, and water-binding properties. Casein microspheres prepared by glutaraldehyde cross-linking have been used for the oral delivery of anticancer drugs including doxorubicin and mitoxantrone (Willmott *et al.,* 1992; Knepp *et al.,* 1993). From our lab Sahu *et al.* reported the delivery of curcumin molecule using casein micelles (CMs) to cancer cells. Curcumin formed a complex with CMs through hydrophobic interactions with binding constant of 1.48 x 10^4 M^{-1}. Cytotoxicity determination revealed that the IC50 of free curcumin and the CM-

curcumin complex were 14.85 and 12.69 mM respectively, in HeLa cells (Sahu *et al.*, 2008). Sneharani *et al.* reported that curcumin has the ability to bind to alpha-s1-casein at two binding sites through by hydrophobic interactions without any change in alpha-s1-casein conformation which enhanced the bio-stability of curcumin (Sneharani *et al.*, 2009).

1.4.11.2 Human serum albumin (HSA) and bovine serum albumin (BSA)

HSA and BSA are the most widely studied carrier proteins which have been reported to serve as carriers of curcumin.The binding constants have been shown to vary from 10^5 to 10^4 M^{-1} for curcumin-HSA interaction. Two binding sites were identified for curcumin at HSA molecule and the binding was mostly favored by hydrophobic interactions and hydrogen bonding. The estimated enthalpy change was-13.6 kcal/mol for curcumin-HSA formation (Barik *et al.*, 2007; Priyadarsini, 2009; Zsila *et al.*, 2003; Mandeville *et al.*, 2009; Kunwar *et al.*, 2006). It was suggested that binding to HSA suppresses the degradation of curcumin molecule (Leung and Kee, 2009).

In a study, Bourassa *et al.* found that curcumin binds BSA through hydrophilic and hydrophobic interactions, with a binding constant of $3.33 \pm 0.8 \times 10^4$ M^{-1}. It was found that curcumin binding altered BSA conformation. Further studies indicated that the binding of curcumin was in the close vicinity of Trp212 and Trp134 residues (Bourassa *et al.*, 2010). In another study, Sahoo *et al.* investigated the interaction of isoxazolcurcumin and diacetylcurcumin with BSA and found that both the molecule preferentially docked to the hydrophobic subdomain near Trp213 residue (Sahoo *et al.*, 2008a).

1.4.11.3 Fibrinogen

Fibrinogen is a soluble blood plasma protein which plays a major role in blood clotting. In a study, it was found that curcumin has strong binding affinity to the hydrophobic domains of fibrinogen (with binding constant of 10^5 M^{-1}). The binding inhibited curcumin degradation due to hydrolysis. These authors suggested that the stabilization effects of fibrinogen may enable curcumin to maintain its medicinal properties (Leung and Kee, 2009).

1.4.11.4 Immunoglobulin (Ig)

Human serum immunoglobulin (Ig) is an important transport protein for drugs. In a study with spectroscopic techniques, two binding sites for curcumin were characterized with the average affinity constant of 1.17 x 104 M^{-1}. Molecular docking study with IgG, suggested hydrogen bonding between curcumin and His35, Tyr91, Ala92, Tyr94, Arg96, Tyr98, Tyr99 and residues (Liu *et al.*, 2004; Liu *et al.*, 2008).

1.4.11.5 β-Lactoglobulin (β-LG)

β-LG is a low molecular weight whey protein which can transport small hydrophobic molecules (Kontopidis *et al.*, 2004). The interaction of curcumin with β–LG was investigated by biophysical techniques; it was found that curcumin interacts with β–LG with an association constant of 1.0 x 10^5 M^{-1} at pH 7.0, through hydrophobic contacts. Further analysis by molecular docking, suggested that methoxy phenyl moiety of curcumin interacted with the aromatic amino acid residues of central calyx of β-LG. Enhanced stability of curcumin molecule by β-LG suggested its potential as a carrier

molecule (Sneharani *et al.*, 2010). In another study with curcumin and diacetylcurcumin, tryptophan residues (Trp19 and Trp61) were reported critical for the binding (Mohammadi *et al.*, 2009).

1.4.12 Interaction with multidrug resistance proteins

Curcumin and its derivatives have been reported to have multidrug resistance (MDR) reversing activity. In a study, Limtrakul *et al.* investigated the ability of tetrahydrocurcumin to overcome the multidrug resistance (MDR) of human cancers. It was found that tetrahydrocurcumin inhibits the efflux function of P-glycoprotein (P-gp or ABCB1), mitoxantrone resistance protein (MXR or ABCG2) and multidrug resistance protein 1 (MRP1 or ABCC1) (Limtrakul *et al.*, 2007). In another study, Li *et al.* reported that curcumin inhibit multi-drug resistance-associated protein 5 (MRP5) mediated drug efflux from the cell (Li *et al.*, 2011).

1.4.13 Interaction with metals

Turmeric powder can be used to remove Cu(II) from aqueous solution as it contain compounds acting as sequestering agent for toxic metals (Qayoon *et al.*, 2009).. Based on structure- function relationship studies, three sites in curcumin have been ascertained to which metals bind (**Figure 1.5**). Two of these sites are contributed by the phenolic and methoxy groups on the two benzene rings and the third site is due to the presence of 1,3-diketone system between the rings (Ahsan *et al.*, 1999). Chena *et al.* confirmed this by resonance light scattering (RLS) technique where they observed enhancement in the intensity of Cu(II) with increase in curcumin concentration (Chena *et al.*, 2009).

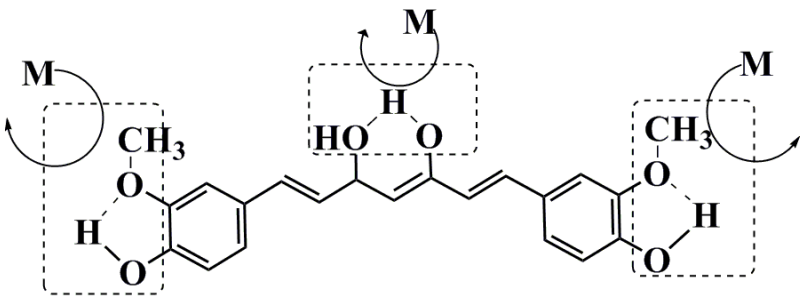

Figure 1.5 *Three metal binding sites (M) in curcumin: two of these sites are contributed by the phenolic and methoxy groups on the two benzene rings and the third site is due to the presence of 1,3-diketone system between the rings.*

Curcumin helps in decreasing amyloid aggregation or oxidation induced neurotoxicity by chelating copper/iron ions which exists in high concentration in Alzheimer's disease. Spectroscopic studies suggested that curcumin have high affinity towards Cu^{2+} or Fe^{2+}, both of which at least bind two curcumin molecules whereas Zn^{2+} showed little binding. When curcumin was added to cultured liver cells there was increase in the mRNA level of ferritin and α-GST but the protein level of ferritin was declined due to inhibition of translation which normally happens when iron chelators are present (Baum and Ng, 2004). A similar study suggested the effect of curcumin on transferrin receptor1 and iron regulatory protein activation as an indicator of iron depletion due to iron chelation by curcumin (Jiao *et al.,* 2006). Borsari *et al.* demonstrated that that β-diketo moiety of the curcumin and diacetylcurcumin is involved in metal chelation (Borsari *et al.*, 2002).

Some studies have suggested that curcumin produces ROS (O_2^-, H_2O_2) in the presence of metals such as Cu(II) and Fe(II) which cause DNA damage in supercoiled circular plasmid DNA (pUC18 and pBR322) as a result of that the molecules become open circular. It was proved that ROS are involved in cleavage (Chakraborty *et al.,* 2004;

Yoshino *et al.,* 2004; Ahsan *et al.,* 1998; Iwasaki *et al.,* 2011; Kong *et al.,* 2009; Sun and Zhong, 2006; Senthil and Sarojini, 2008). Similar results were reported by Urbina-Cano *et al.,* when Balb-C mouse lymphocytes were treated with curcumin-Cu(II) complex. It was found that at high concentration (50 µM) curcumin alone induces DNA strand breaks, the presence of copper increases the DNA damage (Urbina-Cano *et al.,* 2006). Human prostate cancer cells (LnCaP, PC3 and DU145) treated with heteroleptic palladium(II) complex (**Figure1.6**) of curcumin also exhibits ROS-induced DNA damage (Valentini *et al.,* 2009).

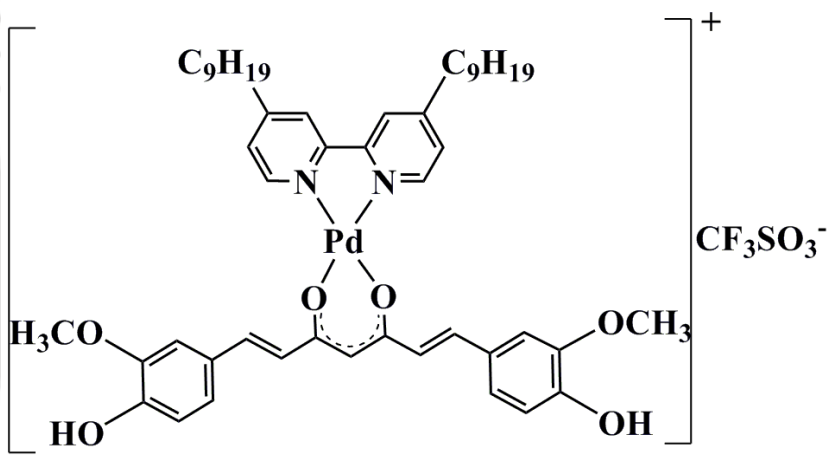

Figure 1.6 *Chemical structure of heteroleptic palladium(II) complex of curcumin which exhibits ROS-induced DNA damage.*

In vitro studies have shown that Cu(II)-curcumin complex causes oxidation of guanine residues at C8 and increases DNA damage in proportion to Cu(II) concentration (Serpia *et al.,* 2010; Sakano *et al.,* 2002; Loft *et al.,* 2006). It was seen that there is an induction of DNA damage by dietary curcumin upon copper accumulation in Long-Evans Cinnamon (LEC) rats through the formation of nuclear and mitochondrial etheno-DNA

adducts (Nair *et al.*, 2005).

Song *et al.* synthesized and characterized rare earth metal complexes with curcumin and 1,10-phenanthroline-5,6-dione (**Figure 1.7**). The general formula of the complexes was REL_3L' (RE = samarium (Sm), europium (Eu), and dysprosium (Dy), L=curcumin, L'= 1,10-phenanthroline-5,6-dione) To study the interaction of complexes with DNA, they treated pBR322 plasmid DNA (0.37 µM) with the varied concentration (0-0.08 µM) of complexes at physiological pH and temperature. The result suggested that SmL_3L' can cleave plasmid DNA at physiological pH and temperature through oxidation of bases. It was found that the cleavage process was sensitive to pH and optimum temperature for cleavage was 37 °C (Song *et al.*, 2009).

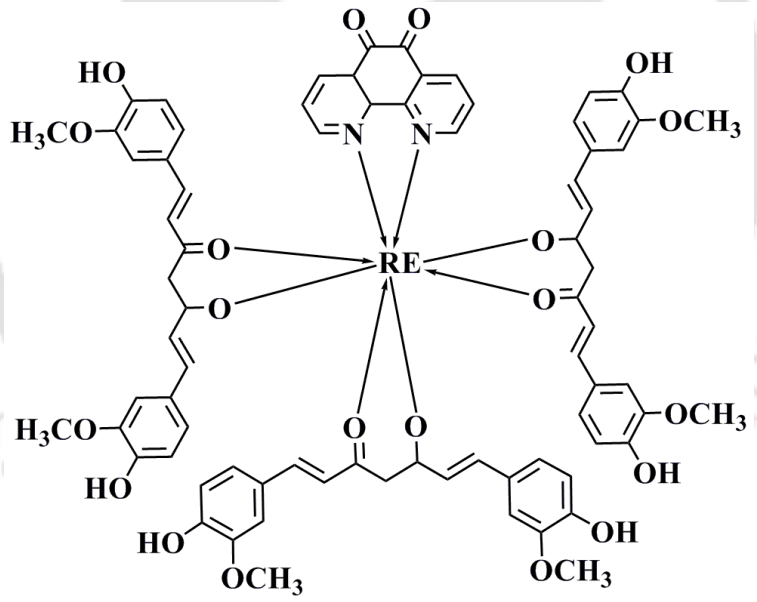

Figure 1.7 *Chemical structure of rare earth metal (RE) complexes of curcumin with 1,10-phenanthroline-5,6-dione which can cleave plasmid DNA at physiological pH and temperature.*

1.4.14. Interaction with nucleic acids

1.4.14.1 As major and minor groove binder

In general interactions of small molecules like curcumin with DNA have three common binding modes: (i) electrostatic interaction, which is due to the negatively charged sugar-phosphate backbone, (ii) hydrophobic binding against minor or major grooves of DNA, preferentially binding to AT rich regions (Bera *et al.,* 2008) and (iii) intercalation between the stacked base pairs of native DNA (Blackburn and Gait, 1990). Thus small molecules interacting with double stranded DNA (dsDNA) can be classified according to their binding modes as groove binders (non-intercalators) and intercalators. In a study Ahsan *et al.* showed that the curcuminoids prevents *EcoRI* and *HindIII* from digesting at the respective restriction sites by directly binding to AT rich base pairs in the sequences whereas allow the restriction digestion by *SmaI* and *BamHI* both of whose sequence are GC rich (Ahsan *et al.,* 1999). However, it is not known if curcumin also inhibits the activity of *EcoRI* and *HindIII* directly. Circular dichroism and absorption spectroscopy techniques along with molecular modelling studies have proven that curcumin binds to the minor groove of the DNA double helix (Zsila *et al.,* 2004b). Independent studies investigating the interaction between curcumin and DNA, suggested that higher curcumin concentration causes conformational changes in DNA double helix (Serpi *et al.,* 2010; Zhang *et al.,* 2009; Wang and Xue, 2010). In another study, *Wang et al.* found that curcumin- cetyltrimethylammonium bromide (CU-CTAB) complex form CU-CTAB–nucleic acid ternary complex (*Wang et al.,* 2007).

It was found that curcumin binds to DNA through thymine O_2 group in the minor groove and through guanine and adenine N7 in the major groove, as well as to the backbone PO_2 group. RNA binding occurs via uracil O_2 and guanine and adenine N7 atoms as well as the backbone phosphate group. Interestingly, the interaction of curcumin

was stronger with DNA than RNA (Nafisi *et al.*, 2009).

Fourier Transform Raman Spectroscopic study at physiological pH on curcumin-dGMP interaction at varying concentrations showed that at low concentration, curcumin/dGMP (1/50) interaction is mainly through backbone PO_2 group. At higher concentration curcumin (1/10) interact with guanine (N7) (Senthil *et al.*, 2009). Furthermore, biophysical techniques and docking studies of binding of curcumin derivatives such as dimethoxycurcumin, isoxazolcurcumin, diacetylcurcumin and a triglycylcurcumin derivative (**Figure 1.8**) with calf thymus DNA showed that these derivatives bind to the minor groove preferentially at AT rich region (Bera*et al.*, 2008; Kunwar *et al.*, 2011; Sahoo *et al.*, 2008b; Kumar *et al.*, 2002). Others have also confirmed that curcumin and its derivatives are not intercalators but minor groove binders by performing ethidium bromide, 4'-6-Diamidino-2-phenylindole (DAPI) and Hoechst 33258 displacement assays (Bera *et al.*, 2008; Kunwar *et al.*, 2011; Sahoo *et al.*, 2008b).

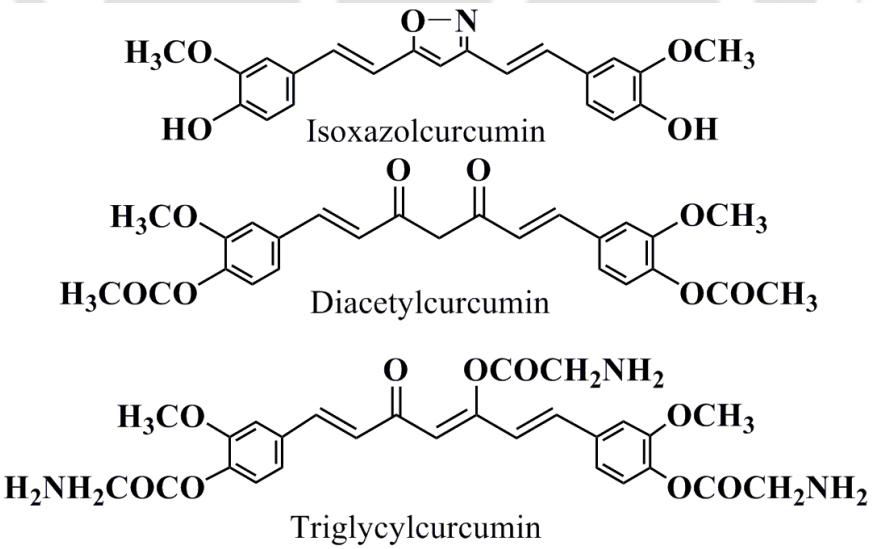

Figure 1.8 *Chemical structures of isoxazolcurcumin (IOC), diacetylcurcumin (DAC) and triglycylcurcumin which preferentially binds to the AT rich minor groove.*

Binding studies of Al-curcumin complex (ACC) ([Al(curcumin) $(EtOH)_2$]$(NO_3)_2$) (**Figure 1.9**) with DNA performed using multi-spectroscopic and voltametric techniques showed that ACC binds to DNA in non intercalating mode to the AT base pairs rich minor groove. Analysis indicated a strong and direct binding of ACC to thymine (O_2) and adenine (N7) of DNA bases located in the minor groove and weak electrostatic interaction of ACC with the phosphate backbone (Ahmadia *et al.*, 2011).

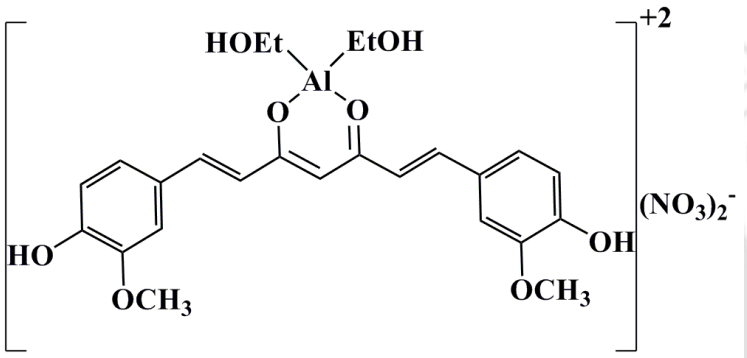

Figure 1.9 *Chemical structure of Al-curcumin complex (ACC) ([Al(curcumin) $(EtOH)_2$]$(NO_3)_2$) which directly binds to thymine (O2) and adenine (N7) of DNA bases located in the minor groove.*

In a study examining the interaction between DNA and mononuclear transition metal (Cu(II), Co(II), Ni(II)) complexes of macrocyclic tetraaza diacetylcurcumin (**Figure 1.10**), it was found that these complexes bind through the minor groove. Amongst these, Cu(II) complex was found to have the highest degree of interaction (Rajesh *et al.*, 2012).

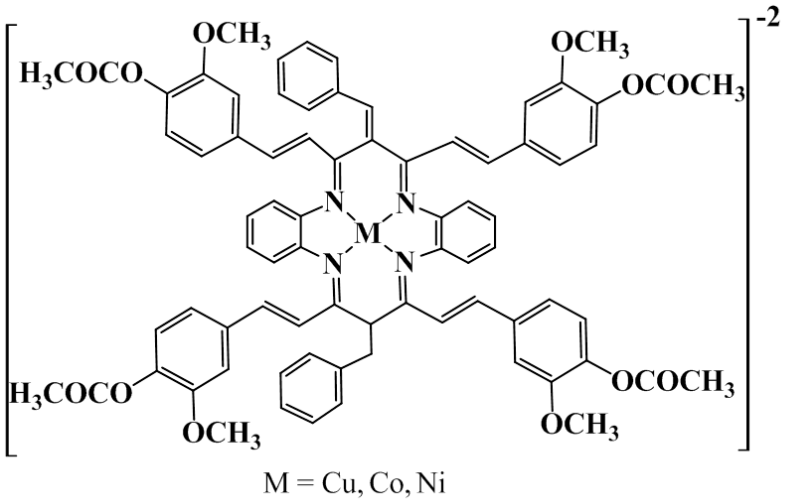

Figure 1.10 *Chemical structures of transition metal complexes of macrocyclic tetraaza diacetylcurcumin which bind to the DNA minor groove.*

In-silico studies looking into the interaction of DNA with curcumin as well as its derivatives either in pure form or their metal complexes provide supporting evidences complementing the experimental findings. Comparative docking studies of curcumin and nicotine on consensus sequence ["GGGCATGCCTAGGCATGCC"] of human p53 gene showed that they bind to thymidine 6 of the p53 gene with free energy change of -272.75 and -163.74 kcal/mol respectively suggesting that binding of curcumin on DNA is more stable than nicotine. Curcumin also interacts with nicotine with free energy change of -129 kcal/mol and reduces the availability of nicotine for DNA binding suggesting that curcumin has potential to protect DNA by not only competitively binding to it directly but also by binding to nicotine (Banerjee *et al.*, 2010).

In another study of curcumin with two DNA duplexes [$d(GTATATAC)_2$ and $d(CGCGATATCGCG)_2$] followed by molecular simulations and free energy analysis, predicted that curcumin binds in the minor grooves of AT-rich sequences of DNA. However unlike the known minor groove binders (netropsin and distamycin) where the

binding is mainly by electrostatic interaction, it was found that binding of curcumin are mainly favoured by van der Waals and hydrophobic interactions (Koonammackal *et al.*, 2011; Khan *et al.*, 2012).

Caruso *et al.* synthesized and characterized ruthenium–arene complex of curcumin (p-cymene)-Ru-(demethoxy-curcuminato)-chloro (**Figure 1.11**). Docking studies of this complex with a guanine rich B-DNA decamer predicted dipolar interaction of Ru with N7(guanine) and was confirmed with electrospray ionization mass spectrometry. This showed excellent activity on the colorectal tumor cell line HCT116 (IC50 = 13.98 ± 1.503 μM) (Caruso *et al.*, 2012).

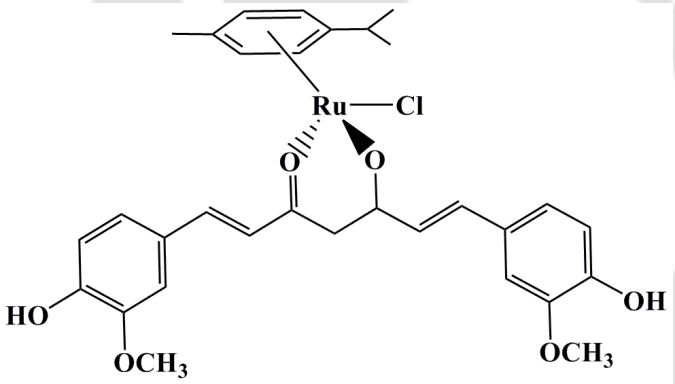

Figure 1.11 *Chemical structure of ruthenium-arene complex of curcumin found highly active against the colorectal tumor cell line HCT116.*

1.4.14.2 As DNA intercalator

In contrast to studies reported in above section, curcumin was found to act as intercalating agent through the unknown clastogenic mechanism that may cause topoisomerase II poisoning. It was found that curcumin at higher concentration (50 μM) acts similar to anticancer agent etoposide and forms a ternary complex with DNA and topoisomerase II enzyme preventing re-ligation of DNA strand. This causes error in DNA synthesis and

promotes apoptosis of cancer cells (Snyder and Arnone, 2002; Martín-Cordero *et al.*, 2003). In another study with immobilized dsDNA, curcumin was found to bind DNA electrostatically at low ionic strength and intercalated at high ionic strength. In UV-visible spectroscopic study curcumin-Fe(II)/Curcumin-Fe(III) complex interacted with salmon sperm DNA in an intercalating mode (Gao *et al.*, 2010; Gao *et al.*, 2011).

Using two newly synthesized heteroleptic pentacoordinated Zn(II) complexes (**Figure 1.12**) containing 4,4'-disubstituted-2,2'-bipyridines as main ligand and curcumin as ancillary ligand Pucci *et al.* demonstrated that the interaction modes of curcumin and curcumin-Zn(II) complexes with dsDNA favour their alignment perpendicular to the DNA axis through base stacking pi-pi interactions between aromatic rings suggesting a partial inter-base intercalation. In vitro studies of curcumin and curcumin-Zn(II), curcumin showed strongest growth inhibition in prostate cancer cell lines. However curcumin-Zn(II) complexes showed the strongest growth inhibition in LAN-5 neuroblastoma cell line (Pucci *et al.*, 2012).

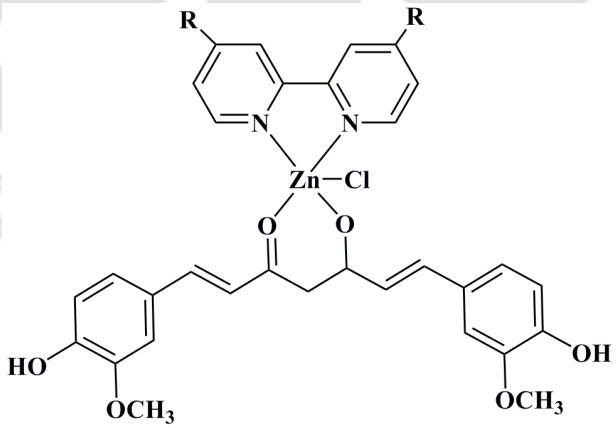

Figure 1.12 *Chemical structures of heteroleptic pentacoordinated Zn(II) complexes of curcumin containing 4,4'-disubstituted-2,2'-bipyridines (R= C_9H_{19} and OH) which interact with DNA in an intercalating mode.*

Several curcumin and/or its derivatives and their metal complexes interact directly with DNA either by binding to the minor groove or as an intercalating agent. Researchers suggested that curcumin is a "double edged sword": having potential for the development of minor groove binding drug for cancer therapeutics but at the same times it may cause DNA damage in the cell at high concentrations (Acharya *et al.,* 2010; Marathe *et al.,* 2011). It is already known that cancer cells are more susceptible than normal cells. On the other hand genomic instability is evolving as a hallmark of cancer (Rajendran *et al.,* 2011; Sadikovic *et al.,* 2008; Louhimo *et al.,* 2012). It would be interesting to investigate the DNA binding potential of curcumin derivatives as a lead molecule for therapeutic implications.

1.5 RESOURCE DATABASES AVAILABLE FOR MOLECULAR MODELING STUDIES

Pubchem, ChEMBL, Zinc Database, BindingDB, DrugBank and Chemspider are the mostly used databases which provide the comprehensive information on small molecules and their molecular targets (Seiler *et al.,* 2008). Pubchem database is maintained by the National Center for Biotechnology Information (NCBI), along with bioassays results (Wheeler *et al.,* 2007). ChEMBL, BindingDB and DrugBank are the databases with provide the information of small molecules as well as their molecular targets (Wishart *et al.,* 2006; Liu *et al.,* 2007; Willighagen *et al.,* 2013). Zinc Database and Chemspider provides physicochemical properties along with 2D and 3D structures (Irwin and Shoichet, 2005; Hettne *et al.,* 2010). For macromolecules such as proteins and DNA, Protein Databank (PDB) is the most comprehensive database available.

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CHAPTER 2

Development of a curcumin resource database (CRDB)

2.1 INTRODUCTION

Curcumin (diferuloyl methane) is a hydrophobic polyphenol derived from rhizome of perennial herb turmeric (*Curcuma longa*) which belongs to the ginger family (Zingiberaceae) native to tropical South Asia (Padhye *et al.,* 2010). The medicinal properties of turmeric were known to ancient India and China. Since the time of Ayurveda (1900 BC), numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions (Aggarwal *et al.,* 2007). It was used as an anti-inflammatory agent, in poultices applied locally to relieve pain and in the treatment of medical conditions such as skin infections, pulmonary and gastrointestinal systems, aches, wounds, sprains and liver disorders (Ravindram *et al.,* 2007). It was also used as a dietary spice and as a coloring agent in foods and textiles (Menon *et al.,* 2007). Curcumin and its analogs have been described in thousands of published research articles over the past few decades, studying its antimicrobial, antioxidant, anti-inflammatory, cancer chemopreventive and chemotherapeutic properties (Shishodia *et al.,* 2007; Jagetia and Aggarwal, 2007; Goel *et al.,* 2008; Anand *et al.,* 2007). Recently, these molecules have also been found active in prevention of heart disease and to combat with degenerative neurological diseases (including Alzheimer's, Parkinson's diseases and multiple sclerosis) and autoimmune diseases (Srivastava and Mehta, 2009; Kim *et al.,* 2012; Bright, 2007). Curcumin analogs have been reported to interact with a huge number of molecular targets like signaling molecules, transcription factors, growth factors, receptors, adhesion molecules, pro-inflammatory enzymes and protein kinases (Aggarwal *et al.,* 2007; Gupta *et al.,* 2011). Researchers have designed many synthetic analogs to improve the binding of these molecules to molecular targets by adding various functional groups in aromatic rings and linker region (Anand *et al.,* 2008). There is no database available which provides comprehensive information of curcumin analogs, its molecular

targets and patents. In the present work, we developed a database of curcumin analogs, its molecular targets and patents.

2.2 MATERIALS AND METHODS

The logistics based on which CRDB has been created is shown schematically in **Figure 2.1.** The database offers an integrated and curated repository of curcumin analogs and their molecular targets. An easy-to-use web interface allows a remote user to retrieve data through interactive web forms. Details of the database design, data sources, query options and other features of the database are described in the following subsections.

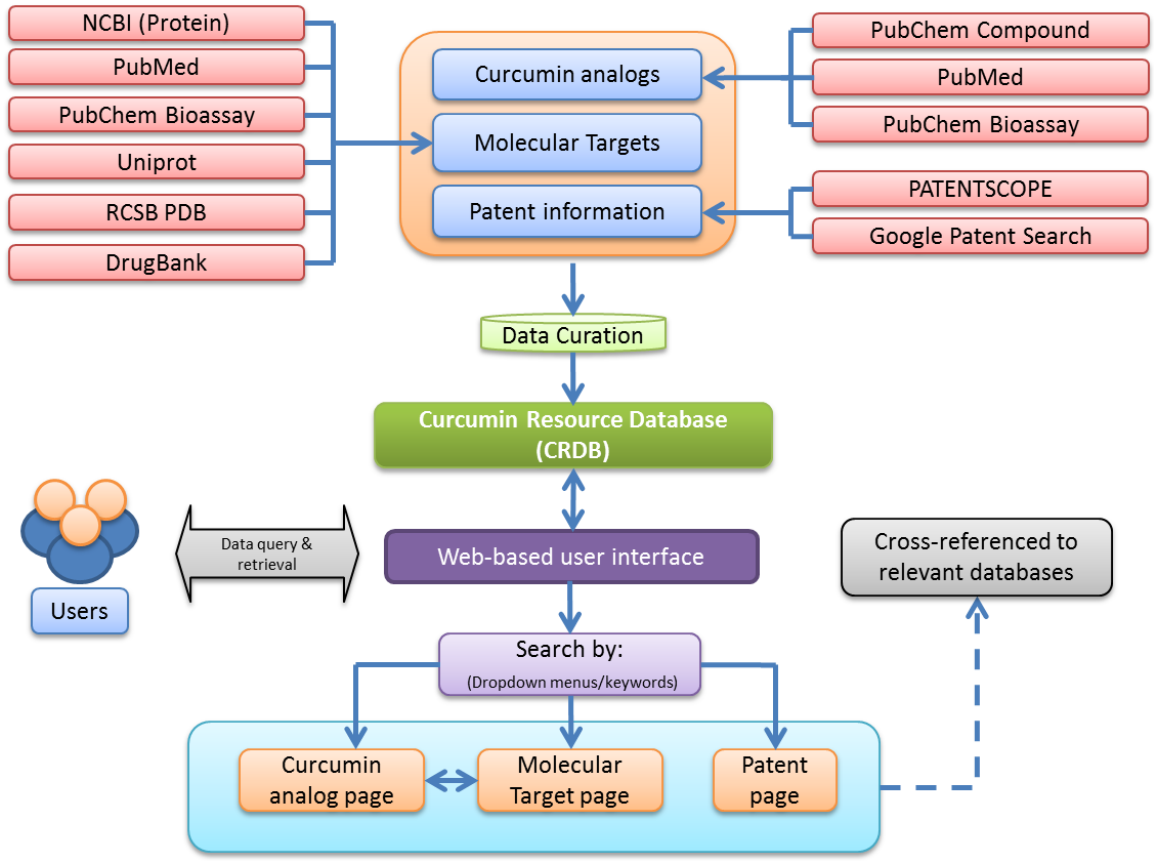

***Figure 2.1** Schematic representation of CRDB*

2.2.1 Database design and implementation

The database is designed and implemented using flat files based content management system PmWiki which is written in PHP and distributed under the General Public License. The web interface is comprised of a collection of web applications/web forms. Link to the "Browse Analogs/Molecular targets/Patents" and "Search CRDB" pages are given on the left sidebar of the "Home Page" which is shown in **Figure 2.2** and **2.3** respectively. The search page of the database serves as the gateway to query the database contents dynamically as instructed by the user through various options (dropdown menu/keyword search). The data collected from different sources are initially stored in manually curated Microsoft Excel spreadsheets and uploaded to the flat file based database. The database works well with Microsoft Internet Explorer, Google Chrome and Mozilla Firefox.

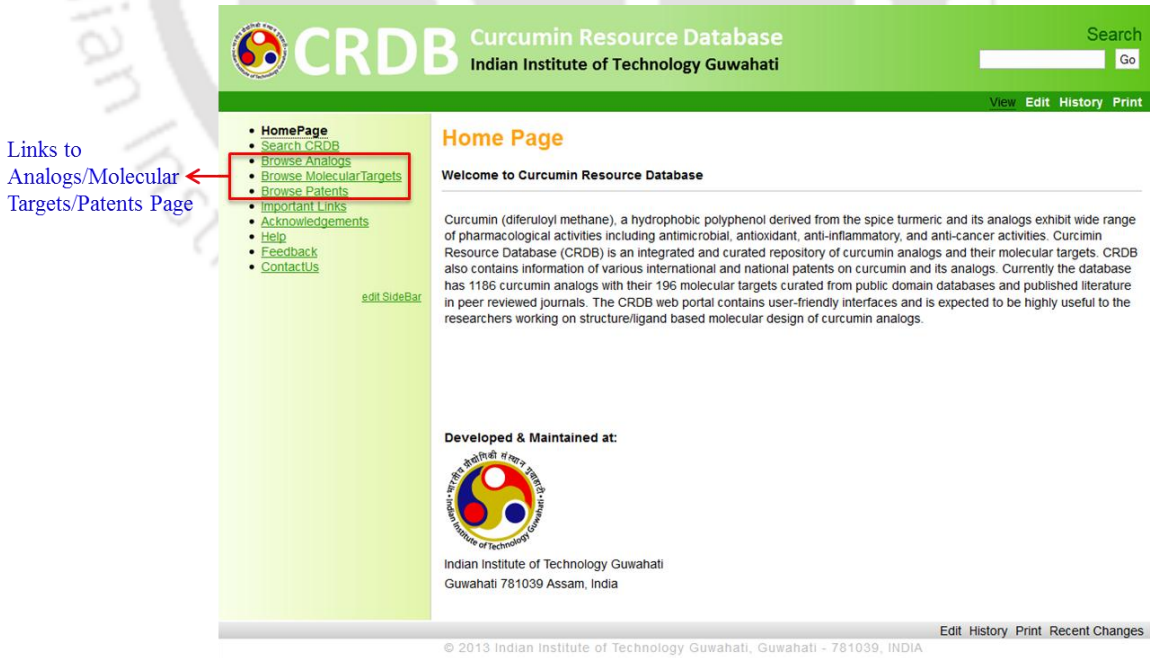

Figure 2.2 *Home Page showing links to Analogs/Molecular Targets/Patents Page*

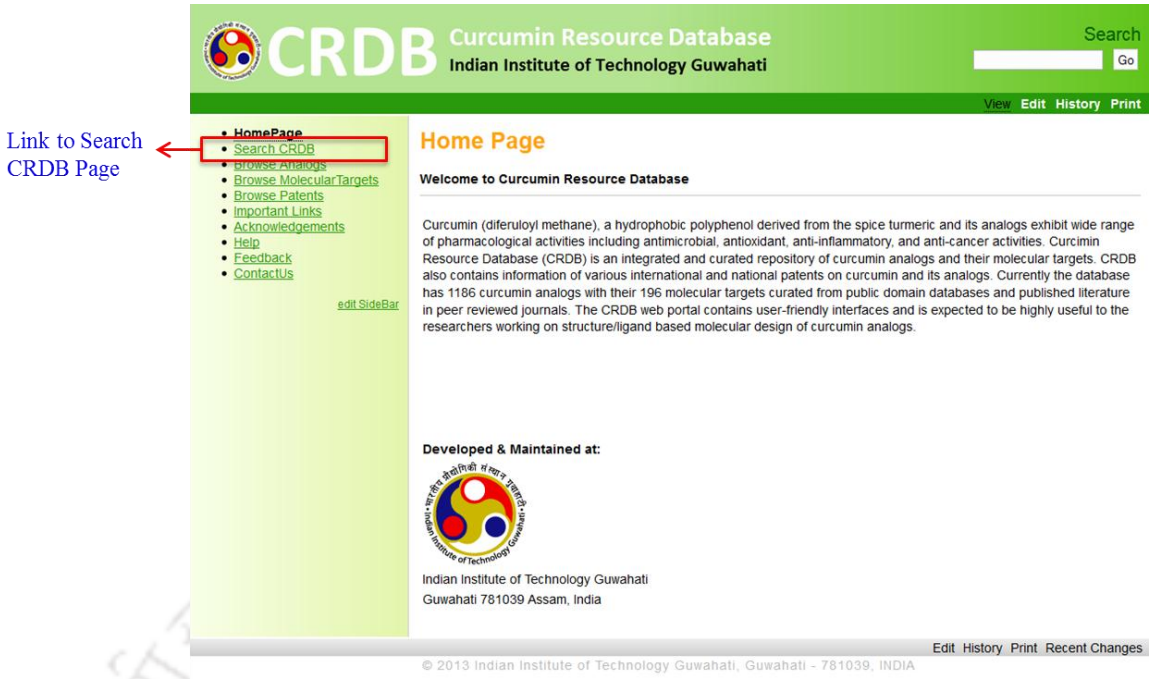

Figure 2.3 *Home Page showing link to Search CRDB Page*

2.2.2 Data content and curation

The primary source of data is public domain databases (PubChem, NCBI protein database, UniProt and PATENTSCOPE) and peer-reviewed published reports in PubMed. All the data sources are duly referred to respective bibliographic pages and hyperlinked. Currently the database has 1186 curcumin analogs with their 196 molecular targets and information of 490 international and national patent documents curated from public domain databases and published literature in peer reviewed journals. Redundancyin the database has been removed manually.

2.2.3 Query options

Database can be either browsed through “Browse Analogs/Molecular targets/ Patents” pages as shown in **Figure 2.4** or it can be navigated from the “Search CRDB” page through dropdown menus shown in **Figure 2.5**. Text based search on specific group of pages can be also performed through “Keyword Search” option available on “Search CRDB” page as shown in **Figure 2.6**. Detailed guidelines are available on the ‘Help Page’ to facilitate effective usage of the database.

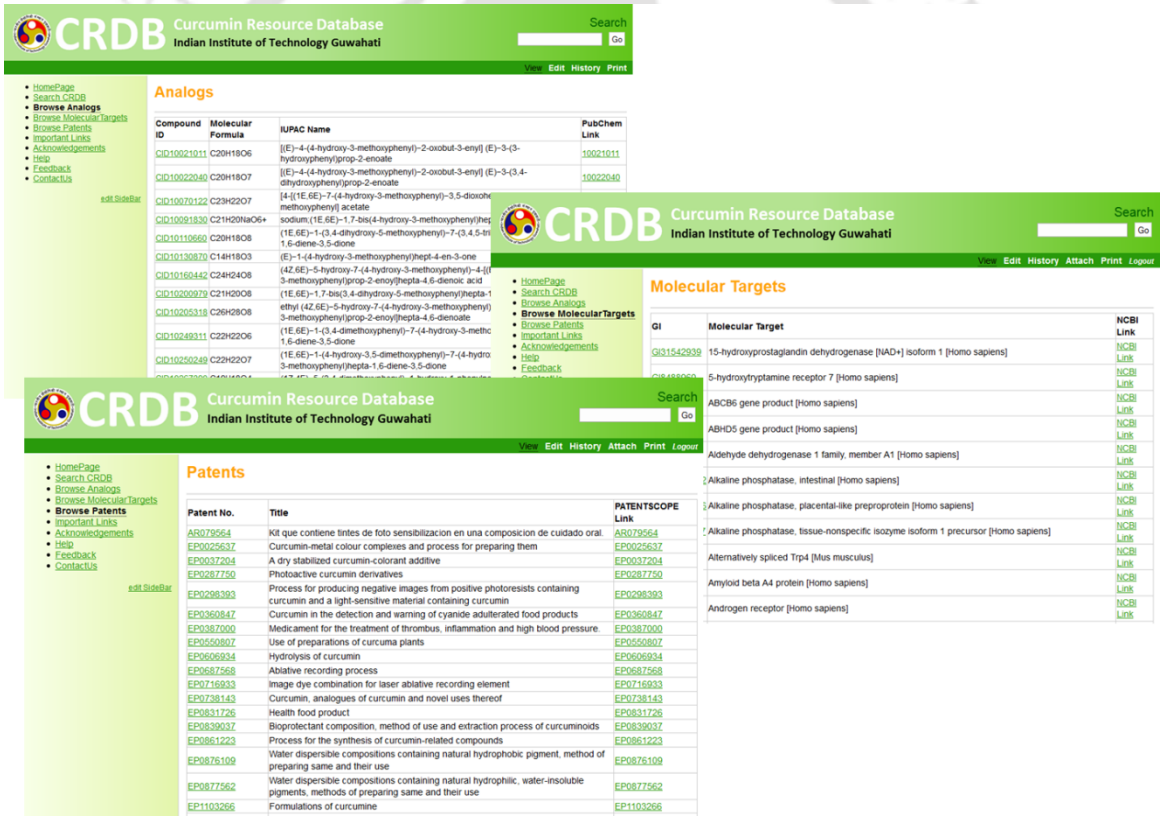

Figure 2.4 *Browse CRDB through Analogs/Molecular Targets/Patents Pages*

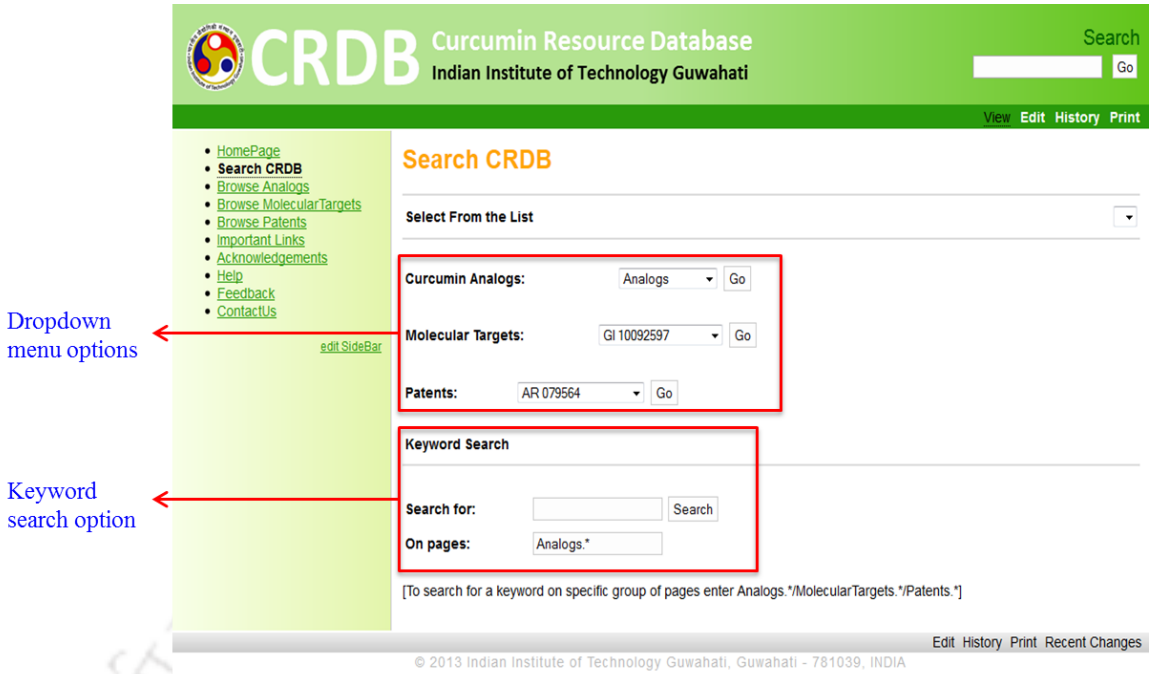

Figure 2.5 *Search CRDB Page showing dropdown menus and keyword search option.*

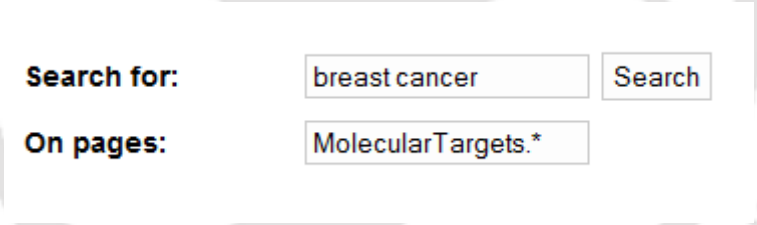

Figure 2.6 *Keyword based search on specific group of pages.*

An analog page shows detail information of physicochemical properties of a molecule (**Figure 2.7**). 3D structure of the molecule can be downloaded in SDF file format and the information of molecular targets against which the moleculeis active can be viewed. All the information has been cross-linked to the relevant databases.

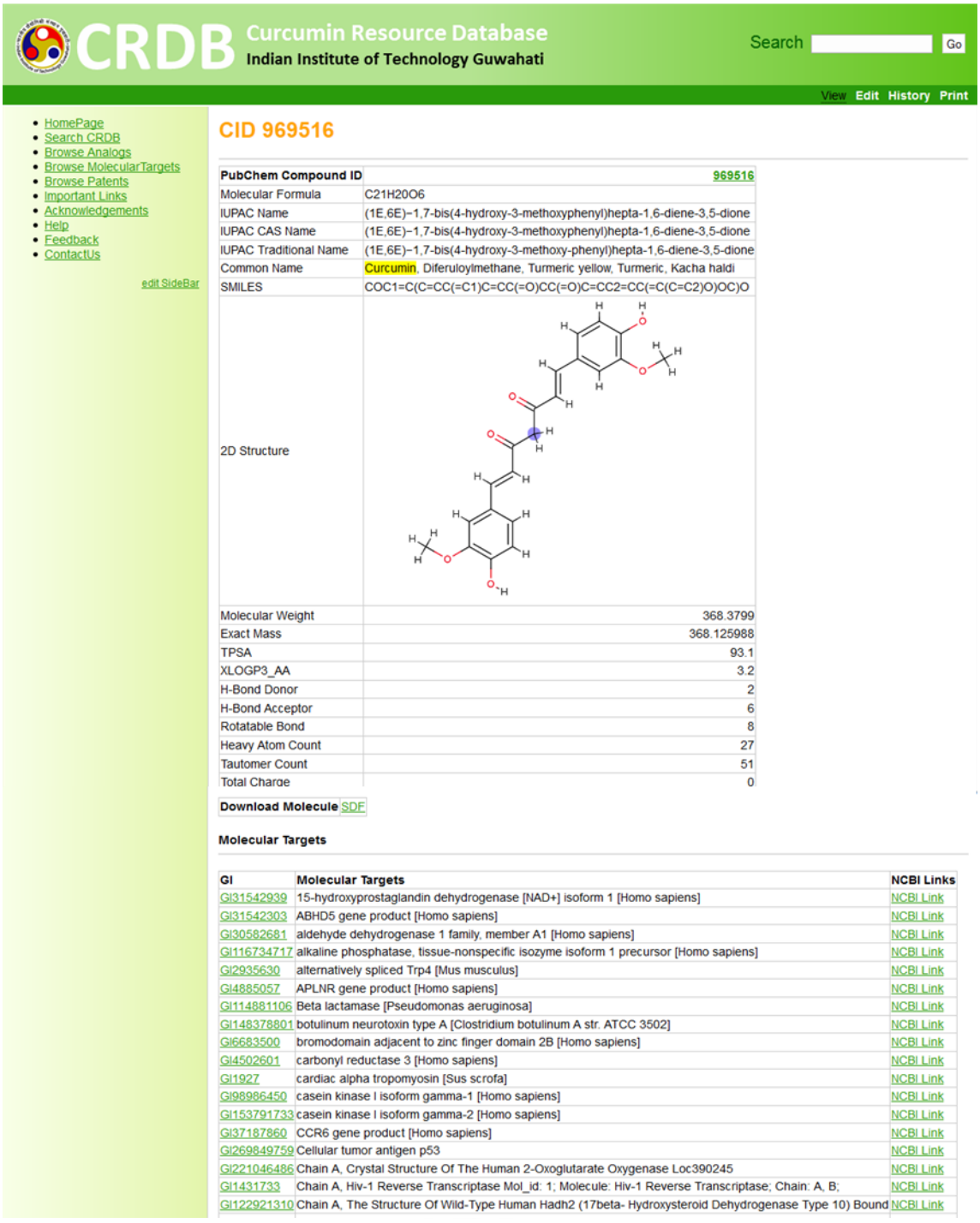

Figure 2.7 *An example of analog page showing detail information of physicochemical properties, link to download the 3D structure of the molecule in SDF file format and the molecular targets against which the analog has been found active in in vitro studies with links to the relevant databases.*

Molecular target page provides the information of protein sequence which could be downloaded by the user through a link in FASTA format. Links to various other relevant databases such as NCBI Protein, CCDS, Gene ID, HGNC, HPRD, OMIM, UniProt, PDB and DrugBank have been provided. Curcumin analogs active against that particular molecular target have also been given with their active concentrations. The disease in which the molecular target has therapeutic potential has been also provided. An example of a molecular target page is shown in **Figure 2.8.**

Patent pages provide the information of international or national patents on curcumin analogs and its implications. Abstract is provided on the patent page and links to the relevant databases such as PATENTSCOPE and Google Patent Search have been given. The patents which are not in English language, were translated into English using Google translate online tool. Example of a patent page is shown in **Figure 2.9.**

2.2.4 Data submission and updates

There is a provision for submission of new data in the database. Currently submission can be done by sending curated information to CRDB via email (crdb.iitg@gmail.com). However, data will be accepted based on their publication in peer-reviewed journal. All updates would be incorporated in the updated versions of the database planned to be released every 6 months interval depending on the volume of new data available.

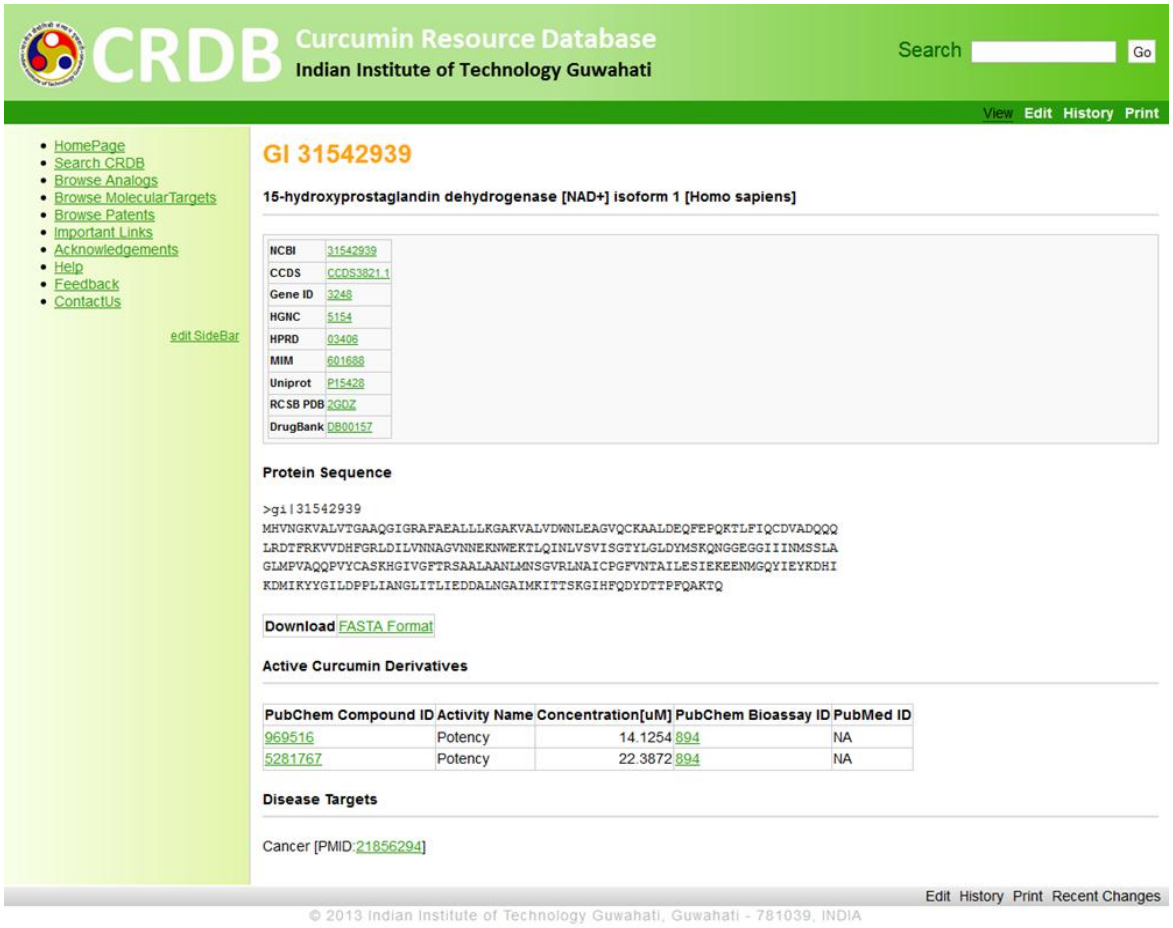

Figure 2.8 *An example of molecular target page showing detail information of protein sequence, link to download the protein sequence, curcumin analogs which are active against the molecular target and the disease targets with links to the relevant databases.*

2.2.5 Database availability

The database is publicly available free of cost without any license fees or requirement of prior registration through the web link www.iitg.ac.in/ubora/crdb. Portable version of the database has been also prepared using open source package Server2Go. Users can request for portable version of the database through e-mail.

Figure 2.9 *An example of patent page showing abstract and links to the relevant databases.*

2.3 SALIENT FEATURES OF THE DATABASE

At present, CRDB represents one of the most data-intensive repositories for curcumin analogs. It can be used as a platform to investigate the interaction of curcumin analogs to its various molecular targets and for structure and ligand based molecular design of curcumin analogs for therapeutic applications. Further enrichment of the database for this purpose would depend on the inputs from the researchers and we plan to make an effort towards this goal.

2.4 CONCLUSIONS

CRDB is an integrated and curated repository of curcumin analogs their molecular targets and patents curated from public domain databases and published literature in peer reviewed journals indexed in PubMed. Database can be browsed through dropdown menus and text based keyword search. Provisions have been made for deposition and regular updation of the database. The CRDB web portal has been made freely available and with user-friendly interfaces. It is expected to be highly useful to the researchers working on structure as well as ligand based molecular design of curcumin analogs.

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CHAPTER 3

Computational studies on inhibition of NF-κB p50 subunit by curcumin natural derivatives

3.1 INTRODUCTION

NF-κB is an inducible mammalian transcriptional factor belonging to the rel family. It exists predominantly in dimeric form either as a homodimer (p50/p50) or heterodimer (p50/p65). In the cytoplasm, its nuclear localization signal (NLS) is masked by ankyrin repeats of Inhibitor kappa B (IkB) proteins thereby preventing it from entering the nucleus and initiating transcription (Thanos and Maniatis, 1995; Beg and Baldwin, 1993). NF-κB normally remains sequestered in the cytoplasm by the IkB family of proteins. Various physical, chemical and biological stimuli induce IkB kinase to phosphorylate IkB which subsequently undergoes proteasome dependent degradation and results in activation of NF-κB (**Figure 3.1**). Activated NF-κB then enters the nucleus and transcribe genes by binding to κB site (consensus sequence GGGRNNYYCC, where N = any base, R = purine, and Y = pyrimidine) (Verma *et al.*, 1995; Hayden and Ghosh, 2004). NF-κB also turns on expression of IκB which represses NF-κB by a feedback inhibition. In case of tumor cells, NF-κB remains active due to the mutation in NF-κB encoding gene or in IkB gene both of which lead to dysfunction in sequestration of NF-κB in cytoplasm (Wulczyn *et al.*, 1996; Baeuerle and Baltimore, 1996). NF-κB also regulates various other genes involved in a variety of human diseases like inflammation, acquired immune deficiency syndrome (AIDS), cancer, asthma, atherosclerosis, septic shock and arthritis. It is therefore a major target for drug development (Garg and Aggarawal, 2002). It has been reported that curcumin can interrupt directly in the binding of NF-κB to its consensus DNA sequences in vitro (Han *et al.*, 2002). However, no information on the site of interaction is reported yet.

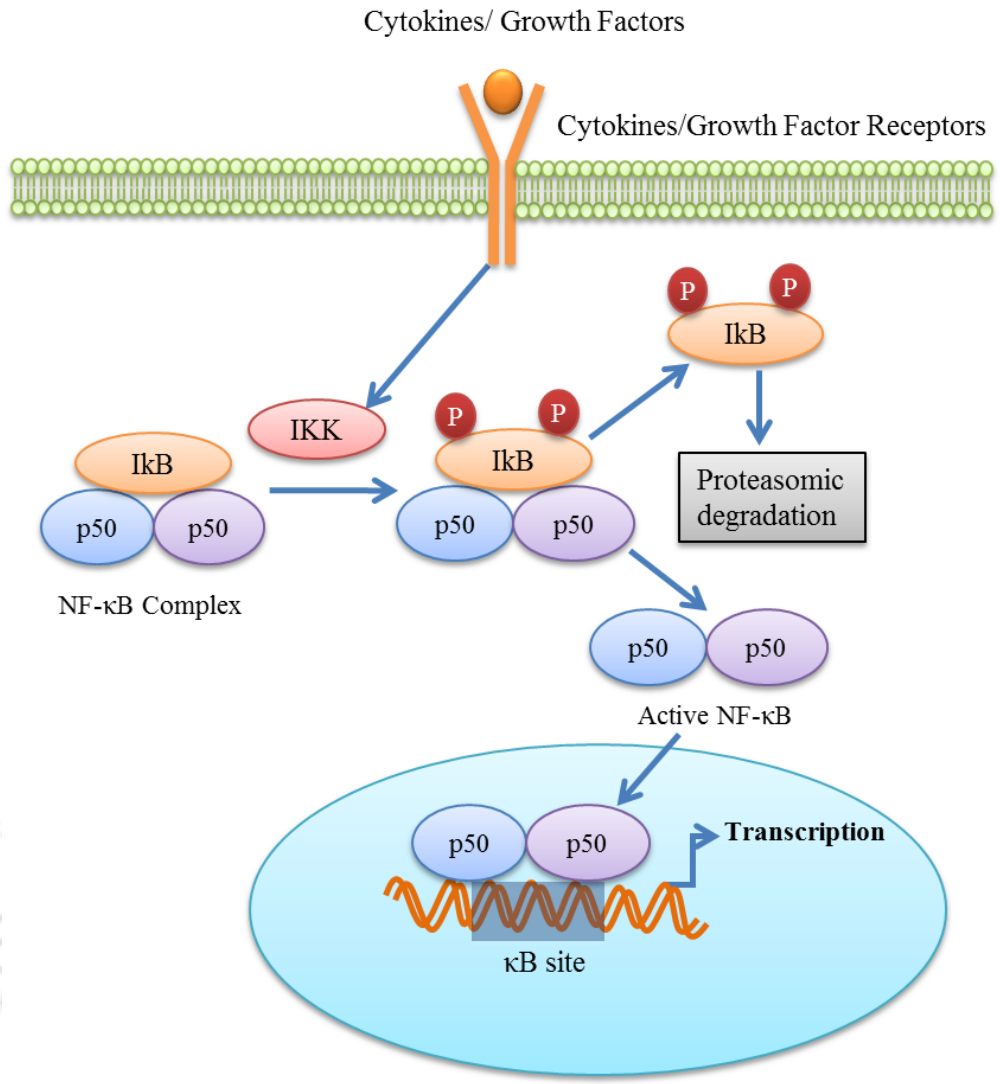


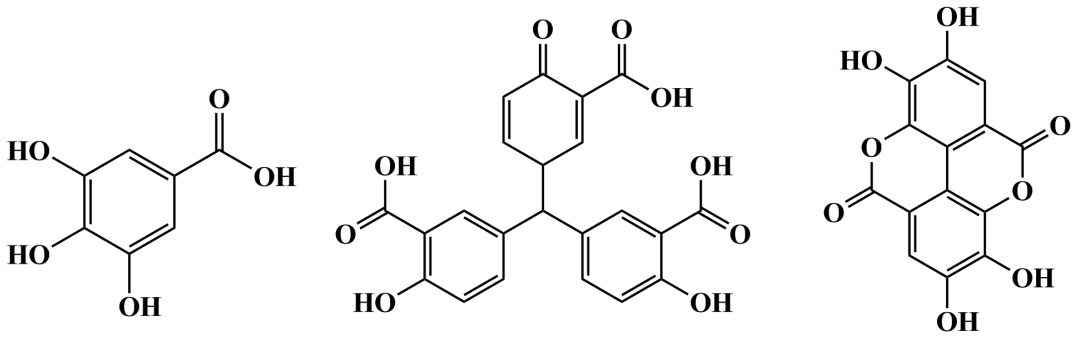
Figure 3.1 *NF-κB pathway.*

3.2 MATERIALS AND METHODS

3.2.1 Preparing small molecules

2D structures of curcumin derivatives (**Figure 1.2**) and known NF-κB inhibitors (**Figure 3.2**) were drawn by ACD/ ChemSketch (Freeware) and then converted to 3D structures in MDL Molfiles format. Using OpenBabel 2.2.3 MDL Molfiles were converted to Protein Data Bank (PDB) file format (Sharma *et al.*, 2000; Kim *et al.*, 2006; Edderkaoui *et al.*, 2008). Ligand preparation was done by assigning Gastegier charges, merging non-polar

hydrogens and saving it in PDBQT file format using AutoDock Tools (ADT) 1.5.4 (Sanner, 1999).



Gallic acid Aurine tricarboxilic acid Ellagic acid

Figure 3.2 *Structures of known NF-κB DNA binding inhibitors used in the docking studies.*

3.2.2 Preparing target molecule

X-ray crystal structure for NF-κB dimer complexed with DNA (PDB ID: 1NFK) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb). X-ray crystal structure for NF-κB dimer complexed with DNA shows that Arg56, Tyr57, Lys144, Lys241, Gln274, Arg305 and Gln306 form hydrogen bonds with DNA. The hydrophobic amino acids present nearby binding site in 5 Å region are Val58, Val142, Leu207, Ser240 and Pro243 which can show hydrophobic interactions. Using Swiss-PdbViewer 4.1, NF-κB p50 chain-A is selected and saved in PDB format leaving aside p50 chain-B and other hetero-atoms (water, ions, etc.). Gastegier charges were assigned to NF-κB p50 subunit and saved in PDBQT file format using ADT.

3.2.3 Molecular docking

Preparation of parameter files for grid and docking was done using ADT. Docking was performed with AutoDock 4.2.1 (Scripps Research Institute, USA) considering all the rotatable bonds of ligand as rotatable and protein as rigid (Morris *et al.*, 1998). Grid box size of 80 Å x 80 Å x 80 Å with 0.375 Å spacing was used that include not only the DNA binding region (DBR) but also significant portions of surrounding surface. Docking to macromolecule was performed using an empirical free energy function and Lamarckian Genetic Algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2500000 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80. Hundred independent docking runs were performed for each ligand and protein-ligand complex for lowest free energy of binding (ΔG) confirmation from the largest cluster was written and saved in PDBQT format. These PDBQT files have been converted to PDB file format using Swiss-PdbViewer. Docking results were analyzed using PyMOL 0.99 for possible polar and hydrophobic interactions.

Docking studies were performed at Intel(R) Core(TM) i3 CPU (3.2 GHz) with Linux-based operating system Fedora 8.

3.3 RESULTS AND DISCUSSIONS

To predict the inhibition of p50 subunit by curcumin and its derivatives, molecules were docked over p50 DNA binding region (DBR). The results of docking studies are summed up in **Table 3.1.**

Table 3.1 *Free energy of binding (ΔG) and predicted inhibition constant (KI) estimated with AutoDock 4.2.1 and Interaction of Inhibitors with NF-κB p50 subunit at DNA binding region*

Inhibitors	ΔG (kcal/mol)	KI(μM)	Polar interactions	Hydrophobic residue in 5 Å region
Curcumin sulphate	-8.94	0.28	Tyr57, His141,Asp239, Lys241, Lys272	Phe55, Met205, Leu207, Ala242, Phe307
Aurine tricarboxylic acid*	-7.83	3.56	Glu60, Lys241, Lys272	Phe55, Tyr57, Val142, Leu207, Ala242
Cyclocurcumin	-7.47	3.35	Val142, Lys144, Asp206, Leu207, Asp239	Phe55, Tyr57, Met205, Ala242
Curcumin glucuronide	-7.21	5.19	Val142, Lys144,Asp206, Leu207, Lys241	Tyr57, Cys59, Met205, Val209, Ala242
β-turmerone	-7.20	5.26	Ser63	Tyr57, Val58, Val112, Leu137,Ile139, Leu140
α-turmerone	-7.12	6.01	Ser63	Tyr57, Val58, Cys59, Pro62, Val112, Leu137, Ile139,Leu140
Curcumin (enol)	-7.05	6.82	Val142, Lys144,Asp206, Ser208	Tyr57,Val58, Cys59, Met205, Leu207, Val209
Bisdemethoxycurcumin	-6.90	8.78	Arg54, Glu60, His141,Lys144, Asp239	Phe55, Tyr57, Val142, Leu207, Ala242
Curcumin (keto)	-6.62	13.97	Arg54, Glu60,His141,Leu207,Lys241	Phe55, Tyr57, Met205, Ala242
Demethoxycurcumin	-6.40	20.27	His141, Lys144, Asp206, Leu207,Asp239, Lys241	Phe55, Tyr57,Val142, Val209, Ala242
Ellagic acid*	-6.38	21.13	Tyr57,Val58,Gly65, Ile139	Phe53, Cys59, Leu67, Val112, Leu137, Leu140
Hexahydrocurcumin	-5.92	45.63	Glu60, Lys144, Asp206, Leu207, Lys241	Phe55, Tyr57, Val142, Val209, Ala242
Gallic acid*	-5.85	51.46	Lys241, Asn247,Asp271, Lys272	Ala242, Ala245,Leu248
Hexahydrocurcuminol	-5.76	60.01	VAl54, Ser63, Leu111, Gly119, Ile139	Val112, Val120,Leu137, Leu140,Pro62
Tetrahydrocurcumin	-5.59	80.28	Glu60, His141,Lys144, Met205, Asp239, Lys241	Phe55, Tyr57, Leu207, Ala242

** Known NF-κB inhibitors*

Curcumin sulphate bound to DBR of p50 subunit with ΔG of -8.94 kcal/mol and predicted KI of 0.24 µM (**Figure 3.3**). The sulphate group of curcumin sulphate was found to form polar interaction with side chains of Lys241 and Lys272. Keto group formed polar interaction with side chains of Tyr57 and Asp239 while enol group is in the polar interaction range with His141.

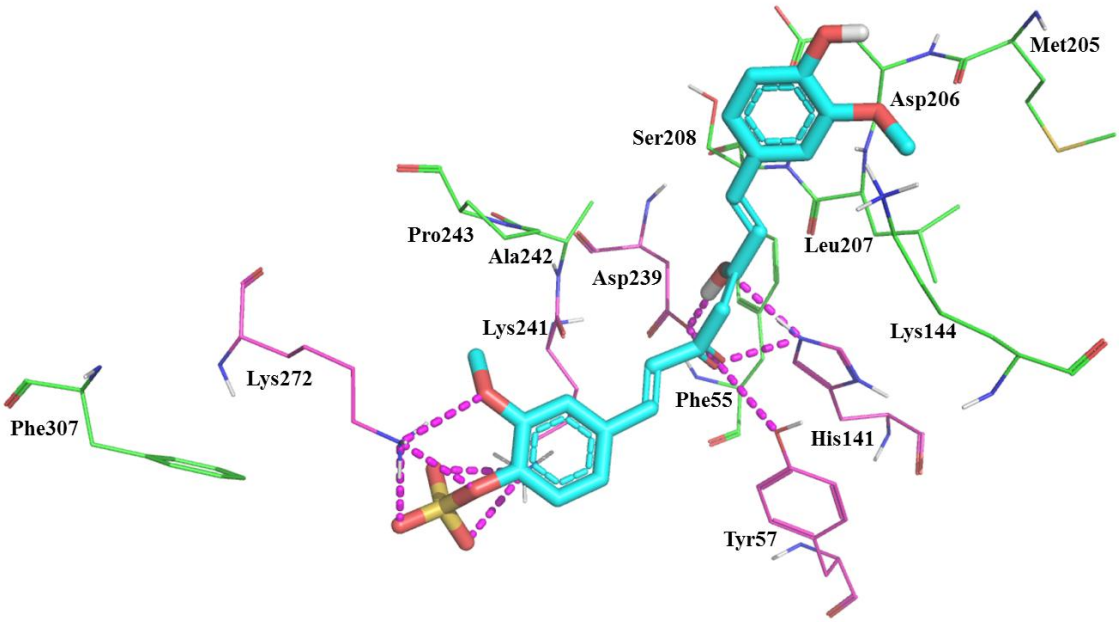


Figure 3.3 *Binding mode of curcumin sulphate showing polar interactions with Tyr57, His141, Asp239, Lys241 and Lys272 (magenta) at DBR of NF-κB p50 subunit.*

Cyclocurcumin bound to the DBR with ΔG of-7.47 kcal/mol and predicted KI of 3.35 µM (**Figure 3.4**). Oxygen of one phenyl group showed polar interaction with Asp239, while other phenyl group showed polar interaction with backbone nitrogen of both the Lys144 and Val142. The oxygen atom of α,β-unsaturated dihydropyranone moiety showed the interaction with Asp206 and Leu207. Curcumin glucuronide bound to

the DBR with ΔG = -7.21 kcal/mol and predicted KI of 5.19 µM. Oxygen of the o-methoxy group showed polar interaction with side chain of Asp206, while phenyl group showed polar interaction with backbone nitrogen of Leu207. Keto and enol group were having interaction with backbone nitrogen atom of Lys144 and backbone oxygen atom of Val142 respectively while glucuronide group was in polar interaction range with side chain of Lys241.

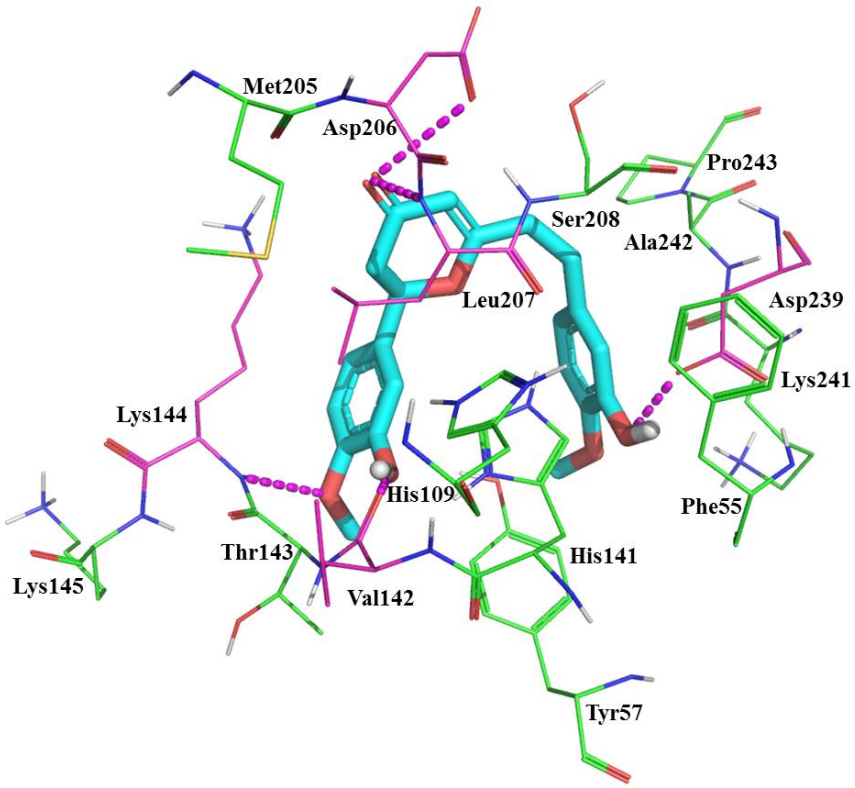


Figure 3.4 *Binding mode of cyclocurcumin showing polar interactions with Val142, Lys144, Asp206, Leu207 and Asp239 (magenta) at DBR of NF-κB p50 subunit.*

We observed that α-turmerone and β-turmerone which are structurally similar except the presence of methyl and methylene group in aromatic ring, keto group of both showed polar interaction with Ser63. α-turmerone bound to DBR with ΔG of -7.12 kcal/mol and predicted KI = 6.01 µM while β-turmerone with ΔG of -7.20 kcal/mol and

predicted KI of 5.26 µM. Curcumin (enol) bound at DBR with ΔG of -7.05 kcal/mol and predicted KI of 6.82 µM (**Figure 3.5**). Oxygen of one phenyl group was having polar interaction with side chain of Asp206 and Ser208. Keto and enol group were having interaction with backbone nitrogen atom of Lys144 and backbone oxygen atom of Val142 respectively.

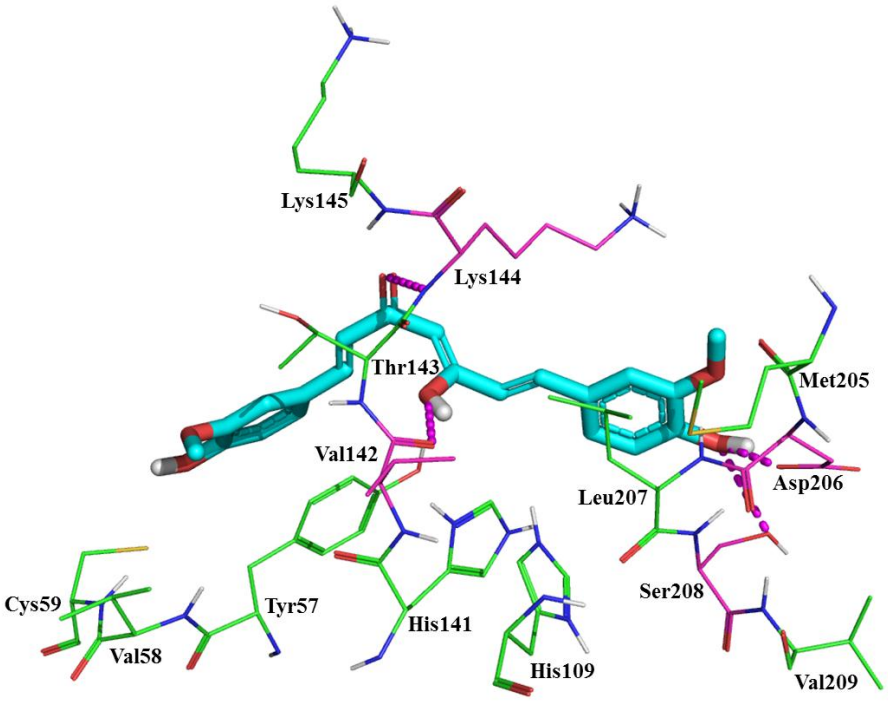


Figure 3.5 *Binding mode of curcumin (enol) showing polar interactions with Val142, Lys144, Asp206 and Ser208 (magenta) at DBR of NF-κB p50 subunit.*

We observed that most of the curcumin derivatives formed polar interactions with Lys144, Asp206, Asp239, Leu207 and Lys241 amongst which Lys144 and Lys241 were the key residues which form hydrogen bond with consensus DNA sequence of κB site suggesting that curcumin derivatives interfere in binding of NF-κB to κB site by interacting with Lys144 and Lys241 at DBR (Jutooru *et al.*, 2010). To compare the

inhibition of NF-κB by curcumin derivatives with known inhibitors, aurine tricarboxylic acid, gallic acid and ellagic acid were docked over p50 subunit out of which aurine tricarboxylic acid showed best binding mode with ΔG of -7.83 kcal/mol and predicted KI= of 3.56 µM (**Figure 3.6**). Amongst curcumin derivatives and known inhibitors thus docked over NF-κB curcumin sulphate showed the best binding mode with lowest free energy of binding while cyclocurcumin, Curcumin glucuronide, β-turmerone, α-turmerone, and curcumin (enol) were the other derivatives with satisfying performance.

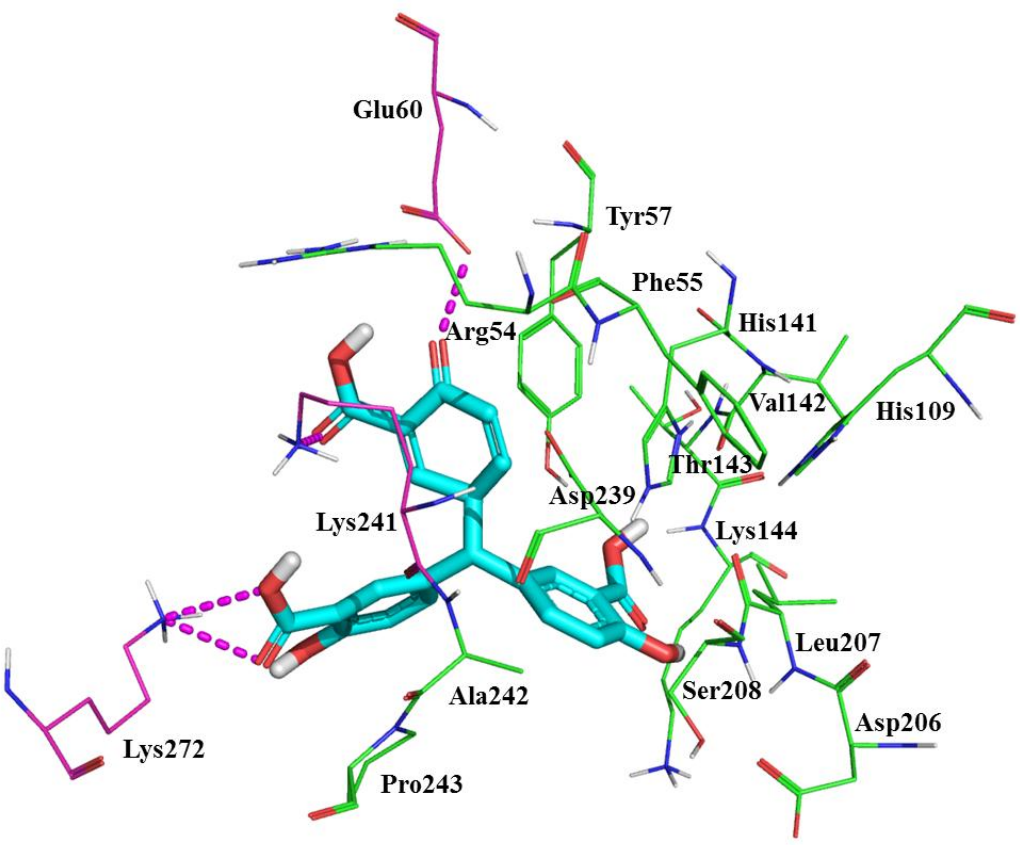


Figure 3.6 *Binding mode of aurine tricarboxilic acid showing polar interactions with Glu60, Lys241 and Lys272 (magenta) at DBR of NF-κB p50 subunit.*

Physicochemical properties were summed up using Molinspiration Property Calculator in **Table 3.2** to evaluate the drug likeness of the inhibitors. According to

Lipinski's Rule of Five, for a drug to show good ADME (absorption, distribution, metabolism, and excretion) properties, its logP should be less than 5, hydrogen bond donor should not be more than 5, hydrogen bond acceptor should not be more than 10 and molecular weight should be less than 500 (Lipinski *et al.*, 2001). All the inhibitors follow Lipinski's Rule of Five except curcumin glucuronide which has molecular weight of 544.509, 6 hydrogen bond donors and 12 hydrogen bond acceptors. For good cell membrane permeability, a molecule should have topological polar surface area (TPSA) less than 140 Å2 (Veber *et al.*, 2002). Curcumin glucuronide has high TPSA of 192.446 hence predicted cell membrane permeability is suboptimal. Curcumin sulphate is having TPSA of 142.428 Å2 hence moderate predicted cell membrane permeability. The remaining inhibitor molecules have TPSA values bellow 140 Å2 and are predicted to have good cell membrane permeability.

Table 3.2 *Physicochemical properties of curcumin derivatives*

Inhibitors	LogP	TPSA ($Å^2$)	Molecular Weight	H-bond acceptors	H-bond Donors	Violations (Lipinski rule)
Curcumin sulphate	-0.444	142.428	447.441	9	2	0
Cyclocurcumin	2.998	85.229	370.401	6	2	0
Curcumin glucuronide	0.91	192.446	544.509	12	6	3
β-turmerone	3.542	17.071	220.356	1	0	0
α-turmerone	3.756	17.071	220.356	1	0	0
Curcumin (enol)	3.048	96.223	368.385	6	3	0
Bisdemethoxycurcumin	3.411	77.755	308.333	4	3	0
Curcumin (keto)	2.303	93.066	368.385	6	2	0
Demethoxycurcumin	3.23	86.989	338.359	5	3	0
Hexahydrocurcumin	2.43	96.223	374.433	6	3	0
Hexahydrocurcuminol	2.613	99.38	376.449	6	4	**0**
Tetrahydrocurcumin	2.99	96.223	372.417	6	3	0

3.4 CONCLUSIONS

The present *in-silico* study provides insights into the inhibition of NF-κB p50 subunit by curcumin and its natural derivatives. The involvement of residues like Lys144, Asp206, Asp239, Leu207 and Lys241 seems to play an important role in binding of curcumin and its natural derivatives to the DBR. Curcumin sulphate was predicted to be the most potent inhibitor amongst all the derivatives and known inhibitors (aurine tricarboxylic acid, gallic acid and ellagic acid) with favorable ADME predictions.

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CHAPTER 4

In-silico studies on inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids

4.1 INTRODUCTION

Mammalian signal transducers and activators of transcription (STAT) is a family of 7 transcription factors (Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b and Stat6) (Bromberg, 2000). These transcription factors are activated in response to cytokines and growth factors including interferons (IFNs), epidermal growth factor (EGF), interleukin 5 (IL5), IL6, hepatocyte growth factor (HGF), leukemia inhibitory factor (LIF) and bone morphogenetic protein 2 (BMP2) which regulate various genes involved in cell growth, proliferation, cell survival and other biological processes (Darnell, 2002; Yu and Jove, 2004). The transcription factors of this family are activated by growth factor receptor tyrosine kinases, Janus kinases or Src family kinases through the phosphorylation of a critical tyrosine residue which leads to the dimerization of two phosphorylated monomers (Bromberg and Darnell, 2000; Bowman *et al.*, 2000). Phosphorylated dimers are translocated to the nucleus where they bind to specific DNA-response elements in the promoter region of target genes, and induce gene expression (Turkson and Jove, 2000; Buettner *et al.*, 2002) (**Figure 4.1**). It has been found that constitutive activation of certain STAT family members, particularly of Stat3 promote dysregulated growth, survival and immune responses which contribute to tumor progression and carcinogenesis (Turkson, 2004; Darnell, 2005). Stat3 dimerization relies on the reciprocal binding of Src Homology (SH2) domain-binding peptide (Pro-pTyr-Leu-Lys-Thr-Lys) of one monomer to another (Shuai *et al.*, 1994; Turkson *et al.*, 2001). It is a critical step in Stat3 activation which presents an attractive target to abrogate Stat3 DNA-binding and to inhibit its aberrant transcriptional activity (Turkson *et al.*, 2004). Interest in development of small molecule and peptide based inhibitors of Stat3 dimerization in the last few years has led to the discovery of inhibitors like Stattic, Sta21 and FLLL32 (McMurray, 2006; Schust *et al.*, 2006).

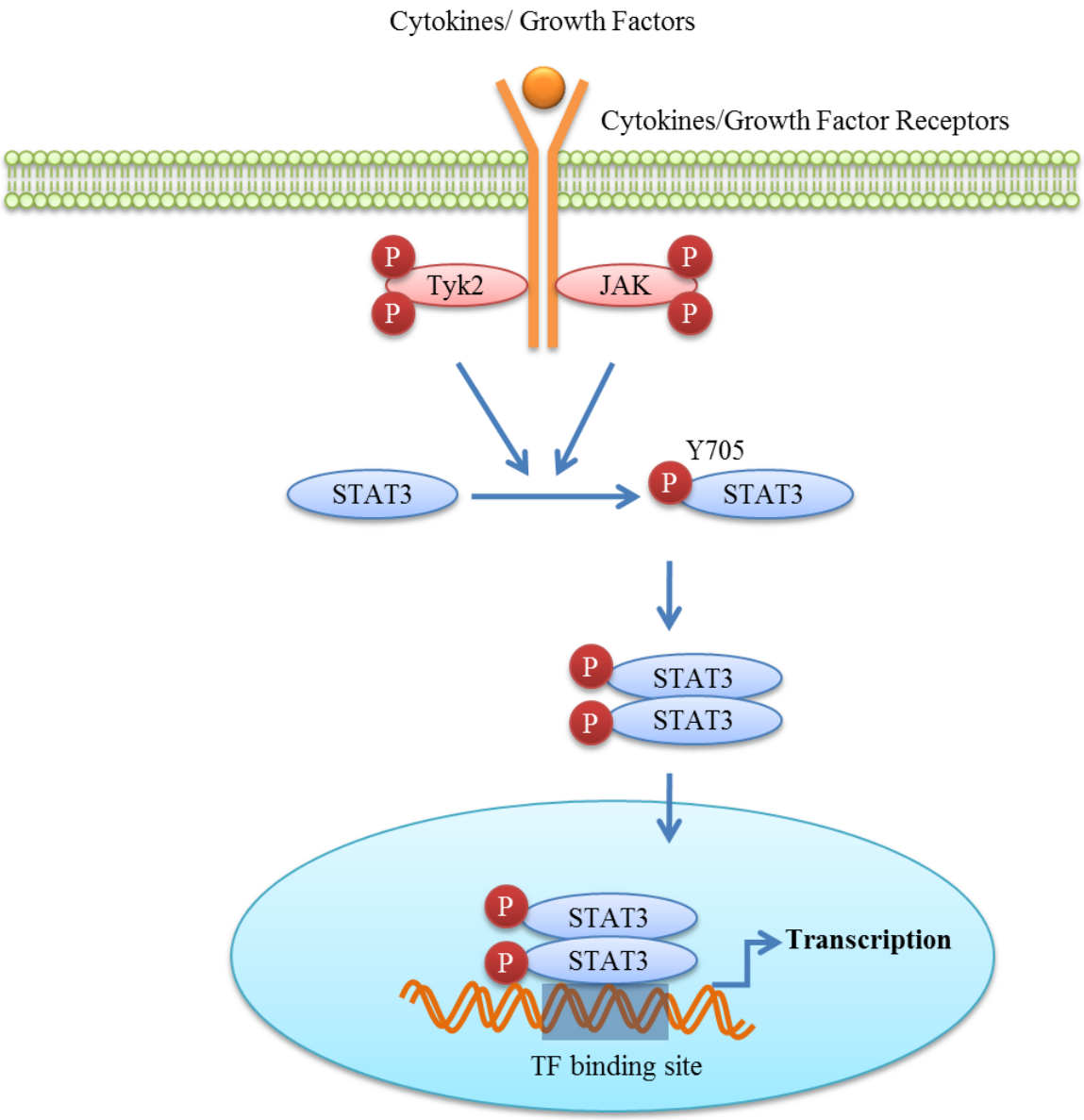


Figure 4.1 *Stat3 pathway.*

Curcumin has been reported to inhibit the Stat3 phosphorylation and DNA binding activity in human cancer cells (Bharti *et al.*, 2003; Alexandrow *et al.*, 2011; Fossey *et al.*, 2011; Bill *et al.*, 2010). In order to improve the pharmacological properties, curcumin was conjugated with various functional groups. Curcumin-amino acids conjugates were also synthesized using different substitution schemes (**Figure 4.2**) which

were tested for antioxidant, antimicrobial, antiviral, antiproliferative and proteasome inhibition activities (Singh *et al.,* 2010; Dubey *et al.,* 2008; Parvathy *et al.,* 2010; Rai *et al.,* 2008; Wan *et al.,* 2010).

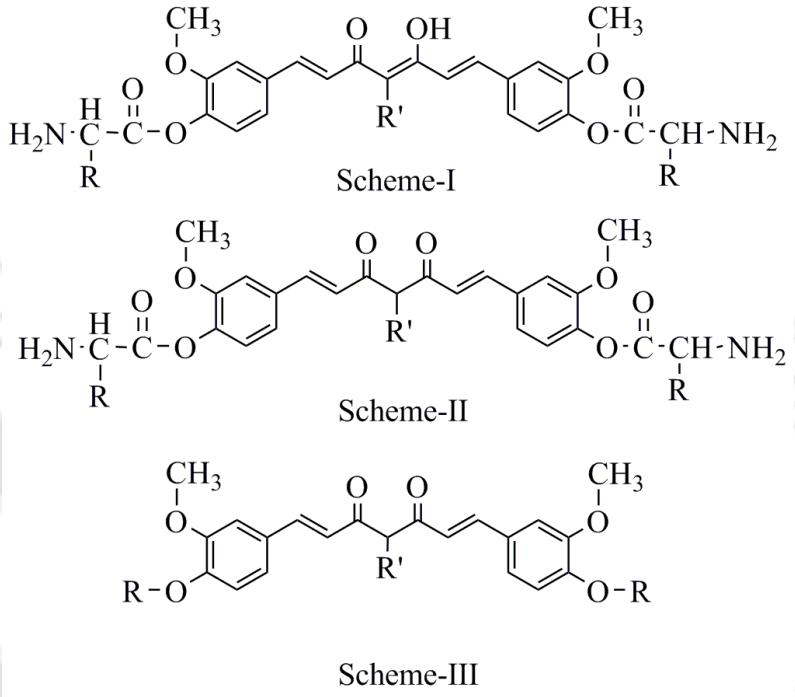
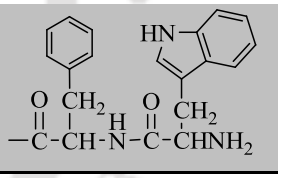


Figure 4.2 *Schemes used for curcumin-amino acid conjugates synthesis by various groups.*

In the present study, we investigate the interaction of curcumin natural derivatives and its conjugates with amino acids (**Table 4.1**) in the pursuance of potential lead molecule for inhibition of Stat3 dimerization using molecular docking over the SH2 domain of a Stat3 monomer (Shao *et al.,* 2004; Song *et al.,* 2005).

Table 4.1 *Curcumin-amino acid conjugates used in the study.*

Compound name	R=
Scheme –I (R'=H) [Parvathy et al., 2010]	
1,7-Bis(4-O-L-leucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH_2CH(CH_3)CH_3$
1,7-Bis(4-O-L-phenylalaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH_2C_6H_5$
1,7-Bis(4-O-L-alaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—CH_3
1,7-Bis(4-O-L-isoleucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH(CH_3)CH_2CH_3$
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH(CH_3)CH_3$
1,7-Bis(4-O-L-serinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—CH_2OH
1,7-Bis(4-O-L-cysteinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—CH_2SH
1,7-Bis(4-O-L-phenylglycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—C_6H_5
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—H
1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH_2CH_2CH_2$— (part of pyrrolidine ring)
Scheme –II (R'=H) [Dubey et al., 2008]	
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	—H
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	—$CH(CH_3)CH_3$
1,7-Bis(4-O-L-glutamatoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	—CH_2CH_2COOH
Scheme –II (R'=$COCH_2NH_2$) [Kumar et al.,2000]	
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-4-glycyl-1,6-heptadiene-3,5-dione	—H
Scheme –III (R'=H) [Singh et al., 2010]	
1,7-Bis(4-O-L-tryptophanylphenylalaninoyl-3-methoxyphenyl)- 1,6-heptadiene-3,5-dione	

4.2 MATERIALS AND METHODS

4.2.1 Preparing small molecules

Curcumin natural derivatives shown in **Figure 1.2**, its conjugates with amino acids and known Stat3 dimerization inhibitors (**Figure 4.3**) were drawn and 3D optimized by MarvinSketch (Free Academic License) and saved in Protein Data Bank (PDB) file format. These small molecules were prepared for molecular docking by merging non-polar hydrogens, assigning Gastegier charges, and saving them in PDBQT file format using AutoDock Tools (ADT) 1.5.6 (Sanner, 1999).

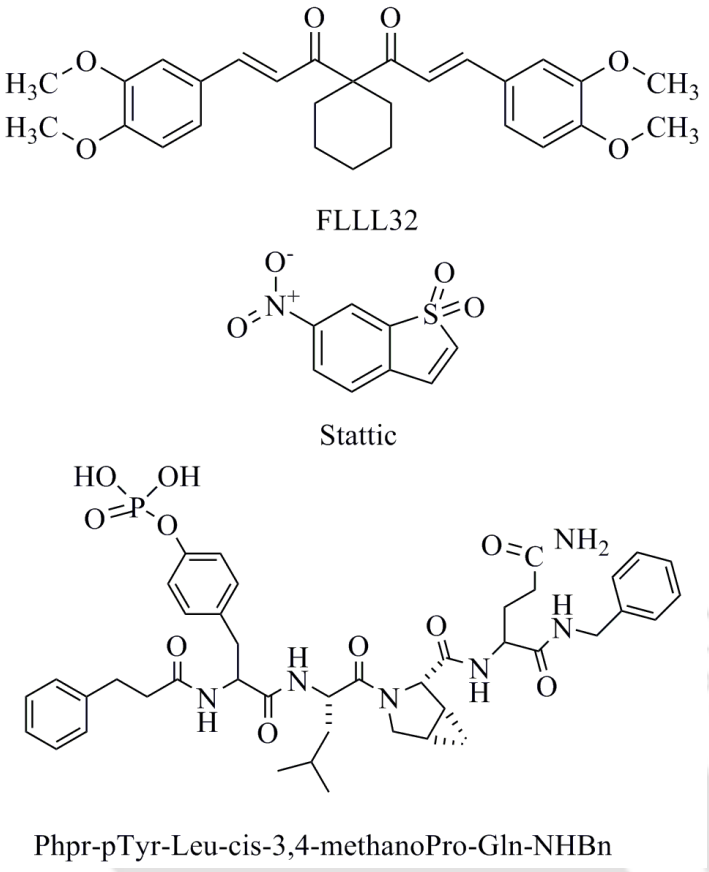


Figure 4.3 *Structures of known Stat3 activation and dimerization inhibitors.*

4.2.2 Preparing target molecule

To investigate the interaction of curcumin natural derivatives and its amino acid conjugates, to identify potent lead molecule for Stat3 inhibition, X-ray crystal structure of Stat3β complexed with DNA (PDB ID: 1BG1) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb) (Becker *et al.*, 1998). For molecular docking, DNA and other hetero-atoms (water, ions, etc.) were removed using PyMOL 0.99, Gasteiger charges were assigned and macromolecule was saved in PDBQT file format using ADT.

4.2.3 Molecular docking

Grid and docking parameter files were prepared using ADT and molecular docking was performed with AutoDock 4.2.1 (Scripps Research Institute, USA) considering all the rotatable bonds of small molecules as rotatable and macromolecule as rigid (Morris *et al.,* 1998). Grid box size of 80 x 80 x 80 Å with 0.375 Åspacing was selected that include the whole SH2 dimerization domain of Stat3 monomer. Empirical-free energy function and Lamarckian Genetic Algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2,500,000 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80 were used to perform molecular docking with macromolecule. Hundred independent docking runs were performed for each small molecule. Protein– small molecule complex for lowest free energy of binding (ΔG) confirmation from the largest cluster was saved in PDBQT format and converted to PDB file format using UCSF Chimera 1.6.1. Docking results were analyzed using PyMOL 0.99 for possible polar and hydrophobic interactions.

Docking studies were performed at Intel(R) Xeon(R) CPU (3.2 GHz) with Linux-based operating system Fedora 15.

4.3 RESULTS AND DISCUSSIONS

Structural analysis of Stat3 showed that it contains four domains, protein interaction domain which helps in cooperative DNA binding, all-alpha domain comprising of a bundle of four antiparallel helices connected by short loops, DNA binding domain comprising of eight-stranded b-barrel and SH-2 dimerization domain comprising of a central three-stranded b-pleated sheet flanked by a helix and two strands (**Figure 4.4**) (Becker *et al.,* 1998; Ren *et al.,* 2009).

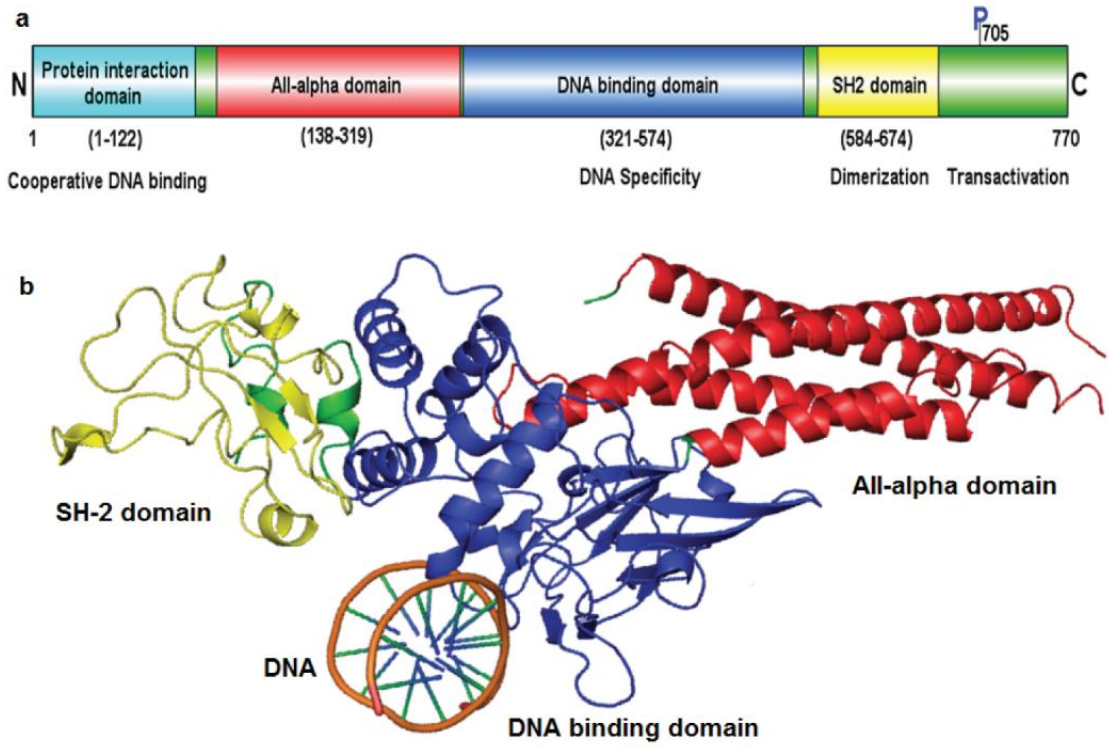


Figure 4.4 *Structure of Stat3 protein.* ***(a)*** *Domain boundaries are shown.* ***(b)*** *Ribbon diagram of the Stat3 monomer-DNA complex (PDB: 1BG1) showing DNA binding domain, SH2-domain and all-alpha domain.*

To predict the inhibition of Stat3 dimerization by curcumin natural derivatives and its amino acid conjugates, these small molecules were docked over SH2 domain of a Stat3 monomer and the results were summarized in **Table 4.2 & 4.3** respectively.

Table 4.2 *Free energy of binding (ΔG) and predicted inhibition constant (KI) estimated with AutoDock 4.2.1 and interactions of curcumin natural derivatives with Stat3 monomer*

Compound name	ΔG (kcal/mol)	KI (μM)	Polar interactions	Hydrophobic residue in 5 Å region
Demethoxycurcumin	-7.80	1.93	Lys591, Arg595, Glu612,Ser613, Ser636	Phe610, Ile634, Val637
Hexahydrocurcuminol	-7.69	2.31	Lys591, Arg595, Glu612, Ser613, Ser636	Phe610, Ile634, Val637
Hexahydrocurcumin	-7.32	4.39	Lys591, Arg595, Glu612,Ser613, Ser636	Phe610, Ile634, Val637
Bisdemethoxycurcumin	-7.19	5.39	Lys591, Arg595, Ser611,Glu612, Ser613, Ser636	Phe610, Ile634, Val637
Curcumin sulphate	-7.11	6.16	Lys557, Ile634	Phe610, Val619,Val637
Curcumin (keto)	-7.09	6.31	Lys591, Arg595, Arg609,Glu612, Ser613,Ser636	Phe610, Ile634, Val637
Curcumin (enol)	-6.94	8.16	Lys591, Arg595, Arg609, Glu612	Phe610, Ile634, Val637
Flll32*	-6.69	12.54	Lys591, Arg609	Phe610, Ile634, Val637
Sta21*	-6.61	14.25	Arg609, Ser636	Ile634, Val637
Tetrahydrocurcumin	-6.49	17.56	Lys591, Arg595, Arg609, Ser613, Ser636	Phe610, Ile634, Val637
Stattic*	-6.45	18.79	Lys591, Arg595	Ile634
Cyclocurcumin	-6.42	19.82	Lys591, Arg609, Gln635	Phe610, Ile634, Val637
Curcumin glucuronide	-5.97	42.40	Lys591, Arg595, Arg609,Ile634	Phe610, Val637
β-Turmerone	-5.39	112.11	Lys591	Ile634, Val637
α-Turmerone	-5.31	127.61	Lys591	Ile634, Val637

**Known inhibitor of Stat3*

Table 4.3 *Free energy of binding (ΔG) and predicted inhibition constant (KI) estimated with AutoDock 4.2.1 and interactions of curcumin-amino acid conjugates with Stat3 monomer*

Compound name	ΔG (kcal/mol)	KI (μM)	Polar interactions	Hydrophobic residue in 5 Å region
1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-6.29	24.55	Glu530, Lys557,Val637	Phe610,Val619
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-5.96	42.93	Glu530,Lys591, Arg595, Ser636	Ile634, Val637
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	-5.51	91.60	Lys591, Arg609,Ile634, Glu638	Phe610,Val637
Phpr-pTYR-LEU-cis-3,4-methanoPRO-GLN-NHBn*	-5.50	93.10	Lys591, Arg595, Arg609	Phe610, Ile634, Val637, Tyr657
1,7-Bis(4-O-L-isoleucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-5.32	125.58	Glu530, Lys557,Ser590, Ser636	Val637
1,7-Bis(4-O-L-tryptophanylphenylalaninoyl-3-methoxyphenyl)- 1,6-heptadiene-3,5-dione	-5.17	162.04	Lys557,Lys591, Glu592, Ser613,Glu638	Phe610, Ile634,Val637, Tyr640
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-4-glycyl-1,6-heptadiene-3,5-dione	-5.00	216.91	Lys591, Arg609,Ser613, Glu638	Phe610,Ile634, Val637
1,7-Bis(4-O-L-phenylalaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.97	226.37	Glu530, Lys557,Ser636, Val637	Phe610, Ile634
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	-4.89	261.36	Glu592, Arg595,Ser636	Ile634, Val637
1,7-Bis(4-O-L-leucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.79	307.46	Glu530, Arg595,Ser636	Ile589, Ile634, Val637
1,7-Bis(4-O-L-alaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.77	317.43	Lys557,Glu612, Glu638	Phe610,Val637
1,7-Bis(4-O-L-serinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.62	407.78	Glu530, Gly558,Ser590, Glu592,Arg593, Glu612	Phe559
1,7-Bis(4-O-L-glutamatoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	-4.57	448.31	Lys557,Lys591, Arg609, Glu638	Phe610,Val637
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.17	871.38	Glu530, Ser636	Leu528, Ile634, Val637
1,7-Bis(4-O-L-phenylglycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-3.75	1790.00	Lys591, Gln635,Ser636	Leu532, Ile634, Val637
1,7-Bis(4-O-L-cysteinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-3.62	2210.00	Ser613, Lys615,Glu616, Ser636	Val619, Ile634, Val637, Tyr640

**Known inhibitor of Stat3*

Demethoxycurcumin bound to SH2 domain with ΔG of -7.80 kcal/mol and KI of 1.93 µM (**Figure 4.5**). Methoxy group of demethoxycurcumin was found to form polar interaction with side chain of Arg595 while the neighboring hydroxyl group was in polar interaction range with main chain of Lys591 and side chain of Arg595. Hydroxyl group present at the other side of the molecule (methoxy group lacking) formed polar interactions with Ser613. Both keto and hydroxyl group present in the linker region were in polar interaction range with Ser636.

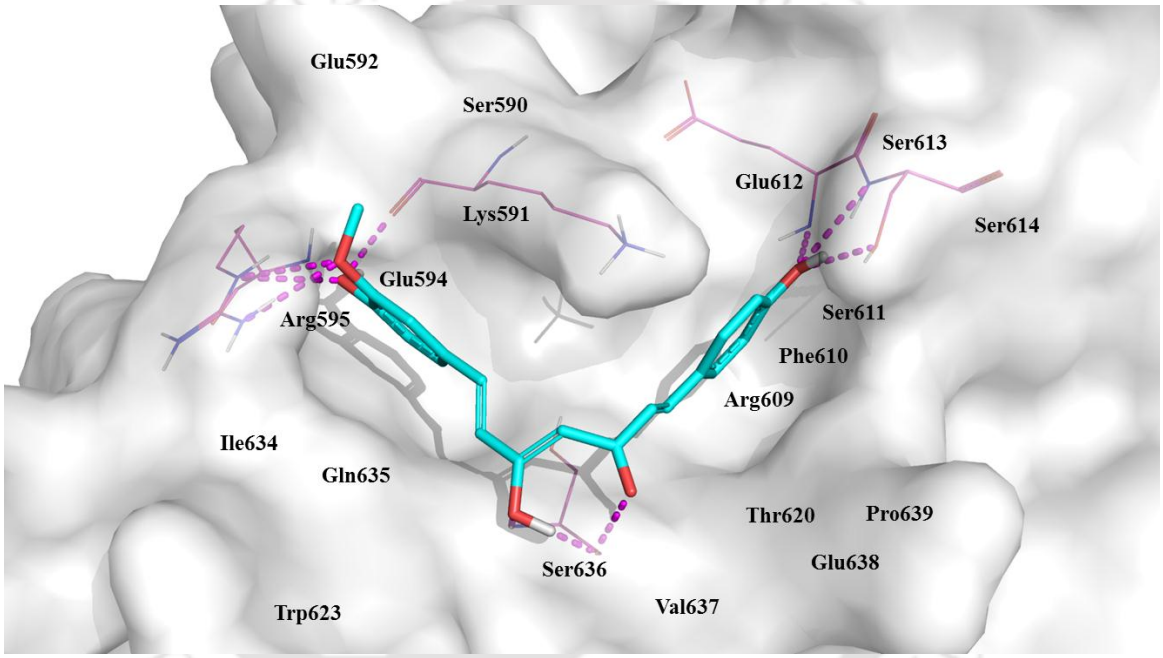


Figure 4.5 *Binding mode of demethoxycurcumin showing polar interactions with Lys591, Arg595, Glu612, Ser613 and Ser636 (magenta) at SH2 domain of a Stat3 monomer.*

It was found that hexahydrocurcuminol bound to SH2 domain with ΔG of -7.69 kcal/mol and KI of 2.31 µM (**Figure 4.6**). One methoxy group of hexahydrocurcuminol was found to form polar interaction with side chain of Arg595 while the neighboring hydroxyl group was in polar interaction range with Lys591 and Arg595. At the other side of the molecule, methoxy group formed polar interactions with Ser613 while hydroxyl

group was in polar interaction range with Glu612 and Ser613. In the linker region, one of the hydroxyl group formed polar interaction with Ser636 while other interacted with side chain of Lys591.

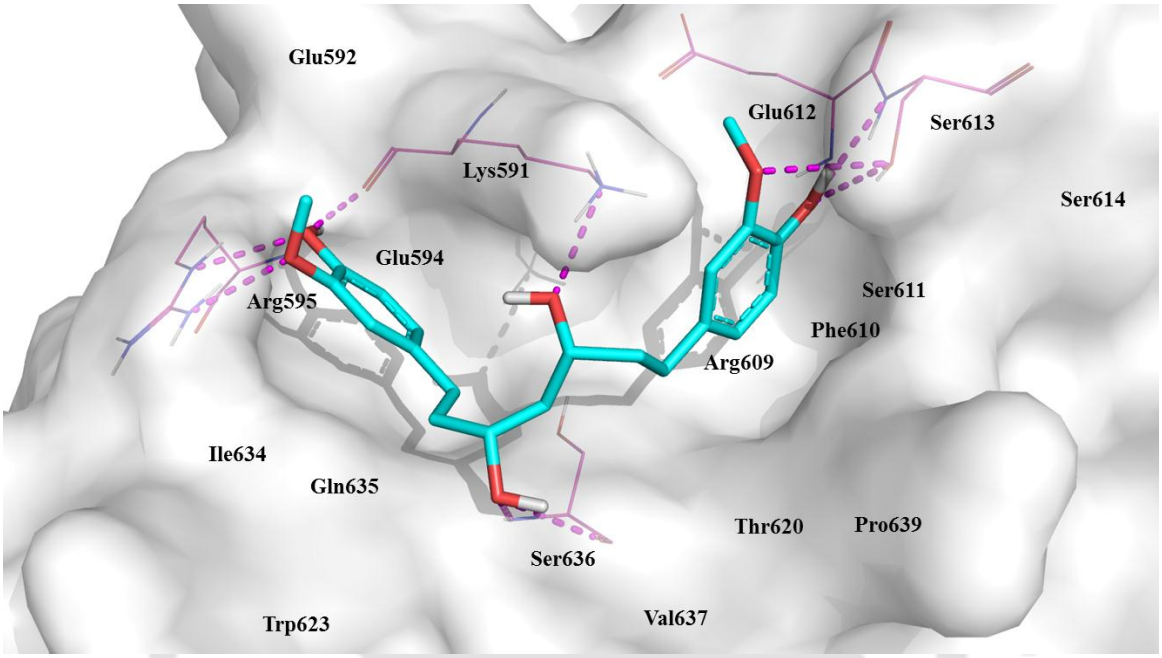


Figure 4.6 *Binding mode of hexahydrocurcuminol showing polar interactions with Lys591, Arg595, Glu612, Ser613 and Ser636 (magenta) at SH2 domain of a Stat3 monomer.*

In contrast to hexahydrocurcuminol, hexahydrocurcumin bound to SH2 domain with ΔG of -7.32 kcal/mol and KI of 4.39 μM (**Figure 4.7**). One methoxy group of hexahydrocurcumin formed polar interaction with side chain of Arg595 while the neighboring hydroxyl group was in polar interaction range with Lys591 and Arg595. Methoxy group at the other side of the molecule formed polar interactions with side chain Lys591 while nearby hydroxyl group was in polar interaction range with Glu612 and Ser613. Hydroxyl group of linker region formed polar interaction with Ser636 while keto group interacted with side chain of Lys591. Amongst known inhibitors FLLL32, static and sta21, FLLL32 bound to SH2 domain with lowest ΔG of -6.69 kcal/mol and KI of

12.54 µM (**Figure 4.8**). Keto groups present in the linker region were found to form polar interactions with Lys591 and Arg609 respectively.

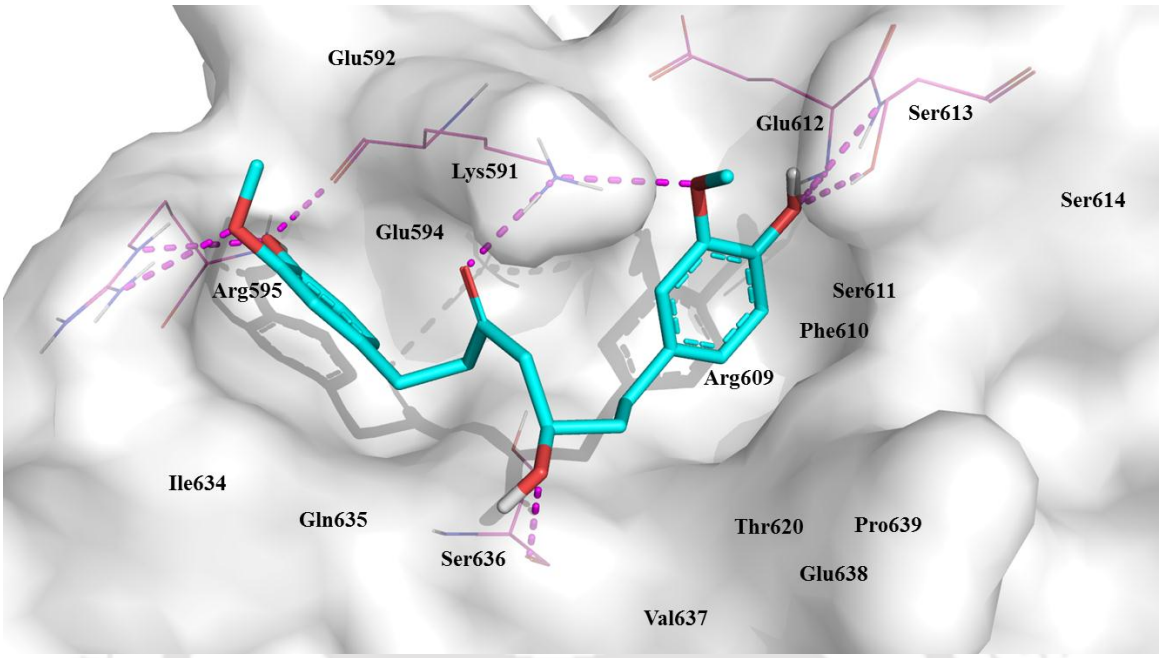


Figure 4.7 *Binding mode of hexahydrocurcumin showing polar interactions with Lys591, Arg595, Glu612, Ser613 and Ser636 (magenta) at SH2 domain of a Stat3 monomer.*

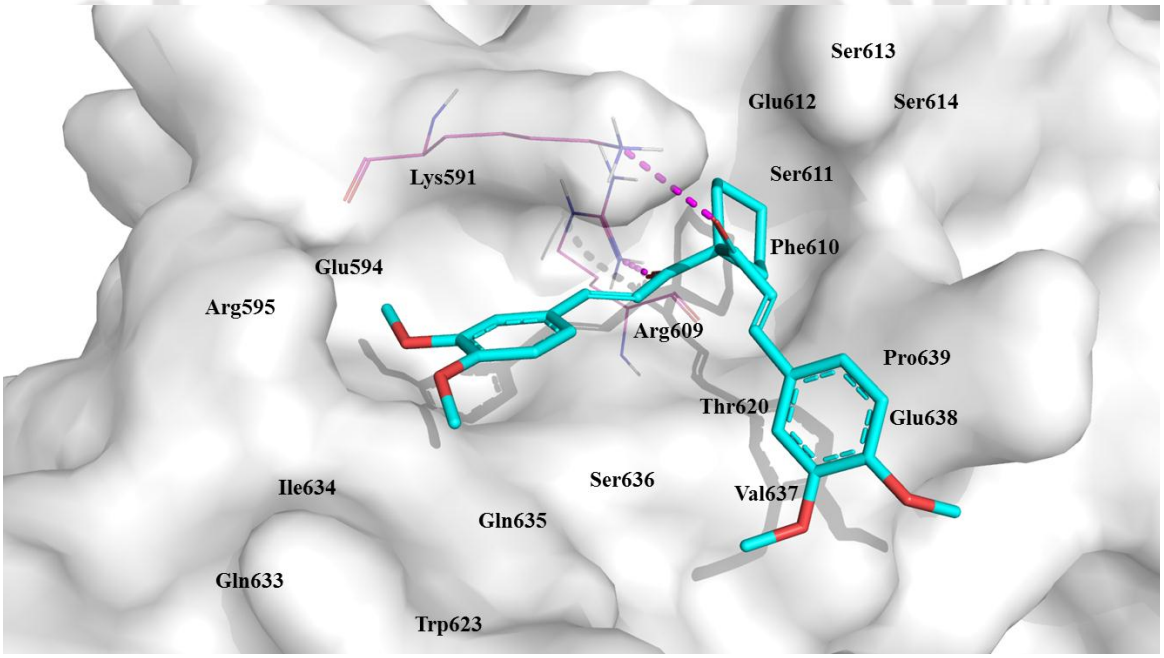


Figure 4.8 *Binding mode of FLLL32 showing polar interactions with Lys591 and Arg609 (magenta) at Stat3 SH2 domain of a Stat3 monomer.*

Amongst the curcumin-amino acid conjugates, curcumin-proline conjugate (1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) docked with lowest ΔG of -6.29 kcal/mol and KI of 24.55 µM (**Figure 4.9**). Prolinoyl group at one side of the small molecule formed polar interaction with Glu530 while at other side it interacted with Val637. The hydroxyl group present in linker region of the conjugate formed polar interaction with Lys557. The peptide based known inhibitor (Phpr-pTyr-Leu-cis-3,4-methanoPro-Gln-NHBn) docked with ΔG of -5.50 kcal/mol and KI of 93.10 µM and formed polar interactions with Lys591, Arg595 and Arg609.

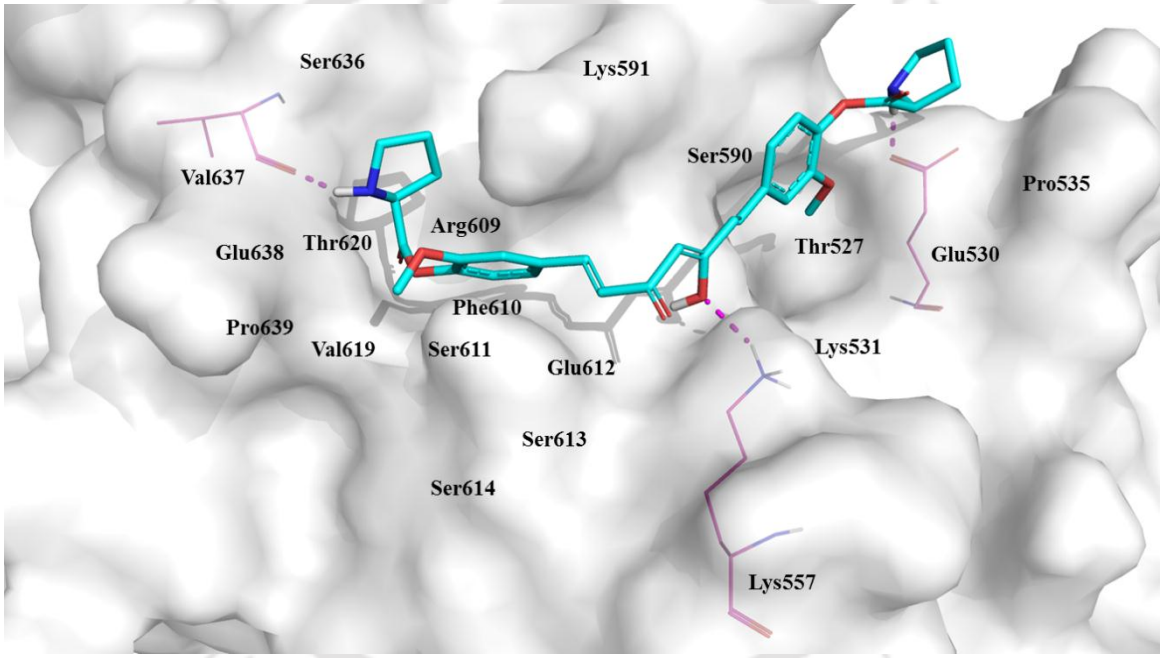


Figure 4.9 *Binding mode of curcumin-proline conjugate (1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) showing polar interactions with Glu530, Lys557 and Val637 (magenta) at Stat3 SH2 domain of a Stat3 monomer.*

Curcumin natural derivatives and its amino acid conjugates bound to SH2 domain through polar interactions with Lys591, Arg609, Ser611, Glu612, Ser613, Ser636 and Val637 among which Lys591, Arg609, Ser611 and Ser613 are the amino acid residues

which remain highly conserved in SH2 domain and play an important role in Stat3 dimerization by forming polar interaction with pTyr-705 residue of other monomer.

4.4 CONCLUSIONS

The present computational study provides insights into the inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids. The involvement of residues like Lys591, Arg609, Ser611, Glu612, Ser613, Ser636 and Val637 play an important role in binding of curcumin natural derivatives and its amino acid conjugates with SH2 domain. Demethoxycurcumin, hexahydrocurcuminol followed by hexahydrocurcumin were predicted to be the most potent inhibitors amongst all the curcumin natural derivatives and known inhibitors (FLLL32, Sta21 and Stattic) docked. Amongst the curcumin-amino acid conjugates curcumin-proline conjugate (1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) was predicted to be the most potent inhibitor of Stat3 dimerization.

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CHAPTER 5

Computational studies on inhibition of Jun-Fos-DNA complex formation by curcumin natural derivatives

5.1 INTRODUCTION

Activator protein-1 (AP1) is a transcription factor consisting of either homo- or heterodimers of the Jun and Fos family proteins (Eferl and Wagner, 2003). It regulates gene expression in response to a variety of stimuli, including environmental stresses, UV radiation, cytokines, and growth factors. AP1 in turn controls a number of cellular processes including proliferation, transformation, inflammation and innate immune response. The Jun and Fos proteins share similar amino acid sequences that comprise the basic DNA binding sequence and the adjacent leucine zipper region by which these proteins dimerize (Curran and Franza, 1988; Johnson and McKnight, 1989; Mitchell and Tjian, 1989). The AP1 transcription factor binds specifically to 12-O-tetradecanoylphorbol-13-acetate (TPA) responsive element 5'-TGAG/CTCA-3' which is commonly referred to as the AP1 site (Lee *et al.,* 1987; Angel *et al.,* 1987) (**Figure 5.1**). C-fos and c-jun genes are autoregulated, the transcription of c-jun is stimulated by its own product and in contrast c-fos is negatively autoregulated (Angel *et al.,* 1988; Angel *et al.,* 1991; Sassone-Corsi and Verma, 1987). AP1 has been found constitutively active in many cancers including breast, ovarian, cervical and lung. Numerous studies have shown that inhibition of AP1 has a profound effect on the behavior of cancer cells and tumors suggesting that AP1 could be a promising target for cancer therapy (Shaulian and Karin, 2002).

Curcumin has been reported to have anti-inflammatory, anti-oxidant and anticancer effects (Ammon and Wahl, 1991, Srimal and Dhawan, 1973; Sharma, 1976; Toda *et al.,* 1985; Satoskar *et al.,* 1986). In vivo administration of curcumin reduced the incidence and size of tumors in mice (Conney *et al.,* 1991; Huang *et al.,* 1992; Huang *et al.,* 1994; Rao *et al.,* 1995). Moreover, curcumin was reported to inhibit proliferation and cell cycle progression in cancer cells (Mohan *et al.,* 2000).

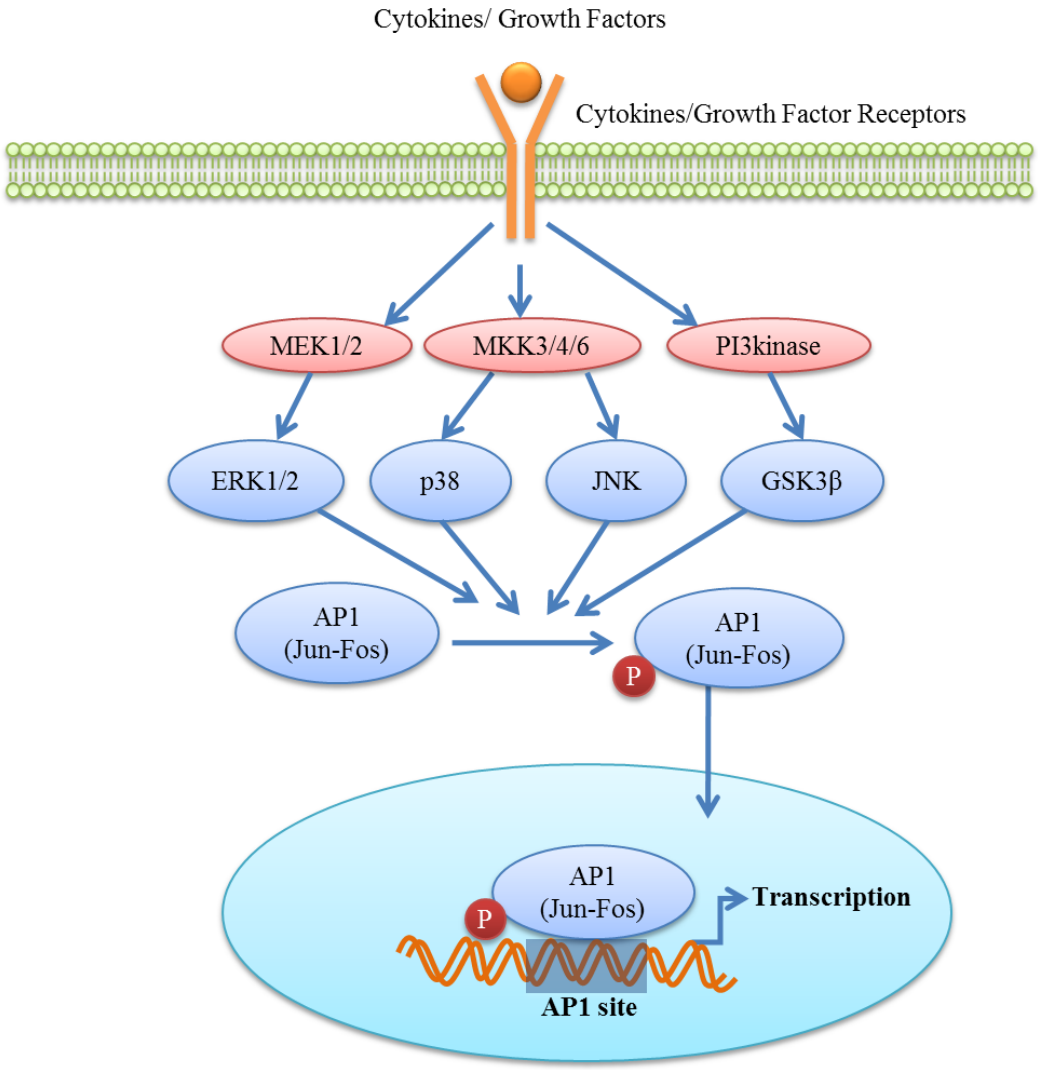


Figure 5.1 *AP1 pathway.*

Curcumin suppresses constitutive AP1 activity in HL-60, Raji and prostate cancer cell lines (LNCaP, PC-3, and DU145) (Huang *et al.*, 1991; Han *et al.*, 2002; Hergenhahn *et al.*, 2002; Mukhopadhyay *et al.*, 2001; Nakamura *et al.*, 2002). Curcumin was also reported to suppress LPS-induced cyclooxygenase-2 gene expression by inhibiting AP1 DNA binding in BV2 microglial cells (Kang *et al.*, 2004). It was confirmed that curcumin directly interact with Jun-Fos dimer and inhibit its binding to DNA (AP1 site) (Park *et al.*, 1998). Some synthetic curcumin derivatives have been discovered as inhibitors of Jun-

Fos-DNA complex formation (Hahm *et al.*, 2002; Park *et al.*, 2005; Kim and Yang, 2004). However, no information on the site of interaction is reported yet. In the present study, we investigate the interaction of curcumin derivatives with Jun-Fos complex by molecular docking studies.

5.2 MATERIALS AND METHODS

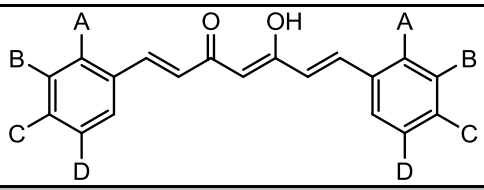
5.2.1 Preparing small molecules

To investigate the interaction with Jun-Fos complex, curcumin natural derivatives (**Figure 1.2**) synthetic curcumin based inhibitors (**Table 5.1**) and other known inhibitors of Jun-Fos-DNA complex formation (**Figure 5.2**) were drawn and 3D optimized using MarvinSketch (Free Academic License) and saved in Protein Data Bank (PDB) file format (Yap *et al.*, 2012). These molecules were prepared for molecular docking by merging non-polar hydrogens, assigning Gastegier charges, and saving them in PDBQT file format using AutoDock Tools (ADT) 1.5.6 (Sanner, 1999).

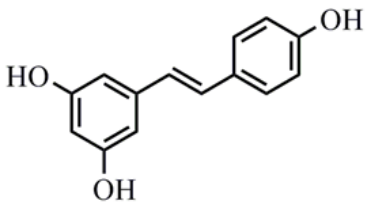
5.2.2 Preparing target molecule

X-ray crystal structure of Jun-Fos-DNA complex (PDB ID: FOS1) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb). For molecular docking, DNA and other hetero-atoms (water, ions, etc.) were removed using PyMOL 0.99, Gasteiger charges were assigned and Jun-Fos complex was saved in PDBQT file format using ADT.

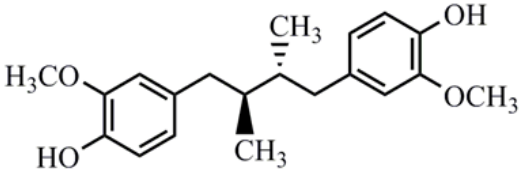
Table 5.1 *Synthetic curcumin based Inhibitors of Jun-Fos-DNA complex formation*



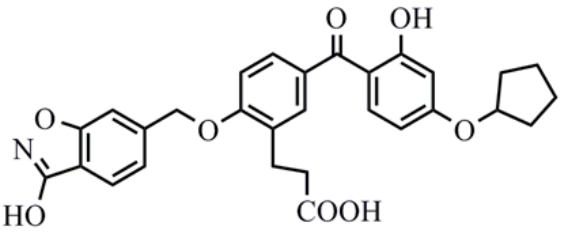
Compounds	A	B	C	D	References
CHC001	H	OCH_3	H	H	[Hahm*et al.*, 2002, Park *et al.*, 2005]
CHC002	H	OCH_3	OCH_3	OCH_3	
CHC003	H	OCH_3	H	OCH_3	
CHC004	H	OCH_3	OCH_3	H	
CHC005	H	H	OCH_3	H	
CHC006	H	H	H	H	
CHC007	H	NO_2	OH	H	
CHC008	H	OH	H	H	
CHC009	H	NO_2	H	H	
CHC010	NO_2	H	H	H	
CHC011	H	H	NO_2	H	
BJC003	H	H	CH_3	H	[Hahm*et al.*, 2002; Kim and Yang,2004]
BJC004	H	NO_2	CH_3	H	
BJC005	H	NO_2	OH	OCH_3	



Resveratrol



Dihydroguaiaretic acid



T5224

Figure 5.2 *Known inhibitors of Jun-Fos-DNA complex formation used in the study.*

5.2.3 Molecular docking

Grid and docking parameter files were prepared using ADT and molecular docking was performed with AutoDock 4.2.1 (Scripps Research Institute, USA) considering all the rotatable bonds of curcumin derivatives as rotatable and Jun-Fos complex as rigid (Morris *et al.*, 1998). Grid box size of 90 x 90 x 90 Å with 0.375 Å spacing was selected that include the whole basic DNA binding sequence and the adjacent leucine zipper region of Jun-Fos complex. Empirical-free energy function and Lamarckian Genetic Algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2,500,000 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80 were used to perform molecular docking. Hundred independent docking runs were performed for each molecule. Curcumin derivative-Jun-Fos complex for lowest free energy of binding (ΔG) confirmation from the largest cluster was written in PDBQT format and converted to PDB file format using UCSF Chimera 1.6.1. Further, these complexes were analyzed using PyMOL 0.99 for possible polar and hydrophobic interactions. All the docking studies were performed at Intel(R) Xeon(R) CPU (3.2 GHz) with Linux-based operating system Fedora 15.

5.3 RESULTS AND DISCUSSIONS

X-ray crystal structure of Jun-Fos-DNA complex shows that Arg140, Asn147, Lys153, Ser154, Arg155, Arg158, Arg268, Asn271, Arg272 and Ser278 are the key residues by which Jun-Fos complex binds to DNA through hydrogen bonding (**Figure 5.3a & b**). To predict the interaction of curcumin derivatives with Jun-Fos complex, natural curcumin derivatives and other known inhibitors of Jun-Fos-DNA complex formation were docked

over DNA binding region (DBR) of Jun-Fos complex and results were summarized in **Table 5.2.**

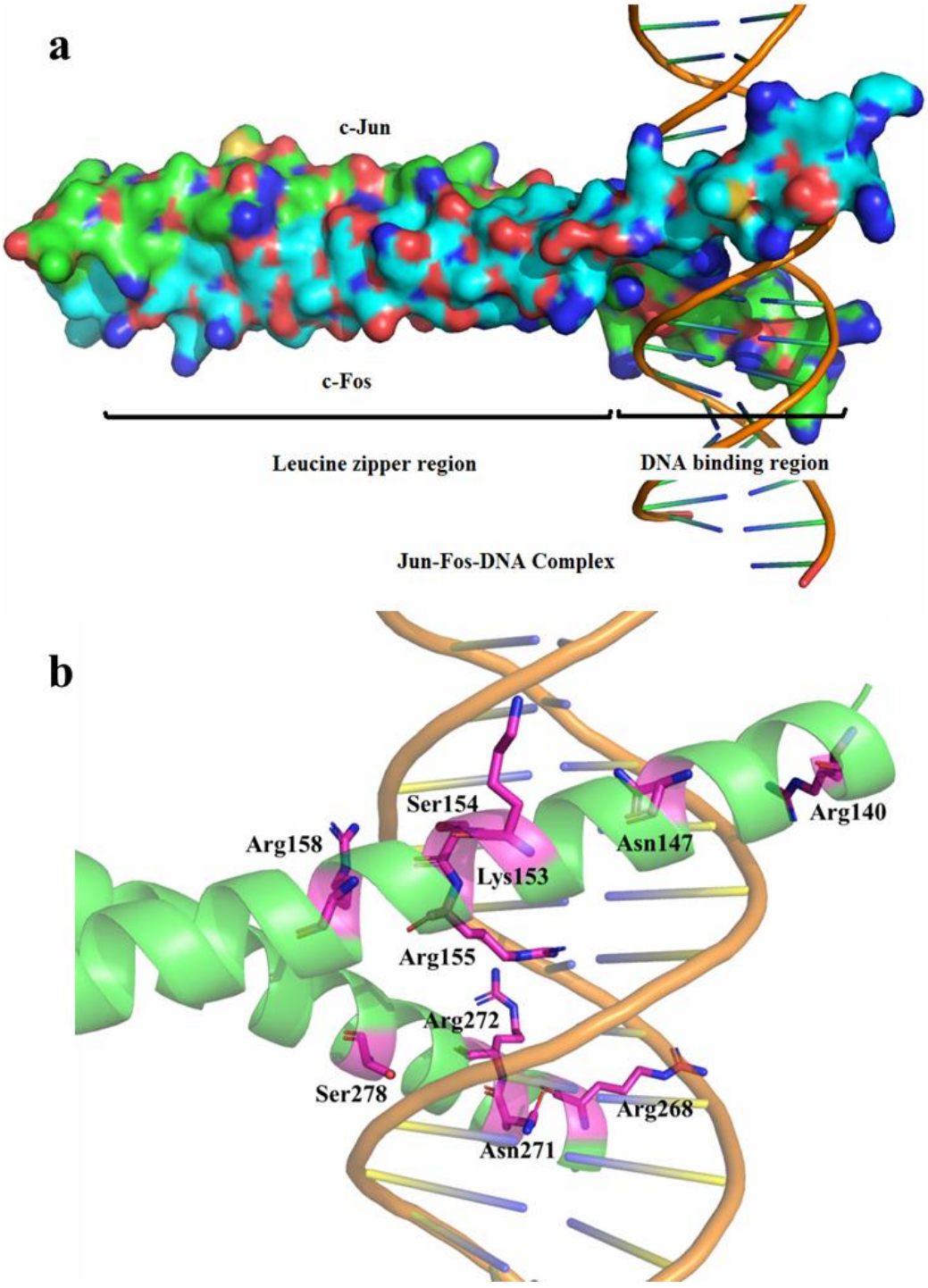


Figure 5.3 *X-ray crystal structure of Jun-Fos-DNA complex (PDB ID: FOS1)* ***(a)*** *showing leucine zipper and DNA binding region* ***(b)*** *showing amino acid residues (magenta) which form hydrogen bond with DNA (AP1 site).*

Table 5.2 *Free energy of binding (ΔG) and predicted inhibition constant (KI) estimated with AutoDock 4.2.1 and interaction of inhibitors with Jun-Fos complex*

Compounds	ΔG (kcal/mol)	KI	Putative Polar Interactions	Hydrophobic Residues in 5Å region
T5224$^{\Psi}$	-9.96	49.64 nM	Arg158, Asn271, Ser278, Arg279, Lys282	Ala274, Ala275, Leu283
CHC011*	-9.59	-93.25 nM	Arg272, Lys282	Ile273, Ala275, Leu283
CHC009*	-9.52	104.26 nM	Arg158	Leu283
CHC007*	-9.15	196.96 nM	Arg155, Arg158, Lys282	Leu283
BJC004*	-9.12	207.86 nM	Lys153	Ala150, Ala151
BJC005*	-8.94	277.86 nM	Arg155, Arg158, Lys280, Lys282	Ala275, Leu283, Ile286
Curcumin sulphate	-8.20	976.64 nM	Arg158, Lys280, Lys282	Leu283
CHC010*	-6.73	11.59 µM	Ser278, Arg279	Ala274, Ala275
CHC008*	-5.86	50.65 µM	Arg155, Arg158, Ser-276, Lys282	Leu283
Cyclocurcumin	-5.75	61.42µM	Arg155, Arg158	Ala151, Leu283
CHC003*	-5.73	62.98 µM	Arg158, Arg279, Lys280, Lys282	Ala275, Leu283
Demethoxycurcumin	-5.72	63.86 µM	Arg155, Arg158, Ser276, Arg279	Ala275, Leu283
BJC003*	-5.69	67.22 µM	Arg158, Arg279	Leu283
CHC004*	-5.66	71.36 µM	Arg158, Lys280, Lys282	Leu283
CHC006*	-5.45	101.79 µM	Arg158, Arg279,	Leu283
CHC002*	-5.32	125.57 µM	Arg155, Arg158, Arg279, Lys280, Lys282	Ala275, Leu283
Bisdemethoxycurcumin	-5.30	130.44 µM	Arg158, Ser276, Arg279	Leu283
Curcumin (keto)	-5.27	136.46 µM	Arg158, Asn271, Arg279, Lys282	Ala274, Ala275, Leu283
Curcumin (enol)	-5.25	141.66 µM	Arg158, Ser276, Lys282	Ala275, Leu283
CHC005*	-5.24	144.93 µM	Arg158	Leu283
CHC001*	-5.19	156.49 µM	Arg158, Lys282	Ala275, Leu283, Ile286
α-Turmerone	-5.13	172.61 µM	Lys282	Ala150, Ala151, Leu283
β-Turmerone	-5.05	197.55 µM	Lys282	Ala275, Leu283
Tetrahydrocurcumin	-5.05	199.62 µM	Arg155, Arg158, Ser276, Arg279	Leu283
Curcumin glucuronide	-4.61	418.23 µM	Arg155, Arg158, Arg279, Lys282	Ala151, Leu283, Ile286
Dihydroguaiaretic acid $^{\Psi}$	-4.43	569.58 µM	Ser278, Arg279	Ala151, Ala275, Leu283
Resveratrol $^{\Psi}$	-4.20	829.30 µM	Ser154, Lys282	Ala151, Leu283
Hexahydrocurcuminol	-4.08	1.02 mM	Arg155, Arg158, Ser276, Lys280, Lys282	Ala275, Leu283
Hexahydrocurcumin	-4.07	1.04 mM	Arg158, Ser276, Arg279	Ala275, Leu283, Ile286

** Synthetic curcumin based inhibitors of Jun-Fos-DNA complex formation*

$^{\Psi}$ Known inhibitors of Jun-Fos-DNA complex formation

Amongst all the natural curcumin derivatives docked to Jun-Fos complex, curcumin sulphate bound with ΔG of -8.20 kcal/mol and predicted KI of 976.64 nM followed by cyclocurcumin and demethoxycurcumin which bound with ΔG of -5.75 and -5.72 kcal/mol and predicted KI of 61.42 and 63.86 µM respectively (**Figure 5.4a**). The binding modes of curcumin sulphate depicted that sulphate and nearby methoxy group present at one aromatic ring of the molecule were in polar contact range with Lys282 however methoxy group present at other side formed polar contact with side chain of Lys280 (**Figure 5.4b**). Keto group present in the linker region was in polar contact range with side chain of Arg158. The binding mode of cyclocurcumin showed that hydroxyl group present at one aromatic ring of the molecule formed polar contact with side chain of Arg155 however at the other side it formed polar contact with Arg158 (**Figure 5.4c**). When demethoxycurcumin docked to Jun-Fos complex, hydroxyl and neighboring methoxy group present at one aromatic ring formed polar contact with side chains of Arg155 and Arg279 respectively while hydroxyl group present at other side of the molecule formed polar contact with side chain of Ser276 (**Figure 5.4d**). In the linker region of the molecule, keto and hydroxyl group were in polar contact range with Arg158 and Arg279 respectively.

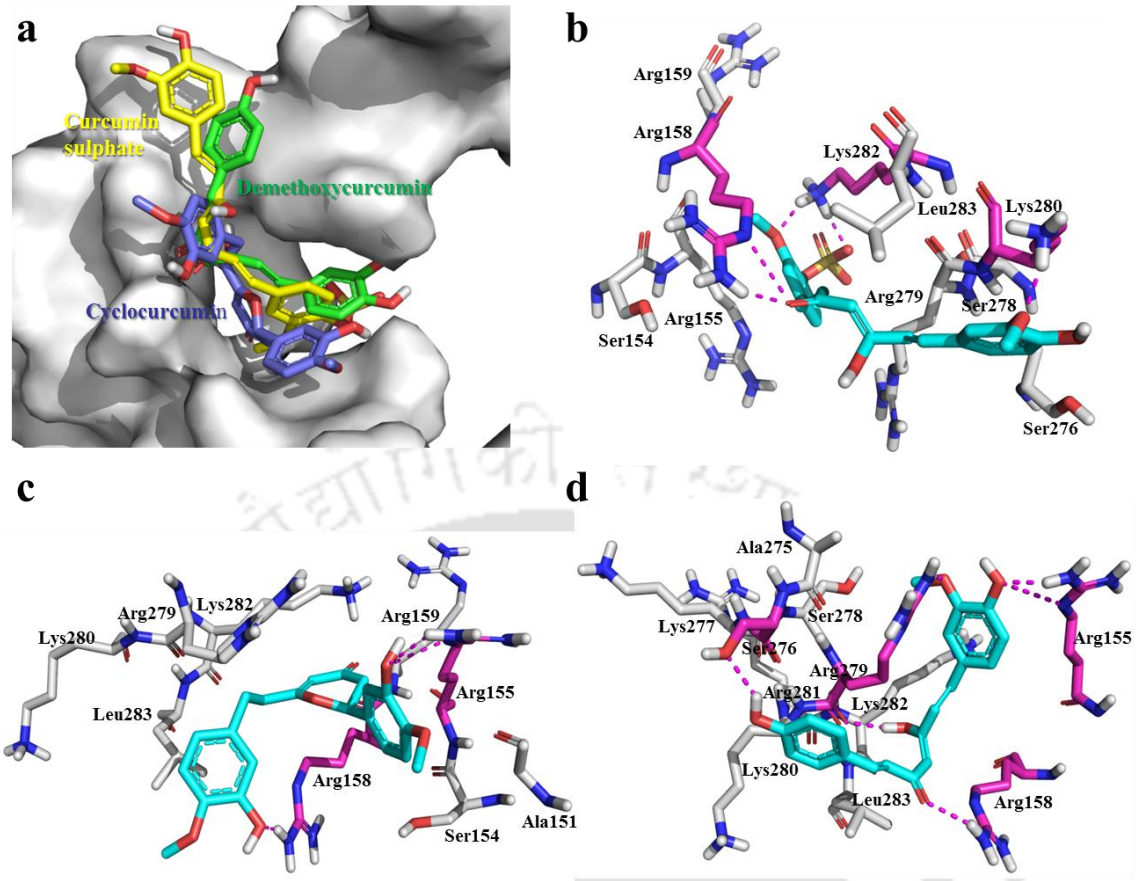


Figure 5.4 *Binding modes of natural curcumin derivatives.* ***(a)*** *curcumin sulphate (yellow), cyclocurcumin (blue) and demethoxycurcumin (green) docked to DBR of Jun-Fos complex* ***(b)*** *curcumin sulphate (cyan) showing polar contacts with Arg158, Lys280 and Lys282 (magenta)* ***(c)*** *cyclocurcumin showing polar contacts with Arg155 and Arg158 (magenta)* ***(d)*** *demethoxycurcumin showing polar contacts with Arg155, Arg158, Ser276 and Arg279 (magenta).*

Amongst the synthetic curcumin based inhibitors, CHC011 bound to Jun-Fos complex with ΔG of -9.59 kcal/mol and predicted KI of 93.25 nM followed by CHC009 and CHC007 which docked with ΔG of -9.52 and -9.15 kcal/mol and predicted KI of 104.26 nM and 196.96 nM respectively (**Figure 5.5a**). Similar results were observed in the in vitro studies by Hahm *et al.* in 2002. The binding mode studies depicted that -NO2 group present at one aromatic ring of the CHC011 molecule formed polar contact with side chain of Arg272 while at other side of the molecule it interacted with Lys282

(**Figure 5.5b**). When CHC009 docked to Jun-Fos complex, keto group present in the linker region of the molecule formed polar contact with side chain of Arg158 (**Figure 5.5c**). Hydroxyl and -NO2 group present at one aromatic ring of the CHC007 molecule formed polar contacts with backbone of Arg155 and side chain of Lys282 respectively while the hydroxyl group present in the linker region of the molecule showed polar contact with side chain of Arg158 (**Figure 5.5d**).

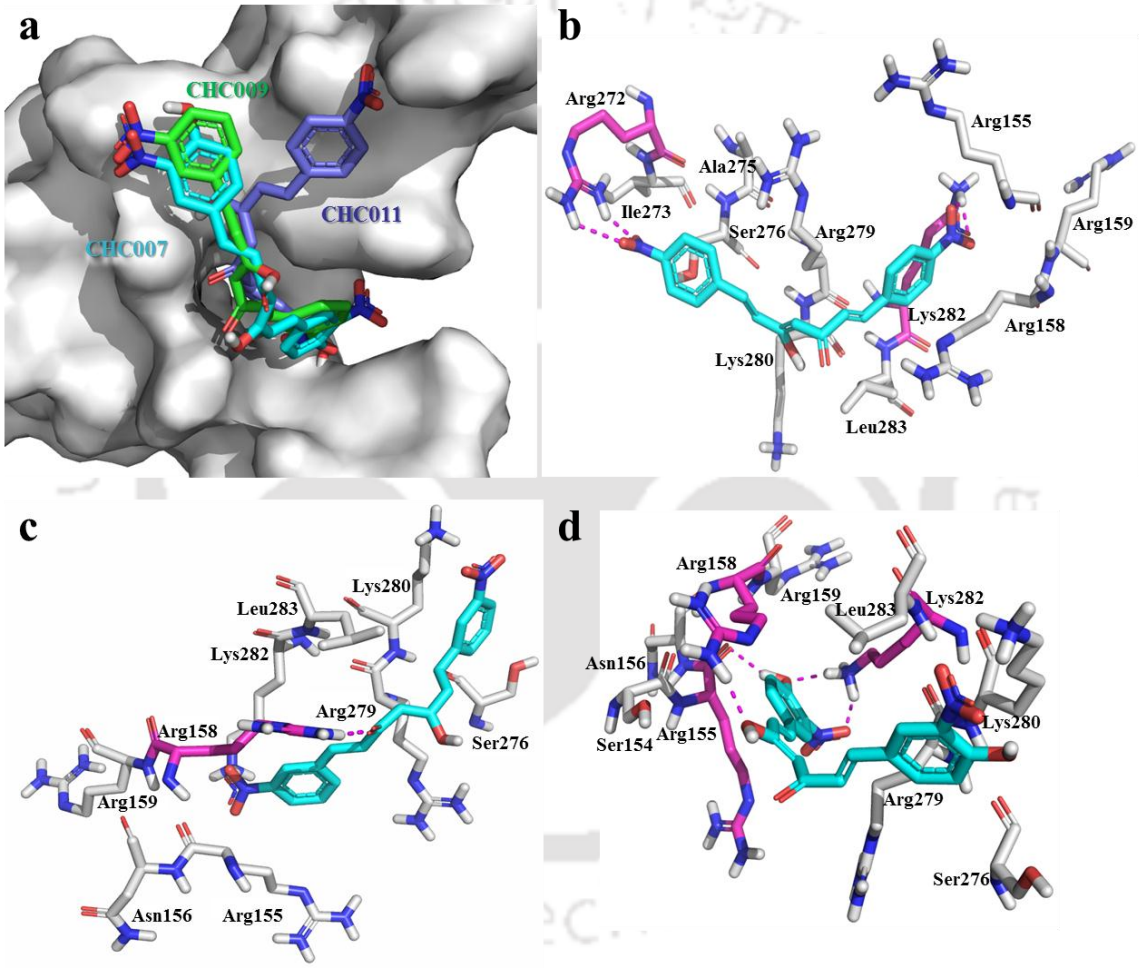


Figure 5.5 *Binding modes ofsynthetic curcumin based inhibitors* ***(a)*** *CHC011 (blue), CHC009 (green) and CHC007 (cyan) docked to DBR of Jun-Fos complex* ***(b)*** *CHC011 (cyan) showing polar contacts with Arg272 and Lys282 (magenta)* ***(c)*** *CHC009 (cyan) showing polar contacts with Arg158 (magenta).* ***(d)*** *CHC007 (cyan) showing polar contacts with Arg155, Arg158 and Lys282 (magenta)*

Amongst the other known inhibitors, T5224 [3-(5-(4-(cyclopentyloxy)-2-hydroxybenzoyl)-2-((3-hydroxybenzo[d]isoxazol-6-yl)methoxy)phenyl)propanoic acid] bound to Jun-Fos complex with ΔG of -9.96 kcal/mol and predicted KI of 49.64 nM followed by dihydroguaiaretic acid and resveratrol which docked with ΔG of -4.43 and -4.20 kcal/mol and predicted KI of 569.58 and 829.30 μM respectively (**Figure 5.6a**). The binding mode studies of T5224 depicted that oxygen atom of cyclopentyloxy group formed polar contact with side chain of Arg158 however nearby hydroxyl group formed polar contact with Arg279. Hydroxyl group of 3-hydroxybenzo[d]isoxazol-6-yl)methoxy group formed polar contact with Asn271 however oxygen atom of its methoxy group formed polar contact with Ser278. Acid group of the T5224 molecule was in polar contact range with Lys282 (**Figure 5.6b**). When docked to Jun-Fos complex neighboring hydroxyl and methoxy groups present at one side of the dihydroguaiaretic acid molecule formed polar contacts with Ser278 and Arg279 respectively whereas the hydroxyl group present at the other side of the molecule formed polar contact with backbone of Arg279 (**Figure 5.6c**). When docked to Jun-Fos complex neighboring hydroxyl groups attached to one of the aromatic ring of resveratrol molecule formed polar contacts with Ser 154 and side chain of Lys282 respectively (**Figure 5.6d**).

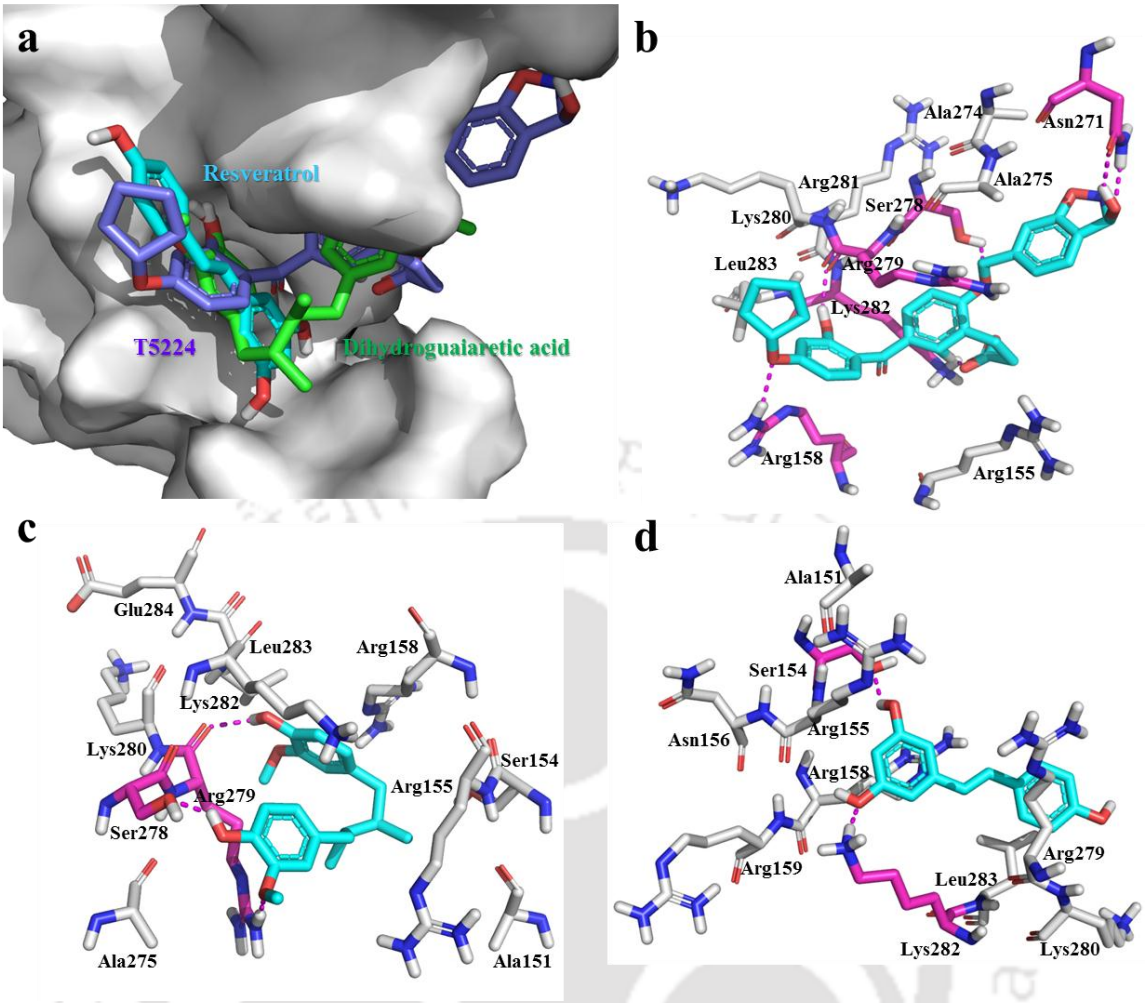


Figure 5.6 *Binding modes of other known inhibitors.* ***(a)*** *T5224 (blue), dihydroguaiaretic acid (green) and resveratrol (cyan) docked to DBR of Jun-Fos complex* ***(b)*** *T5224 (cyan) showing polar contacts with Arg158, Asn271, Ser278, Arg279 and Lys282 (magenta)* ***(c)*** *dihydroguaiaretic acid (cyan) showing polar contacts withSer278 and Arg279 (magenta)* ***(d)*** *resveratrol (cyan) showing polar contacts with Ser154 and Lys282 (magenta).*

We observed that curcumin derivatives form polar contacts preferentially with residues like Arg155, Arg158, Lys276, Arg279, Lys280 and Lys282 when docked to DBR of Jun-Fos complex amongst which Arg155, Arg158 are the key residues by which Jun-Fos complex binds to DNA. The results suggested that interaction of curcumin derivatives with residues like Arg155 and Arg158 could be the possible mechanism by which curcumin derivatives inhibit Jun-Fos-DNA complex formation. Ala151, Ala275,

Leu283 and Ile286 were the hydrophobic residues present at binding site contributing to hydrophobic contacts with inhibitor molecules.

5.4 CONCLUSIONS

The present molecular docking study provides insights into the inhibition of Jun-Fos-DNA complex formation by curcumin derivatives. The involvement of residues like Arg155, Arg158, Lys276, Lys280, and Lys282 seems to play a key role in binding of curcumin derivatives to Jun-Fos complex through polar contacts which prevents its binding to DNA (AP1 site). Ala151, Ala275, Leu283 and Ile286 were the important hydrophobic residues present at binding site. Most of the curcumin derivatives were predicted to be more potent than inhibitors like resveratrol and dihydroguaiaretic acid. Curcumin sulphate was predicted to be the most potent inhibitor amongst all the natural curcumin derivatives docked.

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CHAPTER 6

Computational studies on interaction of curcumin natural derivatives with DNA topoisomerase I and II-DNA complexes

6.1 INTRODUCTION

DNA topoisomerase I (topo I) and II (topo II) are essential ubiquitous nuclear enzymes that manage the topology of DNA during cellular processes such as replication, transcription, recombination, and chromatin remodeling (Gellert 1981; Champoux 2001; Wang 1985). Topo I catalyzes the relaxation of superhelical DNA through cycles of transient single strand cleavage and religation in the DNA duplex while topo II mediates ATP-dependent cleavage of both DNA strands, followed by trans-passing of another double stranded DNA through the transiently broken duplex (Salerno *et al.* 2010). Their critical roles during cellular processes make topoisomerases an attractive drug target against cancer (Wang 2002).

A wide variety of molecules have been discovered as eukaryotic topo I and II inhibitors which are being used for the treatment of human cancers such as lung, ovarian, brain, breast, adrenocortical, testicular cancers, Hodgkin and non-Hodgkin lymphomas. Topo I inhibitors stabilize the cleavable complexes, prevent DNA religation and induce DNA strand breaks (camptothecin, topotecan and irinotecan) (Gupta *et al*. 1997; Champoux, 2000; Staker *et al.* 2002) (**Figure 6.1**). Molecules targeting topo II can be divided into two categories, topo II inhibitors that target the N-terminal ATPase domain of topo II and prevent it from turning over (ICRF-193 and genistein) (Baird *et al.* 2001; Robinson *et al.* 2007) and topo II poisons that encourage the forward cleavage reaction (fluoroquinolones) or prevent the religation of DNA (etoposide, teniposide, doxorubicin and daunorubicin (Chow, 1988; Osheroff, 1989; Beck *et al.* 1993). However, efficacy of these molecules is challenged by poor bioavailability, drug resistance and bone marrow suppression (myelosuppresion) which is a common side effect of chemotherapy (Belani *et al.* 1994).

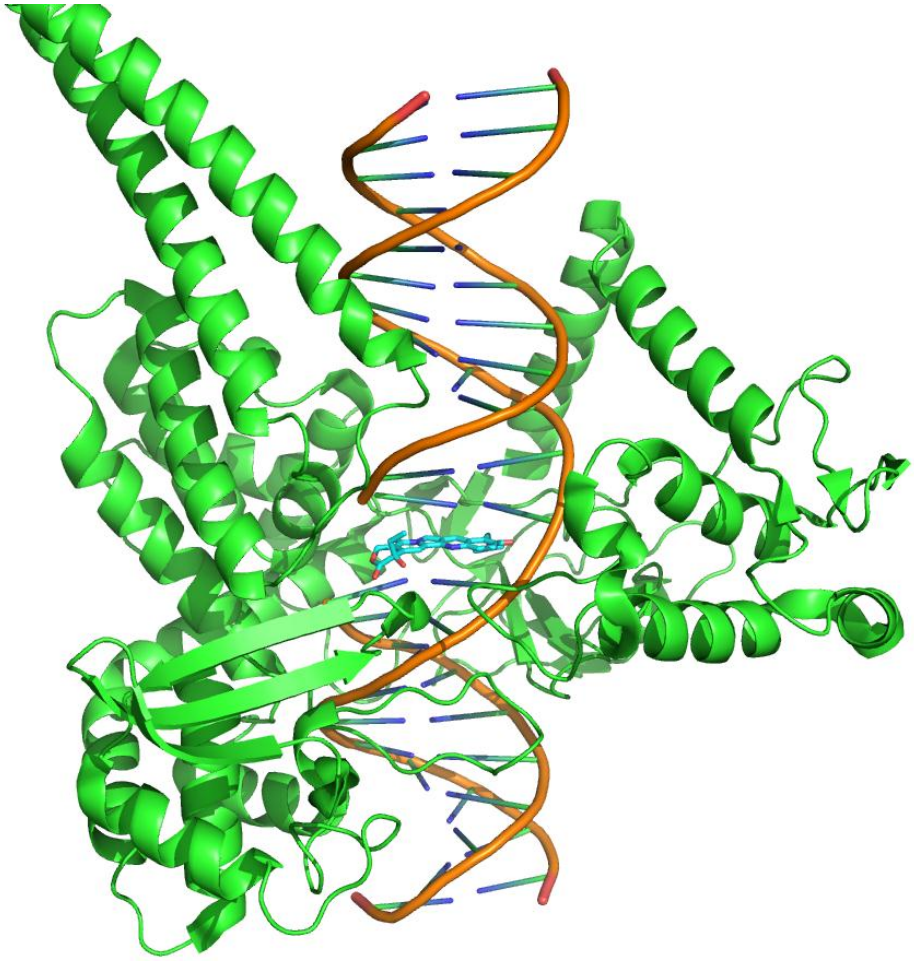

Figure 6.1 *DNA Topoisomerase I in Complex with topotecan.*

In recent studies, curcumin has been reported to induce high levels of topo I and II-DNA complexes activity in various human cancer cell lines including TK-10, MCF-7, UACC-62, HL-60 and K562 cells, the cellular processing converts these complexes into permanent DNA strand breaks that triggers apoptosis (Martín-Cordero *et al.* 2003; Mosieniak *et al.* 2006; López-Lázaro *et al.* 2007). In the present study, we investigated the molecular binding modes of curcumin natural derivatives to human topo I and II by molecular docking studies.

6.2 MATERIALS AND METHODS

6.2.1 Preparing small molecules

Curcumin natural derivatives were drawn and 3D optimized by MarvinSketch (Free Academic License) and saved in PDB file format **(Figure 1.2**). Known topo I and II inhibitors (**Figure 6.2**) were downloaded from PubChem database (http://pubchem.ncbi.nlm.nih.gov/) in SDF format and converted to PDB file format using PyMOL 0.99. Using AutoDock Tools (ADT) 1.5.6 non-polar hydrogens were merged, Gastegier charges were assigned and all the small molecules were saved in PDBQT file format (Sanner 1999).

6.2.2 Preparing target molecules

X-ray crystal structure of human DNA topo I in complex with DNA and topotecan (PDB ID: 1K4T) and Human topo II beta in complex with DNA and etoposide (PDB ID: 3QX3) were obtained from the Protein Data Bank (http://www.rcsb.org/pdb/). Hetero-atoms and drug molecules were removed, Gasteiger charges were assigned and both the macromolecules were saved in PDBQT file format using ADT.

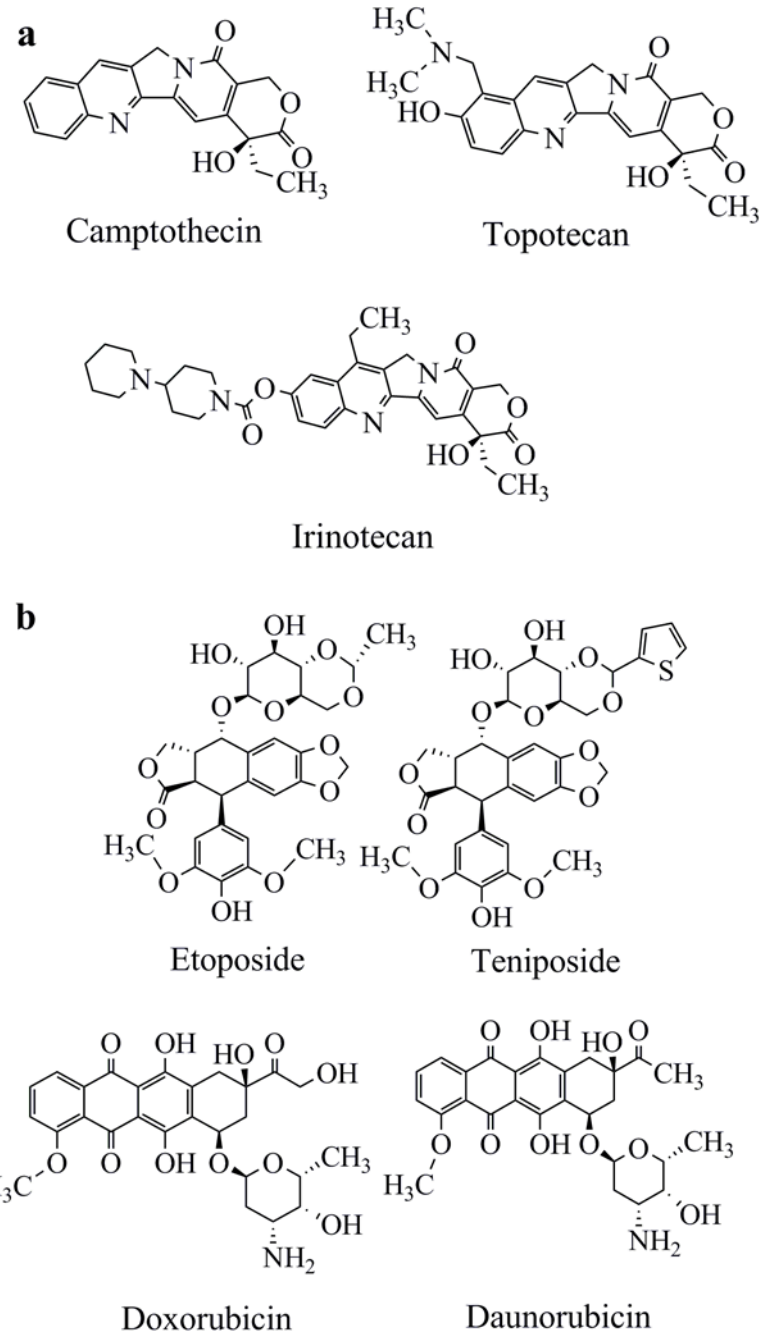

Figure 6.2 *Structures of known inhibitors of* ***(a)*** *topo I and* ***(b)*** *topo II used in the molecular docking study.*

6.2.3 Molecular docking

Grid and docking parameter files were prepared using ADT and molecular docking studies were performed with AutoDock 4.2.1 (Scripps Research Institute, USA) considering all the rotatable bonds of small molecules as rotatable and macromolecules as rigid (Morris *et al.* 1998). Grid box size of 80 x 80 x 80 Å with 0.375 Å spacing centered at the site of DNA cleavage of topo–DNA complexes was selected. Empirical-free energy function and Lamarckian Genetic Algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2,500,000 energy evaluations, a mutation rate of 0.02 and a crossover rate of 0.80 were used. Hundred independent docking runs were performed for each small molecule. Curcumin derivative-topo-DNA complexes for lowest free energy of binding (ΔG) confirmation from the largest cluster were saved in PDBQT format (using ADT) and converted to PDB file format using UCSF Chimera 1.6.1. Docking results were analyzed using PyMOL for putative polar and hydrophobic interactions. All the docking studies were performed at Intel(R) Xeon(R) CPU (3.2 GHz) with Linux-based operating system Fedora 15.

6.3 RESULTS AND DISCUSSIONS

Free energy of binding (ΔG) and putative polar and hydrophobic interactions of curcumin natural derivatives docked to topo I and II-DNA complexes were summarized in **Table 6.1** and **Table 6.2** respectively.

6.3.1 Binding mode analysis of curcumin natural derivatives docked to topo I–DNA complex

Free energy of binding (ΔG) analysis for curcumin natural derivatives docked to topo I-DNA complex demonstrated that curcumin sulphate and cyclocurcumin were the molecules docked with the lowest ΔG of -10.46 and -10.33 kcal/mol amongst all the curcumin natural derivatives while the known inhibitors irinotecan, topotecan and camptothecin docked with free energy of binding of -14.83, -10.75 and -10.68 kcal/mol respectively (**Table 6.1**). Binding modes demonstrated that curcumin sulphate and cyclocurcumin docked at the site of DNA cleavage parallel with the axis of DNA base pairing (similar to topotecan-topo I-DNA complex, PDB ID: 1K4T) and seems to be stabilized by base-stacking pi-pi interactions between aromatic rings with both upstream and downstream base pairs (**Figure 6.3**).

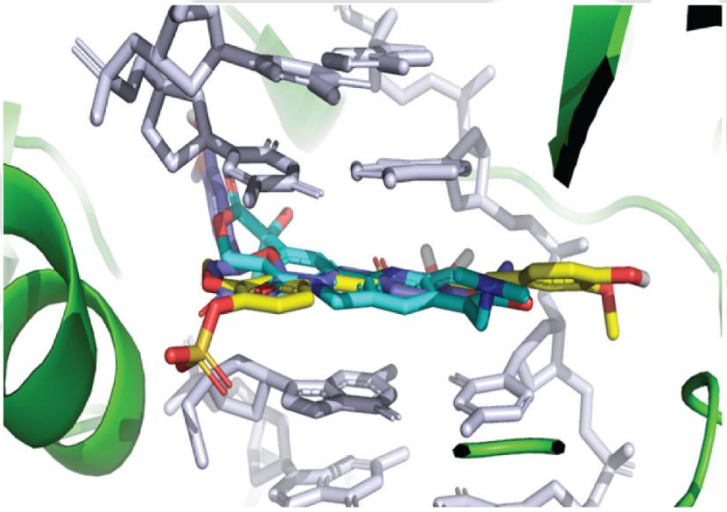

Figure 6.3 *Binding modes of curcumin natural derivatives docked to topo I-DNA complex: Curcumin sulphate (yellow) and cyclocurcumin (purple-blue) docked parallel to the DNA base pairing at the site of DNA cleavage superimposed with topotecan (cyan)-topo I-DNA complex (PDB ID: 1K4T)*

Table 6.1 *Free energy of binding (ΔG) estimated with AutoDock 4.2.1 and interaction of inhibitors with DNA Topoisomerase-I predicted by PyMOL 0.99.*

Compound name	ΔG (kcal/mol)	Putative polar interactions	Hydrophobic residues in 5Å region
Irinotecan*	-14.83	Asp533	Phe361, Ile420
Topotecan*	-10.75	Asp533	--
Camptothecin*	-10.68	Asp533, Arg364	--
Curcumin sulphate	-10.46	Arg364, Lys374, Asn722, Lys751, A113	Leu721
Cyclocurcumin	-10.33	Asn352, Arg364, Asn722, pTyr723, T10, A-113	Ile535, Leu721
Demethoxycurcumin	-8.75	Asp533, Arg364, Asn722, T10, A113	Leu721
Bisdemethoxycurcumin	-8.32	Asn352, Glu356, Lys425, Met428, A114	Ile350, Ile355, Trp416, Ile-424, Tyr426, Ile-427, Leu-429
Tetrahydrocurcumin	-8.06	A113, A114, Lys425, Leu429	Ile-424, Tyr426, Ile427, Met428
Curcumin glucuronide	-8.05	Lys532, Asp533, Lys425, Tyr426, Asn722, C8, T9, A113	Trp416, Ile424, Ile427, Met428, Leu721
Curcumin (keto)	-8.03	Arg364, Lys374, Asn722, A113	Phe361, Leu721
Hexahydrocurcumin	-7.88	Asn352, Arg364, C8, Ile424, Lys425, T10, A113, A114	Tyr426, Met428
Curcumin (enol)	-7.78	Asn352, Arg364, Met428, A113	Ile350, Tyr426, Ile427, Leu429
Hexahydrocurcuminol	-7.5	Arg364, Tyr426, Leu429, Lys436, A113	Ile427, Met428
α-Turmerone	-7.09	Asn722	Leu721
β-Turmerone	-6.84	Asn352	Phe353, Trp416, Ile424, Tyr426, Ile427, Met428

**Known inhibitors of topo I*

Binding mode of curcumin sulphate with topo I-DNA complex suggested that sulphate group form polar interaction with side chain of Lys751 and Asn722 while the neighboring methoxy group was in polar interaction range with Asn722 (**Figure 6.4a**). Methoxy group at the other end of the molecule formed polar interaction with side chain of Lys374. In the linker region of the molecule keto group formed polar interaction with Arg364 while hydroxyl group was in polar interaction range with base A113. Binding mode of cyclocurcumin with topo I-DNA complex showed that hydroxyl group present at one end of the molecule interacted with Lys532 and pTyr723 while at other end it was in polar interaction range with Arg364 and base A113 (**Figure 6.4b**). Keto group of dihydropyranone moiety of linker region form polar interaction with His632 while oxygen atom of dihydropyranone ring was in polar interaction range with base T10.

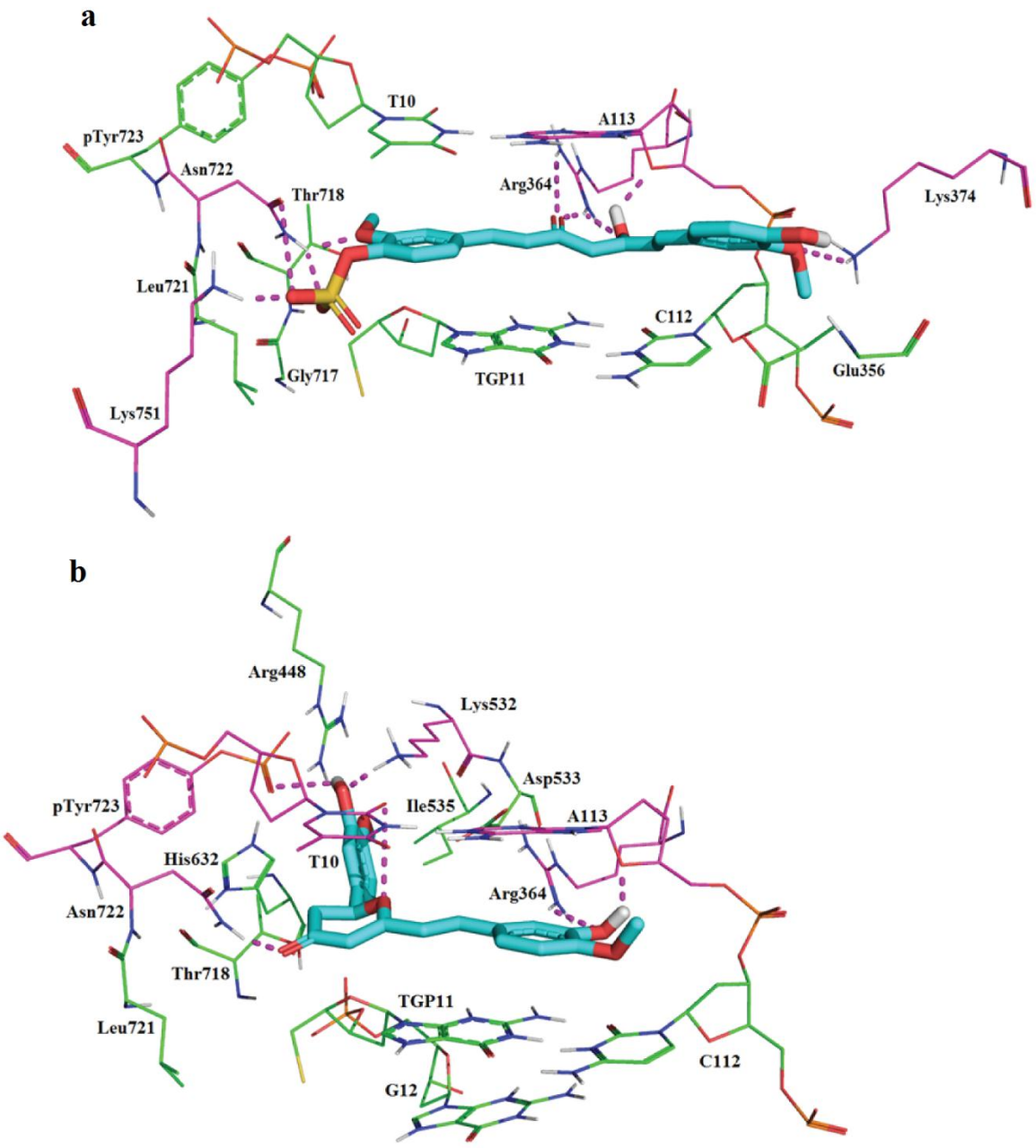

Figure 6.4 *Binding modes of curcumin natural derivatives docked to topo I-DNA complex* ***(a)*** *Binding mode of curcumin sulphate (cyan) depicting polar interactions with residues Arg364, Lys374, Asn722, Lys751 and base A113 (magenta)* ***(b)*** *cyclocurcumin (cyan) depiction polar interactions with residues Asn352, Arg364, Asn722, pTyr723 and bases T10 and A-113 (magenta).*

6.3.2 Binding mode analysis of curcumin natural derivatives docked to topo II–DNA complex

Free energy of binding (ΔG) analysis for curcumin natural derivatives docked to topo II-DNA complex demonstrated that cyclocurcumin and curcumin sulphate were the molecules docked with the lowest ΔG of -11.16 and -9.98 kcal/mol respectively while the known inhibitors teniposide, etoposide, daunorubicin and doxorubicin docked with ΔG of -14.01, -13.49, -9.3 and -9.14 kcal/mol respectively (**Table 6.2**).

Binding modes demonstrated that cyclocurcumin and curcumin sulphate docked at the site of DNA cleavage parallel to the axis of base pairing similar to etoposide-topo II-DNA complex (PDB ID: 3QX3) as shown in **Figure 6.5**.

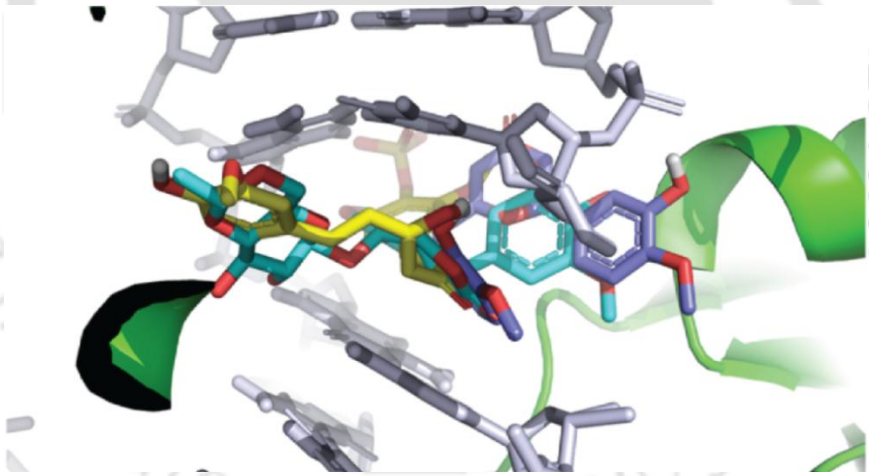

Figure 6.5 *Binding modes of curcumin natural derivatives docked to topo II-DNA complex: Curcumin sulphate (yellow) and cyclocurcumin (purple-blue) docked parallel to the DNA base pairing at the site of DNA cleavage superimposed with etoposide (cyan)-topo II-DNA complex (PDB ID: 3QX3)*

Table 6.2 *Free energy of binding (ΔG) estimated with AutoDock 4.2.1 and interaction of inhibitors with DNA Topoisomerase-II predicted by PyMOL 0.99.*

Compound name	ΔG (kcal/mol)	Putative polar interactions	Hydrophobic residues in 5Å region
Teniposide*	-14.01	Asp479, Gln778, C8, T9, C11, G13	Leu502, Met782
Etoposide*	-13.49	Asp479, Gln778, C8, T9, G13,	Leu502, Met782
Cyclocurcumin	-11.16	Asp479, Ser480, Gln778, T9, G10	Leu502,Tyr821
Curcumin sulphate	-9.98	Arg503, Gln778, T9, A12	Met781, Met782, Tyr821
Curcumin (enol)	-9.74	Asp479, Gln778, T9, G13	Leu502, Met781, Met782
Bisdemethoxycurcumin	-9.48	Asp479, Gln778, T9	Leu502, Met781, Met782
Curcumin glucuronide	-9.42	Glu477, Asp479, Ser480, Gln778, T9, G10, A12	Leu502, Met782
Daunorubicin*	-9.3	Gln778, G13	Met782
Doxorubicin*	-9.14	Gln778, T9, G13	Met781, Met782
Tetrahydrocurcumin	-9.02	Asp479, Gln778, T9	Leu502, Met781, Met782, Tyr821
Demethoxycurcumin	-8.76	Asp479, Arg503, Gln778, T9, G10	Leu502
Hexahydrocurcumin	-8.55	Asp479, Gln778, T9	Leu502, Met782
Curcumin (keto)	-8.04	Asp479, A12	Leu502
β-Turmerone	-7.64	Asp479, Gln778, T9	Leu502
α-Turmerone	-7.51	Asp479, Gln778	Leu502
Hexahydrocurcuminol	-7.45	Asp479, Gln778	Leu502, Met781, Met782

**Known inhibitors of topo II*

Binding mode of cyclocurcumin with topo II-DNA complex showed that hydroxyl group present at one end of the molecule form polar interaction with side chain of Gln778 and base T9 while the neighboring methoxy group formed polar interaction with side chain of Gln778 (**Figure 6.6a**). Hydroxyl group present at other end of the molecule was in polar interaction range with Asp478, Ser480 and base G10. Binding mode of curcumin sulphate with topo II-DNA complex suggested that sulphate group form polar interaction with side chain of Arg503 and base A12 while hydroxyl group present in the linker region of the molecule was in polar interaction range with Gln778 and base T9 (**Figure 6.6b**).

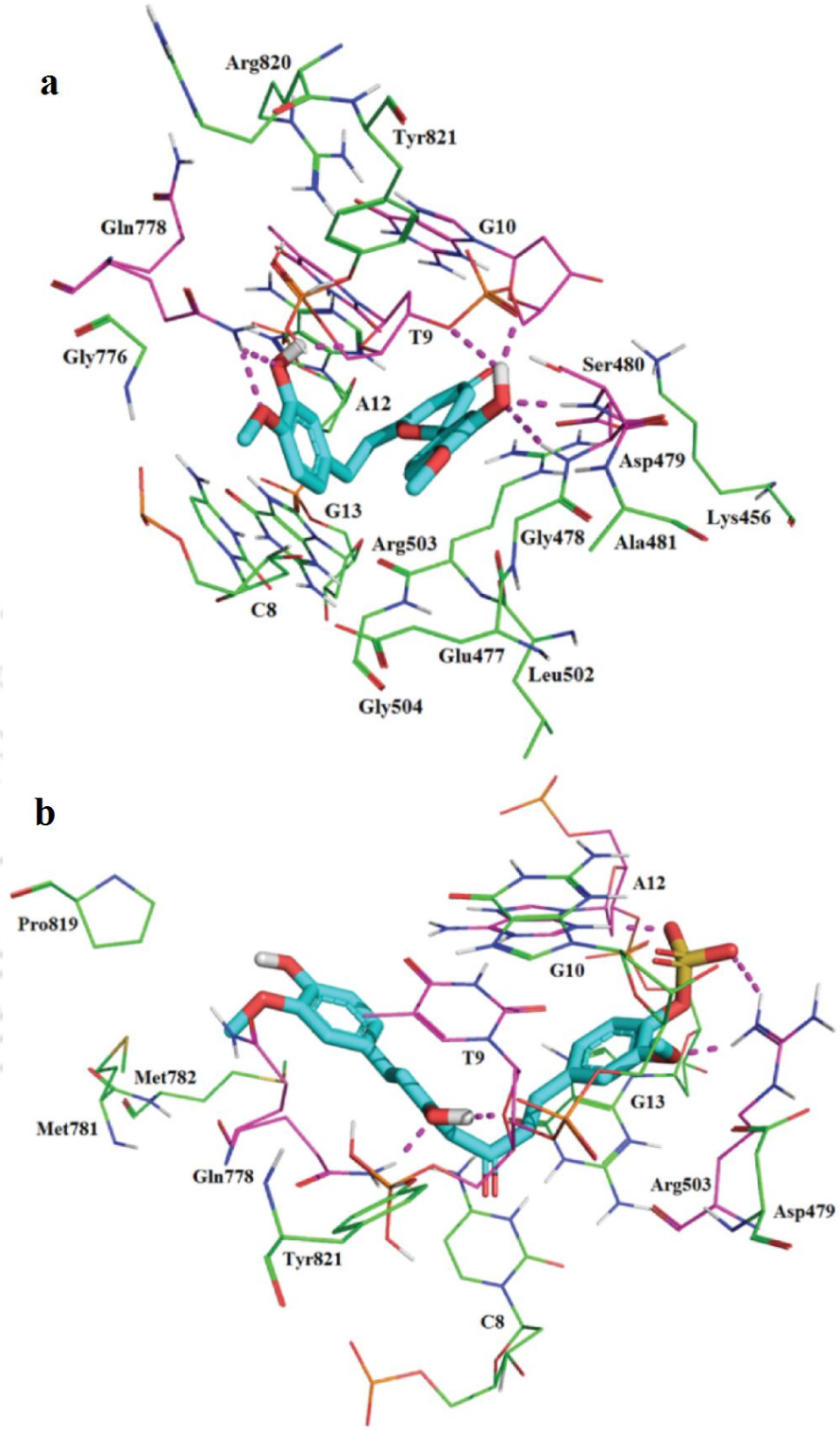

Figure 6.6 *Binding modes of curcumin natural derivatives docked to topo II-DNA complex* ***(a)*** *Cyclocurcumin (cyan) depiction polar interactions with residues Asp479, Ser480, Gln778 and bases T9 and G10 (magenta).* ***(b)*** *Binding mode of curcumin sulphate (cyan) depicting polar interactions with residues Arg503, Gln778 and bases T9 and A12 (magenta).*

Results demonstrated that cyclocurcumin and curcumin sulphate exhibited lowest ΔG when curcumin natural derivatives were docked to topo I and II–DNA complexes. The binding modes of cyclocurcumin and curcumin sulphate were similar to known inhibitors of topo I and II. Both the derivatives docked at the site of DNA cleavage parallel to the axis of DNA base pairing and seem to be stabilized by base-stacking pi-pi interactions between aromatic rings with both upstream and downstream base pairs. Curcumin sulphate and cyclocurcumin docked to topo I-DNA complex with ΔG of -10.46 and -10.33 kcal/mol respectively very close to the known inhibitors of topo I, topotecan and camptothecin (-10.75 and -10.68 kcal/mol respectively) which are being used as chemotherapeutic agents against various types of cancers. When docked to topo II-DNA complex cyclocurcumin and curcumin sulphate exhibited ΔG of -11.16 and -9.98 kcal/mol respectively, less that the known inhibitors of topo II daunorubicin and doxorubicin which docked with ΔG of -9.3 and -9.14 kcal/mol respectively. Residues like Arg364, Asn722 and base A113 (when docked to topo I-DNA complex) and residues Asp479, Gln778 and base T9 (when docked to topo II-DNA complex) seems to play an important role in the binding of curcumin natural derivatives at the site of DNA cleavage. The results suggested that cyclocurcumin and curcumin sulphate have potential to be lead molecules for the development of dual inhibitors targeting topo I and II.

6.4 CONCLUSIONS

In the presented work, we explored the interactions of curcumin natural derivatives with topo I and II-DNA complexes by molecular docking studies. It was found that curcumin natural derivatives docked at the site of DNA cleavage parallel to the axis of DNA base pairing similar to known inhibitors of topo I and II.Cyclocurcumin and curcumin sulphate exhibited lowest ΔG against both topo I and II-DNA complexes suggesting that they

could be the most potent inhibitors amongst all the curcumin natural derivatives docked. Furthermore, these derivatives could be potential lead molecules for development of dual inhibitors of topo I and II.

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CHAPTER 7

Summary & Future prospects

7.1 SUMMARY

The present study was focused on the development of a curcumin resource database and *in-silico* interaction studies with selected targets such as transcription factors (NF-κB, Stat3 and AP1) and DNA topoisomerases I and II.

7.1.1 Development of a curcumin resource database (CRDB)

We developed CRDB, which is an integrated and curated repository of curcumin analogs their molecular targets and patents curated from public domain databases and published literature in peer reviewed journals indexed in PubMed. CRDB can be browsed through dropdown menu and text based keyword search. We made provisions for deposition and regular updation of the database. The CRDB is freely available with user-friendly interfaces. We expect it to be highly useful to the researchers working on structure as well as ligand based molecular design of curcumin analogs.

7.1.2 Computational studies on inhibition of NF-κB p50 subunit by curcumin natural derivatives

In the present work we explored interference of curcumin and its derivatives in the binding of NF-κB to DNA by molecular docking studies.NF-κB is an important transcription factor, involved in many immune, inflammatory and apoptotic responses. Its inhibition could have therapeutic potential for the drug development against cancer and various inflammatory diseases. The involvement of residues like Lys144, Asp206, Asp239, Leu207 and Lys241 seems to play an important role in binding of curcumin and its natural derivatives to the DBR. Curcumin sulphate was predicted to be the most potent inhibitor amongst all the derivatives and known inhibitors (aurine tricarboxylic acid, gallic acid and ellagic acid) with favorable ADME predictions.

7.1.3 *In-silico* studies on inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids

In the present chapter we explored inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids.Stat3 is a mammalian transcription factor which regulates various genes involved in cell growth, proliferation and cell survival. Its constitutive activation contributes to tumor progression and carcinogenesis. Inhibition of Stat3 dimerization which prevents its binding to DNA is a rational strategy that could be translated to potential therapeutic applications. The involvement of residues like Lys591, Arg609, Ser611, Glu612, Ser613, Ser636 and Val637 play an important role in binding of curcumin natural derivatives and its amino acid conjugates with SH2 domain of Stat3. Demethoxycurcumin, hexahydrocurcuminol followed by hexahydrocurcumin were predicted to be the most potent inhibitors amongst all the curcumin natural derivatives and known inhibitors such asFLLL32, Sta21 and Stattic. Amongst the curcumin-amino acid conjugates curcumin-proline conjugate (1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) was predicted to be the most potent inhibitor of Stat3 dimerization.

7.1.4 Computational studies on inhibition of Jun-Fos-DNA complex formation by curcumin natural derivatives

In the present chapter we studied inhibition of Jun-Fos-DNA complex formation by curcumin derivatives through molecular docking.AP1 (Jun-Fos) is a transcription factor which regulates gene expression in response to a variety of stimuli and controls cellular processes including proliferation, transformation, inflammation and innate immune

responses. It has been found constitutively active in several types of cancer including breast, ovarian, cervical and lung cancer. We found that involvement of residues like Arg155, Arg158, Lys276, Lys280, and Lys282 seems to play a key role in binding of curcumin derivatives to Jun-Fos complex through polar contacts which prevent its binding to transcription factor binding site (AP1 site). Ala151, Ala275, Leu283 and Ile286 were the hydrophobic residues present at binding site. Most of the curcumin derivatives were predicted to be more potent than inhibitors like resveratrol and dihydroguaiaretic acid. Curcumin sulphate was predicted to be the most potent inhibitor amongst all the natural curcumin derivatives.

7.1.5 Computational studies on interaction of curcumin natural derivatives with DNA topoisomerase I and II-DNA complexes

In the present work we explored the interactions of curcumin natural derivatives with topo I and II-DNA complexes by molecular docking. DNA topoisomerase I (topo I) and II (topo II) are essential enzymes that solve the topological problems of DNA by allowing DNA strands or double helices to pass through each other during cellular processes such as replication, transcription, recombination, and chromatin remodeling. Their critical roles make topoisomerases an attractive drug target against cancer. We found that curcumin natural derivatives interact at the site of DNA cleavage parallel to the axis of DNA base pairing similar to known inhibitors of topo I and II. Cyclocurcumin and curcumin sulphate exhibited lowest ΔG against both topo I and II-DNA complexes suggesting that they could be the most potent inhibitors amongst all the curcumin natural derivatives docked. Furthermore, results also suggested these derivatives could be potential lead molecules for development of dual inhibitors of topo I and II.

7.2 FUTURE PROSPECTS

We explored the interaction of curcumin natural derivatives with selected targets including transcription factors (NF-κB, Stat3 and AP1) and DNA topoisomerase I and II, which provide insights into the inhibition of these molecular targets by curcumin and its derivatives. Curcumin derivatives seems to be promising lead molecule for therapeutic applications against cancer and other inflammatory diseases which require further extensive studies as summarized below.

- Studies have suggested that curcumin derivatives could be potential lead molecules for development of multi-targeting drugs against complex diseases such as cancer and inflammatory diseases which need extensive further studies.
- Functional group modifications should be done to curcumin derivatives to improve their binding to transcription factors (NF-κB, Stat3 and AP1) which could have therapeutic implications.
- Potential of curcumin derivatives as a lead molecule for development of dual inhibitors of topo I and II should be explored by functional modifications to these molecules followed by in vitro studies.

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List of Publications

Manuscripts in communication

[1] **Kumar A** and Bora U (2013) CRDB: a database of curcumin derivatives their molecular targets and patents (Communicated) - *Research Article*

Manuscripts published

[1] **Kumar A** and Bora U (2013) Molecular docking studies of curcumin natural derivatives with DNA topoisomerase I and II-DNA complexes. *Interdisciplinary Sciences: Computational Life Sciences* ***(Accepted).*** - *Research Article*

[2] **Kumar A** and Bora U (2013) Interactions of curcumin derivatives and its metal complexes with nucleic acids and their implications. *Mini Reviews in Medicinal Chemistry* 13(2):256-64. - *Review Article*

[3] **Kumar A** and Bora U (2012) In silico inhibition studies of Jun-Fos-DNA complex formation by curcumin derivatives. *International Journal of Medicinal Chemistry* vol. 2012, Article ID 316972, 8 pages, 2012. doi:10.1155/2012/316972. - *Research Article*

[4] **Kumar A** and Bora U (2012) Molecular docking studies on inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids. *Bioinformation* 8(20):988-993. - *Research Article*

[5] **Kumar A** and Bora U (2012) In silico inhibition studies of NF-κB p50 subunit by curcumin and its natural derivatives. *Medicinal Chemistry Research* 21(10):3281-3287. - *Research Article*

Other Publications:

1. Babu PJ, Das RK, **Kumar A**, Utpal Bora U (2011) Microwave-Mediated Synthesis of Gold Nanoparticles Using Coconut Water. *International Journal of Green Nanotechnology* 3(1):13-21. - *Research Article*

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

In silico inhibition studies of NF-κB p50 subunit by curcumin and its natural derivatives

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Abstract Nuclear factor-kappa B (NF-κB) is an important transcription factor, involved in many immune, inflammatory, and apoptotic responses. Preventing its binding to DNA is a rational strategy that could be translated to potential therapeutic applications. In this study, the interference of curcumin and its derivatives in the binding of NF-κB to DNA has been explored by in silico studies. Curcumin and its derivatives were docked on the p50 subunit of NF-κB and it was found that most of the compounds formed polar interactions with LYS-144 and LYS-241 which are the key residues involved in binding of NF-κB with DNA at the consensus sequence (κB site) through hydrogen bonding. Molecular docking studies and ADME predictions showed that curcumin sulphate can be a potent inhibitor of NF-κB p50 subunit amongst the natural curcumin derivatives and known inhibitors (aurine tricarboxylic acid, gallic acid, and ellagic acid) docked.

Keywords Curcumin derivatives · Molecular docking · Inhibition constant · Polar interactions · Nuclear factor-kappa B · AutoDock

Introduction

Nuclear factor-kappa B (NF-κB) is an inducible mammalian transcriptional factor belonging to the *rel* family. It exists predominantly in dimeric form either as a homomer (p50/p50) or heteromer (p50/p65). In the cytoplasm, its nuclear localization signal (NLS) is masked by ankyrin repeats of inhibitor kappa B (IkB) proteins thereby preventing it from entering the nucleus and initiating transcription (Thanos and Maniatis, 1995; Beg and Baldwin, 1993). NF-κB normally remains sequestered in the cytoplasm by the IkB family of proteins. Various physical, chemical, and biological stimuli induce IkB kinase to phosphorylate IkB which subsequently undergoes proteosome-dependant degradation and results in activation of NF-κB. Activated NF-κB then enters the nucleus and transcribes genes by binding to κB site (consensus sequence GGGRNNYYCC, where N = any base, R = purine, and Y = pyrimidine) (Verma *et al*., 1995; Hayden and Ghosh, 2004). NF-κB also turns on expression of IκB which represses NF-κB by a feedback inhibition. In case of tumour cells, NF-κB remains active due to the mutation in NF-κB encoding gene or in IkB gene both of which leads to dysfunction in sequestration of NF-κB in cytoplasm (Wulczyn *et al*., 1996; Baeuerle and Baltimore, 1996). NF-κB also regulates various other genes involved in a variety of human diseases like inflammation, acquired immune deficiency syndrome (AIDS), cancer, asthma, atherosclerosis, septic shock, and arthritis. It is therefore a major target for drug development (Garg and Aggarawal, 2002). It has been reported that curcumin can interrupt directly in the binding of NF-κB to its consensus DNA sequences in vitro (Han *et al*., 2002). However, no information on the site of interaction is reported yet.

Curcumin (diferuloyl methane) is a hydrophobic polyphenol derived from the spice turmeric (*Curcuma longa*) having wide spectrum of pharmacological activities. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities

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(Shishodia *et al*., 2007; Jagetia and Aggarwal 2007; Goel *et al*., 2008; Anand *et al*., 2007). Studies with various animal models and human have shown curcumin to be extremely safe even at very high doses (Anand *et al*., 2008a; Maheshwari *et al*., 2006).

Turmeric contains three important analogues curcumin, demethoxycurcumin, and bisdemethoxycurcumin, amongst which curcumin is the most abundant. These three compounds differ in methoxy substitution on the aromatic ring. Whilst curcumin has two symmetric *o*-methoxy phenols linked through the α,β-unsaturated β-diketone moiety, bisdemethoxycurcumin is also symmetric but deficient in two *o*-methoxy substitutions, and demethoxycurcumin has an asymmetric structure with one of the phenyl rings having *o*-methoxy substitution. Commercially available curcumin mixture contains 77% curcumin, 17% demethoxycurcumin, and 3% bisdemethoxycurcumin. Some lesser known curcuminoids from turmeric are α-turmerone and β-turmerone and cyclocurcumin. Structurally, cyclocurcumin differs from curcumin in the β-diketone link. In this molecule, the α,β-unsaturated β-diketone moiety of curcumin is replaced by an α,β-unsaturated dihydropyranone moiety. Metabolites of curcumin reported are tetrahydrocurcumin, hexahydrocurcumin, curcumin glucuronide, and curcumin sulphate having wide spectrum of pharmacological activities (Anand *et al*., 2008b).

Methodology

2D structures of curcumin derivatives (Fig. 1) and known NF-κB inhibitors (Fig. 2) were drawn by ACD/ChemSketch (Freeware) and then converted to 3D structures in MDL Molfiles format. Using OpenBabel 2.2.3 MDL Molfiles were converted to Protein Data Bank (PDB) file format (Sharma *et al*., 2000; Kim *et al*., 2006; Edderkaoui *et al*., 2008). Ligand preparation was done by assigning Gastegier charges, merging non-polar hydrogens, and

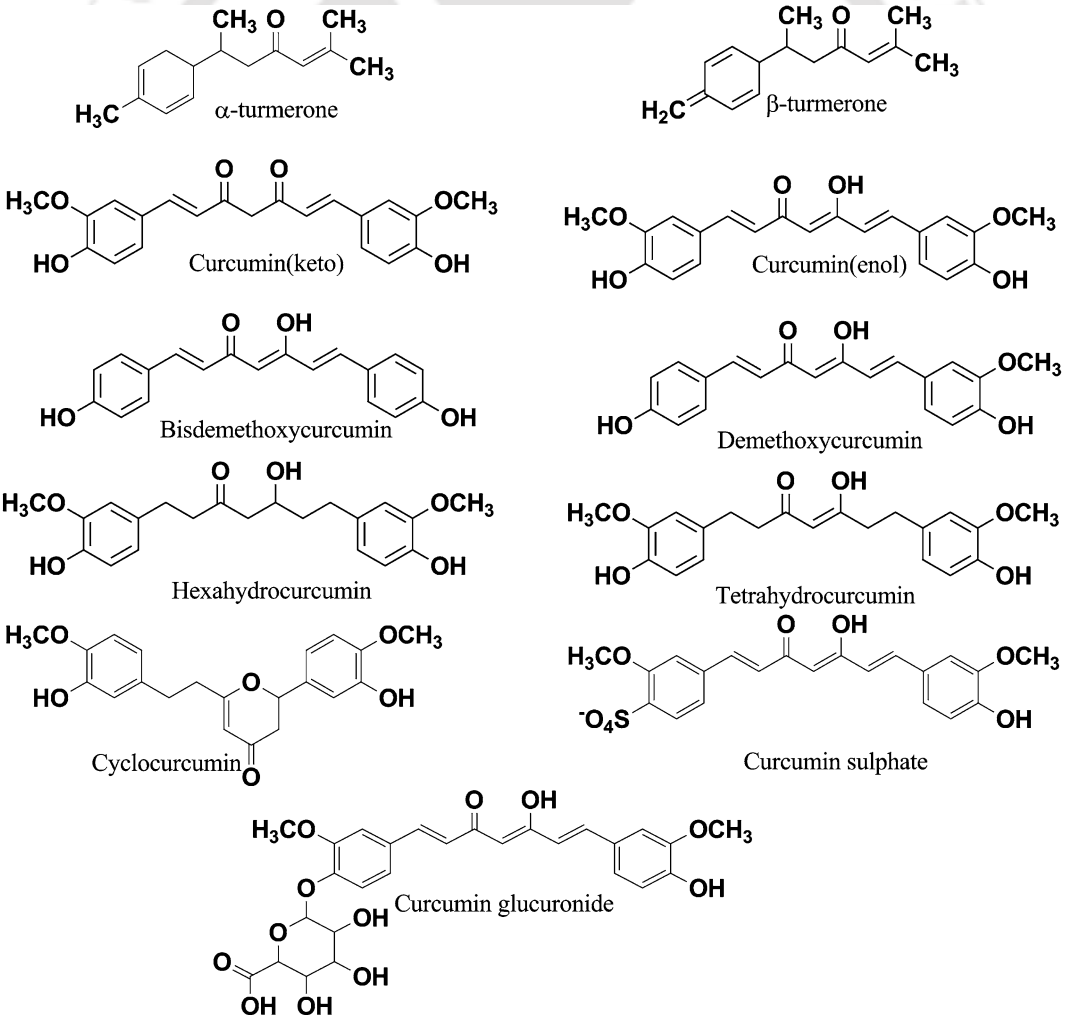

Fig. 1 Structures of curcumin and its natural derivatives used in the docking studies

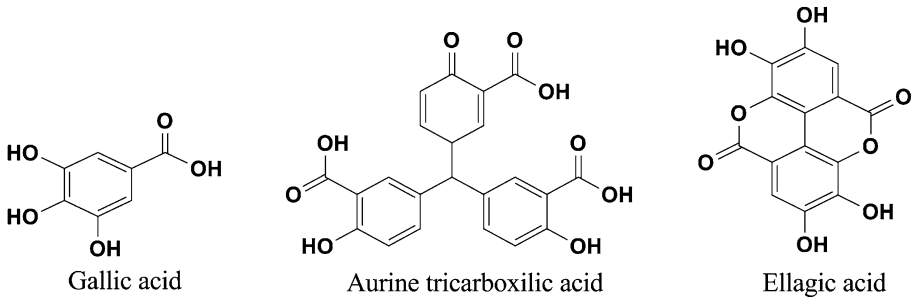

Fig. 2 Structures of known NF-κB DNA binding inhibitors used in the docking studies

saving it in PDBQT file format using AutoDock Tools (ADT) 1.5.4 (Sanner, 1999).

X-ray crystal structure for NF-κB dimer complexed with DNA (PDB ID: 1NFK) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb). X-ray crystal structure for NF-κB dimer complexed with DNA shows that ARG-56, TYR-57, LYS-144, LYS-241, GLN-274, ARG-305, and GLN-306 form hydrogen bonds with DNA. The hydrophobic amino acids present nearby binding site in 5 Å region are VAL-58, VAL-142, LEU-207, SER-240, PRO-243 which can show hydrophobic interactions. Using Swiss-PdbViewer 4.1 NF-κB p50 chain-A is selected and saved in PDB format leaving aside p50 chain-B and other hetero-atoms (water, ions, etc.). Gastegier charges were assigned to NF-κB p50 subunit and saved in PDBQT file format using ADT.

Preparation of parameter files for grid and docking was done using ADT. Docking was performed with AutoDock 4.2.1 (Scripps Research Institute, USA) considering all the rotatable bonds of ligand as rotatable and protein as rigid (Morris *et al*., 1998). Grid box size of 80 × 80 × 80 Å with 0.375 Å spacing was used that include not only the

Table 1 Free energy of binding (Δ*G*) and predicted inhibition constant (KI) estimated with AutoDock 4.2.1 and interaction of inhibitors with NF-κB p50 subunit at DBR

Inhibitors	Δ*G* (kcal/mol)	KI (μM)	Polar interactions	Hydrophobic residue in 5 Å region
Curcumin sulphate	−8.94	0.28	TYR-57, HIS-141, ASP-239, LYS-241, LYS-272	PHE-55, MET-205, LEU-207, ALA-242, PHE-307
Aurine tricarboxylic acid[a]	−7.83	3.56	GLU-60, LYS-241, LYS-272	PHE-55, TYR-57, VAL-142, LEU-207, ALA-242
Cyclocurcumin	−7.47	3.35	VAL-142, LYS-144, ASP-206, LEU-207, ASP-239	PHE-55, TYR-57, MET-205, ALA-242
Curcumin glucuronide	−7.21	5.19	VAL-142, LYS-144, ASP-206, LEU-207, LYS-241	TYR-57, CYS-59, MET-205, VAL-209, ALA-242
β-Turmerone	−7.20	5.26	SER-63	TYR-57, VAL-58, VAL-112, LEU-137, ILE-139, LEU-140
α-Turmerone	−7.12	6.01	SER-63	TYR-57, VAL-58, CYS-59, PRO-62, VAL-112, LEU-137, ILE-139, LEU-140
Curcumin (enol)	−7.05	6.82	VAL-142, LYS-144, ASP-206, SER-208	TYR-57, VAL-58, CYS-59, MET-205, LEU-207, VAL-209
Bisdemethoxycurcumin	−6.90	8.78	ARG-54, GLU-60, HIS-141, LYS-144, ASP-239	PHE-55, TYR-57, VAL-142, LEU-207, ALA-242
Curcumin (keto)	−6.62	13.97	ARG-54, GLU-60, HIS-141, LEU-207, LYS-241	PHE-55, TYR-57, MET-205, ALA-242
Demethoxycurcumin	−6.40	20.27	HIS-141, LYS-144, ASP-206, LEU-207, ASP-239, LYS-241	PHE-55, TYR-57, VAL-142, VAL-209, ALA-242
Ellagic acid[a]	−6.38	21.13	TYR-57, VAL-58, GLY-65, ILE-139	PHE-53, CYS-59, LEU-67, VAL-112, LEU-137, LEU-140
Hexahydrocurcumin	−5.92	45.63	GLU-60, LYS-144, ASP-206, LEU-207, LYS-241	PHE-55, TYR-57, VAL-142, VAL-209, ALA-242
Gallic acid[a]	−5.85	51.46	LYS-241, ASN-247, ASP-271, LYS-272	ALA-242, ALA-245, LEU-248
Tetrahydrocurcumin	−5.59	80.28	GLU-60, HIS-141, LYS-144, MET-205, ASP-239, LYS-241	PHE-55, TYR-57, LEU-207, ALA-242

[a] Known NF-κB inhibitors

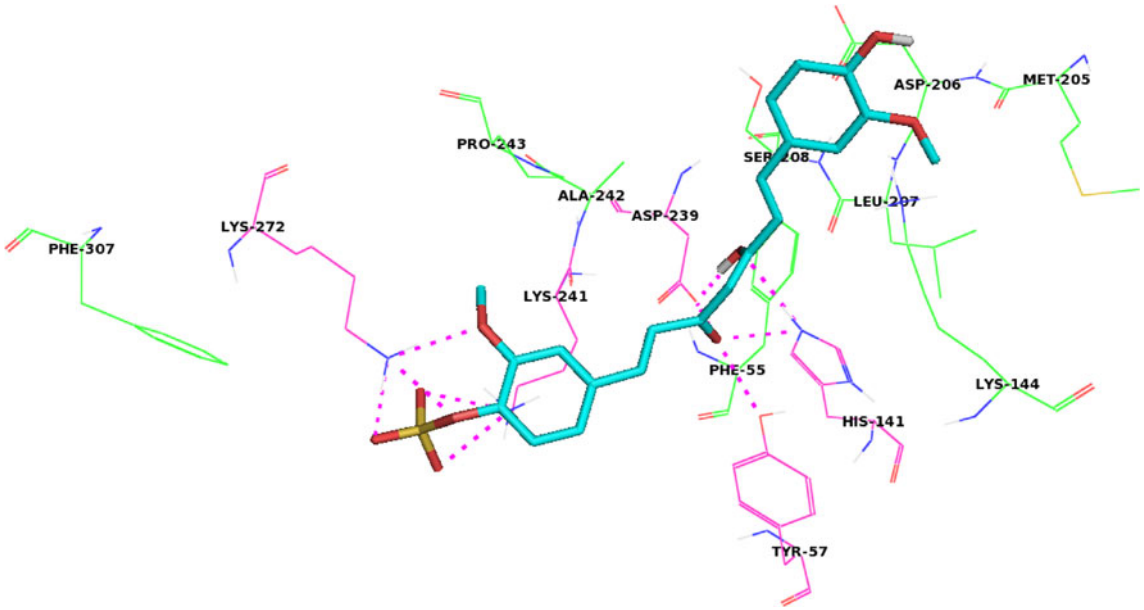

Fig. 3 Binding mode of curcumin sulphate showing polar interactions with TYR-57, HIS-141, ASP-239, LYS-241, LYS-272 (magenta) at DBR of NF-κB p50 subunit

DNA binding region (DBR) but also significant portions of surrounding surface. Docking to macromolecule was performed using an empirical-free energy function and Lamarckian Genetic Algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2,500,000 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80. Hundred independent docking runs were performed for each ligand and protein–ligand complex for lowest free energy of binding (ΔG) confirmation from the largest cluster was written and saved in PDBQT format. These PDBQT files have been converted to PDB file format using Swiss-PdbViewer. Docking results were analyzed using PyMOL 0.99 for possible polar and hydrophobic interactions.

Docking studies were performed at Intel(R) Core(TM) i3 CPU (3.2 GHz) with Linux-based operating system Fedora 8.

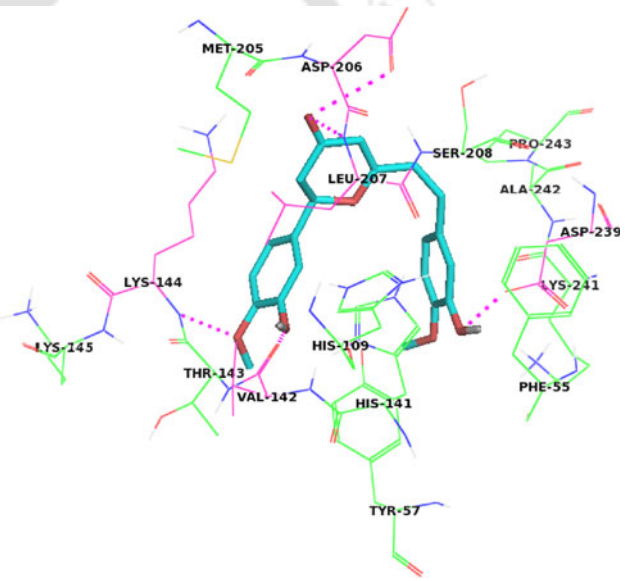

Fig. 4 Binding mode of cyclocurcumin showing polar interactions with VAL-142, LYS-144, ASP-206, LEU-207, ASP-239 (magenta) at DBR of NF-κB p50 subunit

Results and discussions

To predict the inhibition of p50 subunit by curcumin and its derivatives, molecules were docked over p50 DBR. The results of docking studies are summed up in Table 1.

Curcumin sulphate bound to DBR of p50 subunit with ΔG of -8.94 kcal/mol and predicted KI of 0.24 μM (Fig. 3). The sulphate group of curcumin sulphate was found to form polar interaction with side chains of LYS-241 and LYS-272. Keto group formed polar interaction with side chains of TYR-57 and ASP-239 whilst enol group is in the polar interaction range with HIS-141. Cyclocurcumin bound to the DBR with ΔG of -7.47 kcal/mol and predicted KI of 3.35 μM (Fig. 4). Oxygen of one phenyl group showed polar interaction with ASP-239, whilst other phenyl group showed polar interaction with backbone nitrogen of both the LYS-144 and VAL-142. The oxygen atom of α,β-unsaturated dihydropyranone moiety showed the interaction with ASP-206 and LEU-207. Curcumin glucuronide bound to the DBR with $\Delta G = -7.21$ kcal/mol and predicted KI of 5.19 μM. Oxygen of the *o*-methoxy group showed polar interaction with side chain of ASP-206, whilst

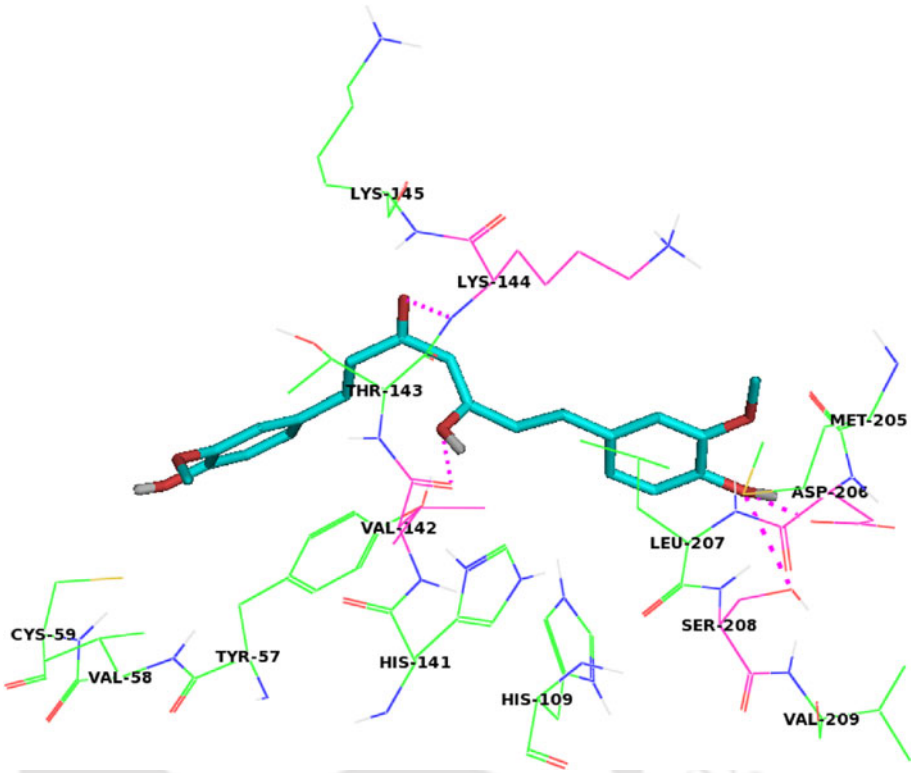

Fig. 5 Binding mode of curcumin (enol) showing polar interactions with VAL-142, LYS-144, ASP-206, SER-208 (magenta) at DBR of NF-κB p50 subunit

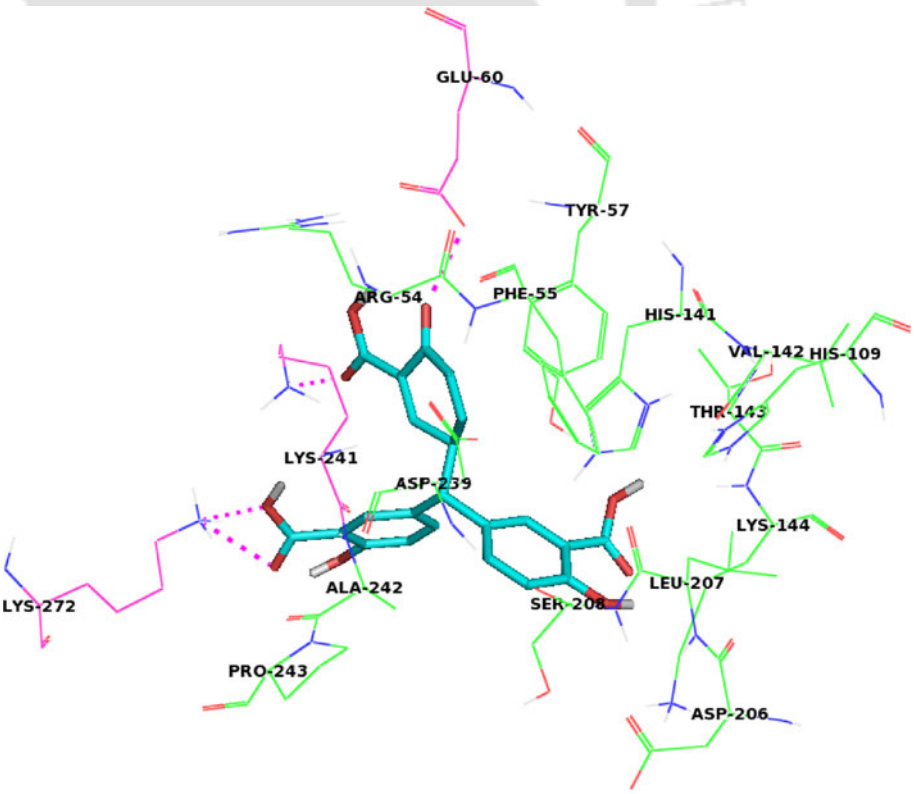

Fig. 6 Binding mode of aurine tricarboxylic acid showing polar interactions with GLU-60, LYS-241, LYS-272 (magenta) at DBR of NF-κB p50 subunit

phenyl group showed polar interaction with backbone nitrogen of LEU-207. Keto and enol group were having interaction with backbone nitrogen atom of LYS-144 and backbone oxygen atom of VAL-142, respectively, whilst glucuronide group was in polar interaction range with side chain of LYS-241.

Table 2 Physicochemical properties of curcumin derivatives

Inhibitors	Log*P*	TPSA (Å^2—)	Molecular weight	H-bond acceptors	H-bond donors	Violations (Lipinski rule)
Curcumin sulphate	−0.444	142.428	447.441	9	2	0
Cyclocurcumin	2.998	85.229	370.401	6	2	0
Curcumin glucuronide	0.91	192.446	544.509	12	6	3
β-Turmerone	3.542	17.071	220.356	1	0	0
α-Turmerone	3.756	17.071	220.356	1	0	0
Curcumin (enol)	3.048	96.223	368.385	6	3	0
Bisdemethoxycurcumin	3.411	77.755	308.333	4	3	0
Curcumin (keto)	2.303	93.066	368.385	6	2	0
Demethoxycurcumin	3.23	86.989	338.359	5	3	0
Hexahydrocurcumin	2.43	96.223	374.433	6	3	0
Tetrahydrocurcumin	2.99	96.223	372.417	6	3	0

We observed that α-turmerone and *β*-turmerone which are structurally similar except the presence of methyl and methylene group in aromatic ring, keto group of both showed polar interaction with SER-63. α-turmerone bound to DBR with ΔG of −7.12 kcal/mol and predicted KI = 6.01 μM whilst *β*-turmerone with ΔG of −7.20 kcal/mol and predicted KI of 5.26 μM. Curcumin (enol) bound at DBR with ΔG of −7.05 kcal/mol and predicted KI of 6.82 μM (Fig. 5). Oxygen of one phenyl group was having polar interaction with side chain of ASP-206 and SER-208. Keto and enol group were having interaction with backbone nitrogen atom of LYS-144 and backbone oxygen atom of VAL-142, respectively.

We observed that most of the curcumin derivatives formed polar interactions with LYS-144, ASP-206, ASP-239, LEU-207, and LYS-241 amongst which LYS-144 and LYS-241 were the key residues which form hydrogen bond with consensus DNA sequence of κB site suggesting that curcumin derivatives interfere in binding of NF-κB to κB site by interacting with LYS-144 and LYS-241 at DBR (Jutooru *et al*., 2010). To compare the inhibition of NF-κB by curcumin derivatives with known inhibitors, aurine tricarboxylic acid, gallic acid, and ellagic acid were docked over p50 subunit out of which aurine tricarboxylic acid showed best binding mode with ΔG of −7.83 kcal/mol and predicted KI = of 3.56 μM (Fig. 6). Amongst curcumin derivatives and known inhibitors thus docked over NF-κB curcumin sulphate showed the best binding mode with lowest free energy of binding whilst cyclocurcumin, curcumin glucuronide, *β*-turmerone, α-turmerone, and curcumin (enol) were the other derivatives with satisfying performance.

Physicochemical properties are summed up using Molinspiration Property Calculator in Table 2 to evaluate the drug likeness of the inhibitors. According to Lipinski's Rule of Five for a drug to show good ADME (absorption, distribution, metabolism, and excretion) properties its log*P* should be less than 5, hydrogen bond donor should not be more than 5, hydrogen bond acceptor should not be more than 10 and molecular weight should be less than 500 (Lipinski *et al*., 2001). All the inhibitors Lipinski's Rule of Five except curcumin glucuronide which has molecular weight of 544.509, 6 hydrogen bond donors and 12 hydrogen bond acceptors. For good cell membrane permeability a molecule should have topological polar surface area (TPSA) less than 140 Å^2 (Veber *et al*., 2002). Curcumin glucuronide have high TPSA of 192.446 hence predicted cell membrane permeability is suboptimal. Curcumin sulphate is having TPSA of 142.428 Å^2 hence moderate predicted cell membrane permeability. The remaining inhibitor molecules have TPSA values below 140 Å^2 and are predicted to have good cell membrane permeability.

Conclusions

The present in silico study provides insights into the inhibition of NF-κB p50 subunit by curcumin and its natural derivatives. The involvement of residues like LYS-144, ASP-206, ASP-239, LEU-207, and LYS-241 seems to play an important role in binding of curcumin and its natural derivatives to the DBR. Curcumin sulphate was predicted to be the most potent inhibitor amongst all the derivatives and known inhibitors (aurine tricarboxylic acid, gallic acid, and ellagic acid) with favourable ADME predictions.

Acknowledgments Anil Kumar thank IITG and MHRD (Government of India) for the fellowship to pursue Ph.D. We thank the developers of the free softwares used for this study and IITG for the infrastructural support.

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Molecular docking studies on inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids

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Abstract:
Stat3 is a mammalian transcription factor which regulates various genes involved in cell growth, proliferation, cell survival and other biological processes. Its constitutive activation promotes dysregulated growth, survival and immune responses which contribute to tumor progression and carcinogenesis. Inhibition of Stat3 dimerization which prevents its binding to DNA is a rational strategy that could be translated to potential therapeutic applications. The present computational study provides insights into the inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids. The involvement of residues like LYS-591, ARG-609, SER-611, GLU-612, SER-613, SER-636 and VAL-637 seems to play an important role in binding of curcumin natural derivatives and its amino acids conjugates with Src Homology (SH2) domain of Stat3 monomer. Demethoxycurcumin followed by hexahydrocurcuminol were predicted to be the most potent inhibitors amongst all the curcumin natural derivatives and known inhibitors (FLLL32, Sta21 and Stattic). Curcumin-proline conjugate (1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) was predicted to be the most potent inhibitor of Stat3 dimerization amongst the curcumin-amino acid conjugates and known peptide based inhibitor (Phpr-pTYR-LEU-cis-3,4-methanoPRO-GLN-NHBn).

Keywords: Curcumin natural derivatives, Curcumin–amino acid conjugates, Stat3 dimerization, Src Homology (SH2) domain, Molecular docking

Background:
Mammalian signal transducers and activators of transcription (STAT) is a family of 7 transcription factors (Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b and Stat6) **[1]**. These transcription factors are activated in response to cytokines and growth factors including interferons (IFNs), epidermal growth factor (EGF), interleukin 5 (IL5), IL6, hepatocyte growth factor (HGF), leukemia inhibitory factor (LIF) and bone morphogenetic protein 2 (BMP2) which regulate various genes involved in cell growth, proliferation, cell survival and other biological processes **[2]**. The transcription factors of this family are activated by growth factor receptor tyrosine kinases, Janus kinases or Src family kinases through the phosphorylation of a critical tyrosine residue which leads to the dimerization of two phosphorylated monomers **[3]**. Phosphorylated dimers are translocated to the nucleus where they bind to specific DNA-response elements in the promoter region of target genes, and induce gene expression **[4, 5]**. It has been found that constitutive activation of certain STAT family members, particularly of Stat3 promote dysregulated growth, survival and immune responses which contribute to tumor progression and carcinogenesis **[6, 7]**. Stat3 dimerization relies on the reciprocal binding of Src Homology (SH2) domain-binding peptide (Pro-pTyr-Leu-Lys-Thr-Lys) of one monomer to another **[8]**. It is a critical step in Stat3 activation which presents an attractive target to abrogate Stat3 DNA-binding and to

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inhibit its aberrant transcriptional activity **[9]**. Interest in development of small molecule and peptide based inhibitors of Stat3 dimerization in the last few years has led to the discovery of inhibitors like Stattic, Sta21 and FLLL32 **[10, 11]**.

Curcumin (diferuloylmethane) is a principal component of Asian spice turmeric with wide range of pharmacological properties which includes antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities **[12]**. Curcumin has been reported to inhibit the Stat3 phosphorylation and DNA binding activity in human cancer cells **[13, 14]**. It has been found that curcumin is extremely safe even at very high doses in various studies with animal models and human **[15]**. In addition to curcumin, turmeric plant contains several other curcuminoids with broad spectrum of pharmacological properties in which demethoxycurcumin and bisdemethoxycurcumin are abundant **[16]**. In order to improve the pharmacological properties, curcumin was conjugated with various functional groups. Curcumin-amino acids conjugates were also synthesized using different substitution schemes which were tested for antioxidant, antimicrobial, antiviral, antiproliferative and proteasome inhibition activities **[17-20]**. In the present study we investigate the interaction of curcumin natural derivatives and its conjugates with amino acids in the pursuance of potential lead molecule for inhibition of Stat3 dimerization using molecular docking over the SH2 domain of a Stat3 monomer.

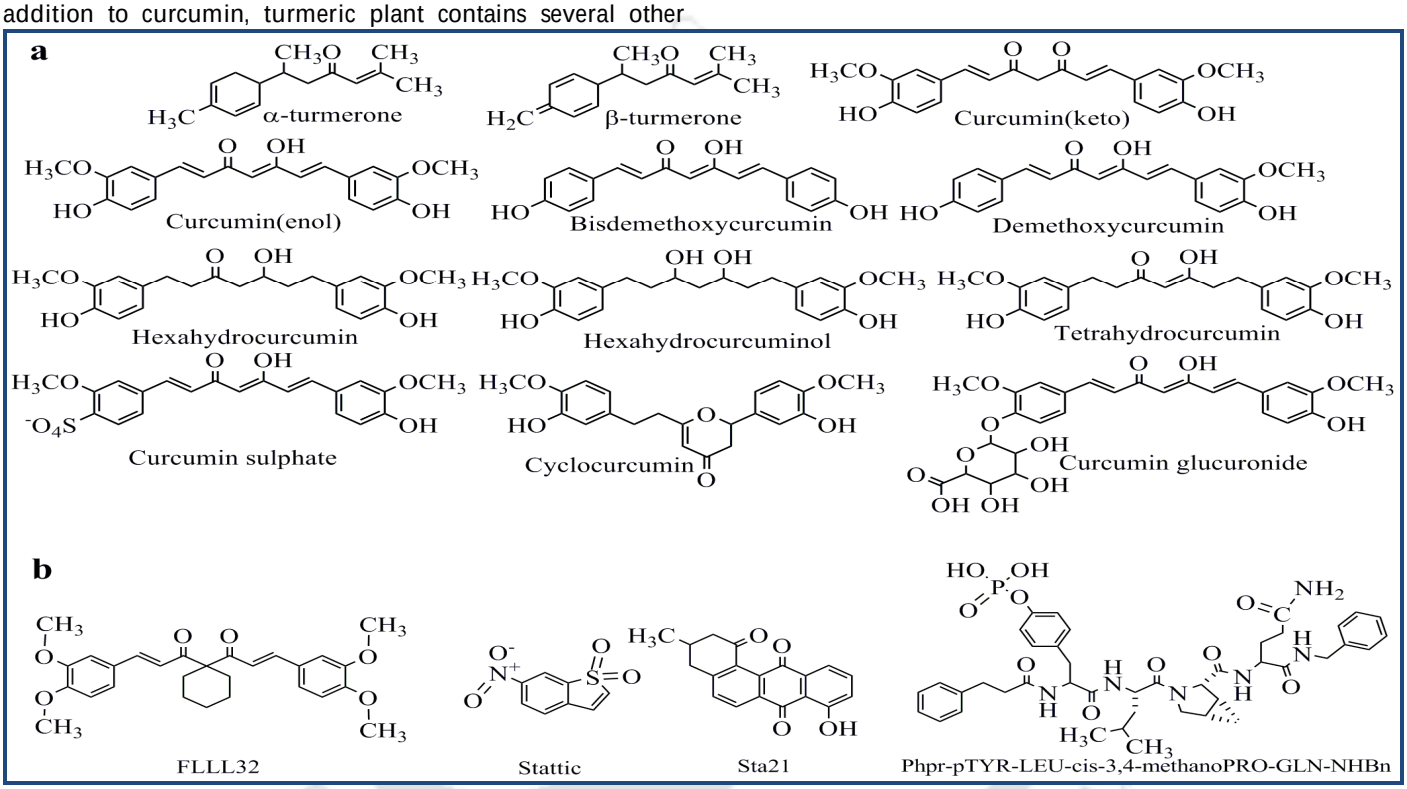

Figure 1: Structures of curcumin natural derivatives and known Stat3 dimerization inhibitors used in the study.

Methodology:

Preparing small molecules

Curcumin natural derivatives **(Figure 1a)**, its conjugates with amino acids **Table 1 (see supplementary material)** and known Stat3 dimerization inhibitors **(Figure 1b)** were drawn and 3D optimized by MarvinSketch (Free Academic License) and saved in Protein Data Bank (PDB) file format. These small molecules were prepared for molecular docking by merging non-polar hydrogens, assigning Gastegier charges, and saving them in PDBQT file format using AutoDock Tools (ADT) 1.5.6.

Preparing Target molecule

To investigate the interaction of curcumin natural derivatives and its amino acid conjugates, X-ray crystal structure of Stat3β complexed with DNA (PDB ID: 1BG1) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb). For molecular docking DNA and other hetero-atoms (water, ions, etc.) were removed using PyMOL 0.99, Gasteiger charges were assigned and macromolecule was saved in PDBQT file format using ADT.

Molecular docking

Grid and docking parameter files were prepared using ADT and molecular docking was performed with AutoDock 4.2.1 (Scripps Research Institute, USA) considering all the rotatable bonds of small molecules as rotatable and macromolecule as rigid. Grid box size of 80 x 80 x 80 Å with 0.375 Å spacing was selected that include the whole SH2 dimerization domain of Stat3 monomer. Empirical-free energy function and Lamarckian Genetic Algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2,500,000 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80 were used to perform molecular docking with macromolecule. Hundred independent docking runs were performed for each small molecule. Protein– small molecule complex for lowest free energy of binding (ΔG) confirmation from the largest cluster was saved in PDBQT format and converted to PDB file format using UCSF Chimera 1.6.1. Docking results were analyzed using PyMOL 0.99 for possible polar and hydrophobic interactions. Docking studies were performed at Intel(R) Xeon(R) CPU (3.2 GHz) with Linux-based operating system Fedora 15.

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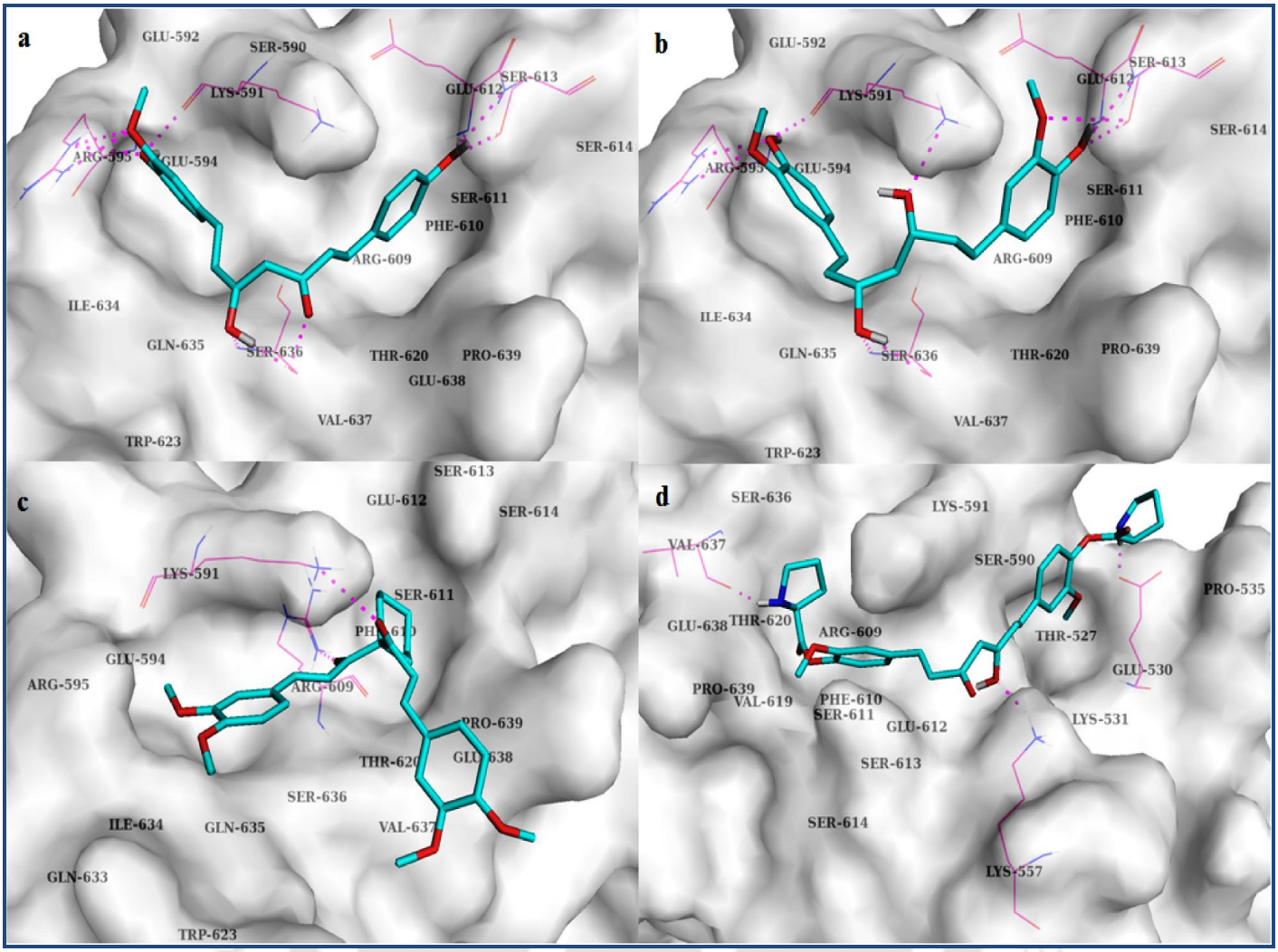

Figure 2: Binding modes of **(a)** demethoxycurcumin **(b)** hexahydrocurcuminol **(c)** FLLL32 **(d)** curcumin-proline conjugate (1,7-Bis (4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) with Stat3 SH2 domain of a Stat3 monomer.

Discussion:

Stat3 monomer contains four domains, a protein interaction domain which helps in cooperative DNA binding, all-alpha domain comprises of a bundle of four antiparallel helices connected by short loops, DNA binding domain comprises of eight-stranded β-barrel and SH-2 dimerization domain comprises of a central three-stranded β-pleated sheet flanked by a helix and two strands. To predict the inhibition of Stat3 dimerization by curcumin natural derivatives and its amino acid conjugates, these small molecules were docked over SH2 domain of a Stat3 monomer and their binding modes were analyzed **Table 2 & 3 (see supplementary material).**

Demethoxycurcumin bound to SH2 domain with ΔG of -7.80 kcal/mol and KI of 1.93 μM **(Figure 2a)**. Methoxy group of demethoxycurcumin was found to form polar interaction with side chain of ARG-595 while the neighboring hydroxyl group was in polar interaction range with main chain of LYS-591 and side chain of ARG-595. Hydroxyl group present at the other side of the molecule (methoxy group lacking) formed polar interactions with SER-613. Both keto and hydroxyl group present in the linker region were in polar interaction range with SER-636.

It was found that hexahydrocurcuminol bound to SH2 domain with ΔG of -7.69 kcal/mol and KI of 2.31 μM **(Figure 2b)**. One methoxy group of hexahydrocurcuminol was found to form polar interaction with side chain of ARG-595 while the neighboring hydroxyl group was in polar interaction range with LYS-591 and ARG-595. At the other side of the molecule methoxy group formed polar interactions with SER-613 while hydroxyl group was in polar interaction range with GLU-612 and SER-613. In the linker region, one of the hydroxyl group formed polar interaction with SER-636 while other interacted with side chain of LYS-591. Amongst known inhibitors FLLL32, static and sta21, FLLL32 bound to SH2 domain with lowest ΔG of -6.69 kcal/mol and KI of 12.54 μM **(Figure 2c).** Keto groups present in the linker region were found to form polar interactions with LYS-591 and ARG-609 respectively.

Amongst the curcumin-amino acid conjugates curcumin-proline conjugate (1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) docked with lowest ΔG of -6.29 kcal/mol and KI of 24.55 μM **(Figure 2d).** Prolinoyl group at one side of the molecule was in polar interaction range with GLU-530 while at other side it interacted with VAL-637. The hydroxyl group present in linker region of the conjugate formed

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polar interaction with LYS-557. The peptide based known inhibitor (Phpr-pTYR-LEU-cis-3,4-methanoPRO-GLN-NHBn) docked with ΔG of -5.50 kcal/mol and KI of 93.10 μM and formed polar interactions with LYS-591, ARG-595 and ARG-609.

Curcumin natural derivatives and its amino acid conjugates bound to SH2 domain through polar interactions with LYS-591, ARG-609, SER-611, GLU-612, SER-613, SER-636 and VAL-637 among which LYS-591, ARG-609, SER-611 and SER-613 are the amino acid residues which remain highly conserved in SH2 domain and play an important role in Stat3 dimerization by forming polar interaction with pTYR-705 residue of other monomer.

Conclusion:

The present computational study provides insights into the inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids. The involvement of residues like LYS-591, ARG-609, SER-611, GLU-612, SER-613, SER-636 and VAL-637 play an important role in binding of curcumin natural derivatives and its amino acid conjugates with SH2 domain. Demethoxycurcumin followed by Hexahydrocurcuminol were predicted to be the most potent inhibitors amongst all the curcumin natural derivatives and known inhibitors (FLLL32, Sta21 and Stattic) docked. Amongst the curcumin-amino acid conjugates curcumin-proline conjugate (1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) was predicted to be the most potent inhibitor of Stat3 dimerization.

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Supplementary material:

Table 1: Curcumin-amino acid conjugates used in the study

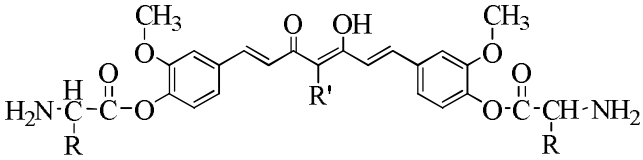
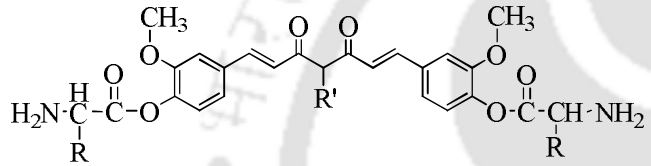
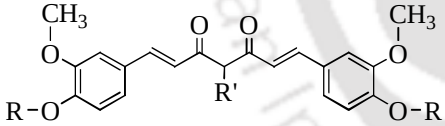
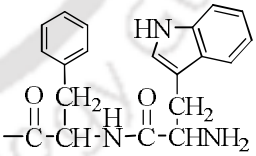
Compound name	R=
Scheme –I (R'=H) [17] 	
1,7-Bis(4-O-L-leucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH_2CH(CH_3)CH_3$
1,7-Bis(4-O-L-phenylalaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH_2C_6H_5$
1,7-Bis(4-O-L-alaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—CH_3
1,7-Bis(4-O-L-isoleucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH(CH_3)CH_2CH_3$
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH(CH_3)CH_3$
1,7-Bis(4-O-L-serinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—CH_2OH
1,7-Bis(4-O-L-cysteinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—CH_2SH
1,7-Bis(4-O-L-phenylglycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—C_6H_5
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—H
1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH_2CH_2CH_2$— (part of pyrrolidine ring)
Scheme –II (R'=H) [18] 	
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	—H
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	—$CH(CH_3)CH_3$
1,7-Bis(4-O-L-glutamatoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	—CH_2CH_2COOH
Scheme –II (R'=$COCH_2NH_2$) [19]	
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-4-glycyl-1,6-heptadiene-3,5-dione	—H
Scheme –III (R'=H) [20] 	
1,7-Bis(4-O-L-tryptophanylphenylalaninoyl-3-methoxyphenyl)- 1,6-heptadiene-3,5-dione	

Table 2: Free energy of binding (ΔG) and predicted inhibition constant (KI) estimated with AutoDock 4.2.1 and interactions of curcumin natural derivatives with Stat3 monomer

Compound Name	ΔG (kcal/mol)	KI (μM)	Polar interactions	Hydrophobic residue in 5 Å region
Demethoxycurcumin	-7.80	1.93	LYS- 591, ARG-595, GLU-612,SER-613, SER-636	PHE-610, ILE-634,VAL-637
Hexahydrocurcuminol	-7.69	2.31	LYS- 591, ARG-595, GLU-612,SER-613, SER-636	PHE-610, ILE-634,VAL-637
Hexahydrocurcumin	-7.32	4.39	LYS- 591, ARG-595, GLU-612,SER-613, SER-636	PHE-610, ILE-634,VAL-637
Bisdemethoxycurcumin	-7.19	5.39	LYS- 591, ARG-595,SER-611,GLU-612,SER-613,SER-636	PHE-610, ILE-634,VAL-637
Curcumin sulphate	-7.11	6.16	LYS-557,ILE-634	PHE-610, VAL-619,VAL-637
Curcumin (keto)	-7.09	6.31	LYS- 591, ARG-595,ARG-609,GLU-612,SER-613,SER-636	PHE-610, ILE-634, VAL-637

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Curcumin (enol)	-6.94	8.16	LYS- 591, ARG-595, ARG-609, GLU-612	PHE-610, ILE-634,VAL-637
FIII32*	-6.69	12.54	LYS- 591,ARG-609	PHE-610, ILE-634,VAL-637
Sta21*	-6.61	14.25	ARG-609, SER-636	ILE-634,VAL-637
Tetrahydrocurcumin	-6.49	17.56	LYS- 591, ARG-595, ARG-609, SER-613,SER-636	PHE-610, ILE-634,VAL-637
Stattic*	-6.45	18.79	LYS- 591, ARG-595	ILE-634
Cyclocurcumin	-6.42	19.82	LYS- 591, ARG-609,GLN-635	PHE-610, ILE-634,VAL-637
Curcumin glucuronide	-5.97	42.40	LYS- 591, ARG-595, ARG-609,ILE-634	PHE-610,VAL-637
β-Turmerone	-5.39	112.11	LYS- 591	ILE-634,VAL-637
α-Turmerone	-5.31	127.61	LYS- 591	ILE-634,VAL-637

*Known inhibitor of Stat3

Table 3: Free energy of binding (ΔG) and predicted inhibition constant (KI) estimated with AutoDock 4.2.1 and interactions of curcumin-amino acid conjugates with Stat3 monomer

Compound name	ΔG (kcal/mol)	KI (μM)	Polar interactions	Hydrophobic residue in 5 Å region
1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-6.29	24.55	GLU-530, LYS-557,VAL-637	PHE-610,VAL-619
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-5.96	42.93	GLU-530,LYS-591,ARG-595,SER-636	ILE-634,VAL-637
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	-5.51	91.60	LYS-591,ARG-609,ILE-634, GLU-638	PHE-610,VAL-637
Phpr-pTYR-LEU-cis-3,4-methanoPRO-GLN-NHBn*	-5.50	93.10	LYS-591,ARG-595, ARG-609	PHE-610, ILE-634,VAL-637,TYR-657
1,7-Bis(4-O-L-isoleucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-5.32	125.58	GLU-530,LYS-557,SER-590,SER-636	VAL-637
1,7-Bis(4-O-L-tryptophanylphenylalaninoyl-3-methoxyphenyl)- 1,6-heptadiene-3,5-dione	-5.17	162.04	LYS-557,LYS-591,GLU-592,SER-613,GLU-638	PHE-610, ILE-634,VAL-637,TYR-640
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-4-glycyl-1,6-heptadiene-3,5-dione	-5.00	216.91	LYS-591,ARG-609,SER-613,GLU-638	PHE-610,ILE-634,VAL-637
1,7-Bis(4-O-L-phenylalaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.97	226.37	GLU-530,LYS-557,SER-636,VAL-637	PHE-610,ILE-634
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	-4.89	261.36	GLU-592,ARG-595,SER-636	ILE-634,VAL-637
1,7-Bis(4-O-L-leucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.79	307.46	GLU-530,ARG-595,SER-636	ILE-589,ILE-634,VAL-637
1,7-Bis(4-O-L-alaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.77	317.43	LYS-557,GLU-612,GLU-638	PHE-610,VAL-637
1,7-Bis(4-O-L-serinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.62	407.78	GLU-530,GLY-558,SER-590,GLU-592,ARG-593,GLU-612	PHE-559
1,7-Bis(4-O-L-glutamatoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	-4.57	448.31	LYS-557,LYS-591,ARG-609,GLU-638	PHE-610,VAL-637
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.17	871.38	GLU-530,SER-636	LEU-528,ILE-634,VAL-637
1,7-Bis(4-O-L-phenylglycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-3.75	1790.00	LYS-591,GLN-635,SER-636	LEU-532,ILE-634,VAL-637
1,7-Bis(4-O-L-cysteinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-3.62	2210.00	SER-613,LYS-615,GLU-616,SER-636	VAL-619,ILE-634,VAL-637,TYR-640

*Known inhibitor of Stat3

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Interactions of Curcumin and Its Derivatives with Nucleic Acids and their Implications

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Abstract: Curcumin (diferuloylmethane) is a yellow polyphenol found in the rhizome of the annual herb turmeric (Curcuma longa) belonging to the family Zingiberaceae. Its interaction with a huge number of molecular targets like cytokines, growth factors, transcription factors, receptors, pro-inflammatory enzymes, protein kinases and adhesion molecules has been studied extensively. Interaction of curcumin with nucleic acids has been the focus of extensive research in recent years. Curcumin is observed to be genotoxic and antigenotoxic agent in time and concentration dependent manner. Curcumin and its derivatives either alone or as metal complexes have been reported to bind directly to DNA. The interactions are mainly as DNA minor groove binding or as DNA intercalating agents. The similarity in the shape of curcumin to DNA minor groove binding drugs is the motivation for exploring its binding to DNA minor grooves. Thus curcumin is a "double edged sword": having therapeutic potential as a minor groove binder but at the same time it may cause DNA damage in the cell at high concentration. The purpose of this review is to summarize the current information related to interaction of curcumin metal complexes and its derivatives with nucleic acids and the implication such interaction can have on therapeutics.

Keywords: Curcumin metal complex, Genotoxic and antigenotoxic agent, Reactive oxygen species (ROS), DNA damage, DNA minor groove binding, DNA intercalation.

1. INTRODUCTION

Turmeric has been used for thousands of years as a traditional medicine, coloring agent and spice in Asian countries. The major curcuminoids found in turmeric are curcumin (77%), demethoxycurcumin (17%) and bisdemethoxycurcumin (3%) [1]. The first chemical characterization of curcumin was done in 1910 [2]. It exists in keto (**1**) and enol (**2**) forms (Fig. **1**) and regarded as the most active constituent of turmeric [3]. Curcumin has been described in hundreds of published papers over the past few decades, studying its antioxidant, anti-inflammatory, cancer chemo-preventive and chemotherapeutic properties [4]. It has been reported to bind a huge number of molecular targets like signalling molecules, growth factors, transcription factors, receptors, pro-inflammatory enzymes, protein kinases and adhesion molecules (Fig. **2**) [5]. In the pursuance of enhanced pharmacological properties, researchers have developed many synthetic curcumin derivatives by adding various functional groups in aromatic rings and linker region of the curcumin molecule to improve the binding of these derivatives to targets through polar interactions [6]. The purpose of this review is to summarize the available scientific knowledge on the interaction of curcumin with nucleic acids which is recently emerging as a field of interest.

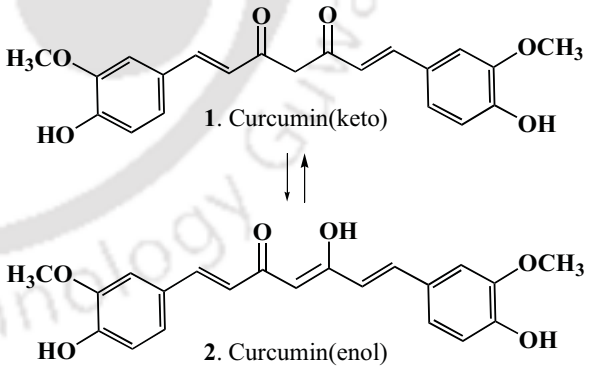

Fig. (1). Chemical structures of keto and enol forms of curcumin.

2. CURCUMIN AS GENOTOXIC AND ANTIGENO-TOXIC AGENT

Several studies have revealed that depending on dose, curcumin can act as an antigenotoxic or genotoxic agent. When curcumin acts as an antigenotoxic agent it scavenges reactive oxygen species (ROS) [7, 8], activates detoxifying enzymes (GPx, GR, G6PDH, catalase, GST and QR) [9] and

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induces the single stranded cleavage repair in DNA [10] while as a genotoxic agent it generates hydroxyl free radicals which attack the deoxyribose moiety of DNA to cause the strand breaks [11, 12]. Independent studies have shown that curcumin at low doses (upto 5µg/ml) is anti-genotoxic but at high doses (>8µg/ml) causes genotoxicity [13-18]. Pre-treatment with curcumin at a low dose (2µg/mL) significantly decreased cyclophosphamide (CPA)-induced micronucleus formation in human hepatoma G2 (HepG2) cells. However, curcumin alone at 8 µg/mL enhanced micronucleus formation. At doses higher than 8µg/mL, curcumin increased the frequency of chromosomal aberrations in HepG2 cells [14]. Curcumin at 10µg/mL induced mutagenic changes and potentiated doxorubicin and gamma radiation-mediated chromosomal aberrations in Chinese hamster ovary cells [13, 19]. Curcumin induces both mitochondrial and nuclear DNA damage with the former being more extensive than the latter [18].

Curcumin-induced DNA damage has been studied in various cell lines, such as mouse-rat hybrid retina ganglion cells, Jurkat T-lymphocytes, colorectal carcinoma HCT116 cells, gastric mucosa cells and human lung cancer cell lines PC-9 [16, 20-23]. These studies have concluded that curcumin has a major contribution in inducing DNA damage at high concentrations. However, the differential behavior of curcumin due to concentration variation is not understood currently and requires further investigations. Else this could turn out to be the Achilles heel of curcumin while finding wide acceptance as an anticancer agent in future inspite of its current promises.

3. CURCUMIN AS METAL CHELATING AGENT

Turmeric powder can be used to remove Cu(II) from aqueous solution as it contains compounds acting as sequestering agents for toxic metals [24]. It has been established that curcumin and its derivatives form complexes with a wide range of metals. These metal complexes have been reported to bind to DNA with high affinity. Based on structure-function relationship studies, three sites in curcumin have been ascertained to which metals bind (Fig. **3**). Two of these sites are contributed by the phenolic and methoxy groups on the two benzene rings and the third site is due to the presence of 1,3-diketone system between the rings [25]. Chena *et al.* confirmed this by resonance light scattering (RLS) technique where they observed enhancement in the intensity of Cu(II) with increase in curcumin concentration [26].

Curcumin helps in decreasing amyloid aggregation or oxidation induced neurotoxicity by chelating copper/iron ions which exist in high concentration in Alzheimer's disease. Spectroscopic studies using $CuCl_2$, $FeCl_2$ and $ZnCl_2$ found that curcumin has high affinity towards Cu^{2+} or Fe^{2+}, where each Cu^{2+} and Fe^{2+} ions appeared to bind at least two curcumin molecules whereas Zn^{2+} showed little binding. When curcumin was added to cultured liver cells, there was increase in the mRNA level of ferritin and α-GST but the protein level of ferritin was decreased due to inhibition of translation which normally happens when iron chelators are present [27]. A similar study has suggested the effect of curcumin on transferrin receptor1 and iron regulatory protein activation as an indicator of iron depletion due to iron chelation by curcumin [28]. Thus curcumin is an iron chelator and modulates the proteins of iron metabolism in cells and tissues.

Borsari *et al.* demonstrated that when curcumin and diacetylcurcumin react with Fe^{3+} in water/methanol 1:1 solution near neutral pH, these molecules form complexes like $FeH_2CU(OH)_2$ and $FeDCU(OH)_2$, {H_2CU=curcumin and DCU= diacetylcurcumin monoanion, respectively} which at basic pH gets ionized to $[FeH_2CU(OH)_3]^-$ and $[FeDCU(OH)_3]^-$. ^1H NMR data suggested that β-diketo

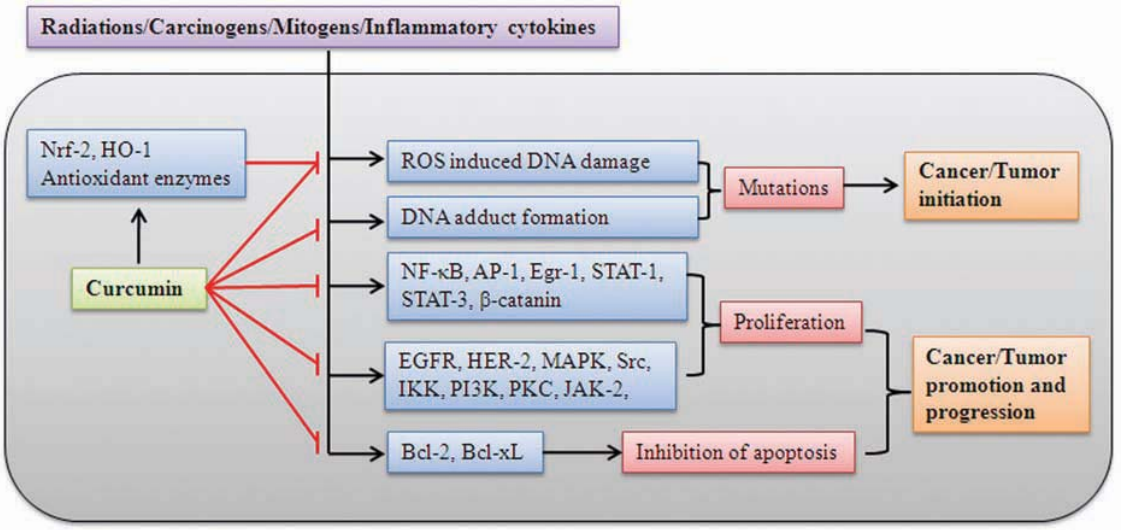

Fig. (2). Anticarcinogenic effects of Curcumin (Abbreviations: Nuclear factor-E2 p45-related factor-2 (**Nrf-2**), Heme Oxygenase-1 (**HO-1**), Nuclear factor kappa-light-chain-enhancer of activated B cells (**NF-κB**), Activator protein 1 (**AP-1**), Early growth response protein-1 (**EGR-1**), Signal Transducers and Activators of Transcription-1 (**STAT-1**), Epidermal growth factor receptor (**EGFR**), Human Epidermal Growth Factor Receptor-2 (**HER-2**), Mitogen-activated protein kinases (**MAPK**), IκB kinase (**IKK**), Phosphatidylinositol 3-kinase (**PI3K**), Protein kinase C (**PKC**), Janus kinase-2 (**JAK2**), B-cell lymphoma-2 (Bcl-2), B-cell lymphoma-extra large (**Bcl-xl**)).

moiety of the ligands is involved in metal chelation under both the pH conditions [29].

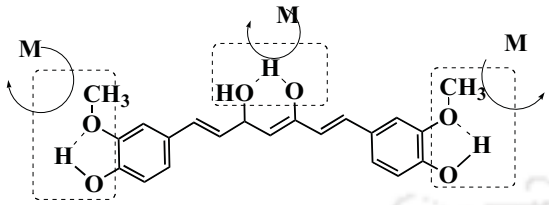

Fig. (3). Three metal binding sites (M) in curcumin: two of these sites are contributed by the phenolic and methoxy groups on the two benzene rings and the third site is due to the presence of 1,3-diketone system between the rings.

4. CURCUMIN METAL COMPLEXES EXIHIBIT ROS INDUCED DNA DAMAGE

Although a limited number of studies have suggested that curcumin produces ROS (O_2^-, H_2O_2) in the presence of metals such as Cu(II) and Fe(II) which cause DNA damage in supercoiled circular plasmid DNA (pUC18 and pBR322) as a result of that the molecules become open circular. It was proved that ROS are involved in cleavage by adding catalase which resulted in inhibition of DNA breakage [10, 30-35]. Similar results were reported when Balb-C mouse lymphocytes were treated with curcumin-Cu(II) complex. It was found that at high concentration (50 µM) although curcumin alone induces DNA strand breaks, the presence of copper increases the DNA damage [36]. Human prostate cancer cells (LnCaP, PC3 and DU145) treated with heteroleptic palladium(II) complex (**3**) (Fig. **4**) of curcumin also exhibit ROS-induced DNA damage [37].

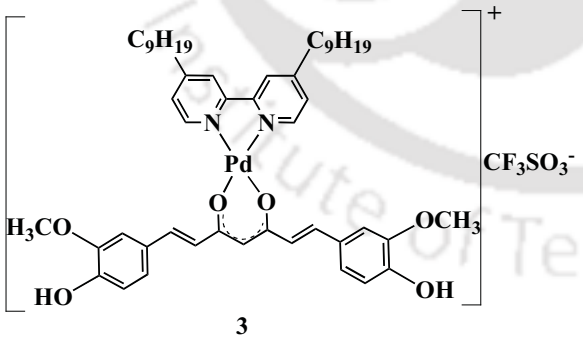

Fig. (4). Chemical structure of heteroleptic palladium(II) complex of curcumin which exhibits ROS-induced DNA damage.

In vitro studies have shown that Cu(II)-curcumin complex causes oxidation of guanine residues at C8 and increases DNA damage in proportion to Cu(II) concentration [38]. Curcumin treated with Cytochrome P450 (CYP 2D6, CYP1A1, or CYP1A2) induced DNA damage in the presence of Cu(II) especially at 5'-TG-3', 5'-GC-3', and 5'GG-3' sequences. The DNA damage inhibited by both catalase and bathocuproine, suggests that reactive species derived from the reaction of H_2O_2 with Cu(I) participate in DNA damage. Formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine by oxidation of guanine was significantly increased by CYP2D6-treated curcumin in the presence of Cu(II) [39, 40]. It was seen that there is an induction of DNA damage by dietary curcumin upon copper accumulation in Long-Evans Cinnamon (LEC) rats through the formation of nuclear and mitochondrial etheno-DNA adducts [41].

Song *et al.* synthesized and characterized rare earth metal complexes with curcumin and 1,10-phenanthroline-5,6-dione (**4**) (Fig. **5**). The general formula of the complexes was REL_3L' (RE = samarium (Sm), europium (Eu), and dysprosium (Dy), L=curcumin, L'= 1,10-phenanthroline-5,6-dione) To study the interaction of complexes with DNA, they treated pBR322 plasmid DNA (0.37 µM) with the varied concentrations (0-0.08 µM) of complexes at physiological pH and temperature. The results suggested that SmL_3L' can cleave plasmid DNA at physiological pH and temperature through oxidation of bases. It was found that the cleavage process was sensitive to pH and optimum temperature for cleavage was 37 °C [42].

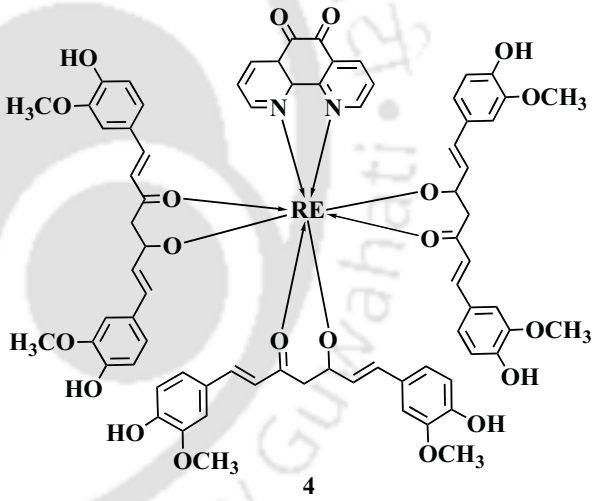

Fig. (5). Chemical structure of rare earth metal (RE) complexes of curcumin with 1,10-phenanthroline-5,6-dione which can cleave plasmid DNA at physiological pH and temperature.

5. CURCUMIN DERIVATIVES AS MAJOR AND MINOR GROOVE BINDERS OF DNA

It is now evident from various studies that curcumin directly interacts with nucleic acids. In general, interactions of small molecules like curcumin with DNA have three common binding modes: (i) electrostatic interaction, which is due to the negatively charged sugar- phosphate backbone, (ii) hydrophobic binding against minor or major grooves of DNA, preferentially binding to AT-rich regions [43] and (iii) intercalation between the stacked base pairs of native DNA [44]. Thus small molecules interacting with double stranded DNA (dsDNA) can be classified according to their binding modes as groove binders (non-intercalators) and intercalators. The potential of curcumin and its natural derivatives

(demethoxycurcumin (**5**) and bisdemethoxycurcumin (**6**)) (Fig. **6**) for DNA binding were studied using restriction digestion of DNA sequences with enzymes such as *EcoRI* (G:AATTC), *HindIII* (A:AGCTT), *SmaI* (CCC:GGG) and *BamHI* (G:GATCC). Analysis showed that the curcuminoids prevent *EcoRI* and *HindIII* from digesting at the respective restriction sites by directly binding to AT-rich base pairs in the sequences whereas allow the restriction digestion by *SmaI* and *BamHI* both of whose sequence are GC-rich [25]. However it is not known if curcumin also inhibits the activity of *EcoRI* and *HindIII* directly.

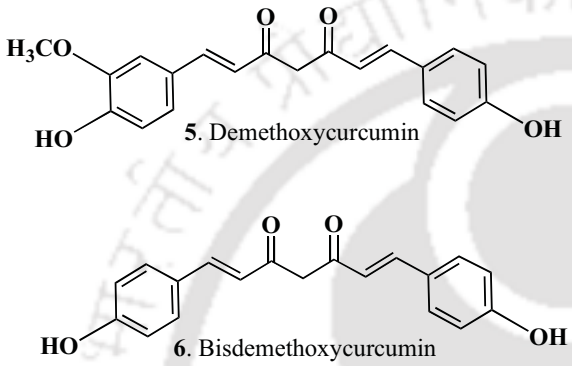

Fig. (6). Chemical structures of demethoxy and bisdemethoxycurcumin which prevent *EcoRI* and *HindIII* from digesting AT-rich restriction sites.

Circular dichroism and absorption spectroscopy techniques along with molecular modeling studies have proven beyond doubt that curcumin binds to the minor groove of the DNA double helix and is proposed to be a promising molecular probe to study biologically important pH and cation-induced conformational changes of nucleic acids [45]. Independent studies investigating the interaction between curcumin and DNA employing various electrochemical methods such as cyclic voltammetry, differential pulse voltammetry and hanging mercury drop electrode (HMDE) using carbon paste electrodes or modified glassy carbon electrode, have suggested that higher curcumin concentration causes conformational changes in DNA double helix [46-48].

Studies on the interaction between yeast RNA and curcumin- cetyltrimethylammonium bromide (CU-CTAB) complex by absorption spectroscopy and ^{1}H NMR spectroscopy suggested that CTAB first forms complex with yeast RNA by its positive charge, which in turn interacts with two carbon atoms of the carbonyls of curcumin through hydrogen bonds and hydrophobic forces and form CU-CTAB–nucleic acid ternary complex [49].

Apart from curcumin binding to the major and minor grooves of the DNA duplex, it binds to RNA bases and to the backbone phosphate group. This was found in a study using FT-IR and UV-visible spectroscopic analysis where no conformational changes were observed upon the interaction of curcumin with the nucleic acids (both DNA & RNA). Instead, it was found that curcumin binds to DNA through thymine O_2 group in the minor groove and through guanine and adenine N7 in the major groove as well as to the backbone PO_2 group. RNA binding occurs via uracil O_2 and guanine and adenine N7 atoms as well as the backbone phosphate group. Interestingly, the interaction of curcumin was stronger with DNA than RNA [50].

Fourier Transform Raman Spectroscopic study at physiological pH on curcumin-dGMP interaction at varying concentrations showed that at low concentration, curcumin/ dGMP (1/50) interaction is mainly through backbone PO_2 group. At higher concentration, curcumin (1/10) interact with guanine (N7) [51]. Furthermore, the UV-absorbance, gel-electrophoresis, fluorescence, CD spectroscopic and docking studies of binding of curcumin derivatives such as dimethoxycurcumin (**7**), isoxazolcurcumin (IOC) (**8**), diacetylcurcumin (DAC) (**9**) and a triglycyl curcumin derivative (**10**) (Fig. **7**) with calf thymus DNA showed that these derivatives do not intercalate but bind to the minor groove preferentially at AT-rich region [43, 52-54]. Others have also confirmed that curcumin and its derivatives are not intercalators but minor groove binders by performing ethidium bromide, 4'-6-Diamidino-2-phenylindole (DAPI) and Hoechst 33258 displacement assays [43, 52, 53].

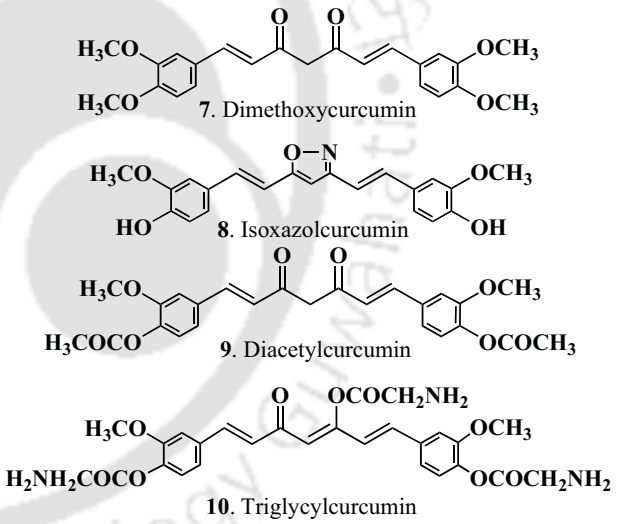

Fig. (7). Chemical structures of dimethoxycurcumin, isoxazolcurcumin (IOC), diacetylcurcumin (DAC) and triglycylcurcumin which preferentially bind to the AT rich minor groove.

6. METAL COMPLEXATION ENHANCES THE GROOVE BINDING AFFINITY OF CURCUMINOIDS

Binding studies of Al-curcumin complex (ACC) ([Al(curcumin) $(EtOH)_2$]$(NO_3)_2$) (**11**) (Fig. **8**) with DNA performed using multi-spectroscopic and voltametric techniques showed that ACC binds to DNA in non-intercalating mode to the AT base pairs rich minor groove. FT-IR analysis showed that there was a major decrease in the intensity of AT bases and minor decrease in the intensities of GC bases and phosphates. This together indicated a strong and direct binding of ACC to thymine (O_2) and adenine (N7) of DNA

bases located in the minor groove and weak electrostatic interaction of ACC with the phosphate backbone [55]. In a study examining the interaction between DNA and mononuclear transition metal (Cu(II), Co(II), Ni(II), Mn(II)) complexes of macrocyclic tetraaza diacetyl curcumin (**12-15**) (Fig. **9**), it was found that these complexes bind through the minor groove. Amongst these, Cu(II) complex was found to have the highest degree of interaction [56].

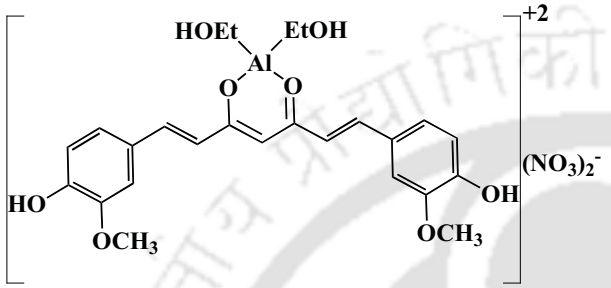

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Fig. (8). Chemical structure of Al-curcumin complex (ACC) ([Al(curcumin) $(EtOH)_2$]$(NO_3)_2$) which directly binds to thymine (O2) and adenine (N7) of DNA bases located in the minor groove.

7. IN-SILICO STUDIES OF CURCUMIN DNA INTERACTIONS

In-silico studies looking into the interaction of DNA with curcumin as well as its derivatives either in pure form or their metal complexes are providing a plethora of supporting evidences complementing the experimental findings. Comparative docking studies of curcumin and nicotine on consensus sequence ["GGGCATGCCTAGGCATGCC"] of human p53 gene showed that they bind to thymidine 6 of the p53 gene with free energy changes of -272.75 and -163.74 kcal/mol respectively suggesting that binding of curcumin on DNA is more stable than nicotine. Curcumin also interacts with nicotine with free energy change of -129 kcal/mol and reduces the availability of nicotine for DNA binding suggesting that curcumin has potential to protect DNA by not only competitively binding to it directly but also by binding to nicotine [57].

Docking studies of curcumin with two DNA duplexes [$d(GTATATAC)_2$ and $d(CGCGATATCGCG)_2$] followed by molecular simulations and free energy analysis of the complexes using molecular mechanic-poisson-boltzmann surface area (MM-PBSA) to assess binding affinity, predicted that curcumin binds in the minor grooves of AT-rich DNA sequences of DNA. However, unlike the known minor groove binders (netropsin and distamycin) where the binding is mainly by electrostatic interaction, it was found that binding of curcumin is mainly favored by van der Waals and hydrophobic interactions [58].

Caruso *et al.* synthesized and characterized ruthenium–arene complex of curcumin (p-cymene)-Ru-(demethoxy-curcuminato)-chloro (**16**) (Fig. **10**). Docking studies of this complex with a guanine rich B-DNA decamer predicted dipolar interaction of Ru with N7(guanine) and was confirmed with electrospray ionization mass spectrometry. This complex was also tested against several tumor cell lines (MCF7, HCT116, A2780, CP8, A549, U87) and showed excellent activity on the colorectal tumor cell line HCT116 (IC50 = 13.98 ± 1.503 μM) [59].

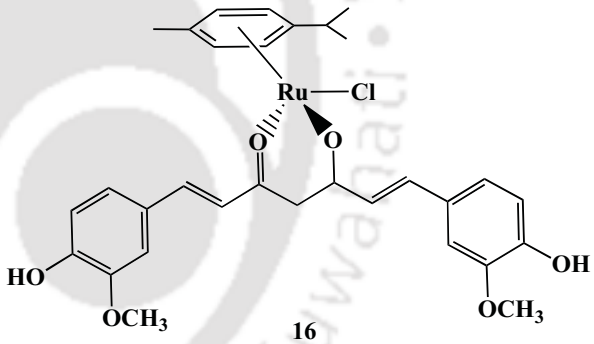

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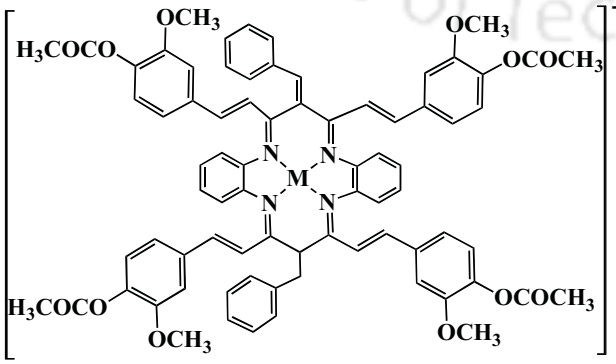
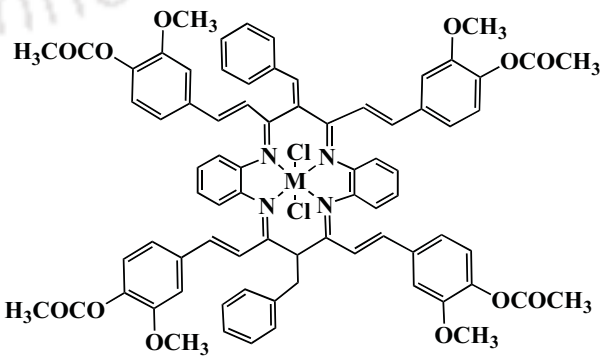
Fig. (10). Chemical structure of ruthenium-arene complex of curcumin found highly active against the colorectal tumor cell line HCT116.



12. M = Cu, **13**. M = Co, **14**. M = Ni



15. M = Mn

Fig. (9). Chemical structures of transition metal complexes of macrocyclic tetraaza diacetyl curcumin which bind to the DNA minor groove.

Table 1. Interaction of Curcumin and Its Derivatives with Nucleic Acids.

S. No	Mode of Action	Biomolecule/Cell Line Used for Study	References
1.	**Curcumin**		
	Prevents DNA damage (2-5µg/ml)	Chinese hamster ovary (CHO) cells, human hepatoma G2 (HepG2) cells & PC12 cells	[12,13,14]
	Prevents micronucleus formation in HepG2 Cells (2µg/ml)	HepG2 cells	[13]
	Causes DNA damage, chromosomal aberration and micronucleus formation (>=8 µg/ml)	Gastric mucosa cells, lymphocytes, CHO cells, HepG2 cells, human colon cancer cell line HT-29, PC12 cells, mouse-rat hybrid retina ganglion cell line, jurkat T-lymphocytes, colorectal carcinoma HCT116 cells & human lung cancer cell line PC-9	[12,13,14,15,16,17, 18,19,20,21,22]
	Acts as topoisomerase II poison (50 µM)	TK-10, MCF-7 & UACC-62 cell lines	[60]
	Binds preferentially to AT-rich region in minor grooves of DNA	Plasmid pBR322, bacteriophage lambda- DNA & calf thymus DNA	[24,44,45,46,47,56, 57]
2.	**Dimethoxycurcumin, bisdimethoxycurcumin, isoxazolcurcumin, diacetylcurcumin & triglycylcurcumin**		
	Bind preferentially to AT-rich minor grooves of DNA	Supercoiled plasmid pBR322, bacteriophage lambda-DNA & calf thymus DNA	[24,42,51,52,53]
3.	**Curcumin in the presence of Cu(II)**		
	ROS dependent DNA damage (50 µM of Curcumin in presence of 10–200 µM Cu(II))	Calf thymus DNA, pBR322 DNA plasmid	[30,33,38,40]
4.	**Curcumin Cu(II) complex**		
	ROS dependent DNA damage (8.14 µM)	CCRF-CEM leukemia cells & pBR322 DNA plasmid	[32,37]
5.	**Curcumin-Al(III) complex**		
	Binds to AT-rich region of minor groove	Calf thymus DNA	[54]
6.	**Curcumin-transition metal complexes [Cu(II), Co(II), Ni(II) & Mn(II)]**		
	Bind to AT-rich region of minor groove (120 µM curcumin Cu(II)complex)	pUC18 DNA	[55]
7.	**Curcumin-Ru-arene complex**		
	Binds to Guanine (N7) residue	Rich guanine B-DNAdecamer (containing only GC alternates)	[58]
8.	**Curcumin-earth metal complexes (Sm, Eu & Dy)**		
	Cause DNA damage (0.08 mM)	Plasmid pBR322 DNA	[41]
9.	**Curcumin-iron complex (Fe(II) & Fe(III))**		
	DNA intercalation	Salmon sperm DNA	[61, 62]
10.	**Curcumin-Zn(II) complex**		
	Partial inter-base intercalation	Synthetic DNA oligomers of sequence (5'-CGCGAATTCGCG-3' and 5'-AGCGACGTCGCT-3')	[63]

8. CURCUMIN AS DNA INTERCALATOR

In contrast to studies reported in section 6 above, curcumin was found to act as intercalating agent through the unknown clastogenic mechanism that may cause topoisomerase II poisoning. It was found that curcumin at higher concentration (50 µM) acts similar to anticancer agent etoposide and forms a ternary complex with DNA and topoisomerase II enzyme preventing re-ligation of DNA strand. This causes error in DNA synthesis and promotes apoptosis of cancer cells [60, 61]. In another study with immobilized dsDNA, curcumin was found to bind DNA

electrostatically at low ionic strength and intercalated at high ionic strength [48]. In UV-visible spectroscopic study, curcumin-Fe(II)/curcumin-Fe(III) complex interacted with salmon sperm DNA in an intercalating mode [62, 63].

Using two newly synthesized heteroleptic pentacoordinated Zn(II) complexes (**17**) (Fig. **11**) containing 4,4'-disubstituted-2,2'-bipyridines as main ligand and curcumin as ancillary ligand Pucci *et al*. demonstrated that the interaction modes of curcumin and curcumin-Zn(II) complexes with dsDNA favor their alignment perpendicular to the DNA axis through base stacking pi-pi interactions between aromatic rings suggesting a partial inter-base intercalation. *In vitro* studies of curcumin and curcumin-Zn(II) complexes against human prostate cancer (DU145, PC3, LNCaP) and neuroblastoma (SHSY-5Y, LAN-5) cell lines were carried out in which curcumin showed strongest growth inhibition in all the prostate cancer cell lines and selectively in the SHSY-5Y neuroblastoma cell line. However curcumin-Zn(II) complexes showed the strongest growth inhibition in LAN-5 neuroblastoma cell line [64].

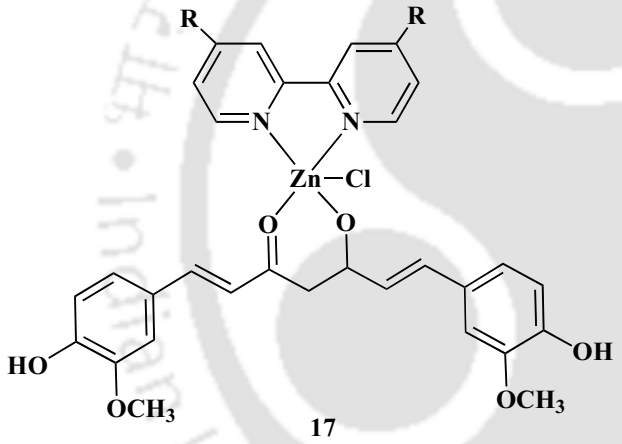


Fig. (11). Chemical structures of heteroleptic pentacoordinated Zn(II) complexes of curcumin containing 4,4'-disubstituted-2,2'-bipyridines (R= C_9H_{19} and OH) which interact with DNA in an intercalating mode.

9. CONCLUSIONS AND FUTURE PROSPECTIVES

Current evidences suggest that curcumin behaves both as a genotoxic and antigenotoxic agent in a time and concentration dependent manner. Several curcumin and/or its derivatives and their metal complexes interact directly with DNA either by binding to the minor groove or as an intercalating agent (see Table **1**). The similarity in the shape of curcumin to DNA minor groove binding drugs such as netropsin and distamycin, is the motivation for exploring its binding to minor grooves of DNA [58, 65]. Curcumin is thus a "double edged sword" having potential for the development of minor groove binding drug for cancer therapeutics but at the same time it may cause DNA damage in the cell at high concentrations [66, 67]. It is already known that cancer cells are more susceptible than normal cells. On the other hand, genomic instability is evolving as a hallmark of cancer [68-70]. Together this provides an opportunity for developing curcumin based novel therapy in which DNA is the target as equally as proteins which are frequently reported in literature. It would be interesting to investigate the DNA binding potential of curcumin derivatives as a lead molecule for therapeutic implications.

CONFLICT OF INTEREST

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

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ABBREVIATIONS

GPx = Glutathione peroxidase

GR = Glutathione reductase

G6PDH = Glucose-6-phosphate dehydrogenase

α-GST = α-glutathione s-transferase

QR = Quinone reductase

dGMP = Deoxyguanosine monophosphate

FT-IR = Fourier transform-infrared spectroscopy

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Research Article

In Silico Inhibition Studies of Jun-Fos-DNA Complex Formation by Curcumin Derivatives

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Activator protein-1 (AP1) is a transcription factor that consists of the Jun and Fos family proteins. It regulates gene expression in response to a variety of stimuli and controls cellular processes including proliferation, transformation, inflammation, and innate immune responses. AP1 binds specifically to 12-O-tetradecanoylphorbol-13-acetate (TPA) responsive element 5′-TGAG/CTCA-3′ (AP1 site). It has been found constitutively active in breast, ovarian, cervical, and lung cancers. Numerous studies have shown that inhibition of AP1 could be a promising strategy for cancer therapeutic applications. The present in silico study provides insights into the inhibition of Jun-Fos-DNA complex formation by curcumin derivatives. These derivatives interact with the amino acid residues like Arg155 and Arg158 which play a key role in binding of Jun-Fos complex to DNA (AP1 site). Ala151, Ala275, Leu283, and Ile286 were the residues present at binding site which could contribute to hydrophobic contacts with inhibitor molecules. Curcumin sulphate was predicted to be the most potent inhibitor amongst all the natural curcumin derivatives docked.

1. Introduction

Activator protein-1 (AP1) is a transcription factor that consists of either homo- or heterodimers of the Jun and Fos family proteins [1]. It regulates gene expression in response to a variety of stimuli, including environmental stresses, UV radiation, cytokines, and growth factors. AP1 in turn controls a number of cellular processes including proliferation, transformation, inflammation, and innate immune response. The Jun and Fos proteins share similar amino acid sequences that comprise the basic DNA-binding sequence and the adjacent leucine zipper region by which these proteins dimerize [2–4]. The AP1 transcription factor binds specifically to 12-O-tetradecanoylphorbol-13-acetate (TPA) responsive element 5′-TGAG/CTCA-3′ which is commonly referred to as the AP1 site [5, 6]. *C-fos* and *c-jun* genes are autoregulated; the transcription of *c-jun* is stimulated by its own product, and in contrast *c-fos* is negatively autoregulated [7–9]. AP1 has been found constitutively active in many cancers including breast, ovarian, cervical, and lung. Numerous studies have shown that inhibition of AP1 has a profound effect on the behavior of cancer cells and tumors suggesting that AP1 could be a promising target for cancer therapy [10].

Curcumin, a dietary spice derived from the plant Turmeric (*Curcuma longa*), is used as a traditional medicine for inflammatory conditions [11]. Further, curcumin has been reported to have anti-inflammatory, anti-oxidant, and anticancer effects [12–15]. In vivo administration of curcumin was found to reduce the incidence and size of tumors in mice [16–19]. Moreover, curcumin was reported to inhibit proliferation and cell cycle progression in cancer cells [20]. Curcumin suppresses constitutive AP1 activity in HL-60, Raji, and prostate cancer cell lines (LNCaP, PC-3, and DU145) [21–25]. Curcumin was also reported to suppress LPS-induced cyclooxygenase-2 gene expression by inhibiting AP1 DNA binding in BV2 microglial cells [26]. It was confirmed that curcumin directly interacts with Jun-Fos dimer and inhibits its binding to DNA (AP1 site) [27]. Some synthetic curcumin derivatives have been discovered

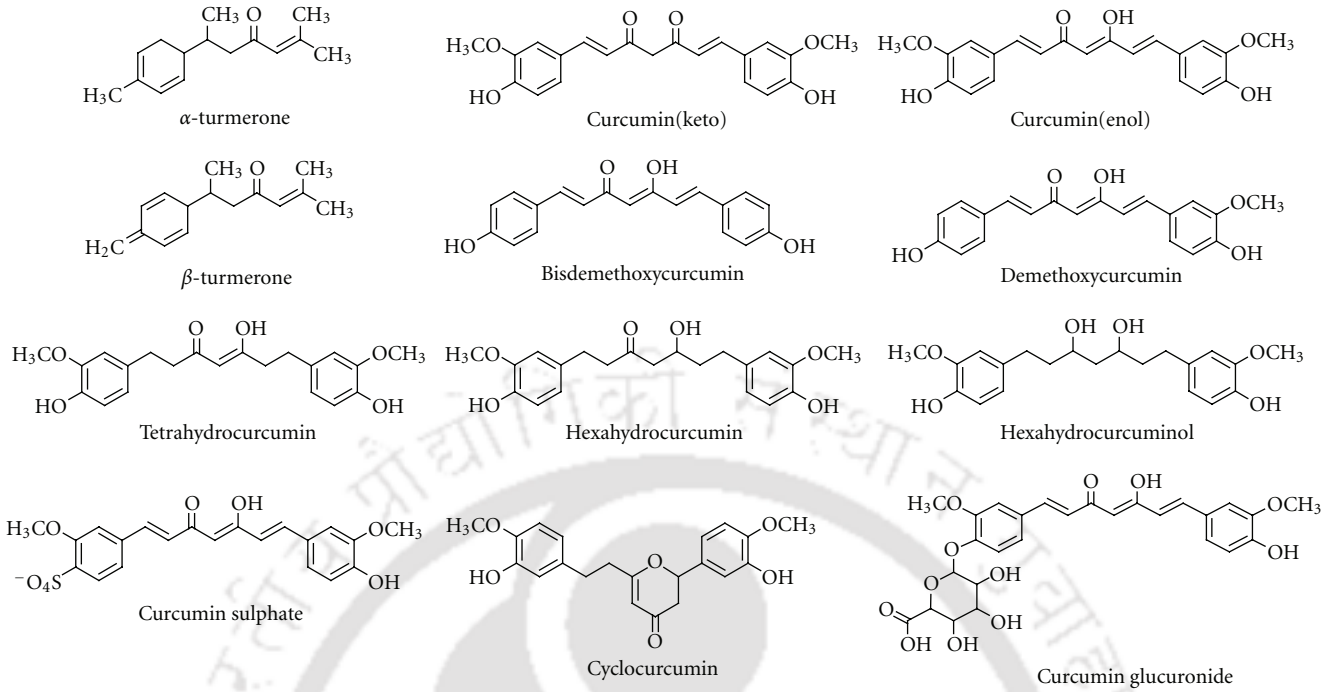


Figure 1: Chemical structures of natural curcumin derivatives.

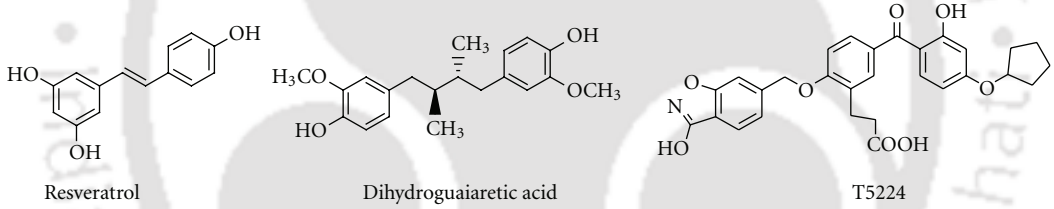


Figure 2: Known inhibitors of Jun-Fos-DNA complex formation used in the study.

as inhibitors of Jun-Fos-DNA complex formation [28–30]. However, no information on the site of interaction is reported yet. In the present study we investigate the interaction of curcumin derivatives with Jun-Fos complex by molecular docking studies.

2. Methodology

To investigate the interaction with Jun-Fos complex, curcumin natural derivatives (Figure 1), synthetic curcumin-based inhibitors (Table 1), and other known inhibitors of Jun-Fos-DNA complex formation (Figure 2) were drawn and 3D optimized using MarvinSketch (Free Academic License) and saved in Protein Data Bank (PDB) file format [31]. These molecules were prepared for molecular docking by merging nonpolar hydrogens, assigning Gastegier charges, and saving them in PDBQT file format using AutoDock Tools (ADT) 1.5.6 [32]. X-ray crystal structure of Jun-Fos-DNA complex (PDB ID: FOS1) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb). For molecular docking DNA and other heteroatoms (water, ions, etc.) were removed using PyMOL 0.99. Gasteiger charges were assigned, and Jun-Fos complex was saved in PDBQT file format using ADT.

Grid and docking parameter files were prepared using ADT, and molecular docking was performed with AutoDock 4.2.1 (Scripps Research Institute, USA) considering all the rotatable bonds of curcumin derivatives as rotatable and Jun-Fos complex as rigid [33]. Grid box size of $90 \times 90 \times 90$ Å with 0.375 Å spacing was selected that include the whole basic DNA-binding sequence and the adjacent leucine zipper region of Jun-Fos complex. Empirical-free energy function and Lamarckian genetic algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2,500,000 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80, were used to perform molecular docking. Hundred independent docking runs were performed for each molecule. Curcumin derivative-Jun-Fos complex for lowest free energy of binding (ΔG) confirmation from the largest cluster was written in PDBQT format and converted to PDB file format using UCSF Chimera 1.6.1. Further, these complexes were analyzed using PyMOL 0.99 for possible polar and hydrophobic interactions. All the docking studies were performed at Intel(R) Xeon(R) CPU (3.2 GHz) with Linux-based operating system Fedora 15.

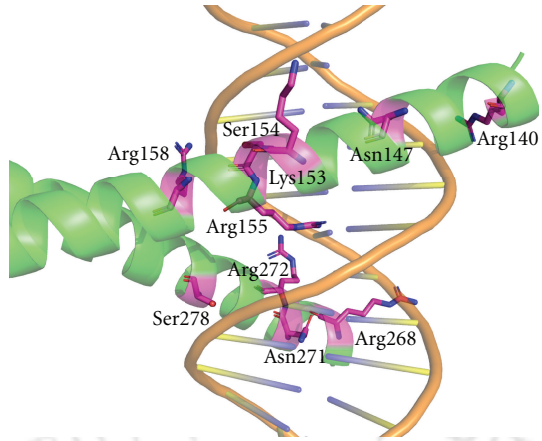


Figure 3: X-ray crystal structure of Jun-Fos-DNA complex (PDB ID: FOS1) showing amino acid residues (magenta) which form hydrogen bond with DNA (AP1 site).

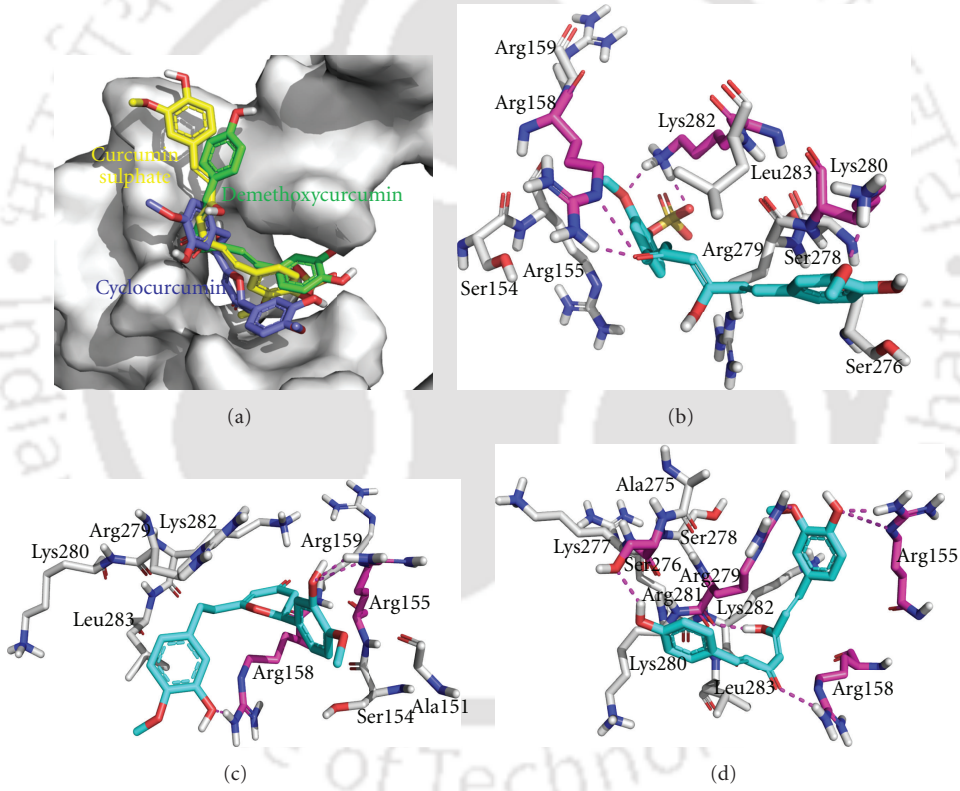


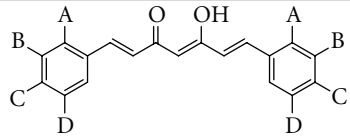
Figure 4: Binding modes of natural curcumin derivatives. (a) Curcumin sulphate (yellow), cyclocurcumin (blue), and demethoxycurcumin (green) docked to DBR of Jun-Fos complex; (b) curcumin sulphate (cyan) showing polar contacts with Arg158, Lys280, and Lys282 (magenta) (c) cyclocurcumin showing polar contacts with Arg155 and Arg158 (magenta); (d) demethoxycurcumin showing polar contacts with Arg155, Arg158, Ser276, and Arg279 (magenta).

3. Results and Discussions

X-ray crystal structure of Jun-Fos-DNA complex shows that Arg140, Asn147, Lys153, Ser154, Arg155, Arg158, Arg268, Asn271, Arg272, and Ser278 are the key residues by which Jun-Fos complex binds to DNA through hydrogen bonding (Figure 3). To predict the interaction of curcumin derivatives with Jun-Fos complex, natural curcumin derivatives and other known inhibitors of Jun-Fos-DNA complex formation were docked over DNA-binding region (DBR) of Jun-Fos complex, and results were summarized in Table 2.

Amongst all the natural curcumin derivatives docked to Jun-Fos complex curcumin sulphate bound with ΔG of -8.20 kcal/mol and predicted KI of 976.64 nM followed by cyclocurcumin and demethoxycurcumin which bound with ΔG of -5.75 and -5.72 kcal/mol and predicted KI of 61.42

Table 1: Synthetic curcumin-based inhibitors of Jun-Fos-DNA complex formation.



Compounds	A	B	C	D	References
CHC001	H	OCH_3	H	H	
CHC002	H	OCH_3	OCH_3	OCH_3	
CHC003	H	OCH_3	H	OCH_3	
CHC004	H	OCH_3	OCH_3	H	
CHC005	H	H	OCH_3	H	
CHC006	H	H	H	H	[28, 29]
CHC007	H	NO_2	OH	H	
CHC008	H	OH	H	H	
CHC009	H	NO_2	H	H	
CHC010	NO_2	H	H	H	
CHC011	H	H	NO_2	H	
BJC003	H	H	CH_3	H	
BJC004	H	NO_2	CH_3	H	[28, 30]
BJC005	H	NO_2	OH	OCH_3	

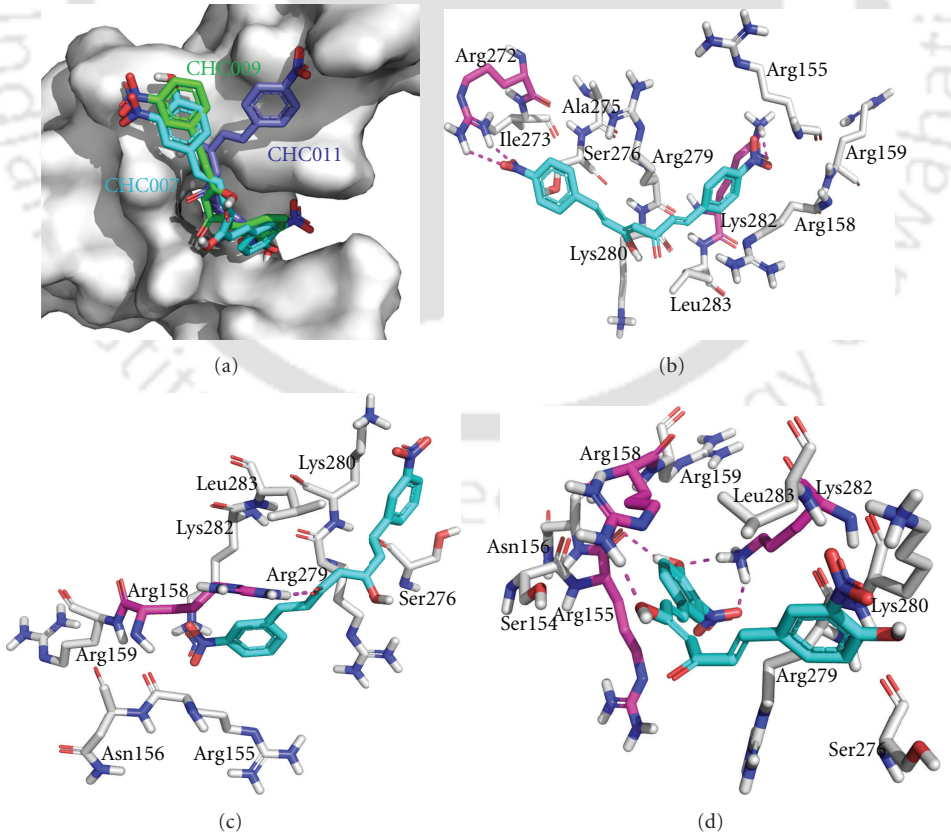


Figure 5: Binding modes of synthetic curcumin-based inhibitors (a) CHC011 (blue), CHC009 (green), and CHC007 (cyan) docked to DBR of Jun-Fos complex; (b) CHC011 (cyan) showing polar contacts with Arg272 and Lys282 (magenta); (c) CHC009 (cyan) showing polar contacts with Arg158 (magenta). (d) CHC007 (cyan) showing polar contacts with Arg155, Arg158, and Lys282 (magenta).

Table 2: Free energy of binding (ΔG) and predicted inhibition constant (KI) estimated with AutoDock 4.2.1 and interaction of inhibitors with Jun-Fos complex.

Compounds	ΔG (kcal/mol)	KI	Putative polar interactions	Hydrophobic residues in 5 Å region
T5224$^{\Psi}$	−9.96	49.64 nM	Arg158, Asn271, Ser278, Arg279, Lys282	Ala274, Ala275, Leu283
CHC011*	−9.59	−93.25 nM	Arg272, Lys282	Ile273, Ala275, Leu283
CHC009*	−9.52	104.26 nM	Arg158	Leu283
CHC007*	−9.15	196.96 nM	Arg155, Arg158, Lys282	Leu283
BJC004*	−9.12	207.86 nM	Lys153	Ala150, Ala151
BJC005*	−8.94	277.86 nM	Arg155, Arg158, Lys280, Lys282	Ala275, Leu283, Ile286
Curcumin sulphate	−8.20	976.64 nM	Arg158, Lys280, Lys282	Leu283
CHC010*	−6.73	11.59 μM	Ser278, Arg279	Ala274, Ala275
CHC008*	−5.86	50.65 μM	Arg155, Arg158, Ser-276, Lys282	Leu283
Cyclocurcumin	−5.75	61.42 μM	Arg155, Arg158	Ala151, Leu283
CHC003*	−5.73	62.98 μM	Arg158, Arg279, Lys280, Lys282	Ala275, Leu283
Demethoxycurcumin	−5.72	63.86 μM	Arg155, Arg158, Ser276, Arg279	Ala275, Leu283
BJC003*	−5.69	67.22 μM	Arg158, Arg279	Leu283
CHC004*	−5.66	71.36 μM	Arg158, Lys280, Lys282	Leu283
CHC006*	−5.45	101.79 μM	Arg158, Arg279	Leu283
CHC002*	−5.32	125.57 μM	Arg155, Arg158, Arg279, Lys280, Lys282	Ala275, Leu283
Bisdemethoxycurcumin	−5.30	130.44 μM	Arg158, Ser276, Arg279	Leu283
Curcumin (keto)	−5.27	136.46 μM	Arg158, Asn271, Arg279, Lys282	Ala274, Ala275, Leu283
Curcumin (enol)	−5.25	141.66 μM	Arg158, Ser276, Lys282	Ala275, Leu283
CHC005*	−5.24	144.93 μM	Arg158	Leu283
CHC001*	−5.19	156.49 μM	Arg158, Lys282	Ala275, Leu283, Ile286
α-Turmerone	−5.13	172.61 μM	Lys282	Ala150, Ala151, Leu283
β-Turmerone	−5.05	197.55 μM	Lys282	Ala275, Leu283
Tetrahydrocurcumin	−5.05	199.62 μM	Arg155, Arg158, Ser276, Arg279	Leu283
Curcumin glucuronide	−4.61	418.23 μM	Arg155, Arg158, Arg279, Lys282	Ala151, Leu283, Ile286
Dihydroguaiaretic acid$^{\Psi}$	−4.43	569.58 μM	Ser278, Arg279	Ala151, Ala275, Leu283
Resveratrol$^{\Psi}$	−4.20	829.30 μM	Ser154, Lys282	Ala151, Leu283
Hexahydrocurcuminol	−4.08	1.02 mM	Arg155, Arg158, Ser276, Lys280, Lys282	Ala275, Leu283
Hexahydrocurcumin	−4.07	1.04 mM	Arg158, Ser276, Arg279	Ala275, Leu283, Ile286

*Synthetic curcumin-based inhibitors of Jun-Fos-DNA complex formation.
$^{\Psi}$Known inhibitors of Jun-Fos-DNA complex formation.

and 63.86 μM, respectively (Figure 4(a)). The binding mode of curcumin sulphate depicted that sulphate and nearby methoxy group present at one aromatic ring of the molecule were in polar contact range with Lys282; however methoxy group present at the other side formed polar contact with side chain of Lys280 (Figure 4(b)). Keto group present in the linker region was in polar contact range with side chain of Arg158. The binding mode of cyclocurcumin showed that hydroxyl group present at one aromatic ring of the molecule formed polar contact with side chain of Arg155; however at the other side it formed polar contacts with Arg158 (Figure 4(c)). When demethoxycurcumin docked to Jun-Fos complex, hydroxyl and neighboring methoxy group present at one aromatic ring formed polar contact with side chains of Arg155 and Arg279, respectively, while hydroxyl group present at other side of the molecule formed polar contact with side chain of Ser276 (Figure 4(d)). In the linker region of the molecule keto and hydroxyl groups were in polar contact range with Arg158 and Arg279, respectively.

Amongst the synthetic curcumin-based inhibitors CHC011 bound to Jun-Fos complex with ΔG of −9.59 kcal/mol and predicted KI of 93.25 nM followed by CHC009 and CHC007 which docked with ΔG of −9.52 and −9.15 kcal/mol and predicted KI of 104.26 nM and 196.96 nM, respectively (Figure 5(a)). Similar results were observed in the in vitro studies by Hahm et al. in 2002 [28]. The binding mode studies depicted that $-NO_2$ group present at one aromatic ring of the CHC011 molecule formed polar contact with side chain of Arg272 while at the other side of the molecule it interacted with Lys282 (Figure 5(b)). When CHC009 docked to Jun-Fos complex, keto group present in the linker region of the molecule formed polar contact

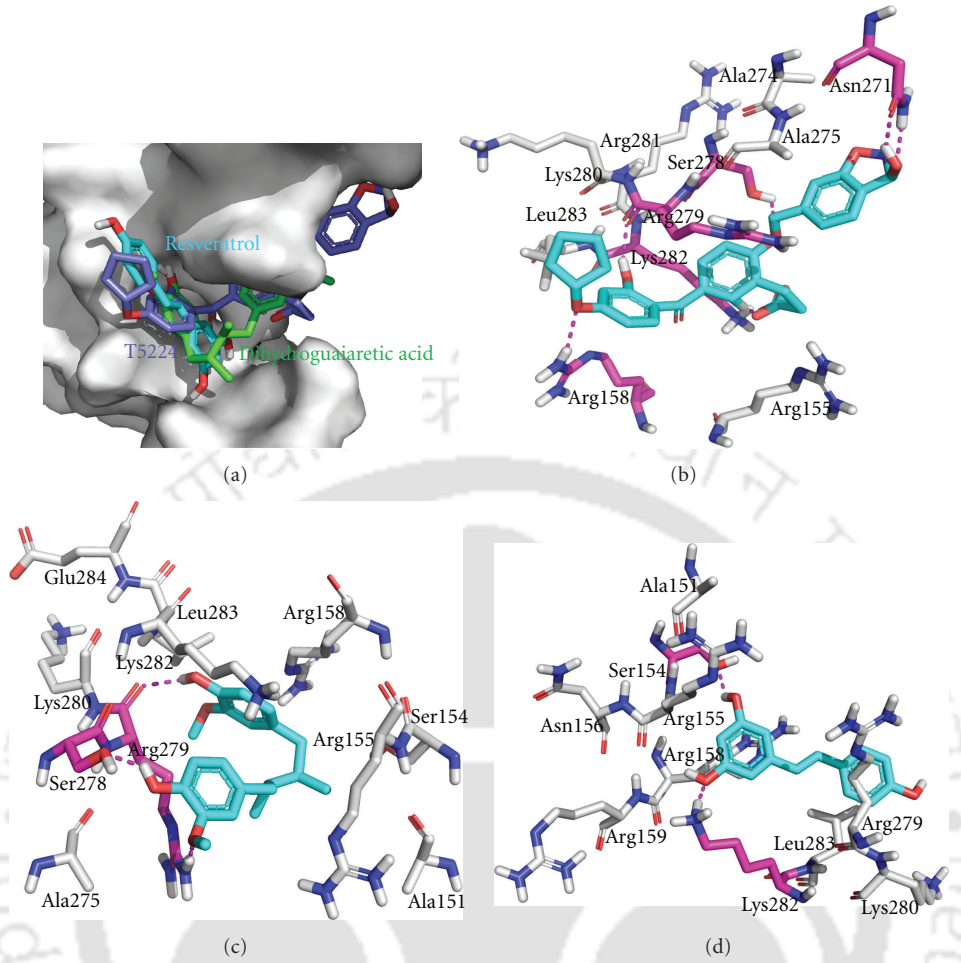


Figure 6: Binding modes of other known inhibitors. (a) T5224 (blue), dihydroguaiaretic acid (green), and resveratrol (cyan) docked to DBR of Jun-Fos complex (b) T5224 (cyan) showing polar contacts with Arg158, Asn271, Ser278, Arg279, and Lys282 (magenta); (c) Dihydroguaiaretic acid (cyan) showing polar contacts with Ser278 and Arg279 (magenta); (d) resveratrol (cyan) showing polar contacts with Ser154 and Lys282 (magenta).

with side chain of Arg158 (Figure 5(c)). Hydroxyl and $-NO_2$ group present at one aromatic ring of the CHC007 molecule formed polar contacts with backbone of Arg155 and side chain of Lys282, respectively, while the hydroxyl group present in the linker region of the molecule showed polar contact with side chain of Arg158 (Figure 5(d)).

Amongst the other known inhibitors T5224 [3-(5-(4-(cyclopentyloxy)-2-hydroxybenzoyl)-2-((3-hydroxybenzo [d]isoxazol-6-yl)methoxy)phenyl)propanoic acid] bound to Jun-Fos complex with ΔG of -9.96 kcal/mol and predicted KI of 49.64 nM followed by dihydroguaiaretic acid and resveratrol which docked with ΔG of -4.43 and -4.20 kcal/mol and predicted KI of 569.58 and 829.30 μM, respectively (Figure 6(a)). The binding mode studies of T5224 depicted that oxygen atom of cyclopentyloxy group formed polar contact with side chain of Arg158; however nearby hydroxyl group formed polar contact with Arg279. Hydroxyl group of 3-hydroxybenzo [d]isoxazol-6-yl)methoxy group formed polar contact with Asn271; however oxygen atom of its methoxy group formed polar contact with Ser278. Acid group of the T5224 molecule was in polar contact range with Lys282 (Figure 6(b)). When docked to Jun-Fos complex neighboring hydroxyl and methoxy groups present at one side of the dihydroguaiaretic acid molecule formed polar contacts with Ser278 and Arg279 respectively, whereas the hydroxyl group present at the other side of the molecule formed polar contact with backbone of Arg279 (Figure 6(c)). When docked to Jun-Fos complex neighboring hydroxyl groups attached to one of the aromatic ring of resveratrol molecule formed polar contacts with Ser 154 and side chain of Lys282, respectively (Figure 6(d)).

We observed that curcumin derivatives form polar contacts preferentially with residues like Arg155, Arg158, Lys276, Arg279, Lys280, and Lys282 when docked to DBR of Jun-Fos complex amongst which Arg155 and Arg158 are the key residues by which Jun-Fos complex binds to DNA. The results suggested that interaction of curcumin derivatives with residues like Arg155 and Arg158 could be the

possible mechanism by which curcumin derivatives inhibit Jun-Fos-DNA complex formation. Ala151, Ala275, Leu283, and Ile286 were the hydrophobic residues present at binding site contributing to hydrophobic contacts with inhibitor molecules.

4. Conclusions

The present molecular docking study provides insights into the inhibition of Jun-Fos-DNA complex formation by curcumin derivatives. The involvement of residues like Arg155, Arg158, Lys276, Lys280, and Lys282 seems to play a key role in binding of curcumin derivatives to Jun-Fos complex through polar contacts which prevents its binding to DNA (AP1 site). Ala151, Ala275, Leu283 and Ile286 were the important hydrophobic residues present at binding site. Most of the curcumin derivatives were predicted to be more potent than inhibitors like resveratrol and dihydroguaiaretic acid. Curcumin sulphate was predicted to be the most potent inhibitor amongst all the natural curcumin derivatives docked.

Acknowledgments

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List of Presentations

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Oral presentations:

[1] **Kumar A.**, Bora U. Seri-Resource Databases. International Consultative Meeting on "Seribiotechnology 2012" (ICMS 2012) organized by Institute of Bioresources and Sustainable Development (IBSD) Imphal, Manipur- 795001, India during 5-7th December 2012.

Poster presentations:

[1] **Kumar A.**, Bora U. Interactions of Curcumin Natural Derivatives with DNA Topoisomerase I and II-DNA Complexes. International Symposium on "Bioengineering 2012" (ISBE2012) organized by IITG Biotech Hub, Centre for the Environment, IIT Guwahati, India on 10th December 2012.

[2] Gogoi N., **Kumar A.**, Utpal Bora U., Mahanta C. Majuli Island Bioresource Database (MIBD). International Symposium on "Bioengineering 2012" (ISBE2012) organized by IITG Biotech Hub, Centre for the Environment, IIT Guwahati, India on 10th December 2012.

[3] Singh D., Raut R., **Kumar A**., Bora U. Development of Seri-Bioresource Database. International Consultative Meeting on "Seribiotechnology 2012" (ICMS 2012) organized by Institute of Bioresources and Sustainable Development (IBSD) Imphal, Manipur- 795001, India during 5-7th December 2012 and received best poster award for the same.

[4] Raut R., Singh D., **Kumar A**., Bora U. Silkworm nucleotide databases. International Consultative Meeting on "Seribiotechnology 2012" (ICMS 2012) organized by Institute of Bioresources and Sustainable Development (IBSD) Imphal, Manipur-795001, India during 5-7th December 2012.

[5] **Kumar A**., Bora U. Inhibition of NF-κB p50 subunit binding to DNA by curcumin derivatives: Computational studies. Short term course on "Cancer Biology 2011: Basic Theoretical Aspects" organized by IITG Biotech Hub, Centre for the Environment, IIT Guwahati, India during 26-27th August 2011.

Workshops attended:

[1] Workshop on "Genome Annotation" organized by IITG Biotech Hub, Centre for the Environment, IIT Guwahati, India on 15th October 2011.

[2] Workshop on "Basic Techniques in Bioinformatics" organized by Bioinformatics facility, Department of Biotechnology, IIT Guwahati, India during 12-14th October 2011.

[3] Hands-on training on “Mammalian Cell Culture Techniques for Toxicity Studies” organized by IITG Biotech Hub, Centre for the Environment, IIT Guwahati, India during 9-17th September 2011.

[4] QIP short term course on "Tools for Bio-resources Conservation" organized by Department of Biotechnology, IIT Guwahati, India during 11-15th July 2011.

Biography

BIOGRAPHY

Anil Kumar is born and brought up in Uttar Pradesh. He completed his High School (10^{th}) in 1998 and Intermediate (12^{th}) education in 2000 from R.B.M.S. Inter College Shankarpur, Raebareli affiliated to Board of High School and Intermediate Education Uttar Pradesh. Subsequently, he joined Feroz Gandhi College, Raebareli affiliated to C.S.J.M. University, Kanpur and completed B.Sc. (Chemistry, Botany, Zoology) in 2003. After qualifying university common entrance test, he joined I.B.S.B.T., C.S.J.M. University, Kanpur, and obtained his M.Sc. in Biotechnology, in 2005. He cleared GATE in 2004 and joined I.I.I.T. Allahabad in July 2005 and completed his M.Tech. in Bioinformatics in 2007. Subsequently, he worked as a Lecturer in Department of Bioinformatics, U.I.E.T., C.S.J.M. University, Kanpur during May 2008 to July 2009.

He joined Ph.D. in I.I.T. Guwahati under the supervision of Dr. Utpal Bora in July 2009. His Ph.D. work focuses on the development of a curcumin resource database and *in-silico* interaction studies with selected targets. His work is published in peer-reviewed international journals, such as Medicinal Chemistry Research, Mini Reviews in Medicinal Chemistry, International Journal of Medicinal Chemistry, Interdisciplinary Sciences--Computational Life Sciences and Bioinformation.