

Repurposing of Nemiralisib as a Potential CDK4 Inhibitor for Breast Cancer Treatment: A Ligand-Based Virtual Screening and Docking Study

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ABSTRACT

As part of the drug repurposing efforts, a pliable PI3K inhibitor Nemiralisib that was developed for use in respiratory diseases was studied for her potential use as a new CDK4 inhibitor in the treatment of breast cancer. Utilization of ligand based virtual screening and molecular docking studies further revealed an appreciable binding affinity of Nemiralisib to CDK4 with a docking score of – 11.1 which is markedly similar to that of an already established CDK4/6 inhibitor, Abemaciclib (-11.5). From structural studies, it was clear that Nemiralisib binds to the CDK4 binding pocket using several residues such as GLU11, ILE12 and ASP97 in a way similar to how Abemaciclib does. Moreover, additional synergy prediction studies using the PISCEScsm platform anticipated the effects of the combination, especially in breast cancer of Nemiralisib with Fulvestrant and Tamoxifen to be more than additive. All these data support the idea that Nemiralisib may be a useful drug to add to the treatment of hormone receptor-positive breast cancer and improve currently existing therapies. This study opens doors of using Nemiralisib in the field of oncology, with an encouraging avenue of treatment for breast cancer patients.

Keywords: Drug Repurposing, Nemiralisib, CDK4 Inhibition, Breast Cancer, Virtual Screening, Molecular Docking, Synergy Prediction, Fulvestrant, Tamoxifen, In Silico Studies, Cancer Therapy, CDK4/6 Inhibitors.

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INTRODUCTION

Drug repurposing is an innovative strategy that seeks to identify new therapeutic uses for existing drugs. This approach offers a faster and more cost-effective route for drug development compared to traditional drug discovery methods, which often require extensive time and resources for safety and efficacy testing.¹ Drug repurposing is especially valuable in oncology, where the urgent need for novel therapeutic agents is critical given the complex and heterogeneous nature of cancer.² By leveraging pre-existing clinical and pharmacological data, researchers can reduce the risk associated with developing entirely new compounds and rapidly transition repurposed drugs into clinical trials for new indications.

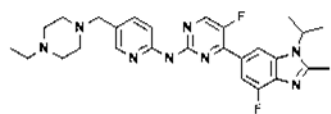
One of these areas is the inhibition of cyclin dependent kinases (CDKs), which play an important role in the cell cycle and cell proliferation. The alterations of CDK functions, particularly CDK4, CDK6 and CDK8 have been reported in many forms of cancers, breast cancer being one of them. Kinase inhibitors have been developed for these enzymes since it is recognized that inhibition of certain pathways axes that promote tumor growth and cell proliferation transverse to allowing such cancer growth is effective in cancer management. The drug Abemaciclib is a typical example of a drug which inhibits CDK4/6 and is FDA approved for HR-positive breast cancer that is non

amplified of HER2. Abemaciclib, being a drug that inhibits division of cancer cells by inhibiting CDK4 and CDK6 makes the drug a significant point of reference for evaluation of other drug inhibitory candidates active against other CDK family members in oncogenesis.³

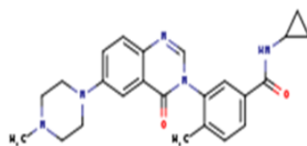
Among various CDKs CDK4 especially becomes a potential target in breast cancer. It is CDK4 that is overexpressed in many cell types, along with other agents that promote the formation of c-Myc oncogenic transactivation complex. CDK4, as a member of the Mediator Complex, is engaged in transcriptional regulation and is able to modulate other pathways related to cancer, such as Wnt/ β -catenin activity, and different oncogenic factors, including Myc. Increased CDK4 levels have been associated with increased tumor aggressiveness and unfavorable outcomes in various malignancies, providing a strong rationale to develop CDK4 inhibitors as treatment options. Nevertheless, selective CDK4 blockers remain in their infancy, which allows for drug repurposing to enhance the discovery of therapies in this area.⁴

Thus, in the current work we concentrate on Nemiralisib, a drug that suppresses the activity of PI3K, which was created to treat chronic obstructive pulmonary disease (COPD) and asthma.⁵ With the expectations of inhibiting the invasive PI3K pathway, which is active in inflammation, Nemiralisib is presently in Phase II clinical

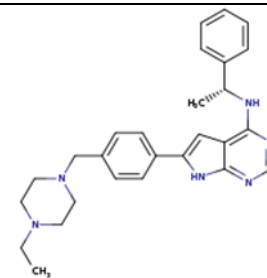
Table 1: Top 10 compound from swissimilarity vertical screening



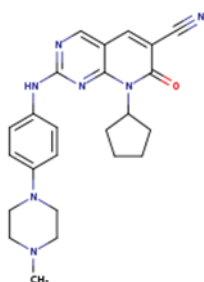
Abemaciclib



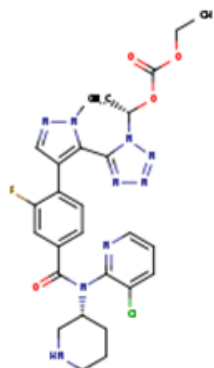
Compound 1



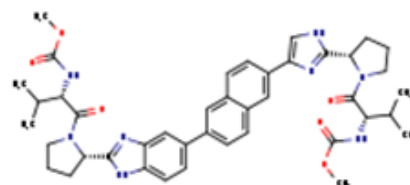
Compound 2



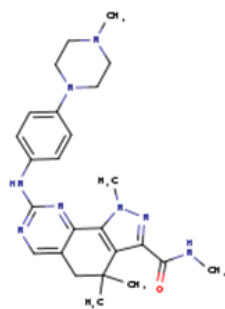
Compound 3



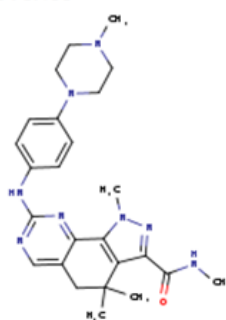
Compound 4



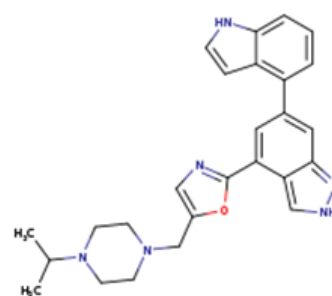
Compound 5



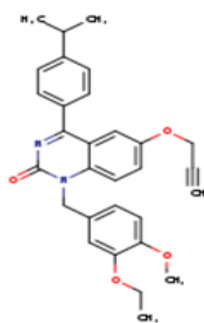
Compound 6



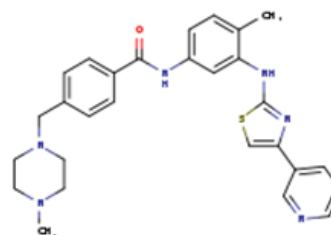
Compound 7



Compound 8



Compound 9



Compound 10

trials, thereby indicating that it has a relatively good safety index.⁶ With that information in mind, we wanted to evaluate how well CDK4 inhibitor activity could be achieved with a drug such as Nemiralisib, which we sought to relinquish in the context of breast cancer treatment. Abemaciclib was chosen as the test ligand because of its demonstrated use in treating breast cancer. Using a molecular editing suite called SwissSimilarity, the

CHEMBL clinic database was searched for similar compounds which led to the identification of Nemiralisib. The binding affinity of Nemiralisib with CDK4 was further confirmed by performing docking studies using the CB-Dock2 server which reported extensive interactions within the active site of CDK4. Furthermore, since Abemaciclib is intended to be combined with standard treatment regimens with drugs such as fulvestrant and tamoxifen to improve efficacy and address resistance, it

Table 2: Docking score obtained after docking in CB-DOCK 2 Server

Sr. No.	Score
Abemaciclib	11.5
Compound 1	-9.8
Compound 2	-7.0
Compound 3	-6.2
Compound 4	10.1
Compound 5	10.1
Compound 6	10.1
Compound 7	10.2
Compound 8	11.2
Compound 9	9.7
Compound 10	-7.5

was also evaluated together with the standard breast cancer agents for synergistic potential. It was therefore envisaged that the combination of Nemiralisib with these agents would yield a synergistic effect suggesting that it may enhance the effects of already existing treatments for breast cancer.

The results of this work provide strong supporting evidence that Nemiralisib is a candidate drug for repurposing for the inhibition of CDK4 in breast cancer. Such repurposing takes advantage of the drug's known pharmacokinetics and safety profile and seeks to reposition the compound for a new indication in cancer therapy when the compound was initially developed for other uses. However, further in vitro and in vivo studies would be important to understand better the potential of Nemiralisib in breast cancer therapy.

METHODS

Virtual Screening

Database Preparation

The ChEMBL_clinic database from SwissSimilarity was carefully filtered in order to eliminate the presence of molecules containing less than six heavy atoms, having a molecular weight exceeding 1500 g/mol, or containing unusual types of atoms. Molecules were subject to structural standardization in the course of which fragments were selected, the molecules were neutralized and protomeric states were calculated. Several different fingerprints were generated namely, FP2, ECFP4, MHFP6, pharmacophore, ErG, E3FP and ES5D all at a pH of 7.4 to be in line with physiological conditions.⁷

Ligand-Based Virtual Screening

As a ligand search reference, the known anticancer activity of Abemaciclib makes it an ideal choice. SwissSimilarity

screening yielded 68 compounds, which were further filtered according to docking scores in order to isolate highaffinity candidates.

Docking Studies

Based on CB-Dock2⁸, we began our docking studies by retrieving the three-dimensional structure of the CDK4 protein from the protein data bank (PDB) for as unobstructed and biologically pertinent model as possible for binding analysis.⁹ The structure that was retrieved was also evaluated and of sufficient quality; there was also a chain-specific targeting for the active form of CDK4 as well. The ligands which were to be used were obtained by writing the SMILES notation in SDF and PDB formats using NovaProLab, a popular molecular modeling docking applications that converts structural data into appropriate formats.¹⁰ Such a preparation enabled architectural alteration in a systematic manner, also ensuring the ligands are in the best conformational states for the purpose of the docking exercise. The cysteine free enzyme and the prepared schematic representation of the ligands were uploaded to the platform called the CB-Dock2 which is interactive blind docking and part of computational core analysis of chemical structures used in prediction of possible binding sites on a given protein.

Labeled binding cavities were visualized by CB-Dock2 within the structure of the protein following a penalization strategy for estimating binding of each ligand to the receptor. This strategy of blind docking permitted visualization of regions on CDK4 that could easily accommodate the test compounds and CB-Dock2 generated possible scoring for each of the regions.

Synergy Prediction Studies

In order to conduct a synergistic prediction study on PISCEScsm, please navigate to the website located at <https://biosig.lab.uq.edu.au/piscescsm/> and collect the SMILES codes for the drugs of your choice for example NEMIRALISIB (which is a drug that has been repurposed) and drugs that are primarily used in the treatment of breast cancers such as Fulvestrant and even Tamoxifen which are available in the site databases of PubChem or ChEMBL.¹¹

RESULTS

Results of Ligand-Based Virtual Screening

Table 1 gives the list of drugs screened with their structures.

Results of Docking Studies

The analysis of docking outcomes rendered in table 2

Table 3: Docking Scores and Protein Interactions found in Abemaciclib and Nemiralisib

Sr. No.	Compound	Docking Score	Pocket	Key Interacting Residues
1	Abemaciclib	-11.5	C2	GLU11, ILE12, GLY13, VAL14, GLY15, ALA16, TYR17, VAL20, ALA33, LYS35, GLU56, VAL72, PHE93, GLU94, HIS95, VAL96, ASP97, GLN98, ASP99, THR102, ASP140, LYS142, GLU144, ASN145, LEU147, ALA157, ASP158, PHE159, GLY160, LEU161, THR177
2	Nemiralisib	-11.1	C2	VAL20, LYS22, ALA33, LYS35, GLU56, VAL72, PHE93, GLU94, HIS95, VAL96, ASP97, GLN98, ASP99, THR102, ASP140, LYS142, GLU144, ASN145, LEU147, ALA157, ASP158, PHE159, LEU161

Table 4: Summary of synergy predictions for Nemiralisib in combination with Fulvestrant and Tamoxifen across different cancer types

Drug Combination	General Prediction	Breast Prediction	Prostate Prediction	Melanoma Prediction	Lung Prediction
Nemiralisib + Fulvestrant	Synergistic	Synergistic	Synergistic	Antagonistic	Synergistic
Nemiralisib + Tamoxifen	Synergistic	Synergistic	Synergistic	Synergistic	Antagonistic

provides relevant information on the binding affinities of the different compounds, which ranked in favor of Abemaciclib, with a binding score of 11.5 for this compound being the highest, thus proving that this compound has great potential in being a CDK4/6 inhibitor. In this analysis, compounds 1 – 10 were evaluated with respect to the aforementioned scores of compounds and their order ranging between – 9.8 and 11.2 was found.

Compound 8, which possessed a score of 11.2, was notably very close to that of Abemaciclib, thus reinforcing its view as an attractive drug to be exploited against the CDK4 target in breast cancer. This indicates that compound 8 has some resemblance with Abemaciclib in terms of both structural and functional attributes, thus becoming a candidate to be repurposed in the treatment of cancer. The results of the docking studies also created an emphasis on the aspect of binding affinity in the search of reactive compounds. The high docking score attained by Abemaciclib reiterates its proven value in the many applications of CDK4/6 inhibition that have been especially in common use in the treatment of hormone receptor-positive breast cancers. The favorable docking scores of compound 8 indicate that this compound might be worth exploring further as a CDK4 inhibitor for possible use in the treatment of breast cancer. Nevertheless, experimental studies including in vitro as well as in vivo assays are warranted to further evaluate the therapeutic effectiveness and safety of this compound. The findings emphasize the role of molecular docking in the search for new drug candidates and assist in drug repositioning for the treatment of cancer.

Nemiralisib exhibited significant docking scores and shared multiple key interactions illustrated in figure 1, 2 and 3 with Abemaciclib, listed in table 3 indicating its potential to inhibit CDK4 similarly.

Results of Synergy Prediction Studies

The application of the PISCEScm platform to evaluate the potential synergies of fulvestrant and tamoxifen combinations has been overall favorable for all cancer types assessed, especially breast cancer. This synergy is indicative of the potential benefits that can be gained from NEMIRALISIB in the treatment of Hormone Receptor-Positive Breast cancer as an add-on therapy.

The results of the synergy predictions for the drug Nemiralisib, when given alongside Fulvestrant and Tamoxifen, are beneficial for the treatment of breast cancer. Nemiralisib in association with Fulvestrant has been shown to exert synergism in malignancies of breast, lung, everyday, and prostate cancers indicating that this drug could potentiate the effects of Fulvestrant in select cases, breast cancer. This is consistent with the use of

Fulvestrant in patients with breast cancer, which is positive for hormone receptor, emphasizing Nemiralisib as a potential additive in situations when patients are likely to benefit from CDK4 inhibition.

Likewise, combining Nemiralisib with Tamoxifen was also synergistic in breast, general as well as melanoma, and prostate cancers, making it more credible as an adjunct therapy in breast cancer management. On the other hand, the antagonistic results obtained in ovarian, colon, and lung cancers suggest that Nemiralisib may not be equally effective in treating all types of cancers and therefore there is need for further investigation to determine the safety and effectiveness of using this drug with other treatments. These results reinforce the belief that Nemiralisib can be used in Nemiralisib, especially with standard treatments for breast cancer.^{12,13}

DISCUSSION

Originally intended as a pharmacological inhibitor of the PI3K for applications in respiratory diseases, Nemiralisib has shown high affinity for binding CDK4, an important kinase involved in breast cancer disease progression (Table 4). Because of our docking scores and also looking at the similarity of the structure to Abemaciclib, we can also view Nemiralisib as a potential CDK4 inhibitor. In addition, the ability of Nemiralisib to predict synergy with other agents such as fulvestrant and tamoxifen in breast cancer treatments further supports the notion of repurposing it for these other uses.^{14,15}

CONCLUSION

In the present study, we evaluated the activity of Nemiralisib, which was initially designed as a PI3K inhibitor for respiratory disorders, in CDK4 directed therapy of breast cancer. Through ligand-based virtual screening, Nemiralisib emerged as the top candidate, and its conformation around CDK4 showed a docking and binding score, which was -11.1 and was comparable to that of Abemaciclib, whose score was -11.5. The binding interactions of Nemiralisib with residues in CDK4 such as GLU11, ILE12, and ASP97 were comparable to those of Abemaciclib, which is known to inhibit CDK4/6. The implication from these results is that Nemiralisib can be used clinically in place of other drugs acting against CDK4 in breast cancer management.

Moreover, studies predicting synergism with the use of PISCEScm platform also showed exceedingly encouraging prediction of the synergism of Nemiralisib with Fulvestrant and Tamoxifen in breast cancer, where such effects were noted in several types of cancers, breast in both combinations and general cancer Nemiralisib +

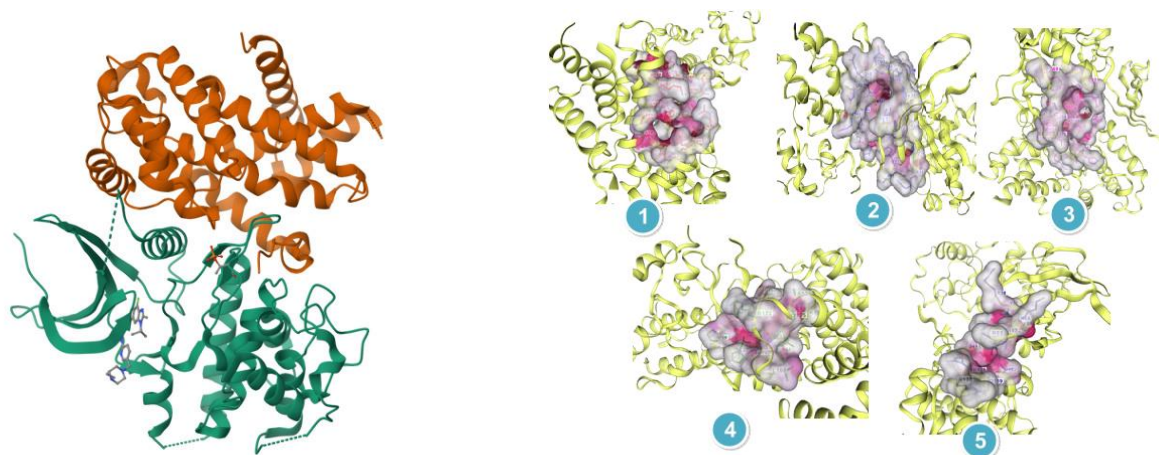


Figure 1: Protein structure (left) and Cavities detected in CDK-8 protein (PDB ID: 7SJ3) (right)

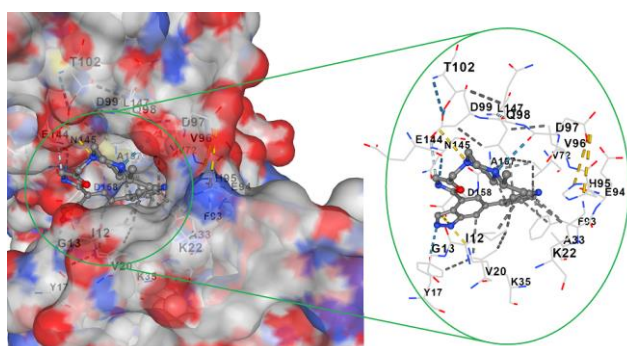


Figure 2: Interaction between CDK-8 and Abemaciclib

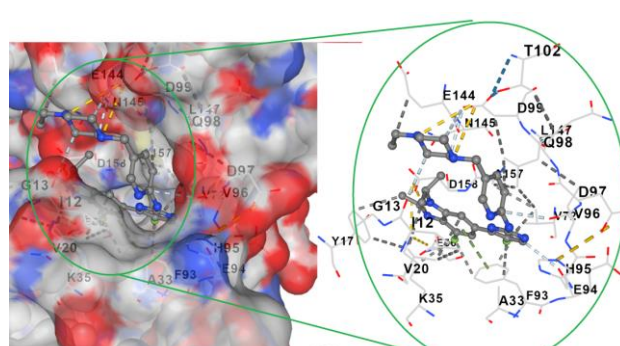


Figure 3: Interaction between CDK-8 and Nemiralisib

Fulvestrant. This validates the notion that these therapies can be combined with Nemiralisib for better outcomes. These results position Nemiralisib as one of the most reasonable candidates that might be used in drug repurposing strategies in oncology, in particular for the complement treatment of hormone receptor-positive breast cancer. Further experimental validation is required to establish its therapeutic efficacy and safety profiles in clinical application.

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