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## MOLECULAR DOCKING OF CURCUMIN ANALOGUES AS SERCA INHIBITORY AGENTS

Curcumin derivatives were virtually screened for inhibitory activity towards SERCA by computational docking. A detailed characterization of the inhibitor binding site at the molecular level and determination of the amino acids involved in interactions with curcumin and its derivatives have been provided. Crucial enzyme/inhibitor interactions were identified by analyzing the docking-predicted binding poses of active compounds. The loss of hydrophilic group by curcumin leads to an increase in binding energy. Some of curcumin derivatives showed better docking energies than curcumin indicating that they could be potent enzyme inhibitors. Additional binding energy was provided by extensive hydrophobic interaction between the hydrophobic parts of the ligands and the nonpolar residues at the binding site. Curcumin derivatives satisfy Lipinski's Rule of five which testifies to their druglikeness (absorption, distribution, metabolism and excretion) and possible pharmacological activity.

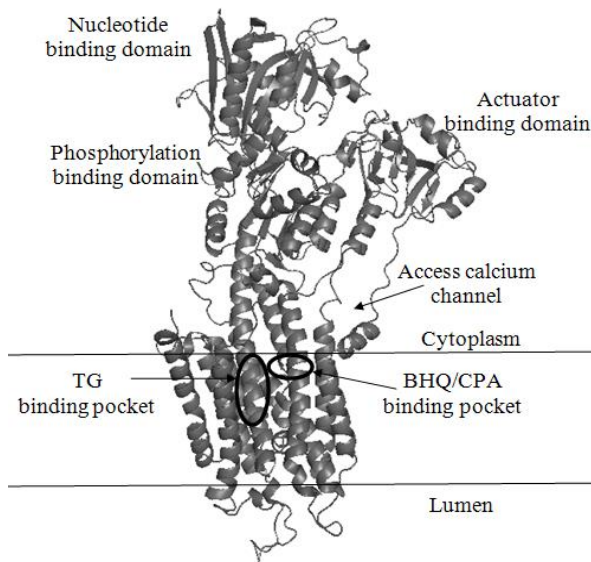
*Keywords:* molecular docking; binding energy; binding site; inhibitors; apoptosis.

**Introduction.** The sarco/endoplasmic reticulum calcium ATPase (SERCA) is an ion transport protein present in the membranes of intracellular calcium storages [1–3]. These storages act as sources for rapid calcium release through calcium-specific channels and ryanodine receptors that constitute an integral part of various signaling pathways. The rapid release of calcium ions triggers a variety of physiologically important functions, such as muscle contraction. Blockage of SERCA leads to malfunction of the calcium homeostasis in living cells and apoptosis. In contrast to chemotherapeutics SERCA inhibitors provoke apoptosis at all stages of the cell cycle. Due to its high potency and specificity, thapsigargin (TG) is the most commonly used SERCA inhibitor [4]. As a natural product of considerable structural complexity, TG is not easily synthesized, being therefore rather expensive agent. Due to significant value as research tools and medicinal potential as an emerging new class of anticancer agents, the development and design of novel SERCA inhibitors has become an area of considerable interest in the recent discovery of a sizeable repertoire of inhibitors with good potency. Examples include the fungal metabolite cyclopiazonic acid (CPA), di-2,5-terbutylhydroquinone (BHQ), terpenolides, clotrimazole, tetrabromobisphenol, and curcumin [5–10]. In addition, inhibitors of SERCA are invaluable tools for the study of the enzyme's physiological functions.

The SERCA is an integral membrane protein that consists of three cytoplasmic domains and ten transmembrane helices (M1-M10) (Fig. 1). A phosphorylation domain contains the invariants ASP 351 residue. A nucleotide binding domain contains the nucleotide binding pocket. An actuator domain communicates the conformational rearrangements that regulate the binding and release calcium [11]. Two inhibitor binding pockets were revealed: the first one is for TG and the second one for BHQ and CPA [4; 7; 9]. The binding site of TG is the cavity surrounded by M3, M5 and M7. TG effectively prevents the conformational changes leading to  $\text{Ca}^{2+}$  binding by immobilization of M3 and of ASN 768 (one of the  $\text{Ca}^{2+}$  ligand residues at site) [4]. BHQ and CPA occupy a pocket surrounded by M1-M4. These compounds inhibit SERCA by

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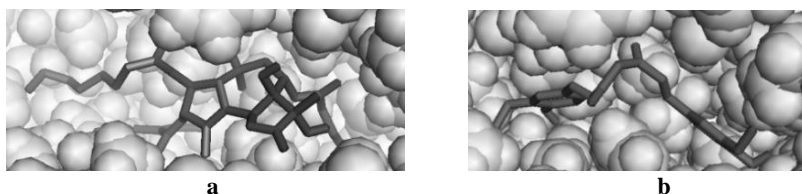
blocking the calcium access channel and immobilizing a subset of transmembrane helices. A binding site for curcumin is not known yet, therefore a mechanism of SERCA inhibition by this compound is not fully understood [2; 10].



**Fig. 1. Structure of SERCA**

Our study was aimed at providing detailed characterization of the inhibitor binding site at the molecular level and identification of amino acids involved in interactions with curcumin. We also undertook virtual screening of forty-three curcumin derivatives by computational docking for inhibitory activity towards SERCA. Crucial enzyme/inhibitor interactions were identified by analyzing the docking-predicted binding poses of active compounds.

**Results and Discussion.** Docking SERCA with curcumin using a grid space involving all amino acid residues of the protein enabled us to find a binding site for curcumin. It is a cavity surrounded by M3, M5 and M7 helices. TG and curcumin binding sites overlap (Fig. 2). It is known AutoDock predicts poses of TG correctly. The RMSD between the docking-predicted and the experimentally observed heavy atom positions of TG is 2.2 Å [12].



**Fig. 2. The TG binding pocket in SERCA with docked (a) thapsigargin, (b) curcumin**

A small grid box involving all residues of predicted binding pocket was used for further docking of curcumin and its derivatives. Docking for curcumin led to a cluster with binding energy -8.7 kcal/mol. It has one hydrogen bond and nine hydrophobic interactions (Fig. 3). The hydrogen bond was formed between hydroxyl of curcumin and carbonyl of hydrophobic amino acid ALA 306 through a distance of 2.0 Å (O-H...O). The hydrophobic bonds were formed between phenyl ring of curcumin and methyl group

of VAL 769, methoxy group of curcumin and methyl group of LEU 260, carbon atoms of heptanoic chain and phenyl ring of PHE 834 and PHE 256.

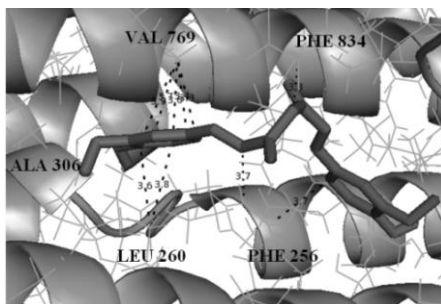


Fig. 3. The minimized energy pose of curcumin in SERCA

To conduct flexible docking runs we allowed the side chains of twelve amino acids closest to the curcumin to be fully flexible. These were PHE 256, PHE 834, VAL 769, VAL 263, ASN 768, ALA 306, ILE 765, ILE 829, LEU 828, LYS 252, LEU 253, LEU 260. The AutoDock predicted drastic changes both in the conformation of side chains (PHE 256, LEU 311, and ASN 101, for instance) and in the position of curcumin, which was severely displaced from the position predicted by rigid-docking, and thus presumed unrealistic. In addition, the decrease in binding energy was observed. These findings are not surprising. It was reported earlier that flexible docking simulations using AutoDock for the TG can lead to erroneous ligand and receptor conformations and are in need of further improvement to be of practical use [12]. Given the lack of improvement in docking performance and increased computation time, simulating a flexible SERCA binding site does presently not offer any real advantage for curcumin.

Docking results for some curcumin derivatives show binding energy of -8.8 – -9.0 kcal/mol (Fig. 4). Additional binding energy was provided by extensive hydrophobic interactions between hydrophobic parts of the inhibitors and nonpolar residues in the binding site (Table 1).

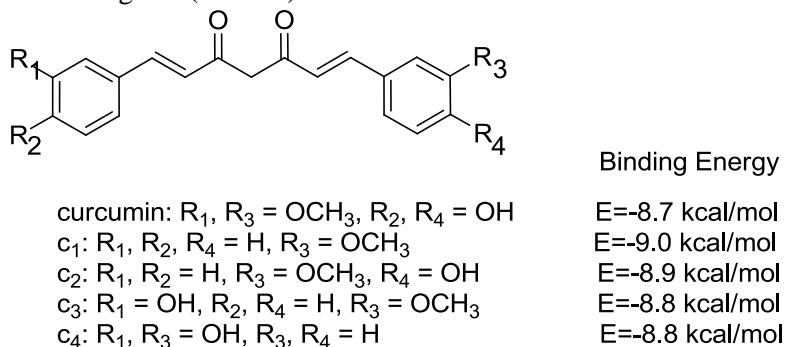


Fig. 4. Docked molecules with the lowest binding energies

Table 1

## Molecular interactions of ligands and binding site residues into SERCA

Residues	Length of bonds (Å)				
	Curcumin	c1	c2	c3	c4
	Hydrophobic bonds				
PHE 256	3.7 (2 bonds)	3.6–3.9 (4 bonds)	3.6 (2 bonds)	3.4–3.8 (4 bonds)	3.7–3.9 (3 bonds)
PHE 834	3.8	–	–	3.7, 3.9	3.9
VAL 769	3.3–3.7 (5 bonds)	3.5–3.8 (3 bonds)	3.4–3.8 (3 bonds)	3.5–3.7 (3 bonds)	3.8, 3.9
VAL 263	–	3.6	3.9	3.7	–
ASN 768	–	3.8	–	3.8	–
ALA 306	–	3.8	3.9	–	–
ILE 765	–	3.7	–	3.3–3.8 (3 bonds)	–
ILE 829	–	3.8	3.7–3.9 (3 bonds)	3.6	3.8 (2 bonds)
LEU 828	–	3.7, 3.8	3.7, 3.9	–	–
LYS 252	–	3.8	–	–	–
LEU 253	–	3.7	3.8	–	–
LEU 260	3.6, 3.8	3.6–3.8 (6 bonds)	3.5–3.8 (3 bonds)	3.8, 3.9	3.4, 3.8
	Hydrogen bonds				
ALA 306	2.0	–	–	–	–
GLU 255	–	–	–	2.8	2.9

Further the molecular descriptor analysis was performed to identify druglikeness of the curcumin derivatives **c1-c4** by Lipinski's Rule of five, which describes molecular properties important for a drug pharmacokinetics in the human body. According to the obtained results (Table 2) the derivatives **c1-c4** obey Lipinski's Rule.

Curcumin was found to have good binding to SERCA enzyme. The curcumin binding site is a hydrophobic pocket surrounded by M3 and M5. It was revealed that curcumin interacts with SERCA mainly through hydrophobic interactions and stacking with PHE 256. These interactions cause the distortion of the phenyl rings. Molecule is pressed against the M5 and M3 helices, thereby probably preventing the movements that are necessary for Ca<sup>2+</sup> binding. Docking runs with a conformationally flexible binding site produced no significant improvement of the results.

Table 2

## Calculated molecular descriptors for c1-c4

Compound	Molecular weight	log P	H-bond donors	H-bond acceptors
<b>c1</b>	306	4.75	–	3
<b>c2</b>	322	4.49	1	4
<b>c3</b>	322	4.49	1	4
<b>c4</b>	308	4.24	2	4
Lipinski's Rule	≤500	≤5	≤5	≤10

The loss of hydrophilic group by curcumin leads to increased binding energy. Some of curcumin derivatives showed better docking energies than curcumin indicating that they could be potent enzyme inhibitors. Curcumin derivatives satisfy Lipinski's Rule of five which testifies to their druglikeness (absorption, distribution, metabolism and excretion) and possible pharmacological activity. Therefore, the compounds **c1-c4** may be potentially effective anticancer agents.

**Computational Methodology.** The three dimensional structure of SERCA protein [PDB: 2C88] was obtained from Protein Data Bank. The structure was determined using X-ray Diffraction. Curcumin, the principal curcuminoid of the popular Indian spice turmeric is used as the ligand. Compound ID: 969516 was retrieved from NCBI

PubChem Compound database. Curcumin analogs were simulated by a variation of substituents on phenyl rings followed by optimization of the structures at the B3LYP/6-31+G\* level using Gaussian 09. The Graphical User Interface program Auto-Dock Tools 1.5.4 was used to simulate the protein and ligands. The grid boxes size was set at 90, 86 and 126 Å (x, y, and z) to include all the amino acid residues that present in rigid macromolecules, and at 18, 26 and 30 Å to include all the amino acid residues that present in binding site. The spacing between grid points was 1.0 Å. Rigid as well as flexible docking was used. The AutoDock Vina 4.0 was used to run and analyze the docking simulations. During the docking process, a maximum of 9 conformers was considered. Autodock results were analyzed to study interactions and binding energy of the docked structure. Hydrophobic and hydrogen bonds and binding distance between atoms were measured for the best conformers. The results were visualized by PyMol.

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## **МОЛЕКУЛЯРНЫЙ ДОКИНГ ПРОИЗВОДНЫХ КУРКУМИНА КАК ИНГИБИТОРОВ Са АТФАЗЫ**

Поиск эффективных ингибиторов Са АТФазы сарко/эндоплазматического ретикулума проведен методом молекулярного докинга. Установлено, что центром связывания куркумина с Са АТФазой является гидрофобный карман, окруженный спиральями М3 и М5. Комплекс куркумин-Са АТФаза образуется за счет взаимодействия лиганда с аминокислотными остатками ALA306, VAL769, LEU260, PHE834 и PHE256. Способность к ингибированию Са АТФазы была проверена для ряда производных куркумина. Показано, что потеря гидрофильных групп в молекуле куркумина приводит к дополнительному связыванию лигандов с гидрофобным карманом белка. Применение правила пяти Липинского к куркумину и его производным для оценки подобия лекарственным веществам (абсорбция, распределение, метаболизм и выделение) не показало ни одного отклонения от правила, которое определяет фармакологическую активность лекарственного вещества в теле.

*Ключевые слова:* молекулярный докинг; энергия связывания; центр связывания, ингибитор; апоптоз.

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## **МОЛЕКУЛЯРНИЙ ДОКИНГ ПОХІДНИХ КУРКУМІНА ЯК ІНГІБІТОРІВ Са АТФАЗИ**

Пошук ефективних інгібіторів Са АТФази сарко/ендоплазматичного ретикулуму проведено методом молекулярного докінгу. Встановлено, що центром зв'язування куркуміна з Са АТФазою є гідрофобна кишеня, оточена спіральями М3 і М5. Комплекс куркумін-Са АТФаза утворюється за рахунок взаємодії ліганду з амінокислотними залишками ALA306, VAL769, LEU260, PHE834 і PHE256. Здатність до інгібування Са АТФази була перевірена для ряду похідних куркуміна. Показано, що втрата гідрофільних груп у молекулі куркуміна призводить до додаткового зв'язування лігандів з гідрофобною кишенею білка. Застосування правила п'яти Липінського до куркуміна та його похідних для оцінювання подібності до лікарських речовин (абсорбція, розподіл, метаболізм і виділення) не показало жодного відхилення від правила, яке визначає фармакологічну активність лікарської речовини у тілі.

*Ключові слова:* молекулярний докинг; енергія зв'язування; центр зв'язування; інгібітор; апоптоз.

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