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Development of Kojic Acid-3-Hydroxyindolin-2-one Conjugates: Structural Characterization and Pharmacological Evaluation

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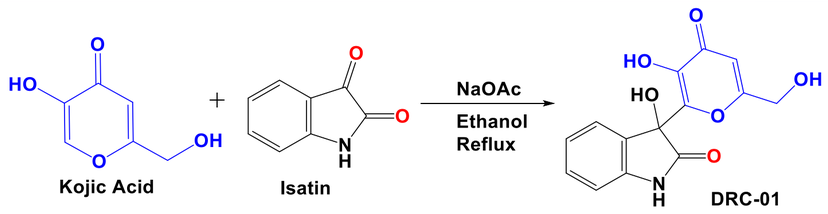
INTRODUCTION & AIM

Kojic acid (KA), a fungal metabolite, is widely studied for its pharmacological potential, particularly as a tyrosinase inhibitor in dermatology. Its 2-hydroxy-4-pyrone structure enables metal chelation, contributing to its antioxidant properties and modulation of oxidative stress pathways. Despite cytotoxic effects against cancer cells, its low potency and bioavailability limit therapeutic applications. 1H-indole-2,3-dione, a human metabolic derivative, exhibits diverse pharmacological activities and is a precursor for bioactive compounds, including anticancer agents. Isatin derivatives induce apoptosis and modulate oxidative stress pathways, making them promising for targeted therapies. However, KA's poor stability, susceptibility to oxidation, and low systemic bioavailability hinder its clinical use. Similarly, Isatin suffers from low solubility and target selectivity, which can lead to potential side effects. Hybridizing KA and Isatin offers a strategy to overcome these limitations. KA's antioxidant and metal-chelating properties complement Isatin's apoptotic and oxidative stress-targeting capabilities, enabling a multifunctional therapeutic approach. This hybridization may enhance solubility, stability, and specificity, improve anticancer efficacy while reducing off-target effects. This study aims to synthesize and evaluate KA-isatin hybrids for their anticancer and antioxidant activities, thereby contributing to the development of multifunctional drugs for cancer and oxidative stress-related diseases.

METHOD



Synthesis of DRC-01: Following Elinson et al. (2022), isatin (2 mmol), kojic acid (2 mmol), and NaOAc (0.2 mmol) were refluxed in ethanol (4 mL) for 3 h. The precipitate was filtered, washed with cold ethanol (2 × 2 mL), dried under reduced pressure, yielding a white solid.

3-Hydroxy-3-[3-hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl]indolin-2-one: White powder; yield 0.445 g (76%); mp: 224-226℃. IR: v = 3194, 3005, 2840, 1726, 1647, 1624, 1581, 1473, 1448, 1246 and 1180. 1H NMR (500 MHz, DMSO-d6) 10.39 (br. s., 1H, NH), 8.99 (br. s., 1H, OH), 7.05 - 7.25 (m, 2H, CHAr), 6.80 - 6.96 (m, 2H, CHAr), 6.75 (d, J = 7.48 Hz, 1H, CH), 6.28 (br. s., 1H, CH), 5.66 (br. s., 1H, OH), 4.33 (br. s., 2H, CH_2OH). 13C NMR (126 MHz, DMSO-d6) d ppm 59.60 (s, 1 C) 74.37 (s, 1 C) 109.03 (s, 1 C) 109.78 (s, 1 C) 121.65 (s, 1 C) 124.07 (s, 1 C) 124.37 (s, 1 C) 130.28 (s, 1 C) 140.96 (s, 1 C) 142.74 (s, 1 C) 146.74 (s, 1 C) 167.65 (s, 1 C) 173.41 (s, 1 C) 175.21 (s, 1 C)ppm. HRMS (+ESI, 121.0 V): m/z (%): M (Calculated) = 289.0586, M+1 (Found)= 290.0649.

The tyrosinase structure (PDB: 2Y9X) was prepared by Biovia Discovery Studio, removing water/ligand and adding hydrogens. Ligands were minimized and docked using AutoDock Vina at the copper active site. Binding poses were ranked by affinity and analyzed for key interactions.

Cell culture: HCT116, MDAMB231, MCF7, and PANC-1 cells were grown in DMEM (4.5 g/L glucose) with 10% FBS and 1% penicillin/streptomycin at 37 °C, 5% CO_2. **MTT assay:** Cells (5×10^3/well) were treated with DRC-01 (1-100 µM) for 72 h. MTT (4 mg/mL) was added for 4 hours, and the formazan was dissolved in DMSO. The absorbance was then read at 570 nm. IC_{50} values were calculated from three experiments.

DPPH assay: A 0.1 mM DPPH solution in methanol was prepared and stored at 4 °C. Test samples and ascorbic acid (50-250 µg/mL) were mixed with DPPH (2 mL DPPH + 1 mL sample) and incubated in the dark at room temperature for 30-60 min. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer.

The antimalarial activity was tested against *Plasmodium falciparum* 3D7 using the SYBR Green I fluorescence assay. Parasites were incubated with different concentrations of the test compound for 72 h, after which SYBR Green dye was added, fluorescence measured, and IC_{50} values calculated.

RESULTS & DISCUSSION

DRC-01 was successfully synthesized and structurally confirmed by FTIR, NMR, and MS. It showed a

The docking analysis of the DRC-01 with Mushroom Tyrosinase (2Y9X) shows a higher tyrosinase binding affinity (-7.4 kcal/mol) than kojic acid (-5.8 kcal/mol). a stable and favorable binding within the active site. The ligand forms a strong hydrogen bond (2.99 Å) with Tyr311, supported by π-σ and π-alkyl interactions (3.75 Å and 4.07 Å), which enhance hydrophobic stabilization. Additional surrounding residues contribute to a complementary electrostatic and hydrophobic environment, helping to anchor the ligand securely in the binding pocket. Overall, the DRC-01 exhibits a moderate to strong binding affinity toward tyrosinase, primarily stabilized by hydrogen bonding and aromatic interactions with Tyr311.

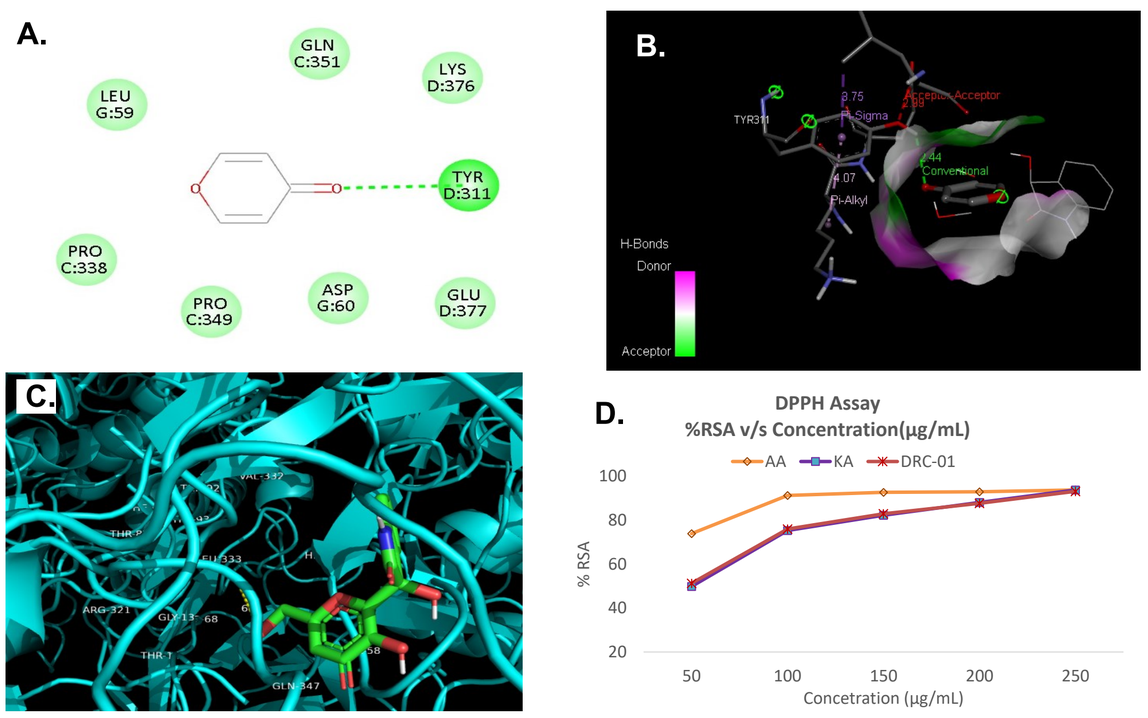


Fig: A. 2D Binding, B. H-Bond acceptor and Donor, C. Ligand-protein Interaction, D. DPPH Assay (AA: Ascorbic acid, KA: Kojic Acid, DRC-01)

The compound exhibited dose-dependent anticancer activity against HCT-116, PANC-1, MDA-MB-231, and MCF-7 cell lines at concentrations above 50 µM. Moderate antioxidant activity with %RSA = 51.26 at 50µg/mL. and antimalarial potential in *P. falciparum* 3D7 (IC_{50} = 54.94 ± 3.11 µg/mL) were also observed, highlighting DRC-01 as a promising multifunctional lead compound.

Sample	MTT Assay IC_{50} (µM)				Antimalarial (µg/mL)
	HCT-116	PANC-1	MDAMB-231	MCF-7	*P. Falciparum* 3D7
DRC-01	>50	>50	>50	>50	54.94 ± 3.11

CONCLUSION

The synthesized kojic acid derivative, DRC-01, demonstrated promising multifunctional bioactivities, including notable anti-tyrosinase, anticancer, antioxidant, and antimalarial effects. Its enhanced binding affinity compared to kojic acid, along with dose-dependent cytotoxicity against multiple cancer cell lines, underscores its therapeutic potential. Although moderate antioxidant and antimalarial activities were observed, further structural optimization could improve potency and selectivity. Overall, DRC-01 represents a valuable lead compound for developing multifunctional agents that target cancer, malaria, and oxidative stress-related disorders, with potential cosmeceutical applications.

REFERENCES

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