Turkish Computational and Theoretical Chemistry

Turkish Comp Theo Chem (TC&TC)

Volume(Issue): 8(4) – Year: 2024 – Pages: 83-92

e-ISSN: 2602-3237

https://doi.org/10.33435/tcandtc.1441494

Received: 22.02.2024 ***Accepted***: 27.05.2024 *Research Article*

Novel Tgase2 Allosteric Site Inhibitors: A Computational Study

Gökhan Erdem Şahay, Nilhan Kızılkanat, Sude Sezgin, Hüseyin Saygın Portakal[1]

Genetics and Bioengineering Department, Izmir University of Economics, Türkiye

Abstract: Tranglutaminase-2 (Tgase2) is one of the primary Transglutaminase enzyme family members having a significant role in Ca2+ -dependent and -independent post-translational modifications. It has been previously reported that Tgase2 has significant regulatory roles over metabolic functions such as signaling pathways, inflammatory response, and wound healing. In particular, many cancer types' prognosis includes over Tgase2 activity since it might induce metastasis through regulating crosslinking of extracellular matrix (ECM) proteins, and tumor proliferation via leading spheroid formation. Considering these fundamentals, discovery of novel chemical compounds to inhibit Tgase2 activity might be a strong approach in cancer treatment. Furthermore, it's known that Tgase2 activity might be inhibited through blocking its allosteric site with chemical compounds. As such, a drug library including 12,111 small compounds were virtually screened to allosteric site of Tgase2. The study has been validated by repetition the strategy with previously discovered inhibitors. Allosteric and active sites of Tgase2 have been demonstrated with protein-protein docking technique. Eventually, recently discovered ligands have been characterized according to their ADME and toxicity profiles. Results have demonstrated that Eltrombopag, Talniflumate, and Lumacaftor drugs might be repurposed in the inhibition of Tgase2 since that they exhibit high binding affinity, ADME, and toxicity properties comparing the known inhibitors.

Keywords: Tgase2, Transglutaminase, Molecular Docking, Virtual Drug Screening, Protein-Protein Docking, ADME and Toxicity

1. Introduction

Transglutaminase (Tgase) enzyme family (also known as protein glutamine g-glutamyltransferase) have significant structural functions through protein cross-linking by covalent bond formations between glutamine and lysine amino acid residues with its totally nine members [1]. Upon their catalytic activities, Tgases may act a pivotal role over tissue integrity and repair, blood clotting, and wound healing by providing a stability to protein complexes' structures, regulating the extracellular matrix (ECM) and participating to various intracellular signaling pathways [2]. Besides their structural functions, since they have prominent roles over prognosis of various autoimmune, inflammatory, and neurodegenerative diseases such as celiac disease, Huntington's and Alzheimer's diseases and various cancer types by regulating apoptosis and inflammatory responses, Tgases have taken great attention of many researches as a therapeutic approach [3].

Among the Tgase members, Transglutaminase-2 (Tgase2) is the most prominent one due to its Ca2+ -dependent and -independent post-translational modification roles. Upon Ca2+ binding, Tgase2 is exposed a conformational change to have transamidase and acyl transferase activity [4]. In similar with other members, as a GTP-binding protein, Tgase2 have many metabolic functions from regulation of signaling pathways, regulating inflammatory response to inducing wound healing and tissue repair [5]. In particular, it's revealed that TNF-α, IL-1, and IL-6 induced NF-κB signaling pathway leads to Tgase2 expression and activity affecting cancer prognosis via regulating ECM proteins' cross-linking patterns and structures.

[1] Corresponding Authors

e-mail: saygin.portakal@ieu.edu.tr

While Tgase2 initiates metastasis through this pathway, it's also proven that cancer cells might manipulate its structural functions in order to create 3D spheroid structures enabling high survival rate [6]. Furthermore, Tgase2 acts significant roles over programmed cell death processes through interacting with biomolecules having apoptotic roles. For instance, besides that interactions of Tgase2 with Cathepsin D (CatD) inhibit apoptosis and provides a convenient proliferative condition to cancer cells, it's also known that stress dependent increased intracellular Ca2+ level induces Tgase2 activity catalyzing apoptotic bodies' formation around the cells undergoing apoptosis [7]. Considering the expression profile as well as metastatic and apoptotic roles of Tgase2, its significant biomarker and therapeutic target potentials for cancer diagnosis and treatment have been reported with various studies [8]. Furthermore, apart from the active site, that allosteric site of Tgase2 might be targeted by small compounds in order to inhibit enzyme activity has been demonstrated with computational studies [9]. Consideringly, a virtual drug screening study aiming the molecular docking of small compounds belonging FDA-Approved Drugs, World-not-FDA Approved Drugs, Drugs in Clinical Trials, and Non-human Metabolites datasets of ZINC15 database to allosteric regulatory site of Tgase2. Furthermore, Tgase2-CatD complex has been created with protein-protein docking studies. Once to repeat molecular docking strategy with known Tgase2 inhibitors, considering the ADME properties, and toxicity profiles, Eltrombopag (ZINC000011679756) from FDA-Approved Drugs, Talniflumate (ZINC000001844627) from World-not-FDA Approved Drugs, and Lumacaftor (ZINC000064033452) from Drugs-in-Clinical Trials datasets have been assessed as most potent Tgase2 inhibitors among the datasets' compounds and the known inhibitors. As such, these compounds used in the treatment of various diseases might be repurposed in Tgase2 activity based diseases with inhibitory activity through recognizing allosteric site of the protein. While the findings point high therapeutic potential of the drugs over Tgase2 activity, they should be verified with further in vitro and in vivo studies.

2. Computational Method

2.1. Protein Preparation

The structure of Transglutaminase-2 (Tgase2) with GTP revealed by X-Ray Diffraction analysis was retrieved from Protein Data Bank (PDB) (PDB ID: 4PYG) with 0.240 observed R value and 0.319 R-value free. The retrieved protein was imported to UCSF Chimera Software version 1.16 and prepared with its Dock Prep module by removing heteroatoms, GTP, and water molecules, adding hydrogens partial charges as well as replacing the side chains by following Rotamer Dunbrack Library 2010 [10].

2.2. Ligand Library Preparation

Drug library consists of 12,111 chemical ligands was designed as combination of Non-human Metabolites (2,313 ligands), FDA-Approved Drugs (1,615 ligands), World-not-FDA Approved Drugs (4,288 ligands), and Drugs-in-Clinical Trials (3,897 ligands) datasets of ZINC15 database. The ligands were imported to PyRx Virtual Screening software and prepared by its energy minimization package [11].

2.3. Virtual Drug Screening and Validation

The prepared ligands were molecularly docked to GTP binding site of Tgase2 by following grid box parameters of x: 16.61, y: -4.074, and z: 2.169 coordinates with 20x20x20 dimensions by using Autodock Vina Package of PyRx Virtual Screening tool [12]. Once to complete molecular docking based virtual drug screening with the designed library, .csv files including the binding affinity, rmsd/ub, and rmsd/lb results were exported to reveal ligands with highest binding affinity and lowest rmsd values. The .pdb files of the modes of docked ligands' with highest binding affinities and 0 rmsd/ub and 0 rmsd/lb values were exported in order to analyze their interactions with the protein in BioVia Discovery Studio Visualizer software. Furthermore, molecular docking strategy was validated with re-docking of GTP and docking of ERW1041E, GK921, and Cysteamine known Tgase2 allosteric site inhibitors that had been previously developed (Pubchem IDs: 16094751, 56682080, and 6058), by following same experimental procedure.

2.4. Protein-Protein Docking with HDock

In order to analyze the expression profiles of Tgase2 with other cytoplasmic proteins, protein-protein docking study was carried out with Tgase2 and Cathepsin D (CatD) whose binding properties has been recently discovered in breast cancer prognosis. Once to crystal structure of CatD was retrieved from PDB Databank with 4OD9 PDB ID, it was prepared with protein preparation protocol. The possible interacting amino acid residues of both Tgase2 and CatD were predicted with SPPIDER II online server [13], and protein-protein docking was carried out with HDock online server [14] by using the predictions as amino acid restrictions. The intermolecular interactions of best scored protein complex were analyzed with PyMol Visualizer software.

2.5. ADME, and Toxicity Analysis

The drugs with higher binding affinity to allosteric site of Tgase2 comparing the GTP and known inhibitors were filtered according to their ADME (adsorption, distribution, metabolism, and excretion) properties considering Lipinski's rule of five which is describing ideal drugs as possessing have less than 500 kDa molecular size, less than 5 hydrogen bond donor, less than 10 hydrogen bond acceptor, and higher than 5 ClogP value as physicochemical features [15]. While the ADME properties of related ligands had been analyzed with SwissADME server [16] by using their SMILES codes as an input, the toxicity profiles were investigated with OSIRIS Property Explorer software [17].

3. Results and discussion

A virtual drug screening study has been carried out to discover novel Tgase2 allosteric site inhibitors by molecular docking of a drug library consists of totally 12,111 chemical ligands from Non-human Metabolites, FDA-Approved Drugs, World-not-FDA Approved Drugs, and Drugs-in-Clinical Trials datasets of ZINC15 database. Once to reveal binding affinities of docked ligands, best 20 ligands' ADME properties have been analyzed to filter considering Lipinski's rule of five. Furthermore, the interacting amino acid residues of Tgase2 with the related drugs have been analyzed with BioVia Discovery Studio Visualizer software. The results including the best 20 ligands including binding affinities, dataset names, interacting amino acids, and Lipinski's rule of five convenience are listed in Table 1. As such, it's revealed that while ZINC000242548690 has the highest binding affinity for Tgase2 allosteric site, it exhibits 3 violations for Lipinski's rules which is depicting the low potential of this non-human metabolite to be an ideal drug in the treatment of diseases that are encountered in human body. Considering the ADME properties, it's discovered that Eltrombopag (ZINC000011679756) from FDA-Approved Drugs, Talniflumate (ZINC000001844627) from World-not-FDA Approved Drugs, and Lumacaftor (ZINC000064033452) from Drugs-in-Clinical Trials datasets have the highest binding affinities among the ones following Lipinski's rules exactly. The chemical structures of these drugs are demonstrated in Figure 1.

Table 1. 20 best compounds from virtual drug screening study targeting Tgase2 allosteric site and their dataset, binding affinities, Lipinski's rule of five ADME properties, and the amino acid residues that are interacted with.

Ligand ZINC Code	Dataset	Binding Affinity (kcal/mol)	Lipinski's Rule of Five	Interacting Amino Acids
ZINC000242548690	*NonHuman-metabolites*	*-9.1*	*3 Violations (MW>500, NorO>10, NHorOH>5)*	*MET475, ALA474, ALA172, PHE174, MET483, VAL479, TYR583, LEU582, ARG580, LYS173, ARG418*
ZINC000012358610	*Drugs in Clinical Trials*	*-8.7*	*3 Violations (MW>500, MLOGP>4.15, NHorOH>5)*	*LYS 173, PHE 174, ARG 478, GLY 480, GLN 481, SER 482, MET 483, ARG 580, TYR 583*
ZINC000011679756	*FDA Approved Drugs*	*-8.6*	*Yes*	*LYS 425, PHE 174, MET 483, VAL 479, ARG 580, ASP 581, LYS 173*
ZINC000001844627	*World-Not-FDA*	*-8.6*	*Yes*	*SER 482, PHE 174, MET 483, LYS 173, TYR 583, LEU 582, SER 171, ASP 581, VAL 479,*

				ARG 580, ARG 478, GLY 480
ZINC000253685418	*Drugs in Clinical Trials*	*-8.5*	*2 Violations (MW>500, NorO>10)*	*ALA 172, LYS 173, PHE 174, ARG 418, LYS 425, ARG 476, VAL 479, MAT 483*
ZINC000064033452	*Drugs in Clinical Trials*	*-8.5*	*Yes*	*LYS 425, LYS 173, ASP 581, ARG 580, MET 483, VAL 479, PHE 174*
ZINC000043207566	*World-Not-FDA*	*-8.4*	*Yes*	*LYS 173, PHE 174, VAL 479, MAT 483, ARG 580, ASP 581, LEU 582*
ZINC000003915519	*Drugs in Clinical Trials*	*-8.4*	*2 Violations (MW>500, MLOGP>4.15)*	*ALA 172, LYS 173, PHE 174, ILE 175, ARG 476, ARG 478, VAL 479, SER 482, MAT 483, ARG 580, LEU 582, LEU 584*
ZINC000103591666	*Drugs in Clinical Trials*	*-8.4*	*2 Violations (MW>500, MLOGP>4.15)*	*LYS 173, PHE 174, ILE 175, VAL 479, GLY 480, GLN 481, SER 482, ARG 580, ASP 581, LEU 582,*
ZINC000137337444	*Drugs in Clinical Trials*	*-8.4*	*2 Violations (MW>500, MLOGP>4.15)*	*LYS 173, PHE 174, ILE 175, ARG 478, GLY 480, ARG 580, LEU 582, TYR 583*
ZINC000003932831	*Drugs in Clinical Trials*	*-8.4*	*2 Violations (MW>500, MLOGP>4.15)*	*LYS 173, SER 482, PHE 174, MET 483, VAL 479, LEU 582, TYR 583, ARG 580*
ZINC000043120334	*Drugs in Clinical Trials*	*-8.3*	*2 Violations (MW>500, MLOGP>4.15)*	*LYS 173, PHE 174, ARG 478 , VAL 479, ARG 580*
ZINC000043203371	*Drugs in Clinical Trials*	*-8.3*	*2 Violations (MW>500, MLOGP>4.15)*	*LYS 173, PHE 174, LEU 424, LYS 425, VAL 479, MET 483, ARG 580, TYR 583*
ZINC000068205977	*Drugs in Clinical Trials*	*-8.3*	*Yes*	*LYS 173, PHE 174, ARG 478, VAL 479, ARG 580*
ZINC000602986377	*Drugs in Clinical Trials*	*-8.3*	*2 Violations (MW>500, NorO>10)*	*LYS 173, PHE 174, ILE 175, ARG 478, VAL 479, GLY 480,ARG 580, LEU 582, TYR 583*
ZINC000003993855	*FDA Approved Drugs*	*-8.2*	*Yes*	*ARG 478, ARG 580, VAL 479, LYS 173, LEU 582, TYR 583, MET 483, PHE 174, SER 482*
ZINC000095618689	*World-Not-FDA*	*-8.2*	*1 Violation (MW>500)*	*LYS 173, PHE 174, ARG 476, ARG 478, ARG 479, SER 482, MET 483, ARG 580, LEU 582, TYR 583*
ZINC000095618690	*World-Not-FDA*	*-8.2*	*1 Violation (MW>500)*	*LYS 173, PHE 174, ARG 476, VAL 479, ARG 580, LEU 582, TYR 583*
ZINC000000577115	*World-Not-FDA*	*-8.2*	*1 Violation (MLOGP>4.15)*	*PHE 174, LEU 582, LYS 173, ASP 581, TYR 583, VAL 479, SER 171, ARG 580, MET 483, ARG 478*
ZINC000000601275	*World-Not-FDA*	*-8.2*	*Yes*	*LYS 173, SER 482, ARG 580, VAL 479, GLY 480, AG 478, MET 483, PHE 174, SER 171, TYR 583, ASP 581, LEU 582*

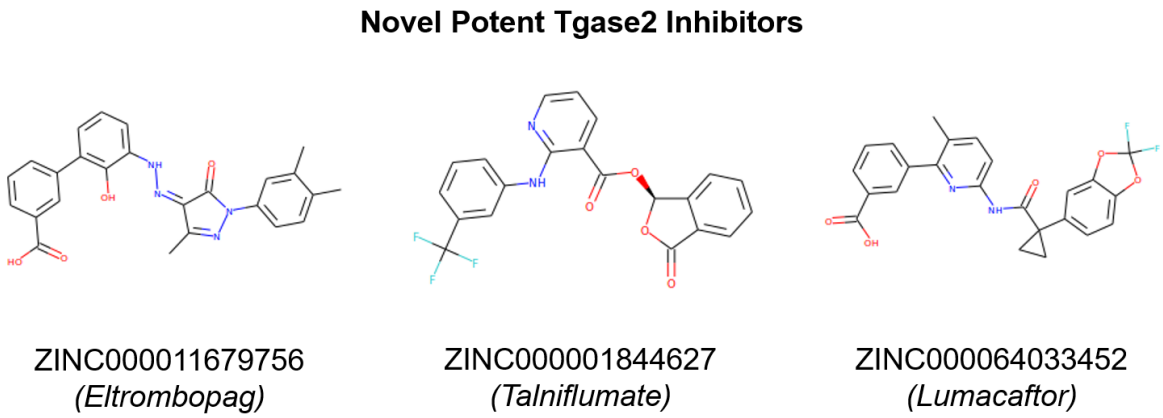

Figure 1. Chemical structures of the novel discovered compounds with highest score and following Lipinski's rule of five to inhibit Tgase2 allosteric site.

Table 2. The results of validation study including known Tgase2 inhibitors as well as GTP.

PubChem ID	Binding Affinity (kcal/mol)	Lipinski's Rule of Five	Interacting Amino Acids
16094751 (ERW1041E)	*-7.0*	*Yes*	*LYS 173, PHE 174, ARG 478, VAL 479, GLY 480, SER 482, ARG 580*
135398633 (GTP)	*-6.6*	*3 Violations (MW>500, NorO>10, NHorOH>5)*	*LYS 173, PHE 174, ARG 476, ARG 478, VAL 479, GLY 480, SER 482, MET 483, ARG 580, LEU 582, TYR 583*
56682080 (GK921)	*-6.3*	*Yes*	*PHE 174, ARG 478, VAL 479, GLN 481, SER 482, MET 483*
6058 (Cysteamine)	*-2.5*	*Yes*	*MET 483, ARG 580, TYR 583*

While there are only few chemical ligands that might recognize allosteric site of Tgase2, it's known that GTP is primary chemical compound to activate Tgase2 through binding its allosteric site [18]. As such, the molecular docking experimental procedure of the study has been validated by repetition the strategy with GTP and the ligands that have been recently produced. The data including the results of the validation studies are listed in Table 2. The findings have demonstrated that recently designed chemical ligands are following the Lipinski's rule, yet their binding affinities are lower than the novel possible Tgase2 inhibitors. Furthermore, it's revealed that while GTP might interact with Tgase2's PHE 174, ARG 476, ARG 478, VAL 479, GLY 480, SER 482, MET 483, ARG 580, and TYR 583 via conventional hydrogen bond, carbon hydrogen bond, salt bridge, attractive charge, unfavorable donor-donor, pi-sigma, pi-pi stacked, and pi-alkyl interactions in retrieved crystal structure, it might interact with LYS173, PHE 174, ARG 476, ARG 478, VAL 479, GLY 480, SER 482, MET 483, ARG 580, LEU 582, and TYR 583 residues via conventional hydrogen bond, carbon hydrogen bond, unfavorable positive-positive, unfavorable donor-donor, pi-pi stacked, and pi-alkyl interactions in re-docked form. The similarities in the interactions of Tgase2 with the re-docked GTP and the one in crystal structure point the sufficiency of developed molecular docking method. In addition, while re-docked GTP has exhibited only -6.6 kcal/mol binding affinity for allosteric site of Tgase2, the higher affinities of discovered results indicate the drugs' possible efficiencies to Tgase2 inhibition in human body for targeting the same region comparing with GTP.

Among the screened ligands Eltrombopag (ZINC000011679756) has exhibited highest binding affinity with totally convenience to Lipinski's rule of five. While Eltrombopag has been approved by FDA in 2014 in the treatment of aplastic anemia, today it's declared as standard for the treatment of aplastic anemia by National Institute of Health (NIH) [19,20]. The binding affinity of the drug has been recorded as -8.6 kcal/mol for allosteric site of Tgase2, and it's

revealed that it may interact with LYS 173, PHE 174, LYS 425, VAL 479, MET 483, ARG 580, and ASP 581 amino acid residues of Tgase2 via conventional hydrogen bond, pi-carbon, pi-pi stacked, and pi-alkyl interactions. These findings indicate the repurposing potential of this thrombopoietin receptor agonist in the inhibition of Tgase2 [21]. Furthermore, Talniflumate (ZINC000001844627) from World-not-FDA Approved Drugs dataset has the second highest binding affinity amongst the drugs with no violation. Currently, this anti-inflammatory molecule is used in asthma, chronic pulmonary disease (COPD), and cystic fibrosis treatments as mucin regulator [22].

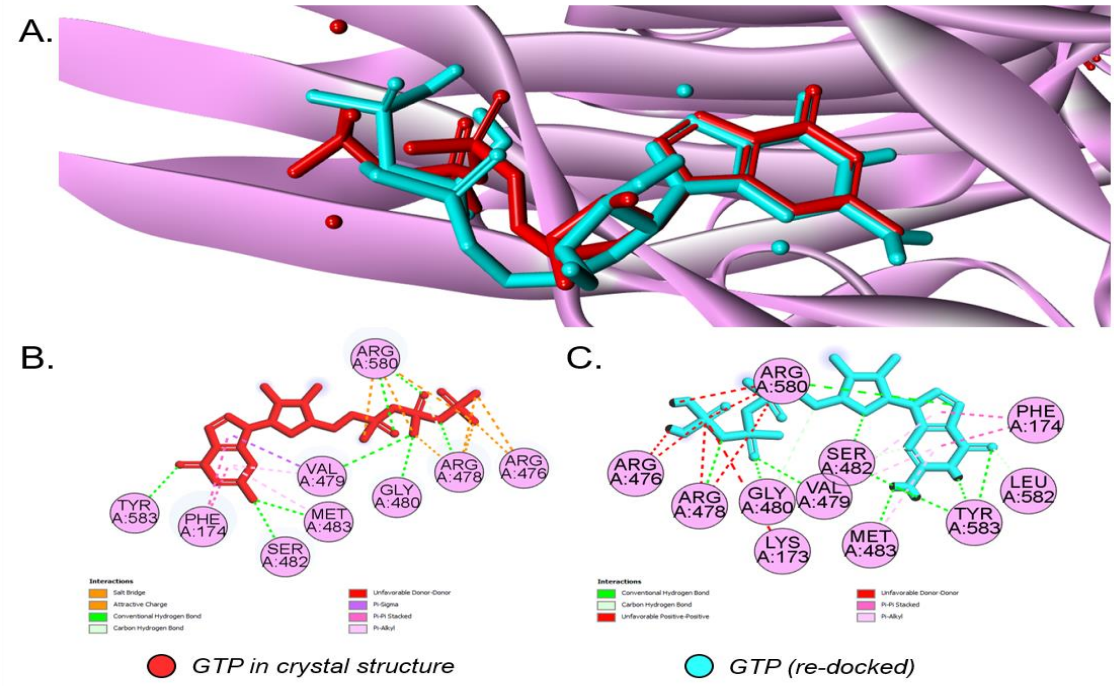

Figure 2. 2D and 3D demonstration of the interactions of GTP with Tgase2 in both; A) crystal structure, and B) re-docking form.

While the first clinical study of Talniflumate had been completed in 2001, it was approved by Argentina in the treatment of cystic fibrosis [23]. The virtual drug screening results have put forward that Talniflumate has -8.6 kcal/mol binding affinity to allosteric site of Tgase2. In addition, computationally, it's interacting with SER 171, LYS 173, PHE 174, ARG 478, VAL 479, GLY 480, SER 482, MET 483, ARG 580, ASP 581, LEU 582, and TYR 583 amino acids of Tgase2 via conventional hydrogen bond, carbon hydrogen bond, halogen (Fluorine), pi-pi stacked, alkyl, and pi-alkyl interactions. Additionally, Lumacaftor (ZINC000064033452) drug which is in Phase II Clinical trials for cystic fibrosis patients with F508del mutation [24] has exhibited third highest binding affinity to allosteric regulatory site of Tgase2 without any Lipinski's rules' violation. While Lumacaftor has -8.5 kcal/mol binding affinity to target region of Tgase2, it's revealed that it might interact with LYS 173, PHE 174, LYS 425, VAL 479, MET 483, ARG 580, and ASP 581 amino acid residues via conventional hydrogen bond, carbon hydrogen bond, unfavorable acceptor-acceptor, pi-carbon, pi-pi stacked, and pi-alkyl interactions. Besides that the findings are demonstrating the repurposing potentials of aforementioned drugs for Tgase2 inhibition, the ADME properties are listed in Table 3 and 3D as well as 2D illustrations of their computational interactions with Tgase2 are demonstrated in Figure 3. Furthermore, while interacting amino acids of Tgase2 exhibit similarities for all docked chemical compounds, the interactions of Tgase2 with Cat D protein have been analyzed via protein-protein docking study carried out in HDock online server. It's known that Tgase2-Cat D interactions promote Cat D depletion causing reduced level of apoptosis

in cancer prognosis (7), to target allosteric site of Tgase2 inhibits its activity to block cancer prognosis via enhancing the apoptosis. As such, totally 20 protein complexes have been created with HDock study, and best one of them with -130.14 docking score, 0.4020 confidence score, and 65.42 Ligand RMSD (Å) have been analyzed in PyMol software. Accordingly both allosteric site and active site of Tgase2 have been demonstrated with modelling of Tgase2-CatD protein complex (Figure 4).

Once to reveal possible repurposable drugs from designed library, ADME and toxicity properties of best three drugs and the ligands that had been previously discovered were investigated with SwissADME online server and OSIRIS Property Explorer tool. The data including ADME and toxicity profiles of the compounds are demonstrated in Table 3. Considering the results, it has been shown that all compounds share similar solubility and druglikeness properties. As a poorly soluble ligand Eltrombopag has quite lipopihilicity, high gastrointestinal tract (GI) absorption, no blood brain barrier (BBB) permanency, and has no CYP isoform inhibitory activity except CYP2C9 with possible tumorigenicity and reproductive effects. Talniflumate has the lowest molar refractivity and highest lipopihilicity among all compounds and it has high GI absorption, no BBB permanency, no CYP2D6 and CYP3A4 inhibitory activitiy without any mutagenicity, tumorigenicity, and irritant effects. Lumacaftor has high GI absorption, no BBB permanency, and no toxicity with possible CYP isoform inhibitory activities. Nonetheless, previously discovered inhibitors has lowest lipophilicities and exhibit inefficient ADME properties such as ERW1041E has CYP2C19, CYP2C9, CYP3A4 inhibitory activities with possible mutagenicity, and GK921 has BBB permanency, inhibitory activities for all CYP isoforms and moderate reproductive effect. These results indicate that novel discovered ligands potent to be used in Tgase2 inhibition since that they have higher binding affinities, and more sufficient ADME and toxicity profiles.

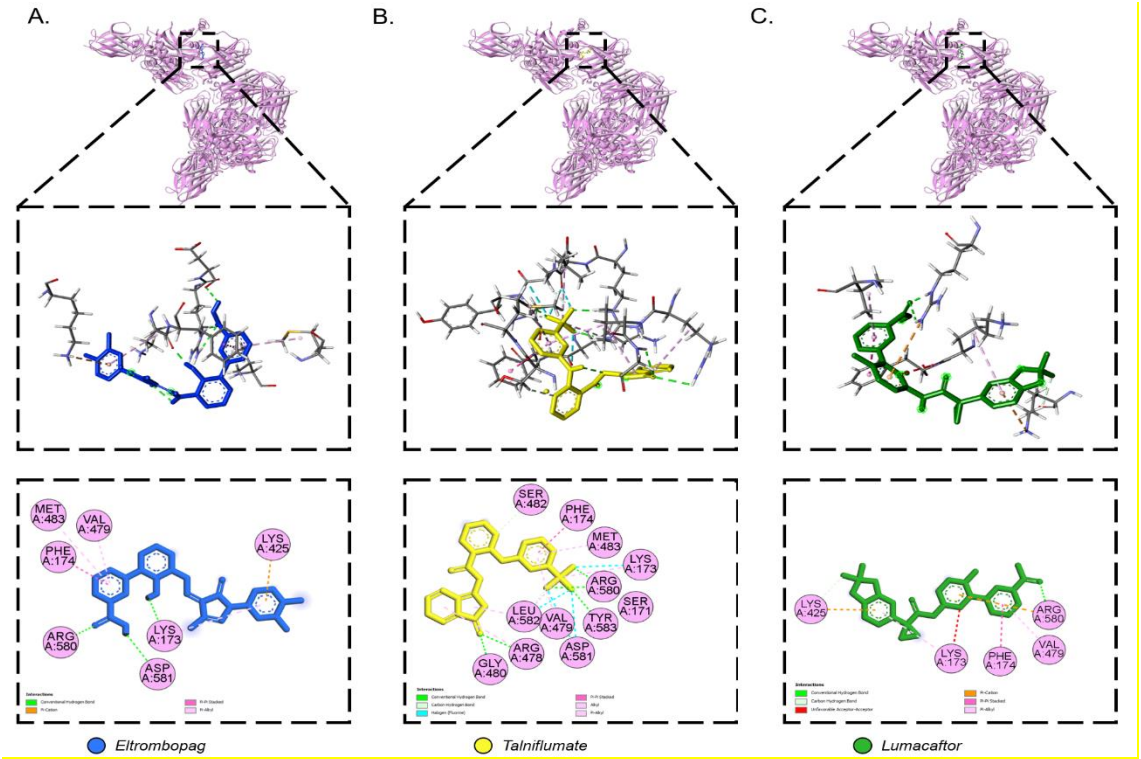

Figure 3. 2D and 3D demonstration of the interactions of recently discovered compounds with Tgase2; A) Eltrombopag, B) Talniflumate, and C) Lumacaftor.

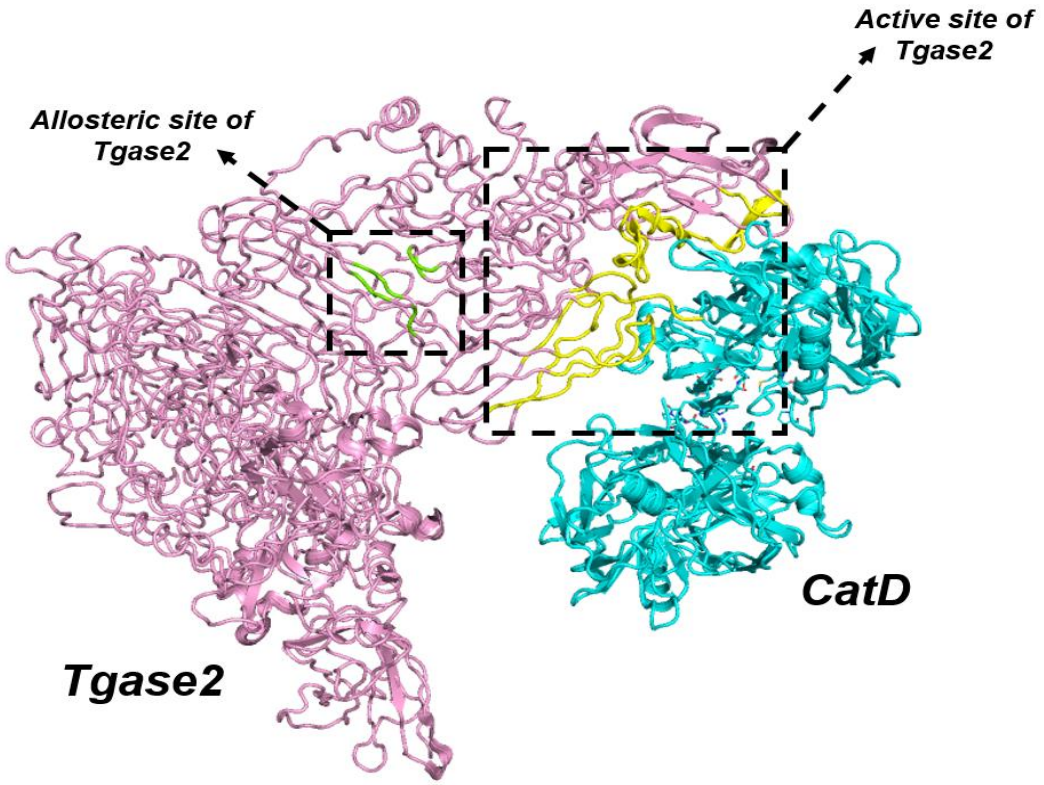

Figure 4. Tgase2-CatD protein complex and functional regions of Tgase2; Green: Allosteric site, Yellow: Active site.

Table 3. ADME and toxicity properties of the discovered compounds as well as two best scored known inhibitor.

ADME and Toxicity Properties						
Properties		**Selected Compounds**			**Inhibitor**	
	Ligand Name	Eltrombopag (ZINC000011679756)	Talniflumate (ZINC000001844627)	Lumacaftor (ZINC000064033452)	ERW1041E	GK921
Physico-chemical properties	**Formula**	C25H22N4O4	C21H13F3N2O4	C24H18F2N2O5	C20H21BrN4O4	C21H20N4O
	Molecular Weight (g/mol)	442.47	414.33	452.41	461.31	344.41
	Molar Refractivity	135.16	98.91	113.98	118.00	104.98
	TPSA (topological polar surface area)	114.59	77.52	97.75	93.12	51.14
Lipophilicity	**Log $P_{o/w}$ (iLOGP)**	2.97	2.82	3.08	3.25	3.79
	Log $P_{o/w}$ (XLOGP3)	4.77	5.27	4.44	2.52	3.36
	Log $P_{o/w}$ (WLOGP)	3.61	5.70	5.33	2.04	2.60
	Log $P_{o/w}$ (MLOGP)	2.97	3.77	2.69	1.35	2.48
	Log $P_{o/w}$ (SILICOS-IT)	4.36	3.98	4.78	2.82	3.88
	Consensus Log *P*o/w	3.74	4.31	4.06	2.39	3.22
Solubility	**Log *S* (SILICOS-IT)**	-7.45	-7.54	-7.81	-5.50	-6.51
	SILICOS-IT Solubility (mg/ml)	1.58e-05	1.18e-05	6.97e-06	1.45e-03	1.05e-04
	SILICOS-IT Solubility (mol/l)	3.56e08	2.85e-08	1.54e-08	3.13e-06	3.05e-07
	Solubility Class	Poorly soluble	Poorly soluble	Poorly soluble	Moderately soluble	Poorly soluble

Druglikeness	**Druglikeness**	-3.38	-8.76	-4.12	-3.24	-4.62
	Drug-score	0.07	0.16	0.17	0.28	0.32
Pharmacokinetics	**GI absorption**	High	High	High	High	High
	BBB permeant	No	No	No	No	Yes
	P-gp substrate	No	No	Yes	Yes	No
	CYP1A2 inhibitor	No	Yes	Yes	No	Yes
	CYP2C19 inhibitor	No	Yes	Yes	Yes	Yes
	CYP2C9 inhibitor	Yes	Yes	Yes	Yes	Yes
	CYP2D6 inhibitor	No	No	Yes	No	Yes
	CYP3A4 inhibitor	No	No	Yes	Yes	Yes
Toxicity	**Mutagenicity**	No	No	No	Yes	No
	Tumorigenicity	Yes	No	No	No	No
	Irritant Effects	No	No	No	No	No
	Reproductive Effects	Yes	Yes	No	No	Yes

4. Conclusions

Tgase2 is the most prominent transglutaminase enzyme family member since it has Ca2+-dependent and -independent post-translational modification roles regulated by conformational change causing transamidase and acyl transferase activity. Furthermore, Tgase2 have many metabolic functions such as regulation of signalling pathways and inflammatory response. In addition, many distinct cancer types' prognosis depends on Tgase2 activation leading the metastasis via regulation of ECM proteins' crosslinking and inhibiting apoptotic pathways via recognition of various apoptotic enzymes such as CatD. These knowledge make Tgase2 a potent biomarker and drug target in cancer diagnosis and treatment. Furthermore, that the inhibiting the allosteric site of Tgase2 with small compounds may inhibit its activity has been demonstrated with in silico studies. As such, a virtual drug screening study aiming to screen FDA-Approved Drugs, World-not-FDA Approved Drugs, Drugs in Clinical Trials, and Non-human Metabolites datasets of ZINC15 database to allosteric regulatory site of Tgase2 has been carried out with this publication. Furthermore, Tgase2-CatD complex has been created with protein-protein docking studies. Virtual drug screening has been validated with repetition the study with previously discovered compounds. Virtual drug screening, and characterization studies including ADME and toxicity analysis have demonstrated that that Eltrombopag (ZINC000011679756) from FDA-Approved Drugs, Talniflumate (ZINC000001844627) from World-not-FDA Approved Drugs, and Lumacaftor (ZINC000064033452) from Drugs-in-Clinical Trials datasets have the highest potential to be repurposed since that they have highest binding affinities with exactly obeying to Lipinski's rule of five and few toxicities comparing the known inhibitors. Considering the findings the discovered drugs' efficiencies in the inhibiting of Tgase2 activity should be analyzed with in vitro and in vivo studies to open novel gates in the Tgase2 based diseases' treatment.

References

[1] S. Beninati, M. Piacentini, The transglutaminase family: An overview, Amino Acids 26 (2004) 367–372.

[2] B. O. Odii, P. Coussons, Biological functionalities of transglutaminase 2 and the possibility of its compensation by other members of the transglutaminase family, Sci. World J. 2014 (2014) 7–9.

[3] L. Lorand, S. E. Iismaa, Transglutaminase diseases: From biochemistry to the bedside, FASEB J. 33 (2019) 3–12.

[4] G.E. Kim, H. H. Park, Structures of human transglutaminase 2: Finding clues for interference in cross-linking mediated activity, Int. J. Mol. Sci. 21 (2020) 35–38.

[5] D. Park, S. S. Choi, and K. S. Ha, Transglutaminase 2: A multi-functional protein in multiple subcellular compartments, Amino Acids 39 (2010) 619–631.

[6] L. Huang, A. M. Xu, and W. Liu, Transglutaminase 2 in cancer, Am. J. Cancer Res. 5 (2015) 2756–2776.

[7] E. Kırmızıay, R. Demir, C. Öğütçü, and H. S. Portakal. Discovery of Repurposable Drugs in the Combination Therapy of Breast Cancer : A Virtual Drug Screening Study, Turkish Comp. Theo. Chem. 8 (2024) 40–53.

[8] S. Kim, New Insights into Development of Transglutaminase 2 Inhibitors as Pharmaceutical Lead Compounds, Med. Sci. 1 (2018) 1–11.

[9] N. Kim et al., Allosteric inhibition site of transglutaminase 2 is unveiled in the N terminus, Amino Acids 50 (2018) 1583–1594.

[10] E. F. Pettersen et al., UCSF Chimera — A Visualization System for Exploratory Research and Analysis, J. Comput. Chem. 25 (2004) 1605-1612.

[11] S. Dallakyan, A. Olson, Small-Molecule Library Screening by Docking with PyRx, NY: Springer New York, U.S.A. 2015, 243-250.

[12] O. Trott, A. J. Olson, AutoDock Vina : Improving the Speed and Accuracy of Docking with a New Scoring Function , Efficient Optimization , and Multithreading, J. Comput. Chem. 17 (2011) 295-304.

[13] A. Porollo, J. Meller, Prediction-Based Fingerprints of Protein – Protein Interactions, Proteins 66 (2007) 630-645.

[14] Y. Yan et al., HDOCK : a web server for protein – protein and protein – DNA / RNA docking based on a hybrid strategy, Nucleic Acids Res. 45 (2017) 365–373.

[15] T. K. Karami et al., Eyes on Lipinski ' s Rule of Five : A New '" Rule of Thumb "' for Physicochemical Design Space of Ophthalmic Drugs, J. Ocul. Pharmacol. Ther. 38 (2022) 43–55.

[16] A. Daina, O. Michielin, and V. Zoete, SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7 (2017) 1–13.

[17] https://www.organic-chemistry.org/prog/peo/, January 2017, Accessed: 22.02.2024.

[18] J. Jeon et al., GTP is required to stabilize and display transamidation activity of transglutaminase 2, Biochem. Biophys. Res. Commun. 294 (2002) 818–822.

[19] B. Drexler, J. Passweg, Current evidence and the emerging role of eltrombopag in severe aplastic anemia, Ther. Adv. Hematol. 12 (2021) 1–10.

[20] R. Desmond, D. M. Townsley, C. Dunbar, N. S. Young, Eltrombopag in aplastic anaemia, Semin. Hematol. 52 (2016) 31–37.

[21] S. L. Corman, R. A. Mohammad, Eltrombopag: A Novel Oral Thrombopoietin Receptor Agonist, Ann. Pharmacother. 44 (2010) 1072–1079.

[22] A. Agostini et al., Talniflumate abrogates mucin immune suppressive barrier improving efficacy of gemcitabine and nab - paclitaxel treatment in pancreatic cancer, J. Transl. Med. 21 (2023) 1–15.

[23] N. M. Walker et al., Talniflumate Increases Survival in a Cystic Fibrosis Mouse Model of Distal Intestinal Obstructive Syndrome, J. Pharmacol. Exp. Ther. 317 (2006) 275–283.

[24] D. M. Cholon et al., Efficacy of lumacaftor-ivacaftor for the treatment of cystic fibrosis patients homozygous for the F508del-CFTR mutation, Expert Rev. Precis. Med. Drug Dev. 1 (2016) 235-243.