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Calquiquelignan D from *Attalea butyracea* exhibits selective cytotoxicity against prostate and skin cancer cells with dual ERK2/PI3K α inhibitory activity

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Calquiquelignan D from *Attalea butyracea* exhibits selective cytotoxicity against prostate and skin cancer cells with dual ERK2/PI3K α inhibitory activity

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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.

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

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 non-melanoma 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 pharmacological 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 anti-proliferative 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 analogs 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**, **Fig. 1S–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-dehydrotheaspirone (**6**), *p*-hydroxybenzoic acid (**8**), protocatechuic acid (**9**), vitexin (**11**), and orientin (**12**). The study also identified several tricin-based derivatives, including tricin 4'-*O*-(*erythro*- β -guaiacylglyceryl) ether 7''-*O*- β -D-glucopyranose (**10**), tricin 4'-*O*-(*threo*- β -guaiacylglyceryl) ether (**14**), tricin 4'-*O*-(*erythro*- β -4-hydroxyphenylglyceryl) ether (**15**), tricin 4'-*O*-(*erythro*- β -guaiacylglyceryl) ether (**16**), and the parent flavone tricin (**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 $C_{26}H_{33}O_{10}$ (calcd 505.2073, mass error = 0.19 ppm). ^1H and APT NMR spectra (**Fig. 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 Δ^7 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 (Fig. 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 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'- OCH_3), suggested the presence of a tricetin-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 analogs [26]. The APT and

HSQC spectra (Fig. 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, indicating the presence of a glyceryl moiety. Through analysis of the HMBC spectrum (Fig. 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 tricin 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 μ M against A431 and 22.5 μ M 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 [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 tricrin [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 co-crystallized ligand back into the active site of the target kinase. The co-crystallized 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 co-crystallized 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 co-crystallized 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 co-crystallized 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). 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).

Ligand **15** is lying in the catalytic (kinase) domain [35] 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 CH₂OH 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 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, Fig. 3A and 6A) of the MD simulation (Table 4). 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 sub-micromolar 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 multi-target 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 multi-target 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 triclin 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 CC_{50} of 14.9 μ M. This value is numerically similar to the reference drug, cisplatin (CC_{50} of 13.2 μ M). When tested against the PC3 prostate cancer line, compound **15** displayed a CC_{50} of 22.5 μ M, slightly outperforming cisplatin's CC_{50} 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 pharmacological 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 sub-micromolar enzyme inhibition (0.15–0.44 μ M) and the weaker cellular activity ($\sim$ 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 tricetin 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 (3S). 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 pre-coated 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 Rofaeel. 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 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 L), dichloromethane (DCM) (3 $\times$ 1 L), and ethyl acetate (EtOAc) (5 $\times$ 1 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; $[\alpha]_D^{22}$ of +41 (*c* 0.04, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) nm, 242 (4.19), 219 (4.21); ¹H and ¹³C NMR data, see Table 1; HR-ESI/MS *m/z* 505.2074 [M–H][–] (calcd for C₂₆H₃₃O₁₀ 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 ¹³C NMR data, see Table 2; HR-ESI/MS

m/z 659.1973 $[M+H]^+$ (calcd for $C_{32}H_{35}O_{15}$ 659.1976, mass error = 0.45 ppm) and m/z 681.1790 $[M+Na]^+$ (calcd for $C_{32}H_{34}O_{15}Na$ 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 $\mu\text{g/mL}$ 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 hours 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 minutes. 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 $\mu\text{g/mL}$ 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 above. 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 μ g/ml MBP, 10 μ M 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 μ M 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 μ M ATP, and test compound in assay buffer, incubated for 40 minutes at 30

°C. Ravoxertinib and alpelisib served as reference standards for ERK and PI3K α assays, respectively. IC₅₀ values were determined from concentration-response curves using non-linear 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].

Supporting Information

Figures 1S–45S present NMR spectra of all isolated compounds 1–17, along with HRESIMS confirmation for compounds **7** and **13**. 1S and 2S include spectral data of isolated known compounds **1–6**, **8–12**, and **14–17**. 3S details the analytical parameters governing HR-ESI/MS. 4S documents methodological parameters for molecular docking simulations. 5S specifies parameters controlling the molecular dynamics simulations. Table 1S presents docking results of compound **15** against nine protein kinases in comparison to the corresponding co-crystallized ligands. Table 2S provides a 2D visualization of docked models of compound **15** to nine protein kinases in comparison to the corresponding co-crystallized ligands. Table 3S presents the concentration-response curves and CC₅₀ determinations for cytotoxicity evaluation. Table 4S reports the IC₅₀ values and concentration-response curves for the inhibitory activity of compound **15** against ERK1, ERK2, and PI3K α kinases.

Contributor's statement

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Declarations

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.

Conflict of Interest

The authors declare no competing interests.

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Data availability

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

A Declaration of Generative AI Use

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

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Figures captions

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

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

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 through 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.

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

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 through 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.

Table 1

¹H- and ¹³C-NMR spectral data of compound 7 (CD₃OD, 400, 100 MHz)

Position	δ _C (ppm)	δ _H (ppm), <i>multiplicity</i> , <i>J</i> (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	-

Table 2¹H- and ¹³C-NMR spectral data of compound **13** (DMSO-*d*₆, 400, 100 MHz).

Position	δ_{C} (ppm)	δ_{H} (ppm), <i>multiplicity</i> , <i>J</i> (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'- <i>O</i> -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	3.65, <i>m</i> 3.21, <i>m</i>
6'''	60.9	

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]

^a CC₅₀ values are presented as best-fit values with 95% confidence intervals.

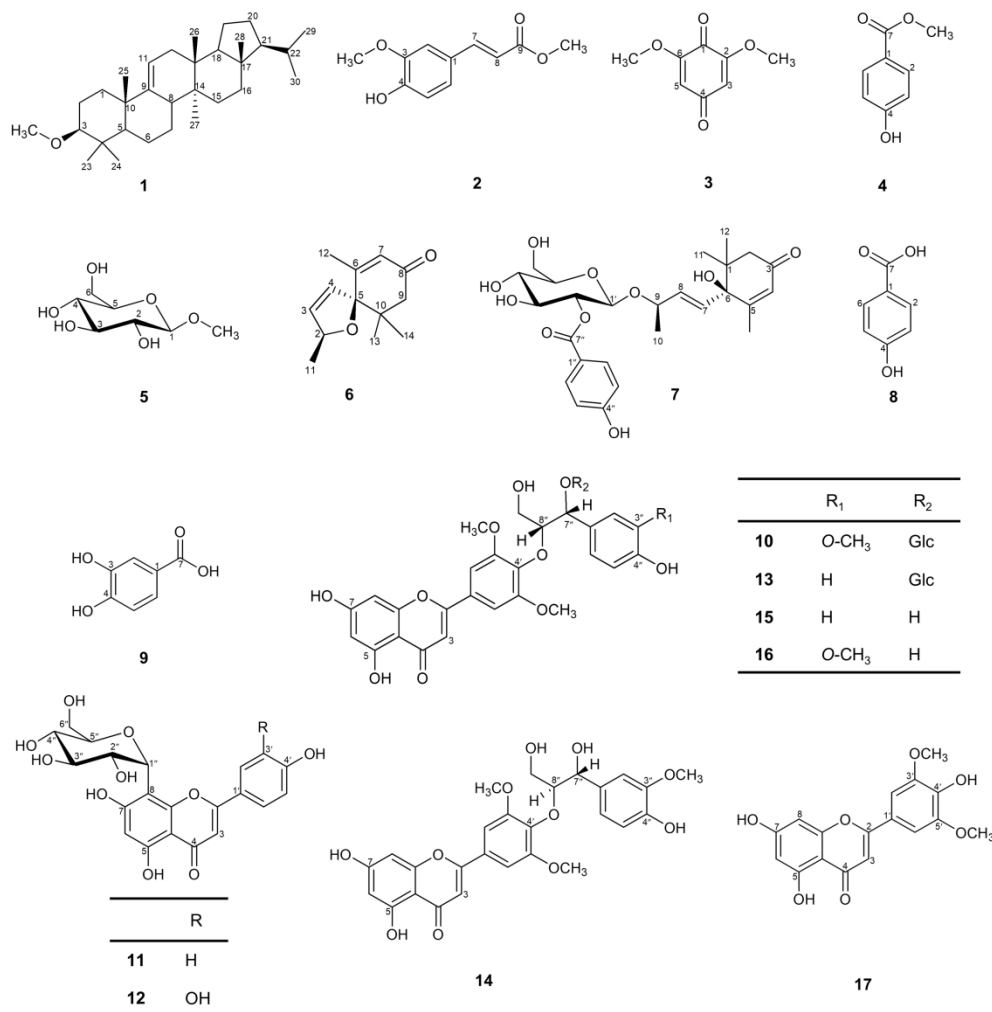
Table 4
Computational binding affinity (ΔG) of ligand 15 – protein complexes

Complex	ΔG (kcal/mol) 100–200 ns
ERK2-ligand 15	−65.52 ± 7.04
PI3Kα-ligand 15	−62.50 ± 5.17

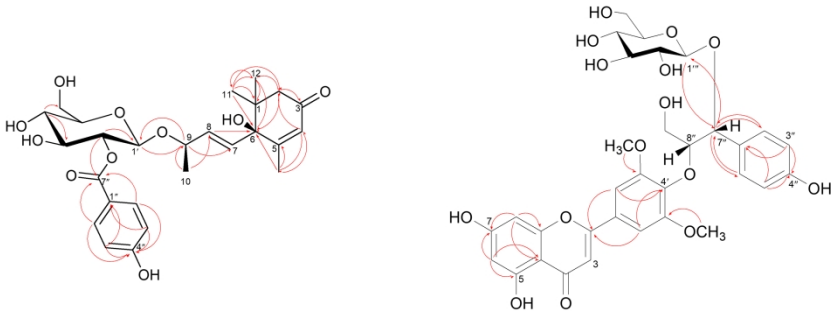
Table 5
Results of Enzyme Inhibition Assays

Compound	ERK inhibition IC ₅₀ (μM/ml) [95% CI]		PI3Kα inhibition IC ₅₀ (μM/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]

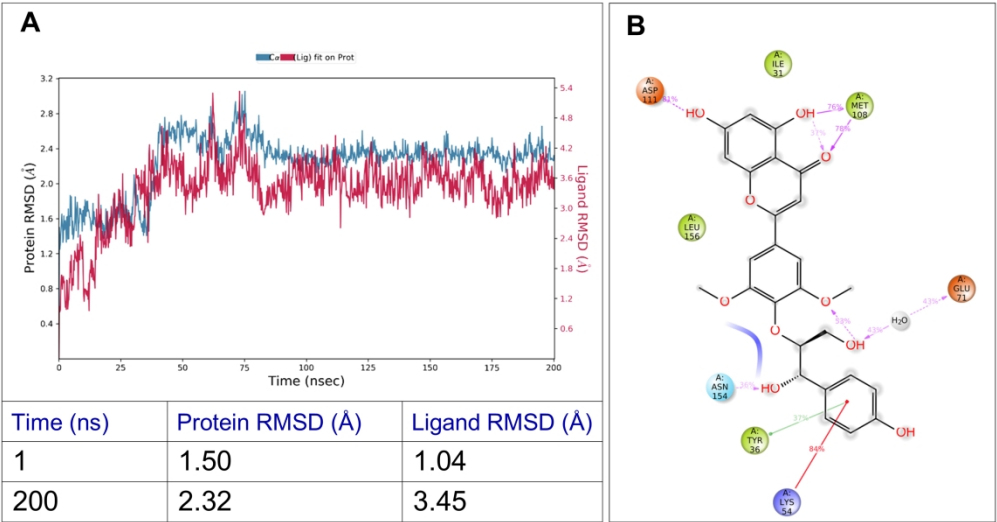
-- Not applicable



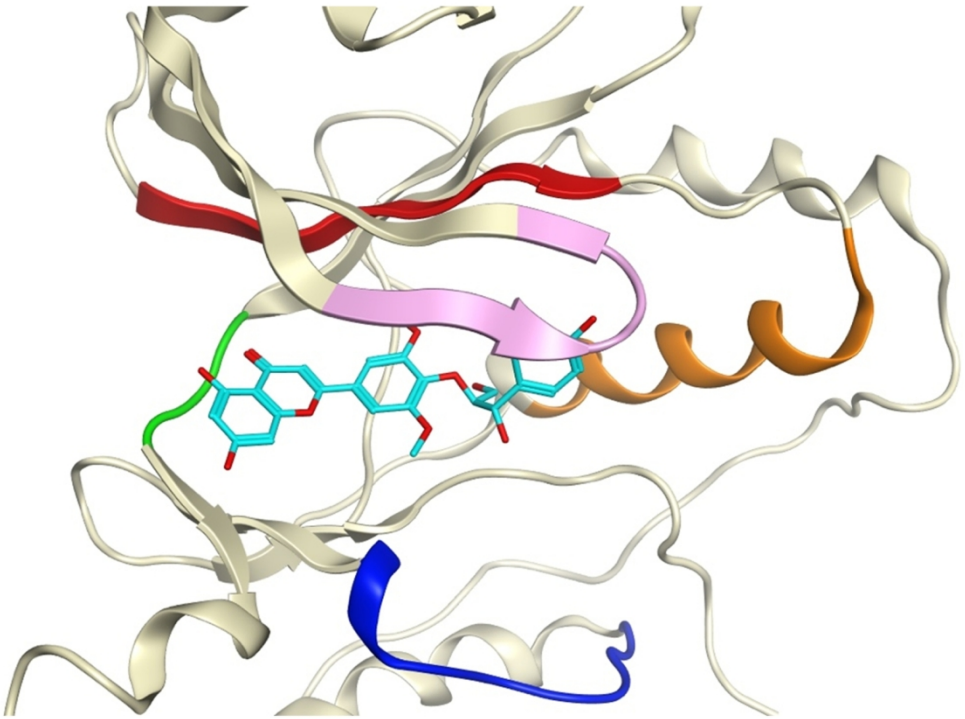
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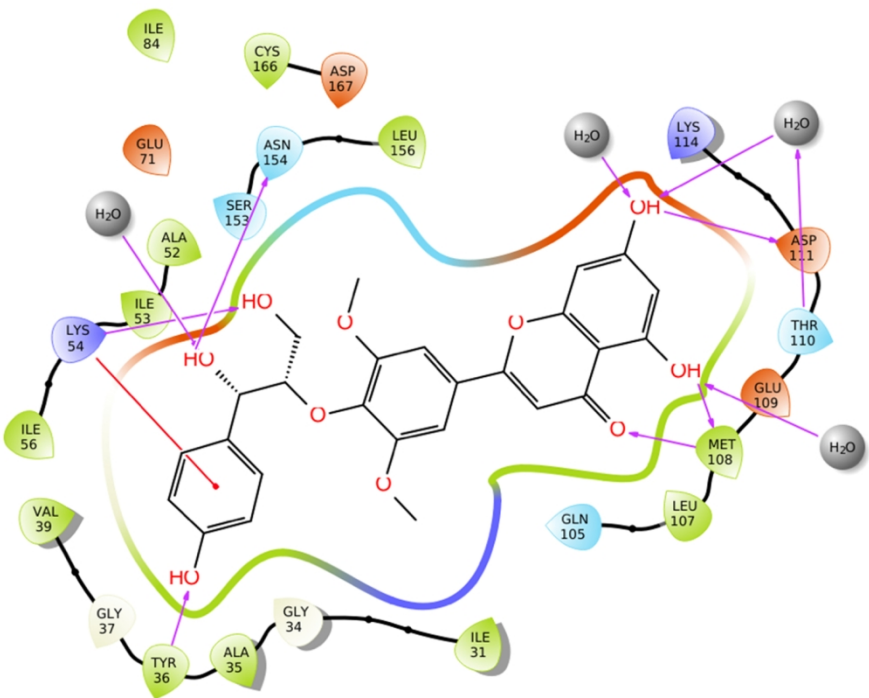
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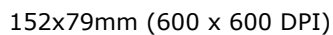
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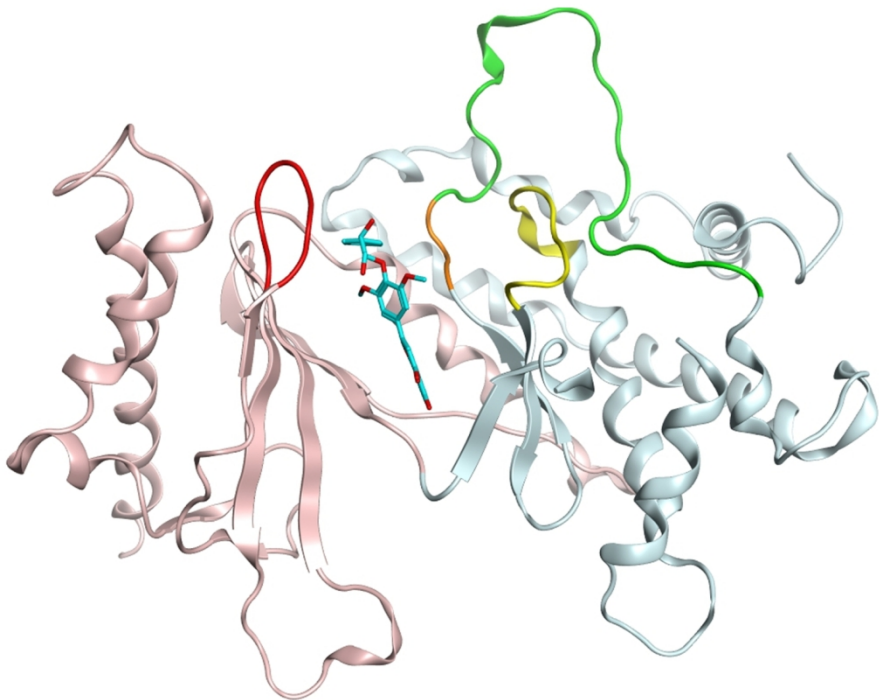


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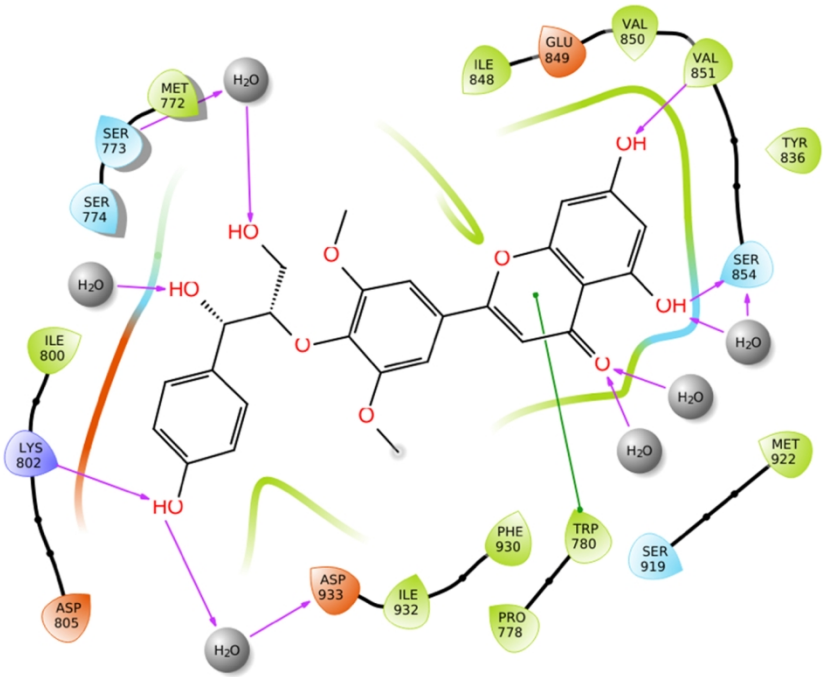


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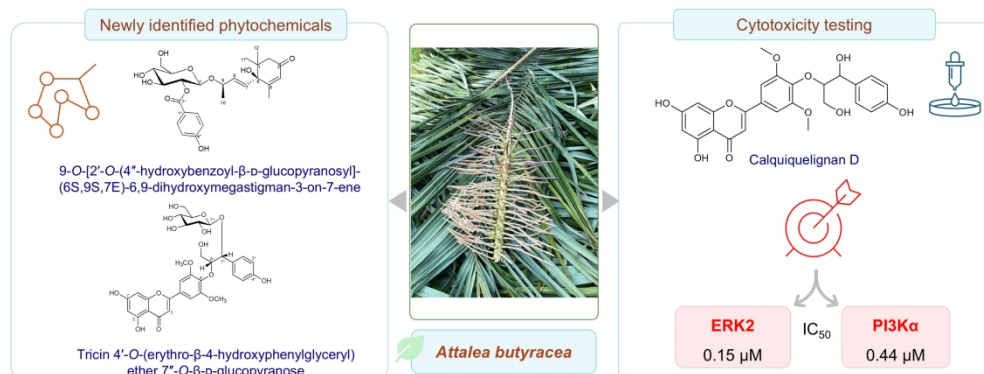




74x54mm (600 x 600 DPI)



74x54mm (600 x 600 DPI)



129x49mm (600 x 600 DPI)

Supporting Information

Calquiquelignan D from *Attalea butyracea* exhibits selective cytotoxicity against prostate and skin cancer cells with dual ERK2/PI3K α inhibitory activity

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For Peer Review

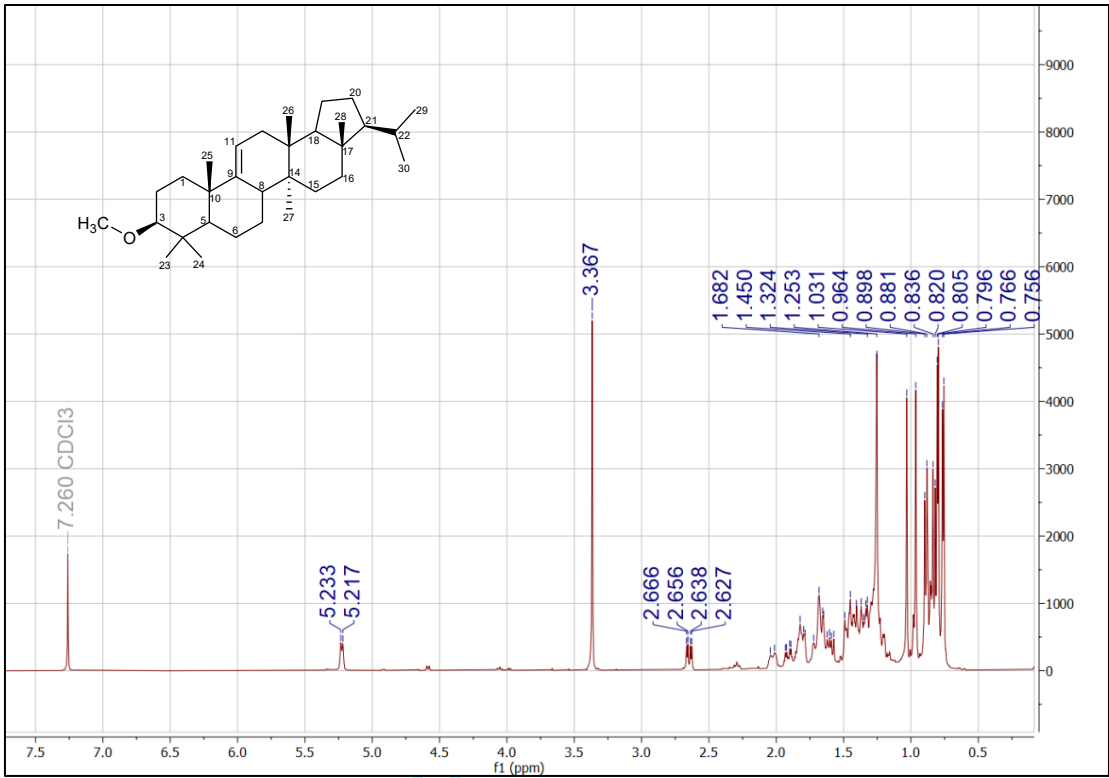


Fig 1S ¹H NMR spectrum of compound 1 (CDCl₃, 400 MHz)

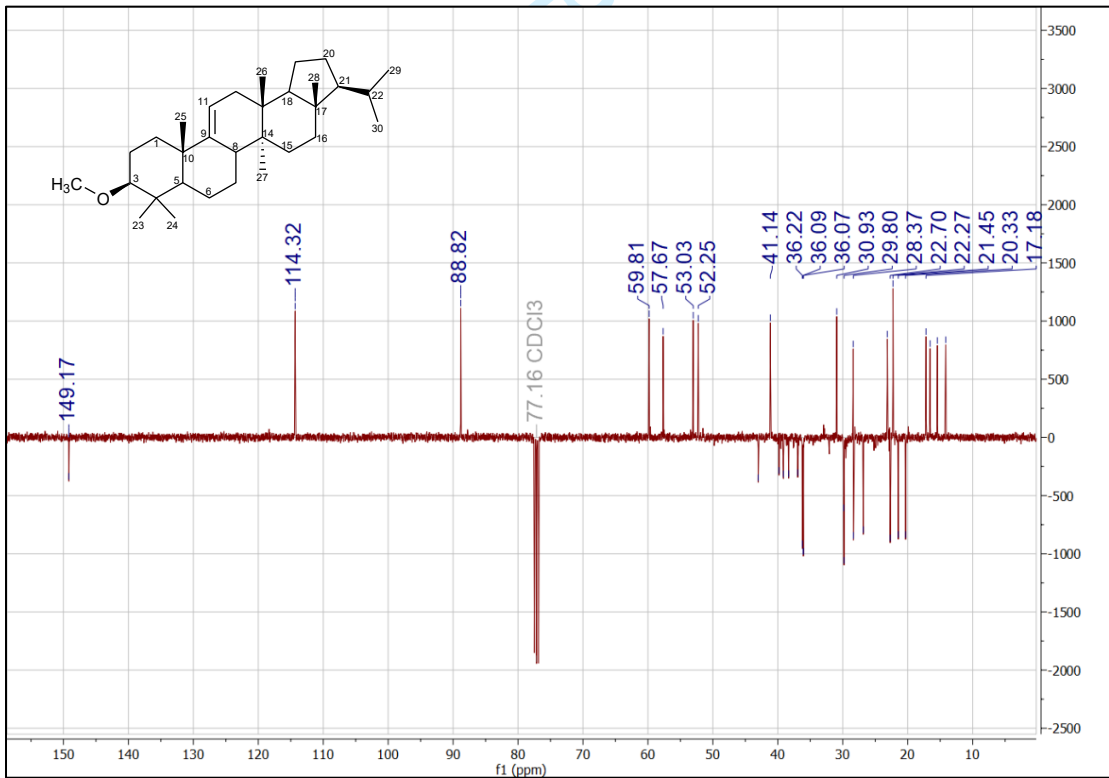


Fig 2S APT spectrum of compound 1 (CDCl₃, 100 MHz)

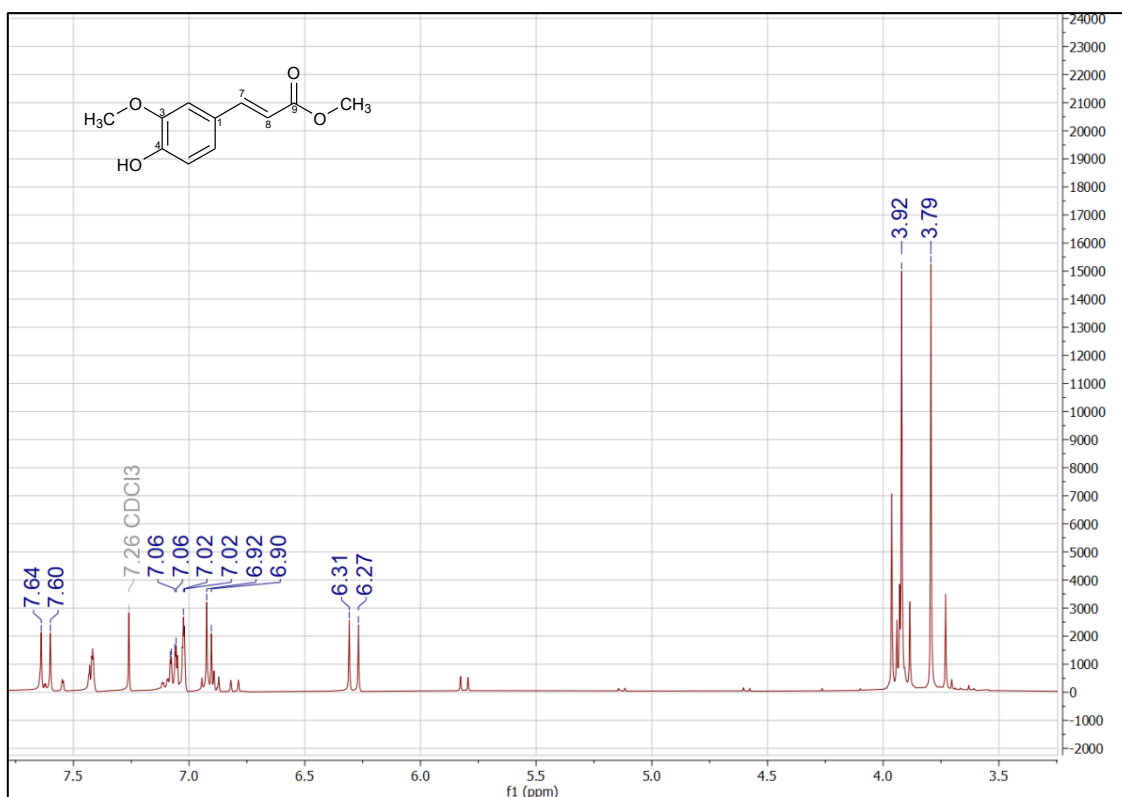


Fig 3S ¹H NMR spectrum of compound **2** (CDCl₃, 400 MHz)

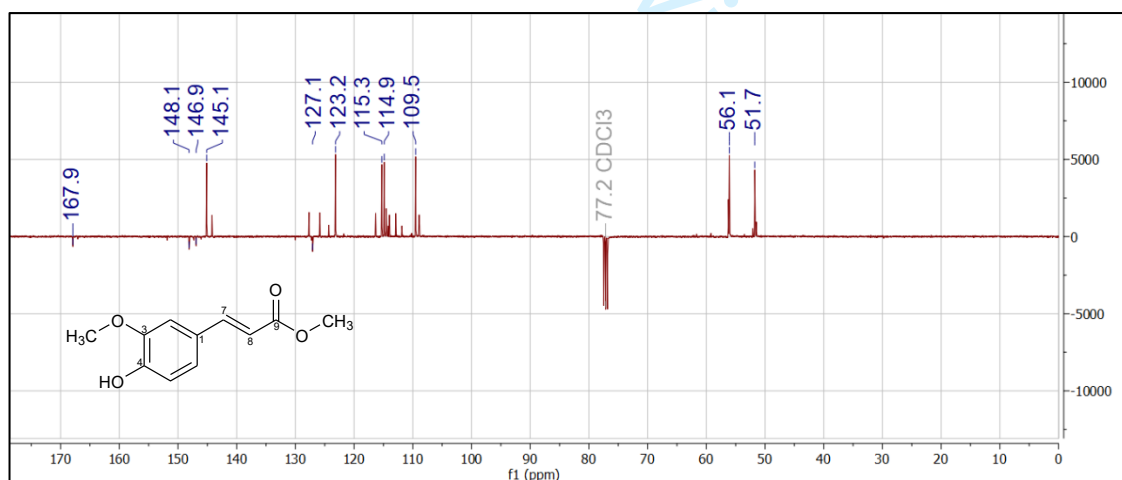


Fig 4S APT spectrum of compound **2** (CDCl₃, 100 MHz)

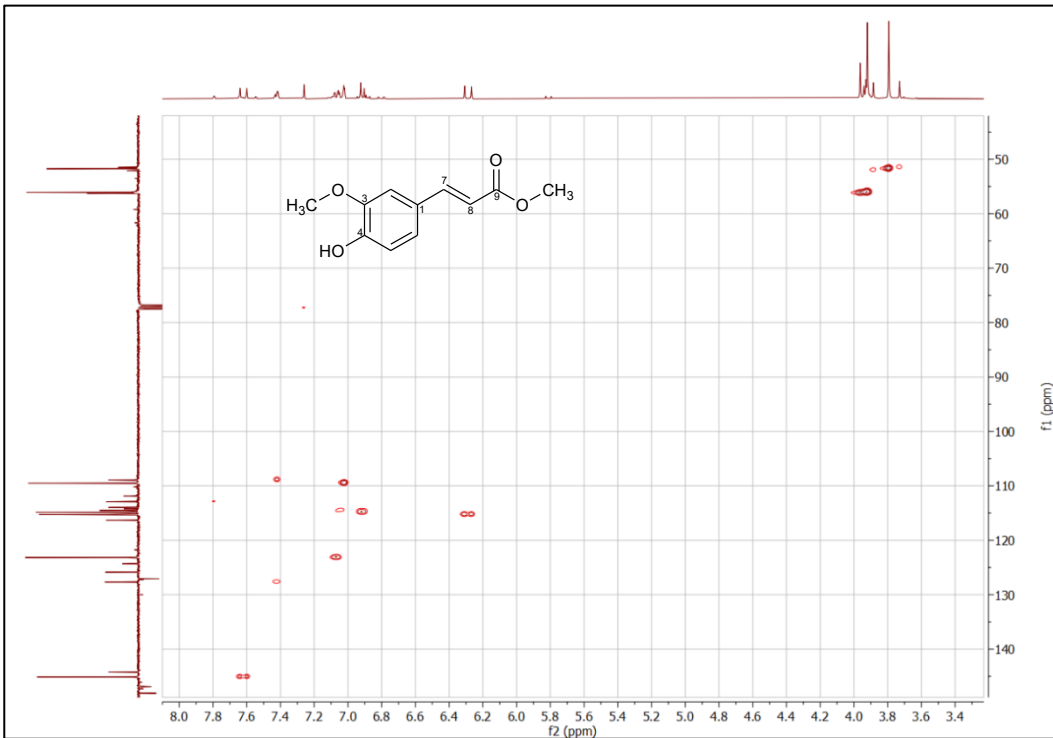


Fig 5S HSQC spectrum of compound 2 (CDCl₃, 400/100 MHz)

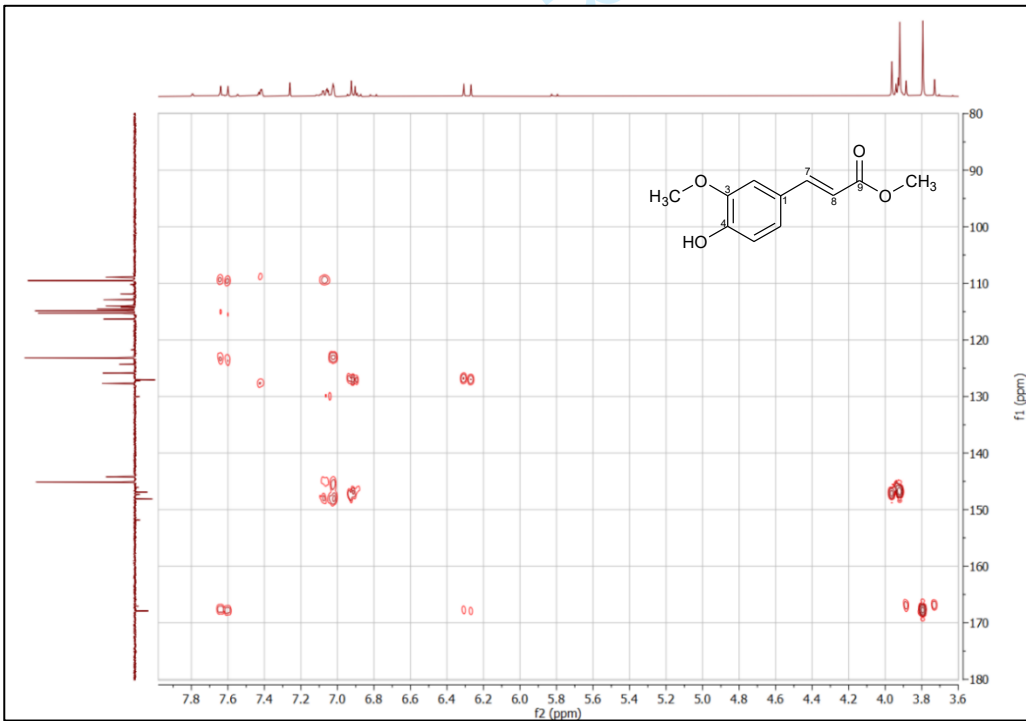


Fig 6S HMBC spectrum of compound 2 (CDCl₃, 400/100 MHz)

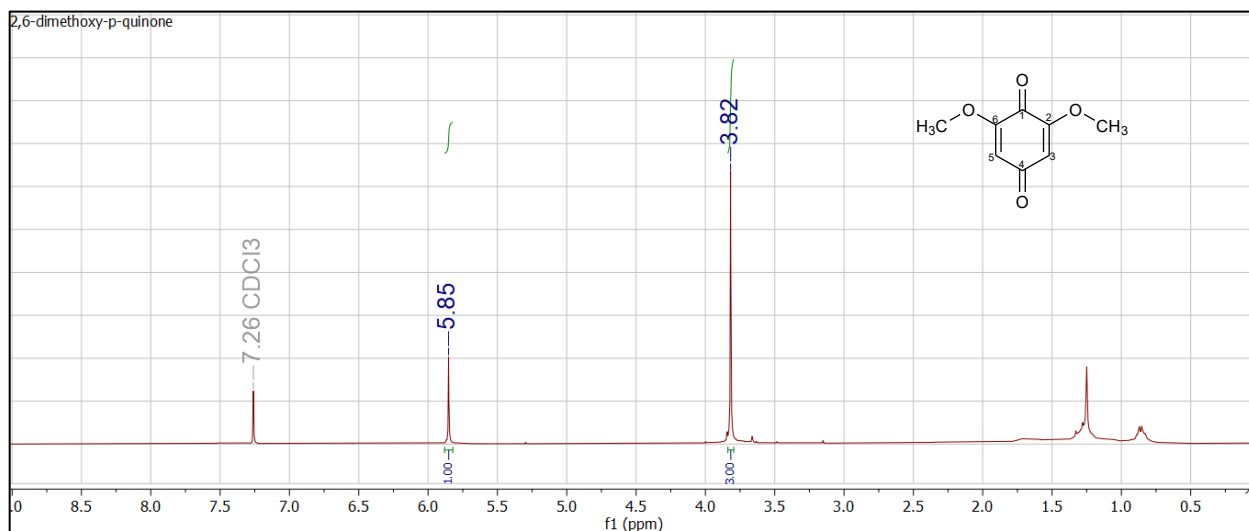


Fig 7S ^1H NMR spectrum of compound **3** (CDCl_3 , 400 MHz)

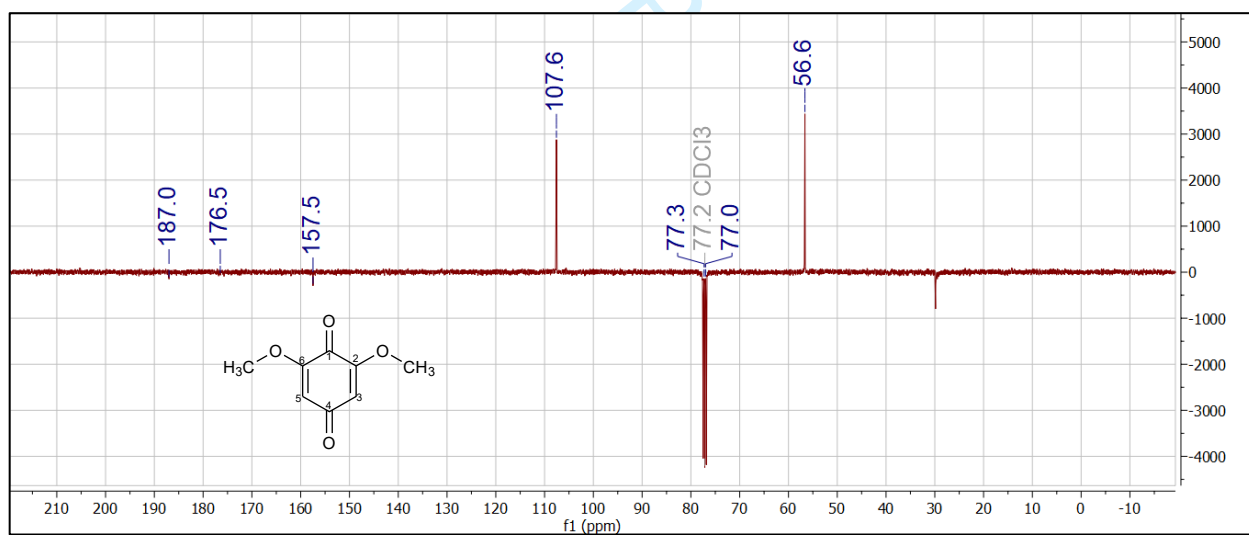


Fig 8S APT spectrum of compound **3** (CDCl_3 , 100 MHz)

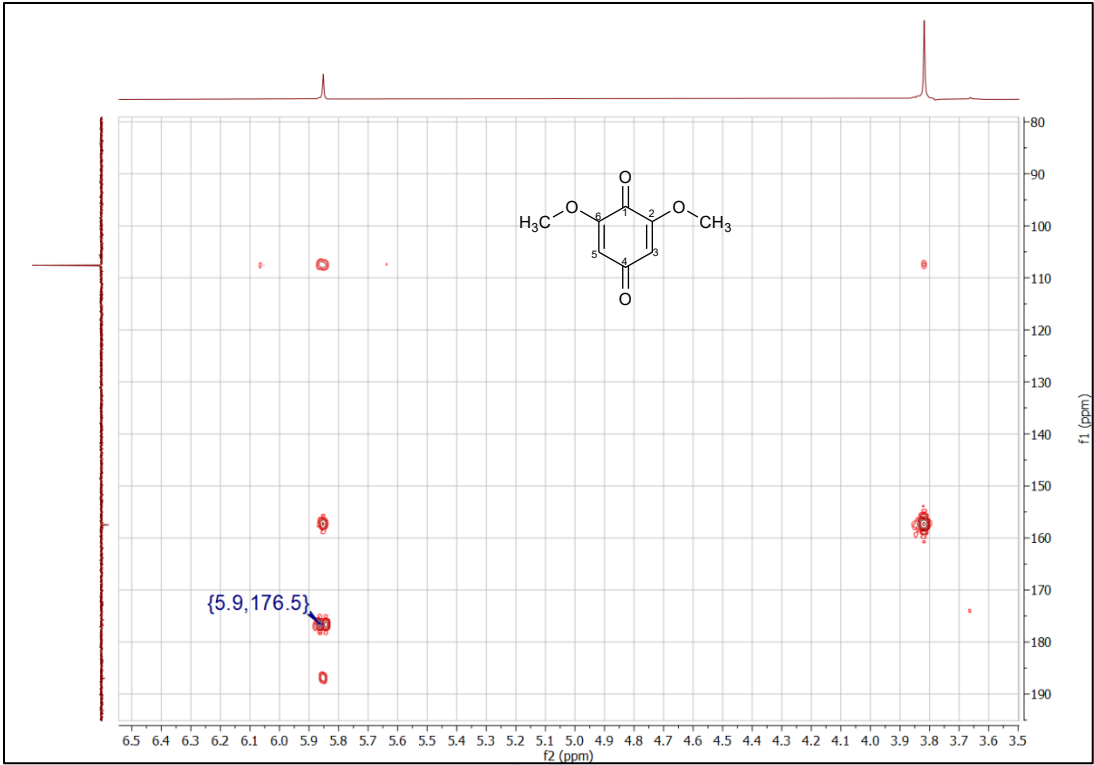


Fig 9S HMBC spectrum of compound 3 (CDCl₃, 400/100 MHz)

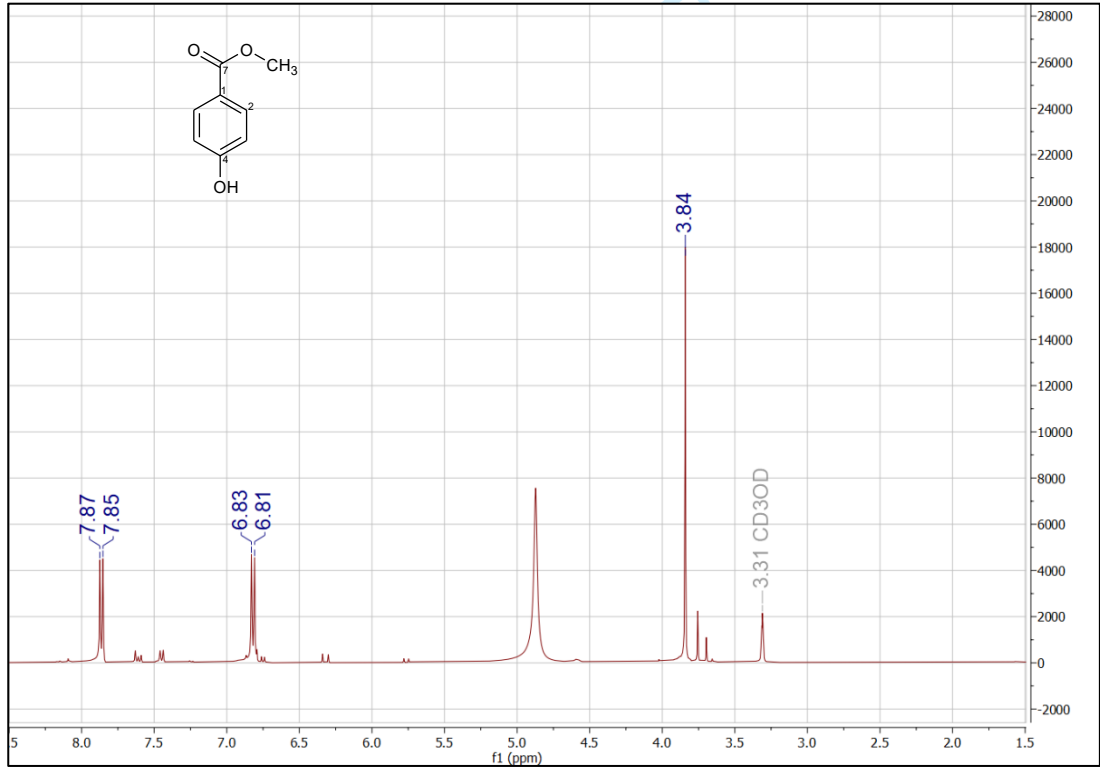


Fig 10S ¹H NMR spectrum of compound 4 (CD₃OD, 400 MHz)

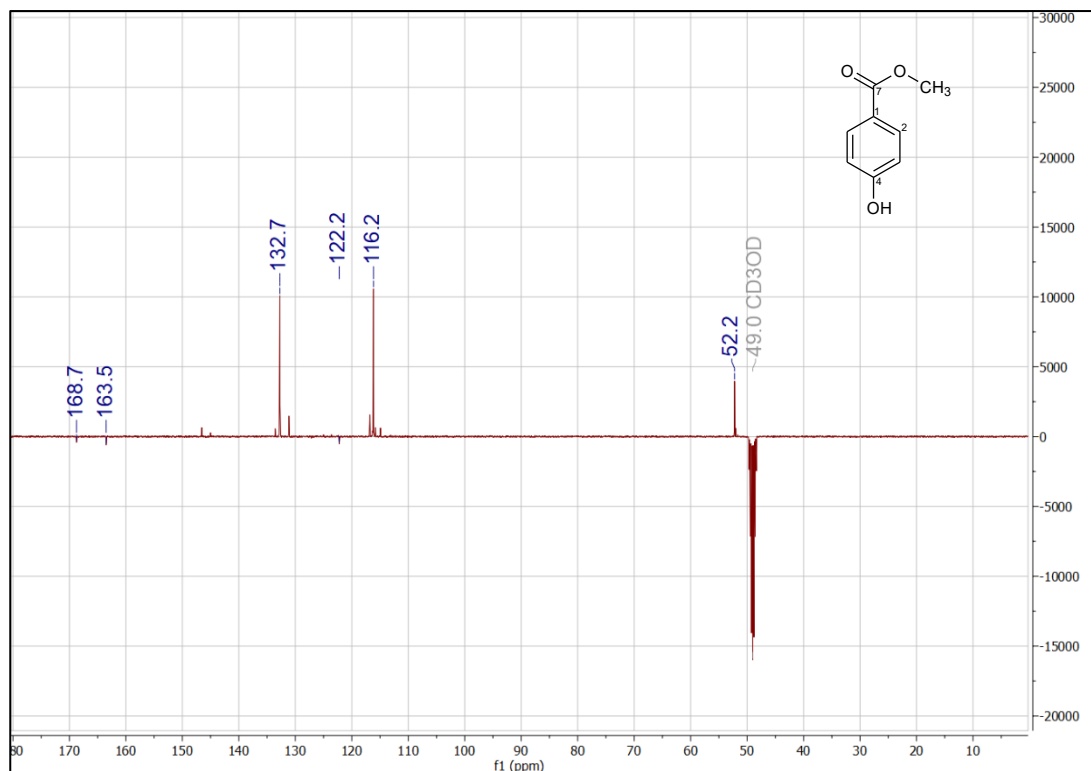


Fig 11S APT spectrum of compound 4 (CD₃OD, 100 MHz)

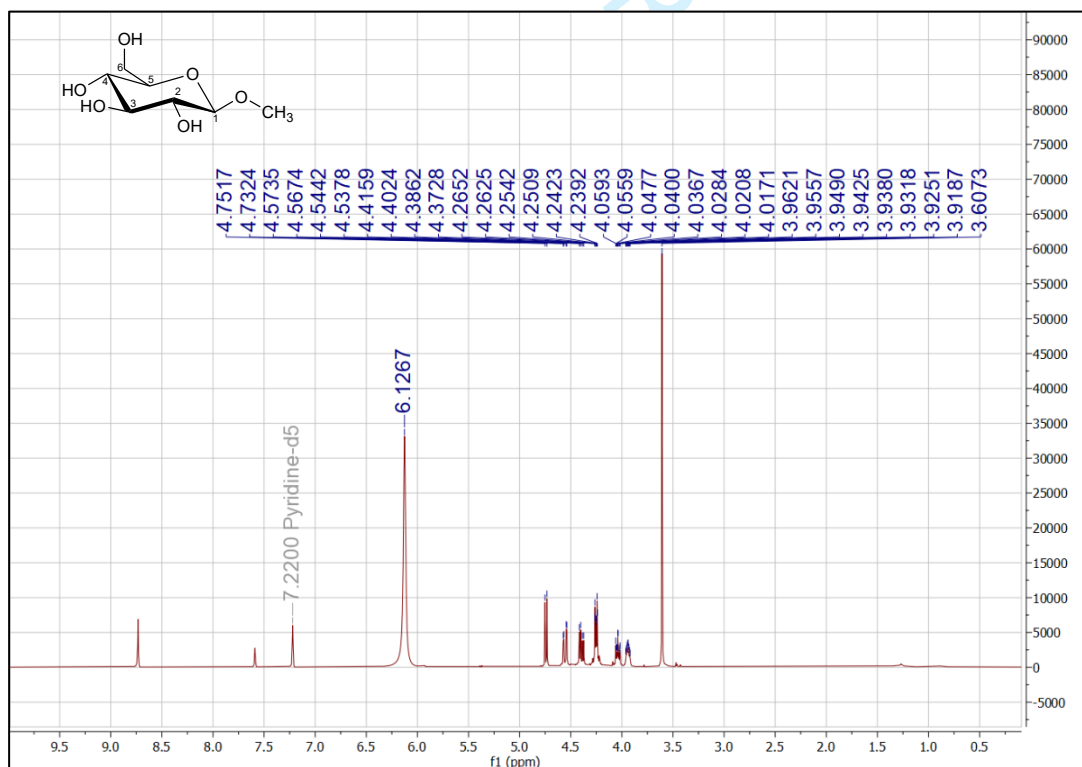


Fig 12S ¹H NMR spectrum of compound 5 (C₅D₅N, 400 MHz)

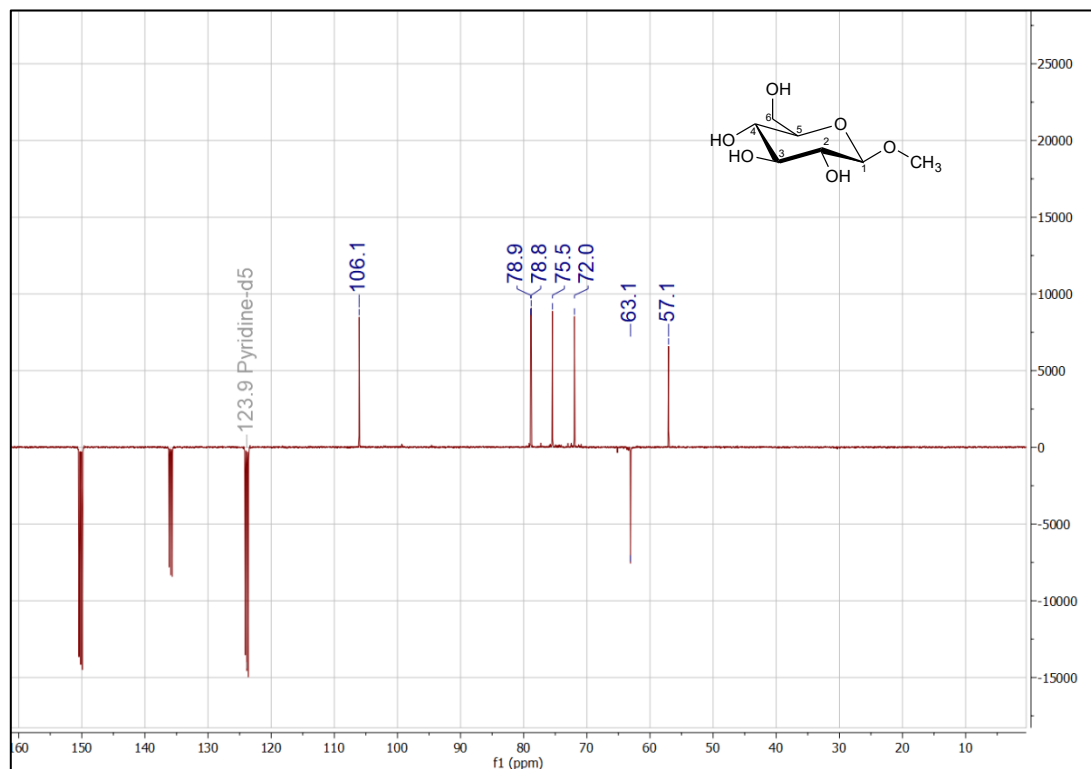


Fig 13S APT spectrum of compound 5 (C_5D_5N , 100 MHz)

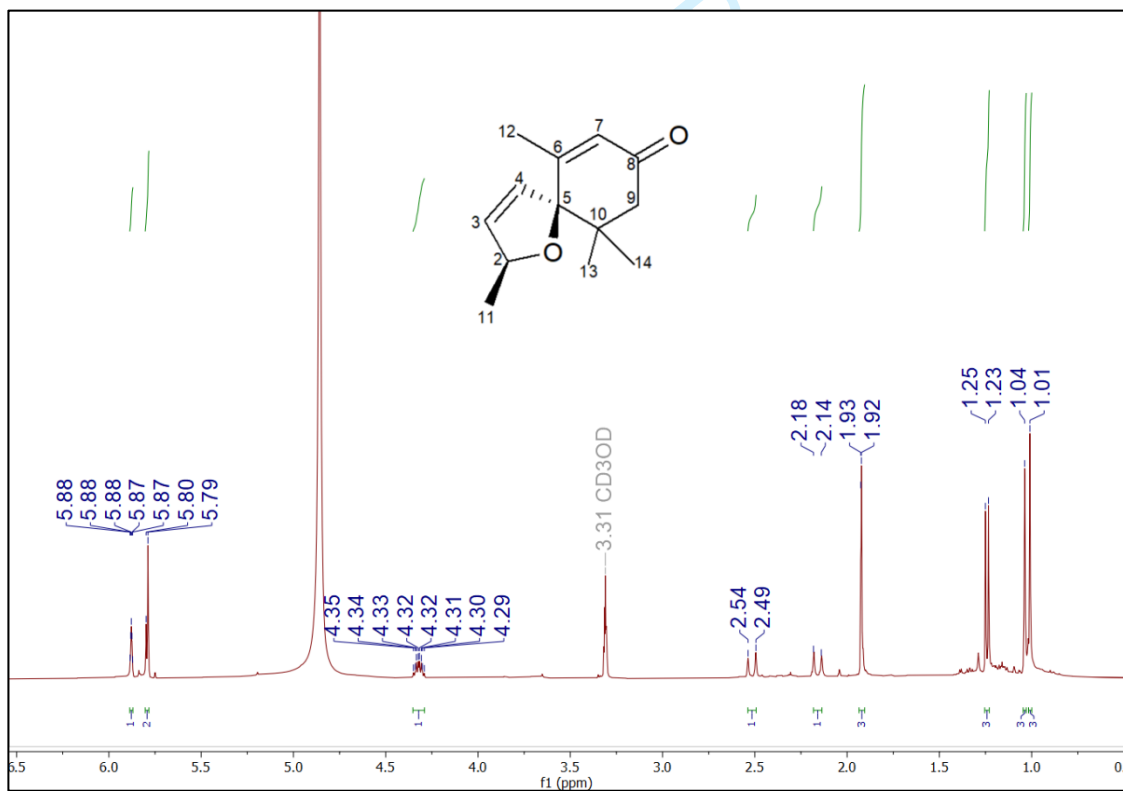


Fig 14S ^1H NMR spectrum of compound 6 (CD_3OD , 400 MHz)

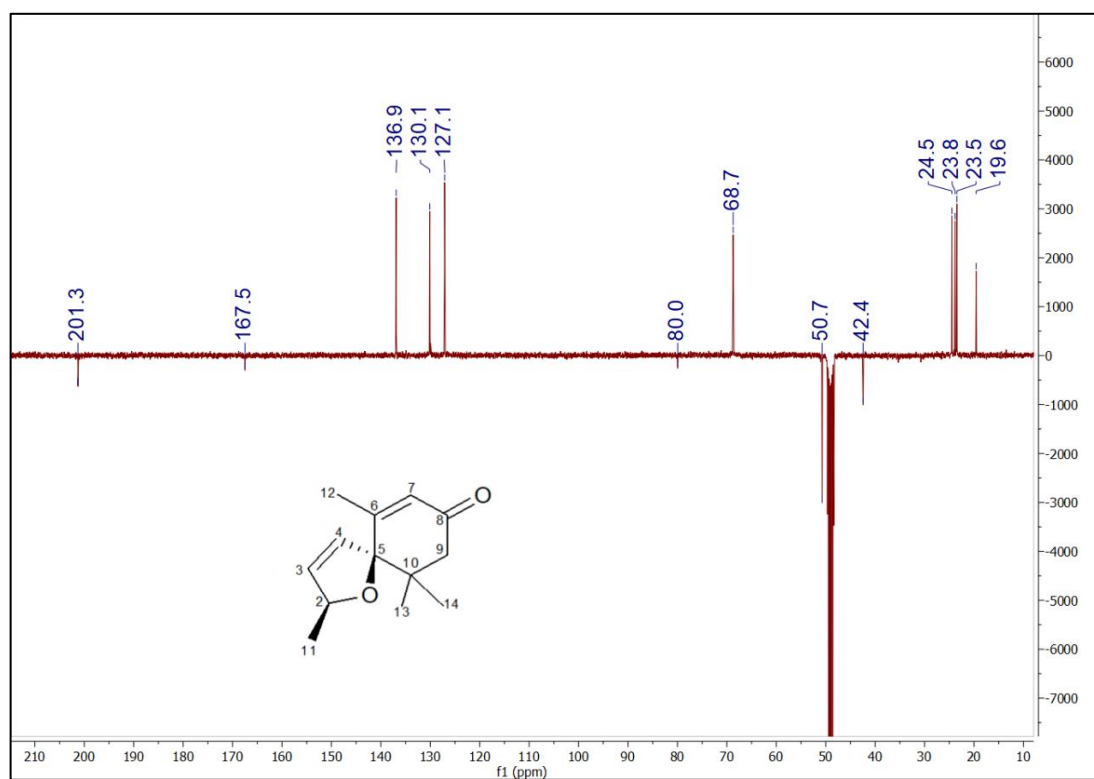


Fig 15S APT spectrum of compound **6** (CD₃OD, 100 MHz)

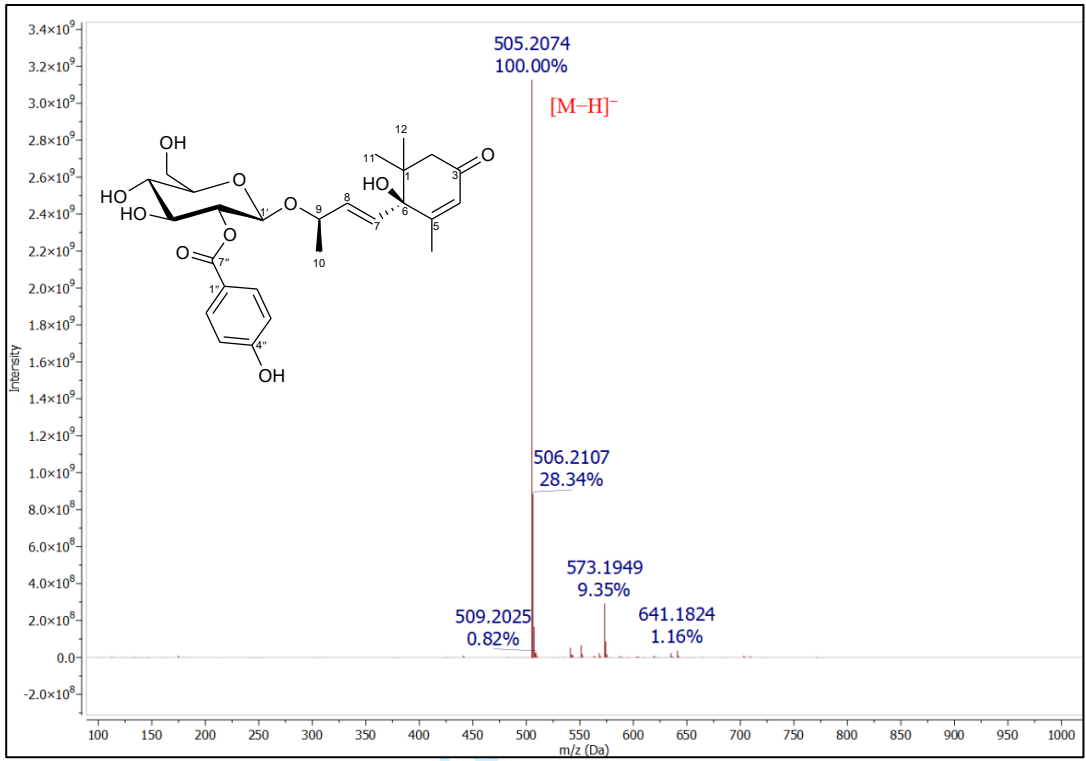


Fig 16S Negative mode HRMS of compound 7

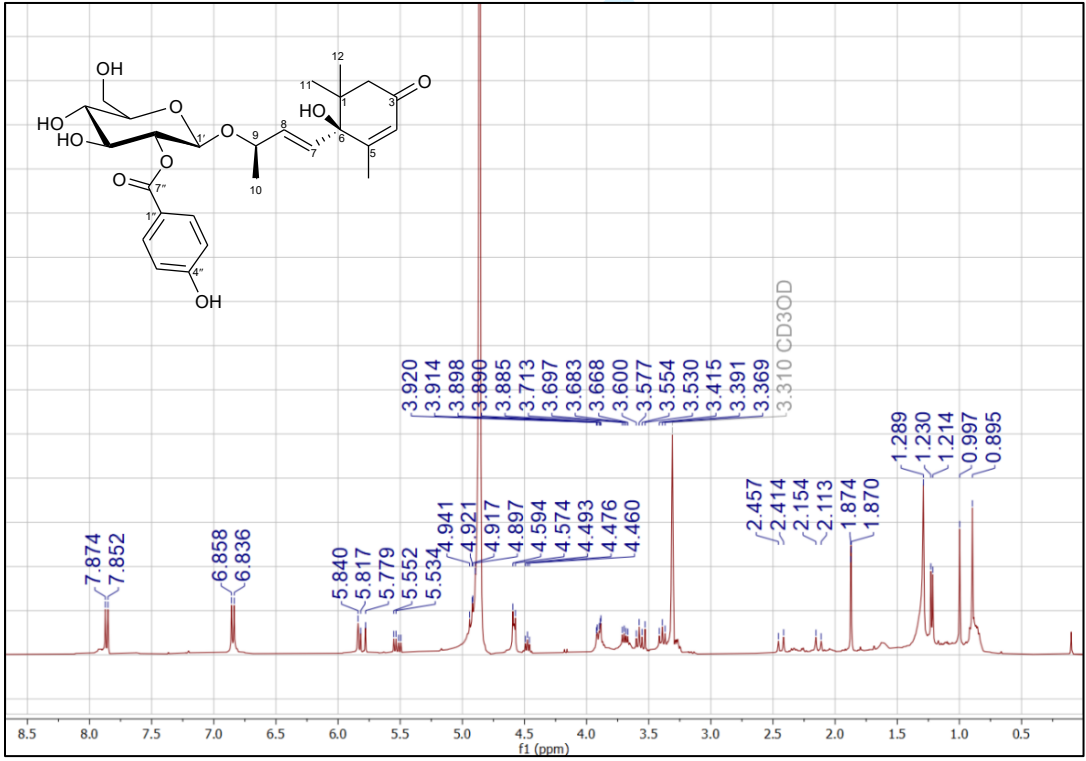


Fig 17S ¹H NMR spectrum of compound 7 (CD₃OD, 400 MHz)

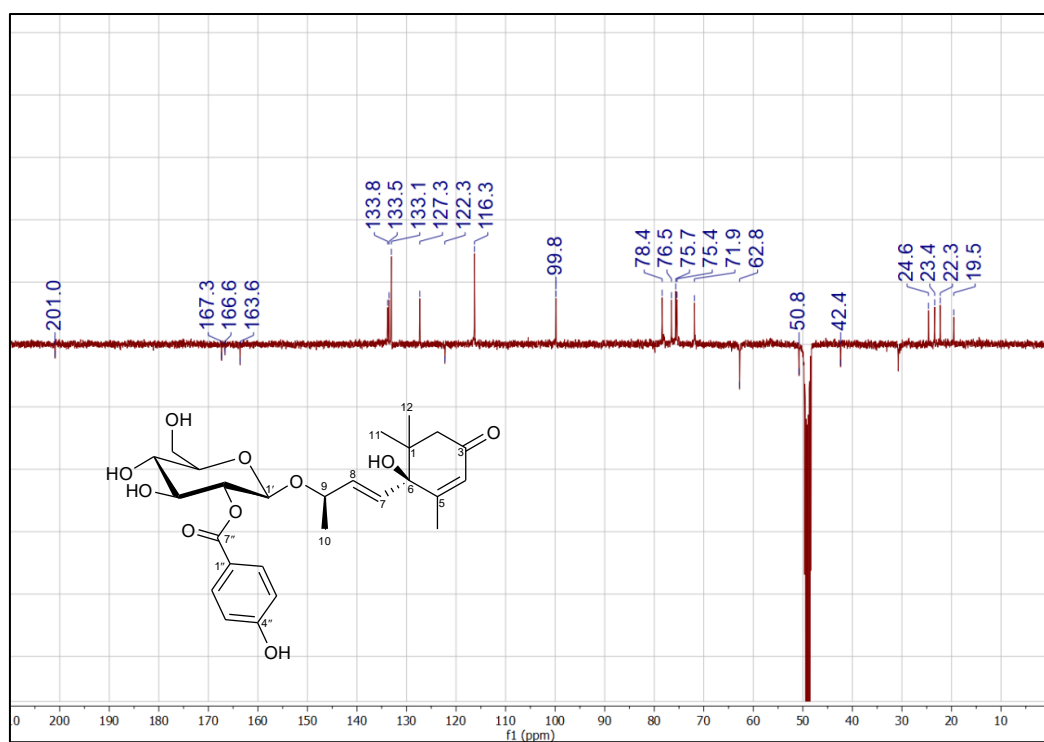


Fig 18S APT spectrum of compound 7 (CD₃OD, 100 MHz)

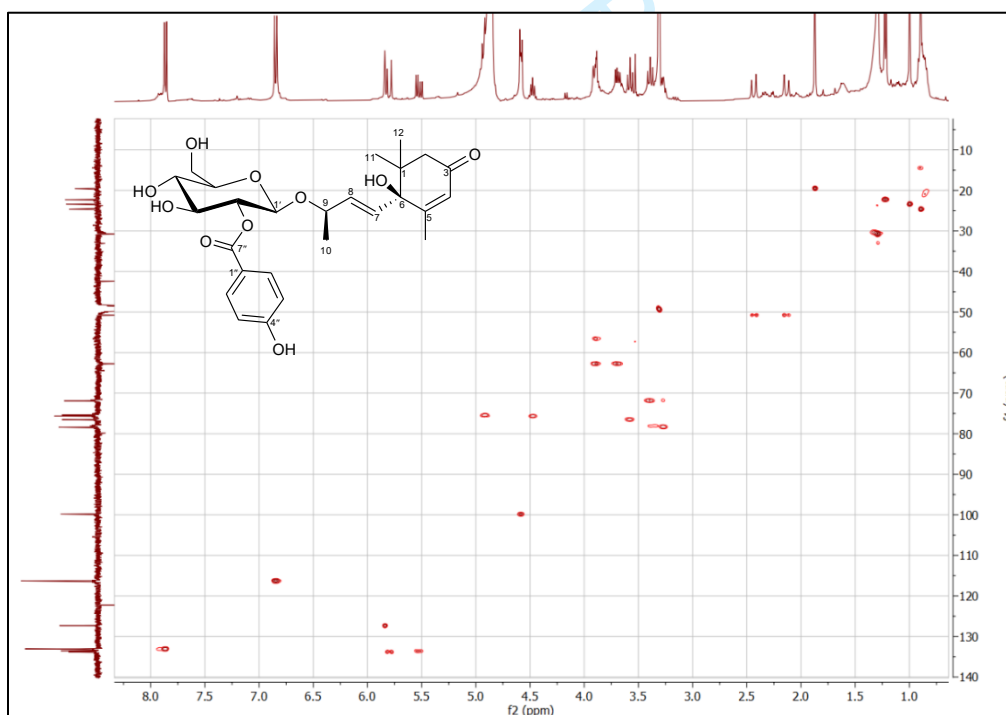


Fig 19S HSQC spectrum of compound 7 (CD₃OD, 400/100 MHz)

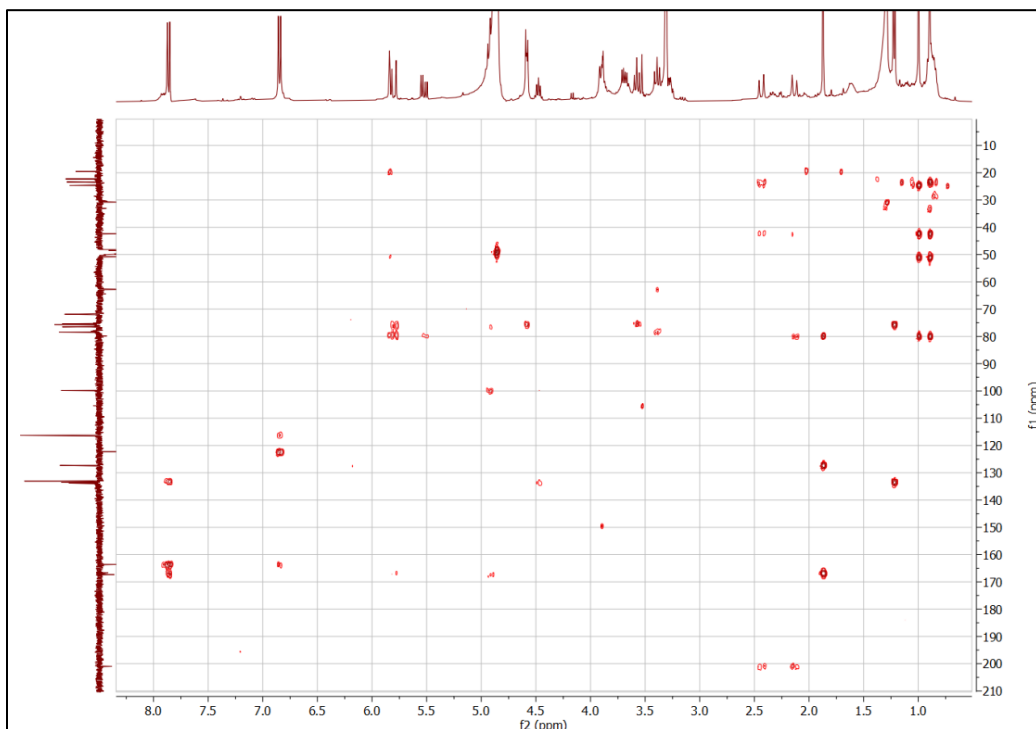


Fig 20S HMBC spectrum of compound **7** (CD₃OD, 400/100 MHz)

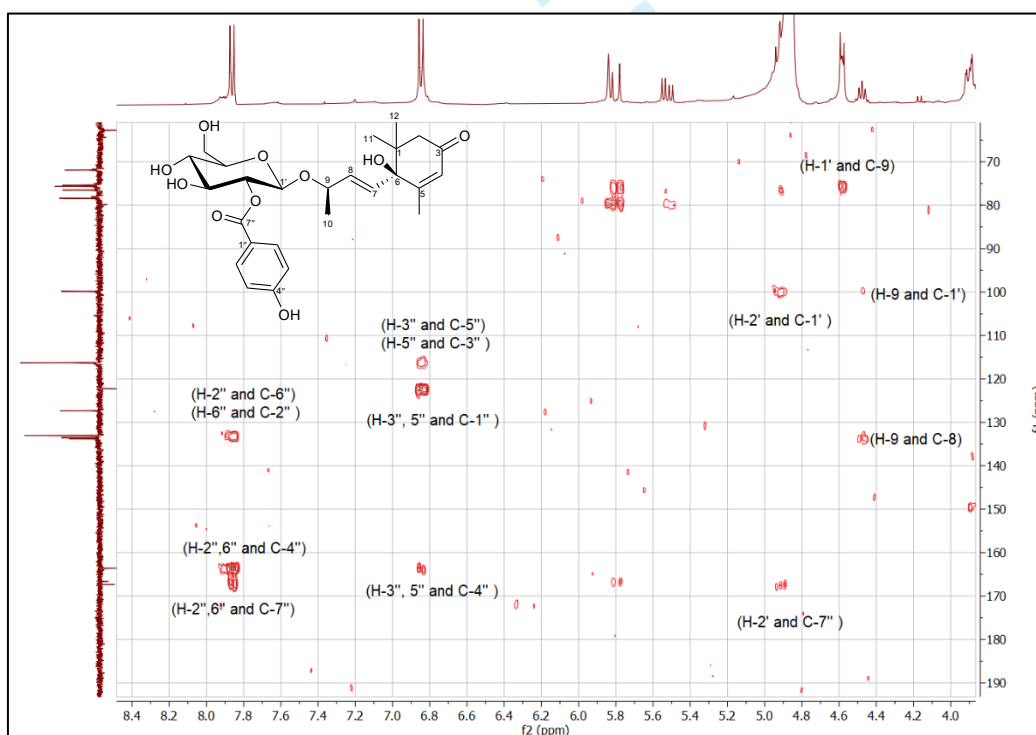


Fig 21S Important correlations observed in the expanded HMBC spectrum of compound **7** (CD₃OD, 400/100 MHz)

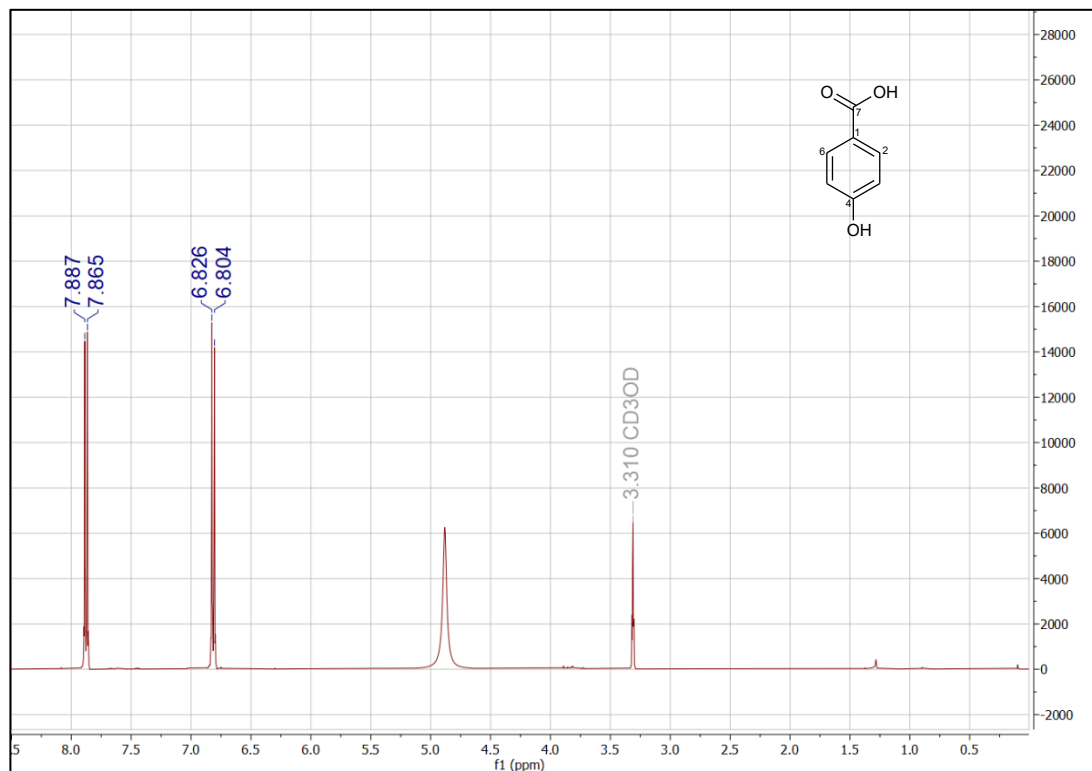


Fig 22S ^1H NMR spectrum of compound **8** (CD_3OD , 400 MHz)

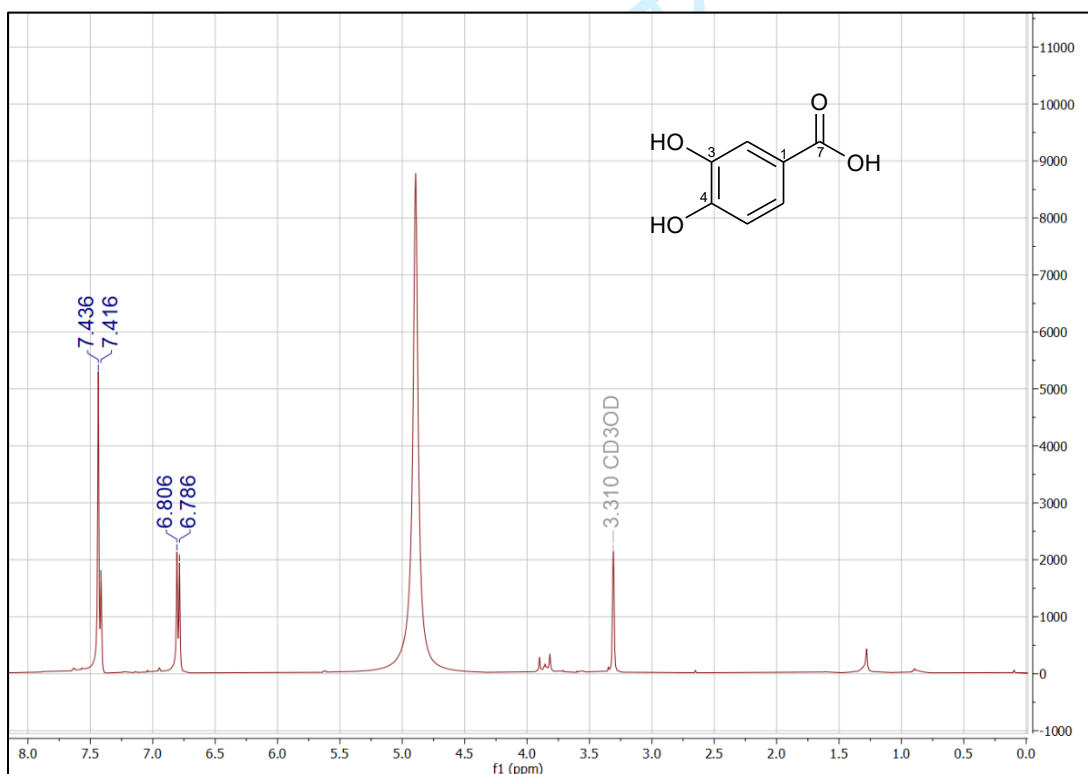


Fig 23S ^1H NMR spectrum of compound **9** (CD_3OD , 400 MHz)

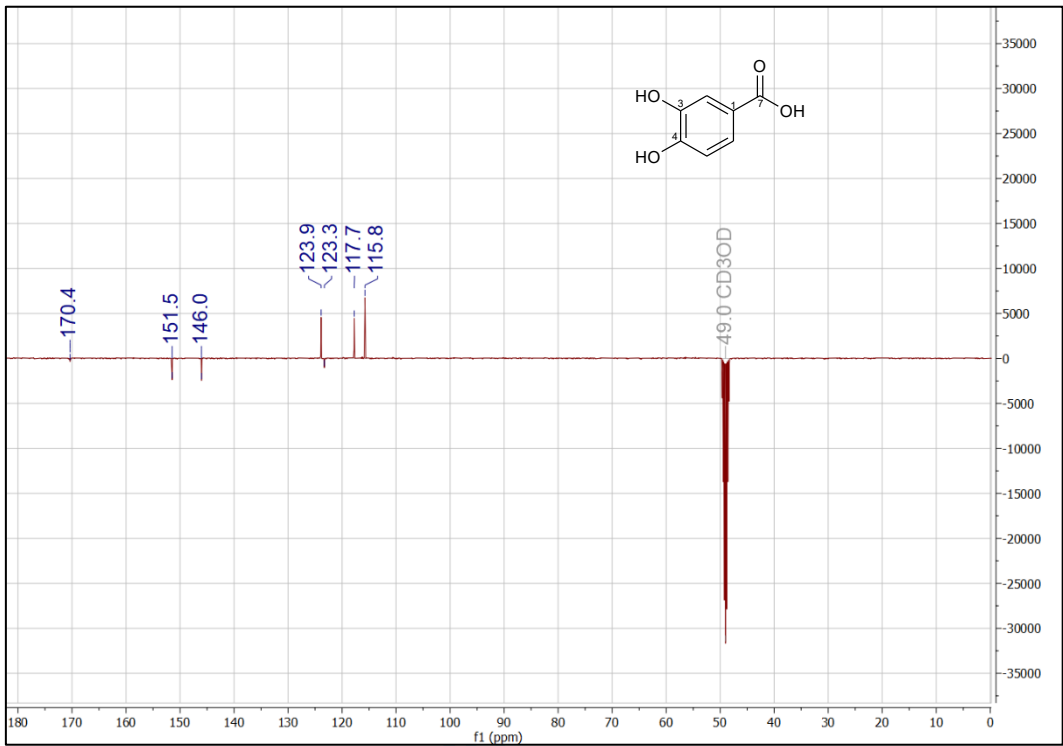


Fig 24S APT spectrum of compound 9 (CD₃OD, 100 MHz)

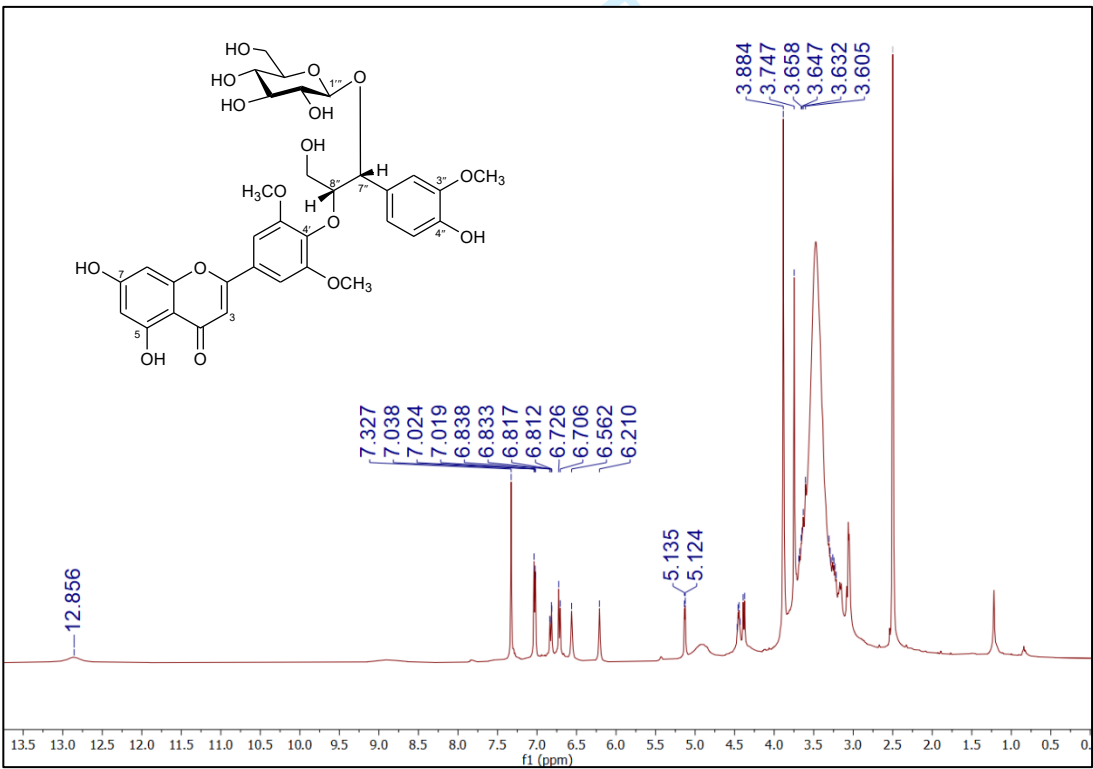


Fig 25S ¹H NMR spectrum of compound 10 (DMSO-*d*₆, 400 MHz)

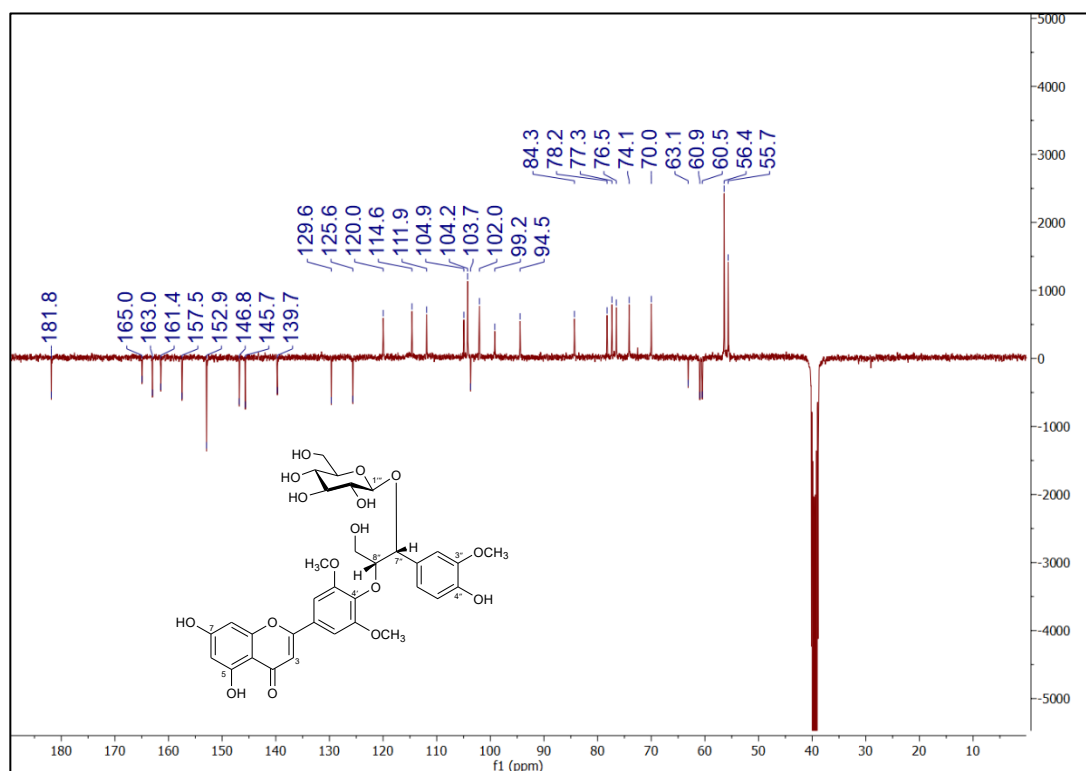


Fig 26S APT spectrum of compound 10 (DMSO- d_6 , 100 MHz)

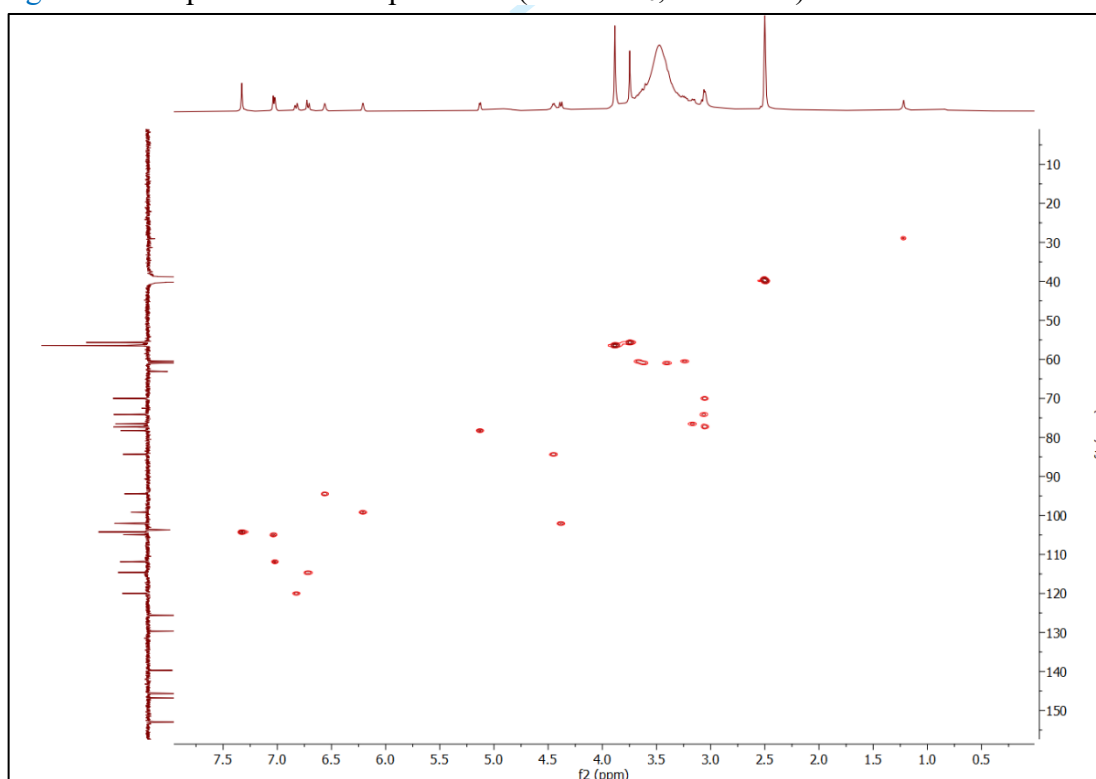


Fig 27S HSQC spectrum of compound 10 (DMSO- d_6 , 400/100 MHz)

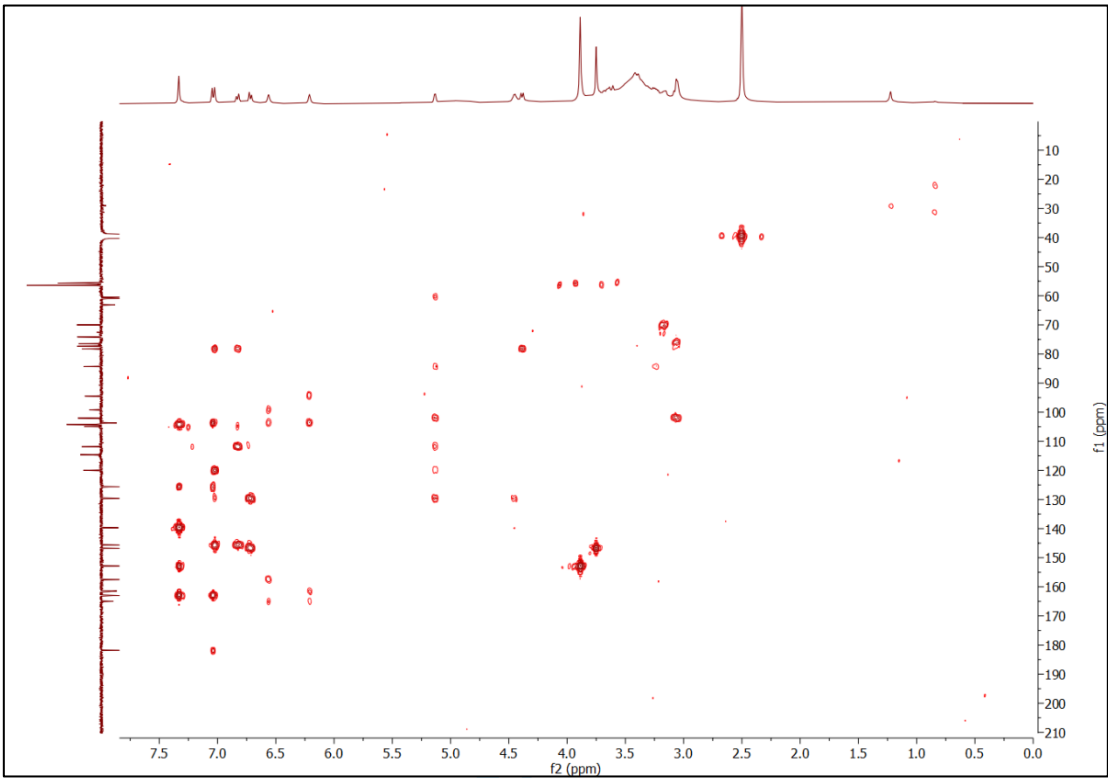


Fig 28S HMBC spectrum of compound **10** (DMSO-*d*₆, 400/100 MHz)

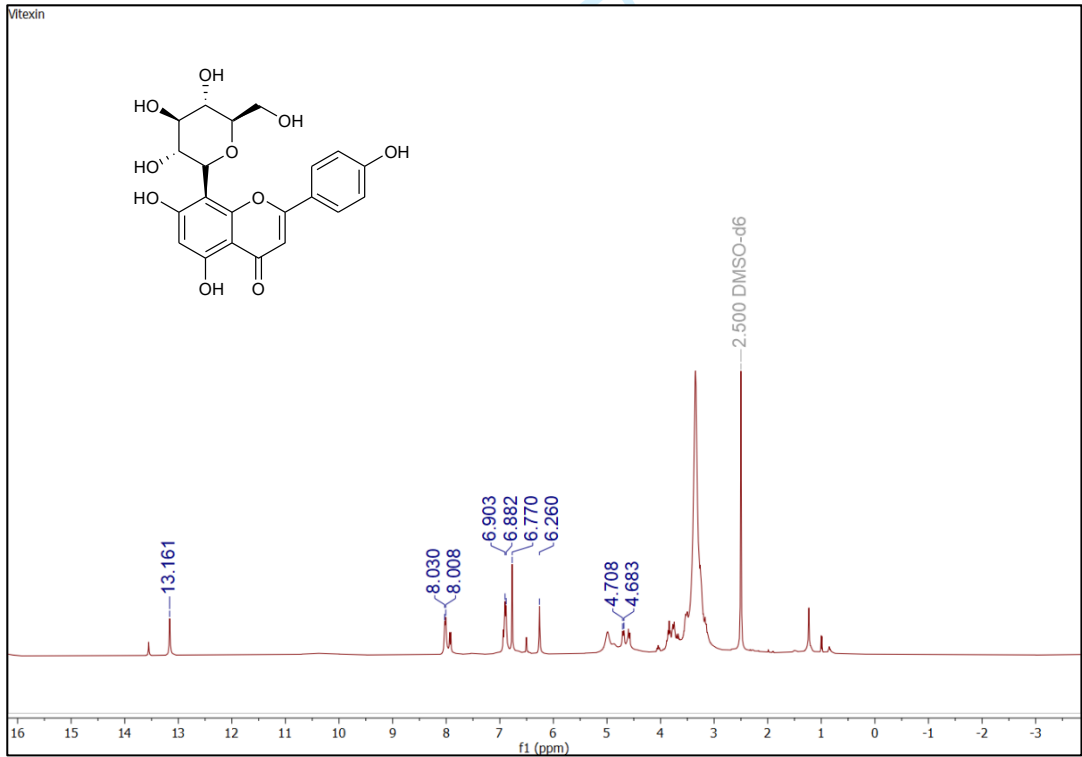


Fig 29S ¹H NMR spectrum of compound **11** (DMSO-*d*₆, 400 MHz)

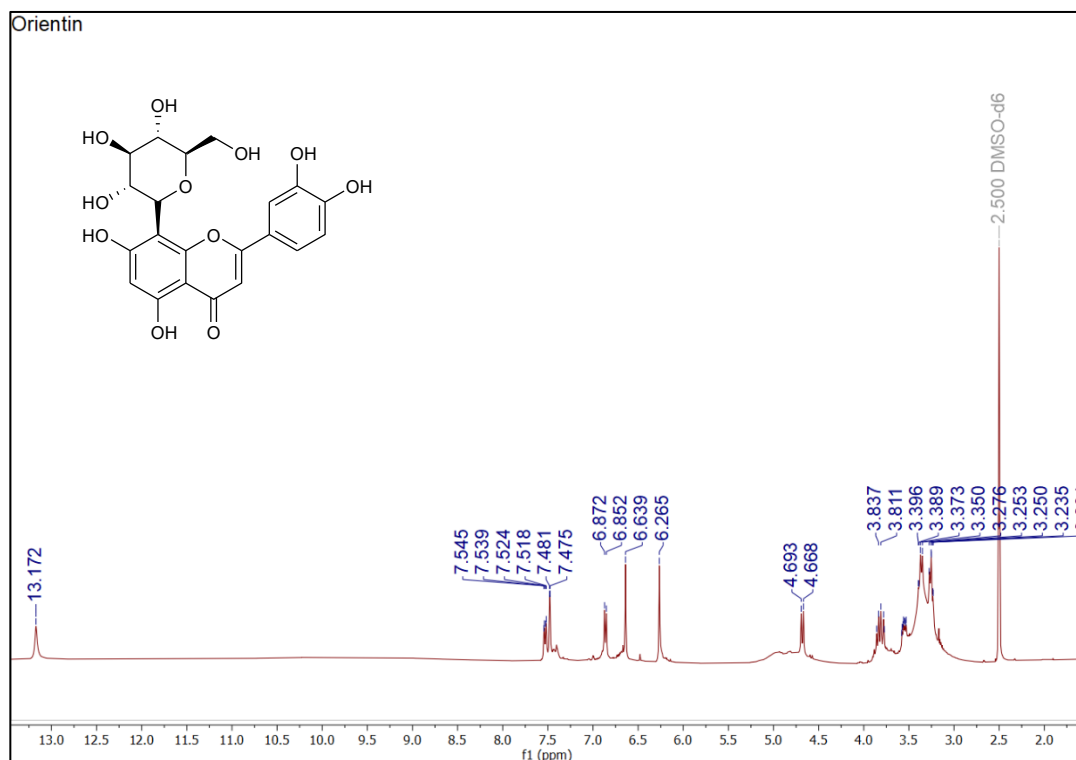


Fig 30S ¹H NMR spectrum of compound 12 (DMSO-d₆, 400 MHz)

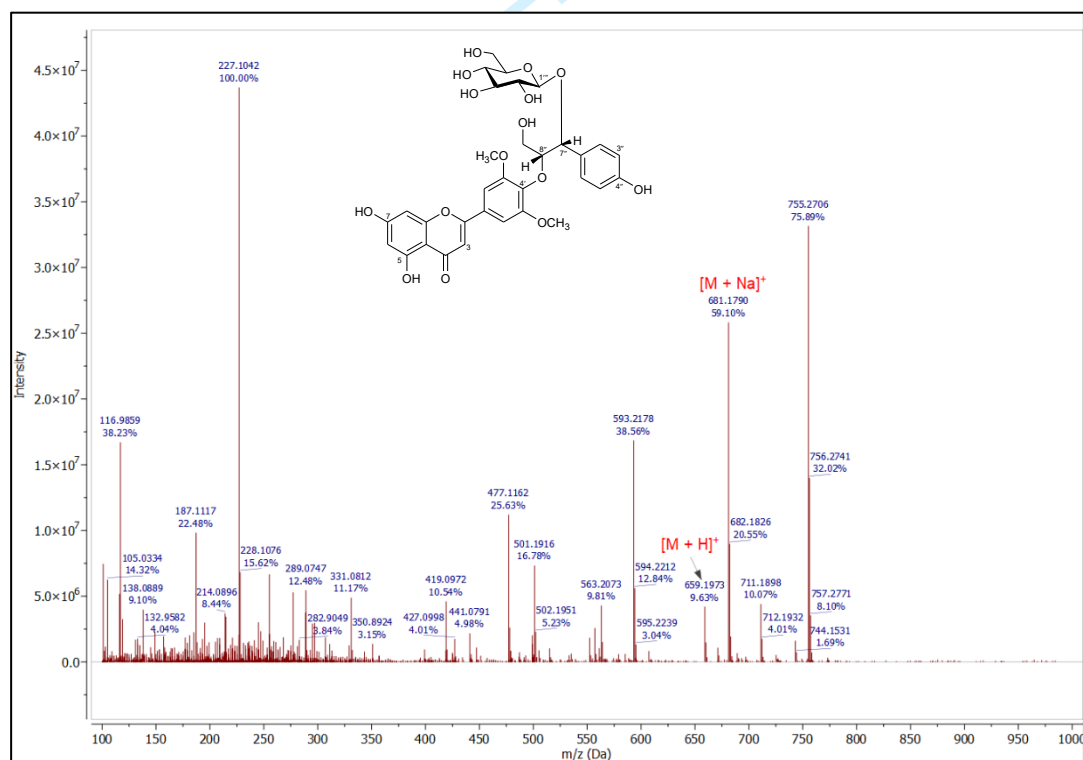


Fig 31S Positive mode HRMS of compound 13

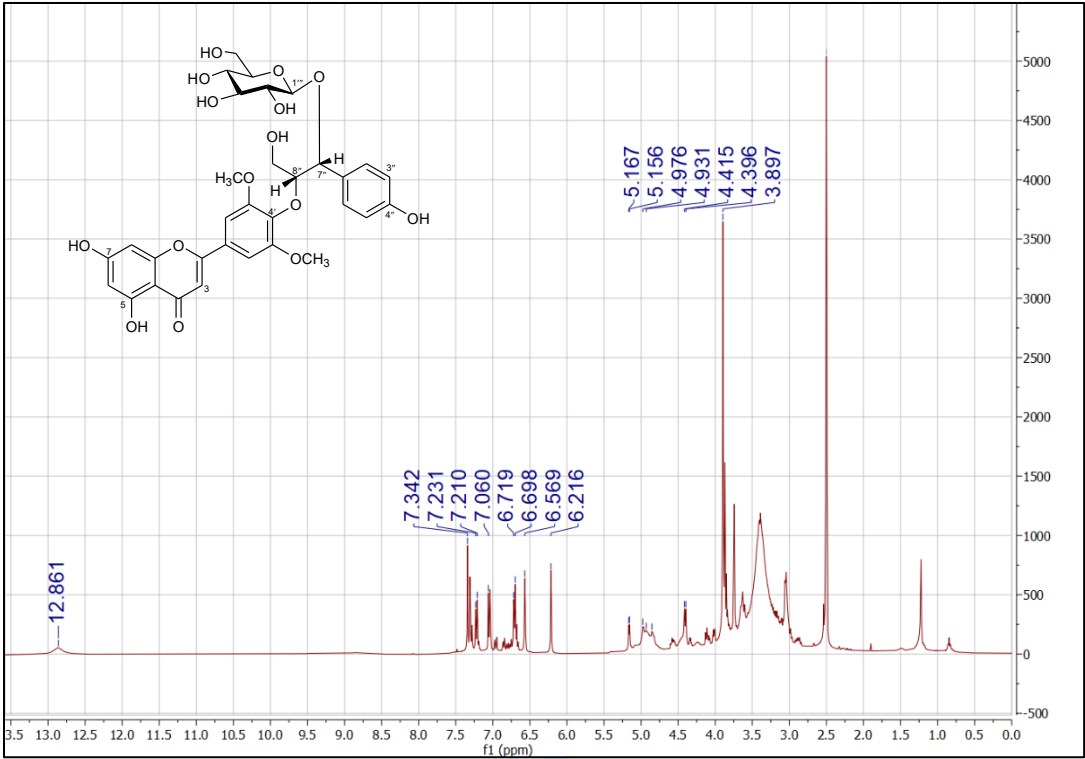


Fig 32S ¹H NMR spectrum of compound **13** (DMSO-*d*₆, 400 MHz)

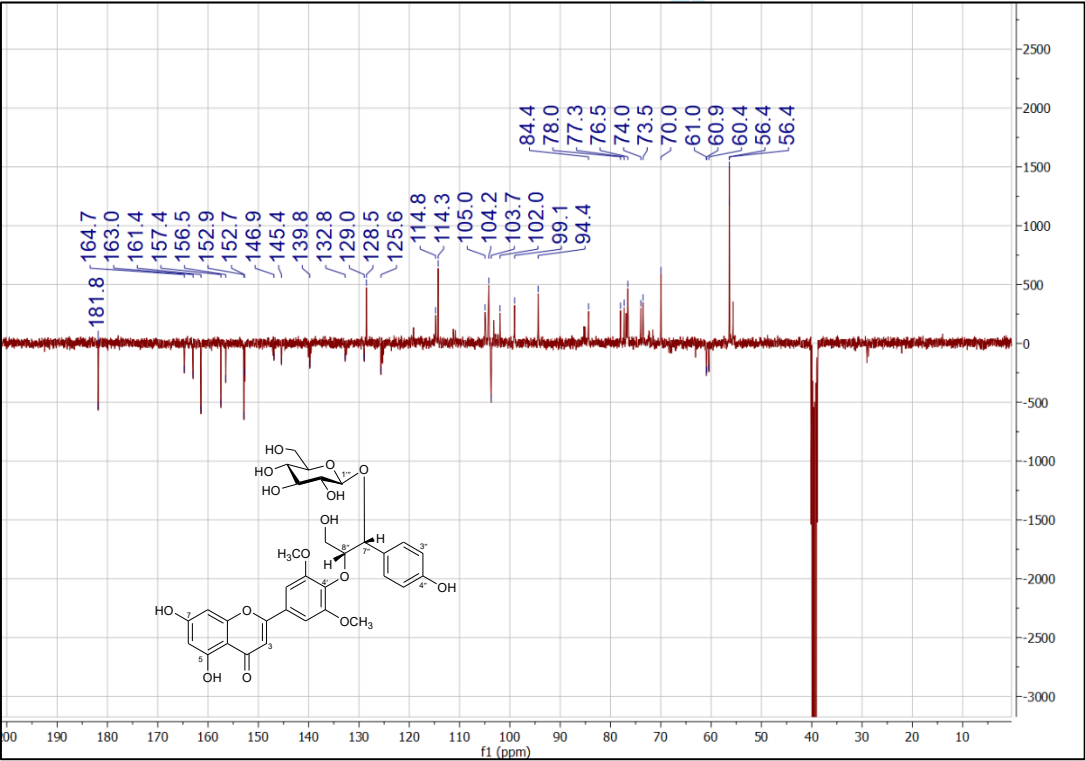


Fig 33S APT spectrum of compound **13** (DMSO-*d*₆, 100 MHz)

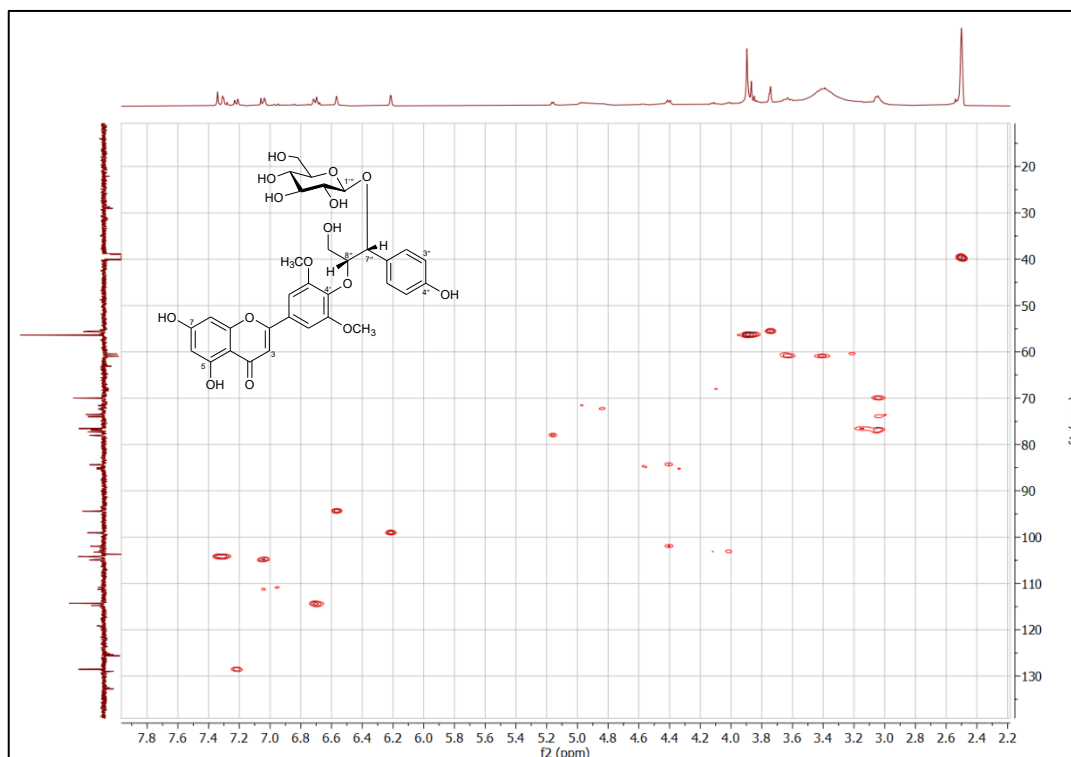


Fig 34S HSQC spectrum of compound **13** (DMSO-*d*₆, 400/100 MHz)

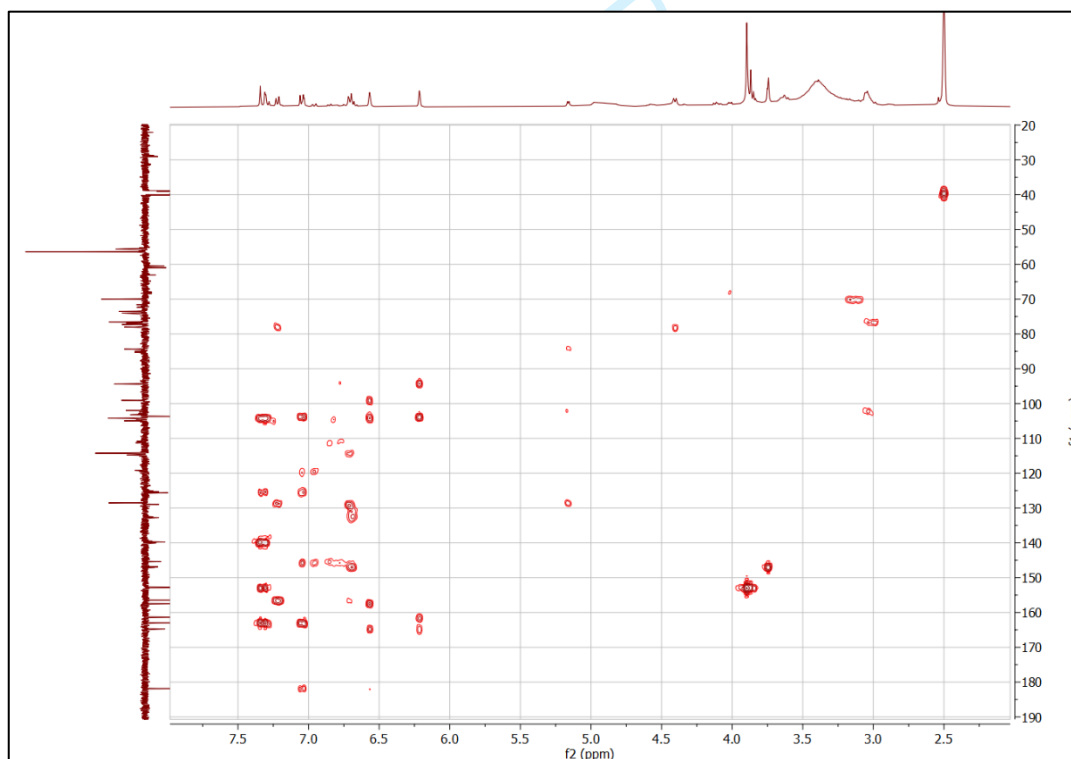


Fig 35S HMBC spectrum of compound **13** (DMSO-*d*₆, 400/100 MHz)

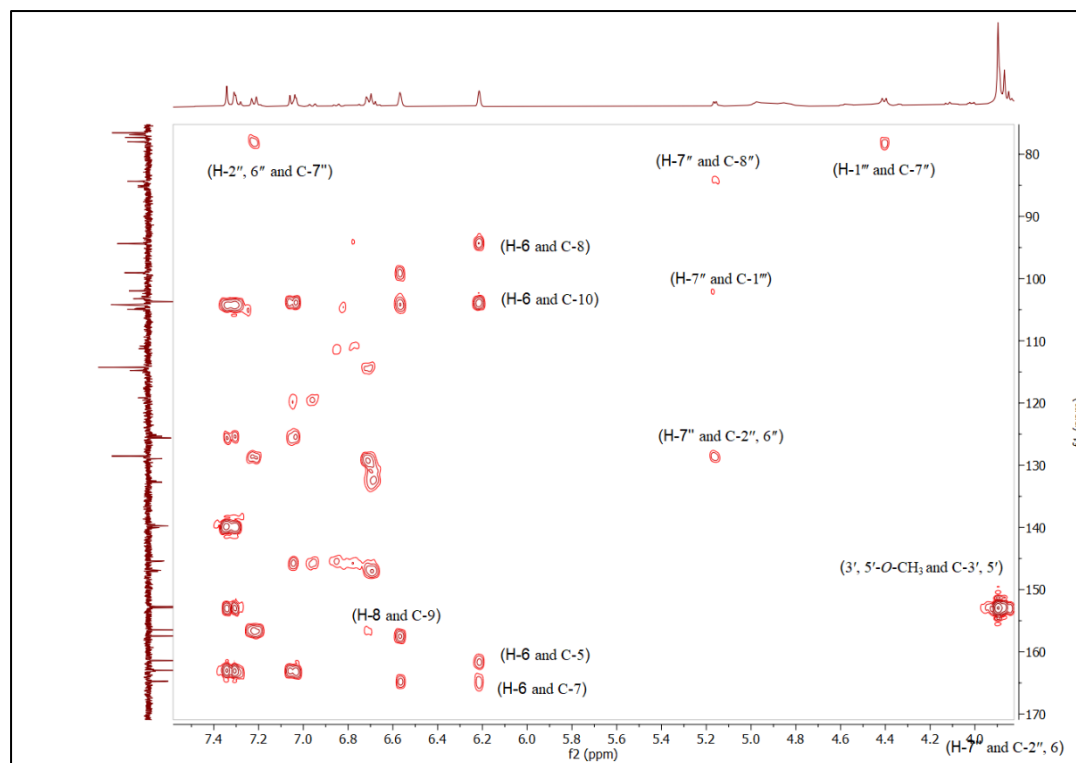


Fig 36S: Important correlations observed in the expanded HMBC spectrum of compound **13** (DMSO- d_6 , 400/100 MHz)

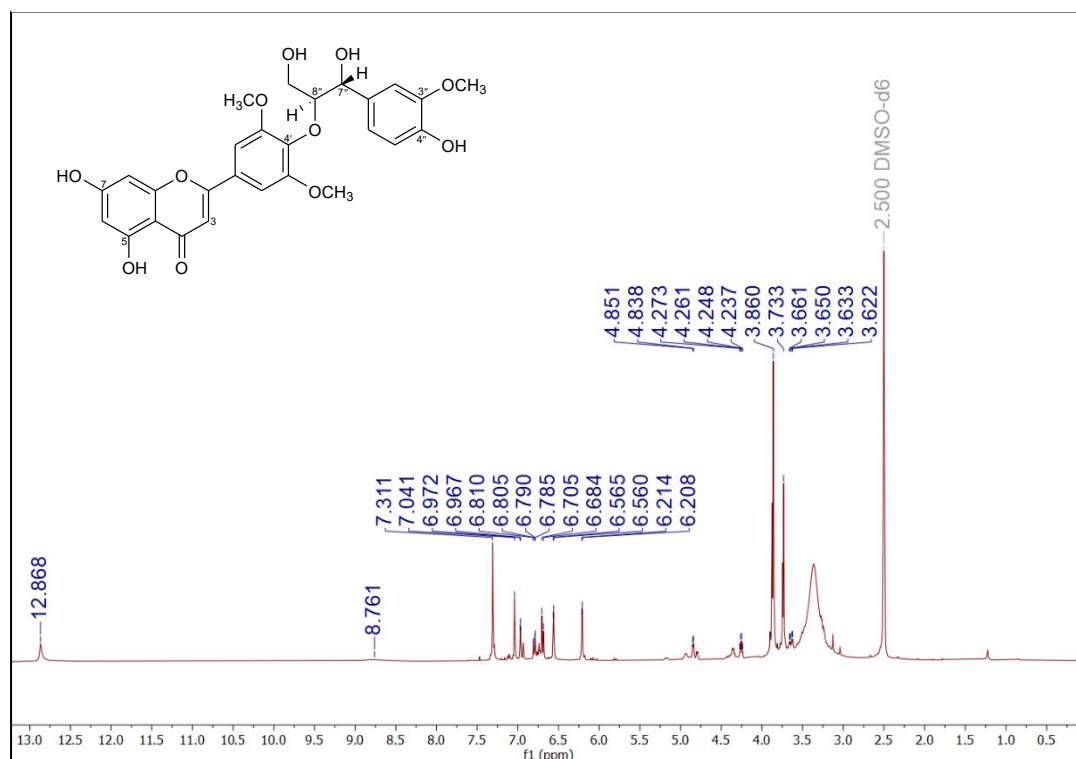


Fig 37S ^1H NMR spectrum of compound **14** (DMSO- d_6 , 400 MHz)

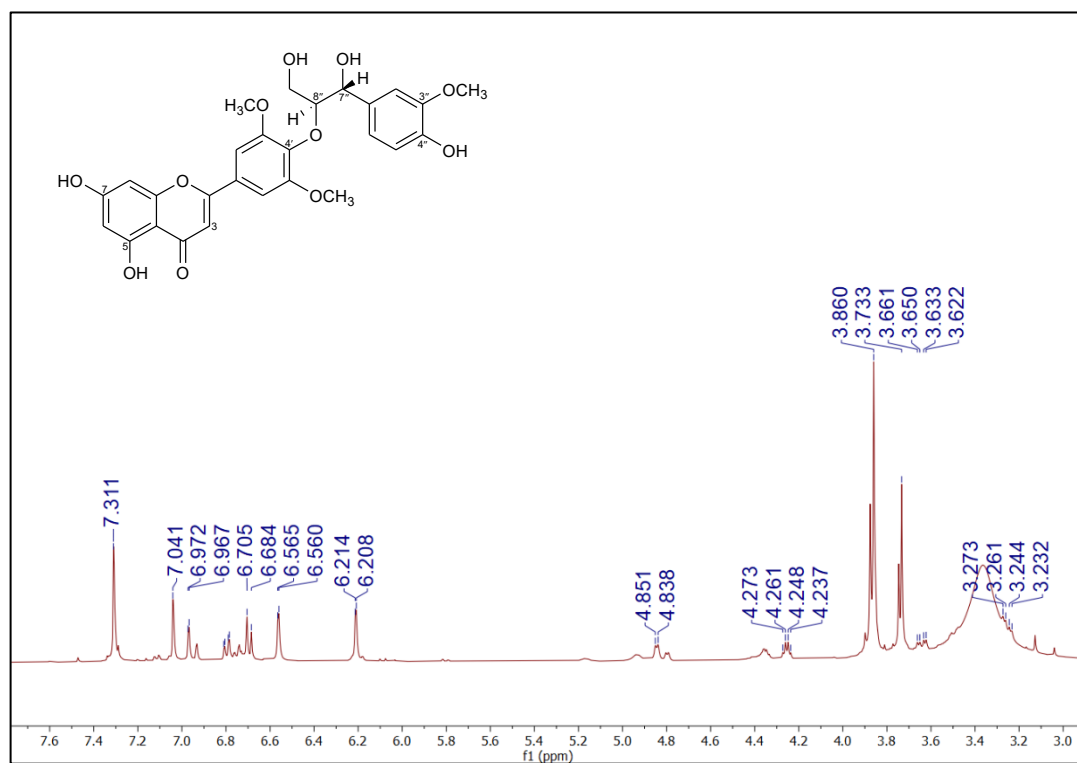


Fig 38S Expanded ^1H NMR spectrum of compound **14** (DMSO- d_6 , 400 MHz)

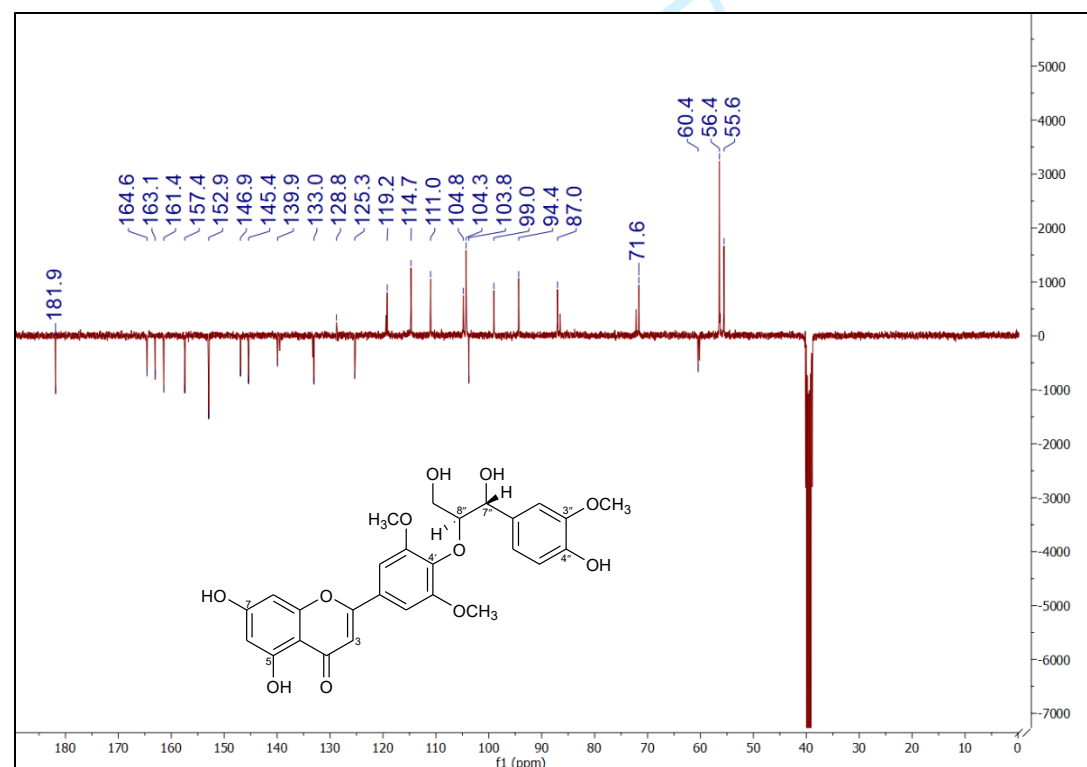


Fig 39S APT spectrum of compound **14** (DMSO- d_6 , 100 MHz)

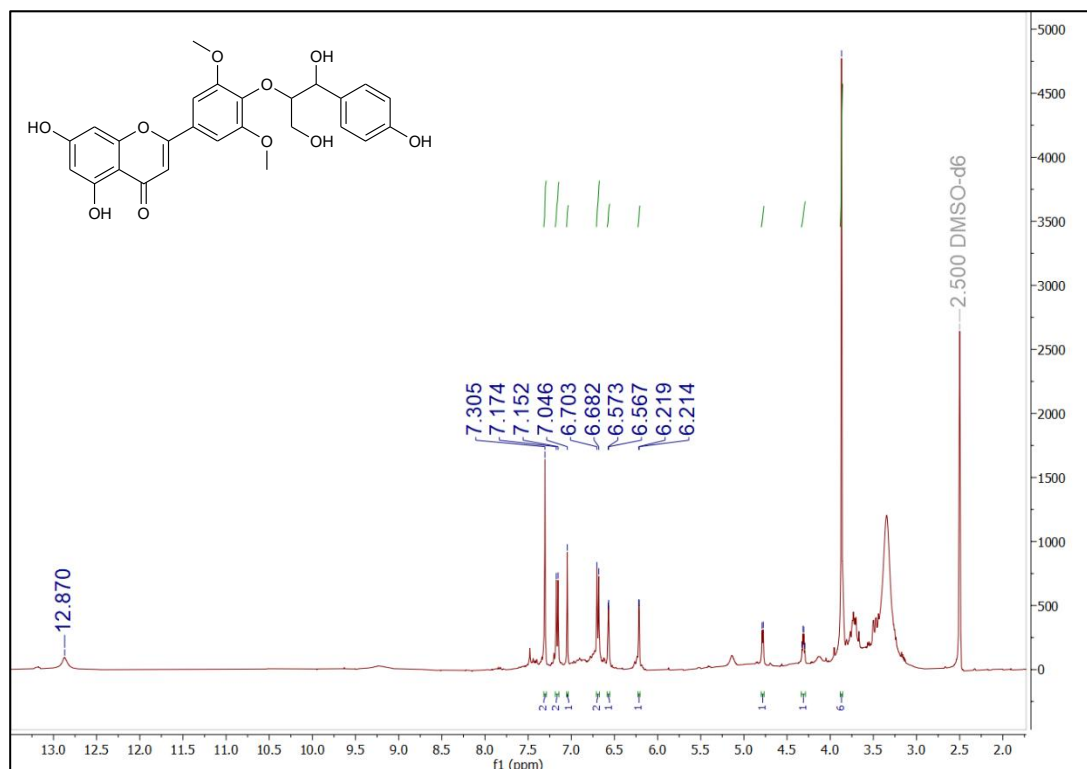


Fig 40S ^1H NMR spectrum of compound **15** (DMSO- d_6 , 400 MHz)

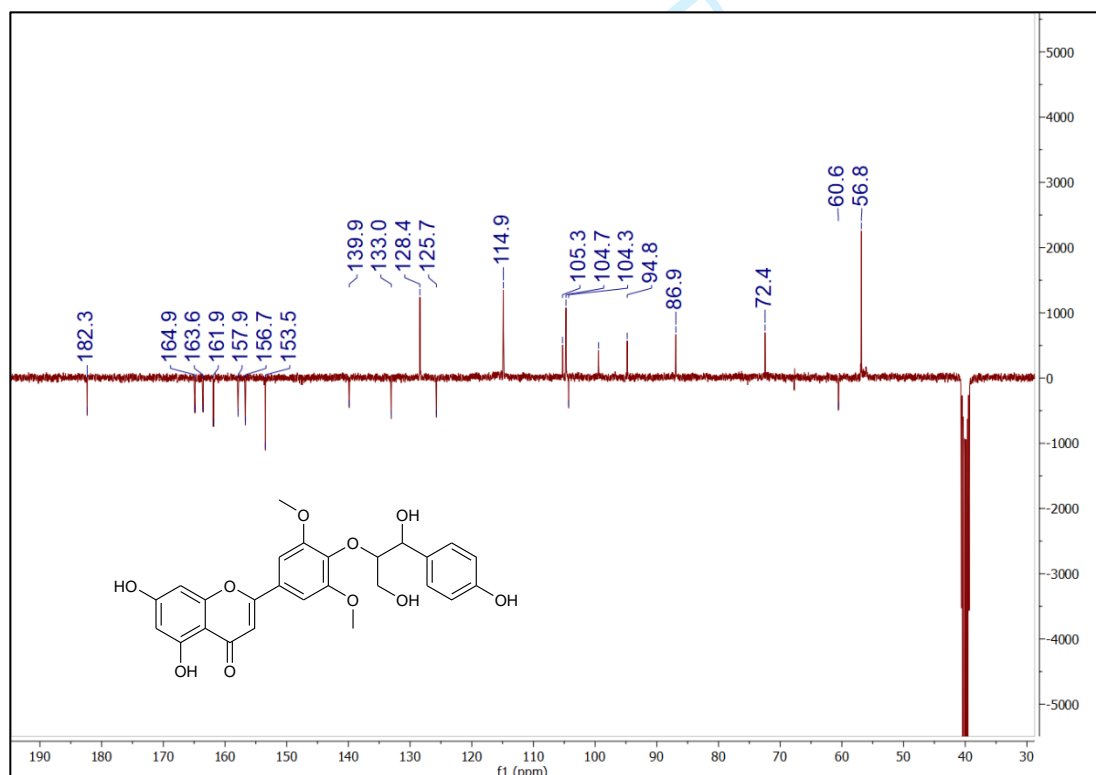


Fig 41S APT spectrum of compound **15** (DMSO- d_6 , 100 MHz)

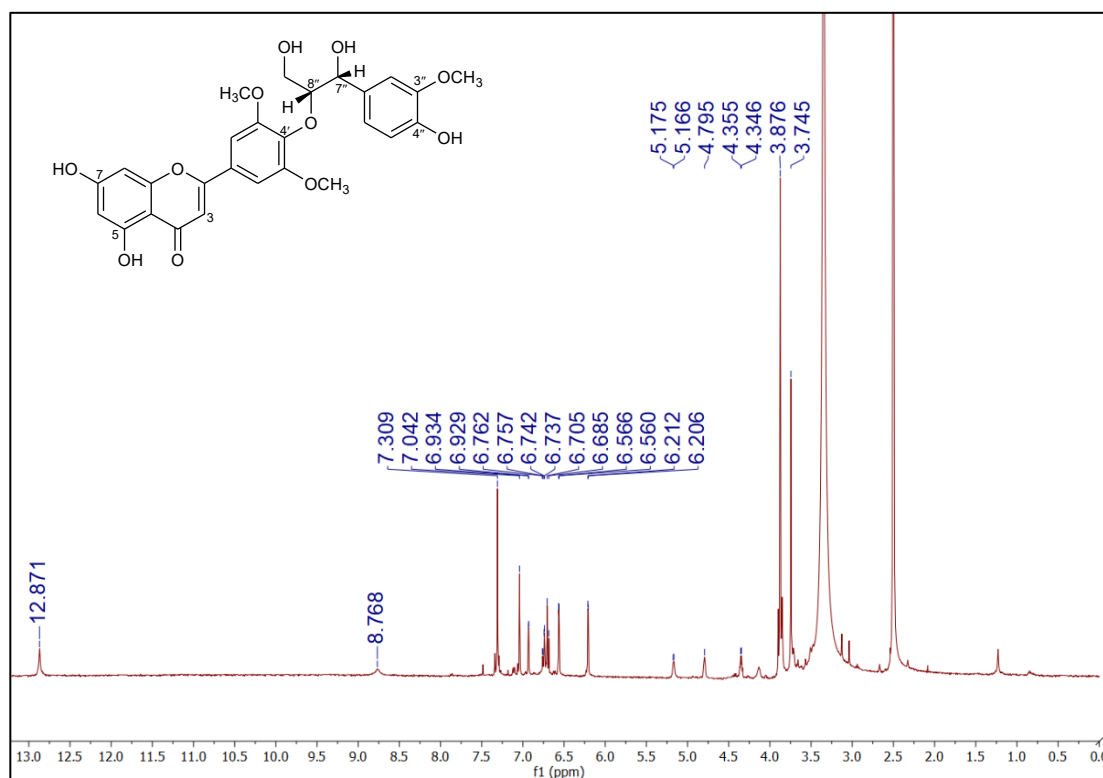


Fig 42S ^1H NMR spectrum of compound **16** ($\text{DMSO}-d_6$, 400 MHz)

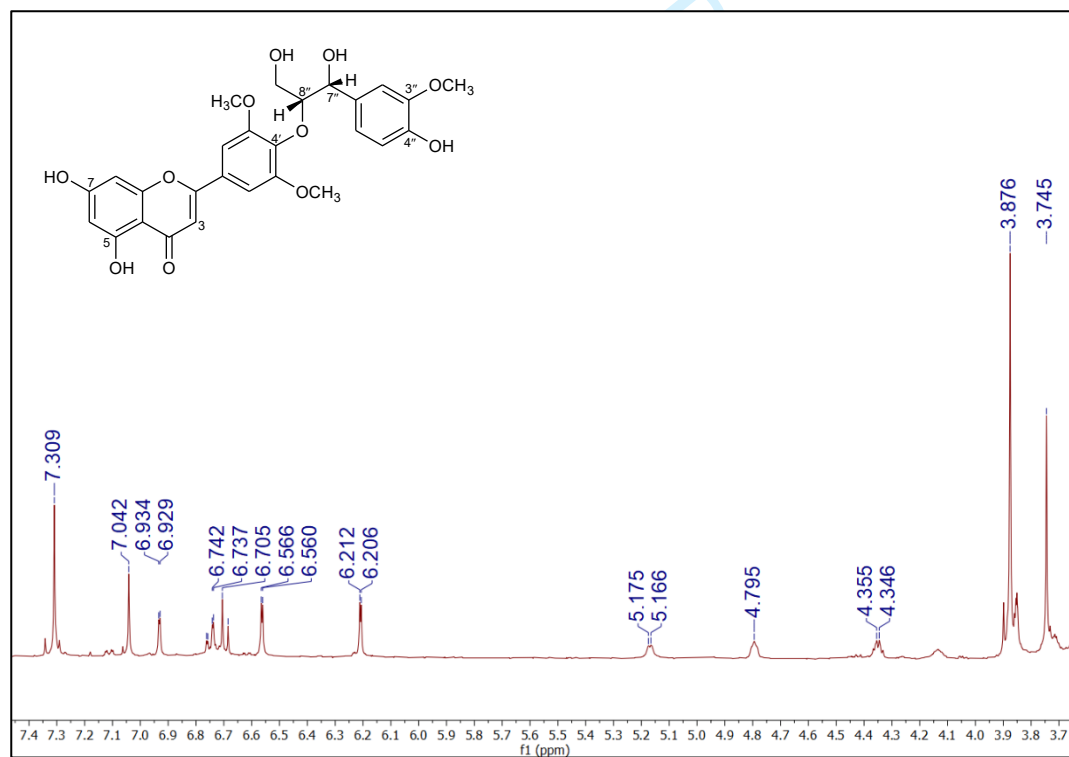


Fig 43S Expanded ^1H NMR spectrum of compound **16** ($\text{DMSO}-d_6$, 400 MHz)

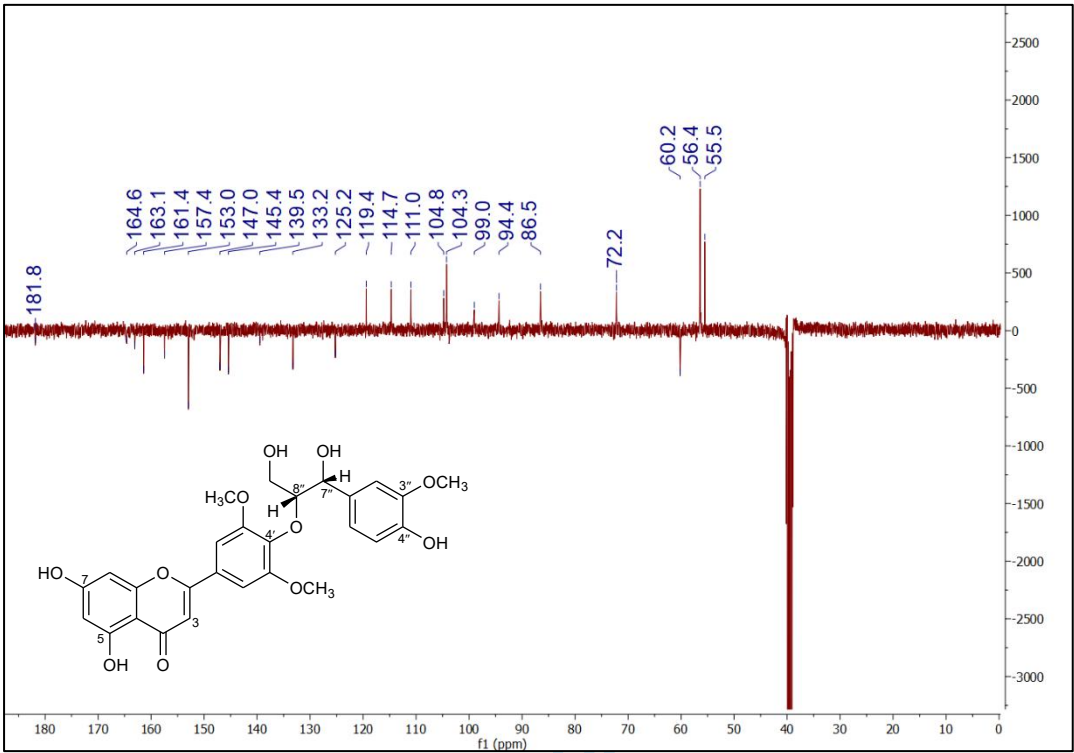


Fig 44S APT spectrum of compound 16 (DMSO-*d*₆, 100 MHz)

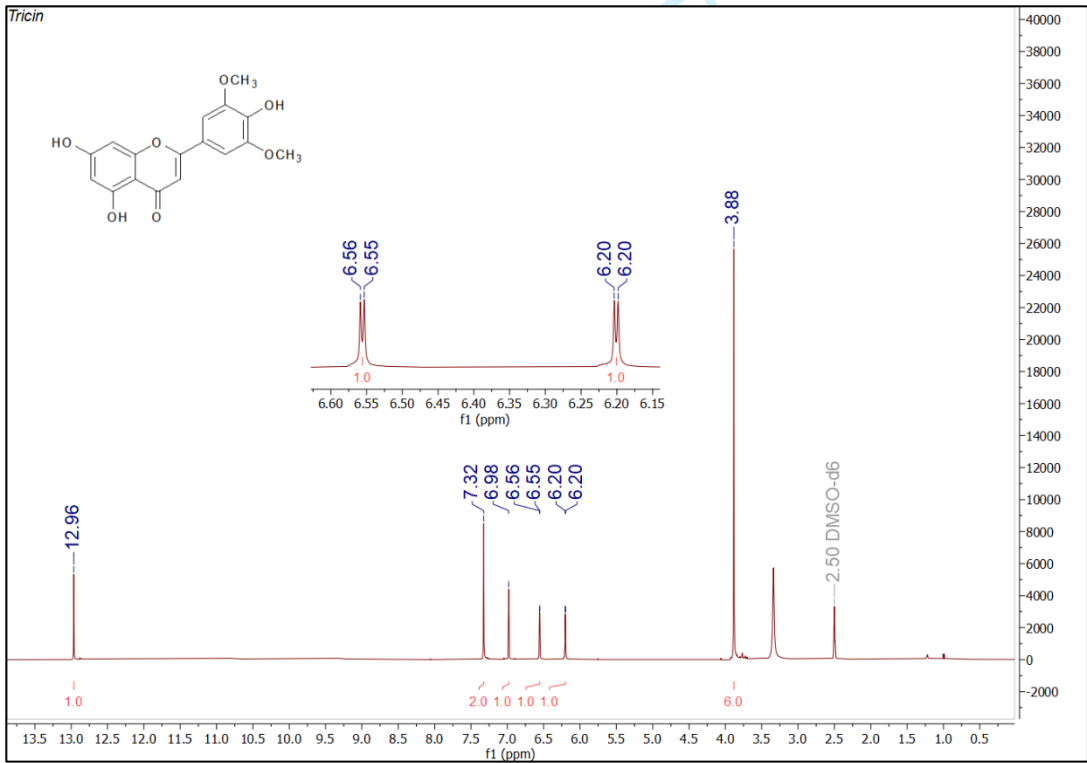


Fig 45S ¹H NMR spectrum of compound 17 (DMSO-*d*₆, 400 MHz)

1S: Spectral data of DCM compounds

Cylindrin (1): white solid. $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ_{H} (ppm): 0.756 (*s*, 3H, CH_3 -27), 0.766 (*s*, 3H, CH_3 -28), 0.796 (*s*, 3H, CH_3 -24), 0.805 (*s*, 3H, CH_3 -26), 0.83 (*d*, $J = 6.4$ Hz, 3H, CH_3 -29), 0.89 (*d*, $J = 6.8$ Hz, 3H, CH_3 -30), 0.96 (*s*, 3H, CH_3 -23), 1.03 (*s*, 3H, CH_3 -25), 2.65 (*dd*, $J = 4$, 11.2 Hz, 1H, H-3), 3.37 (*s*, 3H, 3- OCH_3), 5.22 (*d*, $J = 6.4$ Hz, 1H, H-11). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ_{C} (ppm): 36.07 (C-1), 22.7 (C-2), 88.8 (C-3), 39.1 (C-4), 53.0 (C-5), 21.4 (C-6), 28.37 (C-7), 41.1 (C-8), 149.1 (C-9), 39.8 (C-10), 114.3 (C-11), 36.2 (C-12), 36.2 (C-12), 36.9 (C-13), 38.3 (C-14), 29.80 (C-15), 36.09 (C-16), 43.0 (C-17), 52.2 (C-18), 20.3 (C-19), 29.85 (C-20), 59.8 (C-21), 30.9 (C-22), 28.4 (C-23), 16.5 (C-24), 22.2 (C-25), 17.2 (C-26), 15.4 (C-27), 14.1 (C-28), 22.2 (C-29), 23.1 (C-30), 57.7 (3- OCH_3) [1]

Methyl ferulate (2): white solid. $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ_{H} (ppm): 7.62 (*d*, $J = 16$ Hz, 1H, H-7), 7.07 (*dd*, $J = 2$, 8 Hz, 1H, H-6), 7.02 (*d*, $J = 2$ Hz, 1H, H-2), 6.91 (*d*, $J = 8$ Hz, 1H, H-5), 6.29 (*d*, $J = 16$ Hz, 1H, H-8), 3.92 (*s*, 3H, 4- OCH_3), 3.78 (*s*, 3H, 9- OCH_3). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ_{C} (ppm): 167.9 (C-9), 148.1 (C-4), 146.9 (C-3), 145.1 (7), 127.1 (C-1), 123.2 (C-6), 115.3 (C-8), 114.9 (C-5), 109.5 (C-2), 56.1 (4- OCH_3), 51.7 (9- OCH_3) [2]

2,6-Dimethyl-*p*-quinone (3): Yellow needles. $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ_{H} (ppm): 3.82 (*s*, 6H, $2 \times \text{OCH}_3$), 5.85 (*s*, 2H, H-3, 5). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ_{C} (ppm): 56.6 ($2 \times \text{OCH}_3$), 107.6 (C-3, 5), 157.5 (C-2, 6), 176.5 (C-1), 187 (C-4) (Chen et al., 2022).

Methyl-4-hydroxybenzoate (4): White crystals. $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ_{H} (ppm): 3.84 (*s*, 3H, 7- OCH_3), 6.82 (*d*, $J = 8.0$ Hz, 2H, H-3, 5), 7.86 (*d*, $J = 8.0$ Hz, 2H, H-2, 6). $^{13}\text{C-NMR}$ (100 MHz, CD_3OD): δ_{C} (ppm): 122.2 (C-1), 132.7 (C-2, 6), 116.2 (C-3, 5), 163.5 (C-4), 52.2 (7- OCH_3) [4].

2S: Spectral data of ethyl acetate compounds

Methyl β -D-glucopyranoside (5): White crystals. $^1\text{H-NMR}$ (400 MHz, $\text{C}_5\text{D}_5\text{N}$): δ_{H} (ppm): 4.74 (*d*, $J = 7.7$ Hz, 1H, H-1), 3.94 (*m*, 1H, H-2), 4.04 (*m*, 1H, H-3, H-5), 4.25 (*m*, 1H, H-4), 4.39 (*dd*, $J = 5.4$, 11.88 Hz, 1H, H-6a), 4.55 (*dd*, $J = 2.4$, 11.7 Hz, 1H, H-6b), 3.61 (*s*, 3H, 1- OCH_3). $^{13}\text{C-NMR}$ (100 MHz, $\text{C}_5\text{D}_5\text{N}$): δ_{C} (ppm): 106.1 (C-1), 75.5 (C-2), 78.9 (C-3), 72.0 (C-4), 78.8 (C-5), 63.1 (C-6), 57.1 (1- OCH_3) [5].

3,4-Dehydrotheaspirone (6): Yellowish oily residue. $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ_{H} (ppm): 5.79 (s, 1H, H-7), 5.80 (s, 1H, H-3), 5.88 (m, 1H, H-4), 4.32 (m, 1H, H-2), 2.52 (d, $J = 17.2$ Hz, 1H, H-9a), 2.16 (d, $J = 17.2$ Hz, 1H, H-9b), 1.92 (d, $J = 1.6$, 3H, H-12), 1.24 (d, $J = 6.4$ Hz, 3H, H-11), 1.03 (s, 3H, H-13), 1.00 (s, 3H, H-14). $^{13}\text{C-NMR}$ (100 MHz, CD_3OD): δ_{C} (ppm): 201.3 (C-8), 167.5 (C-6), 136.9 (C-3), 130.1 (C-4), 127.1 (C-7), 80.0 (C-5), 68.7 (C-2), 50.7 (C-9), 42.4 (C-10), 24.5 (C-14), 23.8 (C-11), 23.5 (C-13), 19.6 (C-12) [6]

***p*-Hydroxybenzoic acid (8):** Off-white amorphous powder. $^1\text{H-NMR}$ (400 MHz, CD_3OD) δ_{H} (ppm): 7.87 (d, $J = 8.8$ Hz, 2H, H-3, 5); 6.81 (d, $J = 8.8$ Hz, 2H, H-2, 6). Identical with an authentic sample [7]

Protocatechuic acid (9) Off-white powder. $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ_{H} (ppm): 7.44 (s, 1H, H-2), 7.42 (d, 1H, $J = 8$ Hz, H-6), 6.80 (d, 1H, $J = 8$ Hz, H-5). ^{13}C NMR (100 MHz, CD_3OD): 170.4 (C-7), 151.5 (C-4), 146.0 (C-3), 123.9 (C-1), 123.3 (C-6), 117.7 (C-2), and 115.8 (C-5) (Nguyen et al., 2015).

Tricin 4'-*O*-(erythro- β -guaiacylglyceryl) ether 7''-*O*- β -D-glucopyranose (10): Yellow powder. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ_{H} (ppm): 12.86 (s, 1H, 5-OH), 7.33 (s, 2H, H-2', 6'), 7.04 (s, 1H, H-3), 6.56 (*br s*, 1H, H-8), 6.21 (*br s*, 1H, H-6), 3.88 (6H, s, 3', 5'- OCH_3), 3.75 (3H, s, 3''- OCH_3), 7.02 (d, $J = 2$, 1H, H-2''), 6.83 (*dd*, $J = 2$, 8 Hz, 1H, H-6''), 6.72 (d, $J = 8$ Hz, 1H, H-5''), 5.13 (d, $J = 4.4$, 1H, H-7''), 4.45 (m, 1H, H-8''), 3.67 (*dd*, 4.8, 11.6, 1H, H-9''a), 3.24 (*dd*, 6, 11.6, 1H, H-9''b), 4.38 (d, $J = 7.6$, 1H, H-1'''), 3.05–3.17 (m, H-2'''–H-5'''), 3.61 (m, 1H, H-6''a), 3.39 (m, 1H, H-6''b). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): δ_{C} (ppm): 163.0 (C-2), 104.9 (C-3), 181.8 (C-4), 161.4 (C-5), 99.2 (C-6), 165.0 (C-7), 94.5 (C-8), 157.5 (C-9), 103.7 (C-10), 125.6 (C-1'), 104.2 (C-2', 6'), 152.9 (C-3', 5'), 139.7 (C-4'), 129.6 (C-1''), 111.9 (C-2''), 114.6 (C-5''), 120.0 (C-6''), 146.8 (C-3''), 145.7 (C-4''), 78.2 (C-7''), 84.3 (C-8''), 60.5 (C-9''), 56.4 (3', 5'- O-CH_3), 55.7 (3''- O-CH_3), 102.0 (C-1'''), 74.1 (C-2'''), 77.3 (C-3'''), 70.0 (C-4'''), 76.5 (C-5'''), 60.9 (C-6''') [9].

Vitixin (11): Yellow powder. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ_{H} (ppm): 13.16 (s, 1H, 5-OH), 8.02 (d, $J = 8.4$ Hz, 2H, H-2', 6'), 6.89 (d, $J = 8.4$ Hz, 2H, H-3', 5'), 6.77 (s, 1H, H-3), 6.26 (s, 1H, H-6), 4.70 (d, $J = 10$ Hz, 1H, H-1''), 3.25–3.85 (remaining protons of glucose) [10]

Orientin (12): Yellow powder. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ_{H} (ppm): 13.17 (s, 1H, 5-OH), 7.48 (d, $J = 2.4$, 1H, H-2'), 7.53 (*dd*, $J = 2.4$, 8, 1H, H-6'), 6.86 (1H d, $J = 8$ Hz, H-5'), 6.64 (s, 1H,

H-3), 6.27 (*s*, 1H, H-6), 4.68 (*d*, $J = 10$ Hz, 1H, H-1''), 3.25–3.85 (remaining protons of glucose) [11]

Tricin 4'-*O*-(*threo*- β -guaiacylglyceryl) ether (14): Yellow solid. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ_{H} (ppm): 12.87 (*s*, 1H, 5-OH), 7.31 (*s*, 2H, H-2', 6'), 7.04 (*s*, 1H, H-3), 6.56 (*d*, $J = 2$ Hz, 1H, H-8), 6.21 (*d*, $J = 2$ Hz, 1H, H-6), 3.86 (6H, *s*, 3', 5'- OCH_3), 3.73 (3H, *s*, 3''- OCH_3), 6.97 (*d*, $J = 2$, 1H, H-2''), 6.80 (*dd*, $J = 2, 8$ Hz, 1H, H-6''), 6.69 (*d*, $J = 8$ Hz, 1H, H-5''), 4.84 (*d*, $J = 5.2$, 1H, H-7''), 4.25 (*m*, 1H, H-8''), 3.64 (*dd*, $J = 11.2, 4.4$, 1H, H-9''a), 3.25 (*dd*, $J = 11.6, 4.8$, 1H, H-9''b). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): δ_{C} (ppm): 163.1 (C-2), 104.8 (C-3), 181.9 (C-4), 161.4 (C-5), 99.0 (C-6), 164.6 (C-7), 94.4 (C-8), 157.4 (C-9), 103.8 (C-10), 125.3 (C-1'), 104.3 (C-2', 6'), 152.9 (C-3', 5'), 139.9 (C-4'), 133.0 (C-1''), 111.0 (C-2''), 114.7 (C-5''), 119.2 (C-6''), 146.9 (C-3''), 145.4 (C-4''), 71.6 (C-7''), 87 (C-8''), 60.4 (C-9''), 56.4 (3', 5'- O-CH_3), 55.6 (3''- O-CH_3) (Bouaziz et al., 2002).

Tricin 4'-*O*-(*erythro*- β -4-hydroxyphenylglyceryl) ether (calquiquelignan D) (15): Ivory powder. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ_{H} (ppm): 12.87 (*s*, 1H, 5-OH), 7.31 (*s*, 2H, H-2', 6'), 7.05 (*s*, 1H, H-3), 6.57 (*d*, $J = 2.4$ Hz, 1H, H-8), 6.22 (*d*, $J = 2.4$ Hz, 1H, H-6), 3.87 (6H, *s*, 3', 5'- OCH_3), 7.16 (*d*, $J = 8$ Hz, 2H, H-2'', 6''), 6.69 (*d*, $J = 8$ Hz, 2H, H-3'', 5''), 4.79 (*d*, $J = 5.2$ Hz, 1H, H-7''), 4.32 (*m*, 1H, H-8''), 3.72 (*dd*, $J = 6.4, 12$ Hz 1H, H-9''a), 3.49 (*dd*, $J = 3.6, 12$ Hz, 1H, H-9''b). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): δ_{C} (ppm): 163.6 (C-2), 105.3 (C-3), 182.3 (C-4), 161.9 (C-5), 99.4 (C-6), 164.9 (C-7), 94.8 (C-8), 157.9 (C-9), 104.3 (C-10), 125.7 (C-1'), 104.7 (C-2', 6'), 153.5 (C-3', 5'), 139.9 (C-4'), 133 (C-1''), 128.4 (C-2'', 6''), 114.9 (C-3'', 5''), 156.7 (C-4''), 72.4 (C-7''), 86.9 (C-8''), 60.6 (C-9''), 56.8 (3', 5'- O-CH_3) [13].

Tricin 4'-*O*-(*erythro*- β -guaiacylglyceryl) ether (16): Yellow powder. $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ_{H} (ppm): 12.87 (*s*, 1H, 5-OH), 7.31 (*s*, 2H, H-2', 6'), 7.04 (*s*, 1H, H-3), 6.56 (*d*, $J = 2.4$ Hz, 1H, H-8), 6.21 (*d*, $J = 2.4$ Hz, 1H, H-6), 3.88 (6H, *s*, 3', 5'- OCH_3), 3.74 (3H, *s*, 3''- OCH_3), 6.93 (*d*, $J = 2$, 1H, H-2''), 6.75 (*dd*, $J = 2, 8$ Hz, 1H, H-6''), 6.70 (*d*, $J = 8$ Hz, 1H, H-5''), 4.79 (*d*, $J = 4.8$, 1H, H-7''), 4.35 (*m*, 1H, H-8''), 3.73 (*m*, 1H, H-9''a), 3.50 (*m*, 1H, H-9''b). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): δ_{C} (ppm): 163.1 (C-2), 104.8 (C-3), 181.8 (C-4), 161.4 (C-5), 99.0 (C-6), 164.6 (C-7), 94.4 (C-8), 157.4 (C-9), 103.6 (C-10), 125.2 (C-1'), 104.3 (C-2', 6'), 153.0 (C-3', 5'), 139.5 (C-4'), 133.2 (C-1''), 111.0 (C-2''), 114.7 (C-5''), 119.4 (C-6''), 147.0 (C-3''), 145.4 (C-4''), 72.2 (C-7''), 86.5 (C-8''), 60.2 (C-9''), 56.4 (3', 5'- O-CH_3), 55.5 (3''- O-CH_3) (Bouaziz et al., 2002).

Tricin (17): Yellowish powder. ¹H-NMR (400 MHz, DMSO-*d*₆): δ_{H} (ppm): 12.96 (*s*, 1H, 5-OH), 10.80 (*s*, 1H, 7-OH), 9.32 (*s*, 1H, 4'-OH), 7.32 (*s*, 2H, H-2', 6'), 6.98 (*s*, 1H, H-3), 6.54 (*d*, *J* = 2.4 Hz, 1H, H-8), 6.20 (*d*, *J* = 2.4 Hz, 1H, H-6), 3.88 (6H, *s*, 3', 5'-OCH₃) [14].

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3S: High-resolution mass spectrometry analysis

Samples were run on Hypersil GOLD-C18 column (2.1×150 mm, $3.0 \mu\text{m}$) at a flow rate of 0.3 ml min^{-1} using 0.1% formic acid in water (A) and methanol (B) with the binary gradient conditions 5% B from 0 to 4 min, 5–100% B from 4 to 20 min, and 100% B from 20–22 min. HRMS data were obtained in positive or negative ionization modes using an H-ESI-Ion source with a static spray voltage of 2.5 kV negative ion and 3.5 kV positive ion. Sheath, aux, and sweep gases were applied at 50, 10, and 1 arbitrary units, respectively. The temperature of the ion transfer tube and the vaporizer were set at 325 and 350 °C, respectively. The Orbitrap mass resolution for small molecules (scan range m/z 100–1000) was set to 120 k. At the beginning of each run, EASY-IC mass calibration was used for mass shift correction. Data acquisition and analysis were performed using the Xcalibur v4.6.67.17 software (Thermo Scientific, Bremen, Germany).

4S: Molecular docking simulation

All the molecular modeling calculations and docking simulation studies were performed on a Processor Intel (R) Core (TM) i7-4510U CPU @ 2.00 GHz and 8 GB Memory with Microsoft Windows 10 (64 Bit) operating system using Molecular Operating Environment (MOE); Chemical Computing Group, Canada) as the computational software. The crystal structures of the selected kinases were retrieved from the Protein Data Bank (<https://www.rcsb.org>). All MOE minimizations were performed until a RMSD gradient of $0.01 \text{ Kcal/mol/\AA}$ with the force field (Amber10:EHT) to calculate the partial charges automatically using R-Field solvation. Before simulations, the proteins were protonated and the monomer was isolated. Triangle matching with London dG scoring was chosen for initial placement, then the top 30 poses were refined using induced fit protocol optimized with force field (Amber10:EHT) and GBVI/WSA dG scoring. The output database dock file was created with different poses for each ligand and arranged according to the final score function (S), which is the score of the last stage that was not set to zero

5S: Molecular dynamics simulation

The protein-ligand complexes of ERK2 and PI3K α with ligand **15**, were optimized with protein preparation wizard in Maestro (Desmond Schrödinger Release, 2025), by assigning bond orders, adding hydrogen, and correcting incorrect bond types. A default quick relaxation protocol was used to minimize the MD systems with the Desmond programme. In Desmond, the volume of space in which the simulation takes place, the global cell, is built up by regular 3D simulation

boxes, which was utilized as part of this system for protein interactions. The orthorhombic water box allowed for a 10 Å buffer region between protein atoms and box sides. Overlapping water molecules were deleted, and the systems were neutralized with Na⁺ ions and salt concentration 0.15 M. Force-field parameters for the complexes were assigned using the OPLS_2005 forcefield, that is a 200 ns molecular dynamic run in the NPT ensemble (T = 300 K) at a constant pressure of 1 bar. Energy and trajectory atomic coordinate data were recorded at each 1.2 ns

6S: Binding affinity

Prime/MMGBAS, available in Schrödinger Prime suite, was used to calculate the binding free energy of ligand **15** with ERK2 and PI3Kα

$$\Delta G(\text{bind}) = E_{\text{complex}}(\text{minimized}) - (E_{\text{ligand}}(\text{minimized}) + E_{\text{receptor}}(\text{minimized}))$$

ΔG (bind) values were calculated from each 10 frames of the final 100 ns (frames 500-1000) of the MD simulation. The average generated ΔG was calculated from each energy minimized frame using the equation shown above.

Table 1S: Docking results of compound **15** to nine protein kinases in comparison to the corresponding co-crystallized ligands

PK Target (PDB)	Ligand	Docking <i>S</i> score (Kcal/mol)	RMSD (Å)	HB contacts (Å)	Other contacts (Å)
EGFR (1M17)	Erlotinib	−8.3157	1.5612	Met769 (3.68)	Leu694 (pi-H) (4.27)
	15	−8.4297	1.6174	Met742 (3.32)	Leu820 (pi-H) (4.52) HOH10 (pi-H) (3.49)
VEGFR-2 (4ASD)	Sorafenib	−10.2105	0.5511	Cys919 (2.90) Ile1025 (3.76) Glu885 (2.92) Glu885 (3.36) Asp1046 (2.95) Cys919 (3.66)	Phe1047 (H-pi) (3.71)
	15	−8.4766	1.5674	Asp814 (2.91) Asp1046 (3.24)	Leu889 (pi-H) (3.58)
c-Kit (6GQJ)	F82	−12.7555	0.2027	Glu671 (3.24) Glu640 (2.74) Asp810 (2.99) HOH1179 (3.26) HOH1104 (2.95) Cys673 (3.06)	Phe811 (H-pi) 3.77 Leu595 (pi-H) 4.51 Leu595 (pi-H) 3.79 Leu644 (pi-H) 4.04
	15	−7.5938	1.5599	-----	Val603 (pi-H) 3.96
c-Met (3DKF)	SX8	−9.1621	0.2209	Asp1222 (3.14) Met1160 (3.07)	Met1211 (pi-H) 3.66 Tyr1230 (pi-pi) 3.99 Tyr1230 (pi-pi) 3.70
	15	−8.2957	1.5906	Met1211 (3.23)	-----
PI3K-α (5XGH)	84U	−7.0125	0.7332	Asp810 (2.89) Val851 (2.70)	Trp780 (pi-H) 4.95
	15	−7.5444	1.4691	Asp810 (3.07) Lys802 (3.16) Val851 if ligand PhO [−]	-----
MEK1 (3PP1) Allosteric site	IZG	−9.1367	0.7666	Val127 (3.51) Lys97 (3.17) Asn78 (3.17) Lys97 (2.88) Val211 (2.94)	-----

				Ser212 (3.07)	
	15	-8.1857	1.5162	HOH669 (6.38) Leu74 (16.41) Lys97 (4.28)	-----
MEK1 (3PP1) ATP site	ATP	-13.8641	0.5242	Met143 (3.81) Glu144 (2.83) Lys192 (2.63) Gly77 (3.26) Asn78 (2.94) Ser194 (2.82) Lys97 (3.27) Lys97 (2.79) Met146 (3.07)	MG410 (Metal), 1.82, 1.89, 1.90 Lys192 (Ionic) 2.63 MG410 (Ionic) 1.82, 1.89 Lys97 (Ionic) 3.53, 2.79 MG410 (Ionic) 1.90 Val82 (pi-H) 3.98
	15	-8.8638	1.1935	Met146 (3.03) Met143 (3.80) Gln153 (3.30) Ser194 (2.68)	MG410 (Metal) 2.03 Asn78 (pi-H) 3.80
Akt1 (4GV1)	0XZ	-11.2976	0.4339	HOH642 (2.87) Met281 (3.72) Glu234 (2.70) Met281 (3.47) HOH642 (2.98) Met281 (3.90) Glu228 (2.91) HOH958 (2.76) Ala230 (3.07)	Glu234 (Ionic) 2.70
	15	-7.3250	1.0797	Glu228 (3.51) Asp292 (3.34) HOH958 (2.74) HOH638 (2.66)	Val164 (pi-H) 4.32 Val164 (pi-H) 4.13
MAPK (ERK) (5K4I)	Ravoxert i-nib	-10.1718,	0.3532	Met108 (3.28) Met108 (2.84) Asp167 (2.64) Lys114 (2.83) Met108 (3.29) Lys54 (2.79) HOH531 (2.87) HOH529 (2.72)	Val39 (pi-H) 4.13 Thr110 (pi-H) 4.03
	15	-7.9857	1.3266	Met108 (2.76) Asp167 (3.03) Lys151 (3.36)	Ile31 (pi-H) 4.26 Val39 (pi-H) 4.30
CDK2 (2C6O)	4SP	-9.5734	0.3599	Asp86 (3.39) Asp86 (2.80) Glu81 (2.86) Leu83 (2.80)	-----

				HOH2060 (2.88) Leu83 (3.61) Gln85 (3.27) Asp86 (3.05) Lys89 (3.40) Lys89 (2.90)	
	15	-7.7265	1.0211	Gln131 (2.77) Thr14 (2.84) Ls129 (3.28)	-----

F82: 2-[4-(6,7-dimethoxyquinazolin-4-yl)oxy-2-methoxy-phenyl]~{N}-(1-propan-2-ylpyrazol-4-yl)ethanamide

SX8: 6- {[6-(1-methyl-1H-pyrazol-4-yl)[1,2,4]triazolo[4,3-b]pyridazin-3-yl]sulfanyl}quinoline.

84U: 3-[(4-fluorophenyl)methylamino]-5-(4-morpholin-4-ylthieno[3,2-d]pyrimidin-2-yl)phenol.

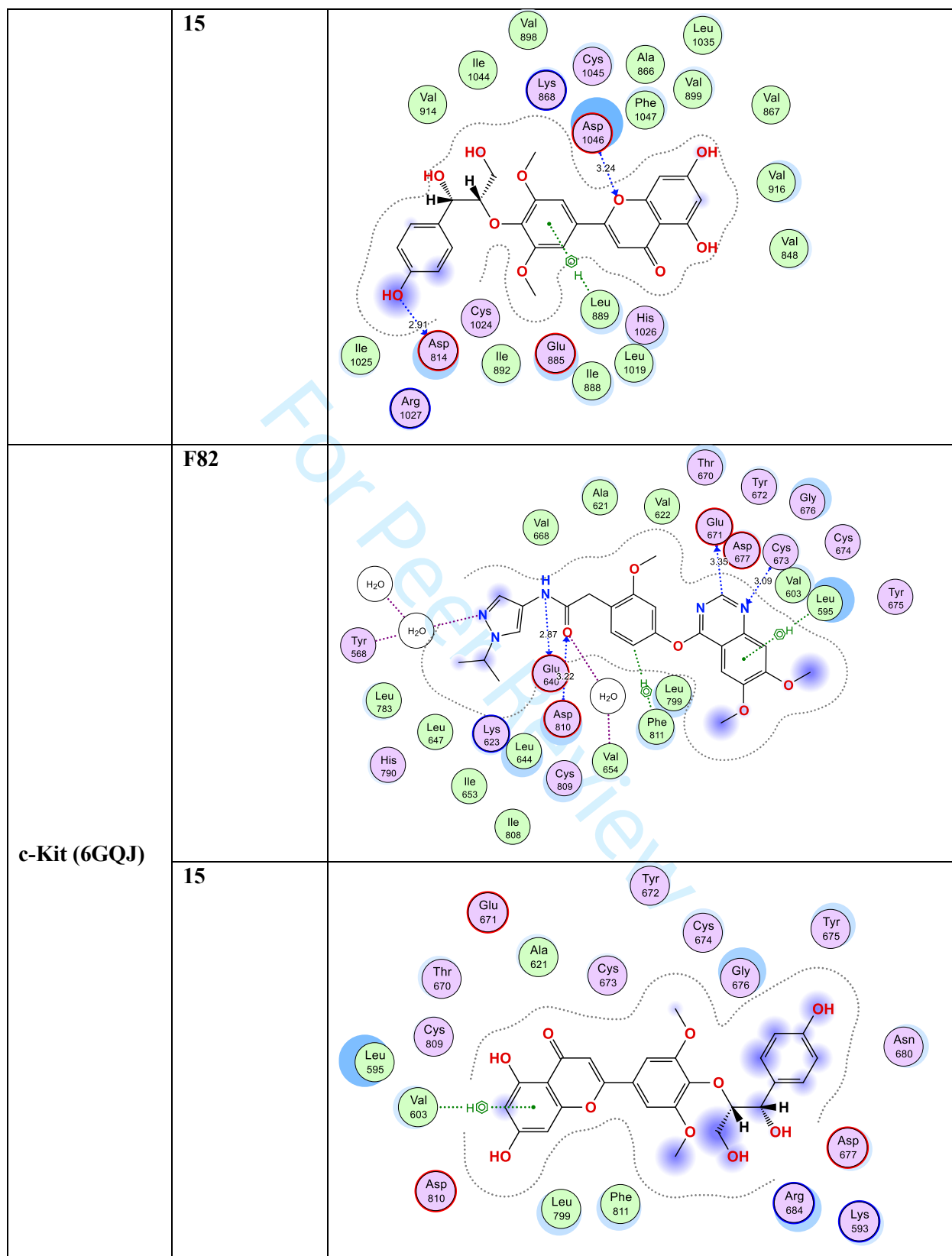
IZG: 3-[(2R)-2,3-dihydroxypropyl]-6-fluoro-5-[(2-fluoro-4-iodophenyl)amino]-8-methylpyrido[2,3-d]pyrimidine-4,7(3H,8H)-dione.

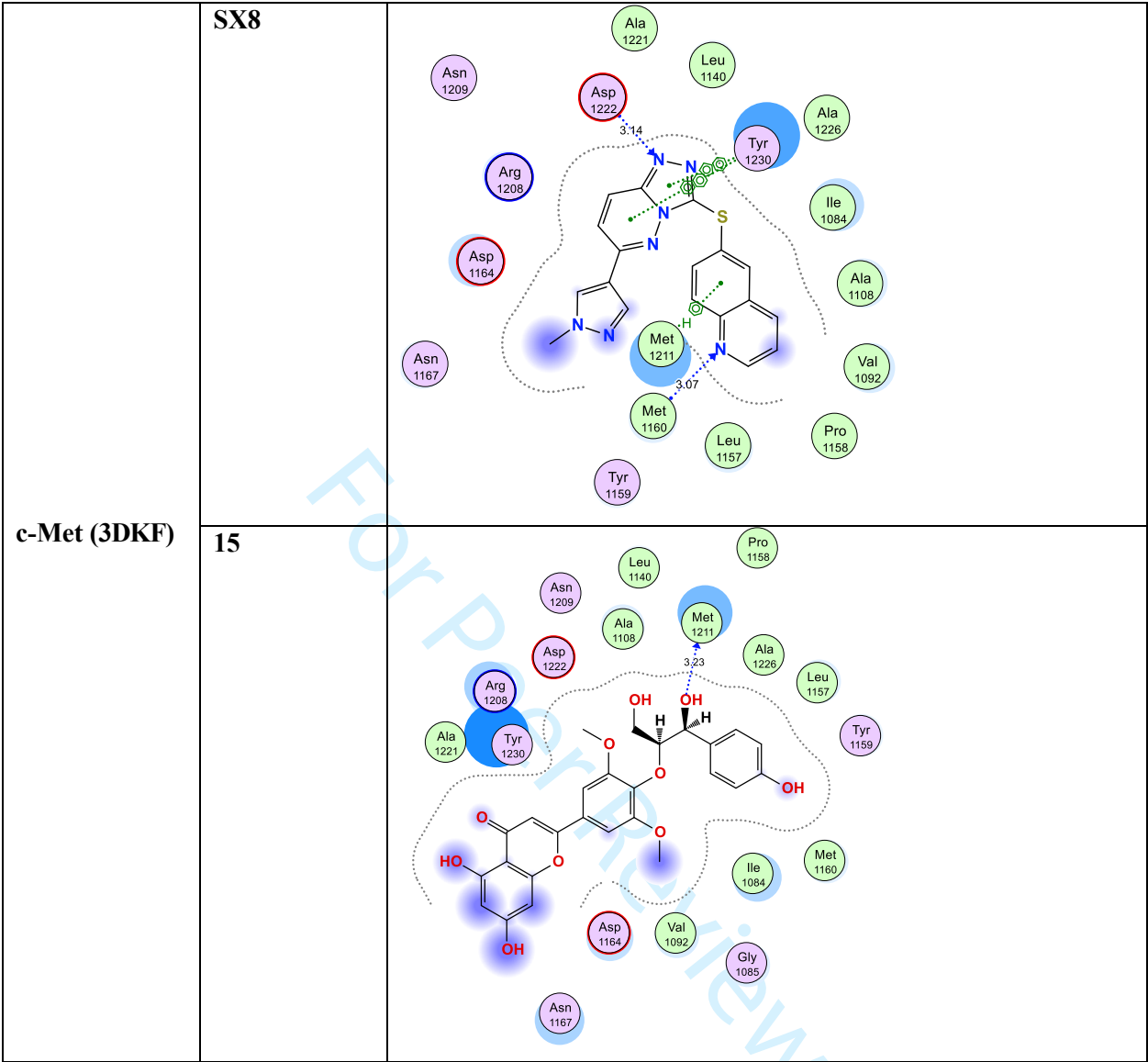
0XZ: 4-amino-N-[(1S)-1-(4-chlorophenyl)-3-hydroxypropyl]-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide.

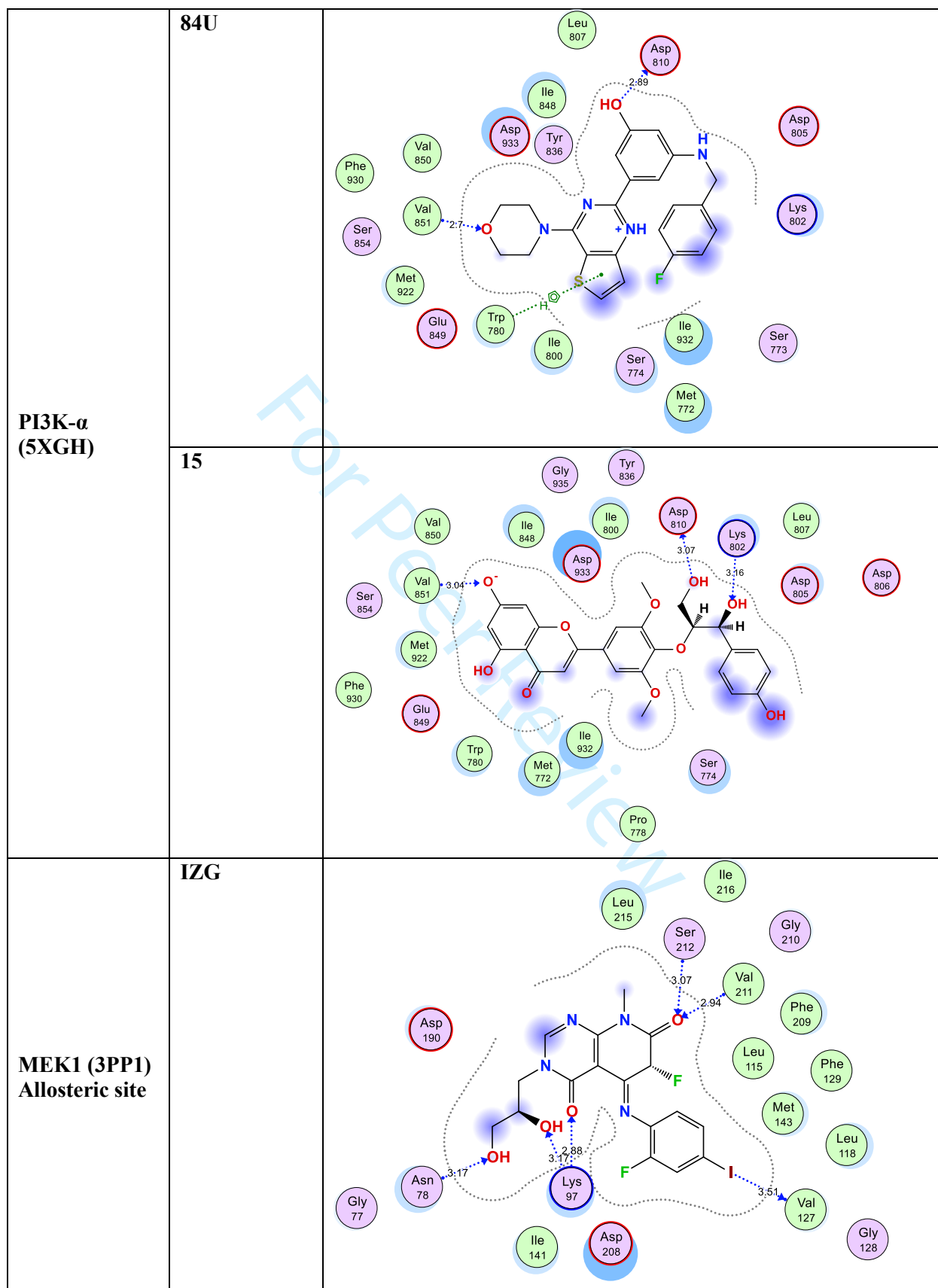
4SP: O6-Cyclohexylmethoxy-2-(4'-sulphamoylanilino)purine.

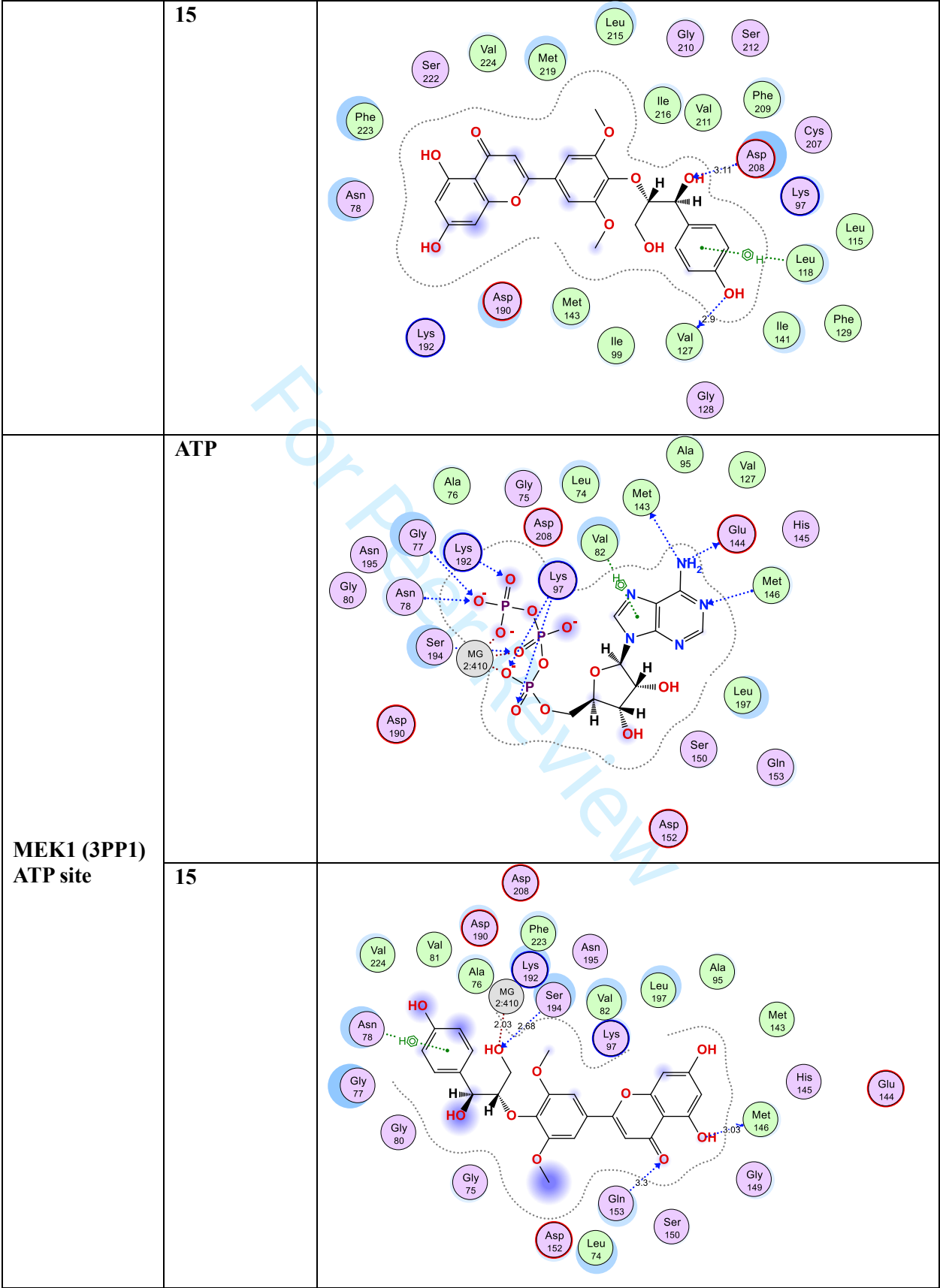
Table 2S: 2D docked models of compound **15** to nine protein kinases in comparison to the corresponding co-crystallized ligands

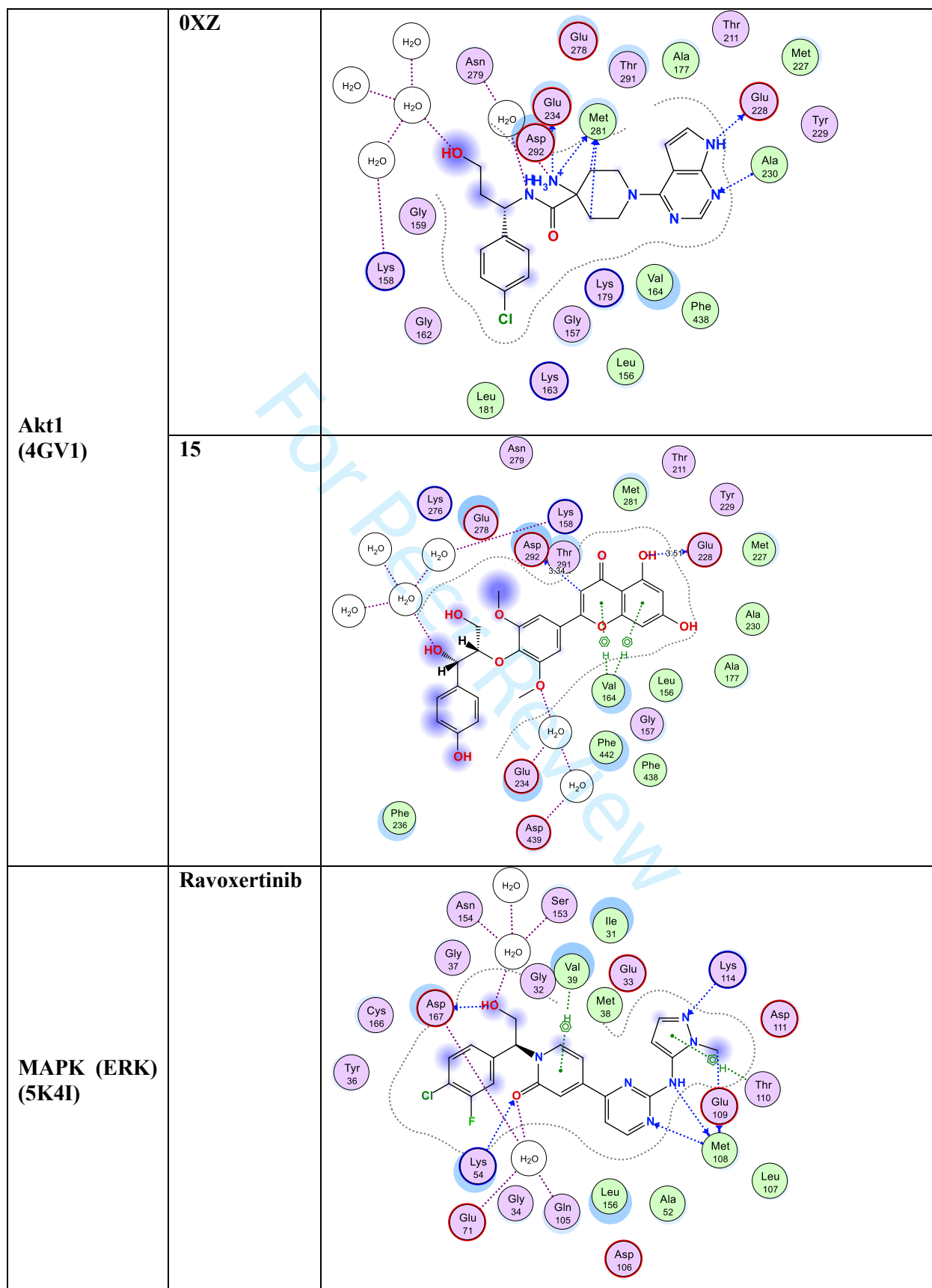
PK Target (PDB)	Ligand	2D model
EGFR (1M17)	Erlotinib	
	15	
VEGFR-2 (4ASD)	Sorafenib	

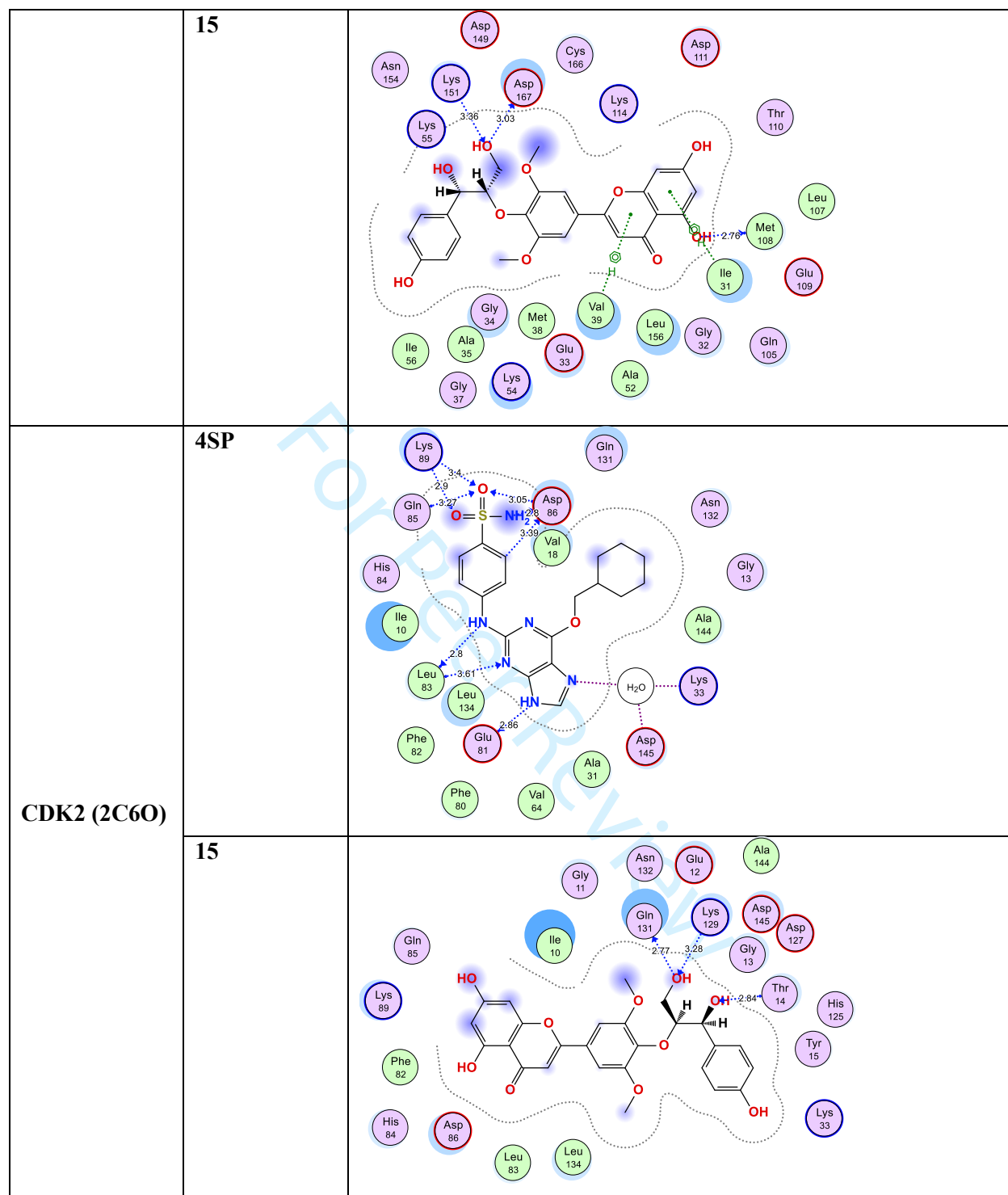












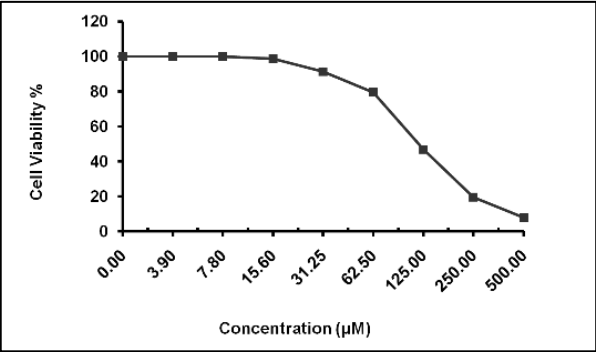
F82: 2-[4-(6,7-dimethoxyquinazolin-4-yl)oxy-2-methoxy-phenyl]~{N}-(1-propan-2-ylpyrazol-4-yl)ethanamide
SX8: 6- {[6-(1-methyl-1H-pyrazol-4-yl)[1,2,4]triazolo[4,3-b]pyridazin-3-yl]sulfanyl}quinoline.
84U: 3-[(4-fluorophenyl)methylamino]-5-(4-morpholin-4-ylthieno[3,2-d]pyrimidin-2-yl)phenol.
IZG: 3-[(2R)-2,3-dihydroxypropyl]-6-fluoro-5-[(2-fluoro-4-iodophenyl)amino]-8-methylpyrido[2,3-d]pyrimidine-4,7(3H,8H)-dione.

0XZ: 4-amino-N-[(1S)-1-(4-chlorophenyl)-3-hydroxypropyl]-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide.

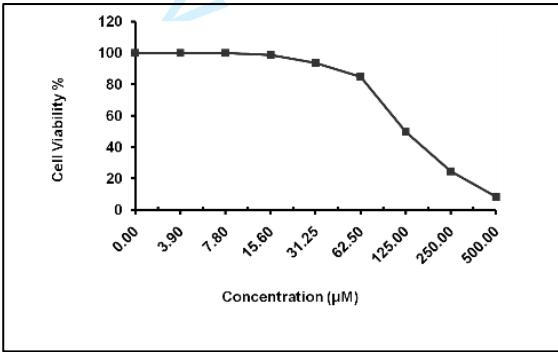
4SP: O6-Cyclohexylmethoxy-2-(4'-sulphamoylanilino)purine.

Table 3S: Concentration-response curves and CC₅₀ determinations for cytotoxicity evaluation against A431, PC3, and HFB4 cells.

A431		
Compound 7		
Sample conc. (μM)	Viability %	Inhibitory %
500	7.89	92.11
250	19.65	80.35
125	46.81	53.19
62.5	79.52	20.48
31.25	91.34	8.66
15.6	98.87	1.13
7.8	100	0
3.9	100	0
0	100	0



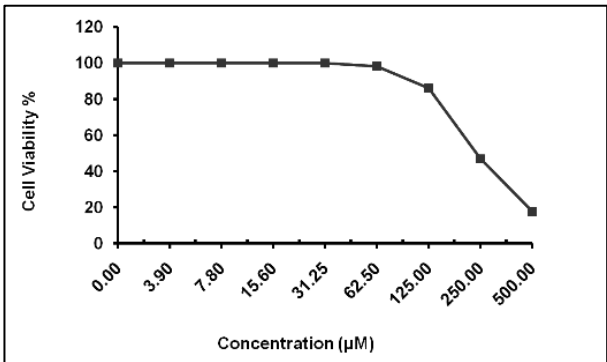
CC₅₀ (μM) [95% CI] = 118.90 [113.1–125.0]

Compound 10		
Sample conc. (μM)	Viability %	Inhibitory %
500	8.42	91.58
250	24.59	75.41
125	49.67	50.33
62.5	84.93	15.07
31.25	93.81	6.19
15.6	98.76	1.24
7.8	100	0
3.9	100	0
0	100	0


CC₅₀ (μM) [95% CI] = 124.42 [114.8–134.9]

Compound 13

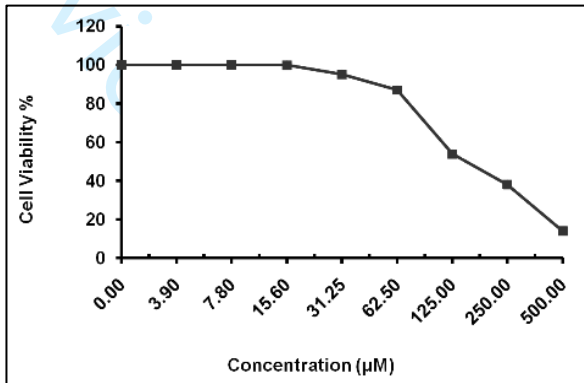
Sample conc. (µM)	Viability %	Inhibitory %
500	17.84	82.16
250	47.05	52.95
125	86.23	13.77
62.5	98.19	1.81
31.25	100	0
15.6	100	0
7.8	100	0
3.9	100	0
0	100	0



CC₅₀ (µM) [95% CI] = 240.59 [227.2–254.8]

Compound 14

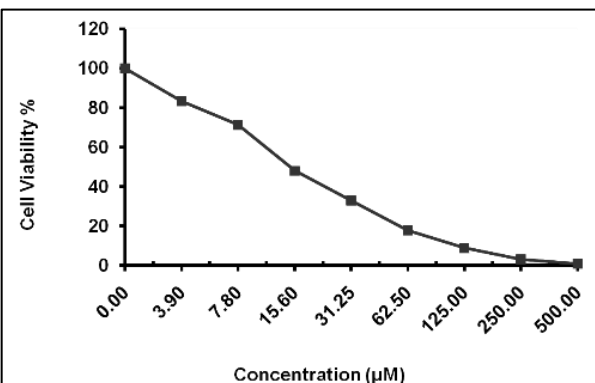
Sample conc. (µM)	Viability %	Inhibitory %
500	13.95	86.05
250	38.16	61.84
125	53.89	46.11
62.5	87.04	12.96
31.25	95.12	4.88
15.6	99.93	0.07
7.8	100	0
3.9	100	0
0	100	0



CC₅₀ (µM) [95% CI] = 155.91 [133.1–182.6]

Compound 15

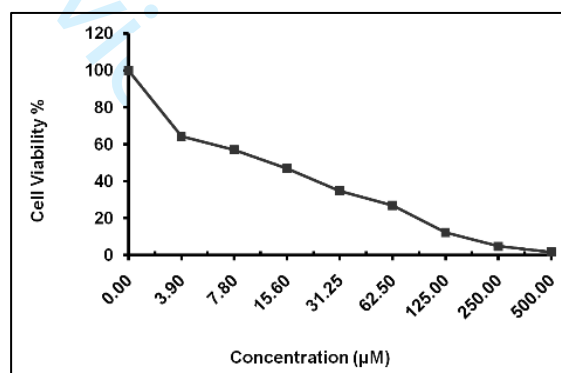
Sample conc. (μM)	Viability %	Inhibitory %
500	0.97	99.03
250	3.12	96.88
125	8.95	91.05
62.5	17.84	82.16
31.25	32.95	67.05
15.6	48.06	51.94
7.8	71.34	28.66
3.9	83.42	16.58
0	100	0



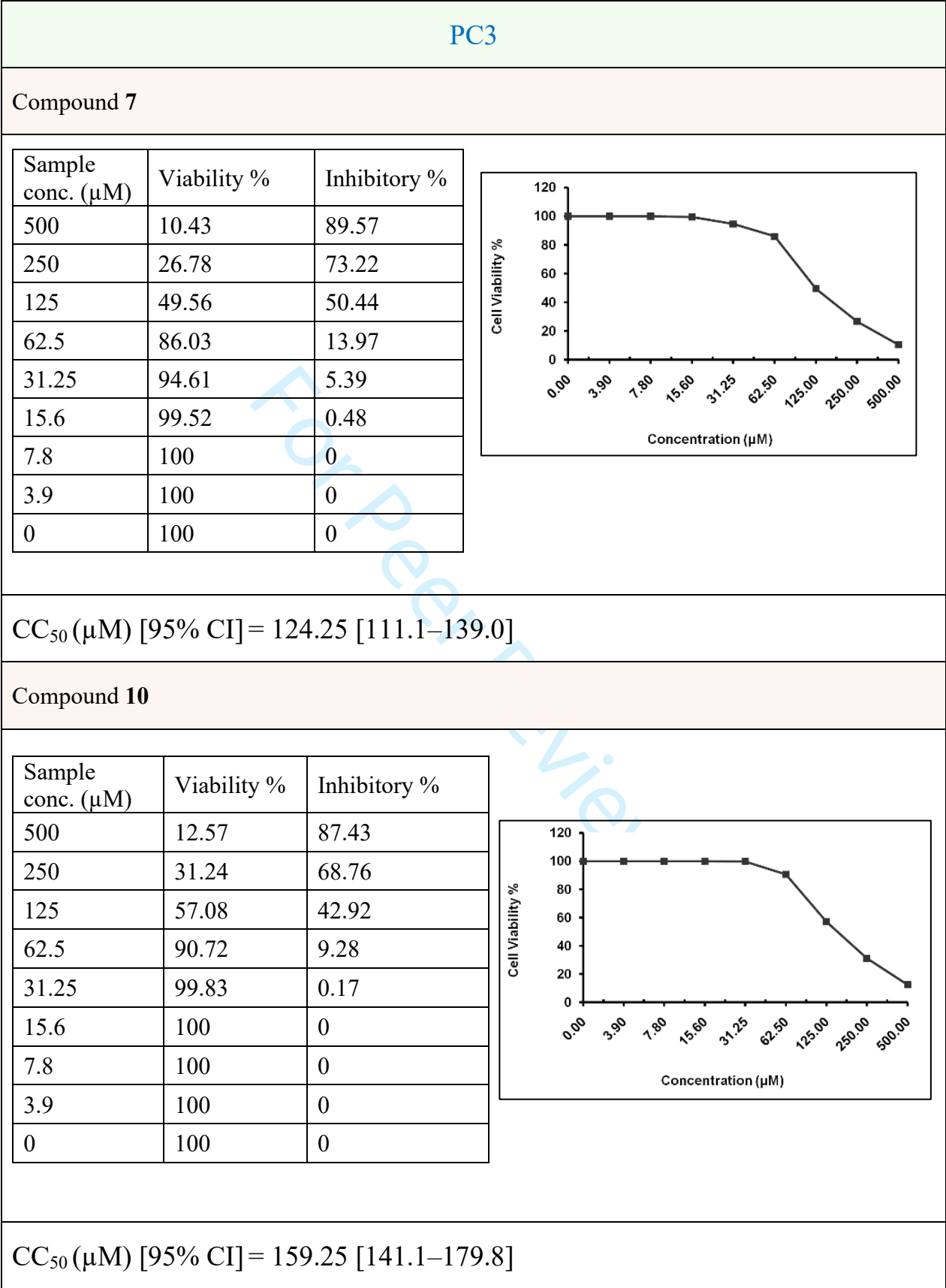
CC_{50} (μM) [95% CI] = 14.95 [13.9–16.1]

Cisplatin

Sample conc. (μM)	Viability %	Inhibitory %
500	1.65	98.35
250	4.82	95.18
125	12.37	87.63
62.5	26.86	73.14
31.25	34.85	65.15
15.6	46.91	53.09
7.8	57.08	42.92
3.9	64.25	35.75
0	100	0

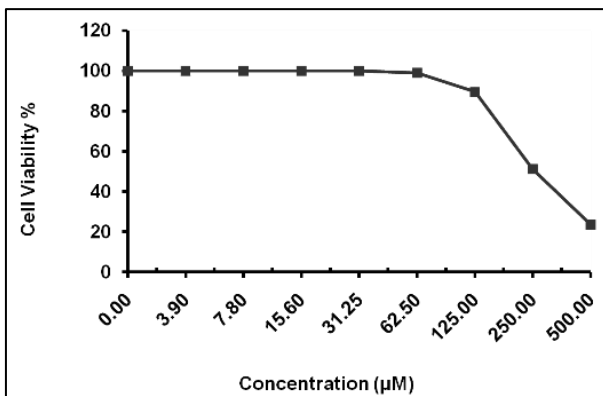


CC_{50} (μM) [95% CI] = 13.23 [10.0–17.5]



Compound 13

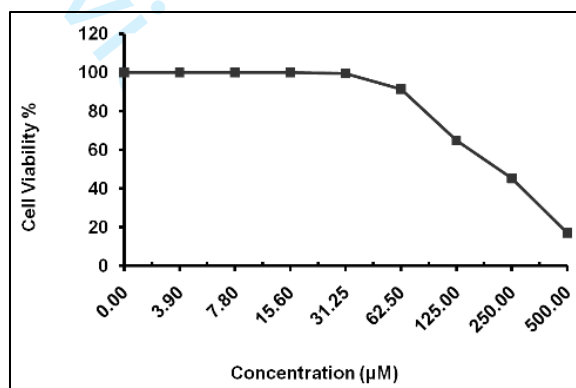
Sample conc. (μM)	Viability %	Inhibitory %
500	23.65	76.35
250	51.34	48.66
125	89.57	10.43
62.5	99.04	0.96
31.25	100	0
15.6	100	0
7.8	100	0
3.9	100	0
0	100	0



CC_{50} (μM) [95% CI] = 262.10 [238.7–287.8]

Compound 14

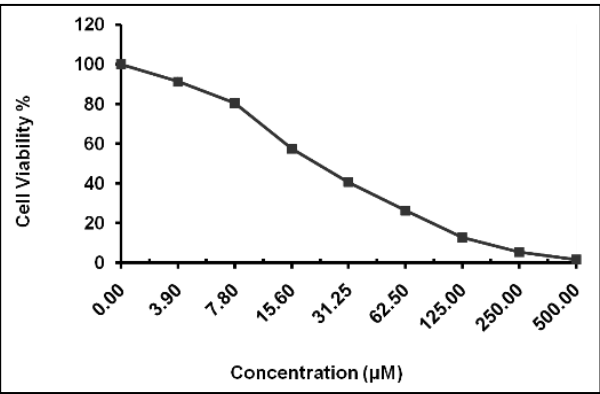
Sample conc. (μM)	Viability %	Inhibitory %
500	17.08	82.92
250	45.31	54.69
125	64.92	35.08
62.5	91.48	8.52
31.25	99.57	0.43
15.6	100	0
7.8	100	0
3.9	100	0
0	100	0



CC_{50} (μM) [95% CI] = 220.10 [194.2–249.4]

Compound 15

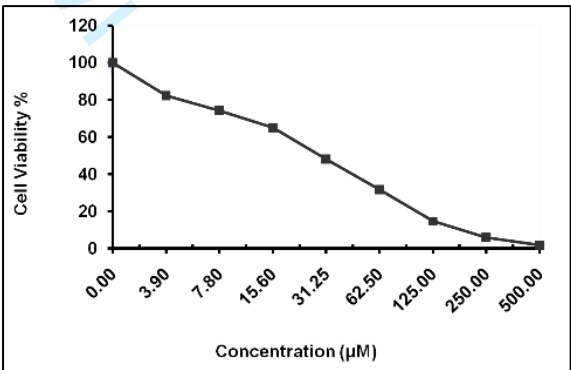
Sample conc. (μM)	Viability %	Inhibitory %
500	1.63	98.37
250	5.49	94.51
125	12.76	87.24
62.5	26.31	73.69
31.25	40.69	59.31
15.6	57.42	42.58
7.8	80.65	19.35
3.9	91.34	8.66
0	100	0



CC₅₀ (μM) [95% CI] = 22.52 [20.1–25.2]

Cisplatin

Sample conc. (μM)	Viability %	Inhibitory %
500	1.78	98.22
250	6.03	93.97
125	14.69	85.31
62.5	31.75	68.25
31.25	48.13	51.87
15.6	65.09	34.91
7.8	74.21	25.79
3.9	82.38	17.62
0	100	0

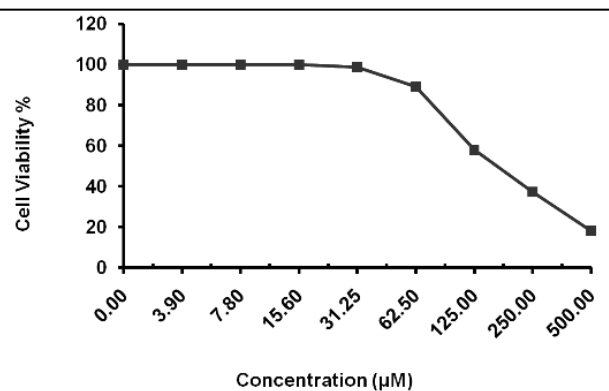


CC₅₀ (μM) [95% CI] = 29.48 [24.6–35.3]

HFB4

Compound 7

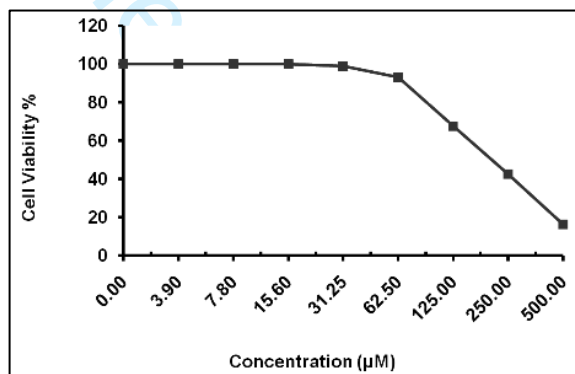
Sample conc. (μM)	Viability %	Inhibitory %
500	18.07	81.93
250	37.41	62.59
125	58.04	41.96
62.5	89.21	10.79
31.25	98.70	1.3
15.6	100	0
7.8	100	0
3.9	100	0
0	100	0



CC_{50} (μM) [95% CI] = 173.72 [150.0–201.2]

Compound 10

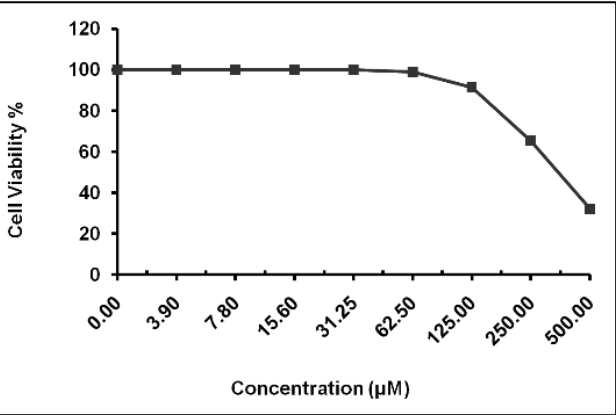
Sample conc. (μM)	Viability %	Inhibitory %
500	16.29	83.71
250	42.71	57.29
125	67.56	32.44
62.5	93.18	6.82
31.25	99.06	0.94
15.6	100	0
7.8	100	0
3.9	100	0
0	100	0



CC_{50} (μM) [95% CI] = 213.33 [195.5–232.8]

Compound 13

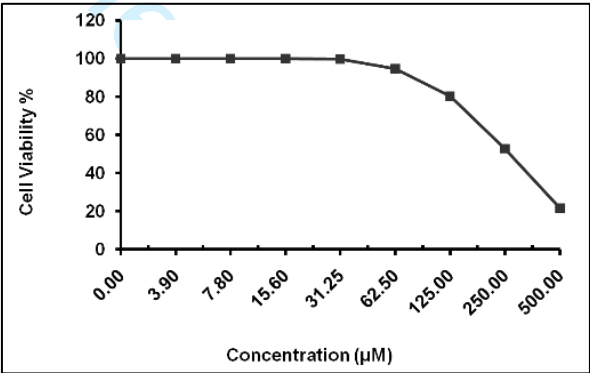
Sample conc. (µM)	Viability %	Inhibitory %
500	32.18	67.82
250	65.46	34.54
125	91.35	8.65
62.5	98.97	1.03
31.25	100	0
15.6	100	0
7.8	100	0
3.9	100	0
0	100	0



CC₅₀ (µM) [95% CI] = 366.14 [352.2–380.7]

Compound 14

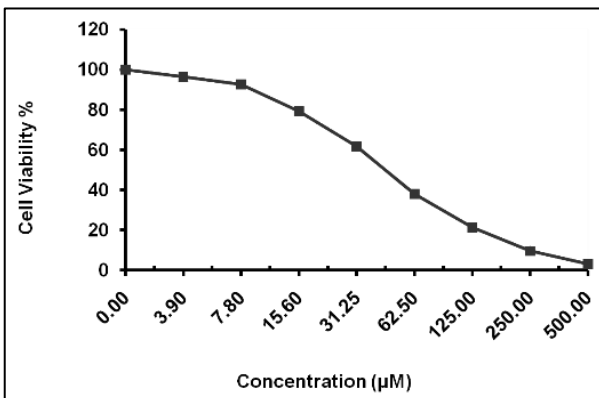
Sample conc. (µM)	Viability %	Inhibitory %
500	21.53	78.47
250	52.84	47.16
125	80.31	19.69
62.5	94.68	5.32
31.25	99.73	0.27
15.6	100	0
7.8	100	0
3.9	100	0
0	100	0



CC₅₀ (µM) [95% CI] = 272.68 [266.0–279.5]

Compound 15

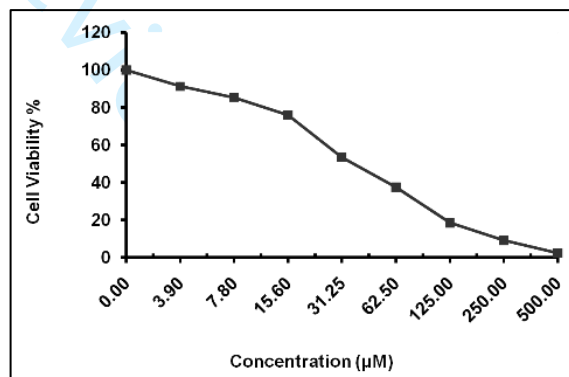
Sample conc. (μM)	Viability %	Inhibitory %
500	3.06	96.94
250	9.62	90.38
125	21.39	78.61
62.5	38.04	61.96
31.25	61.78	38.22
15.6	79.23	20.77
7.8	92.71	7.29
3.9	96.45	3.55
0	100	0



CC_{50} (μM) [95% CI] = 46.76 [44.7–49.0]

Cisplatin

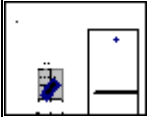
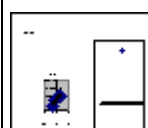
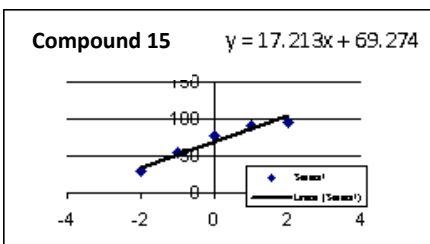
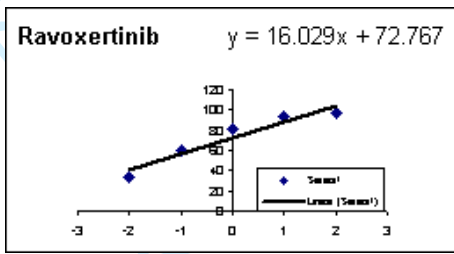
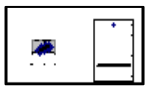
Sample conc. (μM)	Viability %	Inhibitory %
500	2.47	97.53
250	9.26	90.74
125	18.54	81.46
62.5	37.61	62.39
31.25	53.48	46.52
15.6	76.03	23.97
7.8	85.49	14.51
3.9	91.34	8.66
0	100	0

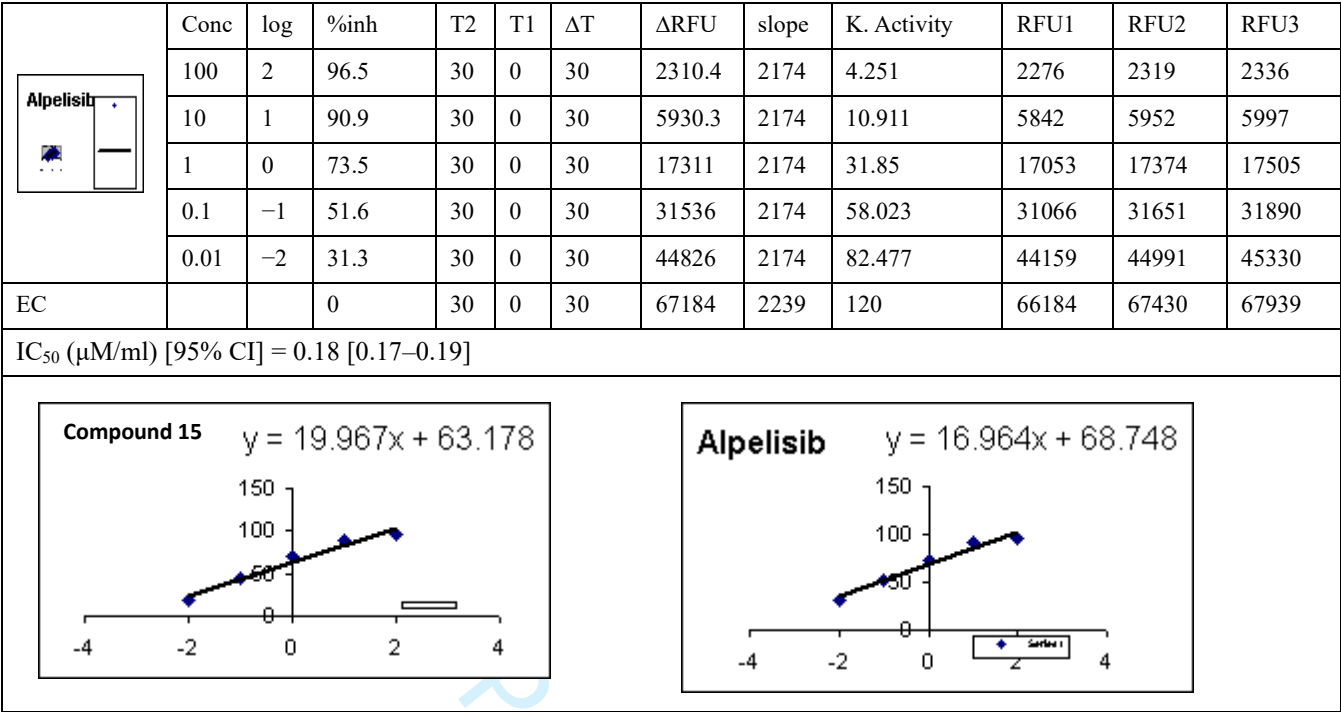


CC_{50} (μM) [95% CI] = 38.19 [34.8–41.9]

Table 4S: Inhibitory activity of compound **15** against ERK1, ERK2, and PI3K α kinases (0.01–100 μ M). Ravoxertinib and Alpelisib were used as reference inhibitors for ERK1/ERK2 and PI3K α , respectively. Concentration-response curves are shown beneath each dataset. EC = enzyme control (0% inhibition baseline).

ERK1												
Compound 15												
	Conc	log	% inh	T2	T1	Δ T	Δ RFU	slope	K. Activity	RFU1	RFU2	RFU3
	100	2	94.9	30	0	30	2964.6	1930	6.14435	2961	2929	3004
	10	1	83.5	30	0	30	9538.7	1930	19.7694	9527	9425	9664
	1	0	62.2	30	0	30	21889	1930	45.3656	21862	21629	22176
	0.1	-1	42	30	0	30	33566	1930	69.5674	33525	33167	34007
	0.01	-2	22.7	30	0	30	44784	1930	92.8168	44729	44251	45372
EC			0	30	0	30	57888	1930	120	57817	57200	58648
IC ₅₀ (μ M/ml) [95% CI] = 0.51 [0.49–0.53]												
Ravoxertinib												
	Conc	log	%inh	T2	T1	Δ T	Δ RFU	slope	K. Activity	RFU1	RFU2	RFU3
	100	2	97	30	0	30	1755.2	1930	3.63763	1753	1734	1778
	10	1	91.6	30	0	30	4872	1930	10.0974	4866	4814	4936
	1	0	76	30	0	30	13889	1930	28.7857	13872	13724	14071
	0.1	-1	61.1	30	0	30	22497	1930	46.6252	22469	22229	22792
	0.01	-2	37.4	30	0	30	36227	1930	75.081	36182	35796	36702
EC			0	30	0	30	57888	1930	120	57817	57200	58648
IC ₅₀ (μ M/ml) [95% CI] = 0.06 [0.058–0.063]												
Compound 15 $y = 18.595x + 61.056$ Ravoxertinib $y = 14.951x + 72.629$												

ERK2												
Compound 15												
	Conc	log	%inh	T2	T1	ΔT	ΔRFU	slope	K. Activity	RFU1	RFU2	RFU3
	100	2	96.5	30	0	30	2135.6	2046	4.17522	2133	2110	2164
	10	1	90.3	30	0	30	5979.4	2046	11.6898	5972	5908	6058
	1	0	77.2	30	0	30	13979	2046	27.3298	13962	13813	14163
	0.1	-1	53.6	30	0	30	28506	2046	55.7303	28471	28167	28880
	0.01	-2	28.8	30	0	30	43699	2046	85.4326	43645	43179	44272
EC			0	30	0	30	61388	2046	120	61312	60657	62193
IC ₅₀ (μM/ml) [95% CI] = 0.15 [0.14–0.16]												
Ravoxertinib												
	Conc	log	%inh	T2	T1	ΔT	ΔRFU	slope	K. Activity	RFU1	RFU2	RFU3
	100	2	97	30	0	30	1843.3	2046	3.60365	1841	1821	1867
	10	1	92.7	30	0	30	4470.5	2046	8.73998	4465	4417	4529
	1	0	80.5	30	0	30	11951	2046	23.364	11936	11809	12108
	0.1	-1	60.8	30	0	30	24089	2046	47.0941	24059	23802	24405
	0.01	-2	32.8	30	0	30	41226	2046	80.5977	41175	40735	41767
EC			0	30	0	30	61388	2046	120	61312	60657	62193
IC ₅₀ (μM/ml) [95% CI] = 0.08 [0.077–0.083]												
 												
PI3Kα												
Compound 15												
	Conc	log	%inh	T2	T1	ΔT	ΔRFU	slope	K. Activity	RFU1	RFU2	RFU3
	100	2	95.8	30	0	30	2760.1	2174	5.0784	2719	2770	2791
	10	1	88	30	0	30	7837.7	2174	14.421	7721	7866	7926
	1	0	70.3	30	0	30	19341	2174	35.586	19053	19412	19558
	0.1	-1	43.7	30	0	30	36697	2174	67.52	36151	36832	37109
	0.01	-2	18.1	30	0	30	53442	2174	98.329	52646	53637	54042
EC			0	30	0	30	65223	2174	120	64252	65462	65955
IC ₅₀ (μM/ml) [95% CI] = 0.44 [0.43–0.46]												
Alpelisib												



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