# Tackling the Moving Mutant Target: Taxifolin Derivatives as Novel Putative E571K Exportin-1 Inhibitors for KRAS-mutant Lung Adenocarcinoma Therapy

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### Abstract

**Aim**: To establish, through molecular modelling, safe and clinically acceptable putative antagonists of E571K-mutated exportin-1 among the bioactive compounds in various parts of *Juglans mandshurica*.

**Methods**: The bioactive compounds were subjected to compendium of druglikeness and leadlikeness filter workflows prior to docking of the resultant compounds into E571K exportin-1 active site using PyRx AutoDock vina to establish their binding affinity and interaction profile. The evolutionary algorithm of Osiris property explorer DataWarrior software as well as lead-likeness filter were employed for generation of novel non-promiscuous analogues of the lead compound with better putative selectivity and clinical acceptability as E571K Exportin-1 antagonists.



**Results**: The findings of this study present taxifolin as the putatively effective and lead-like E571K Exportin-1 inhibitor with high potential of qualifying for clinical evaluation but is associated with high promiscuity tendency in high throughput screening. The evolutionary derivation of novel analogues of the compound, however, results in the generation of putatively non-promiscuous, non-toxic, and lead-like E571K Exportin-1 antagonists with high synthetic accessibility and clinical developability for evaluation in the strategy for treatment of drug-resistant KRAS-mutant lung adenocarcinoma condition.

**Keywords**: KRAS-mutant lung adenocarcinoma, E571K exportin-1, Taxifolin, Molecular modelling.

## 1.0 Introduction

The positive association between the recurring poor prognostic drug resistance and their varied genomic mutations widely emphasizes its clinical relevance in the various chemotherapeutic strategies for reducing cancer-associated morbidities and mortalities<sup>66,67</sup>. This include the missense single nucleotide gain-of-function E571K mutation of exportin-1 gene (XPO1) that thereby codes for positively charged lysine instead of negatively charged glutamate residues in exportin-1 and abnormally elevates its affinity for nuclear export signals (NES)<sup>68,17</sup>.

The deregulation of exportin-1 (also known as chromosomal region maintenance 1), as a major RanGTP-driven exporter of more than 1050 human NES-bearing proteins (including tumour suppressors such as p53, BRCA1 etc.<sup>69</sup> and protooncogenes) as well as several RNA species<sup>70,71</sup> across the cell nucleo-cytoplasmic partition, is an active driver of several oncogenic conditions including the highly untreatable KRAS-mutant lung adenocarcinoma<sup>72,73</sup>.

Although the recurrence of exportin-1 E571K mutation is mostly reported in some haematological malignancies<sup>74,67</sup>, while Taylor *et al.* (2019) opined its rarity in solid tumours; there are currently reported 0.13% of XPO1 E571K mutation cases in a solid tumour such as KRAS-mutant lung adenocarcinoma<sup>53,75</sup>, and developing effective selective inhibitor of nuclear export signals (SINEs) for tackling the isolated E571K-mutated exportin-1 is a plausible strategy with potential significance in improving the prognosis of this highly untreatable condition. Indeed, several currently developed selective inhibitors of nuclear export signals (SINEs), including the clinically evaluated selinexor (KPT-330), KPT-185 etc., has been suggested as effective inhibitors of both the mutated and wild type XPO1 protein but remain limited in potency as single agent and/or safety in single or combination therapy or both<sup>51,18</sup>.

We intend to find novel selective inhibitor (s) of nuclear export signals (SINEs), through molecular modelling, in this study that can effectively inhibit E571K-mutated exportin-1 with better potency and safety profile among the compounds reportedly identified in several studies<sup>32,41,21,35, 61,60,33,62,45,57,28,55</sup> from various parts of *Juglans mandshurica* – a scientifically proven anticancer plant in several tumour cell lines<sup>36,27</sup>. This concept is validated by the fact that natural products are constituted of complex chemotypes with favourable safety profile and promising potential for discovery of novel nuclear export inhibitors<sup>76</sup>.

#### 2.0 Methods

#### 2.1 **Protein Preparation**

The 3D coordinate files of crystal structure of an unliganded human (homo sapiens) chromosomal region maintenance 1 (E571K)-Ran-RanBP1 complex (PDB ID: 6X2O) was retrieved from the

Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (http://www. rcsb.org), visualized using the Schrodinger molecular graphics program PyMol® and prepared using Schrodinger maestro protein preparation wizard. Briefly, the Ran-RanBP1 complex (chain A and B) were deleted while the remainder (chain C) was prepared under OPLS2005 force field at pH of 7.0±2.0. All steric clashes were corrected, hydrogen bond order fixed, while the missing side chains and loops were corrected using prime. Disulfide bonds were created, water molecules beyond 5.00Å were removed from the ionized (Het) groups, waters with less than 3 hydrogen bonds to non-waters were deleted while the bond orders were assigned. The charge cutoff for polarity was 0.25. This was further minimized by addition of polar hydrogens and merging of nonpolar ones using the plugged-in version of open babel software of PyRx AutoDock Vina version 0.8 and Gasteiger charges were computed using AutoDock 4.2<sup>26</sup>.

## 2.2 Ligand Preparation

The simplified input line entry system (SMILES) representations of identified *Juglans mandshurica* compounds<sup>32,41,21,35,61,60,33,62,45,57,28,55</sup> was obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov) while those with unavailable information were designed using ChemAxon Marvin Sketch. These were co-catenated and converted to MDL MOL structure description file format (SDF) using Osiris Property Explorer DataWarrior version 5.2.1. The physicochemical properties of the compounds were calculated and filtered based on the cut-off involving Lipinski's rule, topological surface area (TPSA)  $\leq$  140Å, as well as the number of rotatable bonds  $\leq$  10. The compounds were also filtered further based on their putative tumorigenicity, mutagenicity, irritant properties, as well as druglikeness while the remaining compounds were converted to PDBQT file using PyRx tool to generate atomic coordinates through the optimization algorithm set at the universal force field parameters. The 2D SDF format of the standard compound (KPT-185) evaluated in this study was also obtained from PubChem and prepared with similar workflow.

### 2.3 Ligand Docking

We calculated the binding energies of the obtained compounds with the target binding site by single blind docking simulation using PyRx AutoDock Vina version 0.8 with further energy minimization through the forcefield-based scoring function<sup>77</sup>. The PyRx AutoDock Vina, operating on the basis of assumably fixed three-dimensional target and flexible ligand structure<sup>54</sup>, employs the Broyden-Fletcher-Goldfarb-Shanno method in its multithread gradient optimization approach of iterated local search global optimizer algorithm<sup>44,2</sup>. The grid box resolution was centered at 12.0200 × -38.4570 × 337.6422 along the x, y and z axes respectively at the grid dimension of 25 Å x 25 Å defining the binding site and the comparative analysis of resulting interaction between the obtained Juglans compounds and the standard at the target binding site were obtained within the same docking grid box dimension.

## 2.4 Visualization of Docking Results

The ligand interactions at the target binding pocket and their three-dimension (3D) interaction with the target residues were visualized using Schrodinger Python Molecular graphics interface (PyMol®) version 2.2.0 software while the two-dimension (2D) ligand – residue interactions were obtained using BIOVIA Discovery Suite version 17.2.0.16349. The local minimum energy conformation binding mode of the compounds are regarded as the optimal binding pose at the target binding site in this study<sup>56,46</sup>.

# 2.5 Validation of Docking Study

# 2.5.1 Validation of Docking Protocol

The docking protocol of this study was validated by redocking of the same standard compound into the same target binding site and examining the relative binding pose of the ligands using the Python Molecular graphics interface (PyMol®) software.

# 2.5.2 Validation of Docking Algorithm

The binding energy calculation algorithm applied in this study was validated by comparative analysis of docking scores of the hits and the standard compound (KPT-185) in other different docking algorithms including Molegro virtual docker (PLANTS scoring function), LeDock, and iGEMDock.

# 2.5.2.1 Molegro Virtual Docker (PLANTS Scoring Function)

We employ the iterated simplex search algorithm (based on Nelder-Mead algorithm) with ant colony optimization component<sup>4</sup> of Molegro Virtual Docker for simulation of interaction between the E571K Exportin-1 active site and the hit compounds in this study, while the protein-ligand ANTS (PLANTS) scoring function with GRID<sup>23</sup> was used to calculate the potential energy values on a cubic grid prior to docking.

## 2.5.2.2 LeDock

We docked each of the three hits and the standard (KPT-185) into the target active site and calculated the docking scores through combination of simulated annealing and genetic algorithm for each ligand pose, orientation, and rotatable bond optimization in LeDock<sup>63</sup>, while the scoring function is on the basis of transferable and accurate energy function consisting of van der Waals interaction energy and a unique empirical-based hydrogen bonding energy<sup>64</sup>.

### 2.5.2.3 iGEMDock

The various possible conformations of each of the hits were also obtained through the genetic evolutionary algorithm of iGEMDock<sup>59,25</sup> in which we considered 66 generations per ligand with population size of 200 random individual offspring ligands per generation while the fitness, defined by empirical summation of partial contributory energy of the formed van der Waals, hydrogen bonding, and electrostatic interactions, were calculated for each of the flexibly docked structures at the E571K Exportin-1 active site. We set the ligand hydrophobic and electrostatic preference at 1.00, the number of solutions employed is 2, and only ligands with interactions within the binding site radius of 8.00Å were employed in the GEMDock scoring function calculations that also include the ligand intramolecular energies for total energy calculation in this study.

### 2.6 Pharmacokinetics and Toxicity Profiling

We employed a compendium of workflow involving the web-based predictive tool SwissADME (http://www.swissadme.ch/index.php), and DataWarrior to profile both the hits and the lead compound derivatives in this study for their absorption, distribution, metabolism, excretion, and toxicity properties.

# 2.7 De novo Generation of Lead Compound Derivatives

An evolutionary library of derivatives of the lead compound was built in this study by creating compounds similar to current standard-of-care drugs for 50 generations using the Osiris property explorer DataWarrior software in which the substructures of the parent compound at the turn of each generation were randomly left untouched. The SkelSpheres descriptors were used in the generation of 128 compounds per generation while the maximum of 8 compounds were allowed to survive a generation.

# 3.0 Results and Discussion

# 3.1 Compounds Obtained

The total of 123 compounds reported by some studies<sup>32,41,21,35,61,60,33,62,45,57,28,55</sup> was retrieved in this study while a total of five (5) predictably drug-like and non-toxic compounds (Table 1) were recovered following the pre-filtering workflow (See 2.2) on Osiris property explorer. Compound 30, 31, and 54 were the identified but non-designated compounds from *Juglans mandshurica* with currently no structural information in the PubChem database, the compound with the ChemBL ID: 463247 is tetrahydro-2-(4-hydroxy-3-methoxyphenyl)-6-(alpha,4-dihydroxyphenethyl)-4H-pyran-4-ol while the first-generation SINE compound KPT-185 (PubChem CID: 53495165) was evaluated as the standard compound.

### Table 1: Simplified Molecular Input Line Entry System Representation of Retrieved

Juglans n	nandshurica	Following	Drug-likeness a	and Toxicity Filters
		0		

Compound	SMILES	Drugliken	Mutagenicity	Tumorigenicit	Teratogenicity
		ess		У	
Taxifolin	O=C1c2c(OC(C1O)c1cc(O)c(cc1)	0.44477	None	None	None
	O)cc(cc2O)O				
Malic Acid	O=C(O)[C@@H](O)CC(=O)O	0.70851	None	None	None
Compound	O=C(O)C(O)(C(=O)/C=C/c1ccc(cc	1.0433	None	None	None
30	1)O)C(O)CO				
Compound	O=C1c2c(cccc2)C[C@@H](C1(O)	0.88711	None	None	None
31	0)0				
Compound	O=C1c2c(O)cccc2C2(CC1C(c1c2c	0.75027	None	None	None
54	c(c(c1)O)O)CC(=O)O)O				
ChemBL	O1C(c2cc(OC)c(cc2)O)CC(CC1C(	0.71056	None	None	None
ID: 463247	O)Cc1ccc(cc1)O)O				
<b>KPT-185</b>	FC(F)(F)c1cc(OC)cc(c1)c1nn(cn	-9.3576	High	None	Low
	1)/C=C/C(=O)OC(C)C				

The druglikeness score of a compound, calculated from the compound toxicity descriptors and the degree at which it contains predominantly the fragments frequently present in commercially available drugs, helps validate its potential as a drug candidate<sup>78</sup>. The prefiltered five compounds from *Juglans mandshurica* in this study (Table 1) predictively shows no risk of tumorigenicity, mutagenicity, and teratogenicity and irritant properties with quantitative estimate of druglikeness (druglikeness score) range of 0.44477 to 1.0433 in this study. This, though may not be well balanced with other requisite properties for compound druggability<sup>48,78</sup>, presents them as displaying constitution of at least one of the available core fragments and possible reconstructed fragments generated by the fragmentist-based approach of López-López *et al.* (2019)<sup>78</sup>, involving shredded 3,300 traded drugs and 15,000 commercially available (Fluka) chemicals.

Optimal cell permeability of a compound, and consequentially its bioavailability and potency, is an inverse function of summation of its polar atomic surface area<sup>79</sup>. The total polar surface area (TPSA) of the prefiltered five compounds from *Juglans mandshurica* in this study (Table 2) is in the square angstroms value range of 77.76-135.29, thereby obeying the drug-like model rules of Veber *et al.*  $(2002)^{80}$  and Muegge *et al.*  $(2001)^{81}$ . The cell permeability potential of the compounds however notably precludes, due to their TPSA values greater than 60 square angstroms (Table 2), the potential of central nervous effects and toxicities via their blood-brain barrier impenetrability.

The pivotal balance between a compound molecular weight and other descriptive features of its bioavailability essentially defines its potency and safety profile. This is because increased molecular weight positively correlates with elevated bioactivity but inversely associates with gastrointestinal absorption<sup>78</sup>. The molecular weight of the five compounds (134.09-360.41) retrieved in this study (Table 2) falls within the general consensus range of  $160 \le$  molecular weight < 500 for predictably bioavailable and drug-like compounds<sup>82,83</sup>, as well as represents the fact that

more than 80% of all traded drugs has molecular weight below 450, as demonstrated by López-López *et al.* (2019).

A definable ligand-target specificity is a function of the compound spatial orientation and geometry within the target binding site<sup>19,84</sup>. The molecular shape index range (0.385-0.667) of the retrieved compounds in this study (Table 2) shows the various parts of *Juglans mandshurica* as rich natural sources of compounds with complex chemotypic scaffolds. Furthermore, the modal value range of the retrieved compounds being  $\leq 0.5$  reveals the highest number of the retrieved compounds possess significant three-dimensional (non-flat) shape space coverage (which positively correlates with broad biological activity<sup>50,12</sup> similar to most of current standard-of-care drugs.

The molecular complexity score of a compound, a measure of combination of complexity metrics involving single cluster of its fragment structural fingerprint, accounts for its aromatic ring numbers and sp3 hybridized carbon fractions  $(Fsp3)^{39,85}$ . It is an inverse function of the compound promiscuity potential, and safety profile but positively associates with its clinical trial success rate, and developability<sup>38,37</sup> on a specified scale of  $0.1 - 1.5^{22,85}$ . The molecular complexity score range (0.621-0.997) of the retrieved compounds in this study (Table 2) presents them as having fraction number of chiral sp3 atoms (Fsp3) within the range constituting currently traded drugs; a prerequisite for their potential clinical developability into safe, potent, and E571K Exportin-1-selective inhibitors.

The molecular flexibility scores of the retrieved compounds in this study (Table 2), a function of their complexity, changeable dihedral bond angles, and geometry weighting factors<sup>86,87</sup>, are in the range of 0.136-0.751 on the scale of 0.0 (flexible) to 1.0 (completely rigid). This plays crucial role

in defining the potential relative binding capacities of the compounds in the target active site as a ligand conformational rigidity (due to fewer rotatable bonds) positively correlates with its target binding affinity potential<sup>29,15</sup>.

Molecule Name	Polar Surface Area	Molecular Weight	Molecular Shape Index	Molecular Flexibility	Molecular Complexity
Taxifolin	127.45	304.253	0.50	0.267	0.852
Malic Acid	94.83	134.087	0.67	0.751	0.621
Compound 30	135.29	282.247	0.60	0.563	0.708
Compound 31	77.76	194.185	0.50	0.136	0.761
Compound 54	135.29	356.329	0.38	0.292	0.997
ChemBL ID: 463247	99.38	360.405	0.58	0.512	0.776
KPT-185	66.24	355.32	0.56	0.495	0.810

**Table 2: Physical Properties of Resultant Prefiltered Compounds** 

# 3.2 Ligand Docking

Potency and safety limitations abound around the potential effects of current SINE compounds as E571K-mutated Exportin-1 inhibitors in KRAS-mutant lung adenocarcinoma condition<sup>68,18</sup>. The molecular docking simulation approach is a behavioural characterization of the interaction between potential small molecule inhibitors of druggable targets and the possible biochemistry of their potential effect (s) through phenotypic screening of the drug-like compounds<sup>88</sup>.

The PyRx AutoDock vina screening of the drug-like compounds retrieved from *Juglans mandshurica* in this study (Figure 1) showed compound 54 and the compound with ChemBL ID: 463247 as having relatively higher binding affinities (-5.5 kcal/mol to -5.6 kcal/mol) when compared with that of KPT-185 as the standard compound, thereby suggesting relatively higher bioactivity efficacy potential of these hits as E571K exportin-1 inhibitors. It is also worthy of note that although taxifolin showed comparatively equivalent binding affinity to that of KPT-185 (-5 kcal/mol) in this study (Figure 1); it displays relatively lower molecular flexibility score (Table 2) with the consequential tendency of comparatively advancing its efficacy due to its rigidity-mediated lower entropy loss at the E571K exportin-1 active site.

Cc	ChemBL ID:	463247		folin	mpound 31	Compound 3	0 Malic Aci	d
		5.0	Ē	1.9	-4 6	-4 A -4 2	-4	2.8

# Figure 1: Graphical Representation of Docking Score (in kcal/mol) of Retrieved Compounds

- 3.3 Study Validation
- 3.3.1 Validation of Docking Protocol



## Figure 2: Superimposition of Redocked KPT-185 at the Target Binding Site

The superimposition of relative binding poses of the redocked KPT-185 at the E571K Exportin-1 active site (Figure 2) in this study shows the ligand docking procedure in this study as valid, thereby rendering the various docking scores as accurate.

# 3.3.3 Validation of Docking Algorithm

The results from other docking algorithms (Table 3) further reveals comparative higher binding energies of the hit compounds than that of KPT-185 similar to the findings of the study executed using PyRx AutoDock vina, thereby confirming the binding affinities attributed to each of the hits in this study.

# Table 3: Comparative Scores of Different Docking Algorithms with the Vina Docking Score

Ligand/Algorithm	PyRx AutoDock Vina (kcal/mol)	LeDock (kcal/mol)	Molegro Virtual Docker PLANTS Score (kcal/mol)	iGEMDock (kcal/mol)
Taxifolin	-5.0	-4.39	50.96	-125.56
Compound 54	-5.5	-5.01	-52.30	-126.98
ChemBL ID: 463247	-5.6	-4.37	-49.50	-129.00
KPT-185	-5.0	-4.20	-44.39	-97.74





Figure 3: Representation of Ligand-Target Interaction. (A) Interaction between Taxifolin and E571K Exportin-1 (B) Interaction between Compound 54 and E571K Exportin-1 (C) Interaction between ChemBL ID: 463247 and E571K Exportin-1 (D) Interaction between KPT-185 and E571K Exportin-1

The binding energy and, subsequently, bioactivity of a drug entity at the target active site is defined by the type and number of its interaction within the target active residues<sup>89</sup>. The secondary ammonium ion in the active site lysine residue of E571K Exportin-1 commonly distorts the  $\pi$ electron cloud of the aromatic ring in the three hit compounds (taxifolin, compound 54, compound with ChemBL ID: 463247) as well as KPT-185 in this study, thereby resulting in the formation of electrostatic Pi-cation dipole moment (Figure 3), which might have potential significant role in the mechanism of inhibition of both wild and mutated type of exportin-1 by SINE compounds.

Taxifolin, however, formed an additional hydrophobic Pi-sigma bond and conventional hydrogen bond (Figure 3A) with Lys 360 and Asp 460 residues respectively while both compound 54 (Figure 3B) and compound with ChemBL ID: 463247 (Figure 3C) formed an additional hydrophobic Pialkyl bond with the active lysine 360 residue. It is also noteworthy that taxifolin formed additional conventional hydrogen bond (comparably to KPT-185) with the active site serine residue 467, albeit with additional unfavourable donor-donor bond (Figure 3A) that may however potentially affects the drug-like entity activity stability through repulsive reduction of the ligand-protein complex as deduced by Samant et al. (2019)<sup>90</sup>. Hydrogen bonding interactions (between the E571K Exportin-1 active site lysine 360, aspartate 460, serine 467 residues and the atomic partial charges of taxifolin, compound 54, as well as KPT-185 in this study (Figure 3)) positively associates<sup>90</sup> with significant target-ligand complex stability and bioactivity of the drug-like entities at the target active site. Furthermore, the hydrophobic interactions (Figure 3) of the drug-like entities possess significant putative contributory role in entropy increase-mediated overall binding energy elevation, through displacement of water molecules in ordered layers within 3Å of E571K Exportin-1 active site.

### 3.5 Pharmacokinetics, Toxicity, and Lead-likeness Profile of Hit Compounds

The potency and safety profile of an orally bioavailable small molecule drug entity, a function of its pharmacokinetic profile, is partially defined by its lipophilicity-mediated gastrointestinal permeability and metabolism<sup>5</sup>. This study (Table 4) reveals the three hit compounds, including taxifolin, compound 54, and compound with ChemBL ID: 463247), as highly prone to gastrointestinal (GIT) absorption with comparatively similarity to that of KPT-185. Furthermore, the non-inhibition of cytochrome P450 enzyme (CYP) isoforms 1A2, 2C9, 2C19, 2D6, and 3A4 (metabolizing about 50%-90% of current drugs in the liver<sup>58,9,1</sup> by the three hit compounds (with the exception of compound with ChemBL ID: 463247 on CYP 2D6) have plausible contributory role in the dose-bioavailability balance adjustment and significant toxic metabolite production avoidance of the putative inhibitors en route the action site while the inhibition of these CYP isoforms by KPT-185 (Table 4) may be playing significant role in the development of some of its associated toxicities (due to drug-drug interaction or accumulation) as well as its reportedly poor pharmacokinetic profile by Etchin *et al.* (2012) and Lapalombella *et al.*  $(2012)^{14,31}$ . It is however noteworthy that both the three hit compounds and KPT-185 in this study (Table 4) does not significantly inhibits CYP3A4; an enzyme responsible for degradation of most drug entities in the liver.

The blood brain barrier (BBB), dynamically interfacing the blood and brain, prevents toxic xenobiotics entry into the brain endothelium as well as supply it with requisite nutrients while the P-glycoprotein receptor-mediated transport system plays vital role in the outflux of unwanted xenobiotics from the BBB and several other organs<sup>6,24</sup>. The three hit compounds, unlike KPT-185, does not permeate the blood-brain barrier in this study (Table 4), thereby showing very low potential adverse clinical effects on the brain by the hit compounds in KRAS-mutant lung

adenocarcinoma therapy. Although this study reveals KPT-185 as blood-brain barrier permeant; it is noteworthy to determine the comparative threshold *in vivo* at which it may induce neurotoxic side effects during the disease therapy. Also, this may alternatively associate with its ability to confer neuroprotection against xenobiotics-induced axonal damage as reported by Haines *et al.* (2015)<sup>20</sup>. Only taxifolin, however, showed the properties of significantly less prone to P-glycoprotein receptor-mediated outflux (comparably similar to KPT-185) in this study (Table 4) and can play significant role in its high GIT absorbability-mediated biodistribution model; a feature made pronounced by its non-blood-brain barrier permeability.

Taxifolin	Compound 54	ChemBL ID: 463247	KPT-185
High			High
	High	High	
No			Yes
	No	No	
No			No
	Yes	Yes	
No			Yes
	No	No	
No			Yes
	No	No	
No			Yes
	No	No	
No			No
	No	Yes	
No			No
	No	No	
	Taxifolin         High         No         No	TaxifolinCompound 54HighHighNoNoNoNoNoYesNo	TaxifolinCompound 54ChemBL ID: 463247HighHighHighNoNoNoNoNoNoNoYesYesNoYesNo

# Table 4: Absorption and Metabolic Profile of Hit Compounds

# Table 5: Druglikeness and Lead-likeness Profile of Hit Compounds

Compound ID /Property	Taxifolin	Compound 54	ChemBL ID: 463247	KPT-185
Lipinski's	No	No		
Violation			No	No
Ghose's	No	No		
Violation			No	No
Veber's	No	No		
Violation			No	No
Egan's Violation	No	Yes; 1 violation: TPSA>131.6Å	No	No
Muegge's Violation	No	No	No	No

Lead-likeness	Yes	No (1 violation; MW > 350)	No (1 violation; MW > 350)	No (1 violation; MW > 350)
Bioavailability Score	0.55	0.55	0.55	0.55

# Table 6: Physicochemical, Toxicity, Promiscuity and Synthetic Accessibility Profile of Hit Compounds

Compound ID /Property	Taxifolin	Compound 54	ChemBL ID: 463247	KPT-185
PAINS Alert	1 alert	1 alert	Nil	Nil
Number of	7	7	6	6
Hydrogen				
Bond				
Acceptors				
Number of	5	5	4	0
Hydrogen				
Bond Donors				
Number of	1	2	5	7
Rotatable				
Bond				
Number of	2	2	2	2
Aromatic Ring				
<b>Brenk Alert</b>	1 alert	1 alert	Nil	1 alert
Synthetic	3.51	4.31	4.15	3.29
Accessibility				
Score				

The druglikeness and bioavailability are interdependent features defining the clinical developability of a drug-like entity<sup>91</sup>. The three hit compounds (taxifolin, compound 54, compound with ChemBL ID: 463247) in this study obeys all the current drug-like and oral bioavailability model rules (except compound 54 violating the Egan *et al.* (2000) rule<sup>11</sup> by having topological surface area of greater than 131.6Å (Table 5), therefore revealing them as drug-like compounds with high Caco-2 cell permeability. However, only taxifolin showed predictively high likelihood

of clinical developability (Table 5) as an effective E571K Exportin-1 inhibitor, as the other hit compounds (compound 54 and compound with ChemBL ID: 463247) as well as KPT-185 showed lead-likeness limited by molecular weight greater than 350 in violation of the proposed model by Teague *et al.* (1999)<sup>52</sup>.

The pan assay interference (PAINS) filter of the hit compounds in this study (Table 6), a measure of their potential of exhibiting false positive results in high throughput screening <sup>92,5</sup>, reveals both taxifolin and compound 54 as having high tendency of non-specifically reacting with numerous biological targets rather than specifically on the desirable E571K Exportin-1 while the compound with ChemBL ID: 463247, in similarity to KPT-185, displays significantly low tendency of exhibiting PAINS alert (Table 6). The significantly high promiscuity potential of taxifolin and compound 54, unlike compound with ChemBL ID: 463247 and KPT-185 in this study, shows the duo containing catechol substructure as potential non-selective bioactive response which can therefore restrict their lead-likeness potential as putative E571K Exportin-1 inhibitor for chemotherapy.

The more stringent Brenk model of lead-like compound selection characterized any compound as medicinal that possess both hydrogen bond donor and hydrogen bond acceptor respectively of less than 4 and 7, number of heavy atoms between 10 and 27, ClogP: Clog D ratio of zero to four, limited complexity (definable by less than eight rotatable bonds, less than five ring systems, greater than or equal to two constituted fused ring systems) with the exclusion of reactive and unfavourable groups such as nitros, sulfates, phosphates, 2-halopyridines, and thiols<sup>3</sup>. The violation of Brenk rule by both taxifolin and compound 54 (similar to KPT-185) is as a result of the duo having the cut-off number of 7 hydrogen bond acceptor, more than 4 hydrogen bond donors, as well as containing the reactive catechol as a substructure that characteristically pose

limitations on their safety profile while that of KPT-185 is as a result of possessing the highly reactive non-ring Michael acceptor group that may have contributed to its associated *in vivo* side effects.

The synthetic accessibility score of this study hits (Table 6), based on the primary assumption of positive association between ease of synthesis of compounds and their constituted amount of frequently obtainable chemical moieties<sup>5</sup>, reveals easy synthesis of their multiple analogues at an average score of 3.99 (comparably with 3.29 of KPT-185) in which taxifolin and compound with ChemBL ID:463247 being the easiest and most difficult respectively to synthesize on a scale of 1 (easy to synthesize) to 10 (very difficult to synthesize).

## 3.6 *De novo* Generation of Lead Compound Derivatives

The lead-likeness of taxifolin, among the three hit compounds, in this study (Table 5) presents it as a putative drug-like E571K Exportin-1 inhibitor with high likelihood of qualifying for clinical evaluation and development. Its potential efficacy, however, is limited by its promiscuity tendency in high throughput screening which is a precursor to off-target binding, as well as its violation of Brenk rule of lead-likeness selection due to its constituted catechol substructure. Therefore, exploring the possible chemical space that can be generated from random mutable combination of the compound fragments (based on similar flexophore and dissimilar skelsphere descriptors to that of taxifolin as the parent compound<sup>48</sup> may presents viable approach toward discovery of its better analogue (s) with the most promising property profile as lead-like E571K Exportin-1 inhibitors.

A nature-mimicking evolutionary approach of randomly mutating the taxifolin structure based on skelsphere similarity (through single atom replacements, atom insertions, changes in bond order, ring aromatization, substituents migration etc. with the exclusion of high ring strains-producing mutations) and allowing the 8 best fit child compounds to survive per generation for 50 generations resulted in the generation of 452 novel compounds (having fitness score and skelsphere similarity range of 0.78-1) from which 20 non-toxic, drug-like compounds (molecular weight: 301.3-344.36; skelsphere similarity: 0.80; TPSA: 86.99-124.29; druglikeness score: 0.65-2.88) were obtained following prefiltering based on the procedure and criteria stated in section 2.2.

We evaluated and filtered the resultant 20 novel drug-like taxifolin derivatives based on pan assay interference (PAINS) potential and lead-likeness selection criteria of Brenk *et al.*  $(2008)^3$  in this study and obtained five non-toxic, drug-like novel analogues (Table 7; Figure 4) between generation 24 and 43, having aromatic ring and rotatable bond respectively of 2 and 1 each, molecular flexibility score of 0.20-0.27, molecular weight of 303.27-320.73 (Table 9), TPSA of 86.99-120.11, as well as druglikeness score of 0.70-0.77 (Table 7).

Table 7: Simplified Molecular Input Line Entry System Representation of TaxifolinDerivatives with No Brenk and PAINS Alert

S/N	Compound Name	Generation	SMILES	Druglikeness
1	Compound 220	24	Oc1cc2O[C@H](c3ncc(c(c3)O)O)[C@@H](C(=O)c2c(c1)O)C	0.77
2	Compound 235	26	Oc1cc2O[C@H](c3ccc(c(c3)O)Cl)[C@H](C(=O)c2c(c1)O)C	0.70
4	Compound 250	28	Oc1cc2O[C@H](c3cnc(c(c3)O)O)[C@H](C(=O)c2c(c1)O)C	0.77
5	Compound 294	33	Oc1cc2O[C@H](c3ncc(c(c3)O)O)[C@H](C(=O)c2c(c1)O)C	0.77
6	Compound 379	43	Oc1cc2O[C@@H](c3ncc(c(c3)O)O)[C@@H](C(=O)c2c(c1)O)C	0.77



Figure 4: Structural Representation of Taxifolin Derivatives with No Brenk and PAINS Alert (A) Compound 220 of Generation 24 (B) Compound 235 of Generation 26 (C) Compound 250 of Generation 28 (D) Compound 294 of Generation 33 (D) Compound 374 of Generation 43

# 3.7 Physicochemical, Druglikeness, and Toxicity Properties of Taxifolin Derivatives with No Brenk and PAINS Alert

The resultant five taxifolin analogues with no Brenk and PAINS alert in this study shows high gastrointestinal permeability but possess property, unlike their parent compound (Table 4), which rendered them as substrate for P-glycoprotein receptor (Table 8). This, given the fact that they are non-blood brain barrier- permeable (Table 10), may contributes toward their reduced potential of inducing any side effects associated with the neurological system while aiding in preventing accumulation of the drug entities, via excretion, en route the action site. Also, the analogues,

similar to the parent taxifolin compound (Table 4), display non-inhibition tendency toward the various studied cytochrome P450 isoforms, except compound 235 of generation 26 (Table 8).

The bioavailability score of the analogue compounds with no Brenk and PAINS alert (0.55), in similarity to that of the taxifolin parent compound, also present them as orally bioavailable compounds (Table 9) while obeying the various conventional oral bioavailability and drug-likeness model rules.

Furthermore, the lead-likeness evaluation of these no-Brenk-and-PAINS-alert taxifolin analogues shows them as non-toxic compounds with high tendency of clinical developability (Table 10) while their range of synthetic accessibility score (3.48-3.59) presents them as easy to synthesize (comparably with that of their parent compound) for further preclinical and clinical evaluation.

Compound ID /Property	220	235	250	294	379
Generation	24	26	28	33	43
GI Absorption	High	High	High	High	High
<b>BBB</b> Permeant	No	No	No	No	No
Pgp substrate	Yes	Yes	Yes	Yes	Yes
CYP1A2 Inhibitor	No	No	No	No	No
CYP2C19 Inhibitor	No	Yes	No	No	No
CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 Inhibitor	No	Yes	No	No	No
CYP3A4 Inhibitor	No	Yes	No	No	No

 Table 8: Absorption and Metabolic Profile of Taxifolin Derivatives with No Brenk and PAINS Alert

 Table 9: Oral Bioavailability Profile of Taxifolin Derivatives with No Brenk and PAINS

 Alert

Compound ID /Property	220	235	250	294	379
Generation	24	26	28	33	43
Molecular	303.27	320.72	303.27	303.27	303.27
Weight					
Lipinski's	No	No	No	No	No
Violation					
Ghose's	No	No	No	No	No
Violation					
Veber's	No	No	No	No	No
Violation					
Egan's	No	No	No	No	No
Violation					
Muegge's	No	No	No	No	No
Violation					
CLogP	1.1324	3.031	1.4298	1.1324	1.1324

Bioavailability	0.55	0.55	0.55	0.55	0.55	_
Score						

# Table 10: Toxicity, Lead-likeness, Promiscuity and Synthetic Accessibility Profile ofTaxifolin Derivatives with No Brenk and PAINS Alert

Compound	220	235	250	294	379
<b>ID</b> /Property					
Generation	24	26	28	33	43
PAINS Alert	Nil				
		Nil	Nil	Nil	Nil
BRENK	Nil	Nil	Nil	Nil	Nil
Alert					
Lead-	Yes	Yes	Yes	Yes	Yes
likeness					
Synthetic	3.59	3.48	3.53	3.59	3.59
Accessibility					
Score					

# 3.8 Bioactivity Profile of Taxifolin Derivatives with No Brenk and PAINS Alert

Evaluating the bioactivity probability of the obtained lead-like taxifolin derivatives with no Brenk and PAINS alert in this study (Table 11) characteristically reveals the compounds as highly active ligand of the nuclear receptor such as E571K Exportin-1, when compared with that of KPT-185. Both compound 235 and 250 of generation 26 and 28 respectively exhibits the relatively highest bioactivity against nuclear receptors (Table 11), when compared with KPT-185. It is also noteworthy that although these taxifolin analogues tend to also exhibit moderate activity in modulating ion channel, inhibiting kinase and protease enzymes, as well as actively inhibiting Gprotein coupled receptors and enzymes; their lack of PAINS alert, unlike the parent taxifolin, presents the plausibility of restrictively advancing their bioactivity specifically against the nuclear E571K Exportin-1.

Compound ID	220	235	250	294	379	KPT-185
/Targetability						
GPCR	0.15	0.10	0.20	0.15	0.15	0.11
Ligand						
Ion Channel	-0.06	-0.13	0.19	-0.09	-0.06	-0.07
Modulator						
Kinase	0.11	-0.12	-0.01	0.11	0.11	-0.08
Inhibitor						
Nuclear	0.38	0.58	0.57	0.38	0.38	-0.17
Receptor						
Ligand						
Protease	-0.01	-0.18	-0.10	-0.01	-0.01	-0.19
Inhibitor						
Enzyme	0.33	0.15	0.48	0.33	0.33	0.02
Inhibitor						

Table 11: Bioactivity Score of Taxifolin Derivatives with No Brenk and PAINS Alert

### 3.9 Binding Affinities of Taxifolin Derivatives with No Brenk and PAINS Alert

Comparative evaluation of binding affinity potential of the lead-like non-promiscuous taxifolin analogues as putative E571K Exportin-1 inhibitors against the parent taxifolin compound and KPT-185 in this study (Figure 5) reveals compound 235 of generation 26 as having relatively higher binding affinity (-5.3kcal/mol) than both taxifolin (-5kcal/mol) and KPT-185 (-5kcal/mol) while both compound 220 and compound 379 respectively of generation 24 and 43 shows comparatively equivalent binding affinity with the parent compound and KPT-185 (Figure 5). It is also noteworthy that the characteristic display of highest binding affinity (Figure 5) and highest bioactivity tendency against nuclear receptors (Table 11) by compound 235 of generation 26 in this study, coupled with its lack of Brenk and PAINS alert, may advance the potential of the taxifolin analogue as the most effective, potent, and safe E571K Exportin-1 inhibitor in KRASmutant lung adenocarcinoma therapy.



Figure 5: Comparative Docking Scores of Taxifolin and its Derivatives with No BRENK and PAINS Alert

- 3.10 Interaction Between E571K Exportin-1 and Taxifolin Derivatives with No Brenk and PAINS Alert
- (A)





(B)





(C)





(D)





(E)



Figure 6: Representation of Ligand-Target Interaction. (A) Interaction between Compound 220 and E571K Exportin-1 (B) Interaction between Compound 235 and E571K Exportin-1 (C) Interaction between Compound 250 and E571K Exportin-1 (D) Interaction between Compound 294 and E571K Exportin-1 (E) Interaction between Compound 379 and E571K Exportin-1

Examining the interaction between the non-promiscuous lead-like taxifolin analogues with the binding site of E571K Exportin-1 in this study reveals formation of electrostatic Pi-cation dipole moment (Figure 6) with the active lysine residue 360; a mechanism earlier stated (section 3.4) as having plausibly significant contributory role in E571K Exportin-1 inhibition. This is in addition to compound 220 (Figure 6A), 235 (Figure 6B), and 250 (Figure 6C) of generation 24, 26, and 28 respectively forming Pi-alkyl bond with Leu 363 of E571K Exportin-1 active site; a feature which is notably absent in their parent compound (Figure 3A). Compound 235 of generation 26, however, formed additional Pi-alkyl interaction with the active histidine residue 367 (Figure 6B) that may have contributed toward its relatively higher binding affinity (Figure 5) and led credence to our

earlier deduction (section 3.9) that the compound, among the lead-like non-promiscuous taxifolin analogues in this study, tends toward exhibiting the highest E571K Exportin-1 inhibition for KRAS-mutant lung adenocarcinoma therapy.

### 4.0 Conclusion and Future Perspectives

The E571K mutant of exportin-1 plays pointed role in the recurrently poor prognosis of KRASmutant lung adenocarcinoma through advancement of drug resistance. The development of its potent and clinically acceptable antagonist, therefore, is a provably effective strategy in the treatment of this highly untreatable condition. We discovered novel analogues of taxifolin (obtained from *Juglans mandshurica*) as putatively non-promiscuous and clinically developable exportin-1 inhibitors with better pharmacokinetic properties than the clinically evaluated SINE KPT-185 compound (as the standard) in this study. This study, therefore, has provided evidence in support of assaying for effectiveness, potency and safety metrics of these taxifolin derivatives as E571K Exportin-1 antagonists in both pre-clinical and clinical studies involving KRAS-mutant lung adenocarcinoma chemotherapy.

# **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

### ABBREVIATION

KRAS: Kirsten Rat Sarcoma 2 Viral Oncogene Homolog.

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