# Selective AKR1C3 inhibitors potentiate chemotherapeutic activity in multiple acute myeloid leukemia (AML) cell lines

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**Figure S1. Cytotoxicity of baccharin (1) at 10 $\mu$M concentration in the NCI-60 cancer cell line assay.**

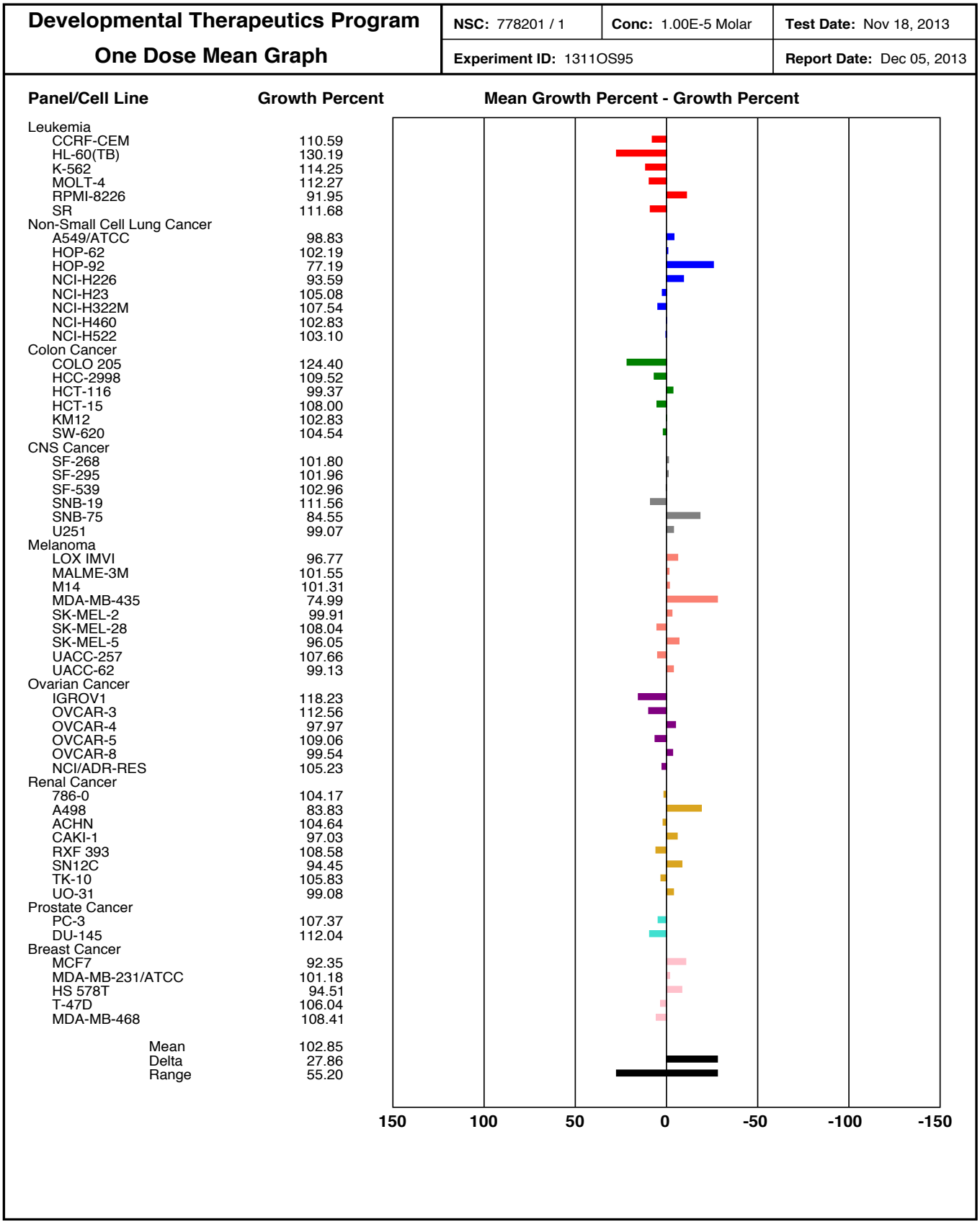


**Figure S2. Baccharin and derivative AKR1C3 inhibitors (2 and 3) do not exhibit cytotoxicity towards human APL HL-60 cells for up to 100 *μ*M concentration at 72 hours.** (A) The natural product baccharin (**1**) exhibits only 20% cytotoxic effect at 300 *μ*M concentration and no effect at 10 *μ*M concentration. (B) *para*-amide derivative (**2**) shows no cytotoxic effect at concentrations up to 100 *μ*M. (C) *meta*-ester derivative (**3**) shows no cytotoxic effect at concentrations up to 100 *μ*M. (D) *meta*-amide derivative (**4**) shows 88% cytotoxic effect at 50 *μ*M but negligible effect at <10 *μ*M. Values are the mean ± S.D, for 3 independent experiments (n = 6). (Statistics: The two-tailed t-test analysis was used to compare the statistical difference between control and treatments, ns – not significant, *** p<0.001, **** p<0.0001).

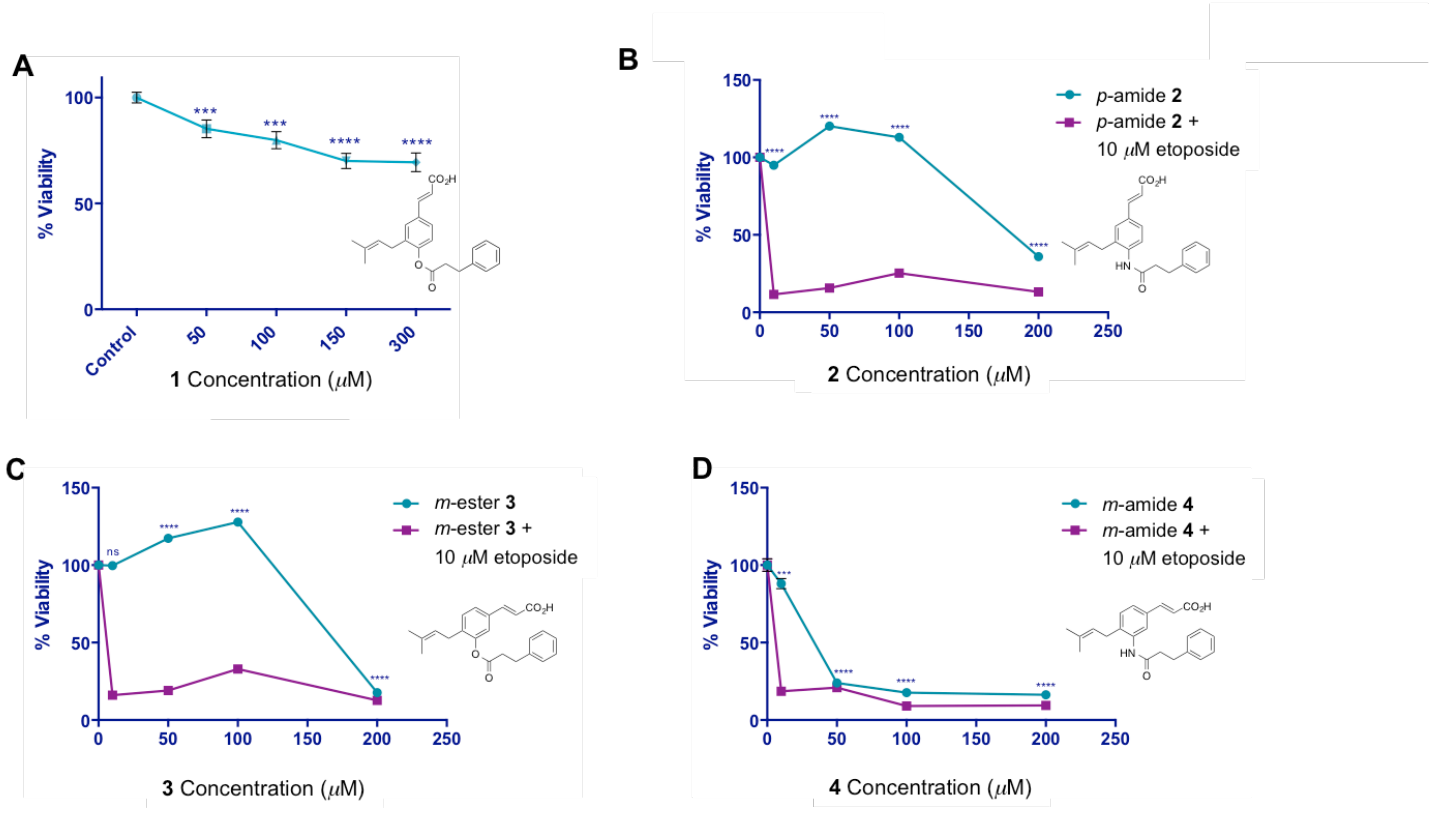


**Figure S3. Expression of AKR1C3 in native HL-60 and KG1a cells with transient upregulation of AKR1C3 in inhibitor (4) treated cells.** (A) AKR1C3 expression of untreated cells and expression upon inhibitor treatment after 48 and 72 hr in HL-60 cells. (B) KG1a cells show considerably higher expression of AKR1C3 as compared to HL-60 cells.

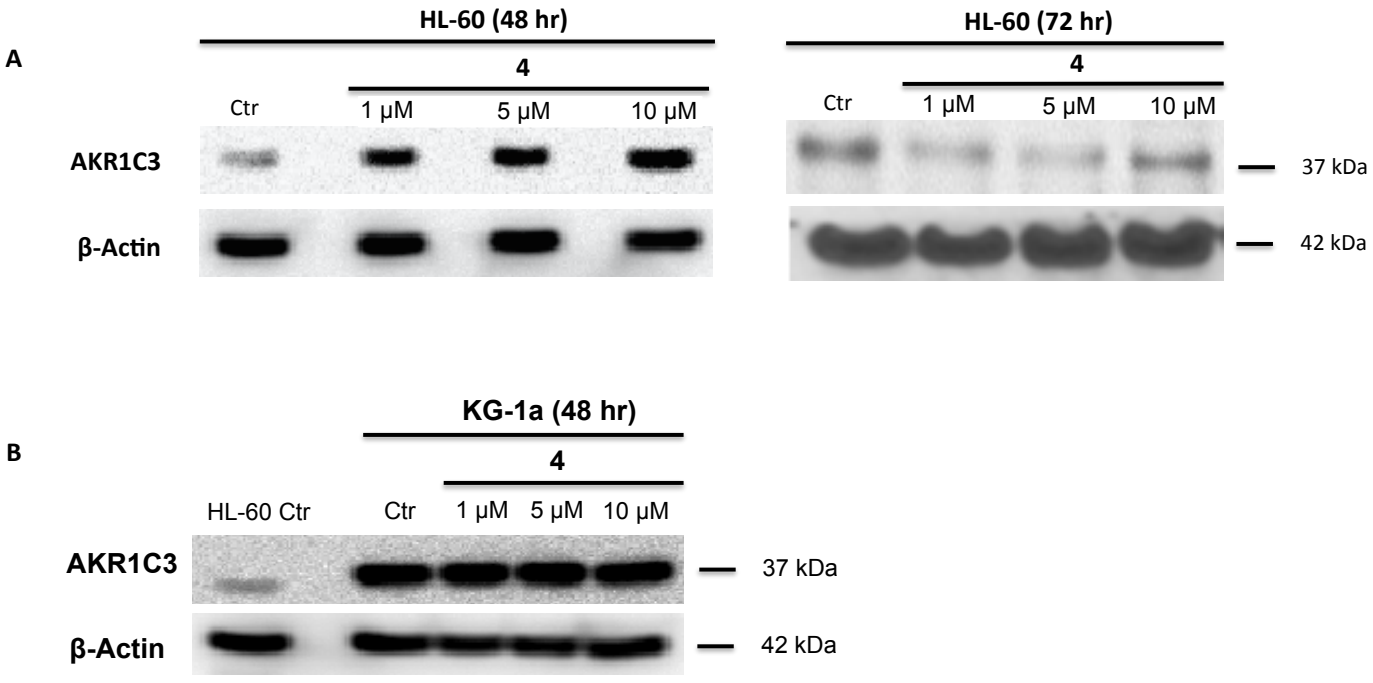


**Figure S4. Baccharin and derivative AKR1C3 inhibitors (2 and 3) do not exhibit cytotoxicity up to 25 *μ*M concentration towards human AML KG1a cells at 72 hours.** (A) The natural product baccharin (**1**) exhibits 28% cytotoxic effect at 50 *μ*M concentration and 4.5% cytotoxic effect at 10 *μ*M concentration. (B) *para*-amide derivative (**2**) shows 15.5% cytotoxic effect at 50 *μ*M concentration and no cytotoxicity at 10 *μ*M concentration. (C) *meta*-ester derivative (**3**) shows 30 % cytotoxic effect at 50 *μ*M concentration and 10% cytotoxic effect at 10 *μ*M concentration. (D) *meta*-amide derivative (**4**) shows 67% cytotoxic effect at 50 *μ*M concentration and 12% cytotoxic effect at 10 *μ*M concentration. Values are the mean ± S.D, for 3 independent experiments (n = 6). (Statistics: The two-tailed t-test analysis was used to compare the statistical difference between control and treatments ns – not significant, ** p<0.01, *** p<0.001, **** p<0.0001).

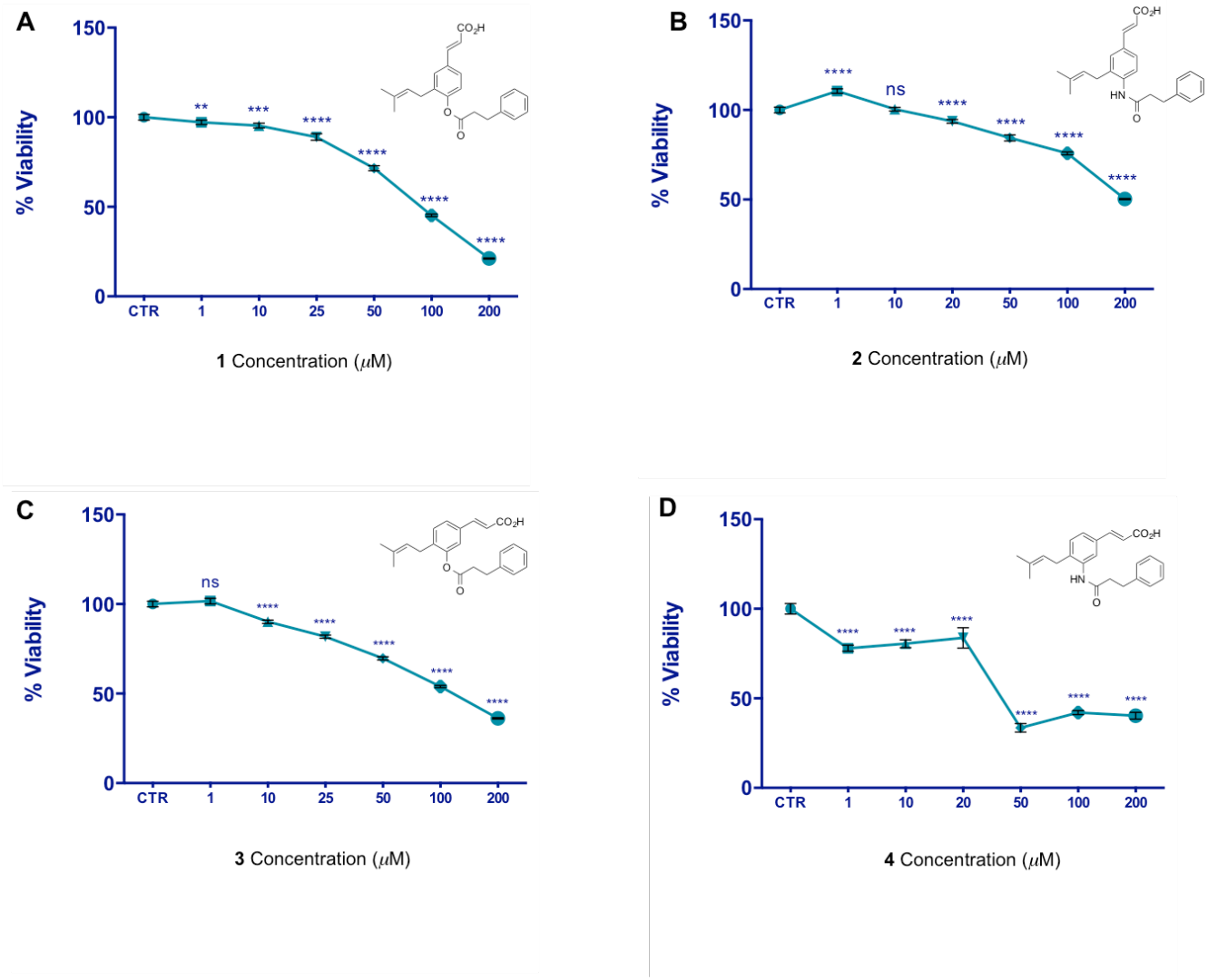


**Figure S5. Dose-response curve of the cytotoxic effect of etoposide in APL HL-60 cells after 72 hours**. Expressed as percentage viable cells as measured by MTS cell viability reagent. Values are the Mean ± S.D, for 3 independent experiments (n = 6). (Statistics: The two-tailed t-test analysis was used to compare the statistical difference between control and treatments, ns - not significant, **** p<0.0001).

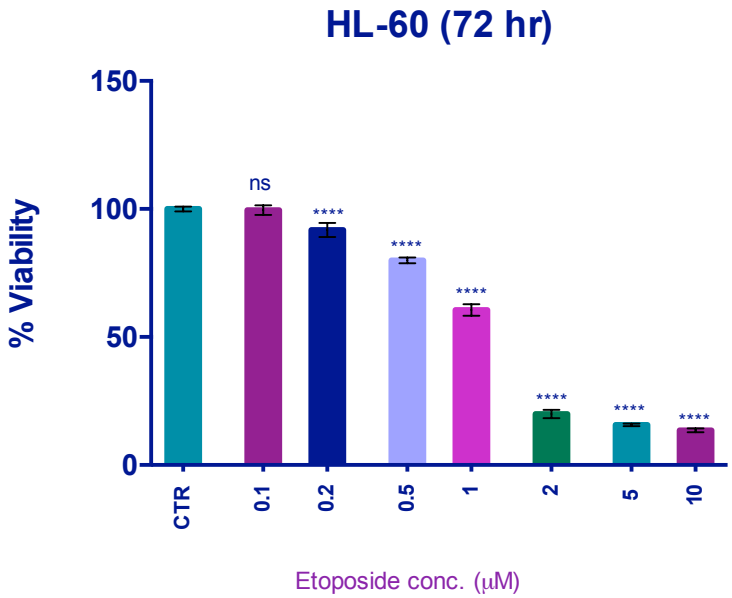


**Figure S6. Dose-response curve of the cytotoxic effect of etoposide in AML KG1a cells after 72 hours.** Expressed as percentage viable cells as measured by MTS cell viability reagent. Values are the Mean ± S.D, for 3 independent experiments (n = 6). (Statistics: The two-tailed t-test analysis was used to compare the statistical difference between control and treatments, **** p<0.0001).

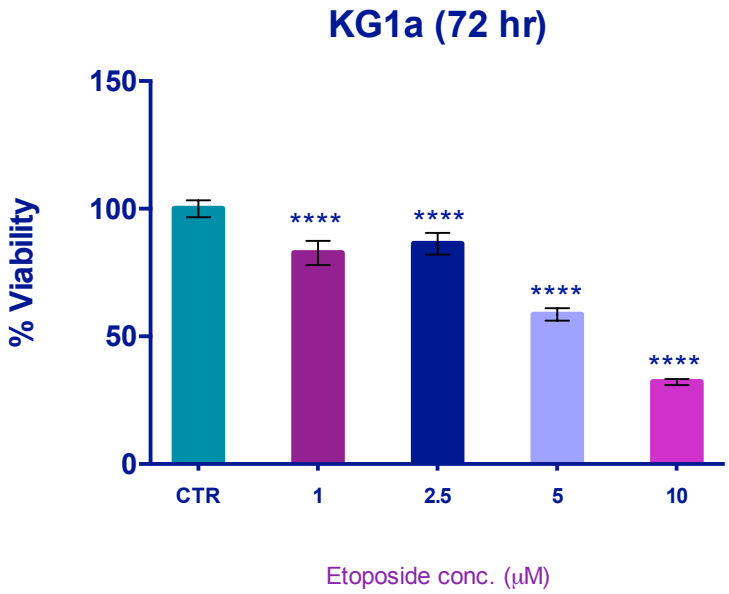


**Figure S7. Drupanin (1a) does not show a synergistic action with etoposide in HL-60 cells.** (A) The low activity AKR1C3 inhibitor drupanin, **1a** ($pIC_{50}$ = 4.8) provides no potentiation of the activity of etoposide beyond an additive effect in HL-60 cells up to 72 hr treatment. Values are the Mean ± S.D, for 3 independent experiments (n = 6). (B) Statistical analysis shows no statistical difference between the observed responses of co-treating HL-60 cells with drupanin and etoposide at 1$\mu$M against the additive effect of individual agents observed at the same concentration (p = 0.8512). (Statistics: The two-tailed t-test analysis was used to compare the statistical difference between control and treatments *** p<0.001, **** p<0.0001, ns – not significant)

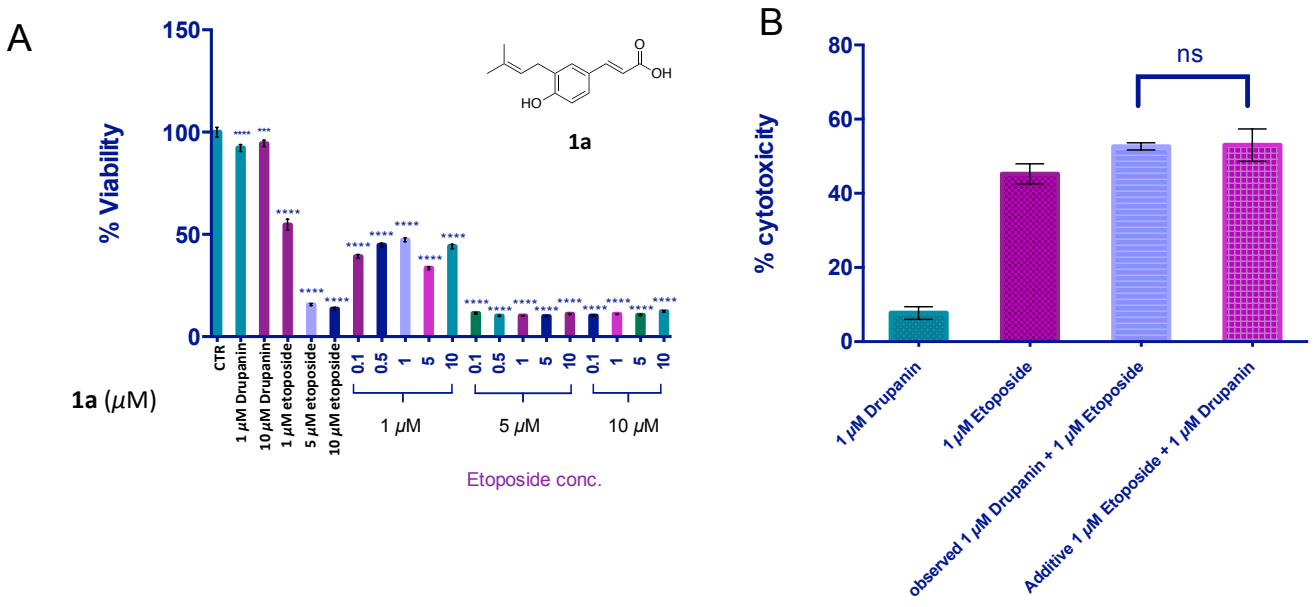
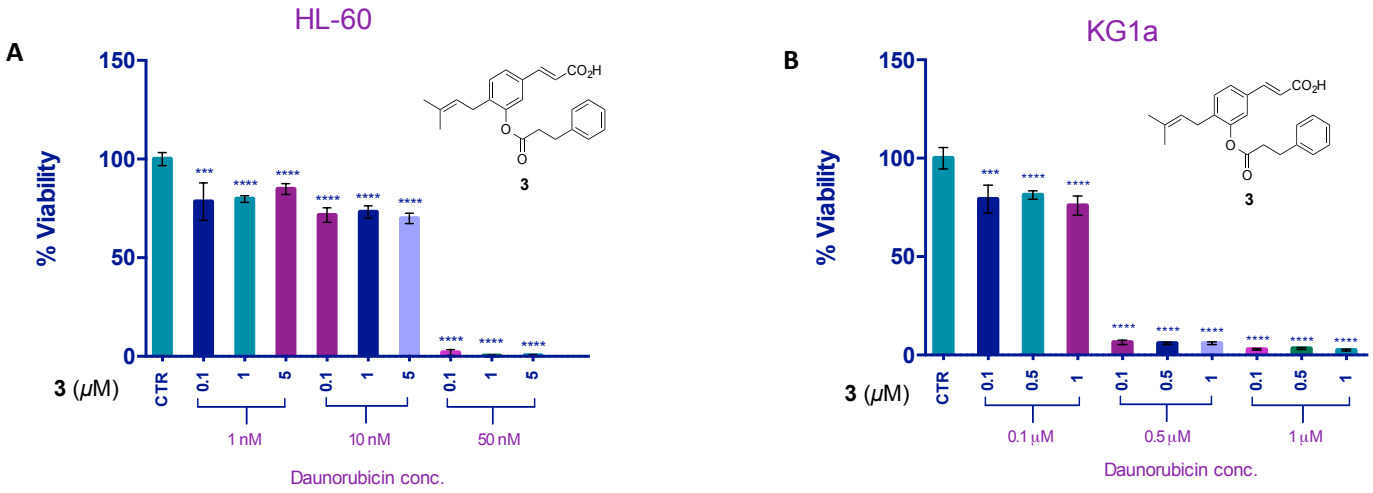


**Figure S8. Synergistic action of AKR1C3 inhibitor 3 with daunorubicin in AML cell lines.** Pre-treatment of compound **3** for 24 hr followed by daunorubicin treatment and incubation for a further 72 hr demonstrates a synergistic drug effect in HL-60 (A) and KG1a (B) cells. C) Quantification of the degree of synergism in AML cell lines.

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| Compound | AKR1C3 $pIC_{50}$ | HL-60 | | | KG1a | | |
|---|---|---|---|---|---|---|---|
| | | CI[a] | DRI[b] | $IC_{50}$* (nM) | CI[a] | DRI[b] | $IC_{50}$* (μM) |
| **3** | 7.1 | 0.09 | 9.98 | 4.16 | 0.08 | 10.3 | 0.17 |
| Daunorubicin | N/A | N/A | N/A | 41.5 | N/A | N/A | 1.77 |

*Calculated for etoposide + AKR1C3 inhibitor
[a]Combination index
[b]Dose reduction index
N/A; Not Applicable

**Methods:**

**Inhibitor screening against AKR1C3 and AKR1C2 assay.**[21] The inhibitory potencies of baccharin derivatives were determined by measuring their inhibition of the $NADP^+$ dependent oxidation of *S*-tetralol catalyzed by purified recombinant AKR1C3 and AKR1C2.[30] The $IC_{50}$ value of each compound was acquired from a single experiment with each inhibitor concentration ran in quadruplicate and directly calculated by fitting the inhibition data to an equation [y = (range) / [1 + (I/$IC_{50}$)S] + background] using Grafit 5.0 software. In this equation, "y" is the initial rate, "range" is the fitted uninhibited value minus the "background", and "S" is a slope factor, and "I" is the concentration of inhibitor. The equation assumes that y falls with increasing "I". The initial rate was obtained by monitoring the formation of NADPH for the first 5 min using a fluorescence plate reader (Synergy 2, BioTek) at 37 ºC (Ex: 340 nm; Em: 460 nm). The reaction solution (200 $\mu$L in each well) was composed of potassium phosphate buffer (100 mM, pH 7), *S*-tetralol (in DMSO), inhibitors (in DMSO), 4% DMSO (total) and enzyme solution (95 nM for AKR1C3 and 86 nM for AKR1C2). The concentration of *S*-tetralol used in this assay for AKR1C3 and AKR1C2 was 165 $\mu$M and 15 $\mu$M respectively, which was equal to the $K_m$ value for each enzyme isoform in order to make a direct comparison of $IC_{50}$ values.

**Adjuvant assay.** HL-60 (ATCC® CCL-240™) and KG1a (ATCC® CCL-246.1™) cells were procured from ATCC and cultured using Isocove's Modified Dulbecco's Media (IMDM) supplemented with 20% Fetal Bovine Serum (FBS), Penicillin/Streptomycin (1%) and maintained at a density of 0.1 – 1 x $10^6$ cells/mL under 5% $CO_2$ at 37$^O$C. To screen the test compounds, cells were seeded at a density of 0.1 x $10^6$ cells/mL in 96 well plates containing 100 $\mu$L cell suspension per well. Stock solutions of the test compounds and etoposide were prepared in DMSO. Cells were treated at the indicated concentrations of test compounds with or without etoposide, limiting the final DMSO concentration to less than 1%. After incubation at 37$^O$C, 5% $CO_2$ for 24, 48 or 72 hr, 20 $\mu$L of MTS reagent (CellTiter

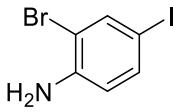
96® AQueous One Solution Reagent) was added to each well and incubated at the above mentioned conditions for 3-4 hr. Plates were read at OD 490 nm on a plate reader and the viability of cells were plotted as percentage of controls.

**Western blotting.** HL-60 and KG1a cells were treated with compound (**4**) over a period of 48 hr at indicated concentrations after which they were harvested and pelleted. The whole cell lysates was prepared in RIPA buffer containing protease and phosphatase inhibitors (1 mM PMSF, 38 µg/ml aprotinin, 2.5 mM $Na_3VO_4$). Samples were incubated on ice for 30 min after which they were sonicated, centrifuged (16000 x g) and supernatant collected. Protein concentration in each sample was estimated following BCA assay protocol by comparing with the BSA standards (Pierce™ BCA protein kit). 40 µg of protein samples containing loading dye (7 µL) were loaded onto 12 % SDS polyacrylamide gel and electrophoresed (80 V, 2 hr). Transferred onto a PVDF membrane overnight (25 V, $4^0$C). Membrane was blocked with 5 % non-fat milk (1 hr) and probed with human anti-AKR1C3 mouse monoclonal antibody (1:500, R&D Systems, MAB7678) and corresponding HRP conjugated anti-mouse secondary antibody followed by immunodetection using VersaDoc™ (MP 5000). Membrane was stripped and re-probed for β-actin (1:5000, Sigma-Aldrich, A5441). Quantity One® software was used to analyze the band intensities and fold change in AKR1C3 enzyme expression was determined based on β-actin controls.

**General chemistry procedures.** All reactions were carried out in oven- or flame-dried glassware under positive nitrogen pressure unless otherwise noted. Reaction progress was monitored by thin-layer chromatography (TLC) carried out on silica gel plates (2.5 cm x 7.5 cm, 200 μm thick, 60 F254) and visualized by using UV (254 nm) or by potassium permanganate and/or phosphomolybdic acid solution as indictor. Flash column chromatography was performed with silica gel (40-63 μm, 60 Å)

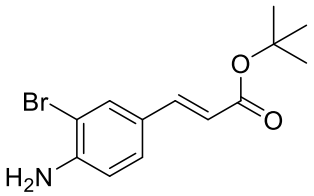
using the mobile phase indicated or on a Teledyne Isco (CombiFlash $R_f$ 200 UV/Vis). Commercial grade solvents and reagents were purchased from Fisher Scientific (Houston, TX), Sigma Aldrich (Milwaukee, WI) or for Prenyl boronic acid pinacol ester, Santa Cruz Biotechnology (Dallas, TX) and were used without further purification except as indicated. Anhydrous solvents were purchased from Across Organics and stored under an atmosphere of dry nitrogen over molecular sieves. $^{1}H$, $^{13}C$, COSY, HMQC and DEPT NMR spectra were recorded in the indicated solvent on a Bruker 400 MHz Advance III HD spectrometer at 400 and 100 MHz for $^{1}H$ and $^{13}C$ respectively with TMS as an internal standard. Multiplicities are indicated by s (single), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), br (broad). Chemical shifts ($\delta$) are reported in parts per million (ppm), and coupling constants (*J*), in hertz. High-resolution mass spectroscopy was performed on a LC/MS IT-TOF (Shimadzu) using an ESI source conducted at the University of Texas at Arlington, Shimadzu Center for Advanced Analytical Chemistry. High-pressure liquid chromatography was performed on a Dynamax HPLC system installed with a Varian pro star UV detector with a Phenomenex® Luna (C18 100A, 250x4.6 mm) column. All samples were assessed to be of >96% purity.

## Experimental



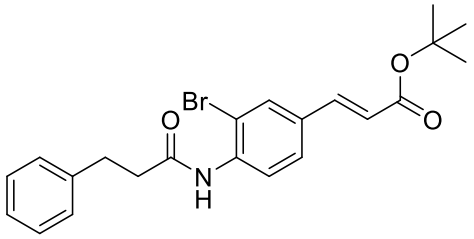

*2-bromo-4-iodoaniline (**6**):* To a solution of 4-iodoaniline (1.1 g, 5.0 mmol), in 6 mL of HOAc, was added a solution of $Br_2$ (250 µL, 4.8 mmol) and the mixture stirred overnight at room temperature. $Et_2O$ was added to the reaction mixture and was washed with brine and saturated aqueous $NaHCO_3$, the organic layer separated and the aqueous layer extracted with $Et_2O$, the combined organic layers were dried ($Na_2SO_4$), filtered and concentrated. Purification by column chromatography (Hexane:EtOAc = 9:1, 4:1, 2:1) provided the title compound as a brown oil (570 mg, 1.9 mmol, 44% based on recovered starting material).

**$R_f$:** 0.46 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; $CDCl_3$):** δ 6.54 (1H, d, *J* = 8.2 Hz, Ar-H), 7.37 (1H, d, *J* = 8.4 Hz, Ar-H), 7.70 (1H, s, Ar-H). **$^{13}$C NMR (100 MHz; $CDCl_3$):** δ 117.3, 131.1, 134.4, 136.9, 139.9, 143.8. **HRMS-ESI:** (*m/z*) calculated for $C_6H_5NBrI$, 297.8723 $[M+H]^+$; found, 297.8709.



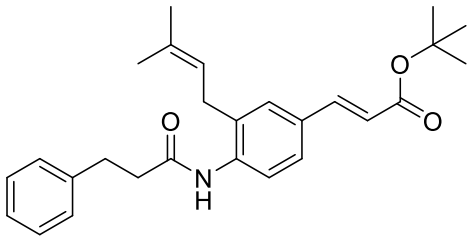

*tert-butyl (2E)-3-(4-amino-3-bromophenyl)prop-2-enoate (**7**):* To a solution of (**6**) (570 mg, 1.9 mmol) in dry toluene (8 mL), was added $PPh_3$ (65.5 mg, 0.2 mmol) and $Pd(OAc)_2$ (47.5%, 60 mg, 0.1 mmol). *tert*-Butyl Acrylate (370 µL, 2.5 mmol) and $NEt_3$ (420 µL, 3.0 mmol) were added and the flask was stirred at reflux overnight. The reaction was allowed to cool and washed with saturated aqueous $NH_4Cl$, brine, extracted with DCM, dried ($Na_2SO_4$), filtered and concentrated. Purification by column chromatography (Hexane:EtOAc = 12:1, 9:1, 4:1) provided the title compound as a brown oil (238.5 mg, 0.8 mmol, 64% based on recovered starting material).

**$R_f$** : 0.23 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; $CDCl_3$,):** δ 1.51 (9H, s, $C(CH_3)_3$), 6.15 (1H, d, *J* = 15.8 Hz, CH), 6.69 (1H, d, *J* = 8.1 Hz, Ar-H), 7.23 (1H, d, *J* = 8.2 Hz, Ar-H), 7.40 (1H, d, *J* = 15.8 Hz, CH), 7.55 (1H, s, Ar-H) **$^{13}$C NMR (100 MHz; $CDCl_3$,):** δ 28.2, 80.2, 109.0, 115.3, 117.0, 126.0, 128.4, 132.4, 142.3, 145.7, 166.6. **HRMS-ESI:** (*m/z*) calculated for $C_{13}H_{16}NO_2Br$, 296.0292 $[M-H]^-$; found, 296.0290.



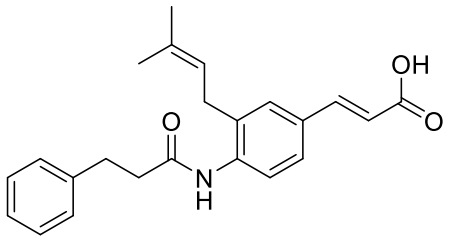

*tert-butyl (2E)-3-[3-bromo-4-(3-phenylpropanamido)phenyl]prop-2-enoate (**8**):* To a solution of (**7**) (290 mg, 1.0 mmol) in dry DCM (5 mL) was added DMAP (13 mg, 0.1 mmol). A solution of $PhCH_2CH_2COCl$ (255 mg, 1.5 mmol) in DCM (2 mL) was added to the mixture followed by addition of $NEt_3$ (420 µL, 3.0 mmol). The solution was heated to $70^0$C and stirred overnight. The reaction was allowed to cool and washed with a saturated aqueous $NaHCO_3$, water and extracted with DCM, dried ($Na_2SO_4$), filtered and concentrated. Purification by column chromatography (Hexane:EtOAc = 12:1, 9:1, 4:1, 2:1) provided the title compound as a brown oil (40 mg, 0.1 mmol, 10%).

**$R_f$:** 0.51 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; $CDCl_3$):** δ 1.54 (9H, s, $C(CH_3)_3$), 2.78 (2H, t, *J* = 7.6 Hz, $CH_2$), 3.09 (2H, t, *J* = 7.6 Hz, $CH_2$), 6.31 (1H, d, *J* = 15.9 Hz, CH), 7.24 – 7.34 (5H, m, Ar-H), 7.44 – 7.48 (2H, m, CH and Ar-H), 7.67 (1H, d, *J* = 1.8 Hz, Ar-H), 8.41 (1H, d, *J* = 8.5 Hz, Ar-H). **$^{13}$C NMR (100 MHz; $CDCl_3$,):** δ 28.1, 31.3, 39.6, 80.6, 120.4, 126.5, 128.0, 128.3, 128.7, 131.4, 136.7, 140.1, 141.2, 165.9, 170.3. **HRMS-ESI:** (*m/z*) calculated for $C_{22}H_{24}NO_3Br$, 430.1012 $[M+H]^+$; found, 430.1014.



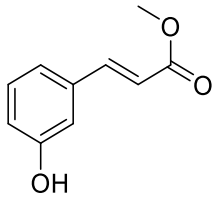
*tert-butyl (2E)-3-[3-(3-methylbut-2-en-1-yl)-4-(3-phenylpropanamido)phenyl]prop-2-enoate (**9**):* To a solution of (**8**) (40 mg, 0.1 mmol) in dry DMF (2 mL) was added $CsCO_3$ (65 mg, 0.2 mmol) and $Pd(dppf)Cl_2$ (16 mg, 0.02 mmol). Prenyl boronic acid pinacol ester (44 µL, 0.2 mmol) was added and the flask was heated at $90^0$C overnight. The reaction was allowed to cool and was filtered through a celite® pad with EtOAc. The solvent was evaporated in vacuo, re-dissolved in DCM and the residual DMF removed by washing with copious amounts of water in DCM, dried ($Na_2SO_4$), filtered and concentrated. Purification by column chromatography (Hexane:EtOAc = 9:1, 4:1) provided the title compound as a transparent oil (20 mg, 0.04 mmol, 44%).

**$R_f$**: 0.30 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; $CDCl_3$):** δ 1.55 (9H, s, $C(CH_3)_3$), 1.74 (3H, s, $CH_3$), 1.77 (3H, s, $CH_3$), 2.63 (2H, t, *J* = 7.3 Hz, $CH_2$), 3.06 (2H, t, *J* = 7.1 Hz, $CH_2$), 3.20 (2H, d, *J* = 6.2 Hz, $CH_2$), 5.14 (1H, t, *J* = 6.4 Hz, CH), 6.31 (1H, d, *J* = 16.1 Hz, CH), 7.23 - 7.33 (6H, m, Ar-H), 7.39 (1H, d, *J* = 8.3 Hz, Ar-H), 7.53 (1H, d, *J* = 16.0 Hz, CH), 8.04 (1H, d, *J* = 7.6 Hz, Ar-H). **$^{13}$C NMR (100 MHz; $CDCl_3$):** δ 18.0, 25.7, 28.2, 31.3, 31.6, 39.7, 80.4, 119.2, 121.3, 122.4, 126.4, 126.8, 128.3, 128.6, 129.4, 134.8, 137.6, 140.5, 143.0, 166.4, 170.1. **HRMS-ESI:** (*m/z*) calculated for $C_{27}H_{33}NO_3$, 418.2388 $[M-H]^-$; found, 418.2384.




*(2E)-3-[3-(3-methylbut-2-en-1-yl)-4-(3-phenylpropanamido)phenyl]prop-2-enoic acid (**2**):* To a solution of (**9**) (20 mg, 0.04 mmol) in dry toluene (10 mL) was added silica gel (3 mL) and the suspension was stirred at reflux overnight. The reaction was allowed to cool and the mixture was filtered after diluting with 20 % MeOH in DCM, dried ($Na_2SO_4$), filtered and the solvent evaporated in vacuo to provide the title compound as a white solid (9.3 mg, 0.02 mmol, 50%).

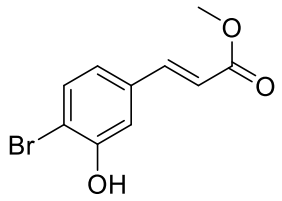
**$R_f$:** 0.1 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; MeOD):** δ 1.70 (3H, s, $CH_3$), 1.77 (3H, s, $CH_3$), 2.72 (2H, t, *J* = 7.0 Hz, $CH_2$), 3.03 (2H, t, *J* = 7.3 Hz, $CH_2$), 3.21 (2H, d, *J* = 6.9 Hz, $CH_2$), 5.19 (1H, t, *J* = 7.0 Hz, CH), 6.42 (1H, d, *J* = 15.8 Hz, CH), 7.14 - 7.30 (5H, m, Ar-H), 7.34 - 7.46 (3H, m, Ar-H), 7.61 (1H, d, *J* = 16.5 Hz, CH). **$^{13}$C NMR (100 MHz; MeOD):** δ 16.6, 24.4, 29.4, 31.3, 37.8, 117.7, 121.3, 125.6, 125.9, 126.1, 127.8, 128.1, 128.1, 128.8, 132.3, 133.2, 136.5, 137.0, 140.6, 144.2, 169.0, 172.6. ***m/z* (ESI):** 364.2 $[M+H]^+$ (47.5 %), 386.3 $[M+Na]^+$ (45 %). **HRMS-ESI:** (*m/z*) calculated for $C_{23}H_{25}NO_3$, 386.1727 $[M+Na]^+$; found, 386.1729.




*Methyl (2E)-3-(3-hydroxyphenyl)prop-2-enoate (**11**):* To a solution of *m*-coumaric acid (1 g, 6 mmol) in methanol (10 mL), was added sulphuric acid (0.6 mL) and the mixture refluxed overnight. The reaction mixture was allowed to cool and washed with brine, water, extracted in DCM, dried

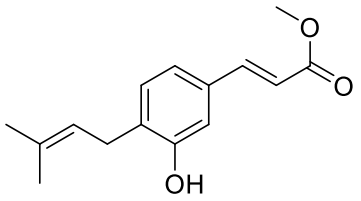
($Na_2SO_4$), filtered and concentrated to provide the title compound as a white solid. (1 g, 5.6 mmol, 93%).

**$R_f$:** 0.29 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz, $CDCl_3$):** δ 3.82 (3H, s, -$OCH_3$), 6.42 (1H, d, *J* = 17 Hz, CH) 6.89 (1H, d, *J* = 8.13 Hz, Ar-H), 7.02 (1H, s, Ar-H), 7.11 (1H, d *J* = 7.5 Hz, Ar-H), 7.65 (1H, d, *J* = 17.0 Hz, CH). **$^{13}$C NMR (100MHz, $CDCl_3$):** δ 51.9, 60.5, 114.3, 119.1, 120.3, 134.0, 134.9, 143.4, 167.0, 171.4. **HRMS-ESI:** (*m/z*) calculated for $C_{10}H_{10}O_3$, 177.0557 $[M-H]^-$; found, 177.0565.



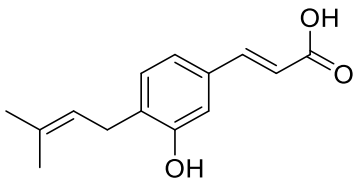

*methyl (2E)-3-(4-bromo-3-hydroxyphenyl)prop-2-enoate (**12**):* To a solution of (**11**) (1 g, 5.6 mmol), in HOAc (8 mL) was added a solution of $Br_2$ (310 µL, 6 mmol) in HOAc (2 mL) and the solution stirred at room temperature for 5 hr. A further solution of $Br_2$ (85 µL, 1.6 mmol) in HOAc (0.9 mL) and the mixture stirred overnight at room temperature. $Et_2O$ was added to the reaction mixture and was washed with brine and saturated aqueous $NaHCO_3$, the organic layer was separated and the aqueous layer extracted with $Et_2O$, the combined organic layers were dried ($Na_2SO_4$), filtered and concentrated. Purification by column chromatography (16% DCM in EtOAc:Hexane = 1:1) provided the title compound as a brown oil (610 mg, 2.3 mmol, 35%).

**$R_f$:** 0.23 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; $CDCl_3$):** δ 3.83 (3H, s, -$OCH_3$), 6.33 (1H, d, *J* = 15.0 Hz, CH), 6.79 (1H, d, *J* = 8.6, Ar-H), 7.12 (1H, s, Ar-H), 7.42 (1H, d, *J* = 8.5 Hz, Ar-H), 7.99 (1 H, d, *J* = 15.9 Hz, CH). **$^{13}$C NMR (100MHz; $CDCl_3$):** δ 51.9, 114.3, 115.3, 119.2, 120.2, 134.0, 143.5, 155.9, 167.2, 171.7. **HRMS-ESI:** (*m/z*) calculated for $C_{10}H_9O_3Br$, 254.9662 $[M-H]^-$; found, 254.9674.



*methyl (2E)-3-[3-hydroxy-4-(3-methylbut-2-en-1-yl)phenyl]prop-2-enoate (**13**):* To a solution of (**12)** (610 mg, 2.3 mmol) in dry DMF (2 mL) was added $CsCO_3$ (456 mg, 1.4 mmol) and $Pd(dppf)Cl_2$ (41 mg, 0.05 mmol). Prenyl boronic acid pinacol ester (330 µL, 1.5 mmol) was added and the flask was heated at $90^0$C overnight. The reaction was allowed to cool and was filtered through a celite® pad with EtOAc, the solvent was evaporated and the residue re-dissolved in DCM. The residual DMF was removed by washing with copious amounts of water in DCM, dried ($Na_2SO_4$), filtered and concentrated. Purification by column chromatography (Hexane:EtOAC = 9:1, 4:1, 2:1, 1:1, 0:1) provided the title compound as a yellow oil (70 mg, 0.3 mmol, 27%).

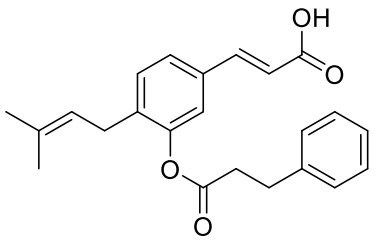
**$R_f$:** 0.23 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; $CDCl_3$):** δ 1.72 (3H, s, $CH_3$), 1.75 (3H, s, $CH_3$), 3.36 (2H, d, *J* = 6.6 Hz, $CH_2$), 3.83 (3H, s, -$OCH_3$), 5.16 (1H, t, *J* = 6.2 Hz, CH), 6.30 (1H, d, *J* = 15.8 Hz, CH), 6.88 (1H, d, *J* = 7.0 Hz, Ar-H), 7.01 – 7.10 (2H, m, Ar-H), 7.42 (1H, d, *J* = 8.5 Hz, Ar-H), 7.99 (1H, d, *J* = 15.8 Hz, CH). **$^{13}$C NMR (100 MHz; $CDCl_3$):** δ 25.6, 31.3, 51.9, 112.9, 117.9, 118.4, 123.0, 131.0, 132.3, 133.5, 133.7, 143.1, 154.5. **HRMS-ESI:** (*m/z*) calculated for $C_{15}H_{18}O_3$, 245.1183 $[M-H]^-$; found, 245.1183.



*(2E)-3-[3-hydroxy-4-(3-methylbut-2-en-1-yl)phenyl]prop-2-enoic acid (**14**):* To a solution of (**13)** (70 mg, 0.3 mmol) in water (6 mL) was added NaOH (100 mg, 2.5 mmol) and the reaction refluxed overnight. The solution was allowed to cool and was acidified with AcOH, the reaction mixture was

extracted with DCM and washed with water, the organic layer was separated, dried ($Na_2SO_4$), filtered and concentrated to provide the title compound as a white solid (70 mg, 0.3 mmol, 99%).

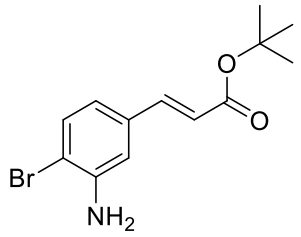
**$R_f$:** 0.10 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; MeOD):** δ 1.77 (3H, s, $CH_3$), 1.75 (3H, s, $CH_3$), 3.36 (2H, d, *J* = 6.6 Hz, $CH_2$), 5.12 (1H, t, *J* = 6.2 Hz, CH), 6.28 (2H, d, *J* = 15.8 Hz, CH), 6.77 (1H, d, *J* = 8.2 Hz, Ar-H), 7.02 (2H, d, *J* = 9.2 Hz, Ar-H), 7.94 (1H, d, *J* = 15.8 Hz, CH). **$^{13}$C NMR (100 MHz; MeOD):** δ 20.5, 28.4, 35.0, 116.1, 121.2, 122.3, 127.3, 134.6, 135.3, 136.4, 137.5, 146.8, 159.5, 173.0. **HRMS-ESI:** (*m/z*) calculated for $C_{14}H_{16}O_3$, 231.1027 $[M-H]^-$; found, 231.1030.




*(2E)-3-[4-(3-methylbut-2-en-1-yl)-3-[(3-phenylpropanoyl)oxy]phenyl]prop-2-enoic acid (**3**):* To a solution of (**14)** (70 mg, 0.3 mmol) in DCM (8 mL) was added DMAP (5 mg, 0.03 mmol), $NEt_3$ (140 µL, 1 mmol) and $PhCH_2CH_2COCl$ (170 mg, 1 mmol) in DCM (2 mL) and the reaction stirred overnight at room temperature. The reaction was washed with saturated aqueous $NaHCO_3$ and water, dried ($Na_2SO_4$), filtered and concentrated. Purification by column chromatography (Hexane:EtOAc = 9:1, 4:1, 2:1, 1:1, 0:1) provided the title compound as a white solid (86.6 mg, 0.2 mmol, 82%).

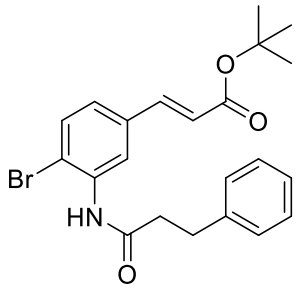
**$R_f$:** 0.10 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; MeOD):** δ 1.72 (3H, s, $CH_3$), 1.77 (3H, s, $CH_3$), 2.90 (2H, t, *J* = 7.2 Hz, $CH_2$), 3.04 (2H, t, *J* = 7.2 Hz, $CH_2$), 3.43 (2H, d, *J* = 6.7 Hz, $CH_2$), 5.15 (1H, t, *J* = 5.9 Hz, CH), 6.29 (2H, d, *J* = 15.7 Hz, CH), 6.94 (1H, d, *J* = 7.2 Hz, Ar-H), 7.16 – 7.33 (7H, m, Ar-H), 7.93 (1H, d, *J* = 15.8 Hz, CH). **$^{13}$C NMR (100 MHz; MeOD):** δ 16.6, 24.4, 30.5, 31.3, 35.3, 119.0, 119.9, 122.3, 122.9, 126.0, 128.14, 128.18, 130.4, 132.3, 134.0, 138.6, 140.2, 141.5, 149.3, 168.5,

171.7. ***m/z* (ESI):** 387.2 $[M+Na]^+$ (100 %), 365.2 $[M+H]^+$ (70 %). **HRMS-ESI:** (*m/z*) calculated for $C_{23}H_{24}O_4$, 363.1602 $[M-H]^-$; found, 363.1609.



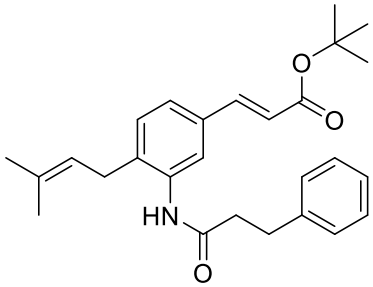

*tert-butyl (2E)-3-(3-amino-4-bromophenyl)prop-2-enoate (**16**):* To a solution of 2-bromo-5-iodo aniline (250 mg, 0.8 mmol) in dry toluene (6 mL) was added $PPh_3$ (26 mg, 0.1 mmol) and $Pd(OAc)_2$ (47.5 %, 24 mg, 0.05 mmol). *tert*-butyl acrylate (160 µL, 1.1 mmol) and $NEt_3$ (210 µL, 1.5 mmol) were added and the reaction was refluxed overnight. The reaction was allowed to cool and washed with saturated aqueous $NH_4Cl$, brine, extracted with DCM, dried ($Na_2SO_4$), filtered and concentrated. Purification by column chromatography (Hexane:EtOAc gradient = 9:1, 4:1, 2:1, 1:1, 0:1) provided the title compound as a white solid (160 mg, 0.5 mmol, 64%).

**$R_f$:** 0.19 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; $CDCl_3$):** δ 1.55 (9H, s, $C(CH_3)_3$), 6.25 (1H, d, *J* = 15.9 Hz, CH), 6.60 (1H, s, Ar-H), 6.92 (1H, s, Ar-H), 7.34 (1H, d, *J* = 8.4 Hz, Ar-H), 7.89 (1H, d *J* = 15.8 Hz, CH). **$^{13}$C NMR (100 MHz; $CDCl_3$):** δ 28.18, 80.7, 113.5, 118.3, 122.4, 131.9, 133.7, 134.9, 142.1, 145.8, 165.8. **HRMS-ESI:** (*m/z*) calculated for $C_{13}H_{16}NO_2Br$, 320.0257 $[M+Na]^+$; found, 320.0243.



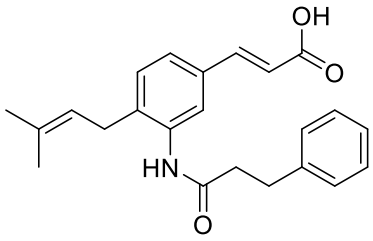

*tert-butyl (2E)-3-[4-bromo-3-(3-phenylpropanamido)phenyl]prop-2-enoate (**17**):* To a solution of (**16**) (160 mg, 0.5 mmol) in dry DCM (2 mL) was added DMAP (8 mg, 0.6 mmol) and a solution of $PhCH_2CH_2COCl$ (170 mg, 1 mmol) in DCM (2 mL) the reaction was heated to $70^0$C and stirred overnight. The reaction was allowed to cool and was washed with saturated aqueous $NaHCO_3$, water and extracted in DCM, dried ($Na_2SO_4$), filtered and concentrated. Purification by column chromatography (Hexane:EtOAc = 12:1, 9:1, 4:1, 2:1, 1:1) provided the title compound as a white solid (200 mg, 0.4 mmol, 88%).

**$R_f$:** 0.32 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; $CDCl_3$):** δ 1.54 (9H, s, $C(CH_3)_3$), 2.68 (2H, t, *J* = 7.4 Hz, $CH_2$), 3.03 (2H, t, *J* = 7.4 Hz, $CH_2$), 6.29 (1H, d, *J* = 15.8 Hz, CH), 7.18 – 7.29 (5H, m, Ar-H), 7.43 (1H, d, *J* = 8.3, Ar-H), 7.76 (1H, s, Ar-H), 7.84 (1H, s, Ar-H), 7.89 (1H, d, *J* = 15.9 Hz, CH). **$^{13}$C NMR (100 MHz; $CDCl_3$):** δ 28.1, 31.4, 39.2, 80.9, 118.7, 119.5, 123.2, 126.4, 128.3, 128.6, 133.5, 134.9, 137.5, 140.4, 141.6, 165.8, 170.8. **HRMS-ESI:** (*m/z*) calculated for $C_{22}H_{24}NO_3Br$, 428.0867 $[M-H]^-$; found, 428.0875.



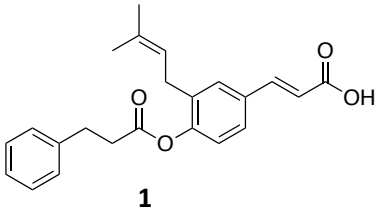
*tert-butyl (2E)-3-[4-(3-methylbut-2-en-1-yl)-3-(3-phenylpropanamido)phenyl]prop-2-enoate (**18**):* To a solution of **(17)** (200 mg, 0.4 mmol) in dry DMF (2 mL) was added $CsCO_3$ (230 mg, 0.7 mmol) and $Pd(dppf)Cl_2$ (40 mg, 0.05 mmol). Prenyl boronic acid pinacol ester (140 µL, 0.62 mmol) was added and the flask was heated at $90^0$C overnight. The reaction was allowed to cool and was extracted over a pad of celite® with EtOAc, the solvent evaporated and re-dissolved in DCM. Residual DMF was removed by washing with copious amounts of water in DCM, the organic layer was dried ($Na_2SO_4$), filtered and concentrated. Purification by column chromatography (Hexane:EtOAc = 9:1, 4:1) provided the title compound as a transparent oil (80 mg, 0.2 mmol, 42%).

**$R_f$:** 0.33 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz; $CDCl_3$):** δ 1.54 (9H, s, $C(CH_3)_3$), 1.73 (3H, s, $CH_3$), 1.74 (3H, s, $CH_3$), 2.67 (2H, t, *J* = 7.4 Hz, $CH_2$), 3.05 (2H, t, *J* = 7.4 Hz, $CH_2$), 3.38 (2H, d, *J* = 6.4 Hz, $CH_2$), 5.16 (1H, t, *J* = 5.6 Hz, CH), 6.27 (1H, d, *J* = 15.7 Hz, CH), 7.11 (1H, d, *J* = 8.0 Hz, Ar-H), 7.22 – 7.35 (5H, m, Ar-H), 7.57 (1H, s, Ar-H), 7.71 (1H, s, Ar-H), 7.84 (1H, d, *J* = 15.7 Hz, CH). **$^{13}$C NMR (100 MHz; $CDCl_3$):** δ 17.9, 25.7, 28.2, 31.5, 31.5 39.2, 80.5, 117.9, 121.8, 122.4, 126.3, 128.3, 128.6, 130.1, 132.7, 133.8, 136.1, 137.1, 140.6, 140.8, 166.3, 170.6. **HRMS-ESI:** (*m/z*) calculated for $C_{27}H_{33}NO_3$, 418.2388 $[M-H]^-$; found, 418.2393.




*(2E)-3-[4-(3-methylbut-2-en-1-yl)-3-(3-phenylpropanamido)phenyl]prop-2-enoic acid (**4**):* To a solution of (**18**) (80 mg, 0.2 mmol) in dry toluene (6 mL) was added silica gel (3 mL) and the suspension stirred at reflux overnight. The reaction was allowed to cool and the mixture filtered, washing with 20 % MeOH in DCM, and the solvent evaporated in vacuo to provide the title compound as a white solid (18.7 mg, 0.05 mmol, 27%).

**$R_f$:** 0.00 (Hexane:EtOAc, 4:1). **$^1$H NMR (400 MHz, MeOD):** δ 1.72 (3H, s, $CH_3$), 1.79 (3H, s, $CH_3$), 2.66 (2H, t, *J* = 7.4 Hz, $CH_2$), 3.01 (2H, t, *J* = 7.4 Hz, $CH_2$), 3.43 (2H, d, *J* = 6.6 Hz, $CH_2$), 5.16 (1H, t, *J* = 5.9 Hz, CH), 6.33 (1H, d, *J* = 15.7 Hz, CH), 7.16 – 7.30 (6H, m, Ar-H), 7.46 (1H, d, *J* = 8.0 Hz, Ar-H), 7.80 (1H, s, Ar-H), 7.96 (1H, d, *J* = 15.7 Hz, CH). **$^{13}$C NMR (100 MHz, MeOD):** δ 16.6, 24.4, 31.3, 31.3, 38.4, 117.5, 119.2, 121.7, 122.6, 125.8, 128.1, 129.9, 132.0, 133.2, 136.9, 137.0, 140.7, 142.3, 168.8, 172.2. ***m/z* (ESI):** 364.2 $[M+H]^+$ (100 %), 386.3 $[M+Na]^+$ (17.5 %). **HRMS-ESI:** (*m/z*) calculated for $C_{23}H_{25}NO_3$, 362.1762 $[M-H]^-$; found, 362.1761.

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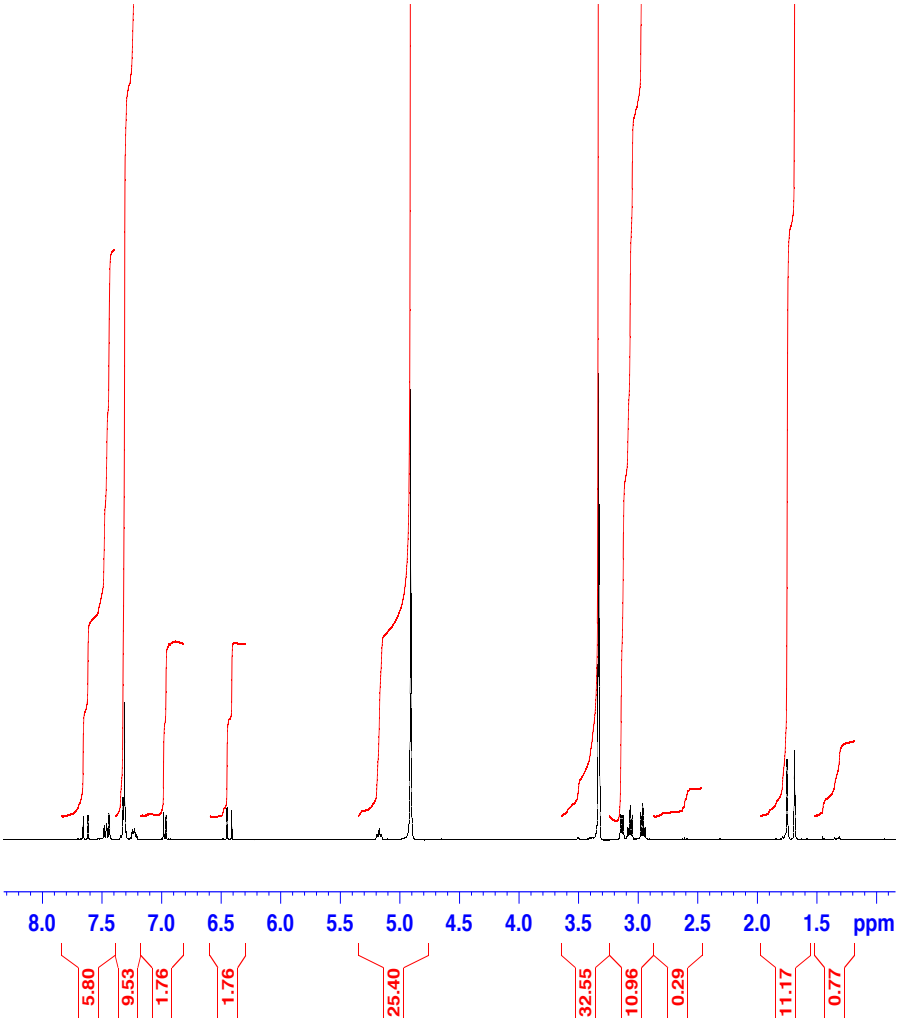


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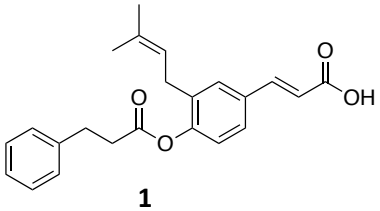


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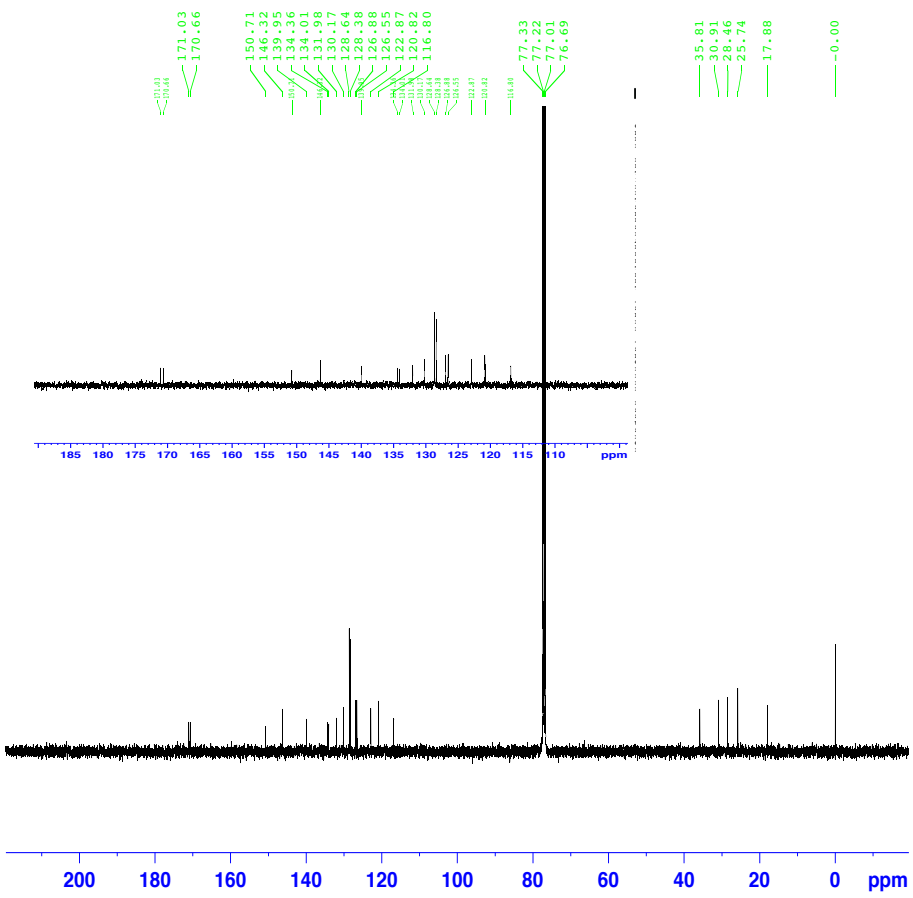


BRUKER

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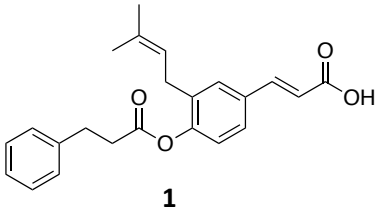


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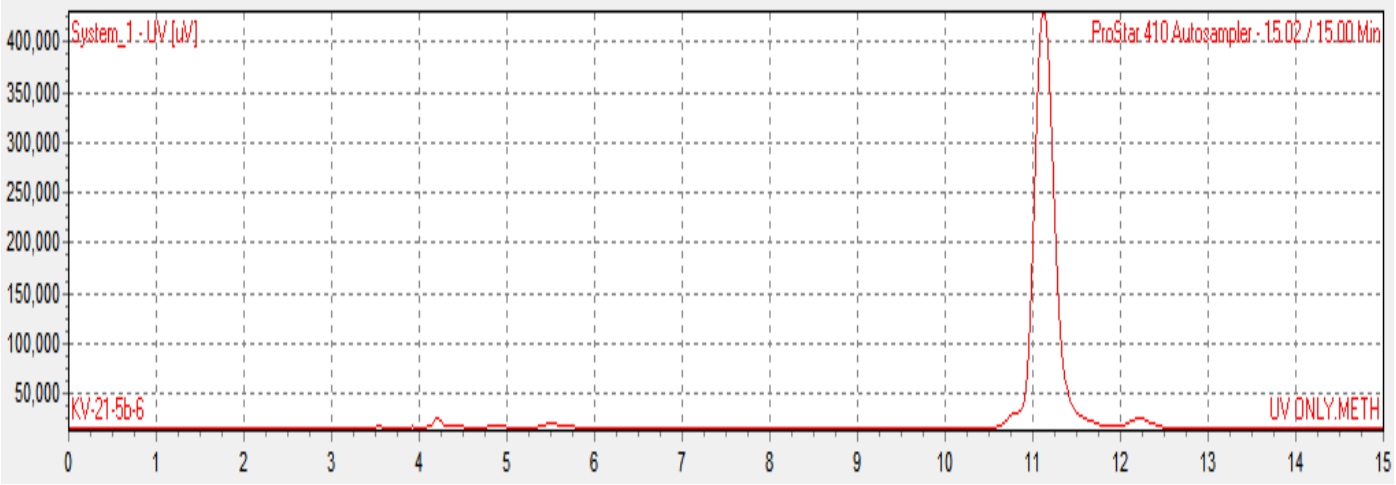


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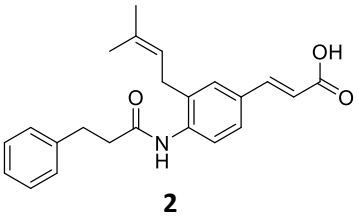


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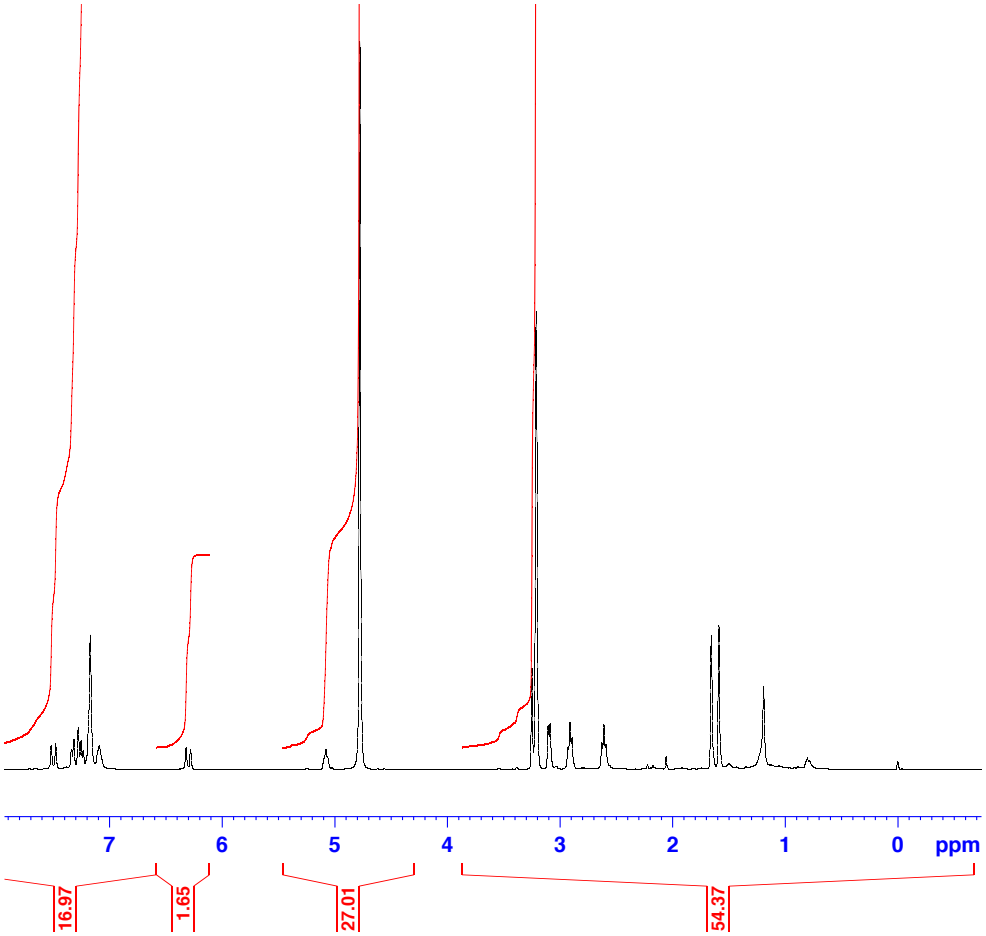


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FIDRES        0.122266 Hz
AQ            4.0894465 sec
RG                 203
DW              62.400 usec
DE               6.50 usec
TE              296.4 K
D1          1.00000000 sec
TD0                  1

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NUC1                1H
P1                15.00 usec
PLW1         9.85000038 W

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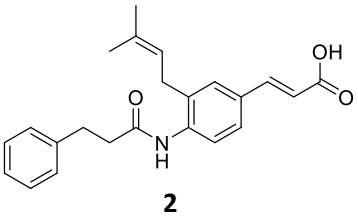
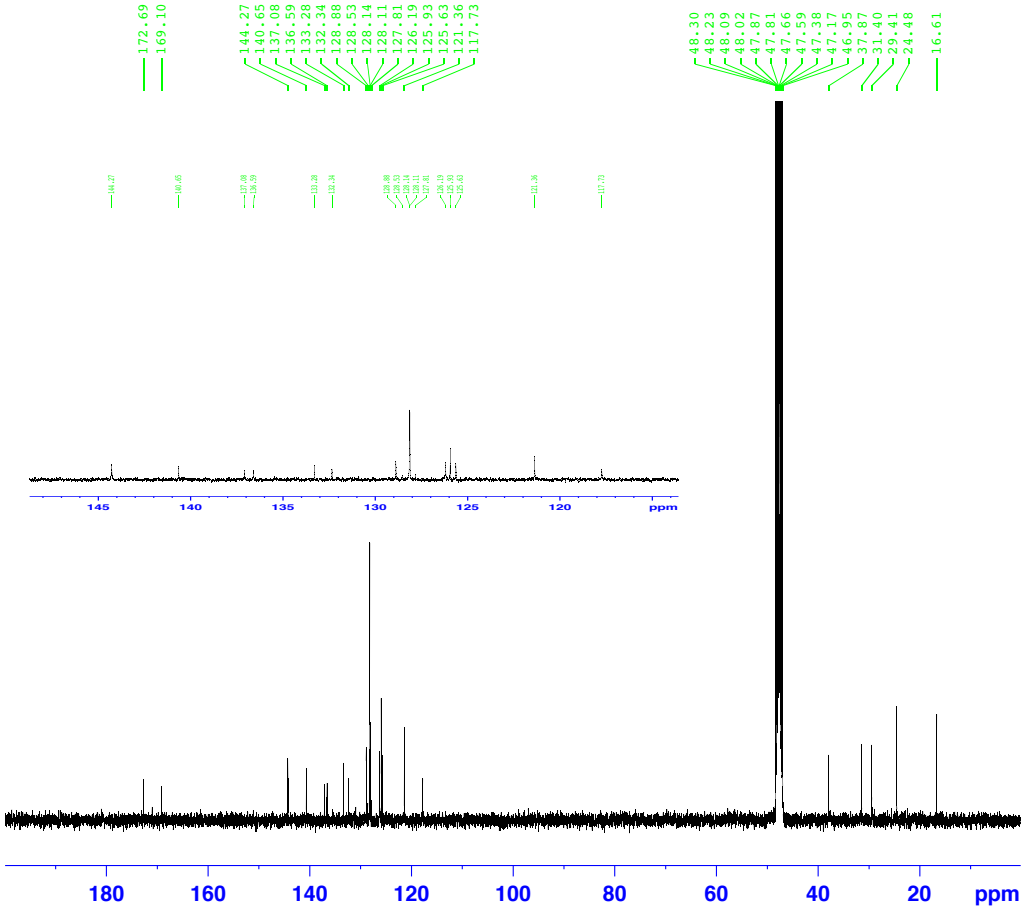
PC                1.00
```




**2**



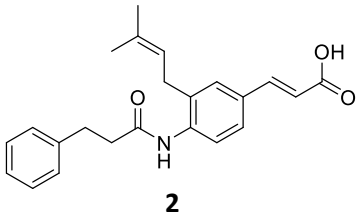

```
Current Data Parameters
NAME        kv-36-1.7(1)
EXPNO                 21
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150601
Time               21.33
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT             MeOD
NS                 4120
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ            1.3631488 sec
RG                  203
DW               20.800 usec
DE                6.50 usec
TE                295.4 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

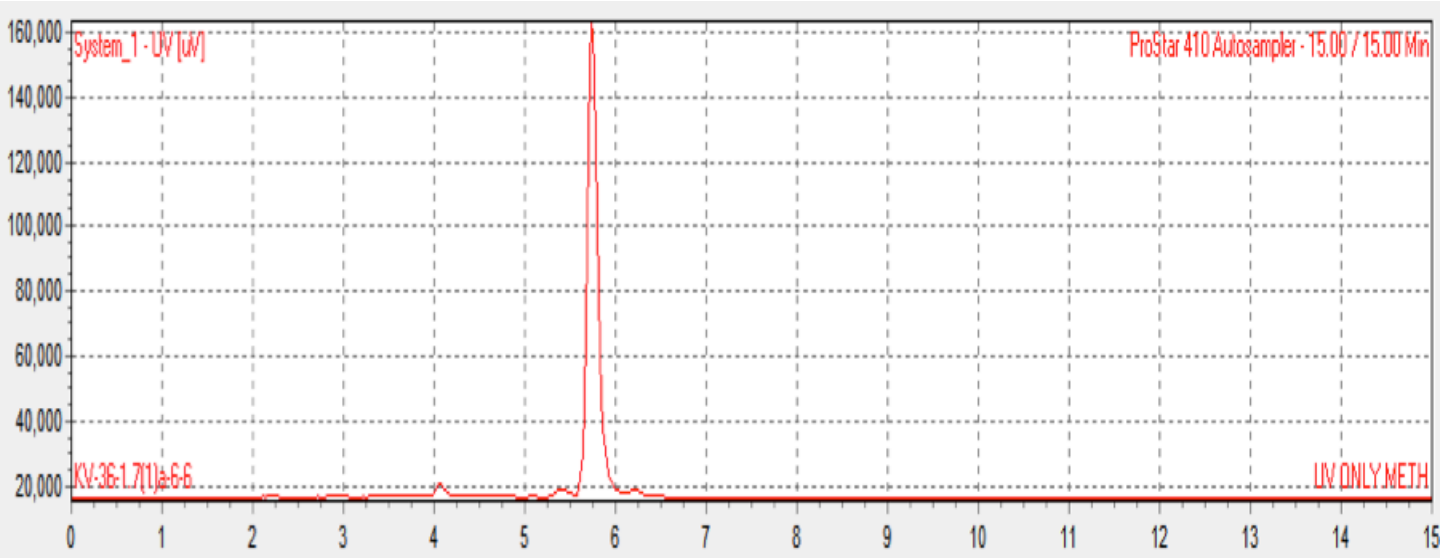
======== CHANNEL f1 ========
SFO1         100.6228293 MHz
NUC1                13C
P1                10.00 usec
PLW1         49.00000000 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                 1H
CPDPRG[2         waltz16
PCPD2             90.00 usec
PLW2         12.00000000 W
PLW12         0.33333001 W
PLW13         0.27000001 W

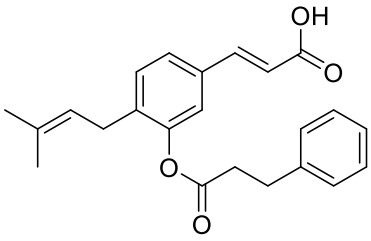
F2 - Processing parameters
SI                 32768
SF           100.6127685 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



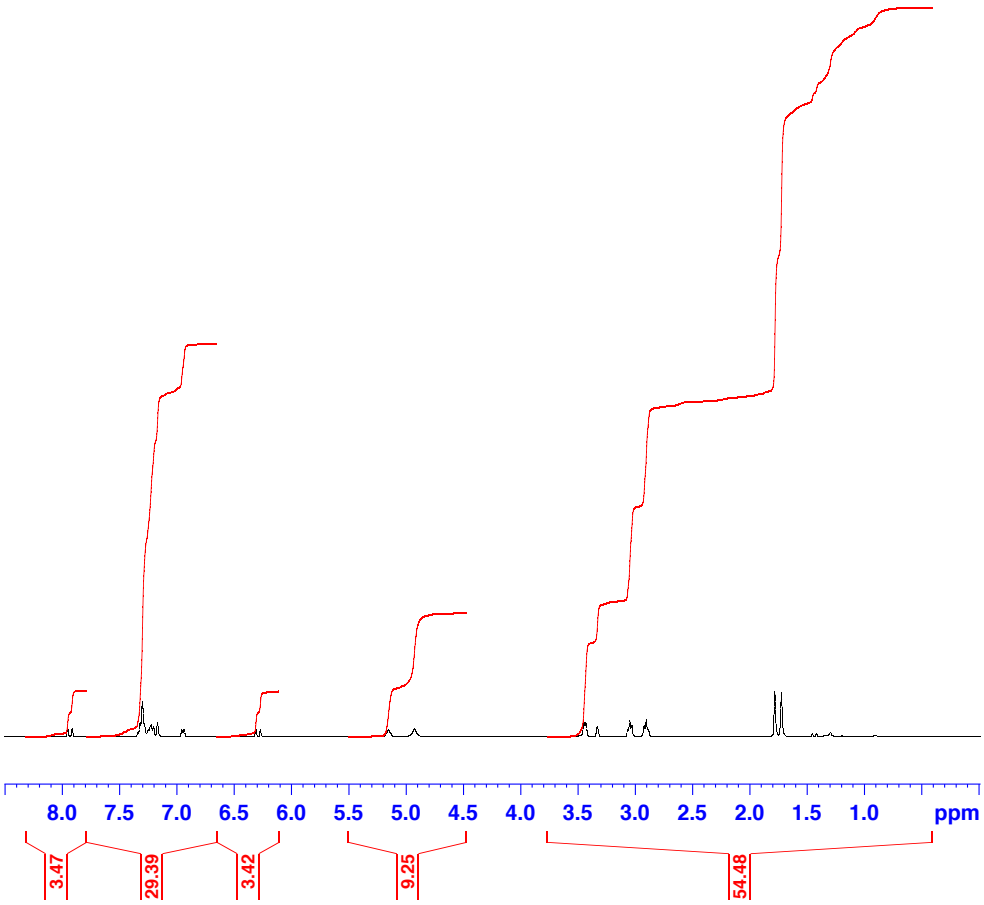

**2**




| | # | Name | Time [Min] | Quantity [% Area] | Height [uV] | Area [uV.Min] | Area % [%] |
|---|---|---|---|---|---|---|---|
| | 2 | UNKNOWN | 4.05 | 1.47 | 3393.8 | 310.8 | 1.474 |
| | 3 | UNKNOWN | 5.40 | 1.19 | 1636.6 | 250.7 | 1.189 |
| | 1 | KV-36-1.7(1)a | 5.73 | 97.05 | 145035.6 | 20462.5 | 97.051 |
| | 4 | UNKNOWN | 6.23 | 0.29 | 682.7 | 60.2 | 0.285 |
| | | | | | | | |
| | Total | | | 100.00 | 150748.7 | 21084.2 | 100.000 |




**3**

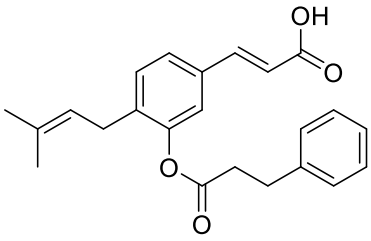



```
Current Data Parameters
NAME                  kv-32-8
EXPNO                      12
PROCNO                      1

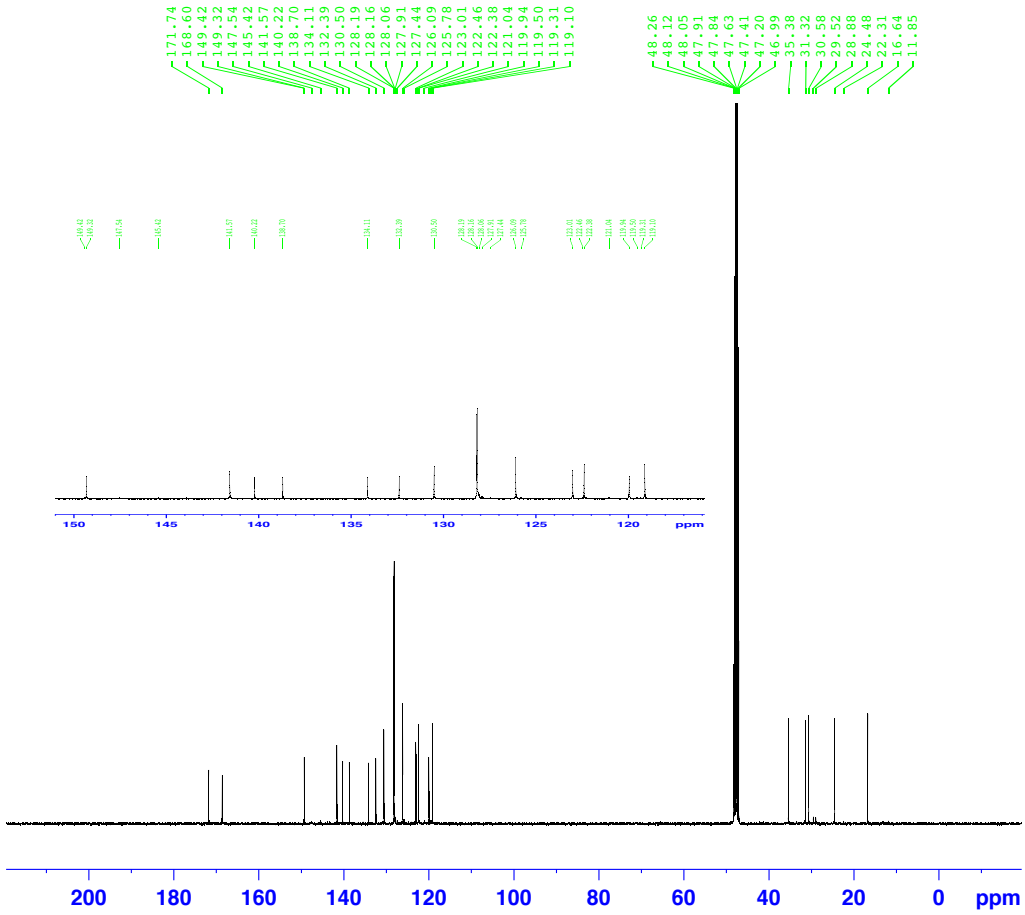
F2 - Acquisition Parameters
Date_               20150215
Time                   20.10
INSTRUM                spect
PROBHD    5 mm PABBO BB/
PULPROG                zg30
TD                    65536
SOLVENT                 MeOD
NS                        16
DS                         2
SWH                 8012.820 Hz
FIDRES              0.122266 Hz
AQ                  4.0894465 sec
RG                       71.8
DW                     62.400 usec
DE                      6.50 usec
TE                     296.0 K
D1                  1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1             400.1324710 MHz
NUC1                       1H
P1                      15.00 usec
PLW1              9.85000038 W

F2 - Processing parameters
SI                        65536
SF               400.1300000 MHz
WDW                         EM
SSB          0
LB                      0.30 Hz
GB           0
PC                      1.00
```




**3**

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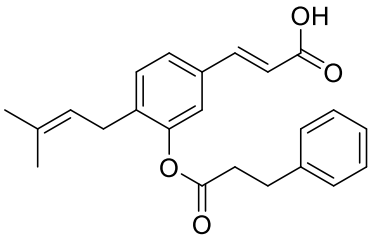
```
Current Data Parameters
NAME                  kv-32-8
EXPNO                      11
PROCNO                      1

F2 - Acquisition Parameters
Date_               20150215
Time                   16.49
INSTRUM                spect
PROBHD    5 mm PABBO BB/
PULPROG                zgpg30
TD                      65536
SOLVENT                  MeOD
NS                       1024
DS                          4
SWH                24038.461 Hz
FIDRES              0.366798 Hz
AQ                  1.3631488 sec
RG                        203
DW                     20.800 usec
DE                      6.50 usec
TE                     297.6 K
D1                 2.00000000 sec
D11                0.03000000 sec
TD0                         1

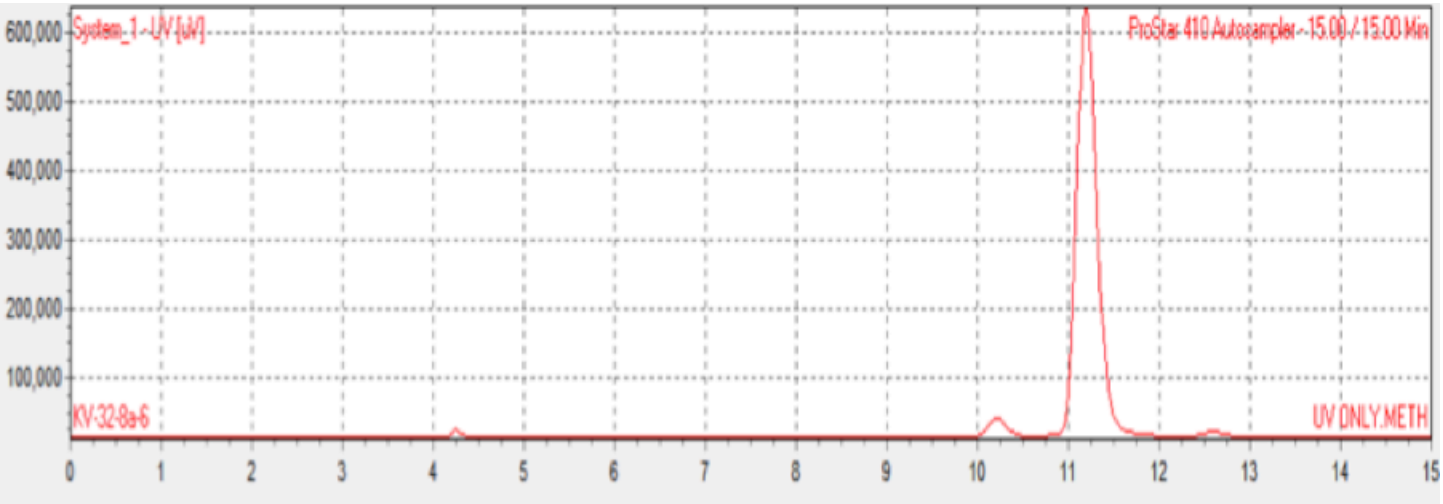
======== CHANNEL f1 ========
SFO1             100.6228293 MHz
NUC1                     13C
P1                     10.00 usec
PLW1            49.00000000 W

======== CHANNEL f2 ========
SFO2             400.1316005 MHz
NUC2                     1H
CPDPRG[2              waltz16
PCPD2                  90.00 usec
PLW2            12.00000000 W
PLW12            0.33333001 W
PLW13            0.27000001 W

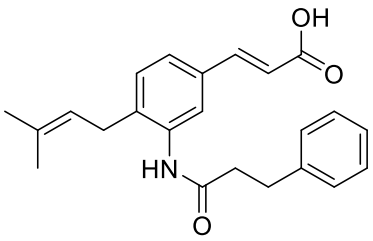
F2 - Processing parameters
SI                       32768
SF               100.6127685 MHz
WDW                         EM
SSB          0
LB                      1.00 Hz
GB           0
PC                      1.40
```



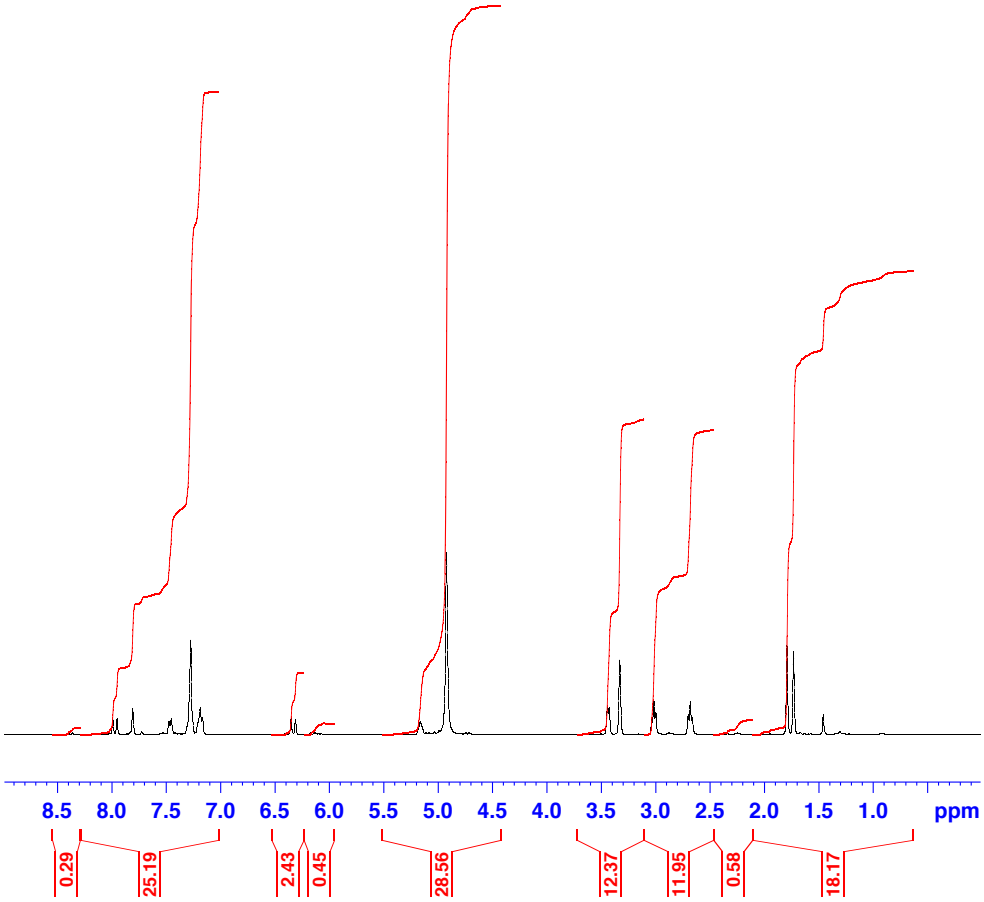

**3**




| | # | Name | Time [Min] | Quantity [% Area] | Height [uV] | Area [uV.Min] | Area % [%] |
|---|---|---|---|---|---|---|---|
| | 4 | UNKNOWN | 4.25 | 0.25 | 6110.4 | 440.5 | 0.254 |
| | 2 | UNKNOWN | 10.22 | 2.37 | 21643.0 | 4114.7 | 2.374 |
| | 1 | KV-32-8a | 11.20 | 97.20 | 620303.3 | 168470.3 | 97.198 |
| | 3 | UNKNOWN | 12.58 | 0.17 | 2251.6 | 301.8 | 0.174 |
| | | | | | | | |
| | Total | | | 100.00 | 650308.3 | 173327.2 | 100.000 |




**4**

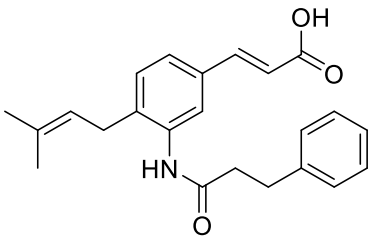



```
Current Data Parameters
NAME                  kv-37-4-a
EXPNO                        10
PROCNO                        1

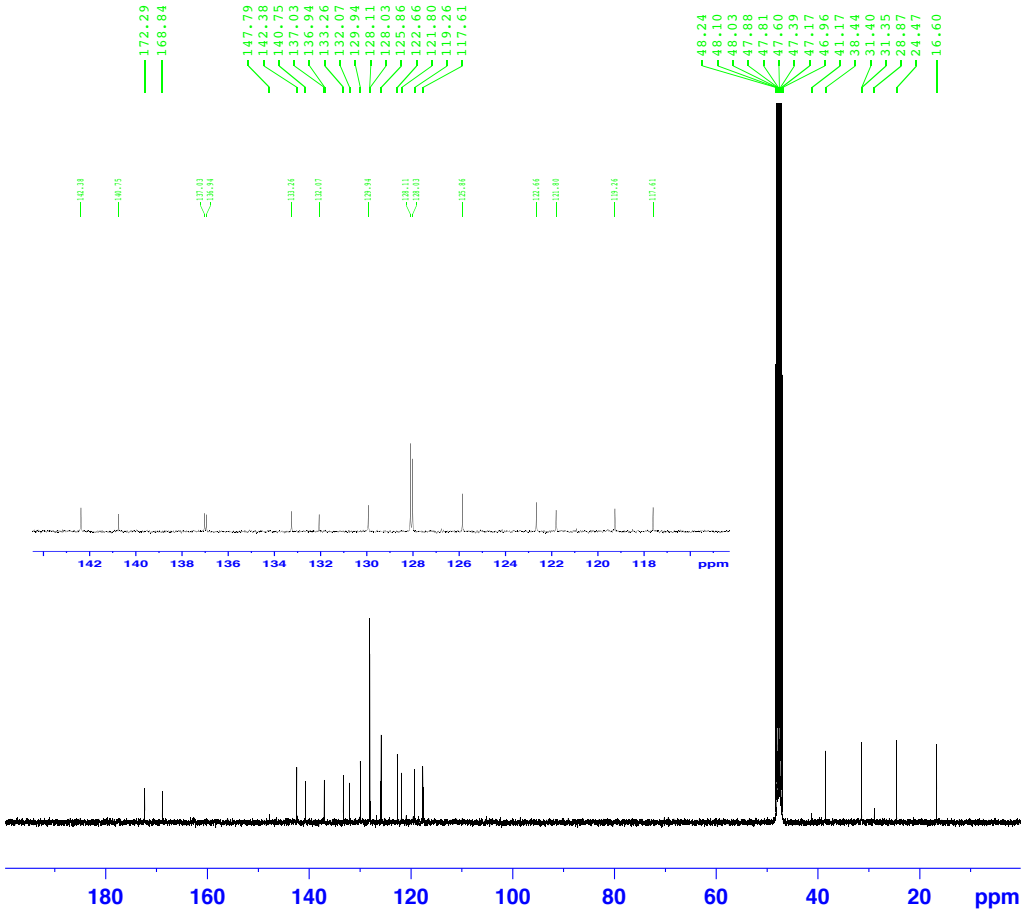
F2 - Acquisition Parameters
Date_                 20150315
Time                     19.06
INSTRUM                  spect
PROBHD    5 mm PABBO BB/
PULPROG                  zg30
TD                      65536
SOLVENT                  MeOD
NS                         16
DS                          2
SWH                   8012.820 Hz
FIDRES                0.122266 Hz
AQ                    4.0894465 sec
RG                        128
DW                       62.400 usec
DE                        6.50 usec
TE                       294.3 K
D1                    1.00000000 sec
TD0                           1

======== CHANNEL f1 ========
SFO1                400.1324710 MHz
NUC1                        1H
P1                       15.00 usec
PLW1                 9.85000038 W

F2 - Processing parameters
SI                          65536
SF                  400.1300000 MHz
WDW                            EM
SSB                 0
LB                            0.30 Hz
GB                  0
PC                            1.00
```




**4**




BRUKER

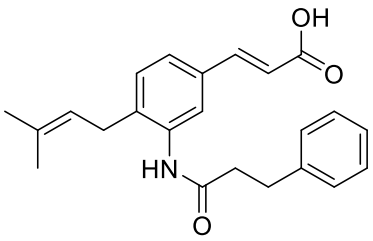
```
Current Data Parameters
NAME                  kv-37-4-a
EXPNO                        11
PROCNO                        1

F2 - Acquisition Parameters
Date_                 20150315
Time                     20.05
INSTRUM                  spect
PROBHD    5 mm PABBO BB/
PULPROG                 zgpg30
TD                       65536
SOLVENT                    MeOD
NS                         1024
DS                            4
SWH                  24038.461 Hz
FIDRES                0.366798 Hz
AQ                    1.3631488 sec
RG                          203
DW                       20.800 usec
DE                        6.50 usec
TE                       295.9 K
D1                   2.00000000 sec
D11                  0.03000000 sec
TD0                           1

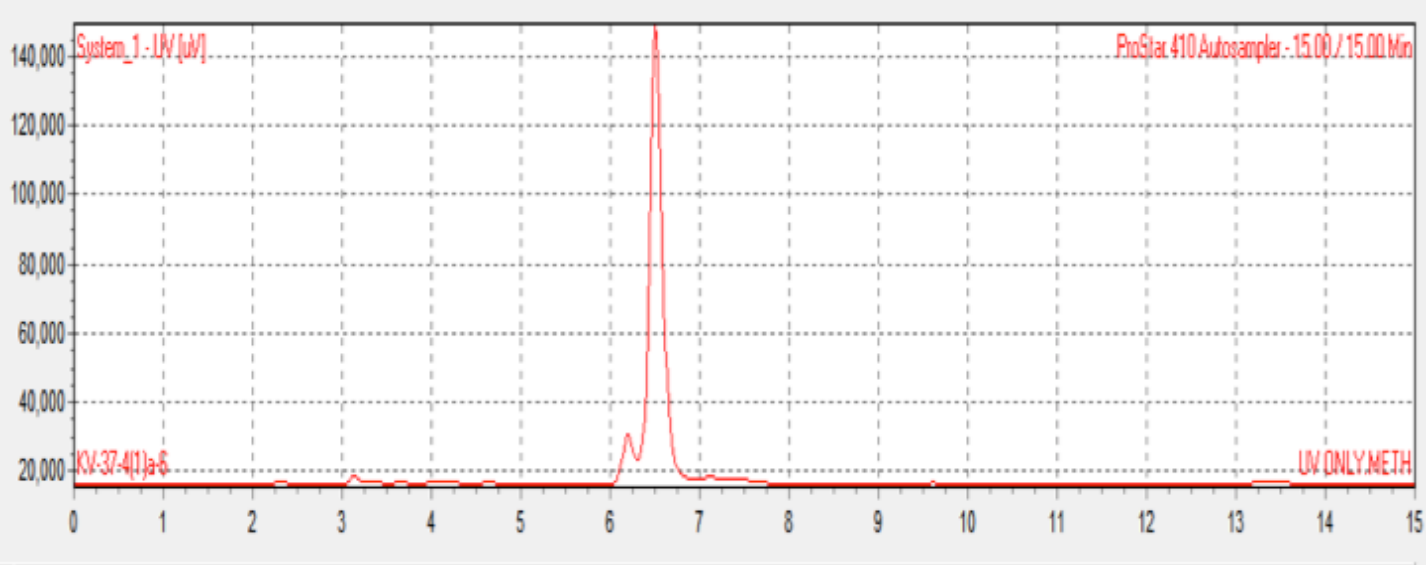
======== CHANNEL f1 ========
SFO1               100.6228293 MHz
NUC1                        13C
P1                        10.00 usec
PLW1               49.00000000 W

======== CHANNEL f2 ========
SFO2               400.1316005 MHz
NUC2                        1H
CPDPRG[2                waltz16
PCPD2                     90.00 usec
PLW2               12.00000000 W
PLW12               0.33333001 W
PLW13               0.27000001 W

F2 - Processing parameters
SI                        32768
SF                 100.6127685 MHz
WDW                          EM
SSB          0
LB                        1.00 Hz
GB           0
PC                        1.40
```




**4**




| | # | Name | Time [Min] | Quantity [% Area | Height [uV] | Area [uV.Min] | Area % [%] |
|---|---|---|---|---|---|---|---|
| | 1 | UNKNOWN | 6.18 | 3.95 | 8725.6 | 922.9 | 3.948 |
| | 2 | KV-37-4(1)a | 6.50 | 96.05 | 130599.3 | 22450.1 | 96.052 |
| | | | | | | | |
| | Total | | | 100.00 | 139324.9 | 23372.9 | 100.000 |