Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2017, Volume 5, Issue 7
ISSN: 2321–3647(online)

Design and Optimization of K-Ras Protein Inhibitors as Anti-Cancer agents using Deltarasin as a Case Study.

Martina Woods[1]*, Claire Shoemake[1]
1.Department of Pharmacy, Faculty of Medicine and Surgery, University of Malta

ABSTRACT

K-Ras serves as an important component of signalling pathways involved in cell cycle control. Proper functioning K-Ras is regulated by phosphodiesterase δ (PDEδ). Deltarasin binds to this prenyl-binding protein thus inhibiting its interaction with K-Ras and hence disrupting Ras signalling. The objective of this study is to use Deltarasin as a template for further iteration of the design of novel drugs with potential clinical use in the management of malignancies. Deltarasin was constructed using SYBYL-X® V1.2, followed by analysis of the critical interactions with the amino acids lining the Ligand Binding Pocket (LBP). Seeds were modelled based on the Deltarasin scaffold and Virtual Screening (VS) was used to identify 'hits', using the same molecule as a template. SYBYL-X®, X-SCORE®, LigBuilder®, Visual Molecular Dynamics (VMD), Accelrys® Draw, Accelrys® Discovery Studio v3.5, Protein Data Bank and ZINCPharmer® were all used to generate results. The main outcome measures of this research project are to discover and optimise *in silico* high binding affinity of PDEδ inhibitory drug molecules, as well as molecule display, Ligand Binding Affinity (LBA) and Ligand Binding Energy (LBE) calculations, seed generation and ultimately *de novo* design. Based on reviewed SAR studies, nine seeds were generated using SYBYL-X® V1.2. The POCKET and GROW algorithm of LigBuilder® V1.2 were used to generate *in silico* molecules for each seed. Surflex-docking in SYBYL-X® V1.2 resulted in five molecules with a total docking score of six or greater. *De novo* molecules created and optimized, present viable leads for high-throughput screening, leading to identification of novel PDEδ inhibitors for use as anti-cancer agents.

Keywords: K-Ras, phosphodiesteraseδ (PDEδ), Deltarasin, *in silico, de novo* molecules.

*Corresponding Author Email: martinawoods94@gmail.com
Received 08 July 2017, Accepted 18 July 2017

Please cite this article as: Woods M *et al.*, Design and Optimization of K-Ras Protein Inhibitors as Anti-Cancer agents using Deltarasin as a Case Study. American Journal of Pharmacy & Health Research 2017.

INTRODUCTION

K-Ras forms part of the Ras family of proteins which serve as important components of signalling pathways involved in cell cycle control, cell adhesion, endocytosis, exocytosis and apoptosis. Proper functioning of this protein is regulated by the prenyl-binding protein phosphodiesteraseδ (PDEδ), which facilitates its diffusion through the cytoplasm. If K-Ras is successfully inhibited, it can no longer bind to this transport protein and hence cannot function properly.[1]

Although a variety of genetic modifications have been identified in pancreatic carcinoma, mutations of K-ras are by far the most commonly occurring mutation. Mutations are seen in over 85% of pancreatic ductal carcinomas. The development of mutations in K-ras appear early in the development of pancreatic cancer, having been observed in precursor lesions within the pancreatic duct. The mutations in K-ras in pancreatic cancer are also unique in that it typically involves codon 12, but may also rarely involve codons 13 or 61.[2] These mutations in K-Ras make it resistant to GAP and as a result lead to constitutive activation of downstream pathways, resulting in altered regulation of cellular proliferation.

Based on the frequency and apparent critical role of K-Ras in pancreatic cancer, several approaches have been developed to indirectly block the functioning of K-Ras. High throughput screening has been performed to identify small molecules that bind to the farnesyl-binding pocket of PDEδ. This yielded several benzimidazole hits. Crystal structure analysis of these compounds in complex with PDEδ revealed that two benzimidazoles bind to the hydrophobic pocket of the prenyl-binding pocket. One molecule is deeply buried and overlaps with the farnesyl-binding site. The second molecule is located close to the binding site that makes main chain contacts with two carboxy-terminal RHEB (Ras homologue enriched in brain) amino acids. Binding of the inhibitors is mediated by hydrophobic interactions and hydrogen bonding between the benzimidazole units and $Tyr^{149}$and $Arg^{61.}$ [3]

Thus far, the most effective inhibitor of PDEδ is Deltarasin, as it has proven to sharply reduce tumour growth in mice. Deltarasin is a small molecule that binds to the farnesyl-binding pocket of PDEδ (Kd=41 nM) in cells thus inhibiting its interaction with K-Ras and hence disrupting Ras signalling.

This study aims to use the Deltarasin molecule as a scaffold for further iterative design of *de novo* molecules capable of inhibiting PDEδ with the possibility of yielding molecules with increased binding affinity to the receptor, improved pharmacokinetic properties and satisfactory

bioavailability.

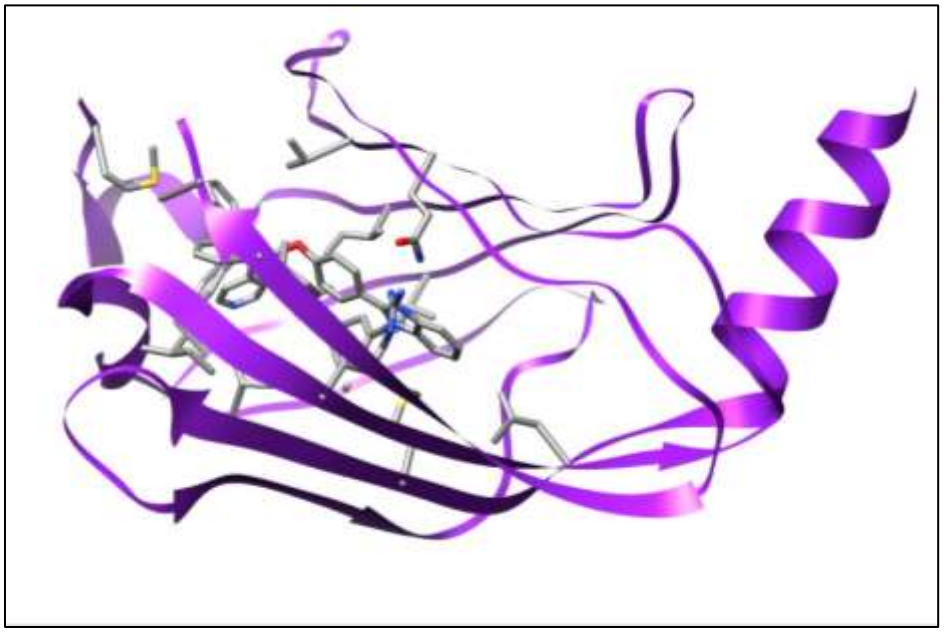


Figure 1: Deltarasin bound to PDEδ rendered in UCSF Chimera® V1.11.2 [1]

PDB Entry – 4JVB [2]

MATERIALS AND METHOD

Using the Protein Data Bank, the crystallographic deposition with the best resolution that described the bound coordinates of PDEδ with a small molecule (rac-2) was selected. [4] This was PDB entry 4JVB, with a resolution of 1.75Å. [5]

Each deposition was visualized and edited in SYBYL-X® v1.2[6] molecular modelling software, prior to Ligand Binding Affinity (LBA) estimation. Water molecules at a distance ≥ 5Å from the LBP were deleted. At the end of the process, a file saved in *.mol2* format containing the bound coordinates of the extracted small molecules and another file saved in *.pdb* format containing the apo receptor, without the deleted water molecules, were generated.

Two different approaches were adopted in this research project; Virtual Screening (VS) and *de novo* drug design.

Approach 1 – Virtual Screening

This first approach is a drug discovery process used to quickly assay the biological or biochemical activity of a large number of drug-like compounds to identify 'hits'.

Following conformational analysis, the optimal conformer of Deltarasin was chosen. ZincPharmer®, [7] the online molecular database which rapidly screens over 2 million compounds, was used to extract small molecules predicted to have a similar shape and electrostatic compositions to the template molecule Deltarasin.

A protomol was generated using SYBYL-X® v1.2[6]. This is intended to mimic the ideal interactions made by a perfect ligand to the protein active site. Next, the original PDB file (4JVB)[5] was retrieved from the RCSB server through SYBYL-X® v1.2[6]. The ligand was extracted from the protein backbone, while the water molecules together with any other ions deemed inessential for ligand stability were removed from the protein. To generate the protomol, the active site was used as placement for the Surflex-Dock process.

The molecules that were identified from ZincPharmer® [7] during the VS process in *.pdb* format, were converged into a single *.sdf* format file using MONA [8], an interactive tool that has been designed to prepare and visualize large small molecule datasets. The molecules were filtered using Lipinski's Rules [9] as inclusive criteria. This means that all generated molecules with molecular weight less than 500, LogP less than 5 and Hydrogen-Bond donors and acceptors less than 5 and 10 respectively were retained on the premise that they would be orally bioavailable.

The *.sdf* files prepared were chosen as Ligand Sources and the Surflex Dock process was run in SYBYL-X® v1.2.[6]

Approach 2 – *de novo* drug design

For this approach, the extracted ligand molecules in *.mol2* format and their respective protein binding pocket *.pdb* files from the first approach, were transferred to X-Score® v1.3 [10], which runs on a Linux Platform. The X-Score® v1.3[10] algorithm was used to measure the LBA (in pKd) of Deltarasin for its cognate receptor. This process was done to establish a baseline affinity against which the LBAs of other seeds would then be compared.

A thorough study of the Structure-Activity Relationship (SAR) for Deltarasin bound to PDEδ was carried out. Discovery Studio v3.5 [11] was used to generate 2D topology maps with the scope of facilitating SAR understanding. All crucial bonds and interactions were retained when creating seeds. Conversely, moieties which were deemed not important for binding to the Ligand Binding Pocket (LBP) were removed and growing sites were created in their place. Four successful seed structures were created based on the acquired SAR.

De novo design was carried out using LigBuilder® v1.2.[10] The POCKET module was used to identify the pharmacophoric structural features and key interaction sites that a hypothetical ligand for a given LBP could occupy. In this study, Deltarasin was used to probe the cognate receptor in *.pdb* file format according to the POCKET algorithm. The resulting data was then used by the GROW module in LigBuilder® v1.2[10] through which *de novo* design was performed. This module constructs ligand molecules for a target protein, in this case PDEδ, by applying the inbuilt growing strategy algorithm. Seeds were allowed to grow alternative side chains. An

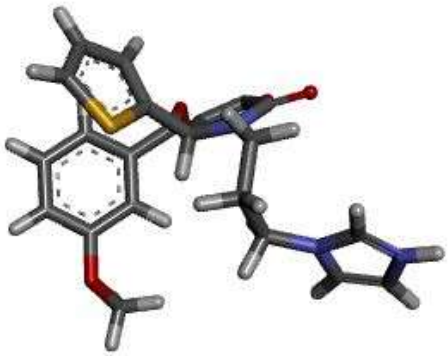
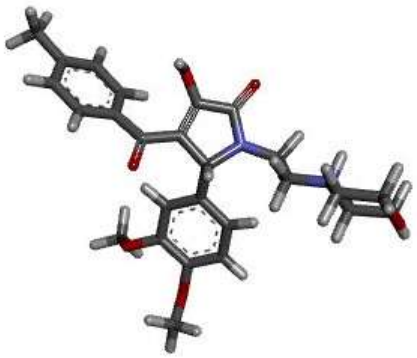
assessment to predict the drug-likeness for the *de novo* ligands was carried out based on the parameters of Lipinski's Rules by transferring each generated *de novo* ligand to Discovery Studio v3.5,[11] which determined Lipinski-Rule-compliance of such novel ligands.

RESULTS AND DISCUSSION

Approach 1

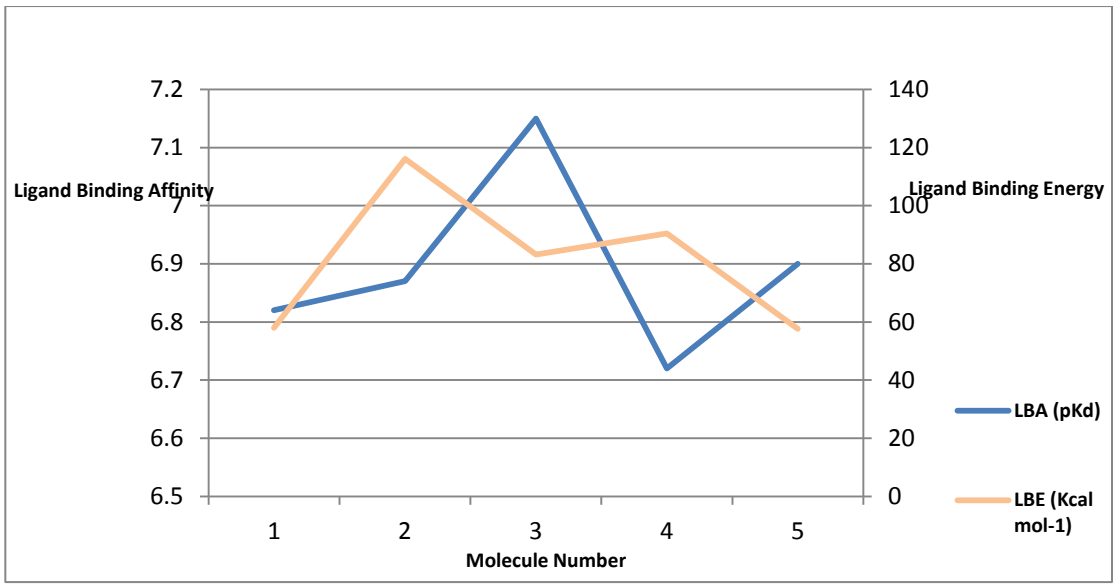
The Surflex Dock Process resulted in a number Lipinski Rule compliant molecules. The top five ligands with the highest *Total Score* are shown in Table 1 below.

Table 1: Top 5 ligand molecules with the highest Total Score

Molecule ID	**3D Structure**	**LBA** (pKd)	**LBE** ($kcalmol^{-1}$)
ZINC05921075		6.82	57.99
ZINC17160093		6.87	116.07
ZINC08438548		7.15	83.20

ZINC08430796		6.72	90.48
ZINC08426403		6.9	57.69

A graph of LBA and LBE vs Molecule Number was plotted for these ligands generated (Graph 1) and the molecule found to have the highest LBA and lowest LBE was ZINC08426403, having an LBA of 6.9.



Graph 1: LBA (pKd) and LBE (Kcalmol^{-1}) vs Molecule Number

Approach 2

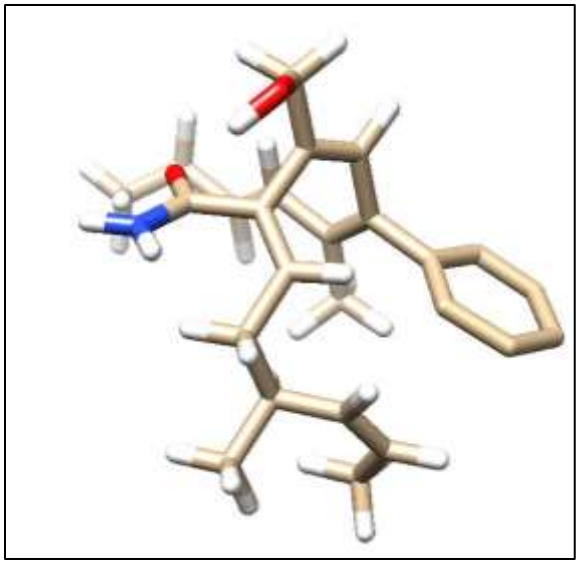
The baseline affinity of Deltarasin for the PDEδ receptor was calculated (in pKd) using X-Score® v1.3 and was found to be 6.67.

Ten seeds were created using SYBYL-X® v1.2,[6] four of which showed successful growth. The number of *Families*, *Lipinski-Rule compliant molecules* and *Highest pKd* can be seen in Table 2 below. A total of 346 Lipinski-Rule compliant molecules were generated by this method.

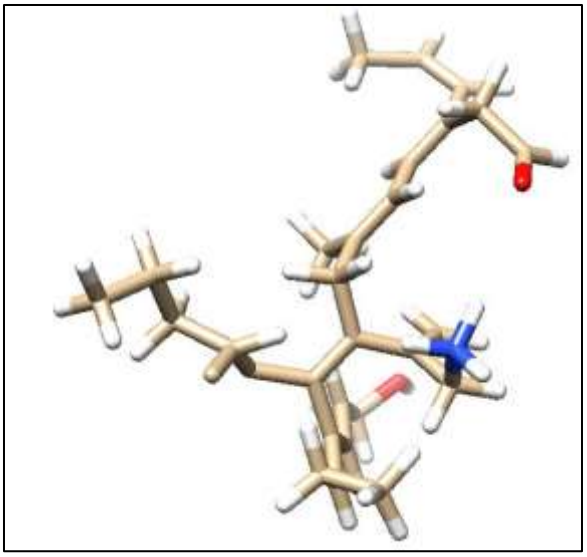
Table 2: Number of Lipinski-Rule compliant molecules from each seed

Seed Number	Number of Families	Number of Lipinski-Rule compliant molecules	Highest pKd
1	3	9	9.83
2	17	94	9.96
3	15	90	9.97
4	9	153	9.97

Two molecules were identified as having the highest binding scores across all families generated from the four different seeds. Seed 3 generated a molecule of molecular weight 374 and a partition coefficient of 4.92 whilst seed 4 yielded a molecule with an equally high pKd of 9.97, a molecular weight of 435 and a LogP of 4.81.



pKd = 9.97
mol. weight = 374 Da
LogP = 4.92



pKd = 9.97
mol. weight = 435 Da
LogP = 4.81

Figure 1: Molecules with the highest binding scores generated from seeds 3 and 4 respectively.

ZINC08426403, generated through the Surflex Dock process, was further analysed relative to the established SAR of Deltarasin to its cognate receptor and through 2D and 3D topology maps, it

DISCUSSION

was concluded that the two phenyl groups are crucial in the binding of inhibitor molecules to the active site. These lipophilic groups extend into the active site of PDEδ and occupy hydrophobic pockets. This explains why the generated *de novo* structures which did not include these highly lipophilic groups had a much lower binding affinity. In fact, the molecules yielded from the second approach, having the highest binding scores also possess highly lipophilic groups which fit comfortably into the highly hydrophobic pockets in the active site.

The *de novo* structures generated by this approach all had a molecular weight and LogP that was significantly lower than that of the parent molecule Deltarasin, potentially leading to better bioavailability and ultimately more effective treatment for the patient.

Limitations

As with any *in silico* drug design study, this project was conducted using a static, rigid LBP, which is very different from *in vivo* modeling and may thus yield different results. This was the major limitation of the freeware used.

CONCLUSION

In conclusion, *in silico* calculated high affinity and improved Lipinski-Rule compliance, make a case for synthesis of the optimal generated molecules. The ability of these molecules to successfully modulate the PDEδ transport protein could then be further validated through molecular dynamic studies and *in vitro* assays.

REFERENCES:

1. Collins M, Pasca di Magliano M. Kras as a key oncogene and therapeutic target in pancreatic cancer. Front. Physio. [Internet]. 2014; 4. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3896882/
2. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. Nature Rev [Internet]. 2003 Jun; 3: 7-13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12778136
3. Zimmermann G, Papke B, Ismail S, Vartak N, Chandra A, Hoffmann M. Small molecule inhibition of the KRAS-PDEδ interaction impairs oncogenic KRAS signalling. Nature [Internet]. 2013 May. Available from: http://www.nature.com/nature/journal/v497/n7451/full/nature12205.html
4. Berman H. The Protein Data Bank: a historical perspective. Acta Cryst Sect A. 2007;64(1):88-95.
5. Zimmermann G, Papke B, Ismail S, Vartak N, Chandra A, Hoffmann M et al. Small molecule inhibition of the KRAS PDEd interaction impairs oncogenic KRAS signalling

[Internet]. Rcsb.org. 2017 [cited 11 July 2017]. Available from: http://www.rcsb.org/pdb/explore.do?structureId=4JVB

6. SYBYL-X Suite [Internet]. Support.certara.com. 2017 [cited 11 July 2017]. Available from: https://support.certara.com/software/molecular-modeling-and-simulation/sybyl-x/
7. Koes David Ryan, Camacho Carlos J. ZINCPharmer: pharmacophore research of the ZINC database. Nucleic Acids Research. [Internet]. 2012; 7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3394271/
8. Hilbig M, Urbaczek S, Groth I, Heuser S, Rarey M. MONA – Interactive manipulation of molecule collections. 2017.
9. Lipinski C.A. Drug-like properties and the causes of poor solubility and poor permeability. Journal of Pharmacological and Toxicological Methods. 2000; 44: 235-249.
10. Wang R., Gao Y., Lai L. LigBuilder: A Multi-Purpose Program for Structure-Based Drug Design. J Mol Model 2000; 6: 498-516.
11. Discovery Studio Visualization [Internet]. Accelrys.com. 2017 [cited 11 July 2017]. Available from: http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php