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AN IN-DEPTH COMPUTATIONAL STUDY FOR EXPLORING STRUCTURAL INSIGHTS INTO THE MOLECULAR INTERACTION MECHANISM OF SPHK1 INHIBITORS

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ABSTRACT : Sphingosine kinase 1 (sphk1) and lipid mediator sphingosine -1 phosphate (S1P) are key players in the survival and proliferation of various types of cancer cells. So, inhibition of sphk1 might be proved a promising strategy to curb and prevent carcinogenesis. In present study, we aimed to examine and compare the therapeutic potential of 08 known inhibitors of sphk1. 2-D structures of all ligands namely-Genzyme 51, Amgen 82, RB005, SKI-178, MPA08, Balanocarpol, SKI-5C, and SK1-I was retrieved from PubChem Compound database and their subsequent conversion into 3-D structures was performed using online software system CORINA. The X-ray crystallographic structure of sphk1 was extracted from RCSB Protein Data Bank. Molecular docking simulation study was carried out by using AutoDock Tools..The docking results revealed that Genzyme 51 showing higher binding energy (-12.5 kcal/mol)and thus, exhibited better binding interaction to sphk1 as compared to others. Moreover, drug-likeness, ADMET properties, and pharmacokinetic features of Genzyme 51 werealso found at par. Our *in silico* findings have explored and compared the therapeutic potential of stated inhibitors and thus, it might be used as an initial lead molecule to improve the efficacy and inhibition potential of new ligand molecules to prevent carcinogenesis.

Key words : Sphk1, Lipinski RO5, ADMET, molecular docking.

INTRODUCTION

Sphingosine kinase-1 (sphk1) has been looked as a crucial target against various types of cancers (Geffken *et al*, 2017). Sphk1 (E.C 2.7.1.91), a diacylglycerol kinase enzyme that is encoded by SPHK1 gene in humans. Originally sphk1, a 49 KDa protein was purified from rat kidney. Sphk1a (MW:42.3 KDa) and sphk1b (MW: 43.2 KDa) are two isoform derived by alternative splicing that differs only by few amino acid residues at their N terminus (Wang *et al*, 2013). It is highly distributed in brain, heart, lung, and spleen. Two variants of sphk have been discovered (Takabe *et al*, 2010) in humans. Sphk1 (MW: 42kDa) and Sphk2 (MW: 63kDa) transcribed from genes located on chromosome 17 and 19 respectively. In contrast to BH3-only protein sphk2, sphk1 promotes cell growth and inhibits apoptosis (Takabe *et al*, 2008). Activated sphk1 by several external stimuli catalyzes an ATP-dependent phosphorylation of sphingosine on its primary hydroxyl group and generates a sphingolipid metabolite S1P, which is a ligand for a family of five specific G-protein coupled receptors (GPCRs) that controls many fundamental processes of cells including survival, growth, differentiation, cytoskeleton rearrangements, motility, angiogenesis, lymphocyte trafficking, and immunity (Maceyka *et al*, 2008). In humans, high expression levels of sphk1 have been reported in many types of tumors and cancer cells such as lung, ovary, colon, breast, uterus, kidney, stomach, and rectum (Hatoum *et al*, 2017) and its elevated expression is associated with drug resistance and is the indication of poor prognosis and reduced survival of patients in cancers (Shimizu *et al*, 2018). Various inhibitors of Sphk1such a SK1-I, SKI-178, SK1-5c, MPA08, Belanocarpol, RB-005, Genzyme 51 and Amgen 82 (Lim *et al*, 2012; Santos *et al*, 2017) have been discovered so far, but to find the best one having plausible bioavailability, pharmacokinetic (PK), and pharmacodynamic (PD) properties is yet to be designed and developed.

SK1-I is a sphk1 selective competitive inhibitor (10ìM) and sphingosine analogue discovered in 2008. According to previous studies, it is efficacious in AML (Acute Myeloid Leukaemia) and glioblastoma xenografts

(Gao *et al*, 2015). Previous studies indicated that treatment with SK1-I inhibited U937 human leukaemia cells by induction of cell death followed by reducing ERK1/2 and AKT signaling (Pitman *et al*, 2016) and also decline S1P levels in U937 human monocytes (Paugh *et al*, 2008) accompanied by concomitant increase in ceramide levels. Moreover, it also reduces S1P levels and metastasis of breast cancer to lungs and lymph nodes (Nagahashi *et al*, 2012).

SKI-5C is also a sphk1 selective inhibitor having IC50 3.3ìM. It reduced colon cancer cells viability by reduction in AKT signaling (Wong *et al*, 2009). Studies have revealed that SKI-5C have modest inhibitory effect on proliferation of U937 cells. It reduced proliferation *in vitro* and induced cell death in MDA-MB-231 and MCF-7 mammary cancer cell line and attenuated growth of tumor in MDA-MB-231 mouse xenograft model (Wong *et al*, 2009).

SK1-178 is a sphk1 selective analogue of SKI-I of Ki 1.3 ìM. In HL-60 cells, it reduced the levels of S1P and increases the formation of ceramide (Dick *et al*, 2015). It induced apoptosis in various cancer cell lines (IC-50 0.1-1.8 ìM). Treated human AML cell line by this inhibitor has shown efficacy by inducing apoptosis via activation of CDK1. It also seems to be effective in mouse models of AML (Dick *et al*, 2015).

Genzyme 51 is one of the best inhibitor of sphk1, discovered at Genzyme with 58nM IC50, but has shown negative response when examined with sphk2 at 10ìM concentration (Santos *et al*, 2015). Pharmacokinetics study in rat after oral administration of this drug revealed 18% bioavailability with half life 7.6h (Santos *et al*, 2015).

RB-005 acts as curative agent for hypertension and other proliferative diseases (Pitman *et al*, 2016). Studies have suggested that RB-005 also induced proteasomal degradation of sphk1 in (PASMC) human pulmonary arterial smooth muscle cells (Back *et al*, 2013).

Amgen 82 is an inhibitor of sphk that blocks the action of human sphk1 and sphk2 with IC50 values of 20 and 114nM respectively. Amgen 82 binds in the hydrophobic region of sphk1 suggested by X-ray crystal structure studies (Santos *et al*, 2015).

In vitro studies of MP-A08 revealed that it blocks sphk1 and sphk2 at 27 and 7ìM Ki respectively and also effective *in vivo*. The treatment of cells with MP-A08 leads to reduction in S1P levels and increment in sph and cer levels via mitochondrial mediated apoptosis in cancer cells (Pitman *et al*, 2016). Moreover, Balanocarpol stimulates PARP cleavage in MCF-7 breast cancer cells (Pitman *et al*, 2016).

To date, *in vitro* studies were conducted on these existing inhibitors but for the first time we have conducted *in silico* studies on these stated inhibitors. Herein, all aforesaid inhibitors were compared and examined on various physicochemical properties like drug-likeness, PK, PD and molecular docking using bioinformatics and computational tools.

MATERIALS AND METHODS

All known compounds along with their target were collected from available literatures. The 3D structure of all stated ligand molecules and target protein sphk1were retrieved from Pubchem database and RCSB PDB respectively. Conversion of 2D structures of ligands into 3D was carried out through Discovery studio visualizer 2.5.

Drug-likeness study

Using Lipinski RO5 screening facility accessible online, the drug-likeness of all known inhibitors of sphk1 was checked (Sharma *et al*, 2018; Sharma *et al*, 2019). This rule estimates the pharmacological and biological activity of the compound thus would make it likely as an orally active drug in humans. Lipinski RO5 states about MW (≤ 500 KDa), HBD (≤ 5), HBA (≤ 10) and lipophilicity (Log P ≤ 5).

ADMET study

It has been reported previously that a number of candidate drugs that are not successful during clinical trials due to the poor ADMET properties. Now days, ADMET properties are considered as one of the most important parameters to select compounds as candidate drugs. PreADMET (http://preadmet.bmarc.org/) a web based tool (Tetko *et al*, 2006) was used for sorting out the compounds based on HIA (Human Intestinal Absorption), BBB (Blood Brain Barrier), PPB (Plasma Protein Binding), CYP2D6, Water solubility, and Toxicity descriptors of ADMET (Table 1). ADMET rule states that compounds that showing HIA levels more than 90% reflect excellent absorption respectively. BBB level reflect penetration level to be very large (0), large (1), normal (2), poor (3) and limitless (4). Compounds that showing PPB levels less than 90% recommends that they are not prone to be exceedingly linked to blood proteins. Compounds should be non inhibitor of CYP2D6 and non carcinogenic.

Docking study

Docking of all the known ligands were performed using the AutoDock tools which is based on Lamarckian Genetic Algorithm for estimating docking score &

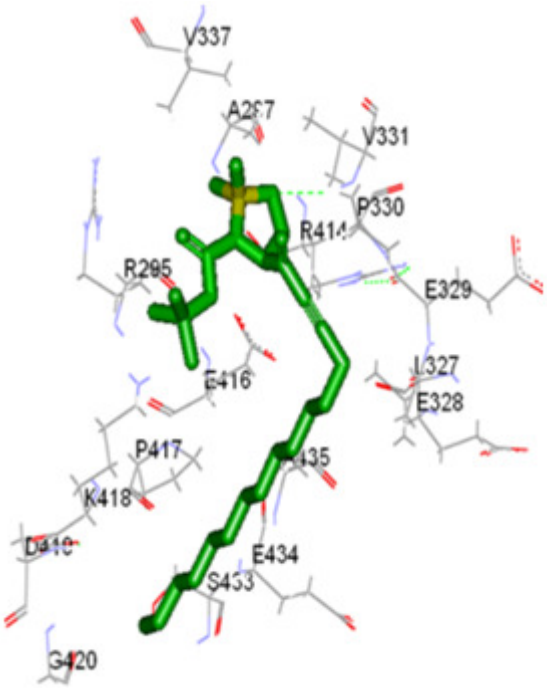

Fig. 1 : Active site residues of sphk1 interacting with SKI-5C via 1 H-bond.

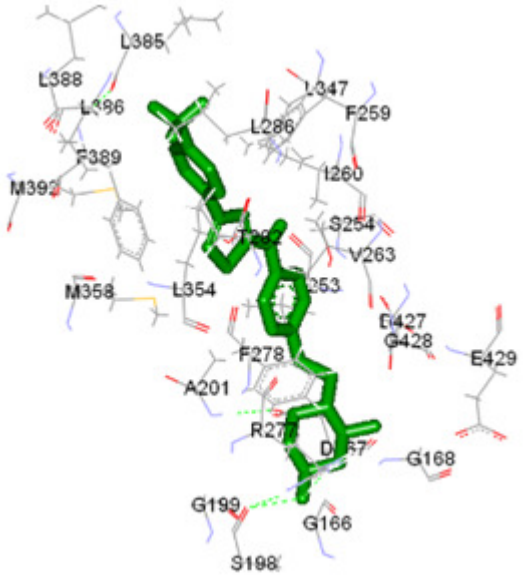

Fig. 2 : Active site residues of sphk1 interacting with Amgen 82 via 2 H-bonds.

inhibitory constant (Sharma *et al*, 2019). The docking protocol comprises preparation of protein, energy minimization, chain selection, heteroatoms removal and exclusion of water molecules by Discovery Studio Visualizer 2.5. All the ligands of sphk1 were prepared and minimized by Discovery Studio Client 2.5. For docking, a grid was set up with 80x80x80 points and all other parameters were set as default for docking. The docked model in the lowest energy cluster was considered as good interaction between ligand and protein (Khan *et al*, 2011; Khan *et al,* 2013;Khan *et al,* 2015; Rehman *et*

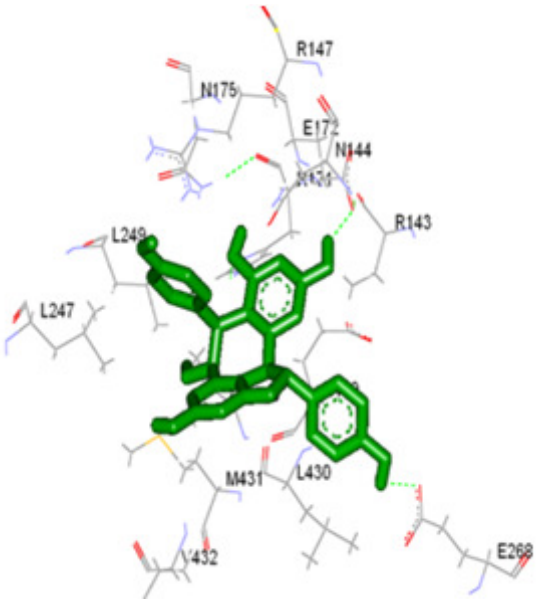

Fig. 3 : Active site residues of sphk1 interacting with Balanocarpol via 3 H-bonds.

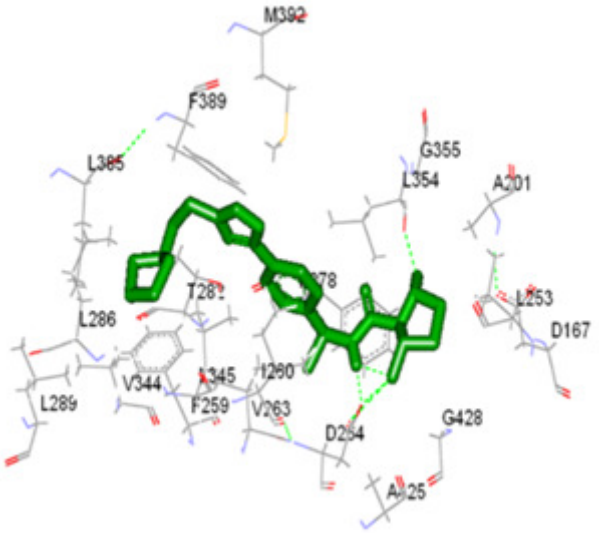

Fig. 4 : Active site residues of sphk1 interacting with Genzyme 51 via 4 H-bonds.

al, 2016; Khan *et al*, 2017). The images of the best docked model of ligands with sphk1 were generated using Discovery Studio Visualizer 2.5.

RESULTS AND DISCUSSION

Analysis of drug-likeness properties

The drug-likeness properties of stated inhibitors were analyzed on the basis of Lipinski RO5 using PreADMET server. Data revealed that all ligand molecules are in the permissible range of Lipinski's parameters (Table 1).

ADMET analysis

A plenty of studies indicated that suboptimal pharmacokinetics and toxicity are the most important causes of leads failure in drug development process. According to the ADMET profiling, all inhibitors were

Table 1 : Analysis of Drug-likeness and ADMET properties.

Inhibitors	Lipinski RO5	HIA	BBB	CYP2D6	PPB	Water Solubility	Toxicity
SKI-5C	Suitable	97.9	5.1	NI*	95.7	0.04	C^
SK1-I	-do-	89.17	1.9	I#	65.98	117.116	NC$
SKI-178	-do-	89.98	0.455	NI	86.86	1.36	C
RB005	-do-	99.8	13.08	I	88.33	14.27	NC
MPA08	-do-	96.33	0.074	NI	100	0.0097	C
Balanocarpol	-do-	84.3	1.08	NI	100	10.8	C
Genzyme 51	-do-	90.4	0.1	I	51.21	23.8	NC
Amgen 82	-do-	93.51	0.84	I	86.14	0.98	NC

*NI: Non Inhibitor #I: Inhibitor ^C: Carcinogenic $NC: Non-carcinogenic

Table 2 : Molecular docking analysis.

Ligands	BE* (kcal/mol)	HB$	Residues involved in molecular interactions
Genzyme 51	-12.5	4	M392, F389, L385, L286, L289, V344, T282, F259, I345, I260, V263, D264, A425, G428, G355, L354, A201, L253, D167
Amgen 82	-10.43	2	L385, L388, L386, F389, M392, M358, L347, L286, F259, I260, S254, V263, D427, G428,E429, G168,G166, S198, G199, D167, R277, A201, F278, T282, L354
RB005	-7.29	1	L405, L288, M392, L385, F389, T282, F259, F278, V263, D264, F260, V376, M358, L345, L354, A201, S254, L253, D427, D167, G428
SKI-178	-7.24	2	M392, F389, L354, A201, L253, D167, G168, D427, D428, E429, R271, F278, R277, D264, I260, V263, L345, T282, F259
MPA08	-5.02	Nil	E172, A146, H145, N144, T140, E141, R142, L169, G168, S165, G166, E429, E268
Balanocarpol	-4.77	3	R147, N175, E172, N144, R171, R143, L249, L247, M431, L430, V432, E268
SKI-5C	-3.47	1	V337, A287, V331, P330, R414, R295, E329, L327, E328, E416, P417, K418, D410, G420, S453, E434
SK1-I	-6.33	1	E328, L327, E329, A435, E434, S433, P330, V331, R414, E416, P417, K418, G420, D419, R295, A297, V337

*BE: Binding Energy $HB: H-bond.

tested for ADMET parameters using PreADMET tool. Our findings depicted that all compounds were following HIA criteria except one. Further, compounds were checked for PPB parameter of ADMET, it was found that out of eight compounds, three compounds (SKI-5C, MPA08 and Balanocarpol) were exempted this criteria. When BBB criteria of ADMET were applied for known compounds of sphk1, it was observed that only two compounds (SKI-5C and RB005) were not suitable for this parameter of ADMET. Subsequently, all known compounds of sphk1 were subjected to water solubility parameter of ADMET and it was noticed that SKI-178 showing satisfactory solubility level. Moreover, aforementioned compounds were tested for CYP2D6 metabolism in which SKI-5C, SKI-178, MPA08 and Balanocarpol were found to be non inhibitor of CYP 2D6. Thereafter, only four molecules (SK1-I, RB005, Genzyme 51 and Amgen 82) were successfully depicted as non-carcinogenic (Table 1).

Docking analysis

Molecular interaction studies were carried out on all the known compounds of sphk1 by using AutoDock tool 4.2. The compound Genzyme 51 shows higher binding energy value (-12.5 kcal/mol)as compared to other inhibitors.

Analysis of hydrogen bond interaction

Interaction of active site residues with the known inhibitors of Sphk1 namely Genzyme51, Amgen 82, Balanocarpol, MPA08, RB005, SK1-I, SKI-5C and SKI-178were analyzed. From the data analysis it was found that the Balanocarpol was found to involve 3 H-bond interactions (Fig. 3). The inhibitor Genzyme 51interacts with residues (Fig. 4) at the active site of sphk1, which involved 4 H-bond interactions. In the case of inhibitors Amgen 82 and SKI-178 that involved 2 H-bond interactions (Figs. 2 and 7). Similarly, the inhibitors RB005, SKI-5C and SK1-I that was bind with the residues involving 1 H-bond interaction (Figs. 6, 1 and 8). Moreover, MPA08 was not involved in any hydrogen bond formation with the target protein sphk1 (Fig. 5). Docking analysis of all inhibitor molecules and residues of sphk1

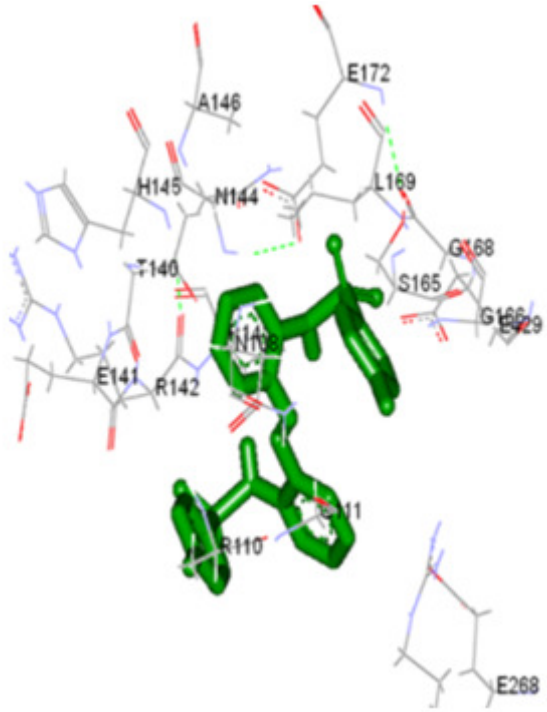

Fig. 5 : Active site residues of sphk1 interacting with MPA08.

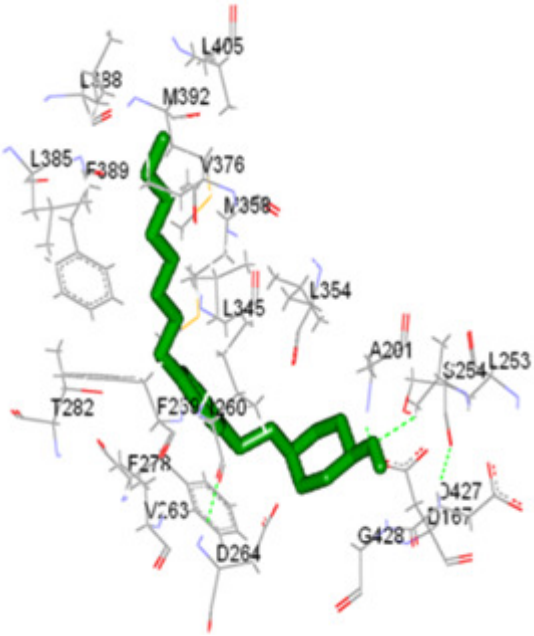

Fig. 6 : Active site residues of sphk1 interacting with RB005 via 1 H-bond.

involved in H-bonding and hydrophobic interactions are shown in Table 2.

CONCLUSION

The inhibition of sphk1 has been well documented, and it widely considered as an effective therapeutic strategy for the treatment of different malignant cancer cells. Despite, the availability of enormous sphk1 inhibitors, the most effective one could be selected on the basis of their selective targeting and good

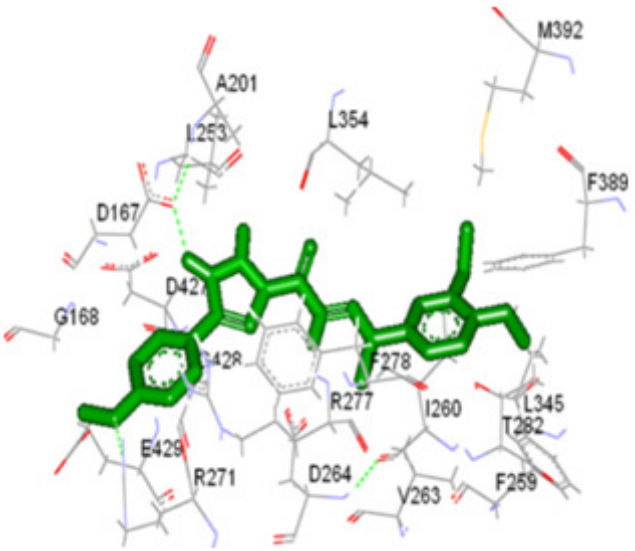

Fig. 7 : Active site residues of sphk1 interacting with SKI-178 via 2 H-bonds.

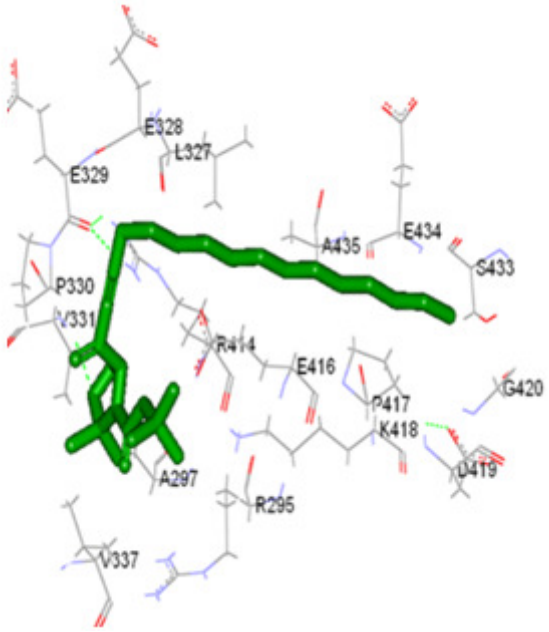

Fig. 8 : Active site residues of sphk1 interacting with SK1-I via 1 H-bond.

pharmacokinetics profiles. This study explores the molecular interaction mechanism of 8 known inhibitors (Genzyme 51, Amgen 82, RB005, SKI-178, MPA08, Balanocarpol, SKI-5C and SK1-I) of sphk1on the basis of their binding and other pharmacokinetics potentials. From docking analysis it was inferred that Genzyme 51 having greater binding propensity with sphk1 apart from showing outstanding physicochemical and pharmacokinetic properties. Moreover, some crucial amino acid residues likeM392, F389, L385, L286, L289, V344, T282, F259, I345, I260, V263, D264, A425, G428, G355, L354, A201, L253 and D167 play key role in proper orientation of Genzyme 51 into the binding cavity

of target protein that was further supported and stabilized by 4 H-bond formation. Thus, Genzyme 51 might be preferred to others as an initial lead molecule in order to design new target specific and more potent drug molecules to recuperate cancer cells and other diseases as well.

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Conflict of interest

The authors declare no conflict of interest and disclosures associated with the manuscript.

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