Prediction of binding site for curcuminoids at human topoisomerase II α protein; an *in silico* approach

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Topoisomerases type I and type II are enzymes that unwind and wind DNA, in order to control the synthesis of proteins. Therefore, targeting topoisomerases is considered to be one of the most promising approaches for the treatment of cancer. Curcuminoids are well-reported anticancer agents. However, the exact localization of the binding site and the potentiating mechanism at molecular level are presently unknown. Blind docking has been performed using AutoDock3 by selecting Lamarckian genetic algorithm and docking using the software Glide supplied by Schrödinger suite. The predicted binding site is at the junction of chains A and B of human DNA topoisomerase II α protein. The interaction site and active-site cavity from blind docking simulation using AutoDock3 and binding site prediction using SiteMap 2.4.107 of Schrödinger module followed by docking simulation using Glide version, 5.6.107 respectively, were found to be similar. These results are also supported by comparative docking of salvicine, a known drug molecule and phosphoaminophosphonic acid-adenylate ester (ANP), a ligand from the crystal structure under similar conditions. The binding affinity of curcuminoids and salvicine is quite similar. In the case of AutoDock3, the binding affinities are in the sequence ANP > bis-demethoxycurcumin > cyclocurcumin > salvicine > curcumin > demethoxycurcumin, and in the case of Glide docking result analysis the sequence is salvicine > demethoxycurcumin > cyclocurcumin > curcumin > bis-demethoxycurcumin.

Keywords: Anticancer agents, curcuminoids, docking, topoisomerases.

CURCUMINOIDS play a significant role in combination therapy of tumours and cancers[1,2]. Curcumin occurs in turmeric as a mixture with three of its analogues, viz. demethoxycurcumin (DMC), bis-demethoxycurcumin (BDMC) and cyclocurcumin (CC), together known as curcuminoids (Figure 1). Curcumin has a flexible C–C chain and its stable form is the trans isomer. It has two phenyl rings at both the ends, and belongs to the diarylheptanoids family. It has strong biological activity against multiple diseases and acts as a lead compound. It is sparingly soluble in water and ether, but is soluble in ethanol, dimethyl sulphoxide and other organic solvents[3].

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However, despite exhaustive work carried out on curcumin no definite drug profile has so far been obtained. Probably the problem lies with its low bioavailability, poor absorption, fast metabolism, efflux from gut and non-selective actions. Moreover, it is not clear whether the therapeutic activities are due to curcumin alone or other curcuminoids, or it is their synergistic action. Human DNA topoisomerase II is over-expressed in blast leukaemia cells[4]. All the topoisomerase II inhibitors are known as topoisomerase II poison and drugs which are marketed as topo poisons, e.g., etoposide, doxorubicin and their analogues. The action of curcumin on this conserved nuclear enzyme topoisomerase II leads to poisoning, finally resulting into the death of the cell. Hence, this is a highly important target for tumour chemotherapy[5].

The anticancerous activity of salvicine and its binding site is known from the DNA cleavage assay performed using human topoisomerase II and docking study. The site of action for salvicine is the ATPase region of human DNA topoisomerase II, which is similar to the etoposide agent[6].

Human DNA topoisomerase II is the target for an increasing number of anticancer drugs that inhibit the enzyme by blocking the reaction that reseals the break in the DNA. Curcumin has been applied on the human cancer cell lines, TK-10, UACC-62 and MCF-7, and their IC_{50} values are 12.16, 4.28 and 3.63 μM respectively. The result shows that possibly this compound acts as a topoisomerase II poison. Also, it was found that curcumin in 50 μM concentration is active in a similar fashion as etoposide (antineoplastic agent). These results suggest that the curcumin initiates apoptosis due to DNA damage induced by topoisomerase II poisoning. The evidence shows that it can be used in cancer therapy, but the initiation mechanism of apoptosis is poorly understood[4]. Topoisomerase II is over-expressed in acute lymphoblastic leukaemia and is almost double in comparison to the normal peripheral blood cells[7]. Human DNA topoisomerases

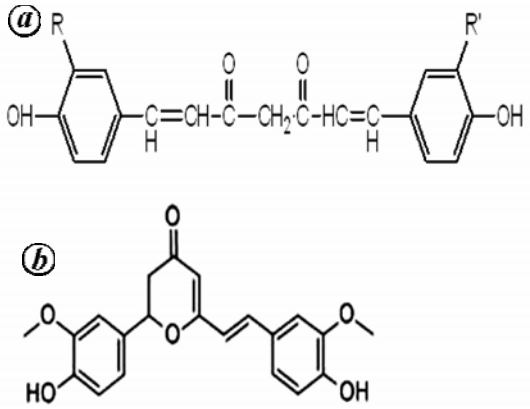

Figure 1. ***a***, Ctructure of curcuminoids (curcumin; R = R′ = –OCH_3; DMC; R = –OCH_3, R′ = H; BDMC; R = R′ = H). ***b***, Cyclocurcumin.

are nuclear enzymes found in all cell types from viruses to man which induce transient breaks in the DNA, allowing DNA strands or double helices to pass through each other[8].

The objective of the present study is to predict the probable binding site for curcuminoids at the ATPase region of human DNA topoisomerase II. For docking we have used two docking softwares, AutoDock3 and Glide (Schrödinger docking module), in order to predict the most accurate inhibitor site. Our findings can be useful in developing new effective drugs against cancer by targeting human topoisomerase II α isoenzyme taking natural curcuminoids as lead molecules.

The three-dimensional X-ray crystal structure of human DNA topoisomerase II α isoenzyme (PDB ID: 1ZXM, resolution = 1.87 Å) was downloaded from the Protein Data Bank[9]. The downloaded protein having two chains A (372 residues) and B (377 residues) with ATPase binding region contains phosphoaminophosphonic acid-adenylate ester (ANP) and magnesium ion (Mg^{++}) as cofactor molecule. It also contains 216 water molecules of crystallization. The protein structure was prepared using the protein preparation module of the Schrödinger software[10]. The co-crystallized ligands and water molecules were removed. Some residues and side-chain atoms are missing from the protein that was modelled using prime modeller followed by refinement and H atoms were added to the structure. The most likely positions of hydroxyl and thiol hydrogen atoms, protonation states and tautomers of His residues, and chi 'flip' assignments for Asn, Gln and His residues were selected by the protein assignment script of Schrödinger. Minimizations were performed until the average root mean square deviation of the non-hydrogen atoms reached 0.3 Å. The final modelled protein was taken as the receptor protein and found to be having the most suitable binding site using sitemap script of Schrödinger[11]. On the basis of priority, site-4 of the protein was chosen for docking with the curcuminoids and salvicine, a known anticancer molecule.

Curcuminoids and salvicine were assigned an appropriate bond order using the LigPrep 2.4.107 script and converted to Maestro file format (Maestro, Schrödinger, Inc.) followed by optimization by means of the OPLS_2005 force field[12].

When the exact location of the binding site/active site is not known within a protein (macromolecule), blind docking can be performed. In this process of docking simulation the entire surface of the protein (macromolecule of interest) is covered using Autogrid to create very large grid maps, with maximum number of points in each dimension, and if necessary, creating sets of adjacent grid maps of volumes that cover the macromolecule[13,14]. The final modelled protein obtained using prime module of Schrödinger suite 10.0 and all selected ligand molecules were prepared as a docking input file for AutoDock3 using MGLTool 1.5.6 program[15]. All single bonds of ligands were allowed to rotate freely and Lamarckian genetic algorithm (LGA) was used to search the energy landscape. The hybrid search technique consists of a global optimizer modified from a genetic algorithm with two-point crossover, random mutation and a local optimizer with a Solis and Wets algorithm. A grid box of highest limit $126 \times 126 \times 126$ points and a grid spacing of 0.375 Å was selected for calculations. Random conditions were used in the settings of seed, initial quaternion, coordinates and torsions. A 0.2 Å step was used for translation and 25° was used for quaternion and torsion. The parameter selected for energy evaluation was 250,000 and the maximum number of generations was 27,000. The rate of gene mutation was 0.02 and the rate of crossover was 0.8. The number of cycles was set to 20. Therefore a total number of 10 docking configurations were determined in each docking calculation. The 'suitable' docking configuration, which was chosen based on the most frequent cluster and the lowest empirical binding free energy was chosen as the 'most active' binding conformation.

Protein–ligand docking studies were performed using Maestro (v7.0.113)[16]. Default parameters were selected with Glide Extra Precision (XP Glide), version 4.5.19 (ref. 17). After complete preparation of the ligands and protein for docking, receptor-grid files were generated. For running the grid-generation module we have scaled van der Waals radii of receptor atoms by 1.00 Å with a partial atomic charge of 0.25. A grid box of size $30 \times 30 \times 30$ Å with coordinates $X = 37.955433$, $Y = -6.749032$ and $Z = 39.920372$ was generated at the centroid of site-4 predicted by sitemap script of Schrödinger suite 10.0. After formation of the receptor-grid file, flexible ligand with rigid receptor docking was performed. Glide generates conformations internally and passes these through a series of filters. A maximum of 100 conformations with the best binding energies were retained for the final analysis, while discarding docked poses with less than 0.01 Å RMSD and 0.01 Å atomic displacement as duplicates. The final energy evaluation was done with Glide Score and the single best pose was generated as the output for a particular ligand.

Thus in this study blind docking using AutoDock3 software and site map for site detection together with docking using Glide simulation software, the binding site for the curcuminoids and the known anticancer drug candidate salvicine at topoisomerase II α isoenzyme was predicted and also the residues involved in different types of interactions were located. The docking results of curcumioids and salvicine are given in Tables 1 and 2. These results show the ranking of ligands based on the free energy for binding and Glide score respectively.

The binding affinity of curcuminoids and salvicine with topoisomerase II α predicted by AutoDock3 and Glide in decreasing order is ANP > bis-demethoxycurcumin > cyclocurcumin > salvicine > curcumin > demethoxycur-

Table 1. Comparative blind docking simulation results of curcuminoids, salvicine and ANP with human DNA topoisomerase II α using AutoDock3

	Curcumin	DMC	BDMC	CC	Salvicine	ANP
Rank	1	1	1	1	1	1
Docked energy (kcal/mol)	–11.09	–10.82	–12.49	–12.67	–11.32	–16.89
Free energy for binding (kcal/mol)	–9.12	–8.78	–10.58	–11.23	–10.34	–14.31

DMC, Demethoxycurcumin; BDMC, bis-demethoxycurcumin; CC, Cyclocurcumin; ANP, Phosphoaminophosphonic acid-adenylate ester.

Table 2. Comparative blind docking simulation results of curcuminoids and salvicine with human DNA topoisomerase II α using glide

Ligand	Glide score	Lipophilic EvdW	HBond	Electro
Curcumin	–7.19	–4.78	–1.96	–0.54
Demethoxycurcumin	–8.12	–5.62	–2.19	–0.19
Bis-demethoxycurcumin	–6.91	–4.59	–1.52	–0.83
Cyclocurcrmin	–7.28	–5.3	–0.014	–0.14
Salvicine	–8.2	–5.27	–2.26	–0.39

Lipophilic Evdw is defined from hydrophobic grid potential and fraction of the total protein-ligand Van der Waals energy. HBond, hydrogen-bonding term; Electro, represents electrostatic rewards.

Table 3. Residues of human DNA topoisomerase II protein interacting with curcuminoids and salvicine using AutoDock3 (highlighted residues are H-bonding interacting residues) and ANP from X-ray crystallized data of the Protein Data Bank

Topoisomerase II residues interacting with					
Curcumin	DMC	BDMC	CC	Salvicine	ANP
Asn91, Asp94, Asn95, Gln97, Arg98, Ile141, Ser149, Lys157, Thr159, Gly164, Gly166, Ala67, Lys168	Asp94, Asn95, Arg98, Lys123, Ser149, Asn150, Lys157, Thr159, Thr215, Ile33B, Tyr34B	Ile88, Asn91, Ala92, Asn95, Arg98, Asn120, Ile125, Thr47, Ser148, Gly164, Lys168, Ile217, Tyr34B	Glu87, Asn91, Asp94, Asn95, Arg98, Asn120, Ile125, Asn150, Gly161, Gly164, Tyr165, Gly166, Thr215, Gln376	Asn91, Asp94, Asn95, Arg98, Ile125, Ile141, Thr147, Ser148, Ser149, Gly164, Ala167, Lys168, Tyr34B	Glu87, Asn91, Asn95, Arg98, Asn120, Ile125, Ile141, Phe142, Thr147, Ser148, Ser149, Asn150, Gly161, Arg162, Asn163, Gly164, Tyr165, Gly166, Ala167, Lys168, Thr215, Gln376, Lys378, Tyr34B

cumin and salvicine > demethoxycurcumin > cyclocurcumin > curcumin > bis-demethoxycurcumin respectively.

The AutoDock3 results (Table 3) show that the residues Asn91, Asp94, Asn95, Arg95, Ile125, Ser149, Gly164 and Lys168 of chain A are the more frequently interacting residues of topoisomerase II with all naturally occurring curcuminoids, salvicine and ANP ligand from the crystal structure pdb 1ZXM. As the active site of topoisomerase II protein contains B chain residues Ile33 and Tyr34. Among B chain residues of active site, DMC interacts with Ile33 and Tyr34, and BDMC, salvicine and ANP interact with residue Tyr34. The strong hydrogen bonding interaction (Figure 2) shows that the residues Asn95, Arg98, Thr147, Lys168 and Thr215 of the A chain, and Tyr34 of the B chain are common in more than one ligand molecule. Also, by analysis of Glide docking results for curcuminoids and salvicine (Table 4) it was found that the Asn91, Asp94, Asn95, Arg98 and Gly164 residues of chain A are the most common residues interacting with curcuminoids and salvicine. Residues Asn95, Arg98 and Thr215 of chain B participate in hydrogen bonding with more than one ligand. The involvement of hydrogen bonds and both chain residues supports that the predicted binding site is at the junction of the chains A and B. The interaction site and active site cavity from blind docking simulation using AutoDock3 and binding site prediction using SiteMap 2.4.107 of Schrödinger module followed by docking simulation using Glide 5.6.107 software respectively, were found to be the same (Figure 3).

These results are also supported by the docking of salvicine, a known drug molecule using blind docking technique applying AutoDock3 and Glide supplied by Schrödinger suite 10.0. On comparing the binding affinity of curcuminoids with salvicine (Tables 1 and 2), it has been found that curcumioids have similar binding affinity

Table 4. Human DNA topoisomerase II protein residues interacting with curcuminoids and salvicine using Glide

Topoisomerase II residues interacting with				
Curcumin	DMC	BDMC	CC	Salvicine
Asn91, Arg98, Ile141, Phe142, Thr147, Ser149, Asn150, Thr159, Gly164, Ala167, Lys168	Ile88, Asn91, Asn95, Arg98, Asn120, Ile125, Ser149, Ala167, Lys168, Ile217, Tyr34B	Arg98, Asn120, Ile141, Thr147, Ser149, Asn150, Thr159, Ala167, Lys168	Asn91, Asn95, Gln97, Arg98, Asn120, Ile125, Ser149, Asn150, Thr215	Asn91, Asn95, Ile118, Asn120, Ile141, Phe142, Ser148, Ser149, Asn150

Highlighted residues are involved in H-bonding interaction with ligands.

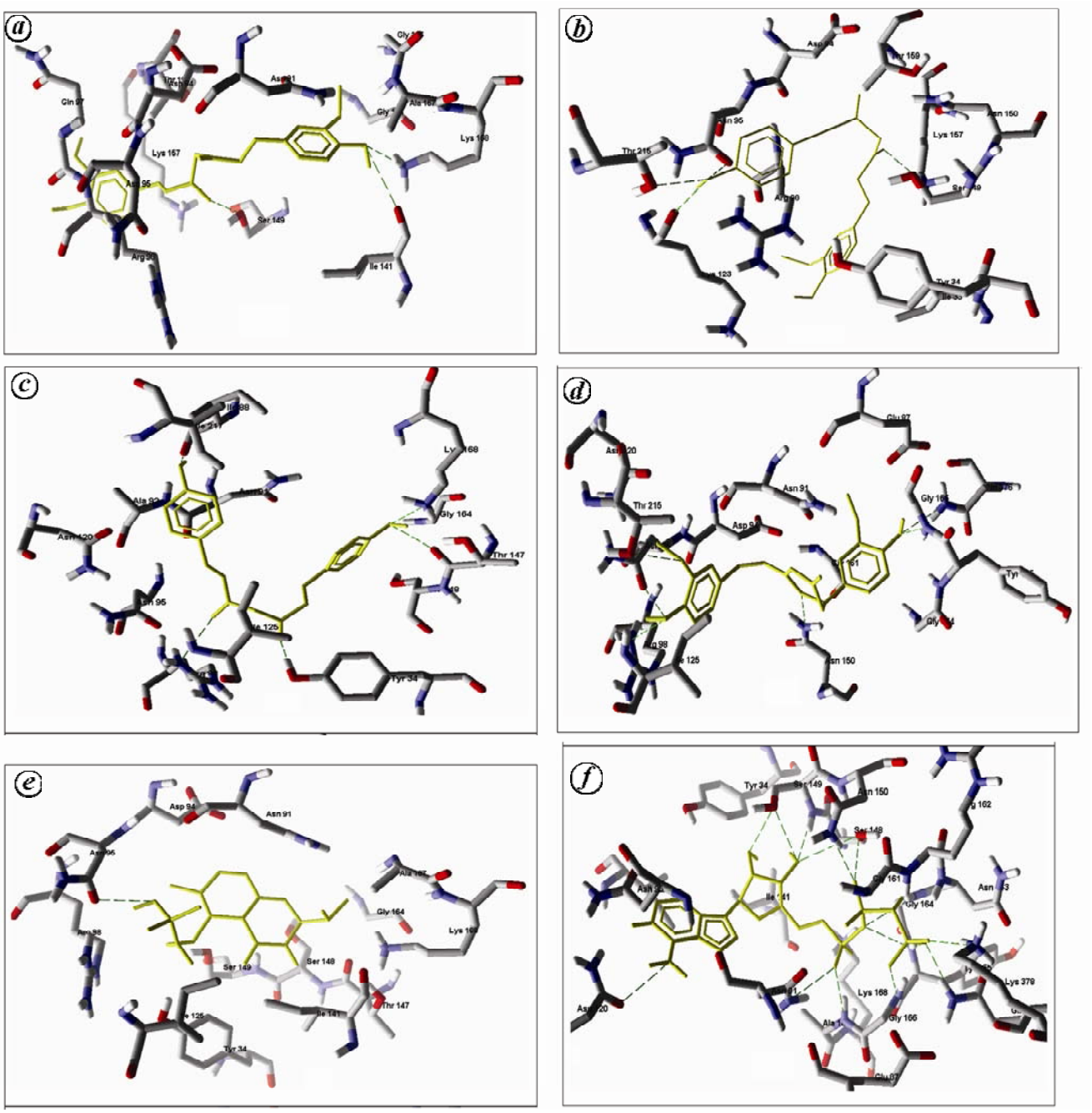

Figure 2. ***a–f***, AutoDock3 results showing the docked conformation of curcumin, demethoxycurcumin, bis-demethoxycurcumin, cyclocurcumin, salvicine and ANP (ligand from the crystal structure) respectively, at the proposed binding site of human topoisomerase II α protein using Molegro Virtual Docker[18].

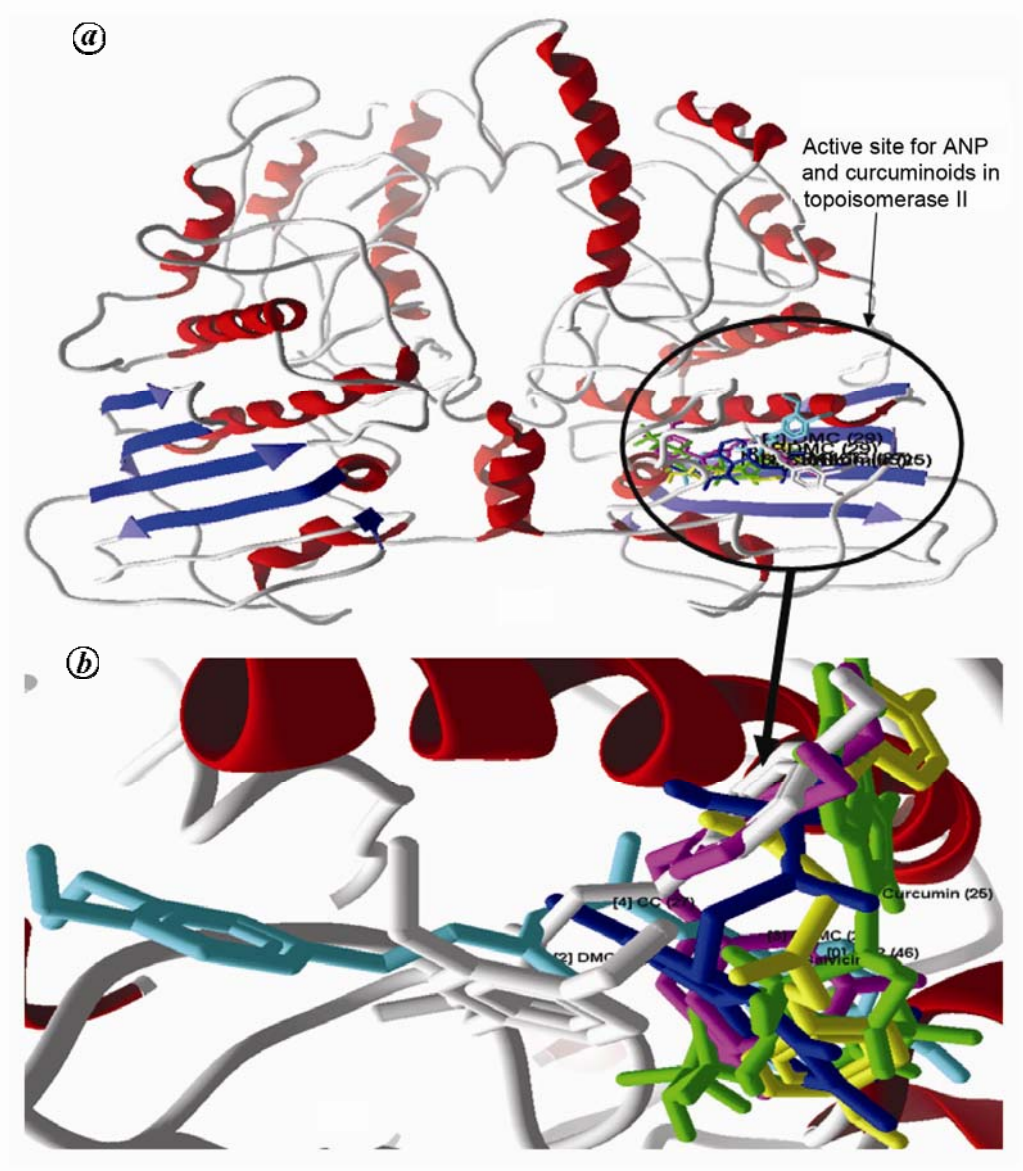

Figure 3 ***a, b.*** Cartoon model representation for bound best conformations of curcuminoids, salvicine and ANP at the junction of the A and B chains of human topoisomerase II (ATPase region) molecule by Molegro Virtual Docker[18].

as salvicine, such as in case of AutoDock the binding affinities are in the sequence ANP > bis-demethoxycurcumin > cyclocurcumin > salvicine > curcumin > demethoxycurcumin, and in case of Glide docking result analysis the order is salvicine > demethoxycurcumin > cyclocurcumin > curcumin > bis-demethoxycurcumin respectively.

The present study using two different docking softwares (AutoDock3 and Glide) has shown that the binding site of curcuminoids at topoisomerase II α is common. However, the energy scoring function and space searching algorithm are different in both docking softwares. And our prediction of binding site for curcuminoids at human topoisomerase II has been also supported by comparing the docking results of salvicine (know inhibitor of human topoisomerase II) and ANP (ligand from the crystal structure lzxm.pdb), bound at the similar active site. On comparing the docking results of AutoDock3 with Glide software, it has been concluded that the naturally occurring curcuminoids compete with ATP for binding. The Glide score and docking energy suggest that the binding affinity is similar to that of salvicine. Thus, curcumin can be taken as a lead molecule for developing novel potent drugs using its predicted biding site. It could either be used alone or in combination with known drugs which act on the DNA catalytic site. These curcumin-based drugs may act singly or synergistically with other known drugs and prove to be more effective for cancer chemotherapy. The added advantage is that curcumin is safe and non-toxic.

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The transmission dynamics of pandemic influenza A/H1N1 2009–2010 in India

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To understand the transmission dynamics of the prevailing pandemic 2009–10, due to the newly emerged pathogen influenza A/H1N1 throughout India, we have analysed the daily reported time-series dataset of the first two waves and a comparative study has been made for different regions of India. In order to quantify the early intensity of the strain, we have estimated basic reproduction number (R_0) through initial intrinsic growth rate method using standard deterministic SEIR model and effective reproduction number (R_t) through the stochastic SIR model using Bayesian inference. The estimate of reproduction number for India is 1.46 with 95% CI (1.15, 1.77), whereas for different states, the reproduction number is between 1.03 and 1.75.

Keywords: Bayesian inference, influenza H1N1, pandemic, reproduction number.

THE presence of the highly pathogenic influenza A/H1N1 virus among human population in several regions of the world, including India, has highlighted the urgent need for taking some efficient preventive measure against the latest pandemic. A novel influenza strain was first detected in Mexico in March 2009, which rapidly spread to different countries of the world[1]. In India, the first case (exogenous) of H1N1 2009–10 pandemic was identified on 17 May 2009 in Hyderabad and then it spread all over the country at varied intensities in different states[2]. As on 17 May 2010, there were 31,924 laboratory-confirmed cases in India and 1525 deaths were reported, i.e. 4.78% of the cases tested positive for influenza A/H1N1 virus[2,3], hence estimating the strength of the epidemic is of great concern.

The basic reproduction number (R_0) is the most common measure of this strength[4,5] to understand the early epidemic situation. In a wholly susceptible population, R_0 is simply the number of secondary cases generated by one primary case. When a fraction p of the population is effectively protected from infection, this quantity is known as the effective reproduction number (R_p) and is related to R_0 by $R_p = (1 - p)R_0$, assuming a well-mixed population[6]. Whereas the effective reproduction number at time t, denoted by R_t, is defined as the actual average number of secondary cases per primary case at time t (for $t > 0$)[7]

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