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PI3K α Inhibitory Activity

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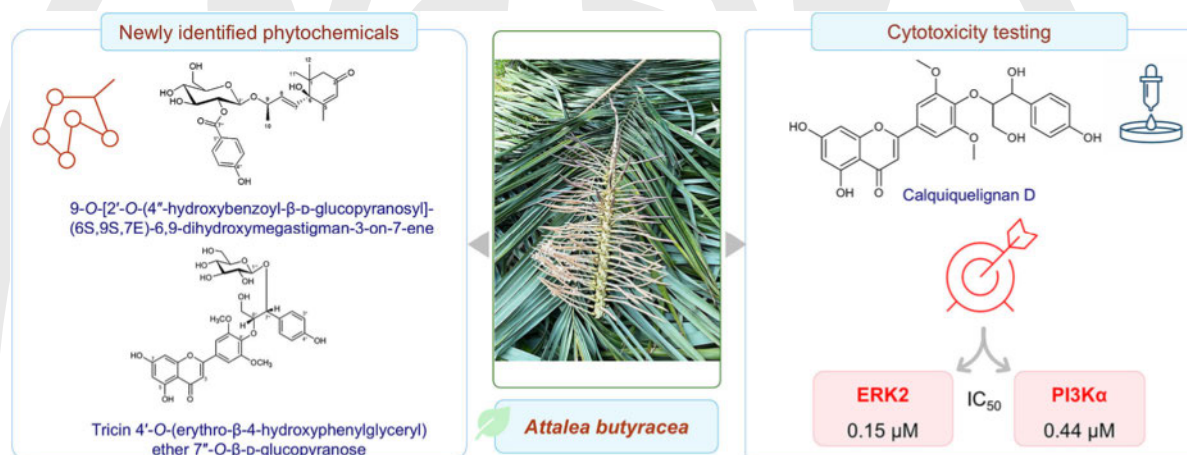
Original Papers

Calquiquelignan D from *Attalea butyracea* Exhibits Selective Cytotoxicity Against Prostate and Skin Cancer Cells with Dual ERK2/PI3K α Inhibitory Activity

Rofida Wahman¹ , Anber F. Mohammed², Claire Simons³, Islam El-Awaad^{1,4}, Shaymaa M. M. Mohamed^{1,5} 

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GRAPHICAL ABSTRACT



ABSTRACT

Skin and prostate cancers pose therapeutic challenges due to drug resistance. *Attalea butyracea*, a Neotropical palm with profound ethnobotanical significance, remains largely unexplored for anticancer constituents. This study aimed to isolate bioactive metabolites from *A. butyracea*, assess their selective cytotoxicity against A-431 and PC-3 cancer cells relative to normal cells, and explore their potential molecular targets. High-Resolution Electrospray Ionization Mass Spectrometry (HR-ESI/MS) and one- and two-dimensional nuclear magnetic resonance (1D/2D NMR) spectroscopy provided structural confirmation. Cytotoxic activity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Computational inverse docking and molecular dynamics simulations identified probable kinase targets, which were subsequently confirmed through enzyme inhibition assays. Phytochemical investigation yielded 17 compounds, including two previously undescribed glycosides, one megastigmane, and one flavonolignan. This marks the first report of flavonolignans within *Attalea*. Among isolated metabolites, calquiquelignan D showed notable activity, with CC₅₀ values of 14.9 μ M (A431) and 22.5 μ M (PC3), comparable to those of cisplatin. Within the ATP pockets of oncogenic kinases, this compound demonstrated stable binding patterns as determined by computational analysis. Enzyme inhibition assays confirmed dual inhibition of ERK2 and PI3K α at nanomolar concentrations. These findings suggest potential dual-pathway modulation of survival signaling. As chemotaxonomic markers with structurally distinct scaffolds, these metabolites merit further kinase-targeted drug development.

Keywords *Attalea butyracea*, Arecaceae, Megastigmane, Flavonolignan, ERK1/2, PI3K, inverse docking

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Introduction

Cancer continues to be a major global threat to human health. Current estimates for 2025 suggest a rising toll, with 20 million new cases and over 10.3 million attributable deaths globally.¹ Within this burden, prostate cancer persists as a leading cause of malignancy-related death in men.² Meanwhile, skin cancers, including melanoma and nonmelanoma types, show increasing prevalence in clinical surveillance.³ Drug resistance remains the primary barrier to successful cancer treatment. Polyphenols represent a promising strategy for overcoming drug resistance. These bioactive phytochemicals can bypass resistance mechanisms through multiple molecular mechanisms.⁴ Addressing this challenge requires exploring the chemical diversity found in nature to identify novel compounds for cancer therapy.

The Western Amazon boasts exceptional palm diversity, featuring species that play essential structural and cultural roles in regional ecosystems. The genus *Attalea* represents a recently diversified Neotropical ancestry whose phylogenetic relationships and biogeographic complexity reflect the dynamic evolutionary history of the Amazon basin.⁵ Ethnobotanical surveys throughout this region document the extensive use of these palms for both medicinal and nutritional purposes.⁶ Species such as *A. butyracea* (Mutis ex L.f.) Wess. Boeris (Arecaceae, wine palm) shows significant commercial potential. The substantial biomass of Amazonian palms further supports their viability as sustainable sources of bioactive compounds for both ecological and economic applications.^{7,8} However, this rich ethnobotanical knowledge requires rigorous pharmacologic validation to meet the standards of evidence-based phytotherapy.

Emerging evidence supports the anti-inflammatory and immunomodulatory capacity of these palm species. *A. speciosa* exhibits notable anti-infective properties through immune system modulation, significantly reducing mortality in methicillin-resistant *Staphylococcus aureus* (MRSA) infection models.⁹ Similarly, bioactive polysaccharides isolated from *Orbignya phalerata* demonstrate potent immunostimulatory effects, acting as robust activators of host defense mechanisms.¹⁰ Furthermore, oils derived from *A. phalerata* and *A. speciosa* exhibit therapeutic efficacy in experimental wound-healing models. Acute oral toxicity evaluations further establish a favorable safety profile, supporting their potential clinical application.^{11,12} While the lipid and polysaccharide fractions are well documented, the polyphenolic fraction of the *Attalea* genus, particularly its potential for flavonolignan structures, remains a critical yet underexplored reservoir for cancer therapeutic intervention.

Flavonolignans, such as the silymarin complex, constitute promising candidates for therapeutic repositioning in contemporary oncology.^{13,14} These compounds exhibit dual mechanisms, downregulating P-gp to sensitize tumors to chemotherapy while simultaneously mitigating liver damage associated with conventional anticancer treatments.¹⁵ Laboratory investigations revealed that silymarin and silibinin markedly suppress cancer cell proliferation in cultured systems.¹⁶ In prostate cancer models, these compounds exhibit potent antiproliferative and apoptotic effects; specifically, pure flavonolignans isolated from *Silybum marianum* have shown differential impacts on cell growth

and the precise regulation of cell cycle molecules.^{17–19} The therapeutic reach of these molecules extends to skin cancer, where they mitigate UVA-induced oxidative damage in keratinocytes and facilitate tumor regression by modulating MAP kinases.^{18,20} A synergy was observed when the flavone tricetin modulated miR-21, thereby enhancing the effects of docetaxel.²¹ Recent mechanistic deep dives and SAR studies have identified flavonol-based analogues as potent inhibitors of critical cancer pathways, specifically targeting CDK2 and mTOR.^{22,23}

Despite the documented nutritional and chemical richness of Brazilian palm fruits, a significant research gap persists at the intersection of Neotropical biodiversity and targeted oncological intervention.²⁴ While the *Attalea* genus is known for its chemical complexity, its specific efficacy against melanoma and prostate carcinoma remains insufficiently synthesized with the established mechanisms of flavonolignans. This investigation addresses this deficit by evaluating the potential of palm-derived compounds as broad-spectrum therapeutic strategies.

Results

The systematic extraction and purification of secondary metabolites from the aerial parts of *A. butyracea* serve as a foundational strategy for identifying structurally unique bioactive scaffolds. This isolation process moves beyond mere observation of traditional botanical uses. Instead, it allows for the precise identification of molecules that regulate oncogenesis. Precise structural elucidation offers the chemical resolution required to map the plant's diverse metabolome. Such detailed characterization transforms crude extracts into viable templates for the development of novel chemotherapeutics.

A comprehensive phytochemical investigation of *A. butyracea* yielded 17 compounds, spanning a chemical spectrum from simple phenolic acids to complex flavonolignan glycosides. The structural identities of the known isolates (**1–6**, **8–12**, and **14–17**) were rigorously confirmed by comparing their NMR spectral data (1S, 2S, **Figs. 15–15S, 22S–30S, 37S–45S**, Supporting Information) with established literature values. These include the triterpene cylindrin (**1**), methyl ferulate (**2**), 2,6-dimethoxy-*p*-quinone (**3**), methyl-4-hydroxybenzoate (**4**), methyl β -D-glucopyranoside (**5**), the megastigmane 3,4-dehydrotheaspiron (**6**), *p*-hydroxybenzoic acid (**8**), protocatechuic acid (**9**), vitexin (**11**), and orientin (**12**). The study also identified several tricetin-based derivatives, including tricetin 4'-O-(*erythro*- β -guaiacylglyceryl) ether 7"-O- β -D-glucopyranose (**10**), tricetin 4'-O-(*threo*- β -guaiacylglyceryl) ether (**14**), tricetin 4'-O-(*erythro*- β -4-hydroxyphenylglyceryl) ether (**15**), tricetin 4'-O-(*erythro*- β -guaiacylglyceryl) ether (**16**), and the parent flavone tricetin (**17**). Notably, the isolation of compound **10** is of high chemotaxonomic significance, as this flavonolignan had previously been reported exclusively in *Zizania latifolia* within the Poaceae family; its identification here represents the first recorded occurrence in the Arecaceae family.

Compound **7** (**Fig. 1**) was isolated as a colorless oil, with a specific rotation $[\alpha]_D^{22}$ of +41 (c 0.04, MeOH). HR-ESI/MS spectrum (**Fig. 16S**, Supporting Information) in negative ion mode revealed a pseudomolecular ion peak at *m/z* 505.2074 [M-H][–], corresponding to the molecular formula C₂₆H₃₃O₁₀ (calcd 505.2073,

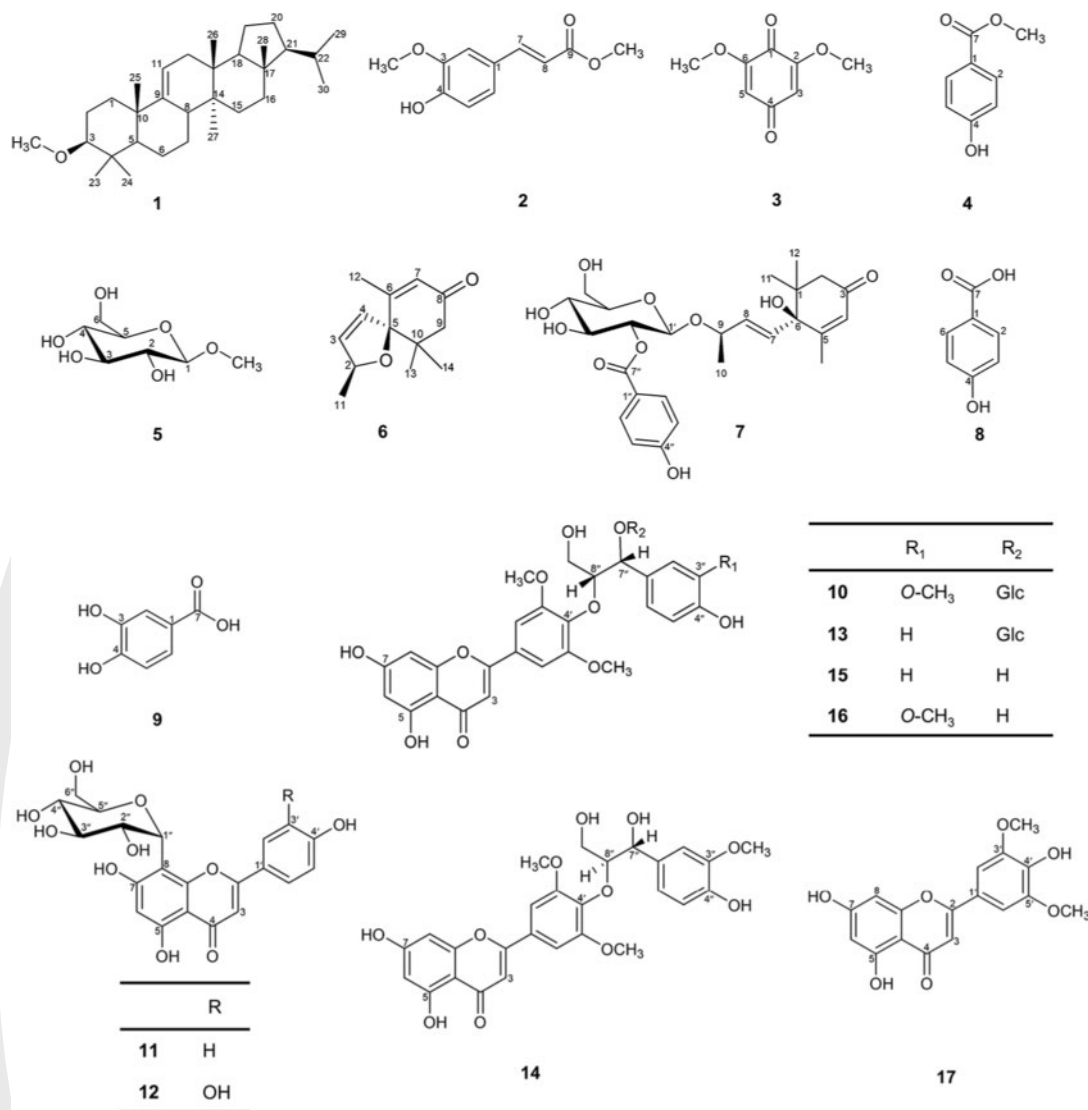


Fig. 1 Compounds isolated from *Attalea butyracea* aerial parts.

mass error = 0.19 ppm). ¹H and APT NMR spectra (**Figs. 17S and 18S**, Supporting Information) showed signals corresponding to a 2'-(4"-hydroxybenzoyl)-β-D-glucopyranosyl moiety. In the ¹H NMR spectrum, two doublet signals at δ_H 7.86 (2H, *d*, *J* = 8.8 Hz, H-2" and H-6") and 6.82 (2H, *d*, *J* = 8.8 Hz, H-3" and H-5") were attributed to a AA'BB' system in a 1,4-substituted benzene ring, assigned to the 4-hydroxybenzoyl moiety. The HMBC spectrum showed a cross peak between the aromatic protons at δ_H 7.86 and the carbonyl carbon (δ_C 166.6, C-7"). Long-range coupling connected this carbonyl group to the sugar proton at δ_H 4.92, assigned to H-2' based on the coupling constants (1H, *dd*, *J* = 8.0, 9.6 Hz) and COSY correlation with the anomeric proton H-1'. This was further supported by HMBC correlation of H-2' with both C-1' and C-3' (**Fig. 2**). Comparative analysis of the NMR data (**Table 1**) with roseoside (a known megastigmane glycoside) suggested that **7** possesses a megastigmane skeleton featuring a carbonyl group at C-3, hydroxyl functional groups at C-6 and C-9 positions, and a

double bond with two substituents. The HSQC spectrum (**Fig. 19S** Supporting Information) confirmed the aglycone moiety as 6,9-dihydroxymegastigman-3-on-7-ene, with full carbon assignments (**Table 1**). Key evidence for the *E*-configuration of the Δ⁷ double bond came from the ¹H NMR coupling constant (*J* = 15.2 Hz between H-7 and H-8), consistent with *trans*-disubstituted olefins. The HMBC spectrum (**Figs. 20S and 21S**, Supporting Information) confirmed the glycosidic linkage at C-9 via the correlation from the anomeric proton at δ_H 4.58 ppm to C-9. Additionally, 1,3-diaxial interactions between the C-6 hydroxyl group and axial protons at C-2 and C-4 induced characteristic downfield shifts, supporting a 6*S* configuration. The absolute configuration at C-9 (9*S*) was established by comparing chemical shifts with known roseoside stereoisomers.²⁵ Identified as 9-*O*-(2'-*O*-(4"-hydroxybenzoyl)-β-D-glucopyranosyl)-(6*S*,9*S*,7*E*)-6,9-dihydroxymegastigman-3-on-7-ene, this molecule represents the first isolation from natural sources, a rare modification that

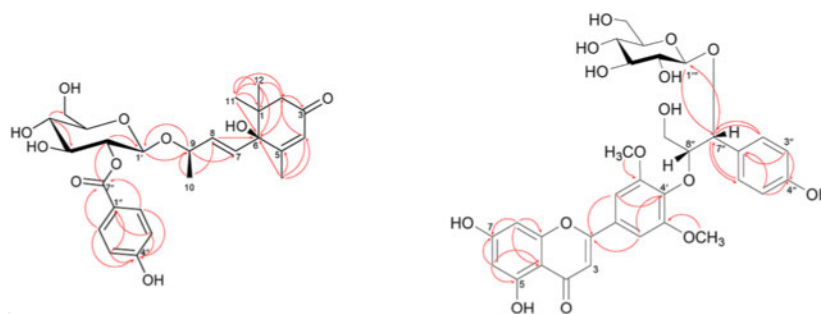


Fig. 2 Key HMBC correlations for compounds **7** (A) and **13** (B)

Table 1 ^1H and ^{13}C NMR spectral data of compound **7** (CD_3OD , 400, 100 MHz).

Position	δ_{C} (ppm)	δ_{H} (ppm), multiplicity, J (Hz)
1	42.4	–
2	50.8	2.13, <i>d</i> , 17.2 2.44, <i>d</i> , 17.2
3	201.0	–
4	127.3	5.84, <i>s</i>
5	167.3	–
6	79.8	–
7	133.8	5.80, <i>d</i> , 15.2
8	133.5	5.52, <i>dd</i> , 7.2, 15.2
9	75.7	4.48, <i>t</i> -like, 6.8
10	22.3	1.22, <i>d</i> , 6.4
11	23.4	1.0, <i>s</i>
12	24.6	0.90, <i>s</i>
13	19.5	1.87, <i>d</i> , 1.6
β -D-Glucopyranose		
1'	99.8	4.58, <i>d</i> , 8.0
2'	75.4	4.92, <i>dd</i> , 8.0, 9.6
3'	78.4	3.27, <i>m</i>
4'	71.9	3.39, <i>t</i> , 9.6
5'	76.5	3.57, <i>t</i> , 9.2
6'	62.8	3.69, <i>dd</i> , 6.4, 12.0 3.90, <i>dd</i> , 2.4, 12.0
4"-Hydroxybenzoyl		
1"	122.4	–
2",6"	133.1	7.86, <i>d</i> , 8.8
3",5"	116.3	6.84, <i>d</i> , 8.8
4"	163.6	–
7"	166.6	–

significantly distinguishes the chemical profile of *A. butyracea* from typical plant metabolomes.

Compound **13** (Fig. 1) was isolated as a yellow amorphous powder, with a specific rotation $[\alpha]_{\text{D}}^{22}$ of +11 (*c* 0.04, MeOH). HR-ESI/MS spectrum in the positive ion mode (Fig. 31S, Supporting Information) revealed a molecular ion peak at m/z

659.1973 $[\text{M}+\text{H}]^+$ (calcd 659.1976, mass error = 0.45 ppm) and a sodiated adduct at m/z 681.1790 $[\text{M}+\text{Na}]^+$ (calcd 681.1795, mass error = 0.73 ppm), establishing the molecular formula as $\text{C}_{32}\text{H}_{34}\text{O}_{15}$. The ^1H NMR spectrum (Table 2, Fig. 32S, Supporting Information) displayed two *ortho*-coupled doublets at δ_{H} 7.22 (2H, *d*, J = 8.4 Hz, H-2", 6") and 6.71 (2H, *d*, J = 8.4 Hz, H-3", 5"), characteristic of a *para*-disubstituted benzene ring. Additionally, five aromatic methine signals resonating at δ_{H} 7.34 (2H, *s*, H-2', 6'), 7.06 (1H, *s*, H-3), 6.57 (1H, *br s*, H-8), 6.22 (1H, *br s*, H-6), along with a methoxy proton signal δ_{H} 3.90 (6H, *s*, 3', 5'-OCH₃), suggested the presence of a tricin-type flavone moiety. Further analysis revealed oxygenated methine protons at δ_{H} 5.17 (1H, *d*, J = 4.4 Hz, H-7") and 4.85 (1H, *m*, H-8") as well as oxygenated methylene protons at δ_{H} 3.63 (1H, *dd*, J = 6.4 Hz, 12 Hz, H-9" a), 3.39 (1H, *overlapped*, H-9" b). Additionally, oxygenated methine and methylene signals for a glucopyranose unit, featuring an anomeric proton signal at δ_{H} 4.40 (1H, *d*, J = 7.2 Hz, H-1") with a large $^3J_{\text{H}1"/\text{H}2"}$ coupling constant of 7.2 Hz, indicating a β -configuration. The relative configuration of the oxygenated chiral methines, H-7" (δ_{H} 5.17) and H-8" (δ_{H} 4.85), was inferred to be of the *erythro* type based on the narrow coupling constant (J = 4.4 Hz) between them. To confirm this, the ^1H NMR spectrum was reacquired in $\text{C}_5\text{D}_5\text{N}$, yielding a slightly reduced coupling constant (J = 3.6 Hz), consistent with reported values for *erythro*-type analogues.²⁶ The APT and HSQC spectra (Figs. 33S and 34S, Supporting Information) revealed a total of 27 carbon signals, including a methoxy carbon resonating at δ_{C} 56.4 (3', 5'-OCH₃), characteristic of a tricin flavone linked to a phenylpropanoid moiety and a hexose moiety. In the down field region, a conjugated ketone carbon δ_{C} 181.8 (C-4), seven oxygenated quaternary carbons [δ_{C} 164.7 (C-7), 163.0 (C-2), 161.4 (C-5), 157.4 (C-9), 152.9 (C-3', 5'), 139.8 (C-4')], two aromatic quaternary carbons [δ_{C} 125.6 (C-1'), 103.7 (C-10)], and five aromatic methine carbons [δ_{C} 105.0 (C-3), 104.2 (C-2', 6'), 99.1 (C-6), 94.4 (C-8)] signals were indicative of a tricin flavone. Furthermore, distinct signals indicative of a *para*-disubstituted benzene were identified, encompassing an oxygenated quaternary carbon at δ_{C} 156.5 (C-4"), an aromatic quaternary carbon at δ_{C} 132.8 (C-1"), and two sets of aromatic methines at δ_{C} 128.5 (C-2", 6") and 114.3 (C-3", 5"). These findings confirm the presence of a 4-hydroxyphenyl moiety. Two oxygenated methine carbons at δ_{C} 78.0 (C-7") and 84.4 (C-8"), and one oxygenated methylene carbon δ_{C} 60.4 (C-9") signals were observed,

Table 2 ¹H and ¹³C NMR spectral data of compound **13** (DMSO-*d*₆, 400, 100 MHz).

Position	δ _c (ppm)	δ _H (ppm), multiplicity, J (Hz)
Tricin moiety		
2	163.0	–
3	105.0	7.06, <i>s</i>
4	181.8	–
5	161.4	–
6	99.1	6.22, <i>br s</i>
7	164.7	–
8	94.4	6.57, <i>br s</i>
9	157.4	–
10	103.7	–
1'	125.6	–
2', 6'	104.2	7.34, <i>s</i>
3', 5'	152.9	–
4'	139.8	–
3', 5'-O-CH ₃	56.4	3.90, <i>s</i>
4'-Hydroxyphenylglyceryl		
1''	132.8	–
2'', 6''	128.5	7.22, <i>d</i> , 8.4
3'', 5''	114.3	6.71, <i>d</i> , 8.4
4''	156.5	–
7''	78.0	5.17, <i>d</i> , 4.4
8''	84.4	4.85, <i>m</i>
9''a	60.4	3.63, <i>dd</i> , 6.4, 11.2
9''b		3.39, <i>m</i>
β-D-Glucose moiety		
1'''	102.0	4.40, <i>d</i> , 7.2
2'''	74.0	3.04–3.15, <i>m</i>
3'''	77.3	
4'''	70.0	
5'''	76.5	
6'''	60.9	3.65, <i>m</i> 3.21, <i>m</i>

indicating the presence of a glyceryl moiety. Through analysis of the HMBC spectrum (**Figs. 35S and 36S**, Supporting Information), a significant cross-peak between the anomeric proton signal at δ_H 4.40 and the oxygenated methine carbon signal at δ_C 78.0 (C-7") was observed, implying a glycosidic bond connecting glucose to the C-7" position of the phenylpropanoid. Additionally, a significant HMBC correlation between δ_H 5.17 and δ_C 128.5 (C-2",6") confirmed the positioning of the 4-hydroxyphenyl moiety at C-7" of the glyceryl moiety (**Fig. 2**). Based on comprehensive spectroscopic analysis, the structure of **13** was determined to be triclin 4'-O-(*erythro*-β-4-hydroxyphenylglyceryl) ether 7"-O-β-D-glucopyranose. This represents the first report for the isolation of this compound from natural sources.

The isolated compounds (**7**, **10**, **13–15**) were evaluated for cytotoxic activity against human skin cancer (A431), prostate cancer (PC3), and normal human melanocyte (HFB4) cell lines using MTT assays. Compound **15** demonstrated the most significant and selective cytotoxic activity, with CC₅₀ values of 14.9 μM against A431 and 22.5 μM against PC3. Calculation of selectivity indices revealed that compound **15** possesses favorable SI values of 3.13 and 2.07 for A431 and PC3, respectively, relative to HFB4 normal cells. While compounds **7**, **10**, **13**, and **14** showed limited efficacy (**Table 3**), the moderate cytotoxicity and selectivity of **15**, targeting malignant phenotypes while sparing healthy melanocytes, make it a significant candidate for deeper mechanistic investigation. It should be noted, however, that the MTT assay carries inherent methodological limitations. Being a redox-based viability assay, it cannot distinguish between cytotoxic and cytostatic effects. Furthermore, non-mitochondrial dehydrogenases and redox-active test compounds may reduce the tetrazolium salt independently of mitochondrial activity, generating false-positive viability signals.

Tricin-based compound **15**, identified by the MTT assay as the most active cytotoxic compound, was exposed to thorough inverse docking experiments utilizing MOE software (2024.0601, Chemical Computing Group ULC, Montreal, QC, Canada)²⁷ to predict its potential target(s) for its cytotoxic activity. For this study, nine kinase proteins were selected, including receptor tyrosine kinases

Table 3 In vitro cytotoxic effects of the tested compounds on cancerous (A431 and PC3) and normal (HFB4) cell lines.

Compound	CC ₅₀ (μM) [95% CI] ^a		
	A431	PC3	HFB 4
7	118.9 [113.1–125.0]	124.2 [111.1–139.0]	173.7 [150.0–201.2]
10	124.4 [114.8–134.9]	159.2 [141.1–179.8]	213.3 [195.5–232.8]
13	240.6 [227.2–254.8]	262.1 [238.7–287.8]	366.1 [352.2–380.7]
14	155.9 [133.1–182.6]	220.1 [194.2–249.4]	272.7 [266.0–279.5]
15	14.9 [13.9–16.1] (SI = 3.13)	22.5 [20.1–25.2] (SI = 2.07)	46.8 [44.7–49.0]
Cisplatin	13.2 [10.0–17.5] (SI = 2.89)	29.5 [24.6–35.3] (SI = 1.29)	38.2 [34.8–41.9]

^aCC₅₀ values are presented as best-fit values with 95% confidence intervals.

[EGFR (1M17), VEGFR-2 (4ASD), c-Kit (6GQJ), c-Met (3DKF)], intracellular signaling kinases [PI3K α (5XGH), MEK-1 (3PP1), AKT-1 (4GV1), MAPK (5K4I)], and cell division kinase [CDK-2 (2C6O)]. These proteins are reported to be essential molecular targets for modulating skin and prostate cancers.²⁸ In addition, they were identified as the cancer-related major pathways influenced by tricin.²⁹ The three-dimensional protein structures were sourced from the Protein Data Bank (PDB) using specific entry codes.³⁰ To ensure the reliability of the docking simulations, we re-docked the original cocrystallized ligand back into the active site of the target kinase. The cocrystallized ligand maintained its native conformation within the binding site, with RMSD values <2 Å. The screening analysis reported the essential amino acid interactions for binding to the selected proteins (as published for each protein), as well as the docking scores for **15** compared with the respective co-crystallized ligands within the protein binding sites. The docking results for **15**, alongside the reference cocrystallized ligands, are documented in **Tables 1S and 2S** (Supporting Information). The three kinases, c-Kit, MEK1 (ATP site), and Akt1, were excluded from further analysis due to compound **15**'s moderate docking scores relative to their respective cocrystallized ligands, suggesting weak or limited interactions to their binding sites. The compound conformation did not touch the pocket interaction surface, which, in reverse, affected its binding score. On the other hand, compound **15** displayed docking scores to the remaining kinases ranging from -8.47 to -7.54 kcal/mol, suggesting a high binding affinity for these proteins. Binding mode inspection revealed that compound **15** missed some key amino acid interactions within the active sites of EGFR, VEGFR-2, c-Met, Mek1 (allosteric site), and CDK2; however, it fulfilled the crucial

interactions for binding to the ERK2 and PI3K α active sites. The compound formed favorable hydrogen bonds and hydrophobic interactions within its binding sites, while maintaining a complementary model aligned with the native conformation of its cocrystallized ligands. An inverse docking study suggested that ERK2 and PI3K α might be the most likely targets modulating the cytotoxic action of compound **15**. Further molecular dynamics simulations were performed to ascertain the stability and free-energy binding calculation of ligand protein complexes to validate the obtained docking results.

The ERK2-ligand **15** complex, generated from the docking experiment, was subjected to a 200 ns molecular dynamics (MD) simulation using the Desmond program within Schrödinger Maestro.^{31,32} The protein ligand complex equilibrated at approximately 95 ns (**Fig. 3A**) with multiple ligand protein interactions over the simulation time (**Fig. 3B**).

Ligand **15** is positioned in the amino-terminal (N)-lobe within the ATP binding pocket (**Fig. 4**). Ligand **15** was observed to form and maintain a hydrogen bond between the phenolic hydroxy and Tyr36 as well as an aryl cation bond between the phenol aryl moiety and Lys54 (**Fig. 5**). Tyr36 is in the glycine-rich (GxGxxG) ATP-phosphate binding loop between the β 1- and β 2-strands, which accommodates the adenine moiety of ATP,^{33,34} while Lys54 is in the β 3-strand and contained within the AxK motif. The two aliphatic hydroxy groups were found to participate in H-bonding interactions with Lys54 and Asn154, which are located in the catalytic loop (**Figs. 4 and 5**). On the other side of the ligand, the 4-keto and 5-phenolic hydroxy groups interact via hydrogen bonds with the catalytic or C-spine residue Met108 positioned between the D-helix and the β 5-strand (**Fig. 4**).

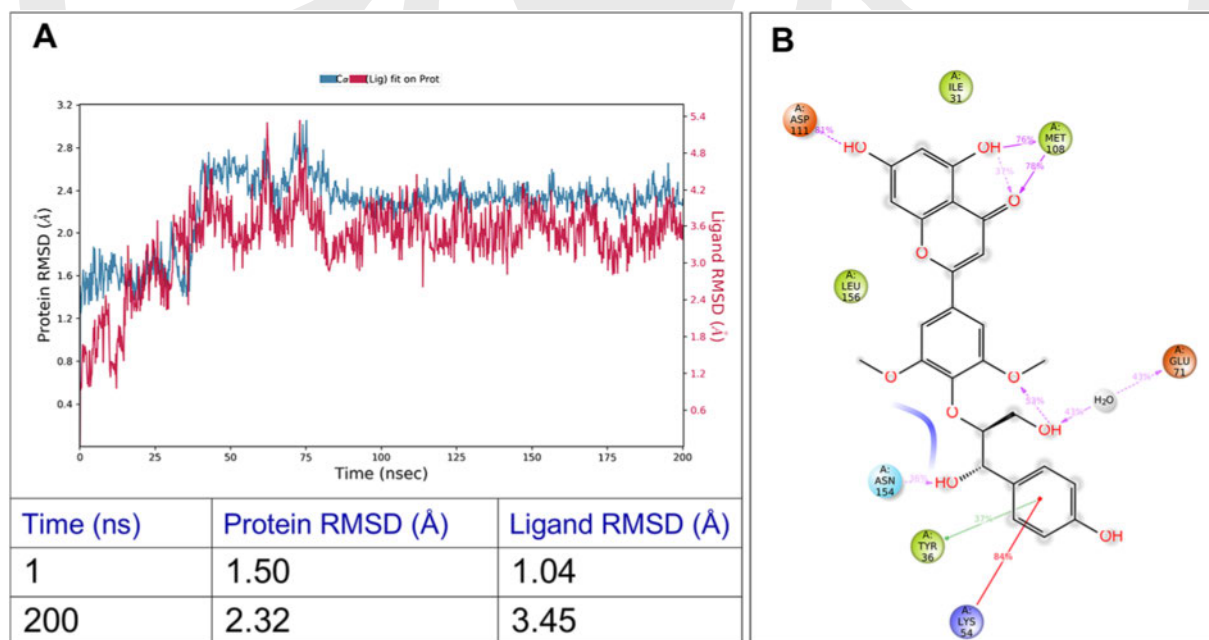


Fig. 3 (A) Protein/ligand RMSD (Å) of protein ligand complexes of **15** with ERK2 over a 200 ns molecular dynamics simulation, and (B) a schematic of the ligand atom interactions with protein residues that occur more than 30% of the simulation time in the selected trajectory (0–200 ns) is shown.

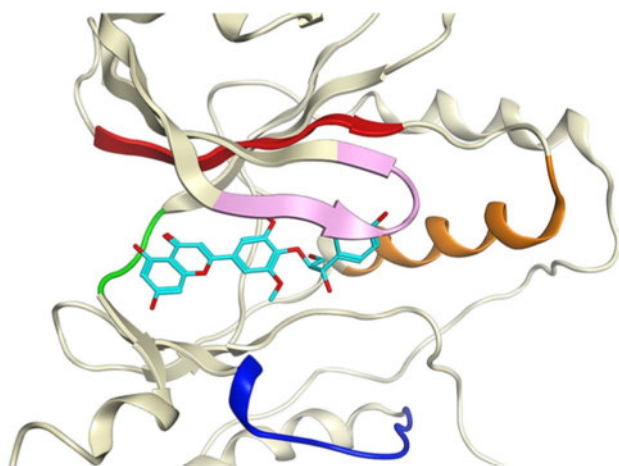


Fig. 4 Ligand **15** (cyan) positioned in the N-lobe of ERK2. Ligand **15** sits in the ATP binding pocket between the glycine-rich loop (pink), the β 3-strand (red), the α C-helix (orange), the catalytic loop (blue), and the loop between the D-helix and β 5-strand (green), which contains the catalytic Met108.

In contrast, the 7-phenolic hydroxy group donates a H-bond contact to Asp111 at the start of the α D-helix (**Fig. 5**).

The PI3K α -ligand **15** complex, generated from a docking study, was also subjected to a 200 ns molecular dynamics (MD) simulation using the Desmond program of Schrödinger Maestro. The protein ligand complex equilibrated at approximately 60 ns (**Fig. 6A**), with multiple ligand protein interactions over the simulation time (**Fig. 6B**).

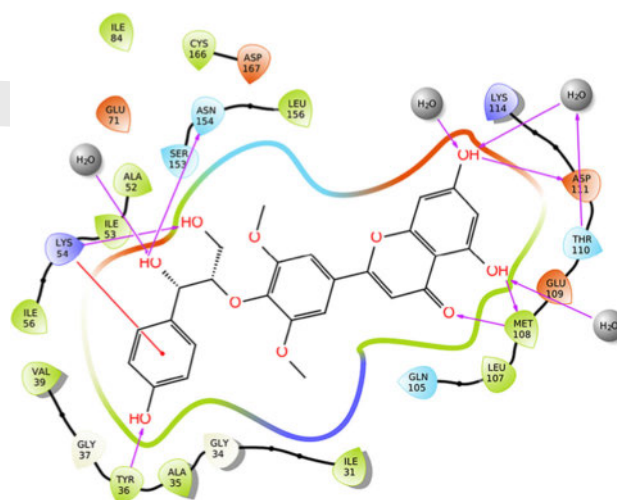


Fig. 5 Ligand **15** binding interaction with ERK2 in the final frame of the 200 ns molecular dynamics simulation.

Ligand **15** is lying in the catalytic (kinase) domain³⁵ of PI3K α , composed of the N-terminal and C-terminal lobes, within the ATP binding pocket (**Fig. 7**). Ligand **15** was found to participate in water bridged H-bond interaction between its phenolic hydroxyl group and Asp933 in the DFG motif of the activation loop,³⁶ as well as H-bonding with Lys802, which is involved in the phosphate transfer reaction (**Fig. 8**).^{36,37} The CH_2OH group forms a water-bridged hydrogen bond with Ser773 in the phosphate-binding loop. On the other side of the ligand, the 4-keto group was observed to form a water-mediated hydrogen

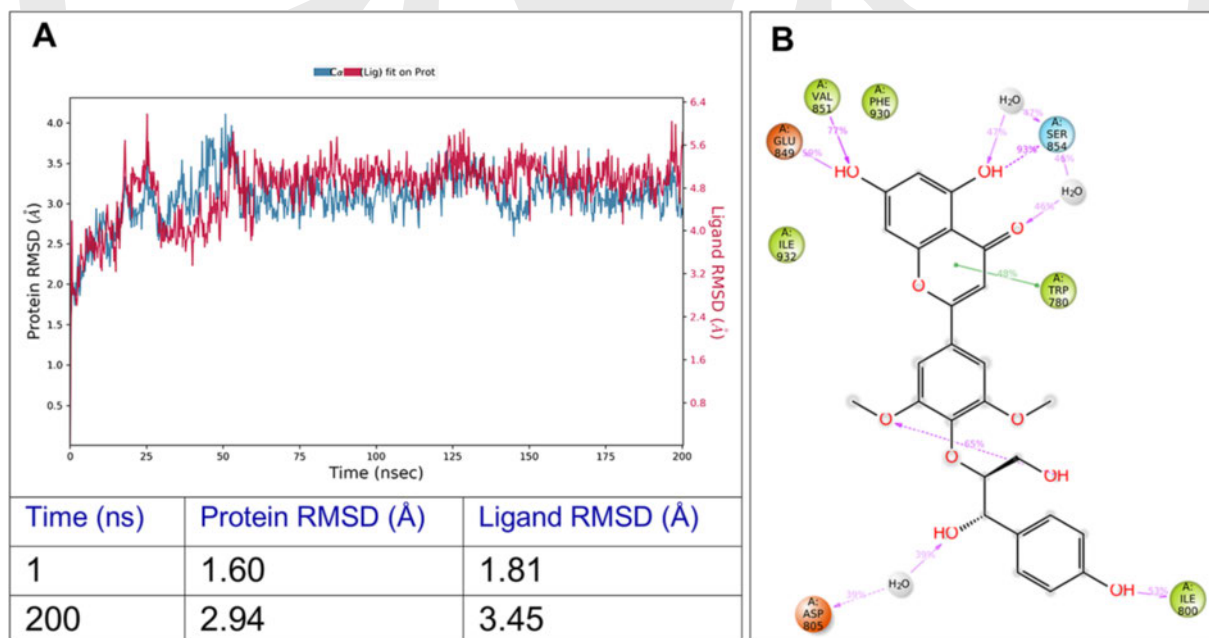


Fig. 6 (A) Protein/ligand RMSD (Å) of protein ligand complexes of **15** with PI3K α over a 200 ns molecular dynamics simulation, and (B) a schematic of the ligand atom interactions with protein residues that occur more than 30% of the simulation time in the selected trajectory (0–200 ns) is shown.

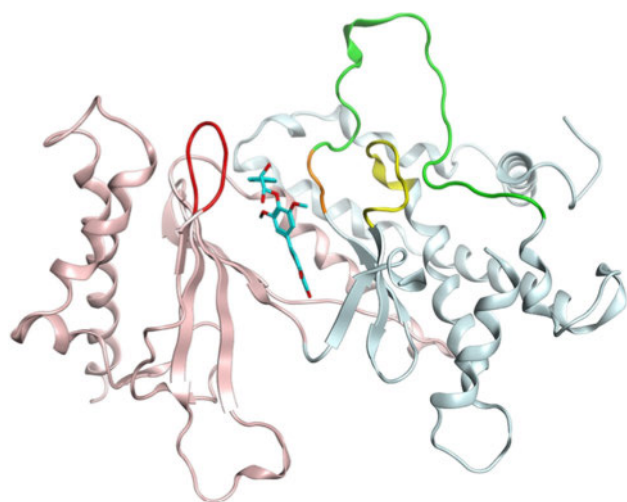


Fig. 7 Ligand **15** (cyan) in the catalytic/kinase domain of PI3K α . Ligand **15** sits in the ATP binding pocket, positioned between the N-terminal lobe (light pink) that contains the phosphate binding loop (red), and the C-terminal lobe (light blue) that contains the conserved DFG motif (orange), activation loop (green), and the catalytic loop (yellow). [For clarity, only the catalytic domain containing the C- and N-lobes is shown.]

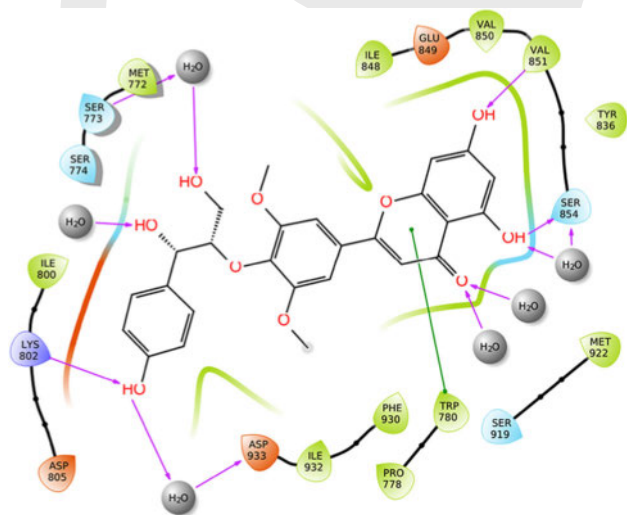


Fig. 8 Ligand **15** binding interaction with PI3K α in the final frame of the 200 ns molecular dynamics simulation.

bond, and the pyran-4-one ring of the chromen-4-one moiety interacts via π - π stacking with Trp780 in the β 4K-strand of the N-lobe. The two hydroxy groups of the chromen-4-one ring participate in hydrogen bonding interactions with Val851, which sits at the interface between the N- and C-lobes, and Ser854 in the β 8K-strand at the beginning of the C-lobe (Figs. 7 and 8).

For the ERK2 and PI3K α ligand **15** complexes, the mean ΔG (bind)³⁸ was calculated from 100–200 ns (from protein-ligand equilibration, Figs. 3A and 6A) of the MD simulation (Table 4).

Table 4 Computational binding affinity (ΔG) of ligand **15**–protein complexes.

Complex	ΔG (kcal/mol) 100–200 ns
ERK2-ligand 15	-65.52 ± 7.04
PI3K α -ligand 15	-62.50 ± 5.17

Table 5 Results of enzyme inhibition assays.

Compound	ERK inhibition IC ₅₀ (μ M/ml) [95% CI]		PI3K α inhibition IC ₅₀ (μ M/ml) [95% CI]
	ERK1	ERK2	
15	0.51 [0.49–0.53]	0.15 [0.14–0.16]	0.44 [0.43–0.46]
Ravoxertinib	0.06 [0.058–0.063]	0.08 [0.077–0.083]	--
Alpelisib	--	--	0.18 [0.17–0.19]
-- Not applicable.			

Ligand **15** showed good binding affinity with both ERK2 and PI3K α , with ΔG values of -65.52 ± 7.04 and -62.50 ± 5.17 kcal/mol, respectively, over the timeframe. These results suggested that ERK1/2 and PI3K α may be plausible dual targets contributing to the cytotoxic activity of **15** and provided a rationale for performing subsequent in vitro enzyme inhibition assays.

The computational predictions were experimentally supported by in vitro kinase inhibition assays, which established compound **15** as a submicromolar dual inhibitor. Compound **15** exhibited IC₅₀ values of 0.51 μ M against ERK1, 0.15 μ M against ERK2, and 0.44 μ M against PI3K α . While the reference standards Ravoxertinib and Alpelisib showed higher absolute potency, Ravoxertinib demonstrated negligible isoform selectivity (0.06 vs 0.08 μ M). In contrast, compound **15** displayed a significant 3.4-fold selectivity for ERK2 over ERK1, highlighting its potential for isoform-preferential modulation (Table 5). These findings confirm the anticipated multitarget inhibitory profile of compound **15** toward ERK1/2 and PI3K α and provide experimental validation consistent with the computational study.

Formal evaluation against Lipinski's Rule of Five reveals that compound **15** satisfies all four criteria, with five hydrogen bond donors, ten hydrogen bond acceptors, and a cLogP of approximately 2, collectively indicating favorable predicted oral bioavailability with no rule violations.

Discussion

The integration of phytochemical structural elucidation with computational and biological validation highlights the strategic value of *A. butyracea* as a source of multitarget inhibitors. Dual inhibition of ERK and PI3K signaling has been proposed as a strategy to overcome adaptive resistance commonly associated with single-target inhibitors.

The identification of compound **10** (tricin 4'-*O*-(*erythro*- β -guaiacylglyceryl) ether 7"-*O*- β -D-glucopyranose) within the Arecaceae provides significant chemotaxonomic insight into the Commelinid clade. Previously, this specific tricin derivative was documented exclusively in *Zizania latifolia*, a member of the Poaceae family.²⁶ The occurrence of such a complex flavonolignan in both Arecaceae and Poaceae suggests that the enzymatic machinery is either an ancestral trait or a highly conserved biosynthetic pathway within the Commelinid clade. This discovery suggests that the biosynthetic pathways responsible for complex flavonolignan assembly are more widely distributed across monocotyledonous families than previously assumed. Furthermore, the identification of the previously undescribed megastigmane glycoside (**7**) and flavonolignan glycoside (**13**) significantly expands the known chemical space for these classes. The presence of rare modifications, such as the hydroxyphenyl substitution at position 2 of the glucopyranosyl unit in compound **7**, suggests the potential involvement of specialized biosynthetic enzymes in *A. butyracea*.

The structure–activity relationship observed for compound **15** indicates that its tricin-based flavonolignan skeleton is essential for its preferential cytotoxicity against A431 skin and PC3 prostate cancer cells. In the A431 cell line, compound **15** exhibited a CC₅₀ of 14.9 μ M. This value is numerically similar to the reference drug, cisplatin (CC₅₀ of 13.2 μ M). When tested against the PC3 prostate cancer line, compound **15** displayed a CC₅₀ of 22.5 μ M, slightly outperforming cisplatin's CC₅₀ of 29.5 μ M. While these figures indicate that compound **15** maintains primary cytotoxic effects comparable to a standard chemotherapeutic, they represent moderate activity by modern pharmaceutical benchmarks. The greater cytotoxic activity of compound **15** compared to its tricin congeners (compounds **10**, **13**, and **14**) may partly reflect the absence of the glucose moiety carried by compounds **10** and **13**, which increases molecular polarity and steric bulk, potentially limiting membrane permeability. In addition, the absence of the vicinal diol functionality seen in compounds **10** and **14**, combined with the dimethoxy substitution pattern on the B-ring side chain of compound **15**, may reduce rotational flexibility and favor a more planar conformation conducive to kinase binding. The observed selective cytotoxicity of compound **15** toward A-431 and PC-3 cell lines, relative to normal HFB-4 melanocytes, may suggest a degree of tumor-selective activity; however, the precise molecular basis of this selectivity remains to be elucidated through dedicated mechanistic studies. Compound **15** showed an SI of 3.13 in A431 cells and 2.07 in PC3 cells. While these values surpass those of cisplatin in the PC3 model (SI = 1.29), they fall short of the rigorous industry standard of SI > 10. The relatively narrow window between cancer cell inhibition and normal melanocyte toxicity suggests a low-to-moderate selectivity profile. Consequently, while the compound serves as a promising lead for further structural refinement, its current pharmacologic profile necessitates careful optimization to enhance its safety margin. Future structural modification studies should focus on increasing the CC₅₀ to reduce off-target effects on healthy tissues.

MD simulations provide the structural rationale for the dual inhibitory activity of compound **15**. In ERK2, the interaction with Lys54 is paramount. The role of Lys54 in ERK2 is to link the α - and β -phosphates of ATP to the C-helix. The formation of a salt bridge

between the β 3-strand Lys54 and the α C-helix Glu71 is essential for the formation of the activated conformation of ERK2.^{33,34} Targeting Lys54 is a hallmark of high-potency inhibitors.³⁹ Simultaneously, compound **15** interacts with Tyr36 in the GxGxxG loop, further stabilizing the complex. In PI3K α , the compound engages Asp933 in the DFG motif and Lys802, the latter being critical for the phosphate transfer reaction.^{36,37} The ability of compound **15** to engage these conserved catalytic residues in both the MAPK and PI3K pathways explains its potent dual inhibition. It reinforces the therapeutic potential of targeting these major cancer signaling pathways with a single, complex scaffold. Testing compound **15** against clinically relevant resistant models, such as vemurafenib-resistant melanoma cell lines, remains an important direction for future investigation.

There is a near 50-fold discrepancy between submicromolar enzyme inhibition (0.15–0.44 μ M) and the weaker cellular activity (~20 μ M). A primary challenge in drug discovery is the frequent discrepancy between compound activity in biochemical assays and cellular models, often driven by factors such as permeability, solubility, and metabolic stability. While the tricin core of compound **15** typically confers favorable bioavailability and higher intestinal absorption compared to simpler flavone structures, its cellular performance may still be limited by suboptimal membrane permeability. We suggest that introducing the *erythro*- β -4-hydroxyphenylglyceryl ether moiety increases lipophilicity but may also raise molecular weight and polar surface area. This structural shift may limit rapid membrane translocation despite enhanced solubility. Furthermore, enzymatic reactions within the cell are influenced by cytoplasmic crowding and physicochemical conditions, which differ markedly from those in simplified, cell-free assay conditions. The identified 50-fold discrepancy in potency likely stems from a combination of factors that attenuate the compound's bioavailability within a cellular environment. One primary consideration is the restricted cellular uptake caused by the amphipathic nature of the flavonolignan scaffold, potentially resulting in a 5–10-fold decrease in internal concentration. Furthermore, metabolic attenuation may play a significant role, as the glyceryl moiety could undergo rapid Phase II metabolism before the molecule effectively engages its enzymatic target, accounting for an additional estimated 5–10-fold reduction in overall activity. Together, these pharmacokinetic barriers provide a plausible explanation for the observed gap between biochemical and cellular assay results.

Despite these cellular bioavailability constraints, the intrinsic physicochemical profile of compound **15** remains encouraging from a drug development perspective. Its properties conform to Lipinski's Rule of Five, predicting favorable oral bioavailability and membrane permeability. These physicochemical properties, therefore, collectively support the candidacy of compound **15** as a drug-like scaffold warranting further preclinical development, with targeted structural modifications aimed at bridging the observed gap between biochemical and cellular potency.

Materials and Methods

General Experimental Procedures

The UV characterizations were performed using the T 80 UV/Vis spectrometer (PG Instruments Limited, Woodway Lane). The NMR

spectra were acquired using a Bruker Avance III spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C -APT) at Zagazig University's Faculty of Science, with tetramethylsilane (TMS) as the internal reference. HR-ESI/MS data were obtained by dissolving the samples in methanol at 10 $\mu\text{g/mL}$. For each analysis, 5 μL was injected into a Vanquish UHPLC coupled to an Orbitrap Exploris 120 mass spectrometer (Thermo Scientific). Comprehensive details regarding the chromatographic gradients, ionization parameters, and software configurations are provided in the supporting information (S5). Various chromatographic materials were employed, including Diaion HP-20 (Sorbent Technologies), MN-polyamide-SC-6 (50–160 μm , Sorbent Technologies), Sephadex LH-20 (Mitsubishi), C18 reverse-phase silica gel (230–400 mesh, Merck), and silica gel G60 (60–120 mesh, Merck). Thin-layer chromatography (TLC) was performed on precoated silica 60 F254 and RP-18 F254 plates (0.25 mm thickness, E-Merck). For biological assays, the A431 (human skin carcinoma), PC3 (human prostate carcinoma), and HFB4 (Human normal melanocytes) cell lines were procured from the American Type Culture Collection (ATCC). Key reagents included dimethyl sulfoxide (DMSO), MTT, and trypan blue dye (Sigma), while fetal bovine serum, RPMI-1640 medium, HEPES buffer, L-glutamine, gentamycin, and Trypsin-EDTA were sourced from Lonza. For enzyme inhibition assays, Chemo-Verse ERK1 Kinase Assay Kit (BPS Bioscience, #82282), ERK2, GST-Tag Recombinant (BPS Bioscience, #40299), and PI3K α (p110 α /p85 α) Assay Kit (BPS Bioscience, #79781) were used.

Plant Material

The wine palm, *A. butyracea* (Mutis ex L.f.) Wess. Boeris, belongs to the Arecaceae family.⁴⁰ The aerial parts of the plant were collected during the flowering season in July 2022 from Aswan Botanical Garden, Aswan, Egypt, and authenticated by Dr. Hafeez Rofa'el. A voucher herbarium specimen (No. 29159) is deposited at the herbarium of Flora and Phytotaxonomy Research, Horticultural Research Institute, Agricultural Research Center, Dokki (Cairo), Egypt. The collected plant materials were air-dried, powdered, and kept at room temperature for subsequent investigation.

Extraction and Isolation

The dried plant material (6 kg) was exhaustively extracted at room temperature with 70% methanol ($3 \times 10\text{ L}$). The resulting extracts were concentrated under vacuum at 40°C, yielding a residue of 212.8 g. This residue was then suspended in distilled water (500 mL) and subjected to successive partitioning with *n*-hexane ($3 \times 1\text{ L}$), dichloromethane (DCM) ($3 \times 1\text{ L}$), and ethyl acetate (EtOAc) ($5 \times 1\text{ L}$), resulting in three distinct fractions. The *n*-hexane-soluble extracts were combined and further concentrated under vacuum to obtain the *n*-hexane-soluble fraction (4.2 g). Similarly, the dichloromethane-soluble extracts were combined and vacuum concentrated to obtain the DCM-soluble fraction (1.8 g). Lastly, the ethyl acetate-soluble extracts were combined and vacuum concentrated to obtain the EtOAc-soluble fraction (8.6 g). The DCM-soluble fraction (1.8 g) was fractionated using silica gel (40 g) column chromatography (CC), eluted with a gradient of hexane-DCM (1:1), gradually increasing the DCM concentration to 100%, to give 8 subfractions, D1–D8.

D1 (68 mg) was chromatographed on silica gel (20 g) by CC using a hexane-DCM (10:1) solvent mixture as the eluent. The DCM concentration was incrementally raised to 10:2 and then to 10:3, yielding compound **1** (6.6 mg). D3 (32.7 mg) was chromatographed using Sephadex LH-20, eluted with (DCM/MeOH 1:1), resulting in the isolation of compound **2** (10 mg). The fractionation of D6 (25.5 mg) was performed by silica gel CC (20 g), eluting with a hexane-DCM (1:1) solvent mixture. Subsequently, purification was performed using Sephadex LH-20 (20 g) with an elution system of DCM/MeOH (1:1), yielding compound **3** (5 mg). D-8 (9.4 mg) was fractionated utilizing Sephadex LH-20 (20 g), and elution was carried out with DCM-MeOH (1:1), yielding compound **4** (6.5 mg). The EtOAc-soluble fraction (8.6 g) was subjected to MN-polyamide-SC-6 (250 g) CC. Elution was performed with 100% water, followed by a gradient of water and methanol (H₂O/MeOH) to 100% methanol, yielding 15 subfractions (E1–E15). E1 (1.04 g) was fractionated using RP (10 g) solid-phase extraction (SPE) CC, initially eluted with 100% H₂O followed by a gradient of H₂O/MeOH 90:10, resulting in the collection of 10 subfractions labeled E1-1 through E1-10. Subsequently, E1-3 (156 mg) was purified using Sephadex LH-20 (100 g) eluted with MeOH/H₂O (7:3) to afford compound **5** (9.5 mg). E3 (340 mg) was subjected to Sephadex LH-20 CC (180 g) to afford compound **6** (5.4 mg). E5 (340.1 g) was fractionated using Sephadex LH-20 CC (180 g) into 7 subfractions E5-1–E5-7. Silica gel SPE CC further purified E5-4 (67.4 g), eluted with DCM/MeOH (13:0.5), to afford compound **7** (25.4 mg). E7 (2.48 g) was chromatographed over Sephadex LH-20 (180 g), eluted with MeOH, to give 9 subfractions (E7-1–E7-9). E7-3 (507.9 mg) was purified over silica gel, eluted with DCM/MeOH (40:1), to afford compound **8** (84.5 mg). E7-4 (357 mg) was chromatographed over repeated silica gel SPE CC, eluted with (DCM/MeOH 50:1) to yield compound **9** (6.6 mg). E8 (840 mg) was chromatographed over Sephadex LH-20 CC (180 g), eluted with MeOH, to obtain a total of 5 subfractions (E8-1–E8-5). E8-2 (257 g) was chromatographed by repeated silica gel SPE CC, eluting with DCM/MeOH (17:1), to yield compound **10** (10.8 mg). E8-5 (27.9 mg) was subjected to further purification using Sephadex LH-20 (50 g) to afford compound **11** (5.6 mg). E11 (89 mg) was fractionated with Sephadex LH-20 (100 g) to afford compound **12** (21 mg). E12 (232.8 mg) was fractionated over Sephadex LH-20 CC (100 g), followed by silica gel (2 g) SPE, eluted with DCM/MeOH (20:1), to yield compound **13** (13.7 mg). E13 (234.1 mg) was chromatographed on Sephadex LH-20 CC (180 g) with MeOH, yielding 4 subfractions (E13-1–E13-4). E13-4 (10.9 mg) was further purified using Sephadex LH-20 CC to yield compound **14** (2.1 mg). E15 (359.9 mg) was chromatographed on Sephadex LH-20 CC (180 g) with MeOH to yield 3 subfractions (E15-1–E15-3). E15-1 (57 mg) was repeatedly purified over Sephadex LH-20 CC (50 g) using MeOH to give compounds **15** (5 mg) and **16** (1.8 mg). E15-2 (64.8 mg) was chromatographed on silica gel (2 g) SPE, eluted with (DCM/MeOH 20:0.5) to give compound **17** (31.1 mg).

Compound 7: colorless oil; $[\alpha]_{\text{D}}^{25}$ of +41 (c 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) nm, 242 (4.19), 219 (4.21); ^1H and ^{13}C NMR data, see **Table 1**; HR-ESI/MS m/z 505.2074 $[\text{M}-\text{H}]^-$ (calcd for $\text{C}_{26}\text{H}_{33}\text{O}_{10}$ 505.2073, mass error = 0.19 ppm).

Compound 13: yellow amorphous powder; $[\alpha]_D^{22}$ of +11 (c 0.04, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) nm, 371 (3.94), 296 (4.21), 234 (4.34); ^1H and ^{13}C NMR data, see [Table 2](#); HR-ESI/MS m/z 659.1973 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{32}\text{H}_{35}\text{O}_{15}$ 659.1976, mass error = 0.45 ppm) and m/z 681.1790 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{32}\text{H}_{34}\text{O}_{15}\text{Na}$ 681.1795, mass error = 0.73 ppm).

In Vitro Cytotoxic Activity Against Skin and Prostate Cancer Cell Lines

The A431 (human skin carcinoma) and PC3 (human prostate carcinoma) cell lines were maintained in RPMI-1640 medium. This medium contained 10% fetal bovine serum and 50 $\mu\text{g}/\text{mL}$ gentamycin. Cells were kept at 37°C in a humidified incubator with 5% CO_2 and passaged bi-weekly to ensure logarithmic growth. For experiments, 96-well plates (Corning) were seeded with tumor cells at a density of 5×10^4 cells per well. After 24 h of attachment, we applied compounds [7](#), [10](#), and [13–15](#). These treatments spanned eight concentrations and were performed in triplicate. Controls included medium or 0.5% DMSO. After 48 h of exposure, we conducted an MTT viability assay. Briefly, the medium was replaced with phenol red-free RPMI-1640 (100 μL). Then, 10 μL of MTT solution (12 mM in PBS) was added. After 4 h, formazan crystals were dissolved in 50 μL of DMSO. The plates were gently agitated and incubated for an additional 10 min. Absorbance was recorded at 590 nm using a microplate reader (TECAN Sunrise, USA). Cell viability (%) was calculated as:

$$\text{Viability} = (\text{OD}_t / \text{OD}_c) \times 100$$

where OD_t represents the mean optical density of treated cells and OD_c that of untreated controls. Concentration–response curves were generated to determine the half-maximal cytotoxic concentration (CC_{50}), defined as the concentration that causes a 50% reduction in cell viability ([Table 3S](#), Supporting Information).⁴¹ Statistical significance among compound-treated cells, the internal negative baseline (0 μM ; 100% viability), and the positive control (cisplatin) was determined using one-way ANOVA with Tukey's post hoc test ($p < 0.05$).

In Vitro Cytotoxicity Evaluation in Normal Melanocytes

Normal melanocytes were grown in DMEM supplemented with heat-inactivated fetal bovine serum 10%, HEPES buffer, L-glutamine 1%, and 50 $\mu\text{g}/\text{mL}$ gentamycin. To ensure logarithmic growth, the cultures were maintained at 37°C with 5% CO_2 . Routine subculturing occurred twice weekly to maintain cell health. Cells were plated at 1×10^5 cells per well in 96-well plates and incubated for 24 h before compound exposure. Test compounds were added in triplicate at eight concentrations, with control wells containing either medium or PBS. After 48 h, viability was assessed using the MTT assay as described previously. CC_{50} values were derived from concentration–response curves using nonlinear regression analysis. GraphPad Prism (version 10.4; GraphPad Software Inc.) performed all calculations. The four-parameter logistic model with variable slope characterized the concentration–response relationship. Confidence intervals were calculated at the 95% level, using asymmetrical confidence intervals.

Molecular Modeling Studies

Molecular Docking

Molecular docking simulations were conducted using the MOE software suite to evaluate ligand binding against kinase crystal structures retrieved from the Protein Data Bank. Comprehensive descriptions of computational hardware, force field parameters, and scoring refinement protocols are provided in the supporting information (4S).

Molecular Dynamics Simulation

The stability of ERK2 and PI3K α complexes with ligand [15](#) was evaluated using the Protein Preparation Wizard in Maestro (Desmond Schrödinger Release, 2025). Comprehensive parameters regarding system solvation, force-field assignments, and trajectory analysis protocols are detailed in the supporting information (5S).

Binding Affinity

The binding free energy of ligand [15](#) with ERK2 and PI3K α was calculated using Prime/MMGBAS, as described in the supporting information (6S).

Enzyme Inhibition Assays

The inhibitory activity of compound [15](#) against ERK1 and ERK2 was evaluated using the ERK1 Kinase Assay Kit and ERK2, GST-Tag Recombinant, respectively. Assays were performed in white 96-well plates using myelin basic protein (MBP) as substrate. Reactions contained 12.5 ng ERK1 or ERK2 enzyme, 200 $\mu\text{g}/\text{mL}$ MBP, 10 μM ATP, and varying concentrations of compound [15](#) in kinase assay buffer (40 mM Tris-HCl, pH 7.4, 20 mM MgCl_2 , 0.1 mg/mL BSA, 50 μM DTT). Following a 45-minute incubation at 30°C, ADP-Glo reagent (Promega, #V6930) was added for quantification of kinase activity by luminescence detection. PI3K α inhibition was assessed using the PI3K α Assay Kit with PI3K lipid substrate. Reactions comprised 5 ng PI3K α enzyme, PI3K lipid substrate, 2.5 μM ATP, and test compound in assay buffer, incubated for 40 min at 30°C. Ravoxertinib and alpelisib served as reference standards for ERK and PI3K α assays, respectively. IC_{50} values were determined from concentration–response curves using nonlinear regression analysis. All experiments were conducted in triplicate ([Table 4S](#), Supporting Information). Statistical significance among inhibition values at each tested concentration of compound [15](#), relative to the enzyme control (EC; 0% inhibition baseline) and the reference inhibitors (Ravoxertinib for ERK1/ERK2; Alpelisib for PI3K α), was determined using one-way ANOVA with Dunnett's post hoc test ($p < 0.05$).

In Silico Physicochemical and Drug-Likeness Evaluation

Drug-likeness of compound [15](#) was assessed against Lipinski's Rule of Five, which defines orally bioavailable small-molecule therapeutics as possessing a molecular weight ≤ 500 Da, ≤ 5 hydrogen bond donors (HBD), ≤ 10 hydrogen bond acceptors (HBA), and a calculated partition coefficient (cLogP) ≤ 5 . The fundamental physicochemical descriptors were calculated using calibrated cheminformatics platforms (SwissADME).⁴²

Abbreviations

APT	Attached Proton Test
CDK2	Cyclin-Dependent Kinase 2
DMEM	Dulbecco's Modified Eagle Medium
EGFR	Epidermal Growth Factor Receptor
ERK1	Extracellular Signal-Regulated Kinase 1
ERK2	Extracellular Signal-Regulated Kinase 2
HR-ESI/MS	High-Resolution Electrospray Ionization Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
MAP	mitogen-activated protein
MAPK	mitogen-activated protein kinase
miR-21	MicroRNA-21
mTOR	mechanistic target of rapamycin
PI3K α	Phosphoinositide 3-kinase alpha
RMSD	Root-Mean-Square Deviation
SAR	Structure–Activity Relationship
VEGFR-2	Vascular Endothelial Growth Factor Receptor-2

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Statements and Additional Information

Conflict of Interest The authors declare that they have no conflict of interest.

A Declaration of Generative AI Use The authors confirm that no Generative AI was used in the preparation of this manuscript.

Contributors' Statement R.M.A.W.: aConceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing. A.M.: Data curation, Formal analysis, Writing—original draft, Writing—review & editing. C.S.: Data curation, Formal analysis, Software, Writing—original draft, Writing—review & editing. I.E.-A.: Data curation, Formal analysis, Writing—original draft, Writing—review & editing. S.M.M.M.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review & editing.

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Ethical Statement All in vitro experiments were conducted using commercially available cell lines purchased from the American Type Culture Collection (ATCC). The cells were maintained and used in strict compliance with the VACSERA (Egyptian Company for Production of Vaccines, Sera and Drugs) institutional biosafety and waste-disposal guidelines.

Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Supplementary Material is available online at <https://doi.org/10.1055/a-2955-9684>

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