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Chemical inhibition of fatty acid synthase: molecular docking analysis and biochemical validation in ocular cancer cells

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Abstract Fatty acid biosynthesis is an attractive target for anti-cancer therapeutics. The ocular cancer, retinoblastoma cells were treated with fatty acid synthase (FASN) enzyme inhibitors: cerulenin, triclosan and orlistat. The IC_{50} and dose-dependent sensitivity of cancer cells to FASN inhibitors decrease in biologic enzyme activity, and cell morphology alterations were analysed. Molecular interactions of enzyme-inhibitor complexes were studied by molecular modelling and docking simulations. The crystal structures of ketoacyl synthase (PDB ID:3HHD) (cerulenin) and thioesterase (PDB ID:2PX6) (orlistat) domains of human FASN were utilized for docking, while for the non-crystallised human FASN enoyl reductase domain (triclosan), homology model was built and used for docking. All three inhibitors showed significant binding energy indicating stable complex formation with their respective FASN subunits. The predicted Ki value of the FASN inhibitors corroborated well with their corresponding anti-cancer effects.

Keywords Fatty acid synthase · Retinoblastoma · Molecular docking · Cerulenin · Triclosan · Orlistat · Cytotoxicity

Introduction

Fatty acid synthase (FASN, EC 2.3.1.85) is a lipogenic multi-enzyme complex (MW 250-270 kDa) that synthesizes the long chain fatty acids from malonyl CoA and acetyl CoA in the presence of NADPH as the reducing equivalent in the cytoplasm of the cells. This enzyme comprises seven catalytic domains and is also found in complex with acyl carrier protein. Each subunit of the enzyme performs a specific function for the synthesis of palmitate—which is the predominant 16-carbon fatty acid. The seven domains are β-ketoacyl synthase (KS), acetyl transacylase (AT), malonyl transacylase (MAT), β-hydroxy acyl dehydratase (DH), enoyl reductase (ER) and β-ketoacyl reductase (KR), and thioesterase (TE) [1].

Retinoblastoma (RB), a cancer of the eye, predominantly occurs in young children, and effective treatment modalities are being investigated and developed. Our lab recently reported the correlation between molecular expression of FASN and RB tumour invasiveness and differentiation, at the level of FASN protein and mRNA expression in fresh tumour tissues, indicating FASN to be a promising diagnostic/prognostic and therapeutic target for retinoblastoma [2]. Cerulenin, triclosan, and orlistat are chemical inhibitors that target various FASN domains and are potential anti-cancer drug candidates [3–5]. For better understanding, the FASN

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structure with its catalytic domains, along with the sites of inhibition by cerulenin, triclosan, and orlistat, have been schematically presented in Fig. 1.

Cerulenin, a mycotoxin, is a potent non-competitive inhibitor of FASN, and it targets the β-ketoacyl synthase domain of the enzyme. It also covalently modifies the active site cysteine, resulting in dead-end inhibition. Cerulenin has been found to inhibit FASN activity and induce apoptotic cell death in breast and colon cancer cells [6]. Triclosan, a broad-spectrum anti-microbial agent, acts by inhibiting the enoyl reductase domain of FASN. This activity has also been associated with its ability to inhibit proliferation of breast cancer cells [4]. Triclosan administration suppressed the rat mammary tumourigenesis suggesting FASN to be a molecular target for cancer chemoprevention [7]. Orlistat is a FDA-approved anti-obesity drug. It is a derivative of a natural product containing a β-lactone moiety that irreversibly inhibits the pancreatic and gastric lipases. Orlistat inhibits the thioesterase domain of the human FASN that has prompted research on its anti-cancer activity [5].

In the present study, three FASN inhibitors (cerulenin, triclosan and orlistat) were evaluated and compared for their anti-cancer effects in retinoblastoma cancer cells in vitro. Molecular modelling and docking studies were performed with the chemical inhibitors for their relative affinity with the respective domains. The in silico analysis of enzyme inhibition was checked for consistency with the differential effect of the inhibitors on the biological activity of FASN, and therapeutic toxicity in ocular cancer cells.

Materials and methods

In vitro studies

Chemicals

Cerulenin, triclosan, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and dimethyl sulphoxide (DMSO) were purchased from Sigma–Aldrich, USA. Orlistat (Xenical^{TM}) was obtained from Roche (Milan, Italy). All the other chemicals were purchased from Merck (Mumbai, India). Cerulenin was solubilized in DMSO, triclosan in acetone and DMSO as previously described [8], and orlistat was solubilised in DMSO/acetone mixture. The drugs were appropriately diluted in the culture medium prior to use.

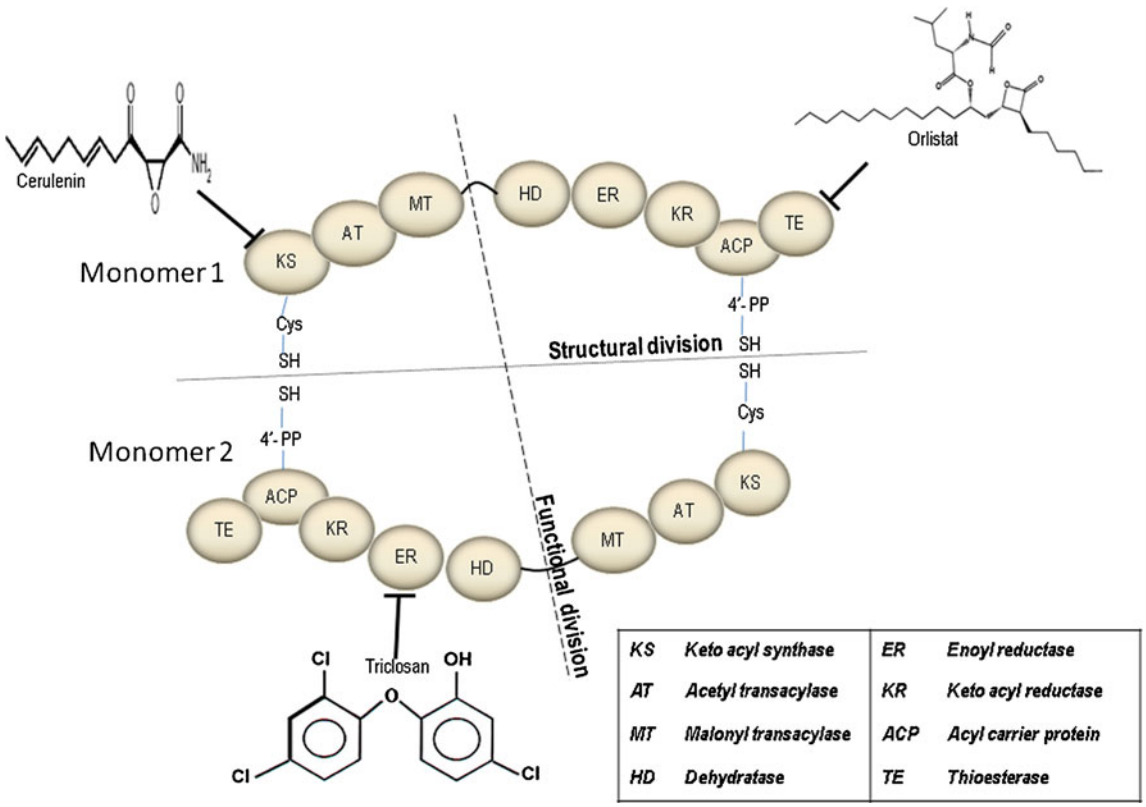


Fig. 1 Schematic depiction of fatty acid synthase enzyme complex and its inhibitors. Structure of FASN enzyme complex (ref 1) is presented with seven catalytic domains, along with the chemical structure of the inhibitors of the respective domains

Cell lines

Human retinoblastoma, Y79, cell line was obtained from the American Type Cell Culture (ATCC, USA), and it was maintained in RPMI 1640 media with 15% FBS in an atmosphere containing 10% CO_2 at 37°C.

MTT assay

Y79 cells were seeded at 5×10^3 cells/well in 96-well plates with 100 µl of culture media and incubated at 37°C for 24 h. The cells were then exposed to varying concentrations of cerulenin, triclosan and orlistat in fresh medium and then incubated for 72 h. At the end of the incubation, 10 µl of MTT (5 mg/ml) was added to the cells with fresh medium and incubated for 4 h at 37°C. The formazan crystals formed were dissolved in 100 µl of DMSO. The colour developed was read at 570 nm. Cell viability (or cell survival) was calculated as: (Test OD/Control OD)×100. The 50% inhibitory concentration (IC_{50}) of the three drugs (cerulenin, triclosan and orlistat) was computed using polynomial regression analysis using Microsoft Excel.

Assessment of cell morphology by phase contrast microscopy

Retinoblastoma Y79 cells treated for 72 h with FASN inhibitors—cerulenin, triclosan, and orlistat—were assessed for morphological changes using a phase contrast microscope (Nikon, Tokyo, Japan).

Estimation of FASN enzyme activity by NADPH oxidation

Approximately 5×10^4 cells were treated with varying concentrations of the three drugs, and the cells were collected and lysed with lysis buffer (pH 7.5) containing 1 mM EDTA, 50 mM Tris HCL, 150 mM NaCl, and 100 µg/ml PMSF. Fifty microlitres of each lysate was taken for the reaction. Background NADPH oxidation was measured initially without the lysate for 10 min. Then the FASN-dependent NADPH oxidation was measured in 1 ml of the reaction mixture containing 200 mM potassium phosphate buffer (pH 6.6), 1 mM DTT, 1 mM EDTA, 0.24 mM NADPH, 30 µM acetyl CoA, 50 µM malonyl CoA and 50 µl cell lysate. The enzyme activity of FASN was then calculated and expressed as nanomoles of NADPH oxidized per minute per millilitre [9].

In silico studies

Molecular modelling and optimization of FASN domains

Human FASN domains: The crystal structure of KS (PDB ID:3HHD), TE (PDB ID:2PX6) domains and the homology model of ER domain were used in this study. The ER domain structure was modelled based on the crystal structure of porcine FASN I (PDB ID:2VZ8) (http://www.pdb.org/pdb/home/home.do) using the MODELLER 9v7 program [10]. Further, all the structures were subjected to geometry optimization using Gromos 43a1 force field through Gromacs4.0 molecular dynamics package [11]. The validation of optimized structures before and after the minimization was carried out with Structural Analysis and Verification Server which implements PROCHECK validation [12]. The optimized and refined structures were used as input for further docking studies.

Optimization of inhibitors

The structural coordinates of the FASN inhibitors cerulenin [Pubchem:5282054] and triclosan [Pubchem:5564] were downloaded from NCBI-Pubchem compound (http://www.ncbi.nlm.nih.gov/pccompound) database in three-dimensional (3D) SDF-file format and then were converted to PDB format using Babel (http://sourceforge.net/projects/openbabel/). For orlistat (hydrolyzed form) [PDB ID:2PX6], the structural coordinates were retrieved from the PDB. The obtained 3D structures in PDB format were further geometry minimized using ProDRG SERVER (http://davapc1.bioch.dundee.ac.uk/prodrg/).

Docking studies of FASN inhibitors

Docking simulations were performed using AUTODOCK 4.0 suite, a molecular docking tool which implements Lamarckian genetic algorithm. In this study, we used semi-flexible docking procedure, in which protein is treated as a rigid molecule whilst the inhibitors, as flexible. The docking grid was fixed to cover the respective catalytic domain of FASN pertaining to the inhibitor binding, as documented in in vitro and in in silico studies. The grid box size in *x*-, *y*-, and *z*-axis was normally set in accordance to the drug binding site [13–16]. The spacing between grid points was fixed as 0.375 Å. During the docking procedure, 100 conformations were considered for each inhibitor for Lamarckian genetic algorithm search, the population size was set to 150 and the individuals were initialized. The maximum number of energy evaluation was set to 2500000 with 27000 as the maximum number of generation, and the maximum number of top individual that automatically survived was set to 1 with mutation rate of 0.02 and over rate of 0.8 (other parameters were set to default). AUTO DOCK 4.0 was compiled and run under Linux operating system [17]. Results were clustered according to the root mean square deviation (RMSD) criterion. To ascertain the correlation between experimentally determined IC_{50} value in cancer cells and the predicted binding energy, the best

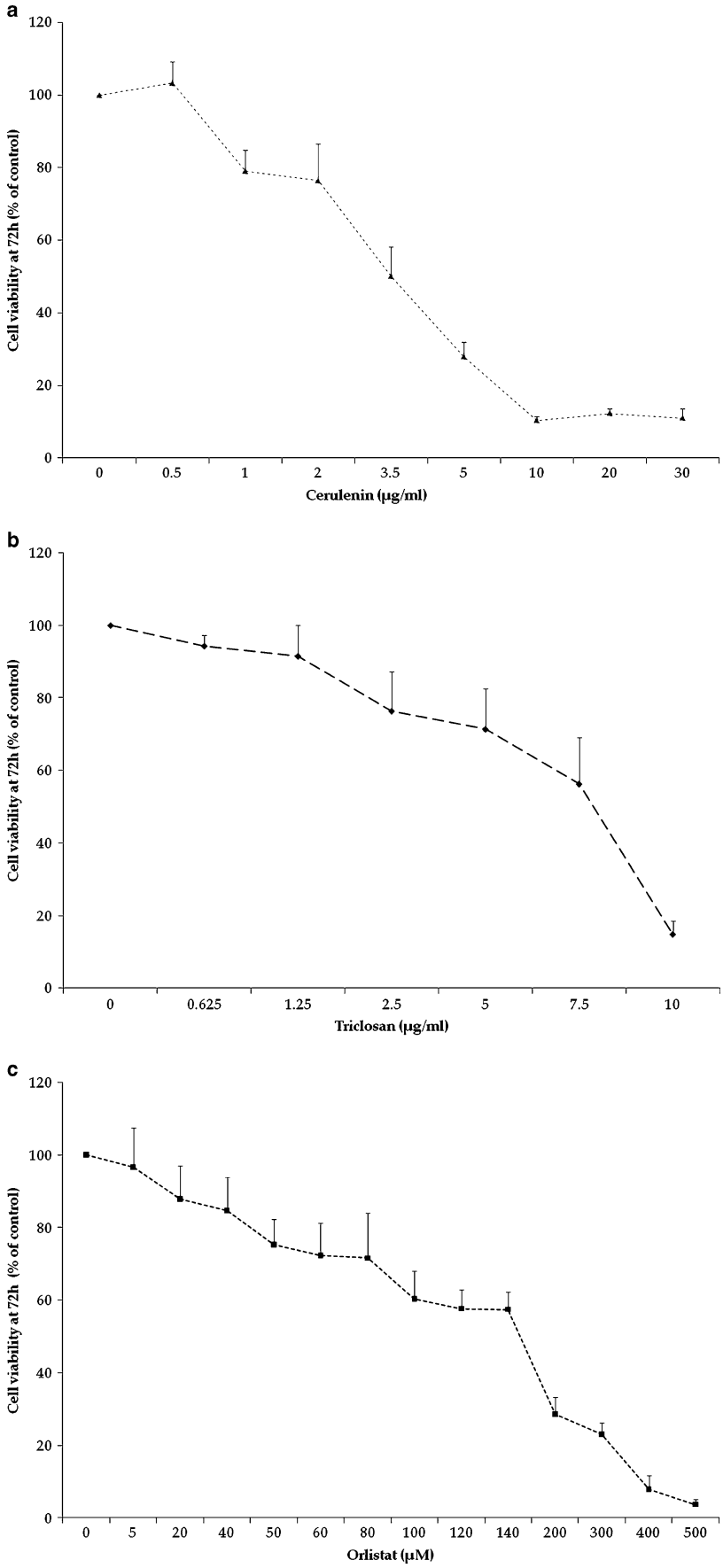


Fig. 2 Cytotoxic effects of FASN inhibitors in cultured retinoblastoma cells. Cytotoxic effects of FASN inhibitors in retinoblastoma cells were evaluated by MTT assay at 72 h with cerulenin (**a**), triclosan (**b**) and orlistat (**c**). IC_{50} was calculated using polynomial regression analysis by Microsoft Excel. Values are expressed as mean ± SD of three separate experiments in triplicate

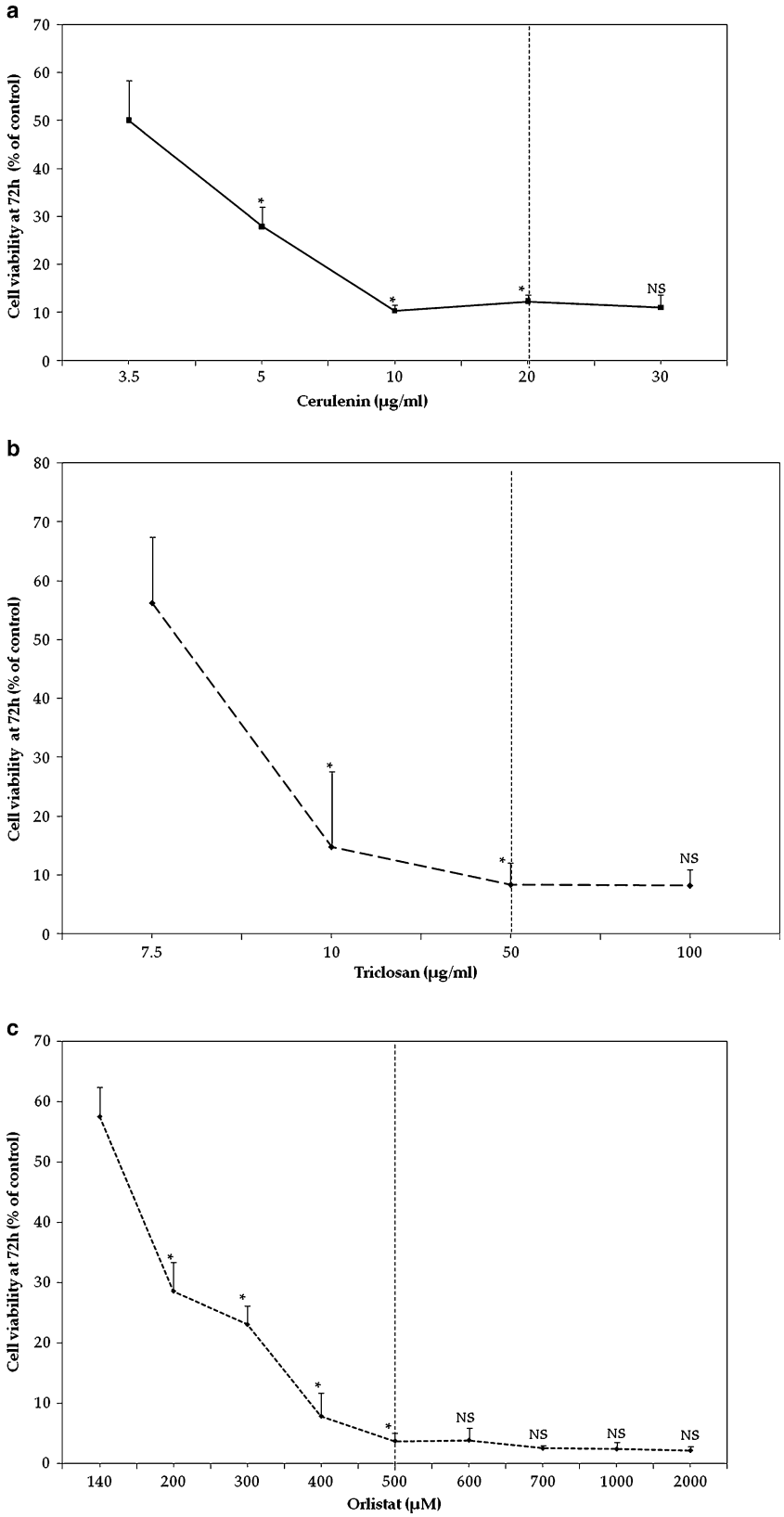


Fig. 3 Sensitivity of FASN inhibitors with multi-fold increase in dosages of drug exposed for 72 h on cultured retinoblastoma cells. Cultured retinoblastoma Y79 cells were treated with multi-fold increase in dosages of each inhibitor cerulenin (**a**), triclosan (**b**), and orlistat (**c**) for 72 h. Dose-dependent decrease in cell viability was observed only until a threshold concentration beyond which the cells were less sensitive to increase in drug concentration. *Indicates significant difference between consecutive dosage of the inhibitor, and *NS* indicates no significant difference

docked conformation of inhibitors was selected based on cluster rank, binding energy and RMSD. The docking results were visualized using Pymol (http://www.pymol.org). Interactions between enzyme domain and inhibitor

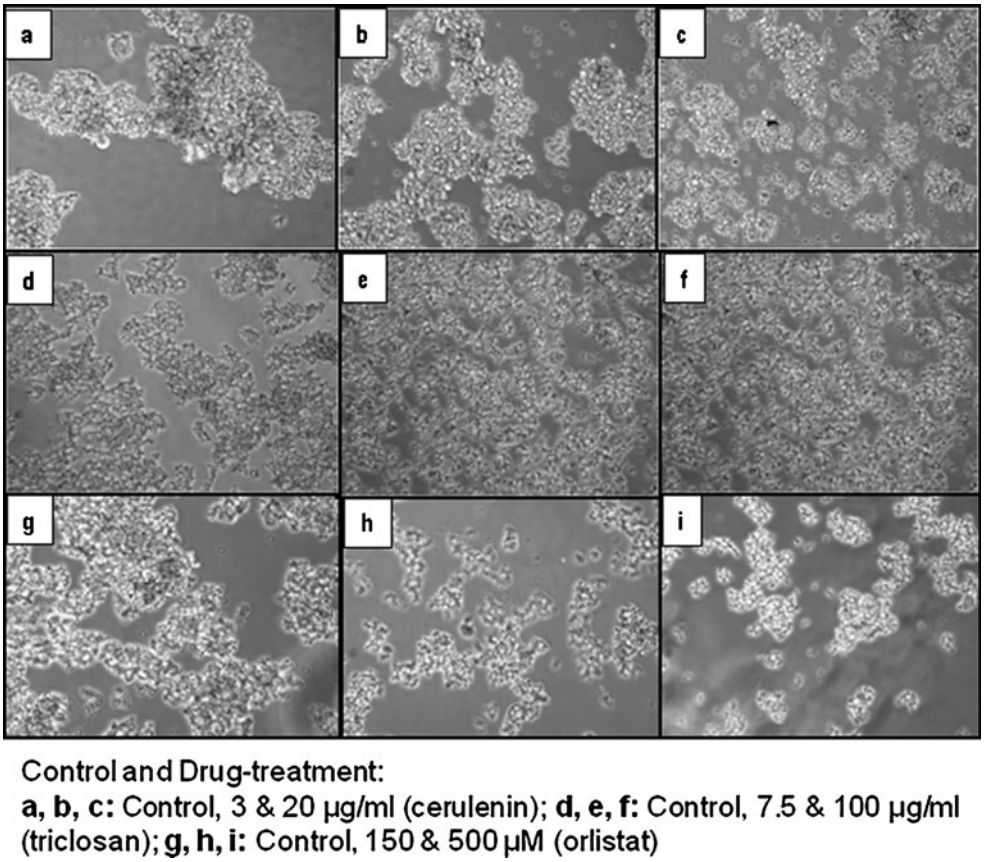


Fig. 4 Morphology of retinoblastoma cells treated with FASN inhibitors (cerulenin, triclosan and orlistat) at different doses and durations. Retinoblastoma cells were treated with the IC_{50} and a higher toxic dosage of cerulenin (**a, b, c:** control, 3 and 20 µg/ml), triclosan (**d, e, f:** control, 7.5 and 100 µg/ml), orlistat (**g, h, i:** control, 150 and 500 µM). The cell morphology was analysed by phase contrast microscopy (magnification, ×100)

were investigated with Ligplot using PDB sum server (http://www.ebi.ac.uk/thornton-srv/database/pdbsum/generate.html).

The Ki or TC_{50}, defined as the theoretical inhibitory constant, was calculated through autodock through the equation: $Ki = \exp(\text{deltaG}/(R \times T))$, where Ki is the inhibitory constant or TC_{50}, delta G is the binding energy, R is the universal gas constant and T is the temperature [18].

Statistical analysis

Values are expressed as mean ± SD. Student's t test was used for comparing experimental groups. Differences between the groups with $P<0.05$ were considered statistically significant.

Results

In vitro studies

Inhibition of cancer cell proliferation by cerulenin, triclosan and orlistat

To demonstrate the effect of FASN inhibitors (cerulenin, Fig.2a; triclosan, Fig.2b; and orlistat Fig.2c) on cell viability, cultured retinoblastoma Y79 cells were treated with different doses of three FASN inhibitors for 72 h. The three drugs showed a dose-dependent decrease in cell viability. Here we report the IC_{50} of cerulenin, triclosan and orlistat to be 3.54 µg/ml, 7.29 µg/ml and 145.25 µM, respectively.

Sensitivity of Y79 cells to increasing concentrations of FASN inhibitors

Figure 3 presents the sensitivity of Y79 cells treated with FASN inhibitors (cerulenin, Fig.3a; triclosan, Fig.3b; and orlistat, Fig.3c) at increasing dosages higher than their respective IC_{50} at 72 h of treatment. It is clear from the graph that the cancer cells respond sharply to increasing concentrations beyond the IC_{50}. However, this dose-dependent sensitivity of the cancer cells diminishes beyond a particular concentration. This is indicated by the dotted line, beyond which increments in drug concentration fail to significantly reduce cell viability ($P<0.05$). Cerulenin showed a significant decrease between consecutive dosages 3.5, 5, 10 and 20 µg/ml; the higher dosage (30 µg/ml) did not show significant difference. We also compared the percentage cell viability difference between 10 and 30 µg/ml, which was insignificant. Triclosan showed significant difference

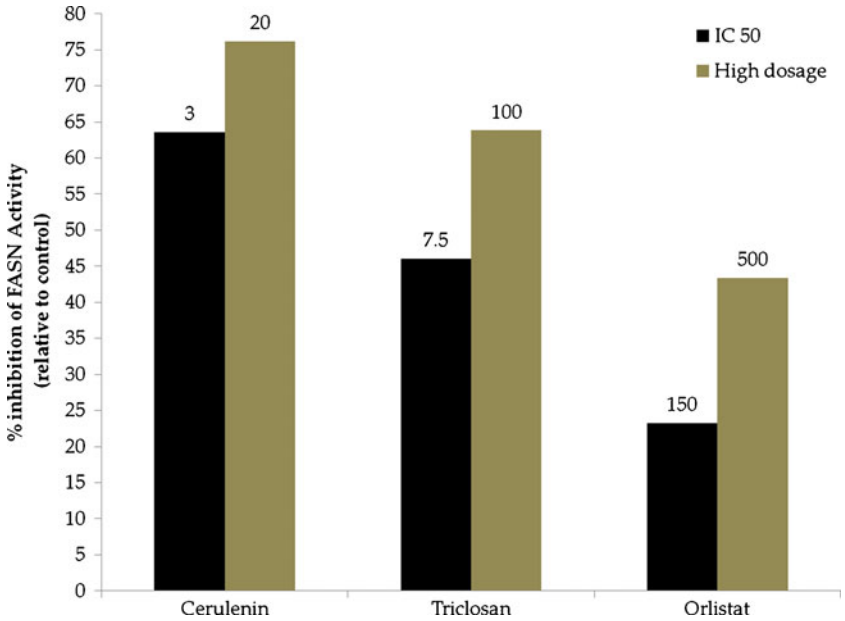


Fig. 5 Inhibition of FASN enzyme activity in ocular cancer cells. Effect of the three inhibitors on FASN enzyme activity on Y79 retinoblastoma cells treated with the IC_{50} and a higher toxic dosage of cerulenin (3 and 20 μg/ml), triclosan (7.5 and 100 μg/ml) and orlistat (150 and 500 μM) for 72 h was measured by NADPH oxidation method at 340 nm. The enzyme activity was calculated as nanomoles of NADPH oxidized per minute per millilitre reaction, obtained from three independent experiments performed in duplicates. FASN activity inhibition was then expressed as percentage decrease in enzyme activity in the inhibitor-treated cells relative to the untreated control retinoblastoma cells. The two dosages of each FASN inhibitor are indicated by the *numbers* placed above each column

between consecutive dosages (7.5, 10 and 50 μg/ml). However, a twofold higher dosage, 100 μg/ml, did not significantly decrease the cell viability. The sensitivity of the cancer cells to increasing dosages of orlistat was in the range 140–400 μM. Beyond 500 μM, the effect of orlistat on cancer cell proliferation was not significant, as the cell viability had already reached less than 5%.

Cell morphology assessment by phase contrast microscopy

Figure 4 reveals the cell morphology alterations in Y79 retinoblastoma cells that were treated with cerulenin (a–c), triclosan (d–f) and orlistat (g–i) for 72 h. Phase contrast microscopic analysis revealed abnormal changes in cell structure when treated with FASN inhibitors at their IC_{50} and as well as at a higher concentration. Particularly, the higher dosage of all the three FASN inhibitors revealed marked cell shrinkage.

FASN enzyme activity assay by NADPH oxidation

Figure 5 shows the inhibition of FASN enzyme activity in Y79 retinoblastoma cells that were treated with cerulenin, triclosan and orlistat for 72 h. All the three anti-lipogenic agents inhibited the FASN enzyme activity in a dose-dependent manner when treated at their respective IC_{50} and higher concentrations, in comparison with the untreated control. However, the extent of inhibition by cerulenin exceeded that of triclosan, which in turn showed a greater degree of inhibition compared to orlistat (cerulenin (64%)> triclosan (46%)>orlistat (23%) at their 50% inhibitory concentrations).

Table 1 Structural optimization and validation analysis using PROCHECK, Gromacs, QMEAN and Pymol

FASN model domain	Ramachandran plot analysis			Energy calculations for models using GROM0S96 potential energy (kcal/mol)	QMEAN score	RMSD (Å)
	Favourable (%)	Allowed (%)	Disallowed (%)			
KS	91.8	7.9	0	−6.40918	0.677	0.075
ER	82.5	16	0	−1.50306	0.617	0.058
TE	91.0	8.9	0	−2.95823	0.733	0.067

KS Ketoacyl synthase, *ER* enoyl reductase, *TE* thioesterase, *RMSD* root mean square deviation

Table 2 Docking characteristics of enzyme-inhibitor interactions compared with in vitro computed cytotoxic IC_{50} in cancer cells

Docked complex	Experimental IC_{50} (μM)	Docking inhibition constant (Ki or TC_{50})	Binding energy (kcal/mol)
KS-cerulenin	15.67	53.93 μM	−5.82
ER-triclosan	25.17	62.73 μM	−5.73
TE-orlistat	145	6.6 mM	−2.97

In silico studies

Homology modelling of ER domain

A suitable template of human FASN ER domain was used to generate 3D models by MODELLER9v7. The stereochemical properties of generated models were validated by the Ramachandran plot using PROCHECK tool.

Validation and refinement

The crystal structures of KS, TE domains and generated best homology model of ER domain were subjected to loop refinement using MODELLER9v7. Further, to optimize the protein geometry, steepest descent energy minimization was performed using the GROMOS 43a1 force field. The refined and optimized models were again validated by the Ramachandran plot, and the final optimization results are tabulated (Table 1). To further examine the refined structures of the FASN domain, the QMEAN tool was used, which scores the structure quality as a function of linear combination of six structural descriptors [19]. The QMEAN score ranges between 0 and 1; higher values are suggestive of plausible models. The RMSD between the main chain atoms of models with its respective templates was also calculated by structural superimpositions by PYMOL, and the corresponding RMSD values are presented in Table 1.

Docking studies of FASN inhibitors

The three inhibitors were found to dock on the documented active sites of FASN, suggestive of predictive accuracy of the semi-flexible docking protocol [13–16]. Table 2 shows the results of the docking experiments, wherein binding energy, theoretical Ki (TC_{50}) between the FASN domains and their inhibitors are compared with the experimental IC_{50} in the ocular cancer cells in vitro. For the purpose of direct comparison, the IC_{50} values of cerulenin and triclosan were converted from micrograms per millilitre to micromolar units.

The hydrophobic interactions and the hydrogen bonding patterns between the FASN catalytic subunit and its respective inhibitor are detailed below.

Cerulenin

Cerulenin was found to dock into the reported active site of KS domain of FASN [13, 14] (Fig. 6a). A careful examination of the binding pocket indicated that cerulenin adopted a position in hydrophobic cage surrounded by Gln78, Ser112, Ser114, Ala160, Cys161, Asn189, Phe200 and Glu333 residues. Gly113, Glu115, Val190 and Arg224 were found to form five hydrogen bonds with cerulenin. These hydrogen bond and hydrophobic interactions stabilized the binding of cerulenin.

Triclosan

We developed an in silico human model for the ER domain of FASN for performing molecular docking studies with triclosan (Fig. 6b) [15]. Triclosan was placed in the binding cavity encased by hydrophobic core: Ser1635, Val1636, Ala1645, Val1648, Pro1649, Ser1653, Trp1807, Arg1808, Asp1635, Gln1815 and Ile1818. In addition, triclosan also formed a hydrogen bond with Trp1811.

Orlistat

The results of orlistat (hydrolyzed form) docking into the reported active site of TE domain [16] is shown in Fig. 6c. The results reveal the hydrophobic interactions between TE domain and orlistat mediated by the following residues: Ile2250, Tyr2307, Asp2338, Glu2366, Ala2367, Phe2370, Phe2423 and leu2427. Four hydrogen bonds were found to be formed with the following residues: Ser2308, Tyr2343, His2481 and Arg 2482.

Discussion

Retinoblastoma tumours develop from the neuro-epithelium of the retina, which differentiates into any retinal cell, including photoreceptors. FASN overexpression has been reported in many cancer types such as breast, prostate and colon [20]. FASN expression in retinoblastoma tumour tissues was correlated with RB tumour invasion and aggressiveness [2, 21]. In the present study, the anti-

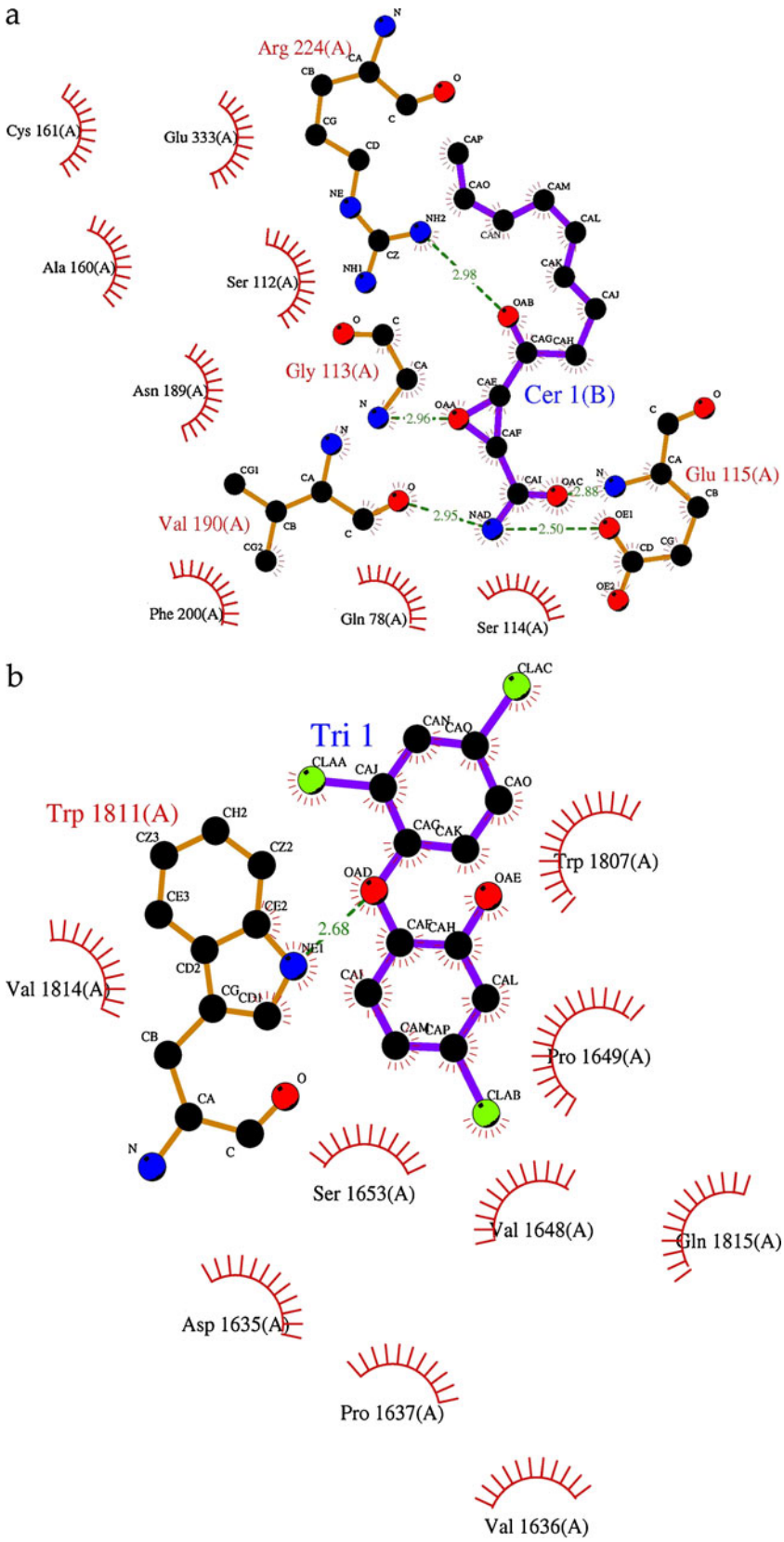
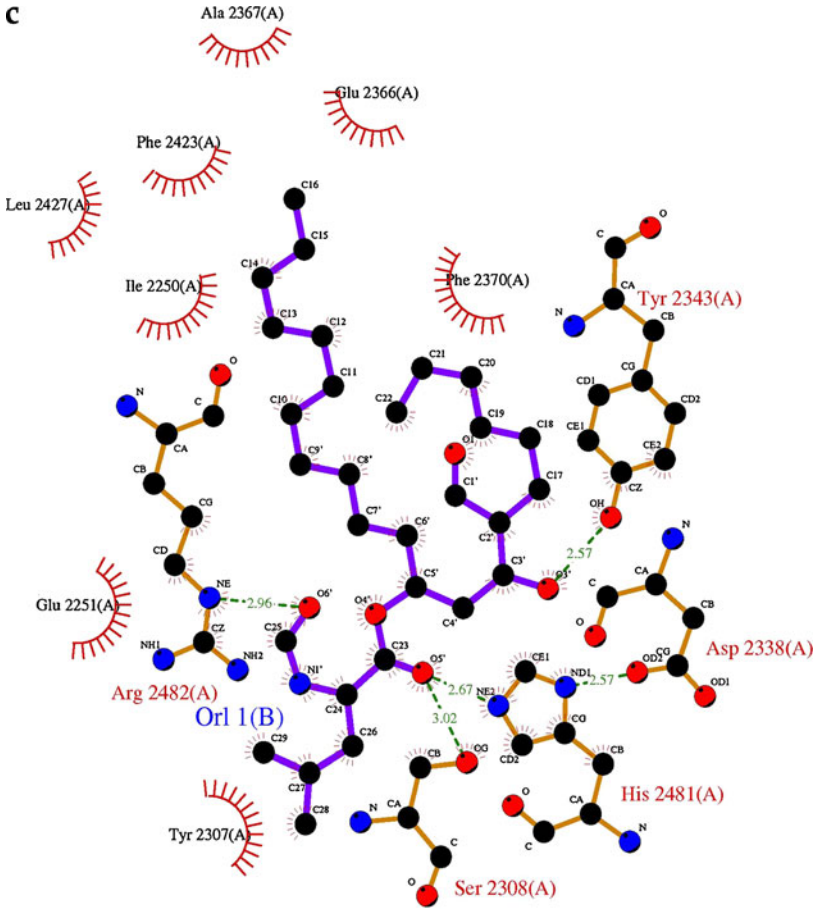


Fig. 6 In silico docking studies of FASN catalytic domains (KS, ER and TE) with their respective inhibitors. Illustration of docked complex for each human FASN domain: KS domain with cerulenin (**a**), ER domain with triclosan (**b**) and TE domain with orlistat (**c**). The atoms of the enzyme and the chemical inhibitors involved in the catalysis are shown as *coloured spheres*: carbon in *black*, oxygen in *red*, nitrogen in *blue* and chlorine in *light green*. Intermolecular hydrogen bonds are shown as *green dashed lines* with their respective bond distances in angstrom units. The FASN inhibitors are depicted with their chemical structure and labelled as: *Cer1* cerulenin, *Tri1* triclosan, *Orl1* (hydrolyzed form) orlistat. These images were generated using PDBSUM server

cancer effects of three FASN inhibitors—cerulenin, triclosan and orlistat—were studied in cultured retinoblastoma (Y79) cells, and the docking characteristics with their specific domains in FASN were analysed for correlation.

Fig. 6 (continued)



The FASN inhibitors showed a dose-dependent decrease in viability of retinoblastoma cancer cells. The IC_{50} of cerulenin, triclosan and orlistat were found to be 3.54 μg/ml, 7.29 μg/ml and 145.25 μM, respectively. Morphological changes in cancer cells treated with increasing dosage of FASN inhibitors substantiated their dose-dependent toxicity. Further, the sensitivity of retinoblastoma cells to the FASN inhibitors at 72 h of exposure was evaluated by increasing the dosage, multi-folds higher than their IC_{50}. All the three FASN inhibitors showed a dose-dependent decrease in cell viability until a particular concentration beyond which increments in concentration did not have any significant effect on cancer cell growth.

FASN inhibitors have been shown to decrease the proliferation of melanoma A-375 cell lines after 24 and 48 h of treatment [22]. Cerulenin induces apoptosis in human cancer cells by inhibiting FASN and causing malonyl-CoA accumulation, CPT-1 inhibition and inhibition of fatty acid oxidation [23]. The crystal structure KS-MAT didomain of human FASN is already available [PDB ID:3HHD], and this structure was used for docking with cerulenin [13]. The docking results revealed the binding energy of the enzyme-inhibitor complex to be −5.82 kcal/mol.

Triclosan inhibited FASN activity and decreased the proliferation of two breast cancer cell lines (MCF-7 and SKBr-3) in a dose- and time-dependent manner, suggesting the enoyl reductase domain of FASN to be an effective anti-cancer target [4]. The enoyl reductase domain of human FASN has not yet been crystallised; therefore, the crystallised ER domain [PDB ID:2VZ8] of porcine was used as template for human ER domain, and further, this model was used for the docking studies with triclosan. Here, we found the binding energy of the enzyme-inhibitor docking complex between the ER domain of FASN and triclosan to be −5.73 kcal/mol.

Orlistat was reported to halt the tumour cell proliferation by arresting the cell cycle at G1/S phase through the retinoblastoma protein pathway in breast cancer cells [5]. The crystal structure of the human TE domain complexed with orlistat is already available (2PX6), in which orlistat was captured in the catalytic triad [16]. Based on this crystal structure information, we performed re-docking using Autodock to calculate the binding energy which was found to be −2.97 kcal/mol. The binding poses were also found to be synonymous in crystallised and docked complexes. Pemble et al. [16] had described the crystal structure of human FASN thioesterase complexed with

orlistat in two forms, an acyl-enzyme intermediate and the hydrolyzed product. The study had revealed the catalytic triad of Ser2308–His2481–Asp2338. The present molecular docking also implicates these amino acid residues in the active site binding with orlistat. All these findings indicate the predictive accuracy of the in silico methods adopted.

The molecular docking performed here predicted the hydrophobic and hydrogen bonding interactions, and the amino acid residues involved in the active site binding between the three FASN domains and their corresponding inhibitors. It is well known that hydrogen bonds and hydrophobic interactions are vital in maintaining the secondary and tertiary structures of biological macromolecules such as DNA and proteins. Hydrogen bonds are also involved in the binding of ligand to active sites of proteins, wherein they contribute to the orientation, recognition and affinity of the ligand [24]. While hydrophobic interactions between lipophilic surfaces of a ligand and hydrophobic areas of its binding site always contribute positively to binding affinity, the contribution of hydrogen bonds to the overall free binding energy depends on the balance of the desolvation energies and the energies of the newly formed hydrogen bonds, where changing a single functionality of a ligand may have very complex consequences [25]. Thus, favourable interactions between a drug and its specific biological target (e.g. a protein) are possible through a perfect geometric fit of the ligand to the binding site, both being in low-energy conformations, a correspondence of the molecular electrostatic potentials, the formation of charged and/or neutral hydrogen bonds between functional groups and hydrophobic interactions between lipophilic surfaces [25].

The theoretical inhibitory constant (Ki or TC_{50}), indicates the molecular affinity between the enzyme active site and the inhibitor, where Ki is inversely related to enzyme affinity. Here, the Ki shown by the FASN inhibitors was in the order cerulenin<triclosan<orlistat. Correspondingly, the extent of inhibition of FASN enzyme activity in the ocular cancer cells in vitro was found to decrease in the order cerulenin>triclosan>orlistat. Further, the therapeutic potential of these inhibitors was assessed by the IC_{50} in ocular cancer cells. The IC_{50} values were in the order cerulenin<triclosan<orlistat, implicating the therapeutic potential in the order cerulenin>triclosan>orlistat. The excellent agreement between the molecular docking analysis and the biochemical analysis presented here shows that greater affinity between the FASN enzyme and its inhibitor confers a better biochemical potential to the inhibitor to exert its anti-cancer effect. By molecular docking studies, Wang et al. had analysed the inhibitory efficacy of novel analogues of C75, using purified FASN from rat liver, that revealed potent anti-tumour activity of a compound, 4-methylene-2-octyl-5-oxo-tetrahydro-thiophene-3-carboxylic acid on HL 60 and HeLa cells, with efficient FASN inhibition [26].

Despite the molecular affinity potentials predicted in silico and confirmed by in vitro assays, the clinical effectiveness of FASN inhibitors in cancer therapy is determined by several factors. For instance, the present analysis indicates cerulenin to be a better cytotoxic agent than triclosan and orlistat. Yet, its reactive epoxide group makes it chemically unstable, limiting its clinical efficacy. This led to synthesis of analogues of cerulenin, such as C75 and C93 that have been tested for their anti-cancer efficacy [27, 28]. In this context, triclosan's inhibition site, ER domain, may be an attractive target for developing anti-FASN metabolites as its blockade is suggested to increase the levels of the enoyl thiolester intermediate that closely resembles cerulenin and C75 [29]. Orlistat inhibits the thioesterase domain, which catalyses the release of the end product, palmitate, from FASN. Orlistat has poor solubility, and low oral bioavailability [30]; hence, alternative formulations may alleviate this limitation and assist in tapping the anti-cancer potential of targeting the TE catalytic domain. Through in silico molecular docking analysis, drugs can be designed to improve binding affinity, therapeutic efficacy and bioavailability through optimized hydrophobic interactions and hydrogen bonding that would stabilize the drug ligands at the target active site [31].

In conclusion, the present study suggests a good correlation between the in silico determined binding energies and Ki of the FASN inhibitors, with the respective extent of inhibition of biologic activity of FASN enzyme and the differential anti-cancer effects shown by the FASN inhibitors in ocular cancer cells studied in vitro. Although some of the domains of human FASN have already been crystallised and reported, there are few domains including human FASN enoyl reductase domain (target for triclosan inhibition) that need to be elucidated. For the present study, however, we have predicted the 3D structure of ER domain based on its sequence homology with porcine ER crystal structure. Molecular docking analysis combined with biological testing will generate effective lead molecules as drug candidates. The nature of amino acid residues in the active site and the types of bonding between enzyme active site and inhibitor will enable the design of inhibitors with suitable functional groups, resulting in better affinity and biological activity. FASN, being a multi-catalytic enzyme complex, has immense potential for drug discovery, as newer chemical molecules targeting any of the seven catalytic domains of FASN can be developed. Chemical modification of existing FASN inhibitors can be explored to improve their therapeutic index. Promising lead molecules generated from structure-based designing have to be tested in vitro and in vivo for key pharmacologic parameters such as bioavailability and metabolic stability, if they have to

translate into clinical success [25]. Further, the FASN inhibitors in combination with conventional cancer chemotherapeutic drugs can be evaluated for their combination index and synergistic effects, which would in turn reduce high-dosage chemotherapy and the associated non-specific toxic side effects [32].

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