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RESEARCH ARTICLE

Screening and Development of Transglutaminase-2 Inhibitors and their Derivative as Anti-lung Cancer Agent by *in silico* and *in vitro* Approaches

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Abstract: ***Aim*:** This study aimed at screening and development of TG2 inhibitors as anti lung cancer agent.

***Background*:** Transglutaminase 2 (TG2) is multifunctional and ubiquitously expressed protein from the transglutaminase family. It takes part in various cellular processes and plays an important role in the pathogenesis of autoimmune, neurodegerative diseases, and also cancer.

***Objective*:** The proposed study focused on screening potent inhibitors of TG2 by *in-silico* method and synthesize their derivative as well as analyse its activity by utilizing an *in-vitro* approach.

***Materials and Methods*:** Molecular docking studies have been carried out on the different classes of TG2 inhibitors against the target protein. Nearly thirty TG2 inhibitors were selected from literature and docking was performed against transglutaminase 2. The computational ADME property screening was also carried out to check their pharmacokinetic properties. The compounds which exhibited positive ADME properties with good interaction while possessing the least binding energy were further validated for their anti-lung cancer inhibition property against A549 cell lines using cytotoxicity studies.

***Results*:** The results of the present study indicate that the docked complex formed by cystamine showed better binding affinity towards target protein, so this derivative of cystamine was formed using 2,5 dihydrobenzoic acid. Invitro results revealed that both molecules proved to be good cytotoxic agents against A549 lung cancer (875.10, 553.22 µg/ml), respectively. Further, their activity needs to be validated on TG2 expressing lung cancer.

***Conclusion*:** Cystamine and its derivative can act as a potential therapeutic target for lung cancer but its activity should be further validated on TG2 expressing lung cancer.

ARTICLE HISTORY

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1. INTRODUCTION

In recent years, lung cancer has posed to be one of the major causes of death worldwide in both men and women [1]. Depending on the tumor size and stage of lung cancer, several therapeutic regimens are available for the treatment of lung cancer. Current treatments like chemotherapy and radiation therapy are associated with several negative side effects. Despite the advancement in cancer treatment, chemoresistance is still a major obstacle, due to which most treatments have failed [2]. Multiple factors are involved in the development of chemoresistance in cancer cells, which include inactivation of chemotherapy drugs, negative regulation of tumor suppressor gene, and upregulated expression of anti-apoptotic and pro-apoptotic proteins.

Transglutaminases (TGs; EC 2.3.2.13) comprises a nine-member family of enzymes; all are involved in the catalysis of Ca^{2+}-dependent protein transamidation [3]. Among them, transglutaminase 2 (TG2) is the most extensively studied protein [4]. TG2 performs diverse physiological functions such as cross-linking activity, GTPase protein kinase and protein disulfide isomerase activities [5]. Moreover, it also exhibits post-translation modification and non-enzymatic

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scaffolding function for cell adhesion [6]. TG2 is located intracellularly, but sometimes it is also present outside the cell in the extracellular matrix. However, abnormal expression of TG2 leads to different diseases, including certain types of cancers. A study revealed that there is a positive correlation between the metastatic status in cancer and TG2 expression [7]. A high level of TG2 expression in various types of cancer cells promotes invasion and drug resistance [8]. This might be due to the induction of epithelial-mesenchymal transition (EMT). Recent studies have demonstrated an increased level of TG2 in pancreatic cancer which promotes tumor growth and drug resistance by affecting extracellular matrix and stromal fibroblasts in the tumor microenvironment [9, 10]. The role of TG2 in cancer development has been extensively studied in breast, ovarian, and pancreatic cancers [11]. This gives some shade of evidence regarding the involvement of TG2 in lung cancer progression. Hence, the present study focused on designing a potent TG2 inhibitor by using an in-silico molecular modeling approach and to validate the same for anti-lung cancer property by utilizing an in-vitro assay. The availability of data, multiple docking studies, molecular dynamics simulations and bioinformatics tools have provided ample scope in the identification of the novel inhibitors against the human TG2, which is a potential target for inhibition of lung cancer progression.

2. METHODOLOGY

In silico molecular modeling investigation was carried out on a machine with computational specifications, Sun Microsystems with Maestro 11.1, Schrodinger, New York, USA workstation.

2.1. Protein Preparation

The crystal structure of human TG2 protein (PDB ID - 4PYG) was obtained from the Brookhaven Protein Data Bank database. The protein structure was processed using Accerlys Discovery studio by removing all non-receptor atoms including water, ion and miscellaneous compounds. The refined and processed structure was saved as a ".pdb" file format and viewed in Discovery studio. The binding site for the inhibitor was searched based on a structural association of template with experimental evidence by using PDBsum supported by a literature survey [12] (Figs. **1-3**).

2.2. Ligand Preparation

The current study covered the analysis of 30 compounds from three classes of TG2 inhibitors selected from the literature, which showed good inhibitory activity (IC50 value) against TG2. The 2D structures of ligands were generated using the 2D Sketcher program available in Marvin sketch, and further, these were prepared by the Ligand Preparation module (LigPrep) [13] (Table **1**).

2.3. ADMET Studies of Compounds

ADMET study is an essential and primary step of drug screening for pharmacokinetic properties. The SWISS ADME tool was used to analyze the properties, including structural analogs; it predicts both significant physical descriptors and pharmaceutically relevant properties. It consists of principle descriptors and physicochemical properties with a detailed analysis of the logP (Octanol/Water), log S, molecular weight, *etc.* It also calculates the analogs depending upon Lipinski's rule of 5 (Lipinski 2001), which is an important parameter for rational drug design [14, 15].

2.4. Molecular Docking Studies

A molecular docking study was performed by Glide. The binding interactions were calculated using a genetic algorithm. The Glide/standard precision (SP), extra precision (X-P), induced-fit docking (IFD), and quantum-polarized ligand docking approaches were used. A grid box was generated at the centroid of binding sites and subjected to three stages of docking using GLIDE v6.7. All the ligands were docked into the binding pocket of a target using Glide/SP, and then complexes with high docking scores were forwarded to the next steps. Finally, all ligands were docked using Glide/XP. In QPLD, initially Glide/XP docking was carried out to generate ten poses per docked compound. The free energy of binding for the best ten leads, selected substrate, and existing inhibitors to their corresponding targets was calculated using Prime/MM–GBSA.

2.5. Molecular Dynamics Simulations

MD simulations have been extensively applied to investigate the conformational changes of a molecule induced by protein-ligand interactions and protein-protein interactions. The docking complex was subjected to MD simulation studies using Desmond v3.8 to check the stability. The complex generated from the top-docking pose of Glide/SP was immersed in an orthorhombic water box and SPC solvent models were used in the preparation of the system; 0.15 M NaCl salt was added to the box to neutralize the used systems. The temperatures and pressures in the short initial simulations were controlled using Berendsen thermostats and barostats, respectively. The relaxed system was simulated for a simulation time of 50 ns (nanoseconds) with a time step of 2 fs (picoseconds), NPT ensemble using a Nose'-Hoover thermostat at 300K and Martyna-Tobias-Klein barostat 1.01325 bar pressure [16].

2.6. Analytical Procedure

2.6.1. Materials and Reagents

All chemicals and reagents were of analytic grade and purchased from Sigma-Aldrich. For analytical experiments, cystamine and 2,4 dihydroxybenzoic acid (2 mM) were purchased. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and horse serum were obtained from Invitrogen; Penicillin/streptomycin (5000 U/mL of penicillin and 5000 mg/mL of streptomycin; Welgene) and L-glutamic acid have been obtained from Sigma. MTT was dissolved in phosphate-buffered saline (PBS; 1 mg/mL). Other general supplies needed for the bioassays were purchased from Fisher Scientific (Itasca, IL, USA).

2.6.2. Synthesis of Cystamine Derivative using 2,5-dihydroxybenzoic Acid

At the laboratory scale, the derivative of cystamine was synthesized using 2,5-dihydroxybenzoic acid. The reaction was catalyzed by the fungal enzyme laccase. Laccases are copper-containing phenol oxidases. They are widely distributed in plants and fungi [17]. Laccase catalyzed reactions of 2,5-dihydroxybenzoic acid complex with cystamine generally proceeded very fast.

For analytical experiments, cystamine (2 mM) and 2,5-dihydroxybenzoic acid (2 mM) were incubated with laccase (activity 0.5 U). Reaction mixtures were incubated with agitation at 200 rpm at room temperature in the dark [18] (Fig. **1**). The reaction was analyzed using a UV spectrophotometer.

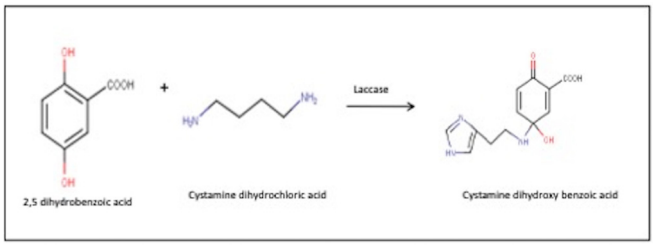

Fig. (1). Synthesis of cystamine derivative in the presence of 2,5 dihydroxybenzoic acid; the reaction has been catalyzed by laccase at room temperature.

2.7. Culture of A549 Cells

Non-small cell lung cancer cell line (A549) was procured from National Centre for Cell Sciences, Pune. The cells were maintained in DMEM supplemented with 10% Fetal Bovine Serum (FBS) and incubated at 37°C under 5% CO_2 in a humidified incubator (NBS Eppendorf, Germany). Cells were utilized for further cytotoxicity experiments during the exponential growth phase.

2.8. Cytotoxicity Assay

To evaluate the cytotoxic property of the cystamine and cystamine-dihydroxybenzoic acid complex, a cytotoxicity assay was performed. The test compounds were identified as being able to decrease the cell viability of A549 cells by decreasing the conversion of MTT to MTT formazan within the cells, which directly reflects inhibition of cell viability. For the assay, 90µL of exponentially growing A549 cells were seeded in a 96-well tissue culture plate, and the plate was incubated at 37°C and 5% CO_2 atmosphere for 24 h. To each well of the 96 well microtitre plate, 200µl of the test compounds at various concentrations (100, 200, 300, 400 and 500 µg/ml from stock) of the cystamine, dihydroxybenzoic acid, and Cystamine-dihydroxybenzoic acid complex, were added. Whereas, cisplatin was used as the standard drug. 200µl of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5mg/ml and incubated for 3 hrs at 37°C and 5% CO_2. Further, 200 µl of dimethyl sulfoxide was added to each well to solubilise the formazan crystals. The absorbance was measured at a wavelength of 570 nm and also at 630 nm. The percentage growth inhibition was calculated, after subtracting the background and the blank, and the concentration of test drug needed to inhibit cell growth by 50% (IC50) was generated from the dose-response curve for the cell line [19].

3. RESULTS

3.1. Structure of Target Protein

The crystal structure of TG2 complexed with the inhibitor has already been reported (PDB ID: 4PYG) [20]. The TG2 is composed of 687 amino acids, with a molecular weight of 77.3 kDa and a resolution of 2.80A. Each monomer has four distinct domains: the amino-terminal ß-sandwich domain consisting of residues Met-1 to Phe-139, the transamidation catalytic core domain consisting of Ala-147 to Asn-460 and two carboxy-terminal ß-barrel domains, which include Gly-472 to Tyr-583 and Ile-591 to Ala-687, respectively [21, 22].

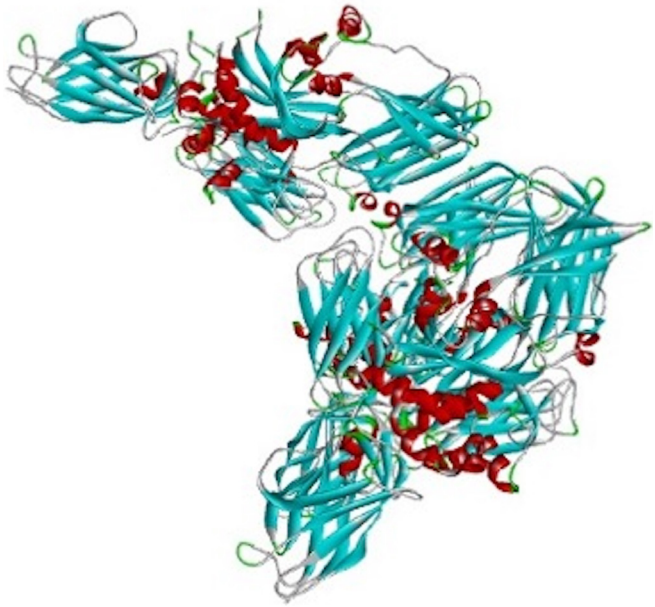

Fig. (2). Crystallographic structure of human TG2 protein (PDB ID-4PYG). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3.2. Binding Site Determination

The binding site information of the TG2 structure was predicted by performing PDBsum and literature survey [23]. The ligand plot obtained from PDBsum showed binding site region of TG2 containing 13 amino acid residues (Fig. **3**) viz., Lys 173, Phe 174, Arg 478, Arg 476, Val 479, Gly 480, Gln 481, Ser 482, Met 483, Arg 580, Leu 582, Tyr 583, Glu 585 of chain A; these residues lined the inhibitor binding site. Thus in the present investigation, residues lining this site were considered for fitting the grid box for molecular docking.

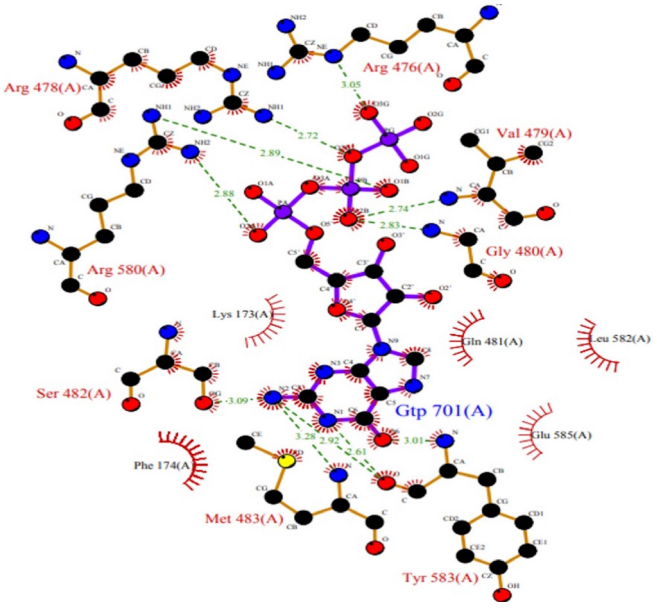

Fig. (3). Amino acid residues lining the inhibitor binding site of TG2. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3.3. Ligand Dataset

The ligand dataset is divided into three categories containing 10 drug molecules each, namely competitive amine inhibitors, reversible inhibitors and irreversible inhibitors [24, 25]. The structures of selected antiviral ligand molecules were downloaded from the PubChem database and the 2D structure was generated using Marvin sketch. The biological activities of the ligands were determined based on their pIC50 values available in the literature. The ligands along with their pIC50 values are shown in Table **1**.

3.4. ADMET Studies of *Ligand*

ADME analysis of selected ligands was predicted for physiological adsorption, distribution, metabolism and excretion properties by using SWISS ADME software. All compounds passed the Lipinski rule of five. Zero violation of the rules was shown by these molecules. Among them, four molecules showed good results as compared to others. The compounds exceeding the cut-off values tend to have solubility and permeability problems which would lead to poor oral bioavailability, hence these molecules have been eliminated from the study. The properties of selected drug molecules are depicted in Table **2**.

3.5. Computational Docking Studies and Free Energy Calculation

Molecular docking determines how closely the lowest energy pose (binding confirmation) can fit and it was predicted by the object scoring function. A 20 x 20 x 20 Å grid was generated around the centroid of binding site residues of four existing inhibitors of human TG2, mainly histamine, cysteamine, spermidine, putrescine. Further, LigPrep, QikProp, Lipinski filter, and Reactive filter, the available constraints of Maestro, were applied to determine the pharmacological properties of obtained leads. Subsequently, systematic Glide docking protocol, high throughput virtual screening (HTVS), standard precision (SP), and extra precision (XP) docking methods were implemented for further refinement of compounds. Glide scoring function (G-score) was used to select the best complex among the selected four existing inhibitors of human TG2.

The cystamine showed better binding energy with the best docking G-score, when compared to the other inhibitors. It exhibited a ΔG binding score of -46.08 kcal/mol, and an XPG score of -5.956 kcal/mol. The residues Lys176, Ile175, Phe174, Lys173, Ser171, Asn484, Met483, Ser482, Glu481, Gly480, Val479, Arg580, Asp582, Leu582, Tyr583, Leu584, Glu585 formed Van der Waals interactions with TG2 (Table **3**).

The human TG2-histamine complex showed an XPG score of -6.238 kcal/mol with a high binding affinity. The human TG2-putrescine complex showed an XPG score of -4.389kcal/mol. The amino acid residues Ser482 and Tyr583 formed two hydrogen bonds with human TG2. While in this case, the spermidine complex exhibited an XPG score of -2.906 kcal/mol. The amino acid residues Gly480 and Tyr583 formed two hydrogen bonds. Finally, comparing the XPG scores of all four known inhibitors of human TG2, cystamine was proposed in the study as the best complex human TG2 (Figs. **4** and **5**).

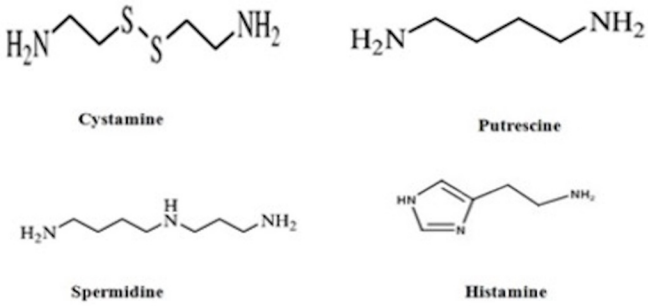

Fig. (4). 2D structures of top four compounds selected as TG2 inhibitors. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3.6. Molecular Dynamics Simulations

The binding orientations of the cystamine-TG2 docking complex were predicted through MD simulations to show a better correlation of their biologically active states among the selected existing inhibitors. The influence of the cystamine-TG2 complex docking complex on the structural and dynamical properties has been clarified by analyzing the trajectory data by using energy plot, RMSD, RMSF, protein-ligand interactions obtained from the 50ns of MD simulation runs. Cystamine-TG2 docking complex energy was found to be consistent at -178500 kcal/mol (Fig. **6**). The complexes

Table 1. The inhibitors of TG2 identified by the literature.

S. No.	Inhibitor Name	Type of TG2 Inhibitor	Molecular Weight gm/M
1.	Putrescine	Competitive amine inhibitors	88.154
2.	Monodansylcadaverine		335.466
3.	5-(biotinamido) pentylamine		328.475
4.	6-diazo-5-oxo-*norleucine*		171.156
5.	Cystamine		152.274
6.	Dermatan sulphate		475.374
7.	Spermidine		145.25
8.	Fluorescein cadaverine		719.61
9.	Histamine		111.14
10.	Spermine		202.34
11.	GMP-PCP	Reversible inhibitors	521.207
12.	LDN-27219		406.49
13.	Tyrphostin 47		220.246
14.	ZM39923		331.45
15.	ZM449829		182.222
19.	**CP4d**		-
20.	Naphthoquinone		158.156
21.	Iodoacetamide	Irreversible inhibitors	184.96
22.	3-halo-4,5-dihydroisoxazole		-
23.	Biotin		244.30
24.	4-aminopiperidine		114.14
25.	Doxorubicin		543.525
26.	ZDON		145.15
27.	KCC009		-
28.	BCNU		214.046
29.	3-bromo-4,5 dihydroisoxazole		149.975
30.	Chloroacetamide		93.51

Table 2. ADME/T properties of top four compounds.

Compound	Molecular weight (130-725)	SASA (300-1000)	Volume (500-2000)	HB-Donar (0-6)	HB Acceptor (0-20)
Histamine	111.14	693.898	576.498	4	3
Cysteamine	152.274	378.153	576.498	4	3
Spermidine	145.25	299.851	355.779	5	3.5
Putrescine	88.154	330.744	1211.119	4	2

of the cystamine-TG2 docking complex were originated in all proper and better binding poses. Average protein-ligand RMSD values of the human TG2 with respect to cystamine were observed to be below 3Å, and complexes were found to be relatively stable throughout the simulation time (Figs. **6** and **7**).

The root mean square deviation (RMSD) and root mean square fluctuation (RMSF) determine fluctuations and characterize local changes in the protein chain of each residue in human TG2. The main key observation that cystamine fell under 1 Å throughout the simulation period indicates that the ligand was very stable and showed good binding with the TG2 during the 50 ns MD run.

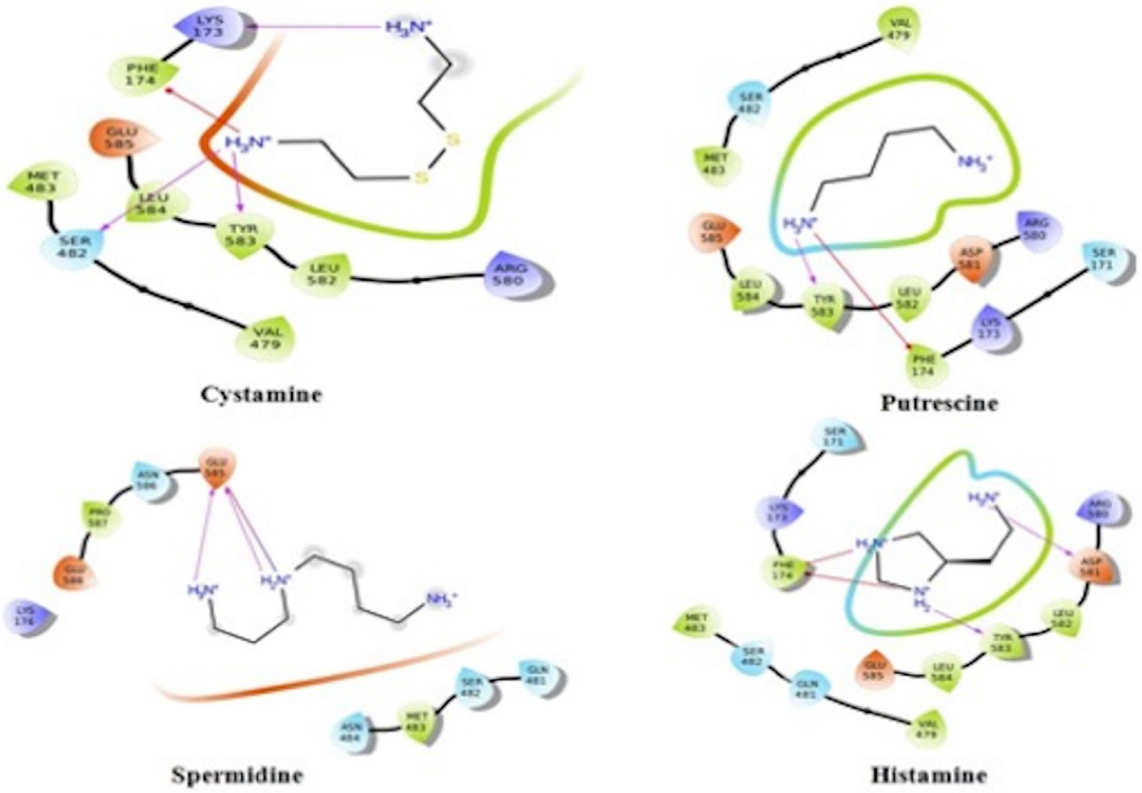

Fig. (5). Molecular interactions of the best four drug molecules with TG2 protein catalytic pocket. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 3. XPG scores of four inhibitors.

Compound	Glide Score	Glide Energy (Kcal/-mol)	Interacted Amino Acids
Cysteamine	-5.96	-46.08	Lys176, Ile175, Phe174, Lys173, Ser171, Asn484, Met483, Ser482, Glu481, Gly480, Val479, Arg580, Asp582, Leu582, Tyr583, Leu584, Glu585
Spermidine	-0.927	-23.18	Ser171, Phe174, Arg478, Val479, Gly480, Gln481, Ser482, Met483, Ser 482
Putrescine	0.898	-18.867	Ser171, Phe174, Arg478, Val479, Gly480, Gln481, Ser482, Met483, Ser 482, Asn 484, Asp581, Leu582 and Tyr583
Histamine	-1.923	4.5	Ser 482, Asn 484, Lys 590, Ile 589, Glu 599, Pro 587, Asn 586, Glu 585, Leu 584, Tyr 583, Lys 176, Phe 174, Val 676, Lys 677

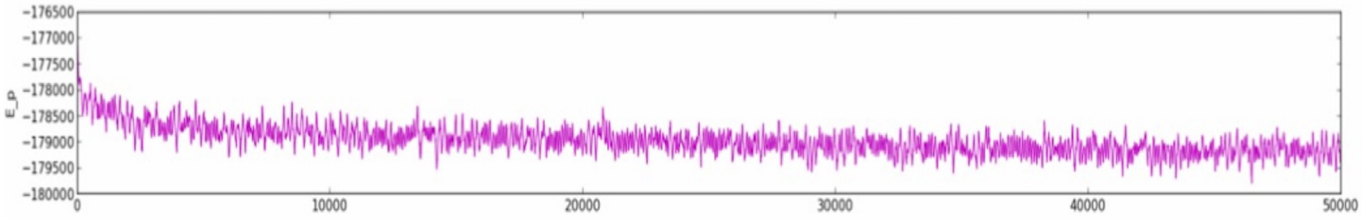

Fig. (6). Energy plot of the cystamine-TG2 docking complex. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

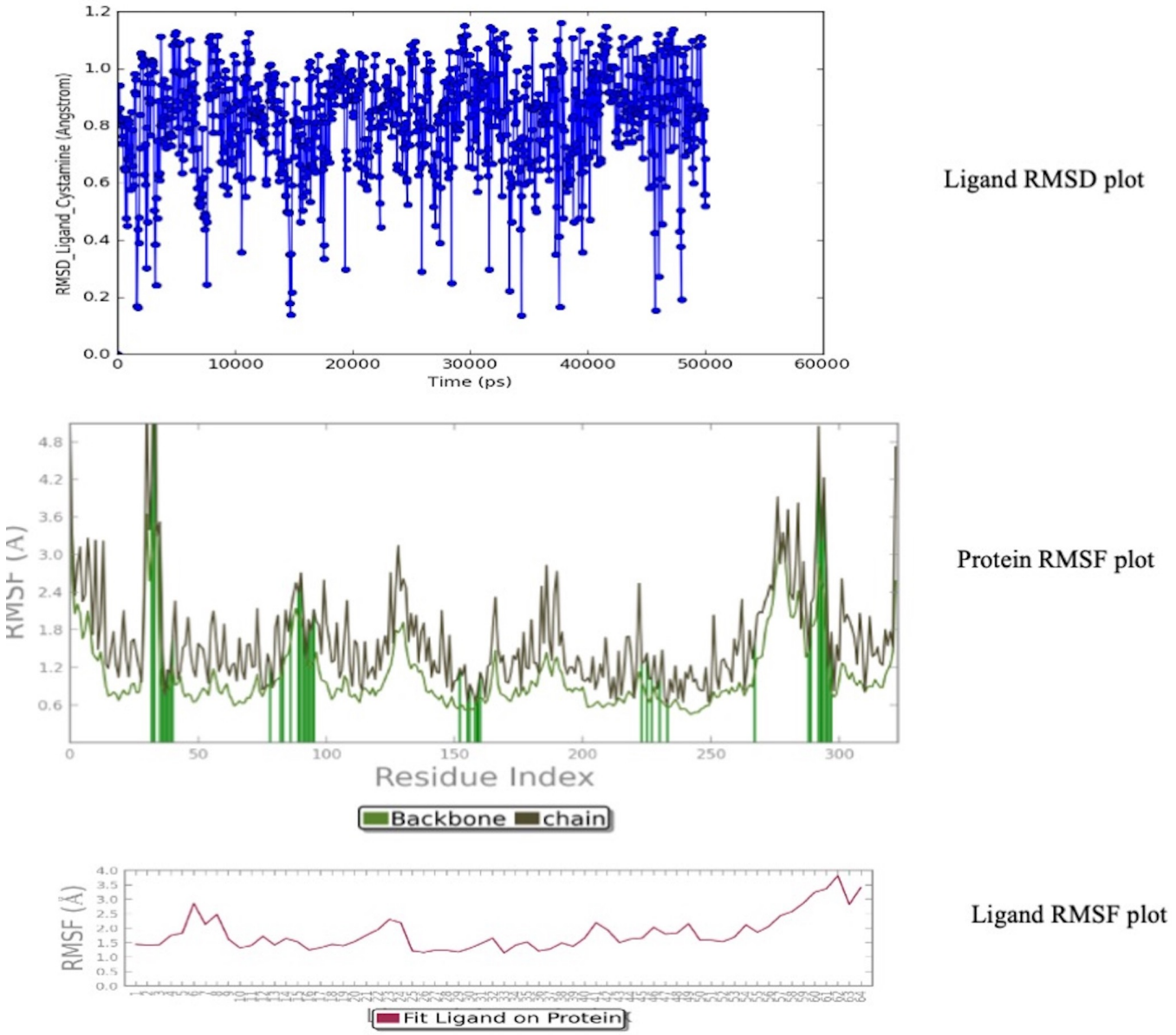

Fig. (7). RMSD of ligand and RMSF of the cysteamine-TG2 complex. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3.7. Cytotoxic Assay

For the cytotoxic assay, three groups were maintained, untreated A549 cell line as the control group, cisplatin-treated as the positive control group, and standard cystamine alone and derivative of cystamine-dihydroxybenzoic acid as the test group. Different concentrations of synthesized molecule was taken to study morphological changes as well as cell toxicity on A549 cell lines. Morphological studies revealed that the treated group and positive control group showed a significant increase in detached cells as an indication of lack of adherence due to cell death when compared to the control group in the culture medium. In cells treated with cystamine, the viability of cells was 67.91% at 100 µg/ml as well as 200µg/ml concentration, and the cell viability decreased as the concentration of cystamine increased. Its IC50 value was determined to be 875.10 µM/ml. While derivative showed the lowest cell viability at 500 µg/ml (56.20%) with an IC50 value of 553.22 µM/ml (Figs. **8** and **9**).

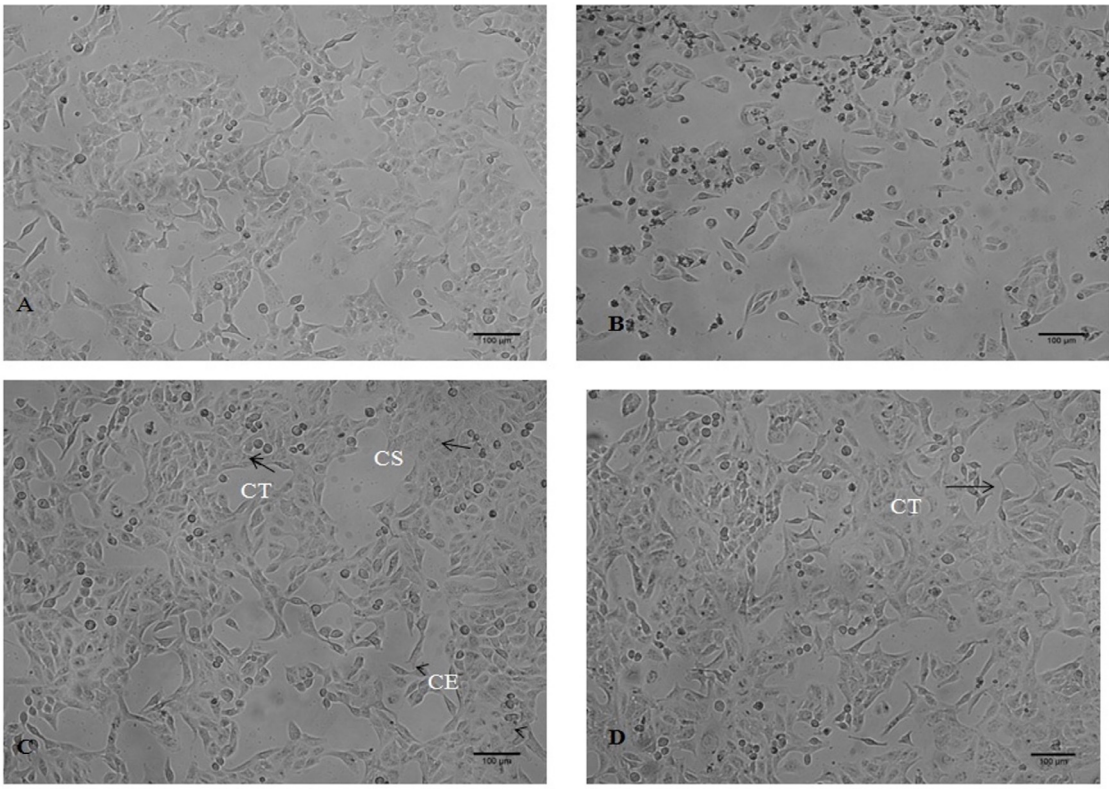

Fig. (8). Cytotoxic effect of different group drug molecules on the A549 cell line. A) Untreated (Control); B) Cisplatine C) Cystamine (100) D) Cystamine-Dihydroxybenzoic acid complex (500µg); CE: Cell expansion; CC: Chromatin Concession; CS: Cell Shrinkage; CT: Cell turgidity. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

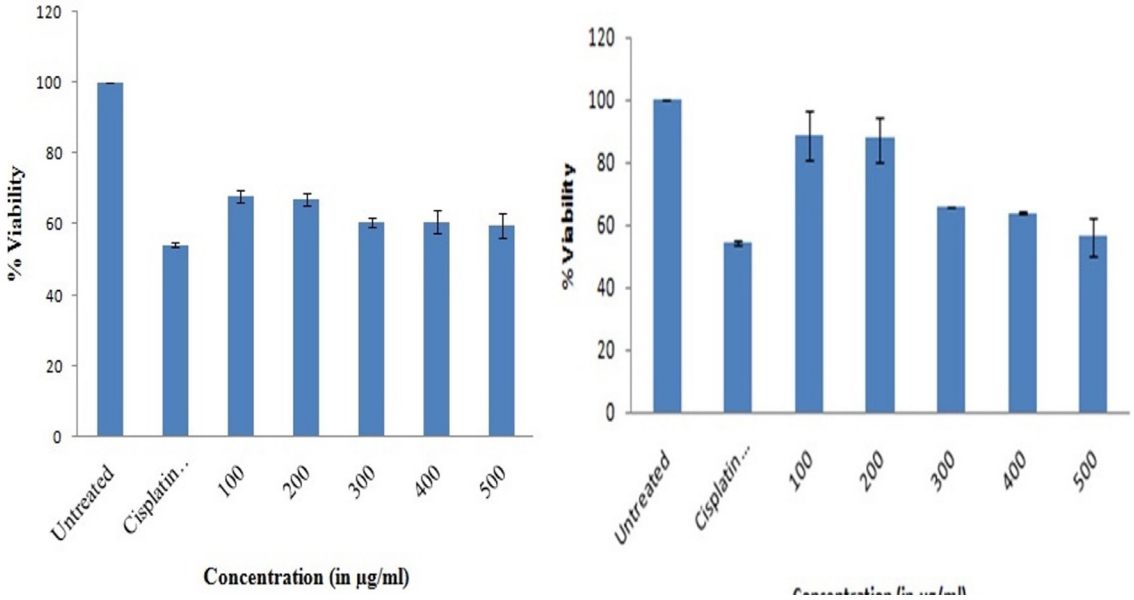

Fig. (9). Graphical representation of the cytotoxic effect of Cystamine and cystamine hydroxybenzoic acid on human A549 lung cancer cell line. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

4. DISCUSSION

The TG2 plays many biological functions and is known to be involved in the pathology of many diseases. Extensive research has been carried out to understand the role of this protein in cancer and neurodegenerative diseases. Structurally, TG2 is composed of 687 amino acid residues and four domains, an N terminal b-sandwich domain, a catalytic domain and two C-terminal b-barrel domains [26, 27]. The existing crystal structure of a protein is complexed with guanosine diphosphate (GDP) and adenosine triphosphate (ATP) [28]. TG2 is believed to play a major role in the metastasis of lung cancer. It has been reported that in proteomic analysis, the expression of TG2 was high in metastatic lung cancer cell lines [29]. As this protein is involved in lung cancer, the development of inhibitors that can reduce the expression of TG2 is necessary for controlling the progression of lung cancer. Virtual screening has become an integral part of the drug discovery process. In the present study, commercially available 30 TG2 inhibitors were selected from three categories with 10 molecules from each retrieved from the chemical database using structure-based virtual screening, and molecular docking along with *in silico* ADMET analysis. The pharmacophore features are the key elements to screen for the best potent small molecules binding to target proteins from publicly available databases. Pharmacophore-based approaches are widely used in virtual screening, de novo design and other applications such as lead optimization and multitarget drug design. ADME screening helps to screen the molecules based on their toxicity properties. The result of ADME revealed that all selected compounds obeyed the Lipinski rule of five. Molecular docking results revealed that the top four hits exactly docked into the active site of TG2. Protein-ligand interactions suggested that the functional groups (residues) mimicked the binding of hits and fit well into the active domain of the TG2 complex. Among the top four molecules, cystamine showed the best molecular interaction with ΔG binding score of -46.08, kcal/mol XPG score of -5.956 kcal/mol and interacted with maximum residues inactive sites. The molecular simulation study showed that the energy of the cysteamine-TG2 docking complex was consistent at -178500 kcal/mol at 50ns. Molecular simulation reveals the actual events happening after the initial stage of binding. Cystamine has been evaluated as the competitive amine TG2 inhibitor. It performed multiple inhibition mechanisms, inhibiting the thiol-dependent protease caspase 3 which resulted in the overexpression of glutathione inside the cells [30]. Usually, competitive amine inhibitors compete with natural amine substrate, thereby inhibiting the activity of TG2 [31]. Apart from cystamine, its reduced form has also been shown to be a competitive amine inhibitor of TG2, such as cysteamine or 2-mercaptoethylamine (MEA). The potency of these inhibitors is measured in terms of kcat/KM, where kcat is the turnover rate and KM is the Michaelis constant [32]. In the present study, the derivative of cystamine was synthesized using 2,5, dihydroxybenzoic acid in the presence of laccase enzyme, and its activity was checked through *in-silico* as well as *in-vitro* studies. 2,4-dihydroxybenzoic acid is a resorcylic acid found in certain food as well as some bacteria [33]. It possesses antioxidant, anti-radical and hydrogen peroxide scavenging activity and is involved in modification of cell signaling and apoptosis process [34]. A different form of dihydroxybenzoic acid is used for the synthesis of pharmacologically active compounds in the presence of a catalyst. In recent years, laccase is being widely used in organic synthesis because of its broad substrate specificity [35]. Laccase is a ligninolytic enzyme mostly found in wood-rotting fungi, molds, insects, and some plants and bacteria [36]. Many phenolic compounds, amino-phenols, polyamines, anilines, aromatic and alkylamines, and benzenethiols are synthesized through laccase [37]. So, in the present study, the derivative of cystamine and dihydroxybenzoic acid complex were synthesized as cystamine-dihydrobenzoic acid complex at room temperature; the reaction was catalyzed by the laccase enzyme. The activity of the newly synthesized derivative was checked through an *in-vitro* study on the human A549 lung cancer cell line. The results indicate that both cystamine as well as cystamine-dihydrobenzoic acid complex proved to be promising agents against lung cancer cell lines. The activity of these compounds should be further validated on TG2 expressing cell line *in vivo*.

CONCLUSION

Computer-aided drug screening provides sufficient scope for the identification of new drug targets against various diseases. In recent years, TG2 has been considered as a therapeutic target being involved in many types of cancers, including lung cancer. Results put forth showed better activity in terms of *in-silico* and *in-vitro* study of cystamine and cystamine-dihydrobenzoic acid complex with TG2. The development of increasingly effective and selective inhibitors of TG2 will facilitate illumination of TG2's role in a variety of human disorders, which may ultimately lead to effective clinical treatments for those disorders. With these conclusive remarks, it may be suggested that these compounds can act as a drug target against TG2 in lung cancer. However, their activity should be valiated by more *in vitro* studies on TG2 expressing cell line and *in vivo* models to declare these compounds as potent drug molecules for the management of lung cancer.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the funding of this study are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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