JOURNAL OF
EMERGING INVESTIGATORS

Article

The design of Benzimidazole derivatives to bind to GDP-bound K-RAS for targeted cancer therapy

Gargi Sharma[1,2], Nisarg Shah[2], Aishwarya Saravanan[1,4], Harman Brah[1]

[1] Aspiring Scholars Directed Research Program, Fremont, California
[2] Round Rock High School, Round Rock, Texas
[3] Del Norte High School, San Diego, California
[4] Sharon High School, Sharon, Massachusetts

SUMMARY

KRAS is a proto-oncogene that plays an important role in cell proliferation and division. It is found to be mutated in approximately 25% of cancer tumors. Several studies have tried to find ways to inhibit the KRAS protein, with modest success. This research aims to design molecules to bind to GDP-bound KRAS to predict how different molecules might bind to an inactivated version of the protein. We observed the binding affinity of various small molecules using UCSF Chimera and Autodock Vina and modified them to enhance their affinity to GDP-bound KRAS. We hypothesized that oxygen additions to the compound would increase hydrogen bonds, which in turn would increase the thermodynamic favorability of binding. We observed that molecules with favorable free-energy binding affinities tended to have more hydrogen bonds between the ligand and the protein, and they were mostly between oxygens in the ligand and various residues on the protein. Certain benzimidazole derivatives, such as Methiazole and Fenbendazole, have been found to inhibit growth of *KRAS*-mutant cells, so we tested several benzimidazole derivatives on GDP-bound KRAS *in silico*. Irbesartan had the most favorable binding affinity after modifications as compared to the other compounds docked on GDP-bound KRAS. The modified versions of Irbesartan can bind to GDP-bound KRAS better than the original Irbesartan.

INTRODUCTION

The leading cause of cancer death in the United States is lung cancer, accounting for 150,000 deaths annually (1). Analysis of the National Human Genome Research Institute Tumor Sequencing Project revealed that the second most mutated gene in lung adenocarcinoma, one of the most frequently diagnosed lung cancers, is Kirsten Rat Sarcoma Virus (*KRAS*) (2, 3). *KRAS* is an oncogene and is part of the RAS family of genes. It plays an important role in cell proliferation and division. Mutations in this gene result in uncontrolled mitogenic signalling, leading to cancerous cell growth (1). Isoforms of RAS include *HRAS*, *NRAS*, and *KRAS*, all three of which are commonly mutated at codons 12 and 61 (4). Mutations at these codons favors binding of GTP and allows for constitutive activation of these proteins, increasing cell proliferation (4). Mutations in each gene are associated with specific types of cancers (4). Out of the three genes, K-RAS is more commonly mutated in cancers, with an overall occurrence of 22% of tumors analyzed in the Catalogue of Somatic Mutations in Cancer (COSMIC) dataset (4).

KRAS plays a role in signal transduction, and it is in the active form when bound to GTP (guanosine triphosphate) and inactive form when bound to GDP (Guanosine Diphosphate) (5). GDP bound to KRAS is displaced by a guanosine exchange factor (GEF) upon sensing an extracellular stimulus, which allows for GTP binding since it is present within the cytoplasm at a higher concentration than GDP (5). KRAS can then activate downstream pathways, such as the mitogen-activated protein kinase pathway (MAP kinase pathway), which is a signal transduction pathway that results in transcription of certain growth-promoting genes (5). KRAS GTPase activity is enhanced by GAP (GTPase Activating Protein), improving its ability to hydrolyze GTP to GDP (6). Deregulation of this process can result through a mutation in KRAS, which can lead to unresponsiveness to GAP proteins and higher association with GTP, resulting in uncontrolled signal transduction (6).

Chemotherapy is one of the most common forms of treatment for cancer (7). Even though it has been effective, chemotherapy also results in many toxicities. Since chemotherapy kills fast-growing cells, it might also affect the growth of healthy cells in the body such as hair cells and cells in the mouth, digestive tract, and reproductive system because they also divide rapidly (8). For this reason, researchers began studying different therapies that result in fewer side effects, such as targeted therapies (9). Targeted therapies attack specific proteins that affect cancer growth as compared to chemotherapy, which targets all fast-growing cells (9). In many cases, this type of therapy aims to inhibit proteins that control cell growth (9).

Early efforts to inhibit KRAS included blocking C-terminal farnesylation at the CAAX motif, a posttranslational modification necessary for protein activity (1). However, this strategy hasn't resulted in favorable outputs in stage III clinical trials, potentially because the protein was alternately being prenylated by geranylgeranyl transferase I (10, 11). Another method of inhibition attempted was to inhibit MEK, a protein activated in the MAP kinase pathway by KRAS. However, the results for non-small cell lung cancer (NSCLC) patients were not very successful (12). A possible method of targeted therapy explored in this paper is binding small molecules to GDP-bound KRAS. Stabilizing the protein in its inactivated state by binding small molecules to it can prevent further malignant growth and may better approximate normal physiology (13). Various compounds were docked on GDP-

https://doi.org/10.59720/23-166

bound KRAS to observe free-energy binding affinities and other molecular interactions such as hydrogen bonds. We focused on benzimidazole derivatives as potential KRAS inhibitors.

Benzimidazole derivatives have been known to exhibit significant activity against HIV, influenza, and human cytomegalovirus (14, 15). They have also been utilized as angiotensin II inhibitors, antimicrobial agents, and potential antitumor agents (14). Benzimidazole derivatives Methiazole and Fenbendazole have been found to inhibit cell proliferation in *KRAS*-mutant cells (16). Therefore, we docked various benzimidazole derivatives on GDP-bound KRAS *in silico* to observe free-energy binding affinities and other molecular interactions.

Ten benzimidazole derivatives were tested using UCSF Chimera and Autodock Vina softwares for protein analysis and docking visualization, respectively. Once the primary testing was completed, Irbesartan had a thermodynamically favorable free-energy binding affinity and was modified and then docked again to test if the binding affinity would become more favorable. In addition, we also modified Methiazole and Fenbendazole since they have shown promise in inhibition of KRAS activity in *KRAS*-mutant cells (16). Binding affinity denotes the molecular interactions between the ligand and protein (17). Therefore, a more negative binding affinity means stronger binding of the molecule to the protein. We hypothesized that making oxygen additions to the compounds would increase hydrogen bonds to the protein, which would in turn increase the free-energy binding affinity. Our hypothesis was based on the observation that some compounds with more thermodynamically favorable binding tended to have several hydrogen bonds between oxygen on the molecule and the GDP-bound KRAS protein.

After modifying and docking Irbesartan, Fenbendazole, and Methiazole, the compound with the lowest, and therefore most thermodynamically favorable binding affinity was Irbesartan, after the addition of two hydroxyl and one carboxyl group and a binding affinity of -9.6 kcal/mol as compared to its previous unmodified compound's binding affinity of -8.1 kcal/mol. Our hypothesis was not proven to be true because while several modifications did result in an increase in the number of hydrogen bonds, this did not always result in an improved binding affinity of the modified compound.

RESULTS

Irbesartan, Cambendazole, Eprosartan, Losartan, Methiazole, Fenbendazole, Telmisartan, Flubendazole, Lansoprazole, and Luxabendazole are benzimidazole derivatives that were first tested for binding to GDP-bound KRAS, the structure of which was obtained from RCSB Protein Data Bank and the molecules were obtained from Pubchem (18,19). They were then modified with an addition of the hydroxyl functional group (**Table 1**). The free-energy of binding to KRAS was computed for each compound. Binding affinities and hydrogen bonds were obtained from Autodock Vina and analyzed in UCSF Chimera (20-22). Binding affinities were determined by averaging the top three results out of ten that was output by Autodock Vina based on the root mean square deviation (RMSD) value and the top three out of the ten corresponding number of hydrogen bonds were averaged as well. The benzimidazole compounds we tested on GDP-bound KRAS were docked in the active site, where

Compounds tested	ΔG of original compound	Hydrogen bonds	ΔG of modified compound	Hydrogen bonds
Irbesartan	-8.1	2	-8.5	9
Cambendazole	-6.8	8	-6.7	13
Eprosartan	-7.6	11	-7.4	16
Losartan	-8	6	-8.4	9
Methiazole	-6.3	8	-6.2	8
Fenbendazole	-8.2	2	-7.9	10
Telmisartan	-8.4	3	-8.2	6
Flubendazole	-8.3	8	-8.5	11
Lansoprazole	-7.6	4	-7.8	5
Luxabendazole	-7.3	12	-8.3	14

Table 1: Binding affinities (ΔG) of original compounds compared to binding affinities of compounds modified with the hydroxyl functional group. Binding affinity is measured in kcal/mol. The binding affinity and number of hydrogen bonds represents the averaged value from the top three values based on the RMSD value output by Autodock Vina. Binding affinities and hydrogen bonds were generated by Autodock Vina and were visualized in UCSF Chimera (20-22). Modified compounds were drawn and optimized in Avogadro (26).

residues TYR32, ALA18, SER17, GLY13, LEU19, and GLY15 are located (**Figure 1**). This was determined by comparing the docked model to KRAS with GDP bound to it and observing the position in relation to the presence of the Mg^{2+} ion (**Figure 1**). Most molecules are bound in this active site.

Our goal was to increase the number of hydrogen bonds through oxygen additions to further increase the thermodynamic favorability of their binding to KRAS. Previous observations of docking results of Irbesartan, Losartan, Flubendazole, Lansoprazole, and Luxabendazole showed that compounds that had better free-energy binding affinities tended to have greater hydrogen bonds between oxygens in the molecules and GDP-bound KRAS within a maximum value 4 angstroms (**Table 1**). After primary modifications of Irbesartan, Cambendazole, Eprosartan, Losartan, Methiazole, Fenbendazole, Telmisartan, Flubendazole, Lansoprazole, and Luxabendazole, we focused on modifications of Irbesartan, Methiazole, and Fenbendazole (**Table 1**). Irbesartan had the most favorable binding affinity

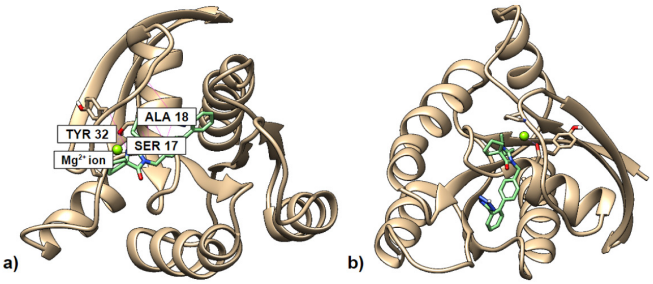


Figure 1: Comparison of GDP-bound KRAS binding to a modified version of Irbesartan with an ester addition versus the unmodified version of Irbesartan. a) Irbesartan with an ester addition docked on GDP-bound KRAS. **b)** Irbesartan docked on GDP-bound KRAS. The green sphere in the structure represents an Mg^{2+} ion. The pink lines depict hydrogen bonds. Protein-ligand complex was visualized in UCSF Chimera and hydrogen bonds were generated after running Autodock Vina (20-22).

https://doi.org/10.59720/23-166

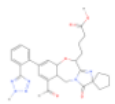
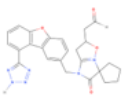
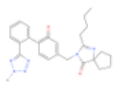
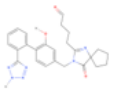
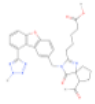
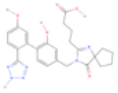
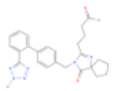
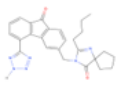
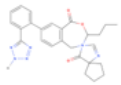
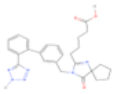
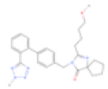
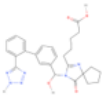
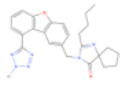
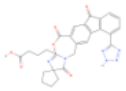
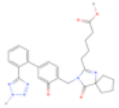
ΔG	Hydrogen bonds	Molecular structure of modified compounds	ΔG	Hydrogen bonds	Molecular structure of modified compounds
-6.6	7		-8.7	9	
-7.9	5		-8.7	11	
-8	9		-8.8	11	
-8.3	8		-8.9	7	
-8.3	6		-9.1	10	
-8.5	8		-9.2	12	
-8.6	5		-9.2	11	
			-9.4	11	

Table 2: Results of modifications that contained only carbon and oxygen that were made to Irbesartan. The binding affinity and number of hydrogen bonds have been averaged from the top three results of each test. ΔG depicts the binding affinity in kcal/mol. The blue letters in the molecular structures of the compounds indicate nitrogen, red indicates oxygen, and gray indicates hydrogen. All docking was done in Autodock Vina and visualization of data in UCSF Chimera (20-22). The modifications to Irbesartan were made in Avogadro (26).

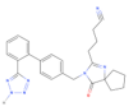
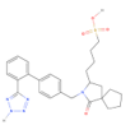
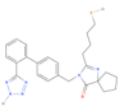
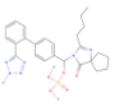
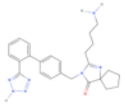
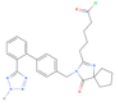
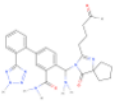
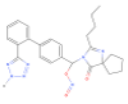
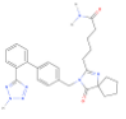
ΔG	Hydrogen bonds	Molecular structure	ΔG	Hydrogen bonds	Molecular structure of modified compounds
-6.8	4		-8.9	14	
-7.8	4		-8.2	15	
-7.9	9		-8.4	7	
-8.1	12		-8.5	9	
			-8.7	12	

Table 3: Results of modifications made to Irbesartan that contained elements other than carbon and oxygen. The binding affinity and number of hydrogen bonds have been averaged from the top three results of each test. ΔG depicts the binding affinity in kcal/mol. The blue letters in the molecular structures of the compounds indicate nitrogen, red indicates oxygen, gray indicates hydrogen, yellow indicates sulfur, orange indicates phosphorus, and neon green indicates chlorine. All docking was done in Autodock Vina and data visualization in UCSF Chimera (20-22). The modifications to Irbesartan were made in Avogadro (26).

out of the ten compounds, and Methiazole and Fenbendazole were utilized to further test our hypothesis because they were found to be promising compounds in inhibiting KRAS in *KRAS*-mutant cells *in vitro* (16). The following functional groups were added to Irbesartan: carboxyl, ester, aldehyde, nitrite, ether, ester, amine, carbonyl, ketone, phosphate group, sulfonate, nitrile, amide, sulfhydryl, acid chloride, and hydroxyl. They were added in different combinations (**Table 2 and 3**).

Compounds with modifications that contained oxygens and nitrogens were found to have a higher hydrogen bond count

The binding affinity of unmodified Irbesartan was -8.1 kcal/mol with 1 hydrogen bond. 17 out of 24 modifications of Irbesartan had improved binding affinities and featured higher numbers of hydrogen bonds than unmodified Irbesartan (**Table 2 and 3**). Modifications that added electron acceptors, particularly oxygen and nitrogen, tended to have a higher number of hydrogen bonds (**Table 2 and 3**). The molecule with the most favorable recorded binding affinity was Irbesartan with the addition of two hydroxyl and one carboxyl groups with a binding affinity of -9.6 kcal/mol and 15 hydrogen bonds. The difference between this modification and unmodified Irbesartan was -1.5 kcal/mol and 14 hydrogen bonds.

Additionally, the average binding affinity of all molecules that contained only carbon and oxygen was -8.5 kcal/mol compared to an average binding affinity of molecules that contained other elements such as nitrogen, phosphate, sulfur, chlorine, which was -8.1 kcal/mol (**Table 2 and 3**). Modifications with carbon and oxygen modifications had a more favorable average binding affinity by a difference of -0.4 kcal/mol. The most favorable binding affinity was -9.6 kcal/mol for a modification that contained only carbon and oxygen, and -8.9 kcal/mol was the most favorable binding affinity for modifications that contained other elements than carbon and oxygen such as nitrogen, phosphate, sulfur, and chlorine (**Figure 2**). In general, compounds with modifications containing only carbon and oxygen had more favorable binding affinities compared to modifications that contained other elements such as nitrogen, sulfur, phosphate. Carboxyl, hydroxyl, ketone, ester, and carbonyl additions resulted in

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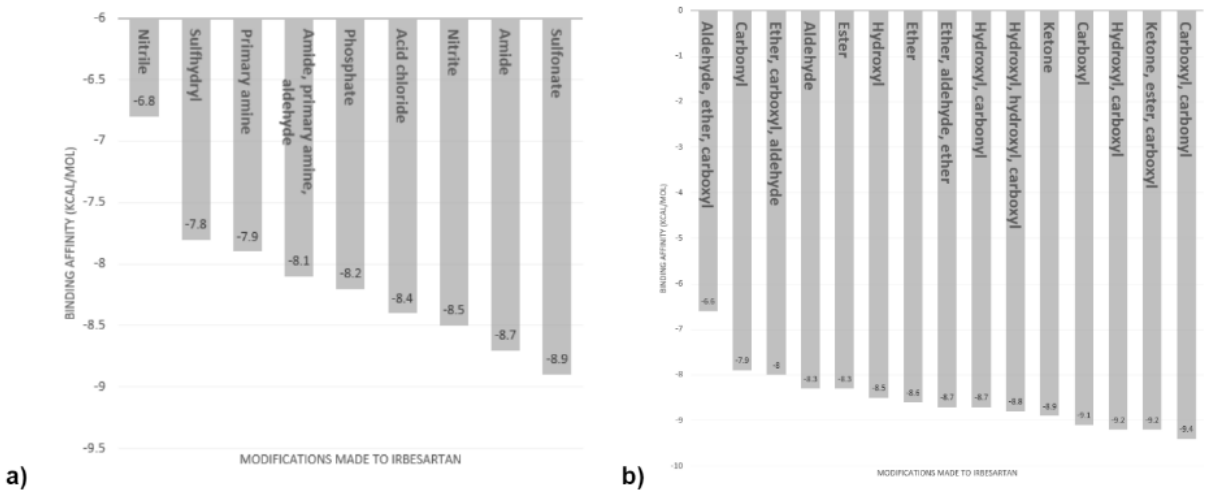

Figure 2: Comparison of binding affinities with different modifications to Irbesartan. The modifications are functional groups that were added to Irbesartan. **a)** Binding affinities in kcal/mol of modifications with functional groups that contained exclusively carbon, oxygen and hydrogen atoms. **b)** Binding affinities of functional groups that had elements other than carbon, oxygen, and hydrogen, such as nitrogen, sulfur, chloride, and phosphorus. In general, the modifications in **(a)** had lower and therefore more favorable binding affinities than those in **(b).** Binding affinities were generated in Autodock Vina and protein-ligand complex was visualized in UCSF Chimera (20-22).

binding affinities lower than -9 kcal/mol, which means they were more favorable than the original Irbesartan compound (**Figure 2**). Most modifications with nitrogen additions, such as nitrile and primary amine groups, had weaker predicted binding affinities (**Table 3**). While some modifications such as the sulfhydryl, nitrile, and primary amine modifications did have a greater number of hydrogen bonds than that of the original Irbesartan compound, not all of them had a greater binding affinity to GDP-bound KRAS compared to Irbesartan (**Table 3**). Therefore, increasing the number of hydrogen bonds did not always result in a more favorable binding affinity.

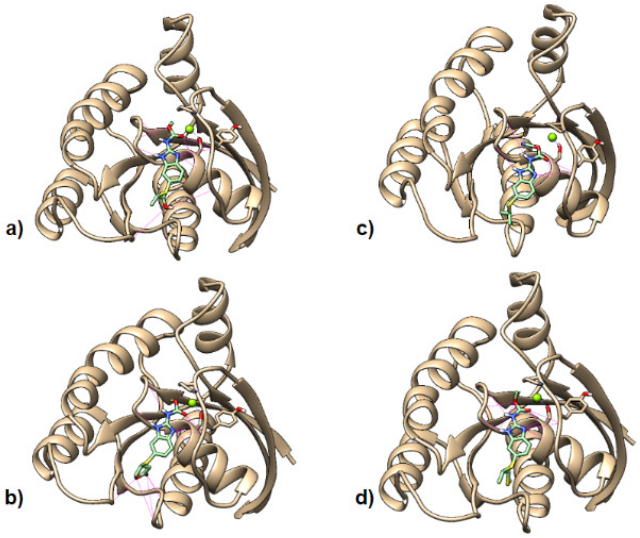

Figure 3: Modifications of the Methiazole compounds. a) Carboxyl functional group addition. **b)** Ester functional group addition. **c)** Nitrite functional group addition. **d)** Sulfhydryl functional group addition. Binding affinities and hydrogen bonds (pink lines) were generated in Autodock Vina and visualized in UCSF Chimera (20-22). Modified compounds were drawn and optimized in Avogadro (26).

Modifications of Methiazole and Fenbendazole show a correlation between oxygen additions and binding affinity

To further test our hypothesis that oxygen additions to the compound would increase hydrogen bonds, which in turn would increase the thermodynamic favorability of binding, we tested modifications of Methiazole and Fenbendazole in addition to Irbesartan. Carboxyl, ester, nitrite, and sulfhydryl groups were added to the compounds. The ester modification of the Methiazole compound had the most improved binding affinity of -7.4 kcal/mol compared to the binding affinity of -6.3 kcal/mol of the original Methiazole compound (**Figure 3 and 4**). The nitrite modification of the Fenbendazole compound had the most improved binding affinity of -8.9 kcal/mol compared to the binding affinity of -8.2 kcal/mol of the unmodified Fenbendazole compound (**Figure 3**). The general trend in the modifications of the Methiazole compound was that modifications with oxygens resulted in a more favorable

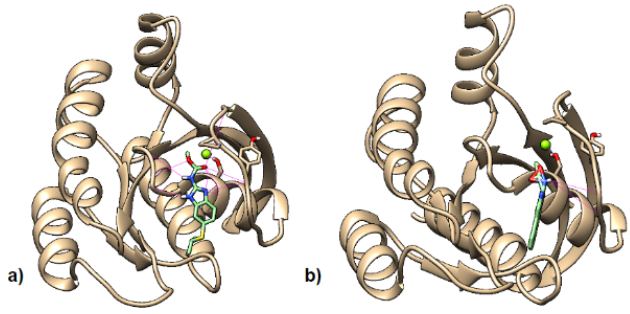

Figure 4: Protein-ligand visualization of Methiazole and Fenbendazole compounds docked on GDP-bound KRAS. a) Methiazole docked on GDP-bound KRAS. **b)** Fenbendazole docked on GDP-bound KRAS. Pink lines in both panels denote hydrogen bonds between the ligand and protein. Both compounds were obtained from PubChem (19). Autodock Vina was used for molecular docking to generate binding affinities and hydrogen bonds and UCSF Chimera was used for visualization (20-22).

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binding affinity to the protein because nitrile, carboxyl, and ester modifications all had a more favorable binding affinity compared to the unmodified Methiazole compound. The nitrile modification had an average binding affinity of -7 kcal/mol and the carboxyl modification had an average binding affinity of -6.8 kcal/mol (**Figure 3**). The sulfhydryl modification had an average binding affinity of -6.3 kcal/mol, which is equal to the binding affinity of Methiazole (**Figure 3 and 4**).

This trend was also observed in the modifications of Fenbendazole compounds, where the nitrile modification had a more favorable binding affinity than that of the unmodified Fenbendazole compound, which had an average binding affinity of -8.2 kcal/mol (**Figure 4**). The carboxyl modification had an average binding affinity of -8 kcal/mol and the ester modification had an average binding affinity of -8 kcal/mol as well (**Figure 3**). The sulfhydryl modification had an average binding affinity of -7.3 kcal/mol, which is lower than the binding affinity of the original Fenbendazole compound (**Figure 4 and 5**). The modification in both Methiazole and Fenbendazole compounds that had the highest and least favorable free energy of binding was the addition of a sulfhydryl functional group (**Figure 3 and 5**).

Regarding hydrogen bonds, no clear correlation between binding affinity and hydrogen bonds was observed, as the modification of Methiazole with a carboxyl group created the greatest number of hydrogen bonds of 14 but resulted in the second lowest average binding affinity out of all four modifications (**Figure 3**). The modifications of the Fenbendazole compound showed a similar pattern, because while the sulfhydryl modification had a greater number of hydrogen bonds than the Fenbendazole compound, it had a less favorable binding affinity (**Figure 5**).

In the docking results we also observed the side chain interactions during hydrogen bonding. The modified molecules commonly interacted with certain residues on the GDP-bound KRAS. We found that TYR 32, ALA 18, and SER 17 were quite frequently interacted with during hydrogen bonding of different modified versions of the Irbesartan compound (**Figure 1**). After modifying various compounds, Irbesartan had the most improved average binding affinity of -9.4 kcal/mol with 11 hydrogen bonds after the addition of carboxyl and carbonyl groups (**Table 2**).

DISCUSSION

We first tested 10 small molecule benzimidazole derivatives on GDP-bound KRAS and recorded their best free-energy binding affinity and number of hydrogen bonds (**Table 1**). We then modified the benzimidazole derivatives with hydroxyl groups and monitored their average binding affinities and hydrogen bonds (**Table 1)** Our premise for modification was to increase hydrogen bonds by adding oxygens. After primary modifications where 10 compounds were modified with a hydroxyl group, Irbesartan had the most improved thermodynamic favorability, and therefore we selected it as the focus of our research in addition to Methiazole and Fenbendazole because they were found to cause cytotoxicity in KRAS-mutant cells in a previous study (16). We designed 24 modifications and added functional groups such as carboxyl, carbonyl, nitrile, and sulfonate functional groups to Irbesartan, Methiazole, and Fenbendazole, and docked the modified molecules on GDP-bound KRAS to record their average binding affinity and hydrogen bonds within 4 angstroms of

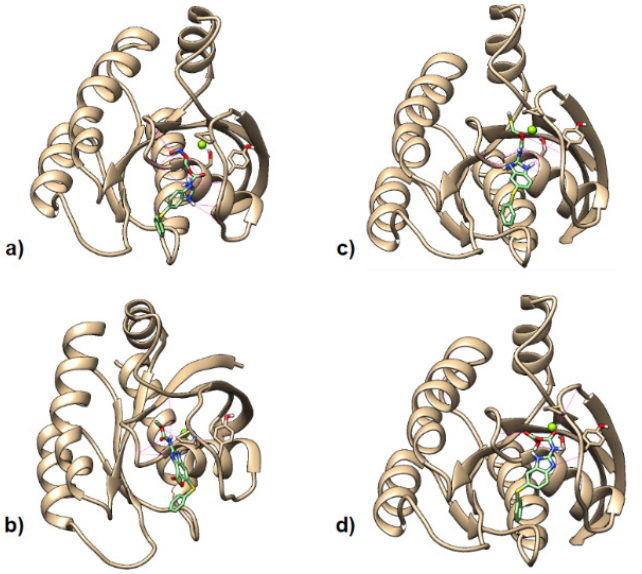

Figure 5: Comparison of KRAS binding to modified Fenbendazole compounds. a) Nitrile functional group addition. **b)** Ester functional group addition. **c)** Sulfhydryl functional group addition. **d)** Carboxyl functional group addition. Binding affinities and hydrogen bonds (pink lines) were generated from Autodock Vina and visualized in UCSF Chimera (20-22). Modified compounds were drawn and optimized in Avogadro (26).

the protein. 17 modified Irbesartan compounds were more thermodynamically favorable than the original Irbesartan compound, suggesting that these modified molecules will have a better binding affinity to GDP-bound KRAS than the original Irbesartan. After various functional group additions to Irbesartan, we observed that modifications with oxygen and nitrogen additions tended to have a higher number of hydrogen bonds compared to other modifications. This was observed in modifications of the Irbesartan compound such as nitrile, carboxyl, amide, and ketone.

In all the modifications, we observed that modifications that added oxygens to the compound generally improved the binding affinity, as seen in modifications of carboxyl, nitrite, ester, ketone, aldehyde, hydroxyl, carbonyl, amide, acid chloride, sulfonate, and phosphate group. A possible reason for this observation may be that they increased hydrogen bonds, which correspond to more molecular interactions between the ligand and GDP-bound KRAS. Greater amount of molecular interactions will increase the strength of binding between the ligand and protein, therefore making the binding affinity between the compound and protein more favorable. Hydrogen bonds generally form between oxygens, nitrogens, fluorine, and hydrogens due to the partial negative and positive charges. This would explain the increase in hydrogen bonds between modified compounds and GDP-bound KRAS observed after the addition of functional groups that contained oxygen and nitrogen.

To further test our hypothesis, we also made some modifications to Methiazole and Fenbendazole, which have previously been found to have cytotoxic effects in KRAS-mutant cells (16). We made four modifications to each compound: a carboxyl addition, ester addition, sulfhydryl addition, and a nitrite addition. However, there was no clear correlation between oxygens and hydrogen bonds because

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the sulfhydryl group addition for Methiazole resulted in the greatest number of hydrogen bonds, but had the least favorable binding affinity (**Figure 3**). Even though the sulfhydryl modification itself doesn't have any oxygens present, the Methiazole compound does have oxygens, which is a likely reason why the modified compound created hydrogen bonds. The addition of the sulfhydryl functional group may have caused a change in the compound's original geometry which possibly allowed the oxygens in the compound to form more hydrogen bonds with GDP-bound KRAS. A probable reason the interaction between the sulfhydryl modified compound and GDP-bound KRAS didn't result in a more favorable binding affinity is because the geometry of the compound may not be complementary to the binding pocket of the protein. Thus, our hypothesis that increasing hydrogen bonds would lead to higher thermodynamic favorability was not true in all cases.

The outputs of modified versions of Irbesartan suggest that there are ways to improve the binding affinity of this compound to GDP-bound KRAS. Increasing the binding affinity of a molecule to a protein can aid in drug development. However, binding affinity is not the sole factor to rely on when researching inhibitors for a protein, as it is possible that *in silico* predicted binding affinities may not be accurate for real-life cell models. *In silico* testing is beneficial because it can predict how compounds might bind to a protein and can help eliminate compounds that don't work well at a faster speed than *in vitro* testing can, and can also save expenses of testing compounds in a laboratory (23). However, this testing may not always be accurate because computer simulations cannot account for all molecular interactions that might occur inside a cell or the side effects a compound might have on the normal functioning of other proteins or organelles. Therefore, *in vitro* testing is still an important method of testing compounds to observe cellular interactions. Binding affinity generated from computer simulations serves as a prediction that can help us understand how the protein and molecule might interact in a cell (24). Docking molecules on GDP-bound KRAS serves as a model of normal physiology and the interactions observed between the unmutated protein with a ligand can help predict how the molecule might bind to a mutated version of the protein, aiding in molecular design.

Using the modified versions of Irbesartan discussed in this paper to inhibit KRAS could represent a form of targeted therapy. In terms of future research, these modified derivatives of Irbesartan could be tested for binding to GDP-bound KRAS *in vitro*. Testing these modified compounds in cells with GDP-bound KRAS will help us understand if the difference in KRAS binding between the unmodified Irbesartan and a modified compound would alter cell proliferation. These molecules could be tested on mutated versions of KRAS proteins to determine the strength and specificity of their inhibition. The three most common KRAS mutations observed in Non-Small Cell Lung Cancer are G12C, G12D, and G12V (25). KRAS is found to be commonly mutated on codons 12, 13, or 61 (25). Therefore, KRAS proteins with mutations in these codons or these amino acids would ideally be most strongly targeted for inhibition.

MATERIALS AND METHODS

Molecular docking was conducted in AutoDock Vina with the help of UCSF Chimera for visualization of the docking (20-22). GDP-bound KRAS was obtained from RCSB Protein Data Bank (PDB ID 4obe), and ligands were downloaded from Pubchem (18, 19). Avogadro was utilized for building molecules with various additional functional groups for the purpose of modifying the molecule and enhancing its binding affinity (26).

Chimera and AutoDock Vina

All molecular graphics and analyses were performed with UCSF Chimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311 (22). UCSF Chimera is a software used for docking visualization. Chimera was utilized for docking visualization and docking preparation (22). GDP-bound KRAS was opened by fetching it by the ID 4obe from RCSB PDB (18). The B-chain of the protein was deleted because the protein in the PDB is a dimer, so both chain A and chain B have the same sequence. Either one of the proteins can be deleted, but we deleted the B chain for consistency in our method. After that, the Dock Prep tool in the Surface/Binding Analysis section is utilized for preparing the protein with all the default settings (27, 28). Once the protein is prepared for docking, the ligand is opened and Autodock Vina is run (20-22). The search volume of the protein was set with the center at [-5.32063, -22.3516, 44.6659] and the size at [46.3556, 44.0087, 43.5389].

AutoDock Vina is a program utilized for molecular docking (20, 21). After docking the molecule, AutoDock Vina provides the free-energy binding affinity and number of intermodal hydrogen bonds, visualized by colored lines. The hydrogen bonds that were noted were within constraints of 4 angstroms. Binding affinity and hydrogen bonds for each compound were averaged from the top three values output in Autodock Vina out of ten based on the root mean square deviation (RMSD) value.

Avogadro

Avogadro is a tool that we used in order to create and edit different ligands to bind with the KRAS protein (26). This software allows us to create various molecular structures with atoms and bonds but can also edit pre-existing molecules (26). After drawing the modified molecule, we optimized the geometry for each molecule with the molecular force field of MMFF94 in the Auto Optimize function which was run until dE = 0 (26). The modified molecule was then tested using the same process as for an unmodified molecule.

Using Avogadro, we modified Irbesartan, Cambendazole, Eprosartan, Losartan, Methiazole, Fenbendazole, Telmisartan, Flubendazole, Lansoprazole, and Luxabendazole with a hydroxyl group and then re-tested the new modified compounds in UCSF Chimera and AutoDock Vina to test binding affinities (**Table 1**). The second stage of docking included 24 modifications to Irbesartan with functional group additions of carboxyl, ester, aldehyde, nitrite, ether, ester, amine, carbonyl, ketone, phosphate group, sulfonate, nitrile, amide, sulfhydryl, acid chloride, and hydroxyl, in different combinations (**Table 2 and 3**). Methiazole and Fenbendazole were also modified to further test our hypothesis with the functional group additions of carboxyl, ester, nitrite, and sulfhydryl groups (**Figure 3 and 5**).

https://doi.org/10.59720/23-166

Received: July 15, 2023
Accepted: November 1, 2023
Published: April 25, 2024

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