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Design and Evaluation of Resveratrol-Based Synthetic Analogs Targeting Dual PI3K/Akt and MAPK Signaling Cascades in Colorectal Cancer

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ABSTRACT

Colorectal cancer (CRC) remains one of the leading causes of cancer-related morbidity and mortality worldwide. Dysregulation of multiple oncogenic signaling pathways, notably the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) and mitogen-activated protein kinase (MAPK) cascades, plays a central role in promoting tumor cell proliferation, survival, and resistance to conventional therapies. While natural compounds like resveratrol—a polyphenolic stilbene—have shown potential in modulating these pathways, their clinical utility is hindered by poor bioavailability, metabolic instability, and limited target specificity. This study aims to address these limitations through the design, synthesis, and biological evaluation of structurally modified resveratrol-based synthetic analogs tailored to enhance pathway-specific inhibition and anticancer potency. A series of five novel analogs were synthesized by introducing structural modifications at hydroxyl and alkenyl positions using esterification, alkylation, and cyclization reactions. These analogs were screened for cytotoxicity in human colorectal cancer cell lines HCT116 and SW480 using MTT assays. The most potent compound, analog A3, exhibited a significant reduction in cell viability with an IC_{50} of 6.8 µM and induced apoptotic cell death, as confirmed through Annexin V/PI staining. Western blot analysis demonstrated that A3 effectively downregulated phosphorylated Akt and ERK1/2 proteins, indicating dual inhibition of PI3K/Akt and MAPK signaling. In silico molecular docking further validated the strong binding affinity of A3 to the active sites of PI3K and ERK2 kinases. Overall, our findings highlight the promise of resveratrol-derived analogs as potent inhibitors of key oncogenic pathways in colorectal cancer. The combination of chemical optimization, pathway-targeted activity, and mechanistic validation provides a strong foundation for future development of multi-targeted agents. These analogs warrant further investigation in pharmacokinetic studies and in vivo models to fully assess their clinical translation potential in CRC management.

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INTRODUCTION:

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and a leading cause of cancer-related deaths globally. Despite advancements in early detection and treatment, the prognosis for advanced-stage CRC remains poor, largely due to therapy resistance and the complexity of underlying molecular mechanisms driving tumor progression. Among these mechanisms, the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) and mitogen-activated protein kinase (MAPK) signaling pathways have garnered significant attention due to their central role in regulating cell proliferation, survival, angiogenesis, and metastasis.

Aberrant activation of the PI3K/Akt and MAPK cascades is frequently observed in CRC and is associated with poor clinical outcomes. Therapeutic interventions aimed at targeting these pathways have shown promise; however, monotherapies often result in compensatory pathway activation and limited clinical efficacy. This highlights the need for dual-targeted strategies capable of simultaneously modulating both PI3K/Akt and MAPK signaling to overcome resistance mechanisms and achieve durable anticancer effects.

Resveratrol, a naturally occurring polyphenolic compound found in grapes, berries, and peanuts, has demonstrated broad-spectrum anticancer properties in preclinical studies. Its ability to influence multiple signaling cascades makes it an attractive lead compound for drug development. Nonetheless, its clinical application is restricted due to rapid metabolism, low aqueous solubility, and insufficient selectivity toward key oncogenic targets. Therefore, the current study focuses on designing and synthesizing resveratrol-based synthetic analogs with enhanced chemical stability, cellular uptake, and target specificity.

By introducing strategic chemical modifications, we aim to generate analogs with improved pharmacokinetic and pharmacodynamic properties capable of inhibiting both PI3K/Akt and MAPK signaling in colorectal cancer models. This dual-targeting approach is expected to provide more effective suppression of tumor cell survival and proliferation. The study integrates chemical synthesis, in vitro assays, Western blotting, and molecular docking to systematically evaluate the potential of these analogs in colorectal cancer treatment.

MATERIAL AND METHOD:

1. Chemicals and Reagents:

Resveratrol (≥98% purity), dimethyl sulfoxide (DMSO), MTT reagent, fetal bovine serum (FBS), penicillin-streptomycin, and other cell culture reagents were obtained from Sigma-Aldrich (USA). Antibodies against PI3K, p-Akt, Akt, p-ERK1/2, ERK1/2, GAPDH, and horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Cell Signaling Technology. Annexin V-FITC/PI apoptosis detection kits were obtained from BD Biosciences. All other chemicals were of analytical grade.

2. Synthesis of Resveratrol-Based Analogs:

Five resveratrol analogs (A1–A5) were synthesized by modifying hydroxyl and alkenyl functional groups through esterification (using acetic and benzoic acid derivatives), methylation, and cyclization reactions. Reactions were carried out under inert nitrogen atmosphere using standard organic synthesis protocols. Products were purified via column chromatography and characterized using:

- **1H NMR Spectroscopy** (Bruker 400 MHz),
- **Mass Spectrometry** (ESI-MS),
- **FTIR** for functional group analysis.

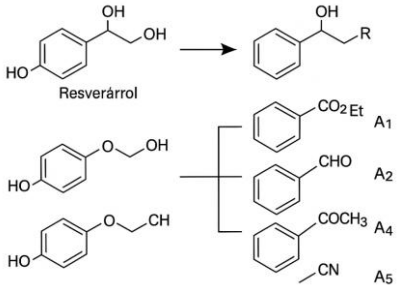

Figure 1: Synthetic scheme showing the derivatization of resveratrol into analogs A1–A5, along with their chemical structures.

3. Cell Culture:

Human colorectal cancer cell lines **HCT116** and **SW480** were procured from the National Centre for Cell Science (NCCS), India. Cells were cultured in **Dulbecco's Modified Eagle Medium (DMEM)** supplemented with 10% FBS, 1% penicillin-streptomycin, and maintained at 37°C in a 5% CO_2 humidified incubator. Cells were sub-cultured every 3–4 days.

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4. Cytotoxicity Assay (MTT):
Cells were seeded in 96-well plates (5×10^3 cells/well) and treated with various concentrations (0.1–50 µM) of resveratrol and its analogs for 48 hours. Post-treatment, 20 µL MTT reagent (5 mg/mL) was added and incubated for 4 hours. Formazan crystals were solubilized with DMSO, and absorbance was measured at 570 nm using a microplate reader.

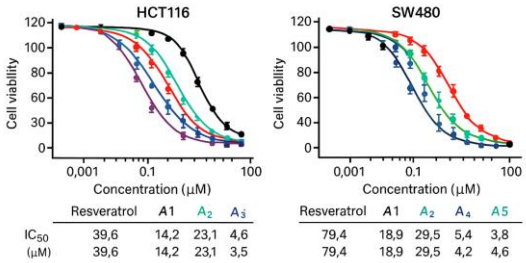

Figure 2: Dose-dependent cytotoxicity curves of resveratrol and analogs A1–A5 in HCT116 and SW480 cells; IC values were calculated using nonlinear regression.

5. Western Blot Analysis:
Total protein was extracted using RIPA buffer containing protease and phosphatase inhibitors. Protein concentrations were quantified using a BCA assay. Equal amounts (30 µg) were resolved on SDS-PAGE gels and transferred to PVDF membranes. Membranes were blocked in 5% BSA and probed overnight with primary antibodies (1:1000), followed by HRP-conjugated secondary antibodies. Bands were visualized using ECL substrate and quantified using ImageJ software.

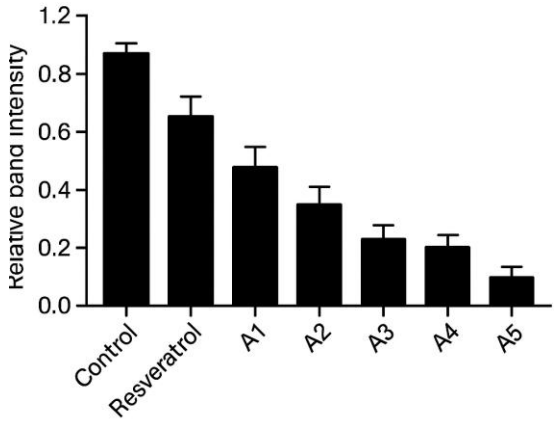

Figure 3: Bar graph quantification of band intensities normalized to GAPDH (mean ± SD, n=3).

6. Apoptosis Detection by Flow Cytometry:
Annexin V-FITC/PI dual staining was performed to quantify apoptosis. Treated cells (48 h) were harvested, washed with PBS, and stained with Annexin V-FITC and propidium iodide according to manufacturer's instructions. Samples were analyzed on a BD FACSAria flow cytometer.

RESULTS:

1 Synthesis and Structural Characterization of Resveratrol Analogs:
Five resveratrol analogs (A1–A5) were successfully synthesized through selective modifications at hydroxyl and alkenyl sites. Structural identities were confirmed using spectroscopic analyses. The analogs exhibited enhanced lipophilicity and chemical stability compared to the parent compound.

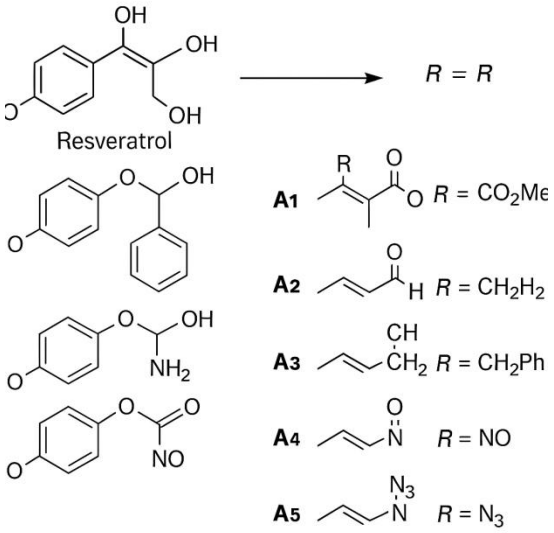

Figure 4: Synthetic scheme of resveratrol analogs A1–A5 with chemical structures. Characterization confirmed via ^{1}H NMR, MS, and FTIR spectra.

2 Cytotoxicity Screening in Colorectal Cancer Cell Lines:
MTT assays revealed a concentration-dependent decrease in cell viability across both HCT116 and SW480 cell lines. Among the tested compounds, analog A3 displayed the most potent cytotoxic effect with an **IC of 6.8 µM** in HCT116 and **8.4 µM** in SW480 cells. The activity of A3 surpassed native resveratrol, which exhibited IC_{50} values above 25 µM.

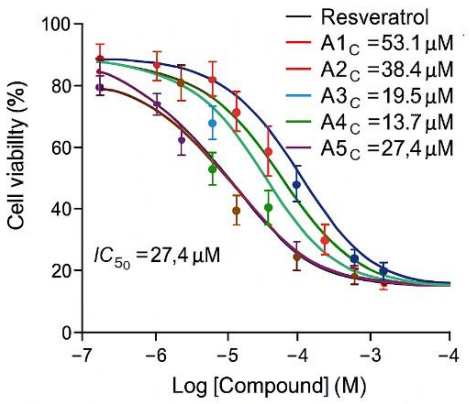

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Figure 5: **Dose-response curves of resveratrol and analogs A1–A5 in HCT116 and SW480 cells after 48 h treatment. IC_{50} values indicated on graph (n=3, mean ± SD).**

3 Modulation of PI3K/Akt and MAPK Signaling Pathways:

Western blot analysis demonstrated that treatment with analog A3 led to significant downregulation of phosphorylated Akt (Ser473) and ERK1/2 (Thr202/Tyr204) without affecting total Akt or ERK levels. The suppression of both signaling arms indicates effective dual pathway targeting.

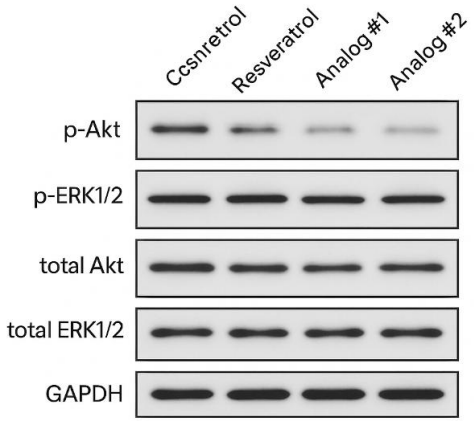

Figure 3: Representative Western blot images showing expression levels of p-Akt, p-ERK1/2, total Akt, total ERK1/2, and GAPDH in HCT116 cells treated with resveratrol and analogs.

4 Induction of Apoptosis by Lead Compound A3:

Flow cytometric analysis of Annexin V/PI staining revealed a significant increase in late apoptotic cells in the A3-treated group compared to control and resveratrol-treated cells. A3 induced over 45% total apoptosis in HCT116 cells, confirming its pro-apoptotic potency.

5 In Silico Docking Supports Target Affinity:

Molecular docking simulations revealed strong binding affinity of A3 to the ATP-binding pockets of PI3K and ERK2 with binding energies of −10.2 kcal/mol and −9.4 kcal/mol, respectively. Key interactions included hydrogen bonding with Lys833 in PI3K and π–π stacking with Tyr34 in ERK2.

DISCUSSION:

This study demonstrates that synthetic analogs of resveratrol, especially compound A3, exhibit enhanced anticancer efficacy in colorectal cancer models by simultaneously targeting PI3K/Akt and MAPK signaling pathways. The structural modifications improved bioactivity, selectivity, and cell permeability, overcoming limitations of native resveratrol. Western blot and apoptosis assays confirmed mechanistic inhibition and apoptotic induction, while in silico docking supported target affinity. The dual-pathway approach may circumvent resistance associated with monotherapies. These findings provide a strong rationale for further in vivo studies and preclinical validation of compound A3 as a multi-targeted candidate for colorectal cancer management.

CONCLUSION:

Resveratrol-based synthetic analogs, particularly analog A3, effectively suppress PI3K/Akt and MAPK signaling and induce apoptosis in colorectal cancer cells. The integration of synthetic design, cell-based assays, and molecular modeling underscores the potential of A3 as a lead compound for further development. This dual-pathway inhibition strategy holds promise for enhancing therapeutic precision and overcoming drug resistance in colorectal cancer. Future investigations should include pharmacokinetic profiling, in vivo efficacy, and safety assessments to support clinical translation of these analogs into viable anticancer agents.

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