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IDENTIFICATION OF CURCUMIN TARGETS IN NEUROINFLAMMATORY PATHWAYS: MOLECULAR DOCKING SCORES WITH GSK-3β, P38 MAPK, COX, ICE AND TACE ENZYMES

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Abstract: In the present study, the multiple targets have been identified in the mediation of anti-inflammatory response of curcumin. The anti-inflammatory pathway of curcumin was identified through docking with of curcumin with various inflammation inducing enzymes like glycogen synthase kinase (GSK-3β), p38 mitogen activated protein kinase (MAPK), COX, interleukin-1β converting enzyme (ICE) and tumor necrosis factor-α converting enzyme (TACE). Theoretical docking study was used for the prediction of the conformation orientation and position (pose) of the bioactive compound into the binding pocket and estimation of effective target-ligand interactions (scoring) was utilized for conformational sampling. The final docked conformations were selected according to their scores. The binding target GSK-3β (-6.44) was found to be more selective for curcumin binding when compared with MAPK (-4.08), COX (-7.35), ICE (-4.02), TACE (-6.38) and their respective native ligand. The binding takes place through hydrogen bonding interactions of curcumin with the amino acids in the substrate enzyme. The key amino acids involved were Vall35, Gln185 and Lys85 in GSK-3β. The binding efficiency of curcumin was compared with a standard molecule GF109203 which showed a docking score of - 4.97. These findings enabled us to identify the keto form of curcumin as a best choice of lead compound to target GSK-3β.

Keywords: curcumin, glycogen synthase kinase (GSK-3β), p38 mitogen activated protein kinase (MAPK), COX, interleukin-1β converting enzyme (ICE), tumor necrosis factor-α converting enzyme (TACE)

Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae). Chemically, curcumin is a bis-α,β-unsaturated diketone (commonly called diferuloylmethane, Fig. 1), which exhibits keto–enol tautomerism having a predominant keto form in acidic and neutral solutions and stable enol form in alkaline medium (1).

Brain inflammation is a serious pathological condition that leads to neurodegenerative diseases. The inflammation is caused by various inflammatory mediators such as cytokines, chemokines and prostaglandins. The activation of microglia and astrocytes induce the expression of these key inflammatory mediators. The inflammatory mediators increase the permeability of blood brain barrier facilitating the invasion of peripheral immune cells that induce the release of potentially toxic molecules leading to cell death in brain (2).

Cytokines are primary mediators of the inflammatory response. During inflammation, the astrocytes and microglia secrete numerous immune cell system modulators such as complement proteins, adhesion molecules, inflammatory cytokines, colony stimulating factors-1 and tumor and growth factors. These factors maintain neuroinflammation by number of mechanisms, which include the activation of PLA2, COX and LOX. The cytokines activates a cascade of protein kinases (MAPK, ERK) and transcription factor such as nuclear kappa B (NFκB) (3). The p38 mitogen-activated protein kinase (p38 MAPK) pathway has been linked to inflammatory pathology in neurological disorder like Alzheimer's disease**.** In addition, the p38 MAPK pathway is a major pro-inflammatory signal transduction pathway activated by oxidants, cytokines, LPS and amyloid β (4). The NFκB is present in the cytoplasm of glial cells attached to

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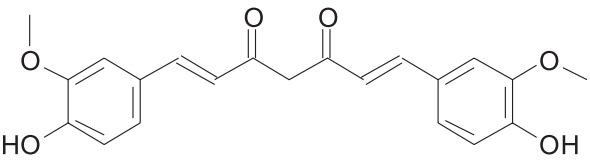
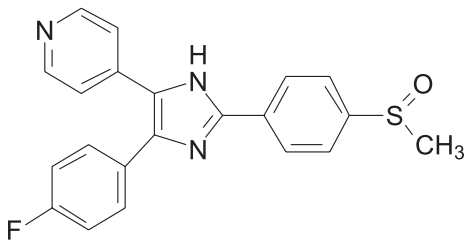
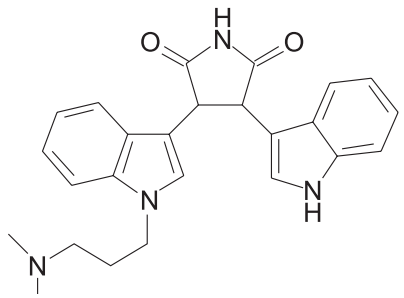
inhibitory protein IκB. Stimulated IκB is rapidly phosphorylated, ubiquitinated and then degraded by proteasomes resulting in the release of active NFκB that mediates the transcription of many genes implicated in inflammatory and immune responses. These genes include COX-2, intracellular adhesion molecule-1, TNFα, IL-1β, IL-6, sPLA2, iNOS and matrix metalloproteinases. The factors like IκB and



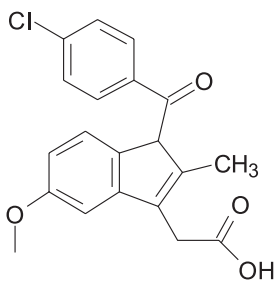
(a) Curcumin – (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione



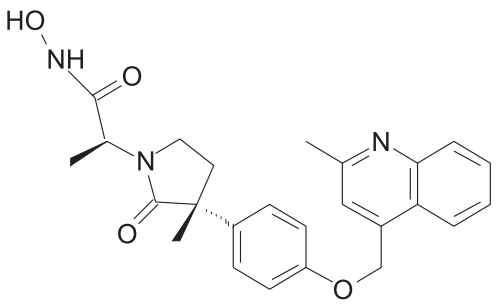
(b) SB203580 – 4-[5-(4-fluorophenyl)-2-[4-(methylsulfonyl) phenyl]-1*H*-imidazol-4-yl]pyridine



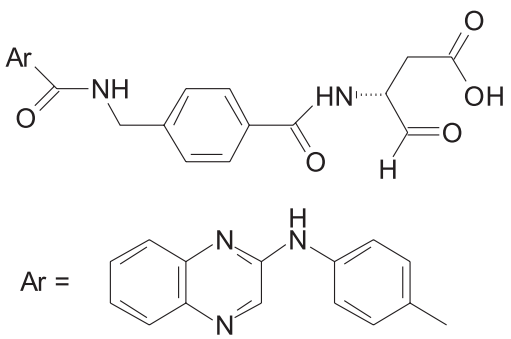
(c) GF109203 – 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide



(d) Indomethacin – 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole



(e) IK682 – (2R)-N-hydroxy-2-[(3S)-3-methyl-3-[4-[(2-methylquinolin-4-yl)methoxy]phenyl]-2-oxopyrrolidin-1-yl]propanamide



(f) 4-Oxo-3-[(6-{[4-(quinoxalin-2-ylamino)-benzoylamino]-methyl}-pyridine-3-carbonyl)-amino]-butyric acid

Figure 1. Selected standard molecules of p38 MAPK, GSK-3β, COX, ICE and TACE enzyme inhibitors

glycogen synthase kinase (GSK-3) tightly regulates NFκB activation (5). Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase highly copious in brain, involved in the regulation of glycogen by insulin and tumorigenesis (6). Molecular cloning revealed that there were two closely-related isoforms, GSK-3α and GSK-3β (7).

Curcumin is a natural product present in turmeric and has been reported as an anti-inflammatory agent acting by inhibition of the inflammatory mediators like arachidonic acid, phospholipase, lipoxygenase, cyclooxygenase-2, leukotrienes, thromboxane, prostaglandins, nitric oxide, collagenase, elastase, hyaluronidase, monocyte chemoattractant protein-1, interferon inducible protein, tumor necrosis factor, and interleukin-12 (8). It also inhibits NFκB (9), p38 MAPK and JNK activities (10). There are several reports explaining the anti-inflammatory effect acting through the inhibition of NF-κB. Few examples include the inhibition of hepatic inflammation in experimental steatohepatitis induced by the methionine and choline deficient diet (11), lipopolysaccharide induced COX-2 gene expression (12), initiation of Janus kinase (JAK)-STAT inflammatory signaling in activated microglia (13) and lipopolysaccharide induced transcription of the MIP-2 promoter reporter gene construction in primary astrocytes (14). Also curcumin is useful in cerebral ischemia through its antioxidant potential (15) and is a promising agent in the treatment and prevention of AD (16). There are several reports of the neuroinflammatory pathways inhibited by curcumin such as the inhibition of Aβ1-40 induced expression of cytokines (TNF-α and IL-1β) and chemokines (MIP-1β, MCP-1 and IL-8) and microglial activation indices related to neurotoxicity, it is a direct target for β amyloid (Aβ) to block the aggregation of fibril formation (17, 18). Curcumin has been used for centuries as a traditional medicine to treat different inflammatory disorders although the anti-inflammatory mechanism is not well defined till now. The various enzymes regulating the neuroinflammation from the results mentioned prompted us to evaluate the anti-inflammatory action of curcumin. In the present study, the inflammatory mediator release and signaling pathway of neuroinflammation were investigated by computer aided molecular docking of curcumin into the binding pocket of the targeted enzymes. The study results enabled us to understand the mechanism of action of curcumin and offered new insights towards the innovation of the potent curcumin analogs for the treatment of neuroinflammation.

EXPERIMENTAL

Docking studies were performed on curcumin with target enzymes (p38MAPK, GSK-3β, ICE, TACE and COX-2) using Glide, version 4.5, Schrödinger, LLC, New York, NY, 2007.

Computational methods with Glide 4.5

All computational studies were carried out using Glide version 4.5, installed in a single machine running on a 3.4 GHz Pentium 4 processor with 1GB RAM and 160 GB hard disk with Red Hat Linux Enterprise version 5.0 as the operating system.

Protein structure preparation in Glide 4.5

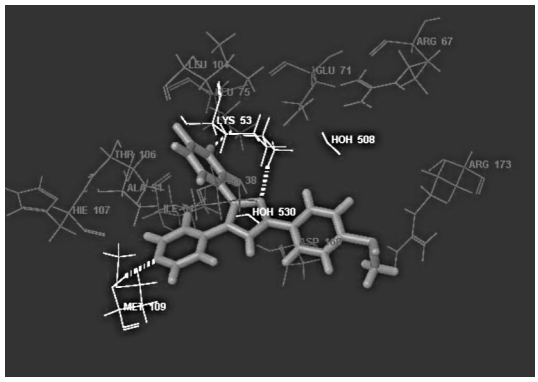
Protein Preparation Wizard of Schrödinger Inc. has been used to prepare protein. The selected target enzymes such as p38MAPK, GSK-3β, ICE, TACE and COX-2 were taken from the Protein Data Bank and their entry codes are 1A9U, 1Q5K, 1rww, 2FV5 and 4COX, respectively, and the water and chain-B were deleted from the proteins. After assigning charge and protonation state, finally the energy minimization was done with *impref*. The minimization was restrained to the input protein coordinates, by a user-selected RMSD tolerance.

Validation of the docking protocol in Glide

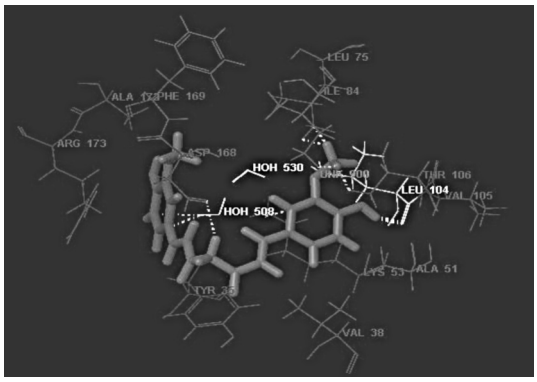
The most suitable method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy poses predicted by the scoring function, which resembles an experimental binding mode as determined by X-ray crystallography. The root mean square deviations (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation of native protein compounds were comparable. This indicates the reliability of the docking method in reproducing the experimentally observed binding mode for target enzyme proteins.

Ligand structure preparation in Glide

Ligand structures were drawn and optimized using Chem. Draw and saved in PDB format. By using the Ligprep utility of Glide, these structures were geometry optimized by utilizing the Optimized Potentials for Liquid Simulations-2007 (OPLS-2007) force field with the steepest descent followed by truncated Newton conjugate gradient protocol. Partial atomic charges were computed using the OPLS-2007 force field.

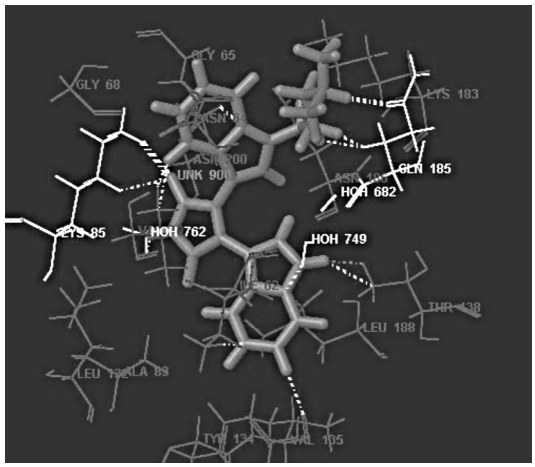


(a) p38α-SB203580(b)

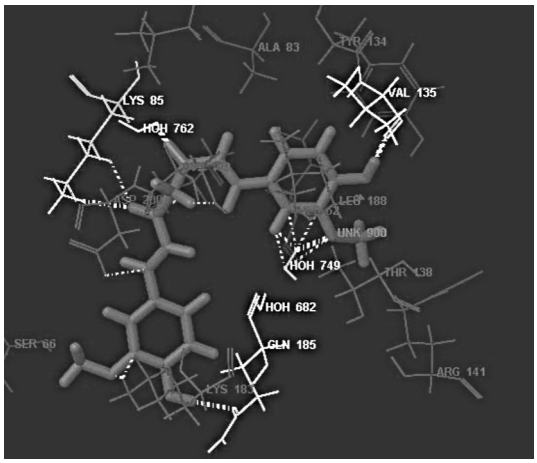


(b) p38α- curcumin

Figure 2. (a) Illustration of the binding mode of co-crystallized compound (SB203580) at the ATP-binding site of p38α MAP kinase by the molecular docking studies and its H-bonding patters (MET 109 and LYS 53) are represented in the stick model. The key H-bonds between SB203580 and p38α MAPK are indicated by the dotted lines. (b) Binding mode of curcumin at the ATP-binding site of p38α MAPK by the molecular docking studies and its H-bonding patters (LEU 104 and LYS 53) are represented in the stick model. The key H-bonds between curcumin and p38α MAPK are indicated by the dotted lines



(a) GSK-3β-GF109203



(b) GSK-3β-curcumin

Figure 3. (a) Illustration of the binding mode of co-crystallized compound (GF109203) at the ATP-binding site of GSK-3β by the molecular docking studies and its covalent bonding patters (VAL 135, GLN 185 and LYS 85) are represented in the stick model. The key covalent bonds between GF109203 and GSK-3 are indicated by the dotted lines. (b) Binding mode of curcumin at the ATP-binding site of GSK-3β by the molecular docking studies and its H-bonding patters (VAL 135, GLN 185 and LYS 85) are represented in the stick model. The key covalent bonds between curcumin and GSK-3β are indicated by the dotted lines

Protein grid generation

All docking calculations were performed using the ”Standard Precision” (SP) mode of Glide Program 4.5. A grid was prepared with the center defined by the co-crystallized native ligands of selected protein targets. During the docking process, the Glide performs a complete systematic search of the conformational, orientation and positional space of the docked ligand, eliminating unwanted conformations using scoring and followed by energy optimization. Finally the conformations are further refined *via* Monte Carlo sampling of pose conformation. Predicting the binding affinity and rank-ordering ligands in database screens was implemented by modified and expanded version of the Glide Score scoring function.

Docking of the ligands in Glide

After validation of the docking method using native compounds of the respective proteins, the curcumin ligand was docked into the same coordinates of the crystal structure. The docked 3D-structure of curcumin ligand against the targeted proteins was scored.

RESULTS AND DISCUSSION

Structure of p38 MAPK–curcumin complex

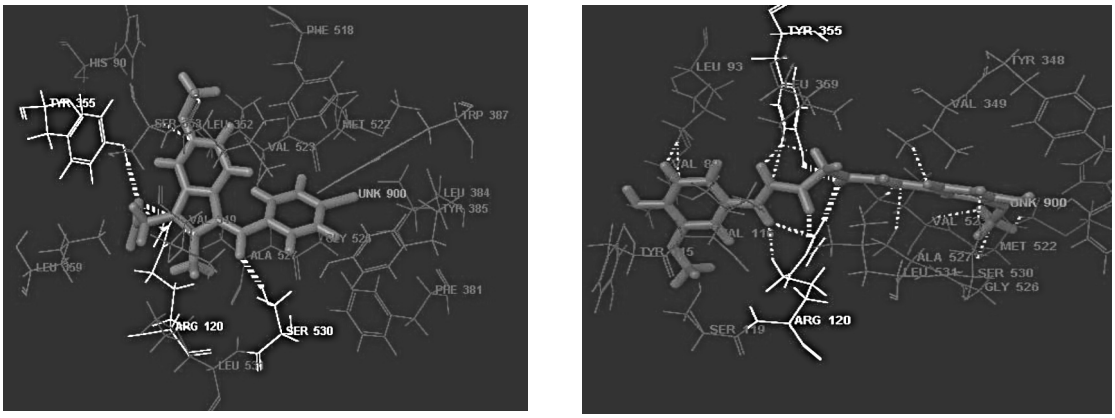
The anti-inflammatory agent pyridinyl imidazole and its analog SB203580 [SmithKline Beecham] are highly potent and selective inhibitors of p38 (19). Compound SB203580 has three rings attached to a central imidazole, which forms a propeller. The pyridine ring of SB1 partially overlaps the adenine ring of ATP in the p38–SB203580 complex. Like ATP, the pyridine ring N atom forms a hydrogen bond with Met109N in the crossover connection. The hydrogen bonding distance between N atom of pyridine with N atom of Met109 was found to be 2.141 Å. Another hydrogen bond forms between the imidazole rings of SB with Lys53 about the distance of 2.099 Å (Fig. 2a). One edge of the fluorophenyl ring makes van der Waals contacts with Thr106 and Leu104 and the opposite edge forms van der Waals contacts with Leu75, phenyl ring interacts with Tyr35 and the glide score of the SB203580 was found to be –7.72. From our present study, one of the phenyl hydroxyl groups of curcumin forms hydrogen bond with Leu104 and the distance between them was found to be 2.449 Å. Methoxy group forms van der Waals contact with Leu75, Ile84 and Thr106 (Fig. 2b). Benzene ring and side chain bridge of curcumin form another van der Waals interaction with Lys53 and Asp168, respectively. Furthermore, no hydrogen bond interaction between Met109 and curcumin was observed, which is essential for competition with ATP at the binding pocket of the enzyme. The investigations on p38 MAPK shows that all inhibitors have hydrogen bond interaction with Met109.

Structure of GSK-3β–curcumin complex

Docking of GF109203 into PDB structure 1q5k of GSK-3β shows that the inhibitor binds through three hydrogen bonds to the backbone of Val135, which is located in the hinge region alongside of the ATP binding pocket of the enzyme. From the studies on GSK-3β, all the ATP competitive kinase inhibitors bind in the adenine binding region and interact with the hinge domain through hydrogen bond interaction and have been essential for enzyme inhibition. The compound GF109203 also forms hydrogen bonding interaction with the key amino acid residues such as Val135, Gln185 and Lys85 in the ATP binding pocket of the enzyme (Fig. 3a). In addition, GF109203 makes van der Waals interaction with the key amino acid residues Lys85, Glu97 and Asp200. The glide score was found to be –4.04. Our docking results show that the phenyl hydroxyl group of curcumin forms hydrogen bonding interaction with Val135 and the other end forms hydrogen bond with Gln185. The hydrogen bond lengths are 1.699 Å and 1.078 Å, respectively (Fig. 3b). Interestingly, the carbonyl side chain of the compound forms third hydrogen bond with Lys85 with a distance of 2.322 Å. Hydrophobic interaction is observed between the carbonyl carbon atom of curcumin and isobutyl side chain of Ile62. Apart from these interactions, curcumin forms van der Waals contacts with the key amino acid residues Asp200, Thy134 and Thr13. The glide score of the curcumin was found to be –6.44. In comparison, the equivalent residues in GSK-3β, the amino acids: Val135, Gly185 and Lys85 interact with two terminal phenyl hydroxyl groups and the side chain carbonyl portion of curcumin. These data suggest that curcumin binds to the ATP-binding pocket of GSK-3β and is competitive with ATP. According to our docking study, the glide score of the curcumin was higher than the native protein compound (GF109203) and also indicated that curcumin is mediating anti-inflammatory action partially *via* inhibiting GSK-3β.

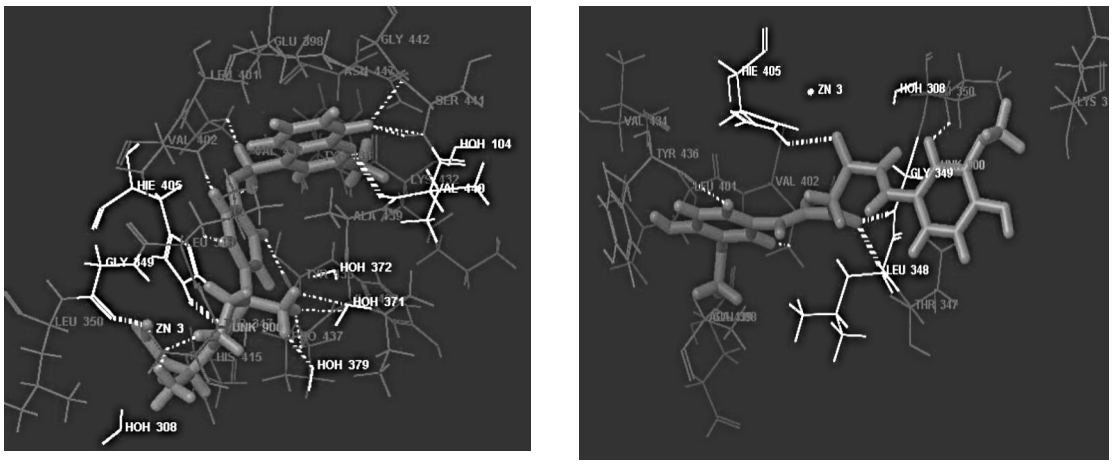
Structure of COX-2–curcumin complex

The standard compound, indomethacin, was docked into the active site of COX-2 successfully. Indomethacin forms hydrogen bonds with Tyr355, Arg120 and the distance was found to be 2.096 Å and 2.122 Å, respectively. These are the key amino acids acting as a gate for ligand entrance to the COX active side (20). Additionally, indomethacin forms a third hydrogen bond with Ser530 with a distance of 1.992 Å (Fig. 4a). Serine is another key amino acid that is selectively acetylated by aspirin (21). In addition to hydrogen bonds, indomethacin is also involved in forming van der Waals interactions with Leu352, Ser352, Val349 and Arg120 and the glide score was found to be –10.70. The docking study of the structure of COX-2 and curcumin shows that the side chain bridge of the first carbonyl group forms hydrogen bond with Tyr355 with a distance of 2.216 Å. Surprisingly, first two carbonyl groups form two separate hydrogen bonds each with Arg120; a distance was found to be 2.137 Å and 2.156 Å (Fig. 4b). The phenyl rings of curcumin are not involved in any stacking interaction against the key residue of the enzymes. However, one edge of the phenyl ring contacts with proteins in COX-2, and is also involved in van der Waals interaction with Val349, Val523, Met522, Ala527 and Ser530. The opposite edge of the phenyl ring forms one contact with Leu93 and the side chain bridge between the two



(a) COX-2-indomethacin (b) COX-2-curcumin

Figure 4. (a) Illustration of the binding mode of standard compound (indomethacin) in the COX-2 active site molecular docking studies and its H-bonding patters (TYR 355, ARG 120 and SER 530) are represented in the stick model. The key H-bonds between indomethacin and GSK-3 are indicated by the dotted lines. (b) Binding mode of curcumin in the COX-2 active site molecular docking studies and its H-bonding patters (TYR 355, ARG 120 and SER 530) are represented in the stick model. The key H-bonds between curcumin and COX-2 are indicated by the dotted lines



(a) TACE-IK682 (b) TACE-curcumin

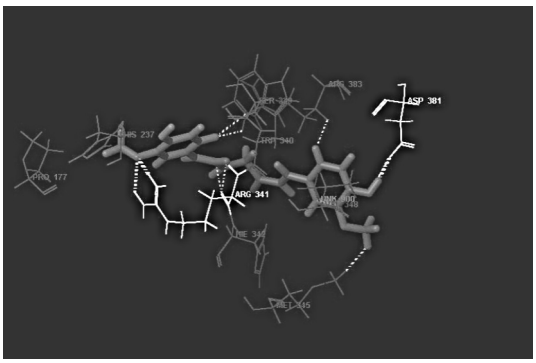
Figure 5. (a) Illustration of the binding mode of co-crystallized compound (IK682) in the TACE active site molecular docking studies and its H-bonding patters (zinc ion, GLY 349, HIE 409 and LEU 348) are represented in the stick model. The key H-bonds between IK682 and GSK-3 are indicated by the dotted lines. (b) Binding mode of curcumin in the TACE active site molecular docking studies and its H-bonding patters (LEU 348, GLY 349 and HIE 405) are represented in the stick model. The key H-bonds between curcumin and TACE are indicated by the dotted lines

phenyl rings makes less contact with the key amino acid residues Tyr355 and Arg120 through van der Waals interactions. The glide score of the curcumin was found to be –7.35 which is less than standard COX inhibitor. However, studies on COX-2 inhibitor reveals that the ligand forms hydrogen bond interactions with Tyr355 and Arg120 necessary to inhibit COX-2 activity (22).

Structure of TACE–curcumin complex

TNF-α converting enzyme (TACE) is a member of the reprolysin family of the metzincin superfamily and converts membrane bound pro-TNF-α to mature and soluble TNF-α. The native compound IK682 was successfully docked into the active site of TACE. The hydroxamate group in the compound forms van der Waals interaction with zinc, the co-

catalytic metal ion in the active site of the enzyme. The compound actively takes part in forming hydrogen bond interaction with the key amino acids Gly349 and Val440 in the enzyme protein and the distance between them was found to be 2.445 Å and 3.102 Å, respectively (Fig. 5a). The phenyl ring forms a third hydrogen bond with His405 in the enzyme and hydrogen bond distance between them was measured as 2.30 Å. Furthermore, the compound surrounded by few residues, such as Ser441, Val439, Leu348, Val434, Tyr436, His415, Ilu438 and Pro437 in the enzyme, contacts through van der Waals interactions and the glide score was found to be –12.75. From our docking study of TACE and curcumin complex it was found that there was no active hydrogen bond interaction with the enzyme by the two phenyl rings of the compound. Hence, the side chain bridge of the two carbonyl groups makes contact through van der Waals interaction with the key amino acids Leu348 and His405 in the enzyme. The hydrogen bond distance between active moieties and the enzyme residues was found to be 2.164 Å and 2.255 Å, respectively (Fig. 5b). Furthermore, third hydrogen bond was also observed between the carbonyl group of the curcumin and Gly349 moiety of the enzyme protein with a distance of 2.335 Å. Hence, curcumin less likely involves contacts with the core residues of the enzymes. There were few van der Waals contacts observed between curcumin and Val402, Tyr436 and Leu350 of the enzyme. Curcumin had no contact with the zinc in the active cavity of the enzyme but made few contacts with the key amino acid residues, which indicates a weak/no inhibitory action on the enzymes as compared with the native compound. Furthermore, the curcumin glide score was found to be –6.38 which are very less as compared with IK682 (23).



ICE-curcumin

Figure 6. Illustration of the binding mode of curcumin in the ICE active site molecular docking studies and its H-bonding patters (ARG 431 and ASP 381) are represented in the stick model.

Structure of ICE–curcumin complex

Interleukin-1β converting enzyme (ICE) catalyzes the proteolytic cleavage of the pro-inflammatory cytokines such as pro- IL-1B and IL-18 to bioactive forms IL-B and IL-18. The native compound was docked in to the active pockets of the ICE successfully. The compound and ICE structure showed the presence of eight hydrogen bonds between the compound and active pockets of the enzyme. There are two separate hydrogen bonds between the compound and the active amino acid residues such as Arg179, Arg349 and His237. Furthermore, the compound made single hydrogen bond interaction against Ser339 and Gly283. In addition to hydrogen bond, compound made fewer van der Waals interactions with Cys285, Val338, Arg341 and Gln283 and the glide score was found to be –7.42. The docking study of curcumin and ICE structure shows that the hydroxyl group of the phenyl ring made only one hydrogen bond with Asp381 and the distance measured was 1.862 Å (Fig. 6). One end of the phenyl ring makes contact with Arg341 and the other end contacts with Arg383 *via* van der Waals interactions. There were other van der Waals interactions observed between the compound and Try340, Met345 and Arg341. The glide score was found to be –4.02. However, all the reported ICE inhibitors showed very strong interaction with active site amino acids Cys285, Arg179 and Arg341 in the protein (24), which was not observed in curcumin and ICE structure. This study reveals that curcumin does not play an inhibitory role on ICE, which is confined additionally by glide score of –4.02.

In this study, we identified that curcumin targets in the neuroinflammatory signaling pathway and mediator release by molecular docking. Theoretical docking study was used for the prediction of conformation orientation and position (pose) of the bioactive compound into the binding pocket and estimation of the tightness of target-ligand interactions (scoring) to guide conformational sampling. The final docked conformations are selected according to their scores. Computational techniques based on molecular docking simulation have now become an essential part of the new drug discovery toolbox. Simulation technique has been used for the identification of novel drug targets by systematically searching for Para logs (related proteins within an organism) of known drug targets and may be able to modify an existing drug to bind to the Para log. The identification of new, clinically relevant, molecular targets is very important in the discovery of innovative drugs.

The binding target GSK-3β (–6.44) was found to be more selective for curcumin binding when compared to MAPK (–4.08), COX (–7.35), ICE (–4.02) and TACE (–6.38) to their respective native ligand. Though, *in vitro* studies show that curcumin acts as a neuroprotective agent both by inhibiting glutamate induced MAP kinase signaling and by cell cycle regulation in HT22 cells. Previous reports have shown that the curcumin exerts anti-inflammatory and growth inhibitory effects in TNF-α treated HaCaT cells through inhibition of NF-κB and MAP kinase pathway (24). However, in our theoretical docking study it was shown that curcumin does not have the ability to inhibit p38 MAPK as it would not compete with the ATP at the binding pocket of the enzyme. The inhibitory activity of curcumin on GSK-3β was shown to be ATP competitive inhibition that takes place through the hydrogen bonding interactions with the key amino acids in the substrate enzyme. The key amino acids involved were Val135, Gln185 and Lys85 in GSK-3β. The binding efficiency of curcumin was compared with a standard molecule GF109203 which showed a docking score of –4.97. Moreover, GSK-3β is one of the targets that have different role and regulation in physiology or pathology. GSK-3β is a cytoplasmic serine–threonine kinase that is involved in insulin signaling and metabolic regulation, as well as in Wnt signaling and the scheme of cell fate during embryonic development. Now, a relatively less familiar member of the family of protein kinases, glycogen synthase kinase-3 (GSK-3), could become a genuine drug target among the protein kinases (25). GSK-3β inhibitors can be classified as direct inhibitors, which interact with GSK-3β, and indirect inhibitors, which increase N-terminal phosphorylation of GSK-3β. Direct inhibitors can be further subdivided into several classes, including lithium, small molecule inhibitors and peptide inhibitors. Lithium was the first selective GSK-3β inhibitor identified and is the only direct GSK-3β inhibitor used clinically. These inhibitors include multiple GSK-3β selective ATP analogs, a smaller group of compounds that do not interact with the ATP binding site and several peptide inhibitors (26). Curcumin falls in the direct ATP competitive inhibitor category from the above classification of GSK-3β inhibitors. Curcumin exhibits ketone-enol tautomerization and there is compelling experimental and theoretical evidence that the enol structure is the most favored due to intra-molecular hydrogen bonding. One of the phenolic hydroxyl groups of curcumin is hydrogen bonded to the nitrogen of Lys-85, while the other phenolic hydroxyl group is hydrogen-bonded to the guanidine of Arg-141. Finally, the enolic-carbon of curcumin seems to share a hydrophobic interaction with the isobutyl side chain of Ile-62. The enolic hydroxyl group of curcumin interacts with the amide carbonyl of Val135 (27). Furthermore, the same studies have reported that the curcumin has potent inhibitory action against GSK-3β with an IC_{50} value of 66.3 nM. However, in this study, the keto form of curcumin has been employed for the theoretical docking study to evaluate the inhibitory activity against the above taken targeted enzyme. Among those enzymes, curcumin has shown the better theoretical score against the GSK-3β. The hydrogen bonds with the key amino acid residue of keto form of curcumin vary from the enol form. The electron rich phenolic hydroxyl group makes hydrogen bond with the amidic carbonyl of Val135. One of the carbonyl groups makes hydrogen bond with nitrogen of Lys85. Finally, the carbonyl carbon of curcumin seems to share a hydrophobic interaction with the isobutyl side chain of Ile-62. These interactions may characterize a keto form of curcumin as a challenger of ATP at the binding pocket of the enzyme to precede inhibitory activity. Moreover, GSK-3 inhibitors protected PC12 cells from thapsigargin-induced apoptosis. Thapsigargin increases $[Ca^{2+}]$, induces apoptotic cell death (chromatin condensation and DNA fragmentation) accompanied by caspase-3 activation in PC12 cells. These findings enable us to identify curcumin as a best choice of lead compound to target GSK-3β. The inhibition of GSK-3β can be utilized for various therapeutic purposes, which includes treatment of neurodegenerative diseases, cerebrovascular disease, bipolar disorders, type II diabetes, cancer and chronic inflammatory disease. Further structure-activity relationships (SAR), quantitative structure-activity relationships (QSAR) and other target identification are in progress in our laboratory.

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