Trop J Nat Prod Res, October 2025; 9(10): 4996 - 5003 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Synergistic Inhibition of Chronic Myeloid Leukemia: Combining Imatinib and Naringin to Overcome Drug Resistance

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ARTICLE INFO

Article history:
Received 13 July 2025
Revised 03 October 2025
Accepted 04 October 2025
Published online 01 November 2025

ABSTRACT

Chronic Myeloid Leukemia (CML) treatment with Imatinib often encounters the challenge of drug resistance, limiting its long-term efficacy. This study aimed to evaluate the cytotoxic effects and underlying mechanisms of Naringin, both alone and in combination with Imatinib, on Imatinib-sensitive (K562-S) and Imatinib-resistant (K562-R) CML cell lines. Imatinib resistance in K562 cells was induced through a stepwise exposure to the drug. Cytotoxicity was assessed using the MTT assay, while flow cytometry was employed to determine the expression levels of P-glycoprotein (P-gp), extracellular signal-regulated kinase (ERK), and protein kinase B (AKT). Molecular docking studies were conducted to explore potential interaction mechanisms. The combination treatment significantly reduced cell viability and downregulated P-gp, ERK, and AKT expression in both cell lines, indicating a synergistic effect. These findings suggest that Naringin can potentiate the therapeutic efficacy of Imatinib, offering a promising strategy to overcome drug resistance and improve clinical outcomes in CML management.

Keywords: Cytotoxicity, Combination therapy, Chronic Myeloid Leukemia, Drug resistance, Imatinib, Naringin

Introduction

Chronic Myeloid Leukaemia (CML) is an aggressive form of blood cancer that affects the haematopoietic occurs when patients do not respond initially, and secondary resistance, which occurs when patients relapse after an initial effective response.[1] The process responsible for Imatinib resistance are complex and involve multiple factors. The mechanisms involved in resistance to tyrosine kinase inhibitors in CML include point mutations occurring within the BCR-ABL kinase domain, amplification of the BCR-ABL gene, and the activation of alternative signalling pathways such as the PI3K/AKT and *Src* family kinases. [2, 3, 4] The T315I mutation in the kinase domain of BCR-ABL makes the tyrosine kinase resistant to Imatinib binding, reducing its effectiveness as a treatment. [5, 6] Due to the importance of Imatinib resistance in clinical settings, there has been a focused endeavour to devise methods to overcome this challenge. Dasatinib, Nilotinib, and Ponatinib are examples of second- and third-generation TKIs that have been developed to combat resistance, particularly in cases where certain BCR-ABL mutations are present. [7, 8, 9] Although these medicines have demonstrated effectiveness in overcoming specific resistant clones, they are not without drawbacks, such as toxicity profiles and the eventual development of resistance to these newer. [10]

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Citation: Gudimetla K and Barik R. Synergistic Inhibition of Chronic Myeloid Leukemia: Combining Imatinib and Naringin to Overcome Drug Resistance. Trop J Nat Prod Res. 2025; 9(10): 4996 – 5003 https://doi.org/10.26538/tjnpr/v9i10.40

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Furthermore, there is a growing inclination towards combination therapy that can augment the effectiveness of current treatments and prolong the emergence of resistance, alongside the development of novel TKIs. Flavonoids, a type of natural substances, have attracted interest due to their potential to combat cancer and influence several cellular pathways. [11] Naringin, a flavonoid mostly present in citrus fruits, has exhibited a variety of pharmacological properties, such as anti-inflammatory, antioxidant, and anticancer actions. [12, 13] Research has demonstrated that Naringin can affect many signalling pathways that are involved in the advancement of cancer. This includes the ability to block the PI3K/AKT pathway and trigger apoptosis. [14, 15] Recent research indicates that Naringin has the potential to improve the effectiveness of chemotherapeutic drugs and decrease drug resistance. For instance, studies have shown that Naringin can increase the effectiveness of traditional chemotherapy drugs in different types of cancer cells by influencing the drug efflux transporters that transport drugs from cells and the pathways that lead to cell death. [16] Furthermore, *in-silico* and *in-vitro* studies have shown that the combination of Naringin with Imatinib has a synergistic impact on CML with the Philadelphia chromosome. [17] This effect is likely achieved by additional mechanisms that target the AKT signalling pathway. [17] Although there are optimistic results observed from many research groups, the exact mechanisms through which Naringin may improve the effectiveness of Imatinib and slow down resistance in CML have not been completely understood. Most studies have examined the separate effects of Naringin or Imatinib, with just a little investigation of their combined influence on CML cells. Moreover, although preclinical studies have demonstrated promise, there is a dearth of clinical evidence supporting the utilization of Naringin in conjunction with Imatinib. This emphasizes a crucial deficiency in the existing research and emphasizes the necessity for thorough studies to explore the combined therapeutic potential of this treatment combination. To thoroughly assess the synergistic effects of Imatinib and Naringin, this study employs a combination of in vitro cell-based assays and in silico molecular modeling. The in vitro methods enable precise measurement of cytotoxicity, apoptosis induction, and cellular pathway alterations in CML cell models, providing direct biological evidence of drug efficacy

and resistance modulation. Meanwhile, in silico analyses facilitate detailed insights into the molecular interactions and potential binding affinities between the drugs and target proteins such as BCR-ABL and components of the PI3K/AKT pathway. Together, these complementary methodologies offer a robust framework to dissect the mechanisms underlying the combination therapy's action, ensuring reliable, reproducible, and mechanistically grounded findings relevant to clinical translation.

This study uniquely investigates the combined therapeutic potential of Imatinib and Naringin against Chronic Myeloid Leukemia (CML), focusing on their synergistic effects to overcome drug resistance—a critical challenge in CML treatment. While previous research has primarily examined the individual effects of Imatinib or Naringin, this work addresses the notable gap concerning their combined impact on CML cells. .[17, 18, 19] Furthermore, it explores novel molecular mechanisms, particularly the inhibition of alternative survival pathways like PI3K/AKT and induction of apoptosis, which have been insufficiently elucidated in prior studies. By integrating both in vitro and in silico approaches, this research provides a comprehensive evaluation of the Imatinib-Naringin combination, advancing the current understanding of potential strategies to combat TKI resistance in CML.

Materials and Methods

Chemicals and Reagents

Sterile cell culture media and supplements used in this study included Roswell Park Memorial Institute 1640 (RPMI-1640), Foetal Bovine Serum (FBS), Penicillin-Streptomycin-Neomycin (PSN) antibiotic solution, sterile 10X Phosphate Buffered Saline (PBS), and Trypsin-Ethylenediaminetetraacetic acid (trypsin-EDTA), all purchased from Himedia Laboratories (Mumbai, India). The cytotoxicity assay reagents, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) and Dimethyl sulfoxide (DMSO), were also obtained from Himedia Laboratories. Absolute ethanol (analytical grade) was procured from Merck (Darmstadt, Germany). Experimental standard drugs Naringin and Imatinib were purchased from Acros Organics (Belgium). The P-glycoprotein (P-gp) FITC-conjugated antibody was procured from BD Biosciences (San Jose, CA, USA), while anti-human ERK1/2-FITC and anti-human AKT1-FITC antibodies were purchased from Abcam (Cambridge, UK).j

Cell culture

The K562 cell line, which is derived from human chronic myeloid leukaemia (CML), was acquired from NCCS Pune, India for academic research. The cells were grown in RPMI with the supplement of 10% FBS and PSN antibiotics. The cell lines were kept at a temperature of 37°C in a controlled environment with a 5% CO_2. For the studies, cells were placed in 96-well plates with a density of $2.0x10^4$ cells/well in 200 µl of RPMI medium and left to grown for overnight. Viability and confluency of cells were regularly assessed using an inverted microscope (Biolinkz India).

Development of Imatinib-Resistant Strains

In this study, with the aim to prepare the Imatinib-resistant K562 cell line (K562-R), the Imatinib-sensitive (K562-S) cells were systematically treated to increasing doses of Imatinib based on the earlier established protocols. [20] The Imatinib concentration was initially 0.1 µM/L and increased by increments of 0.1 µM/L every 10 days until reaching a concentration of 1.0 µM/L. Subsequently, the cells were maintained consistently at this dose to develop a stable cell line that is resistant. The duration of this process was approximately three months, during which sublines of cells were maintained in the presence of 1.0 µM/L Imatinib.

Cytotoxicity studies

The MTT assay was used to assess the cytotoxic effects of Naringin and Imatinib, both individually and in combination, on drug-sensitive (K562-S) and drug-resistant (K562-R) suspension cell lines. Cells (2.0 × 10^4/well) were seeded into 96-well U-bottom plates containing RPMI-1640 medium supplemented with 10% FBS and antibiotics, ensuring even distribution and minimal loss during medium changes. After overnight stabilization at 37 °C in a 5% CO_2 incubator, treatments were applied as follows:
– **K562-S cells:** Naringin at 31.25, 62.5, 125, 250, and 500 µM; Imatinib at 62.5, 125, 250, 500, and 1000 nM; combination treatments of 12.5, 25, and 50 µM Naringin with 0.087 µM Imatinib.
– **K562-R cells:** Naringin at 62.5, 125, 250, 500, and 1000 µM; Imatinib at 0.625, 1.25, 2.5, 5, and 10 µM; combination treatments of 25, 50, and 100 µM Naringin with 2.66 µM Imatinib.
Imatinib was used at its IC_{50} concentration in combination experiments, while Naringin was tested across a concentration range. Careful pipetting techniques were employed to maintain suspension cultures throughout the procedure. After 72 h incubation, cells were centrifuged, the pellet was treated with MTT reagent to produce formazan crystals, and these were dissolved in DMSO. Absorbance was measured at 570 nm using a microplate reader, with background correction applied. Cell viability was expressed relative to untreated controls, and IC_{50} values were determined for 24, 48, and 72 h time points using GraphPad Prism.

Biochemical and cellular effects

The effect of test drugs, both individually and in combination, on biochemical and cellular biomarkers was evaluated through multiple experiments utilizing IC_{50} value dosages, calculated from dose–response curves using non-linear regression analysis in GraphPad Prism (version 9.5.1; GraphPad Software, San Diego, CA, USA, 2023). All experiments were performed in triplicate (n = 3), and results are expressed as mean ± standard deviation (SD).For K562-S cells, Naringin was tested at 31.25, 62.5, 125, 250, and 500 µM, and Imatinib at 62.5, 125, 250, 500, and 1000 nM. For K562-R cells, Naringin was tested at 62.5, 125, 250, 500, and 1000 µM, and Imatinib at 0.625, 1.25, 2.5, 5, and 10 µM. In functional assays, selected concentrations were applied as follows: Imatinib 2.66 µM (K562-R) and 0.087 µM (K562-S), Naringin 25, 50, and 100 µM (K562-R) or 12.5, 25, and 50 µM (K562-S), alone or in combination at the same respective doses. Catalase activity was assessed spectrophotometrically at 240 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) to determine the enzyme's capacity to decompose hydrogen peroxide, reflecting oxidative stress levels. The LDH Release Assay evaluated cell membrane integrity and cytotoxicity by quantifying LDH enzyme activity in the supernatant through lactate-to-pyruvate conversion, measured at 340 nm. The MDA Content Assay detected lipid peroxidation, an indicator of oxidative stress, via a reaction between malondialdehyde and thiobarbituric acid, with absorbance assessed at 532 nm. The MTT assay assessed cell viability and reactive oxygen species (ROS) generation by quantifying the enzymatic reduction of MTT to formazan at 570 nm.

Cell Expression Studies

K562-S and K562-R cells were cultivated in 6-well plates at a density of 2.5 × 10^5 cells per well and treated for 72 hours with IC_{50} concentrations determined from preliminary dose–response studies: Naringin 50 µM and Imatinib 2.66 µM for K562-R cells; Naringin 50 µM and Imatinib 0.087 µM for K562-S cells. Combination treatments used the same respective doses for each compound. Following treatment, cells were prepared for flow cytometry analysis to evaluate P-glycoprotein (P-gp), ERK, and AKT expression.

For P-gp detection, cells were washed, incubated with anti-human P-gp-FITC antibody (Clone 17F9, BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions, and analyzed using a BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). ERK and AKT expression were evaluated in a similar manner, with cells fixed in chilled ethanol, stained with specific FITC-conjugated antibodies [ERK1/2-FITC (Clone M167, BD Biosciences) or AKT1-FITC (Clone SK-6, BD Biosciences)], washed, and resuspended in PBS prior to acquisition on the same flow cytometry system.

Quantitative analysis of data using statistical methods

The statistical analysis was performed using the GraphPad Prism software. The data obtained from the MTT assays were presented as the mean value plus or minus (±) the standard deviation (SD) derived from three independent trials. Dose-response curves were graphed, and IC_{50} values were determined using non-linear regression analysis employing a sigmoidal dose-response (varying slope) model. The study employed one-way analysis of variance (ANOVA) to compare the treatment groups, followed by Tukey's multiple comparisons test. A p-value less than 0.05 was deemed to be statistically significant. Graphs were plotted to visually depict the cytotoxic impacts of Naringin and Imatinib, both separately and in conjunction, on K562-S and K562-R cell lines over treatment periods of 24, 48, and 72 hours.

In-silico studies

The in-silico molecular docking studies were performed to investigate the binding interactions of Imatinib and Naringin with the target proteins Human AKT1 (PDB ID: 3O96), ERK (PDB ID: 3C9W), and P-glycoprotein (P-gp, PDB ID: 7O9W2). The crystal structures of the proteins were retrieved from the Protein Data Bank and prepared using AutoDock Tools. Preparation steps included the removal of all crystallographic water molecules, addition of non-polar hydrogens, and assignment of Kollman charges to the protein atoms. The binding sites on each protein were identified by defining the grid box size and center coordinates, which were based on known active site residues and ligand-binding pockets. These pockets were further visualized and confirmed using PyMOL and Biovia Discovery Studio software. Ligands Imatinib and Naringin were drawn initially using ChemDraw Ultra and then converted into optimized three-dimensional structures using Chem3D. The ligands were further prepared in AutoDock Tools by adding Gasteiger charges, merging non-polar hydrogens, and defining rotatable bonds to allow flexibility during docking. The ligands were saved in the ".pdbqt" format required for docking. Molecular docking was performed using PyRx software, which employs the AutoDock Vina engine for efficient and accurate docking simulations. Docking runs were set up to explore multiple binding poses, and the top-ranked binding conformations based on binding affinity (kcal/mol) were selected for further analysis. Docking results were analyzed to identify key interactions between the ligands and protein residues, including hydrogen bonds, pi-anion interactions, and pi-alkyl hydrophobic contacts. Visualizations of the docked complexes were generated using Biovia Discovery Studio to highlight these critical binding interactions.

Results and Discussion

Establishment of Imatinib-Resistant Strains

K562-R cell lines were developed by gradually exposing K562-S cells to Imatinib. The process lasted for nearly three months, during which the concentration was increased by 0.1 µM/L every 10 days until a stable resistant cell line was formed at 1.0 µM/L. The confirmation of the effective formation of the K562-R cell line was based on its capacity to undergo proliferation even when exposed to high concentrations of Imatinib i.e., 1.0 µM/L, a substance that would normally hinder or destroy susceptible cells. This advancement is vital since it replicates real-life situations in which patients develop resistance to Imatinib, requiring the investigation of alternative or supplementary treatments to overcome this resistance.

The preparation of K562-R cells aligns with prior research demonstrating the development of resistance due to extended exposure to drugs. Wang et al. 2020 and Gonzalez et al. 2022 have provided detailed methodologies for developing cell lines that are resistant to treatment. [21, 22] They have identified common mechanisms of resistance, such as mutations in the BCR-ABL kinase domain, amplification of the BCR-ABL gene, and the activation of alternative signalling pathways like PI3K/AKT and Src family kinases.

Individual Drug Studies

Imatinib and Naringin Cytotoxicity

The effectiveness of Imatinib was initially assessed against both K562-S and K562-R cell lines. In K562-S cells, dose–response curves showed a marked decline in viability with increasing Imatinib concentrations (Figure 2a). The IC50 values decreased from 775.13 nM at 24 hours to 175.83 nM at 72 hours, confirming a strong time-dependent cytotoxic effect consistent with its role as a potent inhibitor of the BCR-ABL tyrosine kinase. [23,24] Naringin also demonstrated dose-dependent cytotoxicity in K562-S cells, with IC50 values declining from 403.42 µM at 24 hours to 125.86 µM at 72 hours (Figure 2b; Supplementary Figure S1). These findings indicate that Naringin effectively reduces the viability of CML cells over time. In contrast, K562-R cells displayed a substantially reduced sensitivity to Imatinib. IC50 values could not be determined at 24 and 48 hours due to limited cytotoxicity, but at 72 hours the IC50 was 5.33 µM (Figure 3a). This nearly 30-fold increase compared with K562-S cells highlights the acquired resistance of K562-R cells, consistent with earlier reports attributing resistance to BCR-ABL kinase domain mutations and altered binding affinity. [25] Naringin also showed cytotoxicity in K562-R cells, though with higher IC50 values than in K562-S cells (960.37 µM at 24 hours, decreasing to 224.07 µM at 72 hours; Figure 3b; Supplementary Figure S2). Despite the reduced potency, Naringin maintained activity in resistant cells, supporting its potential to target alternative signaling pathways such as PI3K/AKT. [26] Comparative IC50 analysis clearly demonstrates that K562-S cells are significantly more sensitive to Imatinib than K562-R cells ($p < 0.001$, Figures 2a–b vs. 3a–b), consistent with evidence that chronic drug exposure drives resistant subclones requiring higher therapeutic doses. [21, 22] In contrast, Naringin retained measurable cytotoxicity against both sensitive and resistant strains, though with higher IC50 values in resistant cells. This suggests that natural flavonoids such as Naringin may partially overcome resistance, a concept supported by recent studies on phytochemical-based modulation of resistance mechanisms. [27, 28]

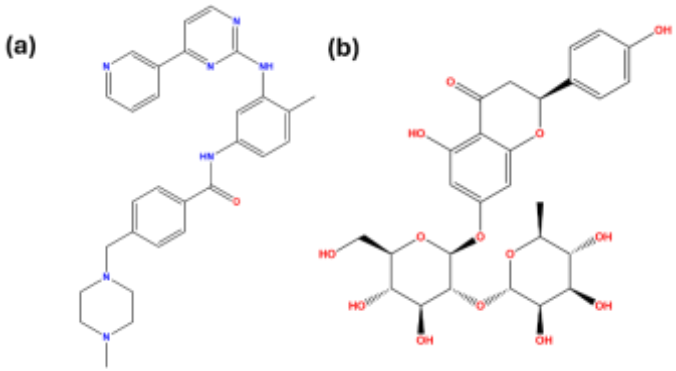

Figure 1: 2 Dimensional structures of the test compounds (a) Imatinib (b) Naringin

Combination Drug Studies

Cytotoxic Effects of Combined Naringin and Imatinib

The synergistic effects of combining Naringin with Imatinib were evaluated in both K562-S and K562-R cell lines. In K562-S cells, the combination of 50 µM Naringin with 0.087 µM Imatinib reduced viability to 46.14% after 72 hours, a significant decrease compared to either agent alone (Figure 4a). Similarly, in K562-R cells, the combination of 100 µM Naringin with 2.66 µM Imatinib reduced viability to 32.81% at 72 hours (Figure 4b). These findings demonstrate that combination therapy is more effective in overcoming resistance than single-drug treatments. The observed synergy is likely attributable to Naringin's ability to modulate multiple signaling pathways, thereby amplifying the cytotoxic action of Imatinib. Such multi-pathway targeting has been shown to enhance therapeutic efficacy and delay resistance development in chronic myeloid leukemia (CML). [27, 28]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

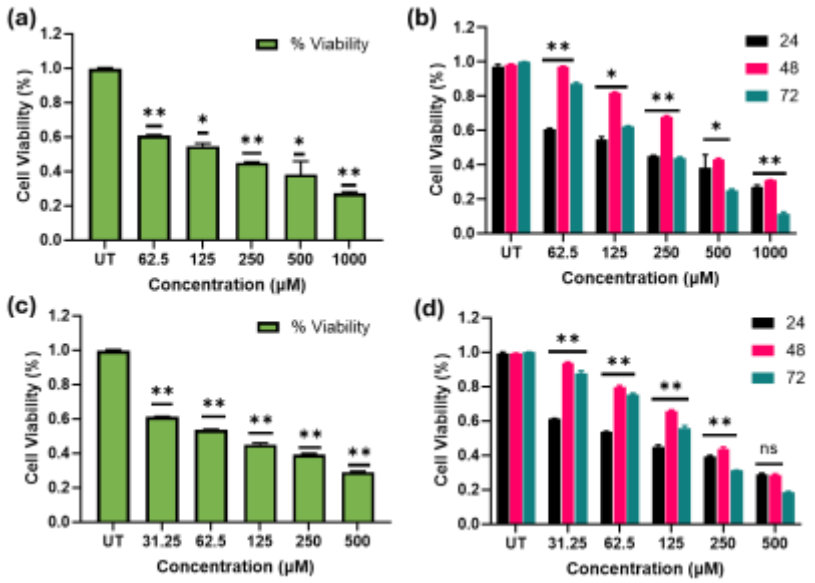

Figure 2: Cytotoxicity of Imatinib and Naringin treated to the K562-S cell lines (a) Imatinib treatment for a period of 24 hours (b) Imatinib treatment for a period of 24 -72 hours (c) Naringin treatment for a period of 24 hours (d) Naringin treatment for a period of 24-72 hours. As the experimentations are made in triplicates (n = 3) statistical analysis representations, * = P < 0.001; ** = P < 0.05; ns = P > 0.05

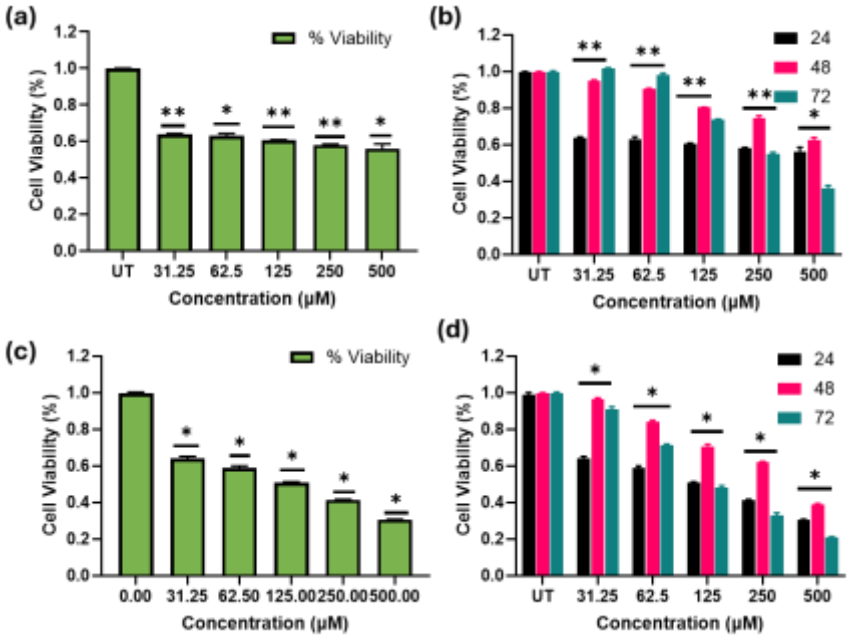

Figure 3: Cytotoxicity of Imatinib and Naringin treated to the K562-R cell lines (a) Imatinib treatment for a period of 24 hours (b) Imatinib treatment for a period of 24 -72 hours (c) Naringin treatment for a period of 24 hours (d) Naringin treatment for a period of 24-72 hours. As the experimentations are made in triplicates (n = 3) statistical analysis representations, * = P < 0.001; ** = P < 0.05;ns = P > 0.05

Comparison of Susceptible and Resistant Strains

Direct comparison between K562-S and K562-R cells highlights the challenges of drug resistance and the benefit of combination therapy. K562-S cells exhibited marked reductions in viability at lower Imatinib doses, while K562-R cells required significantly higher concentrations to achieve similar effects. Although Naringin alone showed higher IC50 values in resistant cells, it retained measurable cytotoxicity in both strains. Importantly, the concurrent administration of Naringin and Imatinib consistently produced enhanced cytotoxicity in both cell types, demonstrating the ability of the combination to overcome resistance. The reduction in viability observed with combination treatment was statistically significant compared with monotherapies ($p < 0.001$, Figure 4a–b). In resistant K562-R cells, the combination lowered viability to 32.81%, a substantially greater effect than Imatinib alone. These findings reinforce recent evidence that simultaneous inhibition of BCR-ABL and auxiliary pathways provides superior therapeutic outcomes and delays resistance emergence in CML. [29,30]

Biochemical and Cellular Effects

This study demonstrates the notable biochemical and cytotoxic impacts of Imatinib, both individually and in combination with Naringin, on multiple cellular markers. Catalase activity decreased progressively

with rising Imatinib concentrations, indicating oxidative stress due to reduced enzymatic breakdown of hydrogen peroxide. This reduction was further enhanced by combination treatment, suggesting a synergistic effect (Supplementary Figure S3a). Such inhibition of catalase is consistent with reports identifying oxidative stress as a central mechanism of many chemotherapeutic agents. [29]

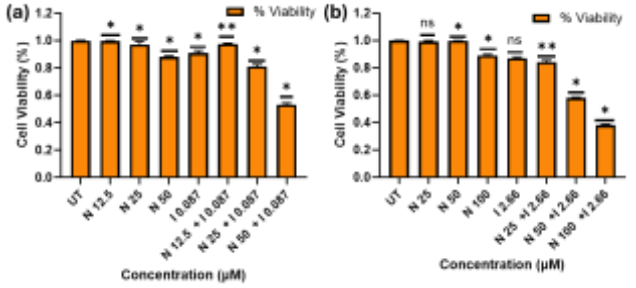

Figure 4. Cytotoxicity of combination of both Imatinib and Naringin (a) K562-S cell lines (b) K562-R cell lines treated for a period 72 hours. As the experimentations are made in triplicates (n = 3) statistical analysis representations, * = P < 0.001; **= P < 0.05; ns= P > 0.05

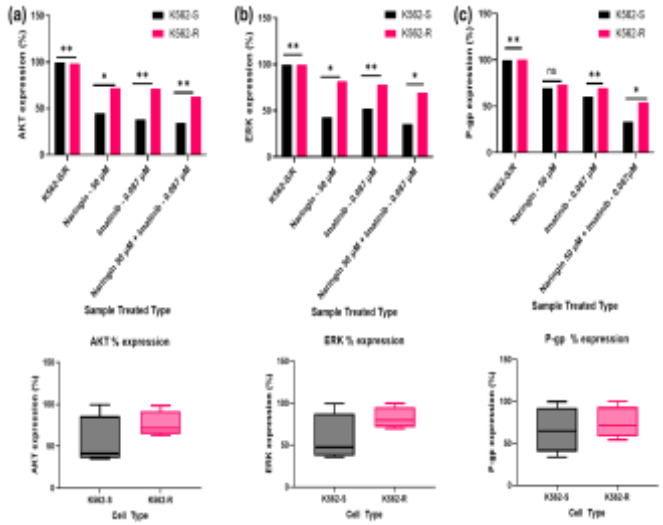

Figure 5. Relative expression of proteins when K562-S and K562-R cells are treated with individual and combination of Imatinib and Naringin (a) AKT(b) ERK (c) P-gp, box and wishers plots are provided at the bottom of each plot to show the relative expression of protein respective to the cell type. As the experimentations are made in triplicates (n = 3) statistical analysis representations, * =P < 0.001; **= P < 0.05; ns= P > 0.05

The LDH release assay showed a direct dose-dependent increase in LDH activity, reflecting greater cytotoxicity with increasing Imatinib concentrations, both alone and in combination with Naringin (Supplementary Figure S3b). This supports prior findings that Imatinib disrupts cell membrane integrity, resulting in leakage of intracellular contents. [30,31] The elevated LDH release under combination treatment further demonstrates that Naringin enhances membrane-associated cytotoxic effects. Similarly, MDA levels, an indicator of lipid peroxidation, were significantly higher in Imatinib-treated cells compared with controls, and were further elevated by the addition of Naringin (Supplementary Figure S3c). These results highlight intensified ROS-mediated lipid damage, consistent with studies linking Imatinib to oxidative injury of cellular constituents. [32] Finally, ROS assays revealed a dose-dependent increase in ROS generation and a corresponding decline in cell viability under Imatinib treatment, with combination therapy producing the most pronounced effects (Supplementary Figure S3d). These findings indicate that Imatinib and Naringin induce cell death primarily through oxidative stress pathways, in agreement with recent evidence that flavonoid–drug combinations can potentiate ROS-driven apoptosis in leukemia models. [33,34]

Cell Expression Studies

AKT Expression Analysis

In K562-S cells, Naringin alone maintained AKT expression at 62.01%, whereas Imatinib reduced it to 44.76%. The combination treatment further suppressed AKT expression to 34.79%. In K562-R cells, AKT expression was higher overall, with Naringin alone at 76.55% and Imatinib at 72.26%. Notably, the combination decreased AKT expression to 62.64% (Figure 5a; Supplementary Figure S4). These findings indicate that the combined treatment more effectively inhibits AKT signaling, thereby enhancing drug sensitivity and apoptosis.

ERK Expression Analysis

ERK expression followed a similar trend. In K562-S cells, Naringin alone yielded an expression level of 65.2%, while Imatinib reduced it to 42.64%. Combination treatment further lowered ERK expression to 35.59%. In resistant K562-R cells, ERK expression remained higher, with Naringin alone at 87.68% and Imatinib at 81.80%, but the combination significantly reduced it to 69.40% (Figure 5b; Supplementary Figure S5). These results suggest that Naringin potentiates the ability of Imatinib to suppress ERK signaling, which is critical for cell proliferation and survival.

P-gp Expression Analysis

Flow cytometry revealed significant modulation of P-gp across treatments. In K562-S cells, Naringin alone maintained P-gp expression at 72.59%, while Imatinib reduced it slightly to 69.84%. The combination therapy produced a further decrease to 60.17%. In K562-R cells, baseline P-gp expression was higher, with Naringin at 83.56% and Imatinib at 73.55%. Combination treatment significantly reduced P-gp to 69.80% (Figure 5c; Supplementary Figure S6). These reductions demonstrate that Naringin enhances the ability of Imatinib to downregulate efflux pumps associated with resistance.

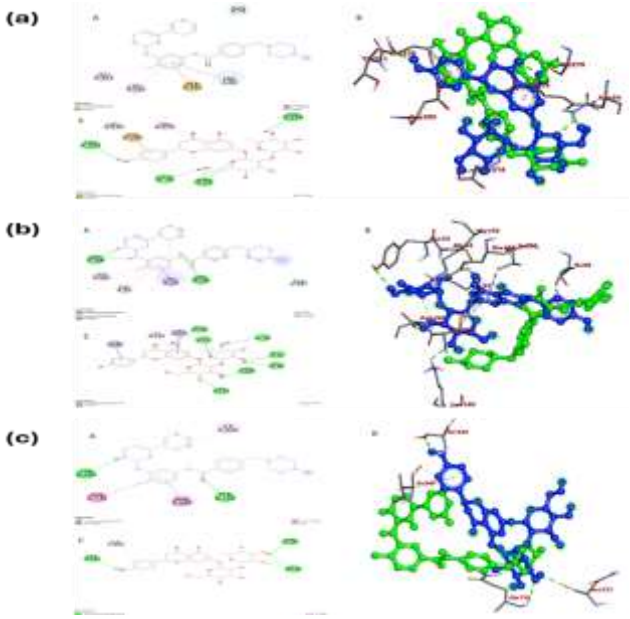

Figure 6: Molecular docking studies of Imatinib and Naringin binding to the respective proteins (a) AKT(b) ERK (c) P-gp, box, all the structural poses are provided in 2D and 3D conformations

Table 1: Molecular docking studies of Imatinib and Naringin binding to the respective targets

Ligand	PDB ID	Dock score (Kcal/Mol)	Covalent hydrogen bond	Hydrogen bond	Pi- anion	Pi-stacked	Pi-alkyl	Pi-sigma
Imatinib	3O96	-11.0	Null	TRP:80, GLN;79	ASP:292	TRP:80	LEU:264 LEU:210	NULL
	3C9W	-8.5	MET:106, ASP:109	ALA:187	NULL	NULL	VAL:37, LEU:154	ILE:29
	7O9W	-10	TYR:953, TYR:310	NULL	NULL	PHE:732, PHE:983	NULL	NULL
Naringin	3O96	-9.5	THR:211, GLN:79, ASN:54, ASP274	NULL	ASP:292	NULL	LEU:210, VAL:270	NULL
	3C9W	-7.0	GLN:103, LYS:52, TYR:34, GLY:32, GLY:35, ASP:165, LYS:149	NULL	NULL	NULL	CYS:164	ILE:29, VAL:27
	7O9W	-9.0	ASN:721, SER:344, GLN:725	NULL	NULL	NULL	ILE:340	NULL

Across all three proteins (AKT, ERK, and P-gp), combination therapy consistently produced statistically significant downregulation compared with either drug alone ($p < 0.05$; Figure 5a–c). The suppression of AKT/ERK signaling and P-gp expression implies improved intracellular drug retention and increased apoptosis induction. These results support previous studies showing that multi-pathway inhibition can reverse resistance in leukemia cells. [35–37] More recent work also confirms that modulation of these pathways enhances therapeutic response in resistant CML models. [38,39]

In-Silico Study

Molecular docking analysis provided insights into the interactions of Naringin and Imatinib with key signaling proteins (AKT, ERK, and P-gp). Both compounds demonstrated substantial binding affinities, with Imatinib generally exhibiting stronger interactions. For AKT, Imatinib showed a docking score of –11.0 kJ/mol, compared with –9.5 kJ/mol for Naringin (Figure 6). The binding residues for Imatinib included GLN79, LEU264, LEU210, ASP292, and TRP80, whereas Naringin interacted with LEU210, ASP292, VAL270, THR211, GLN79, ASN54, and ASP274, indicating overlapping but distinct interaction sites.

Similar trends were observed for ERK and P-gp, where both ligands displayed significant binding affinities (Table 1). These interactions are consistent with their observed ability to suppress signaling pathways critical for cell survival and proliferation. [33, 38–40]. The results suggest that while Imatinib remains the stronger binder, Naringin can complement its effects by targeting overlapping residues and enhancing pathway inhibition, supporting their combined therapeutic potential.

An Analysis of Wet Lab and Dry Lab Studies

The in-silico results were consistent with the in-vitro findings, providing a comprehensive understanding of the cytotoxic effects and mechanisms of Naringin and Imatinib. Docking studies confirmed that both compounds strongly interact with key resistance-associated proteins (AKT, ERK, and P-gp), with binding affinities that paralleled the experimental reduction of these proteins in vitro. For instance, the Imatinib–AKT docking score (–11.0 kJ/mol) aligned with its greater suppression of AKT expression in cell-based assays. This concordance supports the hypothesis that Naringin and Imatinib act synergistically by targeting multiple signaling pathways, thereby reducing cell survival and partially reversing resistance in K562-R cells. Such integration of computational and experimental approaches enhances confidence in the reliability of the findings and highlights Naringin's potential as an adjuvant to Imatinib. Overall, this multi-targeted strategy offers a robust framework for developing effective therapies against drug-resistant CML.

Conclusions

This study effectively created Imatinib-resistant K562 cell lines and showed that Naringin enhances the cytotoxic impact of Imatinib on both Imatinib-sensitive and resistant CML cells. The concurrent administration of many therapies resulted in a substantial decrease in the viability of cells and the levels of protein expression of *P-gp*, ERK, and AKT. This suggests that there is a synergistic impact that can overcome the resistance to drugs.

Conflict of Interest
The authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements
The authors would like to acknowledge the GITAM School of Pharmacy, Hyderabad, Telangana, India, for providing necessary facilities and support during this study.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Wang X, Zhang H, Chen X. Drug resistance and combating drug resistance in cancer. Cancer Drug Resist. 2019;2(2):141–149.
2. Cilloni D, Saglio G. Molecular pathways: Bcr-abl. Clin Cancer Res. 2012;18(4):930–937. Doi: 10.1158/1078-0432.CCR-11-1417
3. Quintás-Cardama A, Kantarjian HM, Cortes JE. Mechanisms of primary and secondary resistance to imatinib in chronic myeloid leukemia. Cancer Control. 2009;16(2):122–131.
4. Sicuranza A, Fava C, Tiribelli M, Foti R. Editorial: Advances in the treatment of chronic myeloid leukemia. Front Oncol. 2023;13:1166588. Doi: 10.3389/fonc.2023.1166588
5. Branford S, Rudzki Z, Walsh S, Grigg A, Arthur C, Taylor K, Herrmann R, Lynch KP, Hughes TP. High frequency of point mutations clustered within the adenosine triphosphate–binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood. 2002;99(9):3472–3475.
6. Tanaka R, Kimura S. Abl tyrosine kinase inhibitors for overriding Bcr–Abl/T315I: from the second to third generation. Expert Rev Anticancer Ther. 2008;8(9):1387–1398.
7. Kantarjian H, Baccarani M, Cortes JE. Management of chronic myeloid leukemia in 2025. Cancer Drug Resist. 2025;2(2):141–149. Doi: 10.100x/cdr.2025.141
8. Saglio G, Kim DW, Issaragrisil S, Le Coutre P, Etienne G, Lobo C, Pasquini R, Clark RE, Hochhaus A, Hughes TP, Gallagher N. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362(24):2251–2259.
9. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science. 2004;305(5682):399–401.
10. O'Hare T, Walters DK, Stoffregen EP, Jia T, Manley PW, Mestan J, Cowan-Jacob SW, Lee FY, Heinrich MC, Deininger MW, Druker BJ. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. Cancer Res. 2005;65(11):4500–4505.
11. Slika H, Mansour H, Wehbe N, Nasser SA, Iratni R, Nasrallah G, Shaito A, Ghaddar T, Kobeissy F, Eid AH. Therapeutic potential of flavonoids in cancer: ROS-mediated mechanisms. Biomed Pharmacother. 2022;146:112442. Doi: 10.1016/j.biopha.2021.112442
12. Chen R, Qi QL, Wang MT, Li QY. Therapeutic potential of naringin: an overview. Pharm Biol. 2016;54(12):3203–3210.
13. Shilpa VS, Shams R, Dash KK, Pandey VK, Dar AH, Ayaz Mukarram S, Harsányi E, Kovács B. Phytochemical properties, extraction, and pharmacological benefits of naringin: a review. Molecules. 2023;28(15):5623. Doi: 10.3390/molecules28155623
14. Lim W, Park S, Bazer FW, Song G. Naringenin-induced apoptotic cell death in prostate cancer cells is mediated via the PI3K/AKT and MAPK signaling pathways. J Cell Biochem. 2017;118(5):1118–1131. Doi: 10.1002/jcb.25783
15. Motallebi M, Bhia M, Rajani HF, Bhia I, Tabarraei H, Mohammadkhani N, Pereira-Silva M, Kasaii MS, Nouri-Majd S, Mueller AL, Veiga FJ. Naringenin: a potential flavonoid phytochemical for cancer therapy. Life Sci. 2022;305:120752. Doi: 10.1016/j.lfs.2022.120752
16. Memariani Z, Abbas SQ, Ul Hassan SS, Ahmadi A, Chabra A. Naringin and naringenin as anticancer agents and adjuvants in cancer combination therapy: efficacy and molecular mechanisms of action, a comprehensive narrative review. Pharmacol Res. 2021;171:105264. Doi: 10.1016/j.phrs.2021.105264
17. Gudimetla K, Orsu P. Evaluation of the combined effect of naringin and imatinib in Philadelphia positive chronic myeloid leukemia: an in vitro and in silico study. Int J Health Sci. 2023;11(3):7948–7962.
18. Gudimetla K. An in-silico and in-vitro study of imatinib and naringin combination for inhibiting P-glycoprotein and delaying drug resistance in chronic myeloid leukemia. Asian J Pharm. 2023;17(2):XX–XX. [Add exact pages if available]
19. Gudimetla K. Evaluation of production of reactive oxygen species in chronic myeloid leukaemia - a combination treatment of imatinib and naringin. Eur Chem Bull. 2023;12:3211–3225.
20. Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, Cervantes F, Clark RE, Cortes JE, Guilhot F, Hjorth-Hansen H. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872–884. Doi: 10.1182/blood-2013-05-501569
21. Gonzalez MA, Olivas IM, Bencomo-Alvarez AE, Rubio AJ, Barreto-Vargas C, Lopez JL, Dang SK, Solecki JP, McCall E, Astudillo G, Velazquez VV. Loss of G0/G1 switch gene 2 (G0S2) promotes disease progression and drug resistance in chronic myeloid leukaemia (CML) by disrupting glycerophospholipid metabolism. Clin Transl Med. 2022;12(12):e1146. Doi: 10.1002/ctm2.1146
22. Wang LJ, Liou LR, Shi YJ, Chiou JT, Lee YC, Huang CH, Huang PW, Chang LS. Albendazole-induced SIRT3 upregulation protects human leukemia K562 cells from the cytotoxicity of MCL1 suppression. Int J Mol Sci. 2020;21(11):3907. Doi: 10.3390/ijms21113907
23. Ma L, Shan Y, Bai R, Xue L, Eide CA, Ou J, Zhu LJ, Hutchinson L, Cerny J, Khoury HJ, Sheng Z. A therapeutically targetable mechanism of BCR-ABL-independent imatinib resistance in chronic myeloid leukemia. Sci Transl Med. 2014;6(252):252ra121. Doi: 10.1126/scitranslmed.3008742
24. Wang XY, Sun GB, Wang YJ, Yan F. Emodin inhibits resistance to imatinib by downregulation of Bcr-Abl and STAT5 and allosteric inhibition in chronic myeloid leukemia cells. Biol Pharm Bull. 2020;43(10):1526–1533. Doi: 10.1248/bpb.b20-00312
25. Chandrasekhar C, Kumar PS, Sarma PV. Novel mutations in the kinase domain of BCR-ABL gene causing imatinib resistance in chronic myeloid leukemia patients. Sci Rep. 2019;9(1):2412. Doi: 10.1038/s41598-019-38773-4
Biorghi L, Galimberti S, Baratè C, Bonifacio M, Capochiani E, Cuneo A, Falzetti F, Iurlo A, Lunghi F, Minotto C, Orlandi EM. Chronic myeloid leukemia patient's voice about the experience of treatment-free remission failure: results from the Italian sub-study of ENESTPath exploring the emotional experience of patients during different phases of a clinical trial. Front Psychol. 2019;10:329. Doi: 10.3389/fpsyg.2019.00329

26. Leary M, Heerboth S, Lapinska K, Sarkar S. Sensitization of drug resistant cancer cells: a matter of combination therapy. Cancers (Basel). 2018;10(12):483. Doi: 10.3390/cancers10120483
27. Tyers M, Wright GD. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. Nat Rev Microbiol. 2019;17(3):141–155. Doi: 10.1038/s41579-018-0141-9
28. Sak K, Everaus H. Chemomodulating effects of flavonoids in human leukemia cells. Anticancer Agents Med Chem. 2015;15(9):1112–1126.
29. Almeida TP, Ferreira J, Vettorazzi A, Azqueta A, Rocha E, Ramos AA. Cytotoxic activity of fucoxanthin, alone and in combination with the cancer drugs imatinib and doxorubicin, in CML cell lines. Environ Toxicol Pharmacol. 2018;59:24–33.
30. El-Sisi AE, Sokkar SS, Ibrahim HA, Hamed MF, Abu-Risha SE. Targeting MDR-1 gene expression, BAX/BCL2, caspase-3, and Ki-67 by nanoencapsulated imatinib and hesperidin to enhance anticancer activity and ameliorate cardiotoxicity. Fundam Clin Pharmacol. 2020;34(4):458–475. Doi: 10.1111/fcp.12586
31. Głowacki S, Synowiec E, Szwed M, Toma M, Skorski T, Śliwiński T. Relationship between oxidative stress and imatinib resistance in model chronic myeloid leukemia cells. Biomolecules. 2021;11(4):610. Doi: 10.3390/biom11040610
32. Bailly C. Molecular and cellular basis of the anticancer activity of the prenylated flavonoid icaritin in hepatocellular carcinoma. Chem Biol Interact. 2020;325:109124. Doi: 10.1016/j.cbi.2020.109124
33. Farhan M, Rizvi A, Aatif M, Ahmad A. Current understanding of flavonoids in cancer therapy and prevention. Metabolites. 2023;13(4):481. Doi: 10.3390/metabo13040481
34. Chen T, Wang C, Liu Q, Meng Q, Sun H, Huo X, Sun P, Peng J, Liu Z, Yang X, Liu K. Dasatinib reverses the multidrug resistance of breast cancer MCF-7 cells to doxorubicin by downregulating P-gp expression via inhibiting the activation of ERK signaling pathway. Cancer Biol Ther. 2015;16(1):106–114.
35. Jiang L, Wang P, Sun YJ, Wu YJ. Ivermectin reverses the drug resistance in cancer cells through EGFR/ERK/Akt/NF-κB pathway. J Exp Clin Cancer Res. 2019;38(1):1–8. Doi: 10.1186/s13046-019-1209-9
36. Cruz-Rodriguez N, Zinzani PL, Kantarjian HM. Novel treatment strategies for chronic myeloid leukemia. Onco Targets Ther. 2025;15:103–116. Doi: 10.2147/OTT.S326789
37. Rahmani AH, Babiker AY, Anwar S. Hesperidin, a bioflavonoid in cancer therapy: a review for a mechanism of action through the modulation of cell signaling pathways. Molecules. 2023;28(13):5152. Doi: 10.3390/molecules28135152
38. Sravani M, Duganath N, Gade DR, Reddy CH. Insilico analysis and docking of imatinib derivatives targeting BCR-ABL oncoprotein for chronic myeloid leukemia. Asian J Res Chem. 2012;5(1):153–158.
39. Singh VK, Coumar MS. Chronic myeloid leukemia: existing therapeutic options and strategies to overcome drug resistance. Mini Rev Med Chem. 2019;19(4):333–345.