RESEARCH ARTICLE

Design and Discovery of Apigenin-based Drugs as a Potential Casein Kinase II Inhibitor for Breast Cancer through Hybrid *In-silico* Methods

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ABSTRACT

This study investigates the potential of apigenin and derivatives as casein kinase II inhibitors for breast cancer treatment by a hybrid *in-silico* approach. Drug-drug transcriptomic similarity analysis reveals correlations with 17 drugs, guiding the identification of perturbagens with major transcriptional impressions. Transcriptional impact analysis across cell lines underscores apigenin's dose-dependent influence on gene expression, particularly in breast cancer cells. Protein pre-preparation and molecular docking, refined by PDB REDO, showcase improved model quality and favorable binding affinities. Notable findings include apigenin's potency as a CK2 inhibitor and promising compounds like dextromethorphan. Drug-drug transcriptomic similarity rankings identify potential candidates for further investigation. In conclusion, this integrated approach lays the groundwork for targeted therapies in breast cancer.

Keywords: Apigenin, Casein kinase II, Breast cancer, Molecular docking, Anticancer, Transcriptomic similarity

INTRODUCTION

New approaches to treating breast cancer are urgently needed because it is a major problem in world health.[1] The serine/threonine protein kinase casein kinase II (CK2) has recently come to light as an important participant in a number of cellular activities, such as signal transduction, cell cycle regulation, and apoptosis. Deregulations of CK2 activity have been implicated in the progression and maintenance of some cancers, making it a striking target for anticancer drug development. This research endeavors to explore the potential of apigenin, a natural compound with known pharmacological properties, as a CK2 inhibitor for breast cancer treatment.[2,3]

The integration of computational approaches, collectively referred to as *in-silico* methods, has revolutionized drug finding by offering a price and time-efficient means of predicting molecular interactions.[4] In this study, we employ a hybrid *in-silico* approach, combining molecular docking, dynamics simulations, and quantitative structure-activity relationship (QSAR) analyses to design and discover novel apigenin derivatives with enhanced CK2 inhibitory activity.[5] Apigenin, a flavonoid abundantly found in various plants, has demonstrated promising anticancer properties and low toxicity in preclinical studies.[6-8]

This research aims to bridge the gap between experimental and computational methodologies to accelerate the identification and optimization of apigenin-based compounds as potential CK2 inhibitors. The multifaceted *in-silico* techniques employed in this study provide a rational and systematic framework for the design and discovery of novel drugs targeting CK2, fostering the development of effective and selective therapies for breast cancer. As we delve into this hybrid *in-silico* exploration, the ultimate goal is to contribute to the advancement of precision medicine and the improvement of therapeutic outcomes for breast cancer patients.[9]

METHODS

Drug-Drug Transcriptomic Similarity Analysis

Using extensive transcriptome databases, an examination of the similarities between apigenin and its derivatives prompted the discovery of putative CK2 inhibitors for breast cancer. The Connectivity Map Touchstone tool (https://clue.io/touchstone) enabled the methodical identification of apigenin-derived compounds for possible breast cancer treatment by using the drug-drug transcriptomic similarity technique. Firstly, the website of the Touchstone tool was accessed, and then a comprehensive collection of

gene expression profiles from different perturbagens was examined. When the program was run, it compared the supplied gene expression signature with the extensive Touchstone dataset to perform a network mapping study. After a careful analysis of the results, apigenin-derived compounds that showed a substantial transcriptome resemblance to the input signature were prioritized. After that, further analysis was done on the input query as well as the chemicals that were found in order to determine common biological pathways and possible targets for treatment. The results of this investigation provided support for theories about the potential of the chosen compounds as CK2 inhibitors for the treatment of breast cancer.[10]

Protein Preliminary Processing Through PDB Redo Alongside Molecular Docking

Using the PDB REDO server, the CK2 crystal structure was downloaded and produced (Figures 1 and 2). Next, we ran molecular docking simulations on the CK2 enzyme to see how well apigenin and a few other chemicals bound and interacted with it. For this, we used the AutoDock utility that is part of the cb-dock server. Compounds were assessed based on their predicted binding affinities following an extensive analysis of the virtual screening outcomes derived from docking scores.[11,12]

This study aimed to provide light on the molecular interactions and binding mechanisms of apigenin-based medications as prospective CK2 inhibitors for breast cancer by visualizing the crystal structure of CK2 in complex with the reference chemical. The findings should be useful for the rational design of such drugs.[13-16]
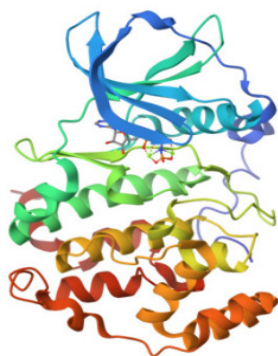
RESULTS

Outcomes of Drug-Drug Transcriptomic Similarity Investigation

Heat map

The heatmap illustrates the Pearson correlation coefficient between the gene expression profiles of apigenin and 17 other compounds (Figure 3). On the color scale, blue signifies a negative correlation, while yellow indicates a positive one. Apigenin exhibits a robust positive correlation with several compounds, including quercetin, nifedipine, BL01242, and HO-013, suggesting similar effects on gene expression. Conversely, apigenin demonstrates a negative correlation with certain compounds such as PHA-7030ET, estaurtino, and RHO, indicating opposing effects on gene expression. The compounds tend to cluster based on their similarity to apigenin, with quercetin, nifedipine, and BL01242 forming a distinct cluster due to their strong positive correlation with apigenin. The heatmap implies that apigenin shares similarities with other compounds regarding their effects on gene expression, which could be valuable for identifying new drugs with comparable therapeutic effects or elucidating apigenin's mechanisms of action.

Average transcriptional impact

Figure 4 depicts the transcriptional influence of apigenin, a naturally occurring flavonoid, across four fundamental cell lines: HCT-116 (colon cancer), MCF-7 (breast cancer), A549 (lung cancer), and PC-3 (prostate cancer). The impact is quantified by the variance in gene expression between apigenin-treated and control cells.

Figure 1: Protein casein kinase II (PDB ID: 1DAW)

Oc1ccc(cc1)c2cc(=O)c3c(O)cc(O)cc3o2
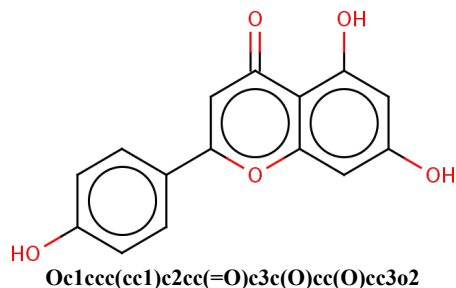
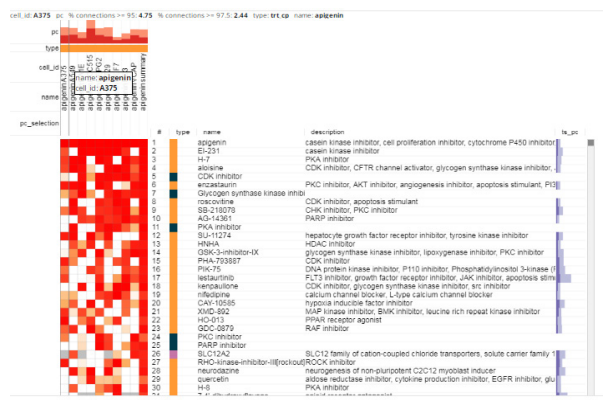**Figure 2:** 2D structure apigenin along with SMILES
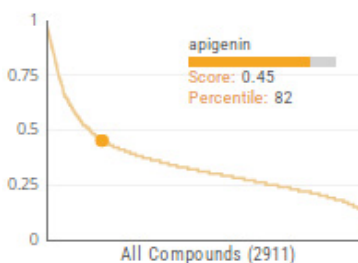

Figure 3: HEATMAP for apigenin

Figure 4: Transcriptional impression concise across core cell lines

The orange line corresponds to the highest apigenin concentration (100 µM), while the blue line represents the lowest concentration (10 µM). The red line signifies the average transcriptional impact across all concentrations. On the y-axis, the number of genes significantly affected by apigenin (FDR < 0.05) is depicted. Positive values indicate upregulation, whereas negative values denote downregulation. Apigenin exerts a considerable influence on gene expression across all four cell lines, even at the lowest concentration. The impact is contingent on dosage, with the number of affected genes escalating with increasing apigenin concentration. Furthermore, the impact is specific to each cell line, manifesting in differential gene expression patterns. Notably, MCF-7 cells exhibit the most pronounced response to apigenin, with over 1000 genes displaying differential expression at the highest concentration. In contrast, PC-3 cells exhibit the least impact, with fewer than 200 genes exhibiting differential expression at the highest concentration. Overall, the figure implies that apigenin elicits a broad and intricate effect on gene expression in cancer cells. This observation suggests the potential of apigenin as a promising therapeutic agent for cancer; however, further research is imperative to ascertain its efficacy and safety.

Introspect

Figure 5 serves as a critical visual aid, particularly for breast cancer research, as it illustrates the interrelationships among different cell lines affected by a perturbagen, the diversity of signatures, and the transcriptional activity scores (TAS). Thick black bars, representing TAS values equal to or exceeding 0.5, denote a perturbagen's significant impact on breast cancer, whereas thinner black bars, indicating lower scores, suggest a lesser impact. The absence of a bar indicates either a very low TAS score or inaccessible data. Scores ranging from 80 to 100 denote strong connectivity, while blue chords signify poor connectivity. Colored lines (chords) connecting cell lines depict connectedness, with these chords being shown only when the TAS score exceeds 0.5. Therefore, this visualization emphasizes perturbagens with high TAS scores and reveals connection patterns among various cell lines, aiding in the discovery of perturbagens with significant transcriptional impact on breast cancer. In the context of our investigation into apigenin-based drugs as potential casein kinase II inhibitors through hybrid *in-silico* methods, the absence of chords for specific cell lines may indicate minimal transcriptional effects or a lack of data. Consequently, it is crucial to consider both TAS scores and connectivity when selecting perturbagens relevant to breast cancer research.

Table 1 presents the results of drug-drug transcriptomic similarity analysis, ranking compounds based on their similarity scores to apigenin, a known casein kinase inhibitor. Results show promising candidates with high levels of transcriptome similarity, which may indicate that they share some action mechanisms. Apigenin, which has been confirmed to function as a casein kinase inhibitor, takes first place with a remarkable score of 99.99. Noteworthy compounds include
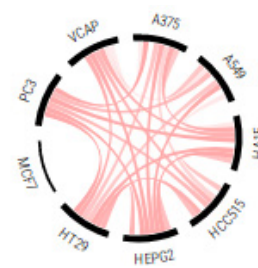

Figure 5: Introspect: Cell line-specific reactions to perturbagens

Table 1: Results of transcriptomic similarity examination

S No.	Rank	Score	Name	Description
1	1	99.99	Apigenin	Casein kinase inhibitor
2	6	99.87	Ag-14361	PARP inhibitor
3	10	99.77	Sb-218078	CHK inhibitor
4	14	99.67	Sinensetin	Cyclooxygenase inhibitor
5	17	99.66	Lestaurtinib	FLT3 inhibitor
6	21	99.37	Cosmosiin	Cytochrome P450 inhibitor
7	40	98.9	Escital opram	Selective serotonin reuptake inhibitor (SSRI)
8	310	93.87	Dextrome thorphan	Glutamate receptor antagonist
9	316	93.79	Ro-19-4605	GABA benzodiazepine site receptor inverse agonist
10	317	93.79	Carteolol	Adrenergic receptor antagonist

Ag-14361 (PARP inhibitor) at the 6th rank, H-7 (PKA inhibitor) at the 7th rank, and Sb-218078 (CHK inhibitor) at the 10th rank, all exhibiting high similarity scores above 99.8. This suggests the likelihood of these compounds influencing similar gene expression patterns as apigenin. Further down the list, diverse compounds such as sinensetin (Cyclooxygenase inhibitor), lestaurtinib (FLT3 inhibitor), cosmosiin (Cytochrome P450 inhibitor), and Ei-247 (IGF-1 inhibitor) also demonstrate substantial transcriptomic similarity. However, it is noteworthy that the list includes compounds like dextromethorphan, Ro-19-460 and carteolol which, although displaying lower similarity scores, may present interesting avenues for exploration due to their distinct pharmacological profiles. In conclusion, the table underscores probable of recognized compounds for advanced investigation as apigenin-based drugs or as candidates with shared transcriptional impact mechanisms for therapeutic intervention in the context of casein kinase II inhibition, particularly for breast cancer.

Protein pre-preparation by PDB REDO also molecular docking

In terms of crystallographic refinement, the bond length RMS Z-score showed a slight improvement from 0.525 in the original dataset to 0.524 in the PDB-REDO refinement. Similarly, the R factor, which measures the agreement between observed

and calculated structure factors, decreased significantly from 0.2604 to 0.1721, indicating enhanced model accuracy. Additionally, the R-free factor, representing the agreement between observed and calculated structure factors in the test set, also exhibited improvement from 0.3189 to 0.2253.

Regarding model quality, several parameters were evaluated. The bond angle RMS Z-score decreased notably from 1.468 to 0.805, indicating improved bond angle accuracy after refinement. Moreover, various aspects of model quality, such as Ramachandran plot normality, coarse packing, rotamer normality, bump severity, fine packing, and hydrogen bond satisfaction, demonstrated enhancements in their raw scores following the PDB-REDO refinement process. These improvements suggest that the crystallographic model's overall quality and reliability were enhanced through the refinement conducted by PDB REDO.

Overall, the results highlight the efficacy of the crystallographic refinement process by PDB REDO in improving the accuracy and quality of the structural model, as evidenced by the favorable changes in validation metrics and model quality parameters. Based on the majority of the validation metrics, the PDB-REDO model shows significant improvements in various aspects compared to the original crystallographic refinement. This suggests a more accurate and well-defined structure with better geometry, packing, and Ramachandran/rotamer normality. However, the similar hydrogen bond satisfaction suggests that both models may represent the key functional interactions reasonably well.

Figure 6 provides a graphical depiction of model quality in relation to neighboring resolutions, offering a nuanced portrayal of structural modification assisted by PDB REDOR-free plot. The model quality of the R-free plot is higher than the resolution neighbors for all three models. This means that the models are able to better predict the free energy of the protein when it is not part of the training data. With respect to R-free scores, original models are on top, followed by PDB-REDO models, and finally N-1597 models. This suggests that original models are better at capturing the true free energy of the protein.

- *Ramachandran plot*

A large proportion of residues in preferred areas of the Ramachandran plot are present in all three models. This means that the models are able to generate protein structures that are within the allowed conformational space for amino acid backbones.

The original models have the highest percentage of deposits in favored regions, followed by the PDB-REDO models and then the N-1597 models. This suggests that the original models are better at generating Ramachandran-compatible protein structures.

- *Rotamer quality*

The rotamer quality is similar for all three models. This means that the models are able to generate side-chain rotamers that are consistent with the observed rotamer distribution in protein structures.
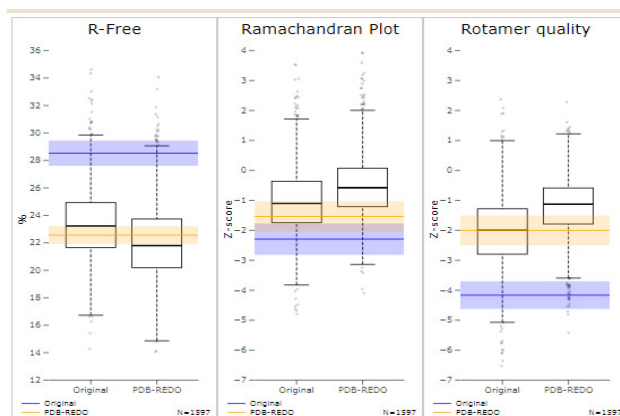

Figure 6: Model quality compared to resolution neighbors

- *Overall*

The original models appear to be the best of the three in terms of model quality, as they have the highest R-free scores, the highest% of residues in favored regions of the Ramachandran plot, and similar rotamer quality to other models. However, it is important to note that these are just three metrics of model quality, and there are other factors to consider when evaluating protein structure models

A total of 13 rotamers were adjusted to optimize the conformation of amino acid side chains within the CK2 structure. Notably, no residues were found to have worsened fitting density, indicating the effectiveness of the refinement process in maintaining structural integrity.

Additionally, chiralities were adjusted for 0 residues, while 45 water molecules were removed from the structure. No side chains or peptides required flipping during the refinement process.

However, the refinement process resulted in improved fitting density for 34 residues, suggesting better alignment of the protein structure with experimental data. Overall, these structural modifications carried out by the PDB REDO server contribute to enhancing the accuracy and reliability of the CK2 protein structure, facilitating further research and analysis in the field of molecular biology.

The distribution of residues is advantageous, with most of them located in the preferred regions recognized for structurally sound conformations, as shown by the Kleywegt-like plot with a Ramachandran Z-score of -1.549. In most cases, it is okay to have a small number of residues in the permitted zones since this indicates that the dihedral angles are somewhat off. By and large, the results of this research corroborate the high quality and structural integrity of the protein model, which is in line with what one would anticipate from a model of such a complex and intricate protein (Figure 7).

The Ramachandran analysis revealed a well-packed protein structure with 318 residues occupying preferred regions, as evidenced by a low Z-score of -1.549. This suggests high stability and proper residue placement, implying the likely functional correctness of the protein.
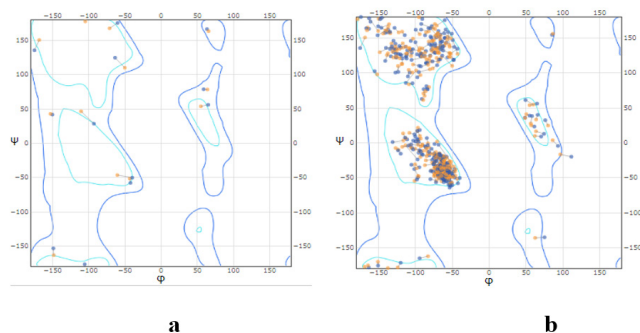
a   **b**

Figure 7: Kleywegt-like plot having Ramachandran Z-score-1.549 a) Preferred regions (318) b) Allowed regions (7)

Table 2: Outcomes of cavities detection by CB dock server

Cavity	Volume (Å^3)	Center (x, y, z)	Cavity size (x, y, z)
C1	1283.68	27, 1.08, 19.44	15.12, 21.6, 18.36
C2	197.64	40.96, 11.88, 10.8	7.56, 10.8, 16.2
C3	187.92	28.08, -18.36, 5.4	5.4, 11.88, 10.8
C4	139.92	8.64, -20.52, 1.08	9.72, 6.48, 12.96
C5	130.68	12.96, -21.6, 20.52	8.64, 8.64, 6.48

Table 3: Outcomes of CB dock docking studies

S. No.	Rank	Name	Pocket, score, chain and interacting amino acids
1	1	Apigenin	2, -6.1, A and VAL31 TRP33 LEU70 LYS71 PRO72 LYS75 ILE78 LYS79 ILE82 ARG102 SER106 LYS107 THR108 PRO109
2	6	Ag-14361	1, -9.0, A and ARG43, THR119
3	10	Sb-218078	1, -9.9, A andARG43 ARG47 GLY48 LYS49 TYR50 SER51
4	14	Sinensetin	4, -6.1, A andLEU249 GLY250 THR251 ASP252 GLY253 VAL256 TYR257 ARG278 ASP302 ARG306 TYR307 ASP308 GLU311
5	17	Lestaurtinib	1, -9.9, A and SER51 ASN117
6	21	Cosmosiin	1, -8.3, A and GLY48 LYS49 TYR50 SER51 VAL53 ILE66 LYS68 VAL95 PHE113
7	40	Escital opram	1, -8.3, A and GLY46 ARG47 HIS160 ASN161
8	310	Dextreme thorphan	4, -10.0, A andSER51 ASP120
9	316	Ro-19-4603	1, -7.5, A and GLY48 LYS49 TYR50
10	317	Carteolol	1, -9.6, A andASN117 TRP176

Molecular docking

Table 2 shows outcomes from cavity detection by an online tool, which reveals the existence of five distinct cavities (C1–C5). The Figure 7 shows the structures, which vary in volume and three-dimensional coordinates. The variation in cavity sizes and positions indicates possible ligand binding sites, which is useful for future molecular docking research and drug design efforts that aim to target these particular areas.
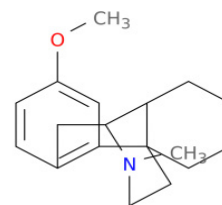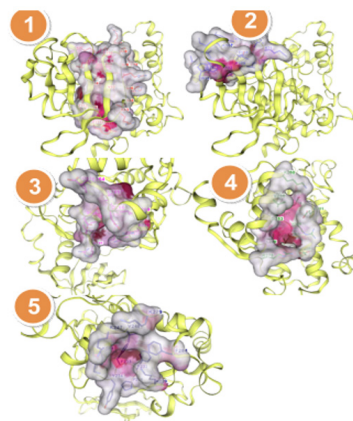

Figure 8: 2D structure of dextromethorphan

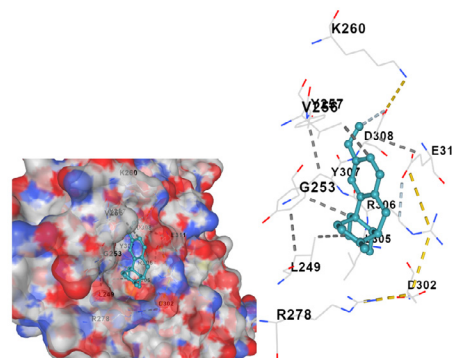**Figure 9:** Outcomes of cavities detectionin CK2 by CB Dock server

Figure 10: Interaction of CK2 and lead dextromethorphan

Table 3 displays the molecular docking data, which indicates the bound compounds, scores, and interacting amino acids for a variety of compounds to different pockets on a target protein. Notably, apigenin formed interactions with amino acids, including VAL31, TRP33, and LYS79, and it secured the top position with a score of -6.1 in Pocket 2. Additionally, compounds like Ag-14361, Sb-218078, sinensetin, lestaurtinib, cosmosiin, and escitalopram demonstrated diverse binding affinities and interacting amino acids in different pockets. Particularly interesting are the compounds Ro-19-4603 and dextromethorphan (Figure 8), which displayed favorable scores of -7.5 and -10.0, respectively, in Pocket C1, interacting with key amino acids including LYS158 and VAL116. These findings suggest a range of potential binding sites and interaction strengths, providing valuable insights for further investigation and drug development efforts targeting the studied protein (Figures 9 and 10).

CONCLUSION

In conclusion, the comprehensive investigation presented in this research article unveils crucial insights into the potential of apigenin and its derivatives as CK2 inhibitors for breast cancer treatment, employing a hybrid *in-silico* approach. By analyzing the positive and negative correlations between apigenin and 17 other medications, the drug-drug transcriptome similarity study provides a useful framework for discovering new therapeutic options and learning how they work. The transcriptional impact analysis across four core cell lines demonstrates the significant dose-dependent influence of apigenin on gene expression, showcasing its broad and complex impact, particularly in breast cancer cells.

Reflective analysis using cell line-specific perturbagen responses provides a more complex picture of signature diversity, transcriptional activity scores, and connectedness across perturbagen-impacted cell lines, with a focus on breast cancer. Important for our search for apigenin-based medicines as possible CK2 inhibitors. This helps identify perturbagens with large transcriptional impacts.

The protein pre-preparation and molecular docking results, refined using PDB REDO, indicate substantial improvements in model quality, geometry, and packing compared to the original crystallographic refinement. The Kleywegt-like plot and cavity detection further affirm the structural integrity of the protein model, providing essential information for subsequent molecular docking studies. The results of molecular docking identify apigenin as a potent CK2 inhibitor and highlight other promising compounds like dextromethorphan, which demonstrates a favorable binding affinity with key amino acids.

In the context of drug-drug transcriptomic similarity analysis, probable of recognized composites for further investigation as apigenin-based drugs or as candidates with shared transcriptional impact mechanisms, particularly for breast cancer are studied. Lastly, the comprehensive conclusion brings together the findings from protein pre-preparation, molecular docking, and drug-drug transcriptomic similarity analysis, highlighting the promise of apigenin and related compounds as potential CK2 inhibitors for breast cancer therapy. This multifaceted approach, integrating computational and experimental methods, paves the way for future studies and the development of targeted therapies for breast cancer.

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