

# Molecular Docking Studies of Curcumin Derivatives with Multiple Protein Targets for Procarcinogen Activating Enzyme Inhibition

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## Abstract

Curcumin derivatives which are very potent antioxidant, free radical scavenger and known inhibitor of dioxygenases have been extensively studied to explore their potential utilization in chemoprevention. The main objective of the present work is to perform a docking analysis of curcumin derivatives: Tetrahydrocurcumin (THC), Bisdemethoxy curcumin (BDC). Docking studies of these were performed using GOLD and AutoDock into a few well validated targets of anticancer therapy (COX-2, PhenolsulphoTransferases, Matrix metalloproteinases (MMPs), P450 and TNF-alpha). A good correlation was observed in binding affinity of THC and BDC against the targets indicating these derivatives are potent procarcinogen activating enzyme inhibitors.

**Keywords:** Docking; Procarcinogen inhibitors; Anticancer therapy targets; Tetrahydrocurcumin; Bisdemethoxycurcumin

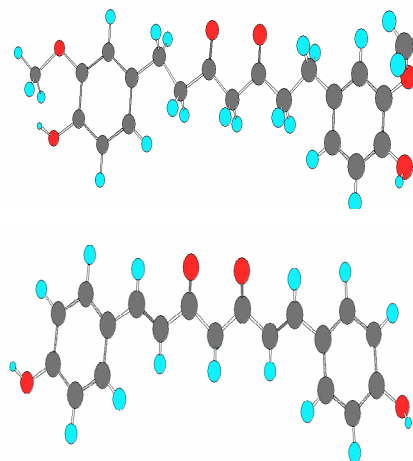
## Introduction

Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3,5-Dione] is the major component of the Curcumin species used as a yellow coloring and flavoring agent in foods. Curcumin has shown anti-carcinogenic activity in animals as indicated by its ability to block colon tumor initiation by azoxymethane and skin tumor promotion induced by phorbol ester TPA. It is proposed that curcumin may suppress tumor promotion by blocking signal transduction pathways in the target cells (Lin and Lin-Shiau, 2001). Curcumin has been demonstrated to have potent antioxidant (Kunchandy and Rao, 1990; Subramanian et al., 1994; Sreejayan and Rao, 1994), anti-inflammatory activity (Huang et al., 1988; Conney et al., 1991; Huang et al., 1997; Liu et al., 1993), to inhibit the carcinogen-DNA adduct (Conney et al., 1991) and tumorigenesis in several animal models (Huang et al., 1992; Huang et al., 1994; Huang et al., 1995; Rao et al., 1995).

As a part of our continuing program to discover procarcinogen inhibitory compounds, curcumin derivatives were studied. Tetrahydrocurcumin(THC) and bisdemethoxycurcumin(BDC) Figure 1, are the reduced form of curcumin, derived from curcuminoids and can also be extracted from the roots of *Curcuma longa*, commonly called turmeric root (Govindarajan, 1980). Tetrahydrocurcuminoids are colorless unlike bisdemethoxycurcumin a yellow curcuminoid which are used in color-free foods and cosmetic products. An antioxidant used in a cosmetic application should have the capability of efficiently quenching any radicals on the surface of the skin. In this context, compound THC displays superior free-radical scavenging ability and also exhibits antioxidant, anti-inflammatory and skin-lightening actions (Sugiyama et al., 1996; Rao et al., 1982) and anticancer activity (Huang et al., 1995). It is thought that the *p*-hydroxy functional groups in THC are responsible for the antioxidant activity and keto groups are responsible for the chemopreventive action of the compound (Rao et al., 1995; Halliwell and Gutteridge, 1985). The crystal structures (Figure 2a and Figure 2b) of THC and BDC have been determined using X-ray crystallography and the results have been extrapolated for docking analysis (Girija et al., 2004).

The concept of docking is important in the study of various

properties associated with protein-ligand interactions such as binding energy, geometry complementarity, electron distribution, hydrogen bond donor acceptor properties, hydrophobicity and polarizability. Since molecules in nature have a tendency to be found in their low energy form, the final configuration should also be of low energy (Pyne and Gayathri, 2005). Understanding these properties is crucial in rationale design of potent inhibitors.



**Figure 1:** Structure of THC and BDC as viewed in Chem3D Ultra with atom coloring.

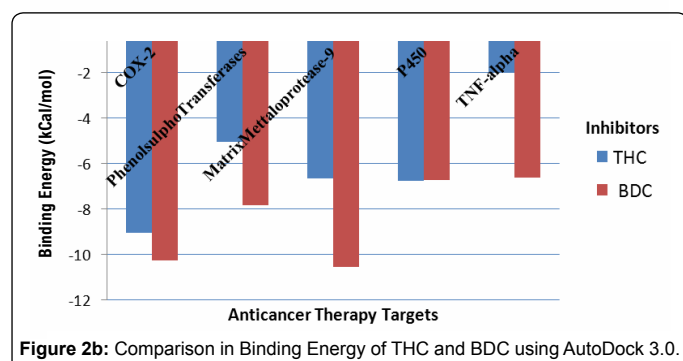
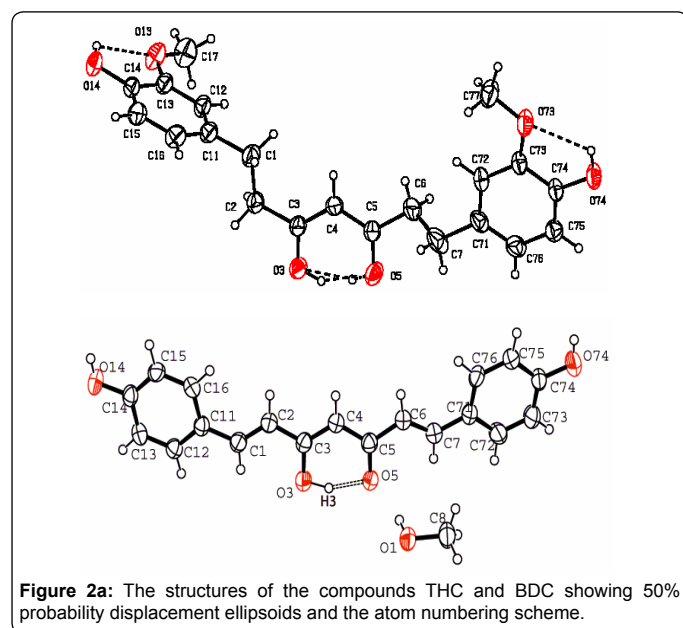
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## Materials and Methods

### Preparation of ligand structures

The small-molecule topology generator Dundee PRODRG 2 server (Schuttelkopf and van Aalten, 2004) is used for ligand optimization, a tool for high-throughput crystallography of protein-ligand complexes which takes input from existing coordinates or various two-dimensional formats and automatically generates coordinates and molecular topologies suitable for X-ray refinement of protein-ligand complexes. CambridgeSoft– ChemOffice 6.0.1 (CambridgeSoft.com, Cambridge, MA, USA) tool used for physicochemical properties of THC and BDC (Table 1).

### Preparation of protein structures

Availability of several experimentally determined three-dimensional structures of COX-2 (1PXX), Phenol sulpho Transferases (1LS6), Matrix metalloproteinases (MMPs) (1GKC), P450 (1OG5) and TNF-alpha (1A8M) co-crystallized with various inhibitors provides an excellent basis for using structure-based approaches for the discovery of new inhibitors. All water molecules and if present, ligands were removed from the proteins for docking studies.

### Protein-ligand interaction using autodock and GOLD

**Autodock (version 3.0):** AutoDock 3.0 includes Lamarckian Genetic Algorithm search engine and an empirical free energy

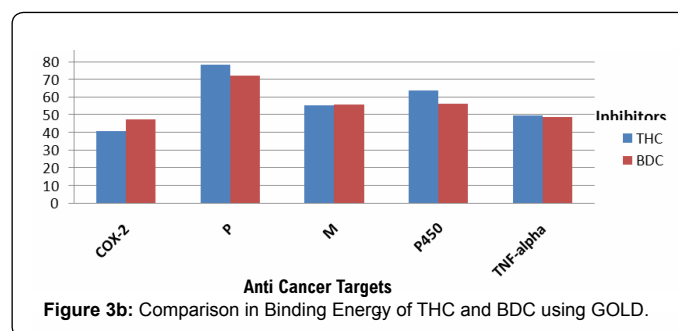
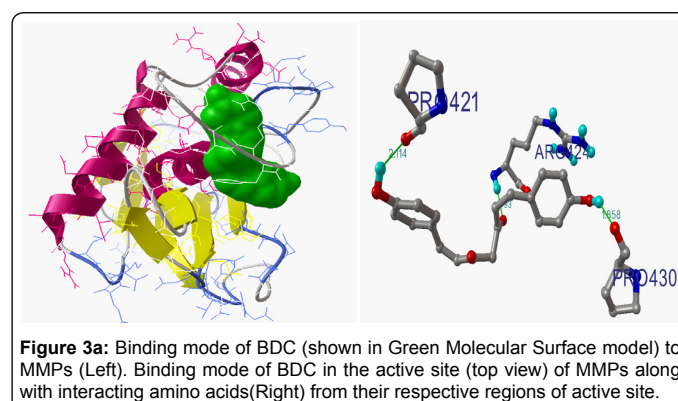
function for estimating binding energy, docking energy, inhibitory constant, intermolecular energy, torsional energy and internal energy. Four binding energy terms were included in the score function: electrostatic, van der Waals, hydrogen bonding and desolvation effect. The binding free energy is empirically calculated based on these energy terms and a set of co-efficient factors (Morris et al., 1998).

Using MGLTools 1.5.1, a grid spacing of 0.374 Å with 60x60x60 points for all Proteins was prepared. The grid was centered around the catalytic cleft of the enzyme for docking. Docking for 100 number of GA run was carried out using Lamarckian Genetic Algorithm (LGA) and all other parameters set to default. The top ranked model in the lowest energy cluster with maximum cluster size was considered for all further interaction studies.

**GOLD (version 2.1.2):** GOLD, which is available through the Cambridge Crystallographic Data Center (CCDC) utilizes a genetic algorithm that was originally described by Jones and colleagues and an evolutionary strategy involving three genetic operators; crossover, mutation and migration (Jones et al., 1997; Jones et al., 1995). It was the first algorithm to be evaluated on a large dataset of complexes, possesses an empirical free energy scoring function that estimates the free energy of binding permitting inhibition constants,  $K_i$  to be calculated. Although initial applications of GOLD and the GA employed provided poor convergence results for hydrophobic ligands, it has recently been validated using a test set of 305 diverse protein-ligand complexes and 72% of the top-ranked solutions were deemed accurate using the authors' self-imposed stringent success criteria (Nissink et al., 2002).

## Results and Discussion

In assessment using AutoDock 3.0, BDC showed better affinity with all anticancer therapy targets than THC. Interaction of BDC with respect to Matrix Metalloprotease (MMPs) is represented. A docking energy of -11.46 Kcal/mol with three hydrogen bonds was



Physicochemical Properties	Tetrahydrocurcumin	Bisdemethoxycurcumin
Boiling Point	787.355 K	747.197 K
Melting Point	771.57 K	669.37 K
Critical Volume	1075.5 Cm.Cm.Cm/mol	887.5 Cm.Cm.Cm/mol
Critical Temperature	969.303 K	946.4 K
Critical Pressure	20.437 Bar	29.155 Bar
Crippens fragmentation method ( Crippens, 1987)		
Log (P)	2.60	2.81
Molar Refractivity	102.09 Cm.Cm.Cm/mol	91.30 Cm.Cm.Cm/mol
Heat of Formation	-870.87 KJ/mol	-307.77 KJ/mol
Gibbs Energy	-445.58 KJ/mol	-72.72 KJ/mol
Ideal gas thermal capacity	438.08 J/mol.K	329.49 J/mol.K
Henry's Law constant	18.2264 log	17.0884 log
Connolly Accessible Area	662.529 ( Å ) squared	574.346 ( Å ) squared
Connolly Molecular Area	360.435 ( Å ) squared	301.153 ( Å ) squared
Connolly Solvent-Excluded Area	307.909 ( Å ) cubed	244.895 ( Å ) cubed
Molecular Formula	C <sub>21</sub> H <sub>24</sub> O <sub>6</sub>	C <sub>19</sub> H <sub>16</sub> O <sub>4</sub>
Molecular Weight	372.41 a.m.u	308.33 a.m.u
Bond Energy	17.923 Kcal/mol	8.163 Kcal/mol
Dipole-Dipole Energy	6.239 Kcal/mol	6.538 Kcal/mol
Non-1, 4 VDW Energy	8.751 Kcal/mol	1.065 Kcal/mol
Stretch Energy	5.041 Kcal/mol	7.567 Kcal/mol
Stretch-Bend- Energy	-0.333 Kcal/mol	-0.129 Kcal/mol
Torsion Energy	-6.825 Kcal/mol	-8.912 Kcal/mol
1, 4 VDW Energy	19.458 Kcal/mol	9.283 Kcal/mol

**Table 1:** Physicochemical properties of THC and BDC.

showed. The hydrogen bond was formed between hydroxyl (H14) of the phenyl ring and carboxyl (O) of hydrophobic amino acid Pro421 by a distance of 2.114 Å (O-H...O) and energy of -0.374 Kcal/mol. Another interaction bridging (O5) of the heptane branch and amine (NH2) of positively charged residue Arg424 with a distance of 1.793 Å (N-H...O) along a minimum energy of -5.492 Kcal/mol. The hydroxyl (H74) of another phenyl ring and carboxyl (O) of hydrophobic amino acid Pro 430 by a distance of 1.858 Å (O-H...O) along with a energy of -1.817Kcal/mol (Figure 3a).

From GOLD also it was observed that BDC showed better affinity with COX-2 and Matrix Metalloproteinase (MMPs) than Phenol sulpho transferases, P450, TNF-alpha anticancer therapy targets though THC showed good affinity to Phenol sulpho transferases, P450 and TNF-alpha (Figure 3b).

## Conclusion

Analysis of these docked ligands with the proteins brought in focus some important interactions operating at the molecular level. The six-membered phenyl ring plays a vital role in holding the molecule at place (binding) at the active site by three important hydrogen bonds. The present study also attempts a 3D-QSAR study on curcumin derivatives. Applying Lipinski's Rule of five to curcumin derivatives to evaluate druglikeness (absorption, distribution, metabolism and excretion), there was no violation of the rule determining drugs pharmacological activity in the body. These studies are expected to provide useful insights into the roles of various substitution patterns on the curcumin derivative and also help to design more potent compounds. The docking studies and 3D-QSAR indicate that substitution of electron-rich compounds may lead to improved biological activity of curcumin derivatives. Thus this study will be useful for the design of novel procarcinogen activating enzyme inhibitors based on docking methods.

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