Supporting Information

Potential Metabolic Activation of a Representative C4-Alkylated Polycyclic Aromatic Hydrocarbon Retene (1-Methyl-7-isopropyl-phenanthrene) Associated With the Deepwater Horizon Oil Spill in Human Hepatoma (HepG2) Cells

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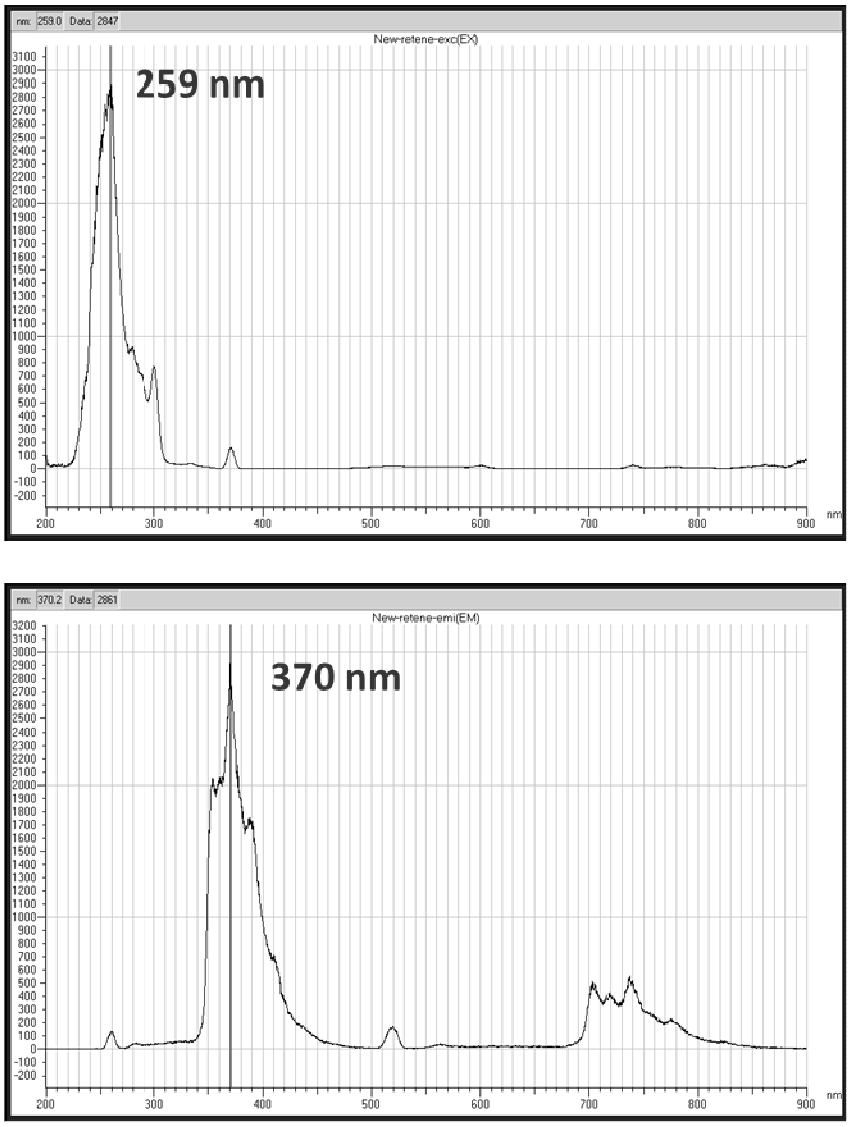


Figure S1. Excitation wavelength and emission wavelength spectra of retene.

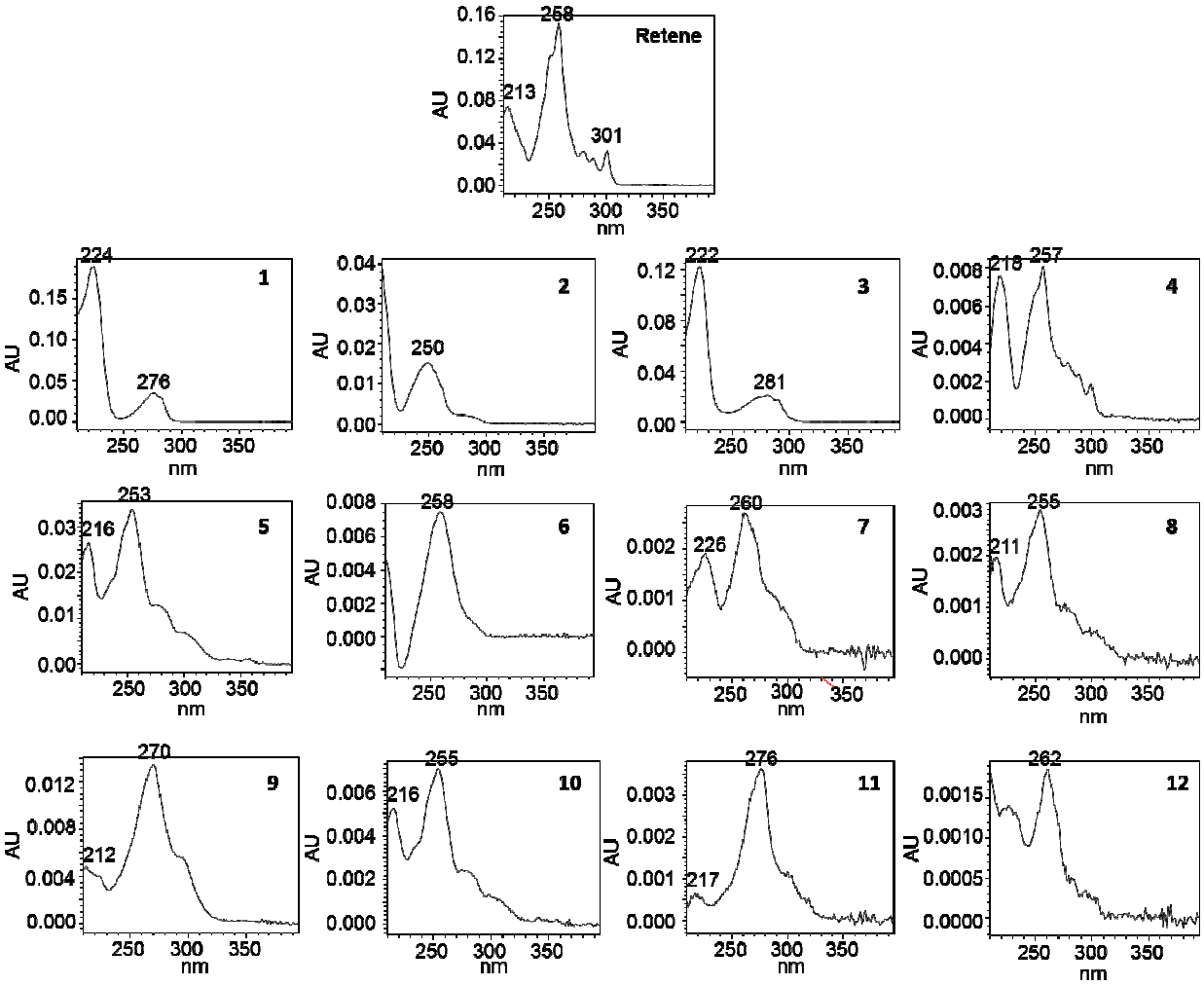


Figure S2. UV spectra of retene and metabolite 1-12 in human HepG2 cells.

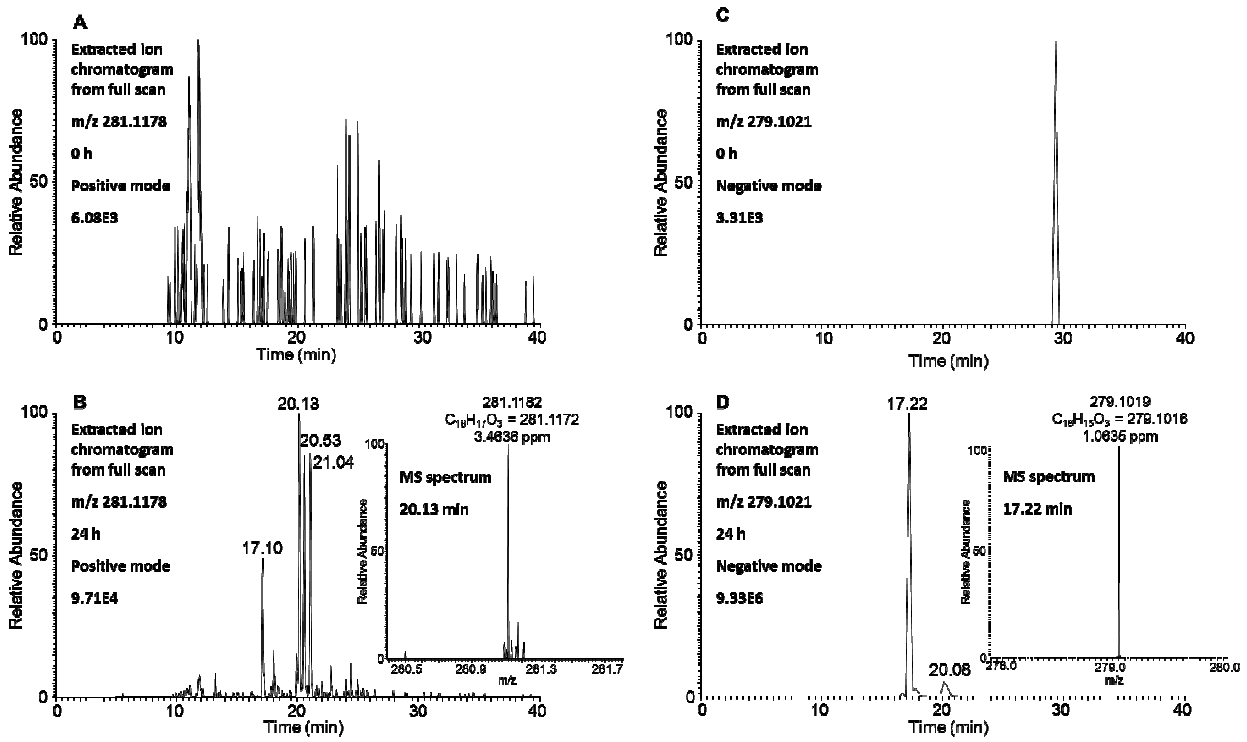
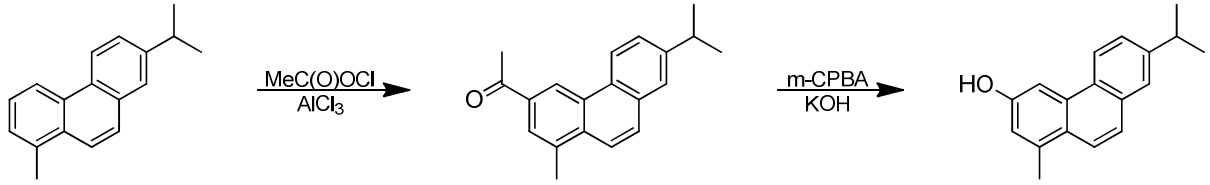


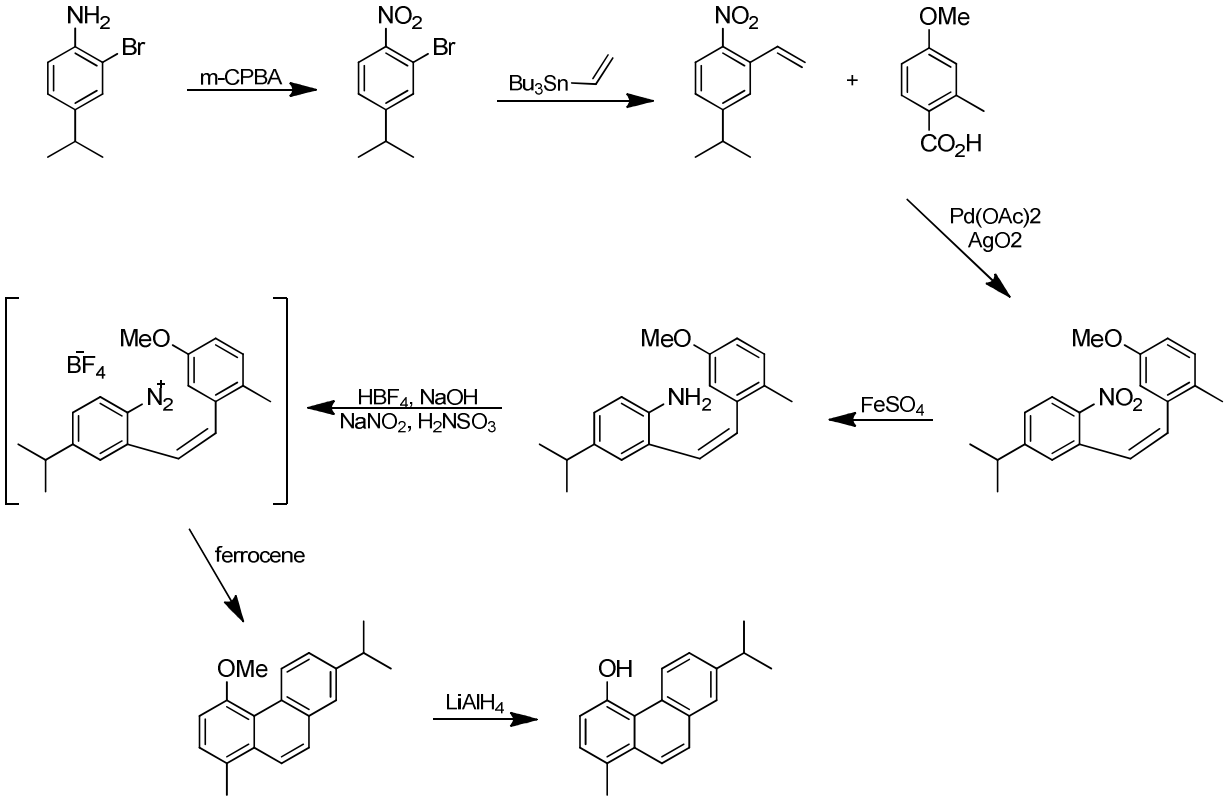
Figure S3. Detection of monohydroxy-retene-diones in human HepG2 cells. (A) Extracted ion chromatogram of Orbitrap full scan in the positive mode at 0 h. (B) Extracted ion chromatogram of Orbitrap full scan in the positive mode at 24 h and MS spectrum of the peak at 20.13 min. (C) Extracted ion chromatogram of Orbitrap full scan in the negative mode at 0 h. (D) Extracted ion chromatogram of Orbitrap full scan in the negative mode at 24 h and MS spectrum of the peak at 17.22 min.

Figure S4. Synthetic routes and details of synthesis of 3-hydroxy-retene, 4-hydroxy-retene and 6-hydroxy-retene.

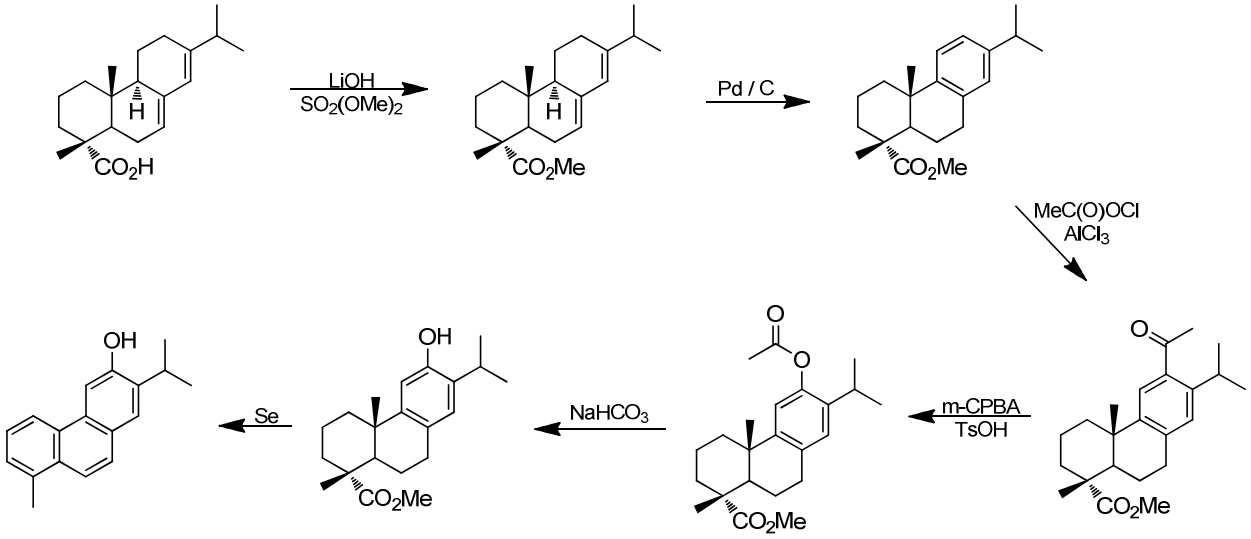
3-Hydroxyretene



4-Hydroxyretene



6-Hydroxyretene



Materials and Methods

Retene was purchased from MP Biomedicals, abietic acid and palladium acetate from Alfa Aesar, nitrobenzene and lithium aluminum hydride from TCI America and sulfamic acid from Ricca Chemicals. Silica gel and all other chemicals and solvents were purchased from Fisher Scientific or Aldrich Chemicals.

Analyses

All NMR data was obtained on a Bruker Ultrashield 300, Avance II, 300 MHz. Negative mode mass spectroscopy data was obtained on a Thermo Fusion Orbitrap ESI-MS. Positive mode mass spectroscopy data was obtained on a Sciex 5800 Maldi TOF/TOF.

3-Hydroxyretene

3-acetylretene:[1] Retene (450 mg, 1.92 mmol) was dissolved in 2 mL nitrobenzene in a 5mL RB flask containing a magnetic stir bar. Acetyl chloride (0.273 mL, 3.84 mmol) was added and the solution was cooled to 0°C while stirring. Aluminum chloride (525 mg, 3.94 mmol) was added slowly and stirring was continued at 5 °C for 5 hrs, allowed to come to room temperature followed by 40 hrs without stirring. The mixture was poured into 10mL ice containing 1.3 mL concentrated hydrochloric acid. The solvent was removed by distillation and the residue treated with activated carbon in ether, the ether was filtered and evaporated. The crude product was purified by silica gel chromatography using 100 % hexane to 20 % methylene chloride / hexane as eluant gradient to give 3-acetylretene (220 mg, 0.796 mmol, 41.5 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): 1.41 (s, 3H); 1.42 (s, 3H); 2.67 (s, 3H); 2.7s, 3H); 3.13 (m, 1H); 7.56 (dd, J=1.9,

6.7 Hz, 1H); 7.68 (d, J=1.9 Hz, 1H); 7.76 (d, J=3.7 Hz, 2H); 7.85 (m, 1H); 8.6 (d, J=8.7 Hz, 1H); 9.03 (s, 1H).

3-hydroxyretene: This compound was made using the Baeyer-Villiger oxidation method.[2] 3-Acetylretene (210 mg, 0.76 mmol) was dissolved in 8 mL chloroform in a 25 mL RB flask containing a magnetic stir bar. 3-Chloroperoxybenzoic acid (mCPBA), (262 mg, 1.52 mmol) was added and the mixture was stirred in the dark for 65 hrs. Thin layer chromatography in 20 % methylene chloride / hexane showed that the reaction was incomplete. A second addition of mCPBA (131 mg, 0.76 mmol) was stirred for 72 hr. The intermediate was purified on a silica gel column using benzene as eluant. The yellow fraction was evaporated to dryness in a 25 mL RB flask. Potassium hydroxide (107mg, 1.9 mmol) in 10 mL ethanol was added and a reflux condenser attached and the mixture refluxed for 15 min. The solvent was evaporated and the residue triturated with 3 mL water and decanted. The aqueous layer was acidified to pH 3 with 10% hydrochloric acid resulting in the precipitation of excess mCPBA which was removed by filtration. The resulting mother liquor (CH_2Cl_2) was evaporated and purified on a silica gel column using 100% hexane to 30% methylene chloride / hexane as eluant to give 3-hydroxyretene (83 mg, 0.332 mmol, 43.6 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.36 (s, 3H); 1.37 (s, 3H); 2.71 (s, 3H); 3.1 (m, 1H); 7.0 (dd, J = 0.9, 1.65 Hz, 1H); 7.50 (dd, J = 2.1, 6.7 Hz, 1H); 7.60 (d, J = 9.0 Hz, 1H); 7.68 (d, J = 1.7 Hz, 1H); 7.83 (d, J = 9.8 Hz, 1H); 7.89 (d, J = 2.3 Hz, 1H); 8.46 (d, J = 8.8 Hz, 1H). MS^- = 249.1; MW = 250.3 g/mol.

4-Hydroxyretene

2-Bromo-4-isopropyl-1-nitrobenzene:[3] 2-Bromo-4-isopropylaniline (10 g, 46.7 mmol) was dissolved in 340 mL toluene in a 1L RB flask fitted with a reflux condenser and a magnetic stir bar. The solution was stirred at room temperature and mCPBA (58.4 g, 339 mmol) was added very slowly and the mixture then refluxed overnight. The mixture was allowed to cool to room temperature resulting in the formation of a precipitate which was removed by filtration. The filtrate was diluted with ether, washed with 250 mL 10% sodium hydroxide, and washed with 250 mL saturated sodium chloride. The ether layer was then dried over sodium sulfate, filtered and evaporated to give 2-bromo-4-isopropyl-1-nitrobenzene (5.3g, 21.71 mmol, 46.5 % yield) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$): 1.26 (s, 3H); 1.28 (s, 3H); 2.96 (h, J = 6.93 Hz, 1H); 7.29 (dd, J = 1.59, 6.51 Hz, 1H); 7.58 (d, J = 1.83 Hz, 1H); 7.81 (d, J = 8.34 Hz, 1H).

4-Isopropyl-1-nitro-2-vinylbenzene: This compound was made using the cross-coupling method described by Soderberg and Shriver.[4] 2-Bromo-4-isopropyl-1-nitrobenzene (3.67 g, 15.04 mmol) was dissolved in 150 mL toluene in a 250 mL RB flask containing a magnetic stir bar under an argon atmosphere. Tributyl(vinyl)tin (4.83 ml, 16.54 mmol), bis(dibenzylideneacetone)palladium (865 mg, 1.504 mmol) and triphenylphosphine (1.617 g, 6.16 mmol) were added under argon to the stirring solution. The mixture was refluxed for 19 hrs, cooled to room temperature, and evaporated to dryness. The residue was dissolved in 300 mL methylene chloride, washed with 10 % ammonium hydroxide (3 x 150 mL), dried over sodium sulfate, filtered and evaporated. The crude product was purified on a silica gel column using 100% hexane to 50 % ethyl acetate / hexane as eluant gradient to give 4-isopropyl-1-nitro-2-

vinylbenzene (1.37g, 7.16 mmol, 47.6 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.27 (s, 3H); 1.30 (s, 3H); 3.00 (h, J = 6.9 Hz, 1H); 5.44 (dd, J = 1, 10.0 Hz, 1H); 5.72 (dd, J = 1.1, 16.2 Hz, 1H); 7.22 (m, 2H); 7.43 (d, J = 2.0 Hz, 1H); 7.90 (d, J = 8.5 Hz, 1H).

(*Z*)-4-Isopropyl-2-(5-methoxy-2-methylstyryl)-1-nitrobenzene: This compound was made using the Heck coupling method.[5] 4-Methoxy-2-methylbenzoic acid (1.043 g, 6.28 mmol), silver oxide (1.454 g, 6.28 mmol), palladium acetate (70mg, 0.314 mmol) and molecular sieves (3 g, 6.28 mmol) were slowly stirred under a nitrogen atmosphere in a 50mL RB flask fitted with a reflux condenser and a magnetic stir bar. 4-Isopropyl-1-nitro-2-vinylbenzene (1.2 g, 6.28 mmol) was dissolved in 15 mL anhydrous dimethylformamide and added under nitrogen to the stirring mixture. The resulting mixture was then heated to 110 °C for 20 hrs, allowed to cool to room temperature and then filtered through celite. The filtrate was diluted with 100 mL water and extracted with ethyl acetate (3 x 75 mL). The combined organic layer was washed with 50 mL saturated sodium bicarbonate, dried over sodium sulfate, filtered and evaporated. The crude product was purified by silica gel chromatography using 100% hexane to 50 % ethyl acetate / hexane as eluant gradient to give (*Z*)-4-isopropyl-2-(5-methoxy-2-methylstyryl)-1-nitrobenzene (410mg, 1.317 mmol, 20.98 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): 1.3 (s, 3H); 1.33 (s, 3H); 2.43 (s, 3H); 3.02 (heptet, J = 6.9 hz, 1H); 3.82 (s, 3H); 6.76 (m, 2H); 7.21 (d, J = 16 Hz, 1H); 7.24 (dd, J = 1.95, 6.3 Hz, 1H); 7.43 (d, J = 16 Hz, 1H); 7.52 (d, J = 1.9 Hz, 1H); 7.56 (d, J = 8.5 Hz, 1H); 7.92 (d, J = 8.5 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$): 20.4, 23.8, 34.5, 55.5, 112.1, 116, 123.8, 125.4, 125.8, 126.6, 127.6, 128.7, 131, 134.2, 138, 146.2, 155, 159.9.

(*Z*)-4-Isopropyl-2-(5-methoxy-2-methylstyryl)aniline: This compound was made by following the reduction method of Wassmundt and Kiesman.[6] Iron (II) sulfate (2.387 g, 8.58 mmol) was dissolved in 18 mL water and 20 mL concentrated ammonium hydroxide in a 100mL RB flask fitted with a reflux condenser and a magnetic stir bar. The mixture was then stirred and heated to boiling resulting in a dark solution with very dark brown precipitate. (Z)-4-isopropyl-2-(5-methoxy-2-methylstyryl)-1-nitrobenzene (405 mg, 1.301 mmol) was dissolved in 18 mL 10% ammonium hydroxide (heated to dissolve) and added to the boiling mixture. The reaction mixture was further boiled 15 min. The mixture was hot filtered through celite to remove the brown solids and the resulting filtrate neutralized with concentrated acetic acid, then extracted with 25 mL methylene chloride. The organic layer was dried over sodium sulfate and filtered. The celite retaining the brown solid was further washed with 50 mL methylene chloride, which was combined with the previous organic layer and evaporated to dryness. The crude product was purified by silica gel chromatography using 100 % hexane to 30 % ethyl acetate / hexane as eluant gradient to give (Z)-4-isopropyl-2-(5-methoxy-2-methylstyryl)aniline (210mg, 0.746 mmol, 57.4 % yield). ^{1}HNMR (300 MHz, $CDCl_3$): 1.24 (d, J = 2.07 Hz, 3H); 1.27 (d, J = 1.83 Hz, 3H); 2.38 (s, 3H); 2.89 (heptet, J = 7.0 Hz, 1H); 3.78 (s, 3H); 6.7 (m, 2H); 6.9 (d, J = 8.19 Hz, 1H); 6.98 (dd, J = 2.07, 6.21 Hz, 1H); 7.03 (d, J = 16.6 Hz, 1H); 7.16 (d, J = 15.8 Hz, 1H); 7.29 (d, J = 1.86 Hz, 1H); 7.6 (d, J = 8.28 Hz, 1H).

4-Methoxyretene: This compound was made by following the diazotization and cyclization method of Wassmundt and Kiesman.[6] Fluoroboric acid (1.416 ml, 11.37 mmol) in a 50 mL RB flask containing a magnetic stir bar was cooled to 0-5°C. A solution of (Z)-4-isopropyl-2-(5-

methoxy-2-methylstyryl)aniline (200mg, 0.711 mmol), sodium hydroxide (85mg, 2.132 mmol), sodium nitrite (147mg, 2.132 mmol) and 10 mL water was added dropwise over 20 minutes to the fluoroboric acid while stirring. Stirring was continued for 50 minutes resulting in the formation of a precipitate. Sulfamic acid (69mg, 0.711 mmol) was added until the solution reacted negatively to starch-iodide paper. The mixture was extracted with 15 mL ethyl acetate, dried over sodium sulfate, filtered and evaporated to dryness. The crude product was purified by silica gel chromatography using 100% hexane to 50% ethyl acetate / hexane as eluant gradient to give the intermediate (Z)-4-isopropyl-2-(5-methoxy-2-methylstyryl)benzenediazonium tetrafluoroborate (170mg, 0.526 mmol, 74.0 % yield), which was used directly in the next step.

Ferrocene (26mg, 0.139 mmol) was dissolved under argon in 10 mL anhydrous acetone in a 25mL RB flask containing a magnetic stir bar. (*Z*)-4-Isopropyl-2-(5-methoxy-2-methylstyryl)benzenediazonium tetrafluoroborate (170 mg, 0.447 mmol) was dissolved in 1 mL anhydrous acetone and added dropwise to ferrocene solution while stirring at room temperature 30 min. The green reaction mixture was added to 10 mL water and the resulting precipitate was filtered and dried. Additional product was obtained by extraction of the aqueous layer with methylene chloride (3 x 30 mL) and combined with the precipitate and evaporated. The crude product was purified by silica gel chromatography using 100% hexane to 70% ethyl acetate / hexane as eluant gradient to give 4-methoxyretene (104mg, 0.393 mmol, 88 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): 1.2 (d, J = 6.3 Hz, 3H); 1.26 (d, d, J = 6.3 Hz, 3H); 1.92 (d, J = 17.7 Hz, 3H); 2.88 (h, J = 6.7 Hz, 1H); 3.76 (d, J = 11.9 Hz, 3H); 6.64 (d, J = 11.3 Hz, 1H); 6.72 (d, J = 9.4 Hz, 1H); 6.94 (d, J = 9.6 Hz, 1H); 7.23 (m, 3H); 7.68 (d, J = 9.4 Hz, 1H).

4-Hydroxyretene: This compound was made using the demethylation method of Amin *et. al.*[7] Lithium aluminum hydride (115 mg, 3.03 mmol) was dissolved under argon at room temperature in 4 mL anhydrous tetrahydrofuran in a 50mL RB flask fitted with a reflux condenser and containing a magnetic stir bar. 4-Methoxy-retene (100mg, 0.378 mmol) was dissolved in 15 mL anhydrous tetrahydrofuran under argon and added dropwise slowly over 5 min. Stirring was continued for 90 mins at 25 °C then 4 hrs at 70 °C. The solution was allowed to cool to room temperature overnight, diluted slowly with water and extracted with ethyl acetate (3 x 20 mL). The organic solution was dried over sodium sulfate, filtered and evaporated. The crude product was purified by silica gel chromatography using 100% hexane to 30% ethyl acetate / hexane as eluant gradient to give 4-hydroxyretene (12mg, 0.048 mmol, 12.8 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): 1.32 (d, J = 2.49 Hz, 3H); 1.35 (d, J = 2.58 Hz, 3H); 2.69 (s, 3H); 2.89 (heptet, J = 6.84 Hz, 1H); 6.58 (d, J = 7.89 Hz, 1H); 6.77 (dd, J = 2.43, 12.48 Hz, 1H); 6.99 (d, J = 8.19 Hz, 1H); 7.26 (m, 1H); 7.33 (d, J = 8.7 Hz, 1H); 7.49 (s, 1H); 7.69 (d, J = 9.54 Hz, 1H).

6-Hydroxyretene

Methyl abietate:[8,9] Abietic acid (6.5 g, 16.12 mmol) was dissolved in 25 mL dimethylformamide in a 50 mL RB flask containing a magnetic stir bar. Lithium hydroxide (1.158 g, 48.4 mmol) was added and the mixture was stirred for 48 hrs resulting in a yellow/orange solution and the formation of a precipitate. Dimethyl sulfate (11.74 mL, 124 mmol) was added and stirring was continued for 72 hrs at room temperature giving a clear orange solution. The mixture was poured into 50 mL water and extracted with hexane (3 x 50

mL). The combined organic layers were washed first with 0.1 N sodium hydroxide, followed by saturated aqueous sodium chloride. The resulting organic layer was then dried over sodium sulfate, filtered and evaporated. The resulting oily residue was triturated with saturated aqueous sodium chloride and the aqueous liquid layer decanted. The oily residue was further dried to give methyl abietate (4.69g, 14.82 mmol, 92 % yield) as an orange oil. ^{1}H NMR (300MHz, $CDCl_3$): 0.81 (s, 3H); 0.99 (d, J=5.5Hz, 3H): 1.0 (d, J=5.6Hz, 3H); 1.2 (m, 2H); 1.24 (s, 3H); 1.56 (m, 3H); 1.8 (m, 5H); 2.05 (m, 3H); 2.2 (m, 1H); 3.62 (s, 3H); 5.35 (m, 1H); 5.76 (s, 1H).

Methyl dehydroabietate:[9] Palladium on carbon (237 mg, 0.111 mmol) was added to methyl abietate (4.69 g, 14.82 mmol) in a 25 mL RB flask, fitted with a reflux condenser and magnetic stir bar. The mixture was stirred and heated to 240-250 °C for 2 hrs. The mixture was allowed to cool to room temperature and diluted with 100 mL 20% ethyl acetate / hexane. The solid palladium was filtered off. The mother liquor was evaporated to give methyl dehydroabietate (4.18g, 13.29 mmol, 90 % yield) as a brown oil. ^{1}H NMR (300MHz, $CDCl_3$): 1.21 (s, 6H); 1.23 (s, 3H); 1.27 (s, 3H); 1.36-1.9 (m, 8H); 2.18-2.32 (m, 2H); 2.79-2.96 (m, 3H); 3.66 (s, 3H); 6.88 (d, J = 1.6 Hz, 1H); 6.99 (dd, J = 1.8, 6.5 Hz, 1H); 7.16 (d, J = 8.16 Hz, 1H).

Methyl 6-acetyldehydroabietate:[10,11,12,13] Methyl dehydroabietate (4.18 g, 13.29 mmol) was dissolved under argon in 40 mL anhydrous carbon disulfide in a 100 mL RB flask with a magnetic stir bar and stirred at room temperature. Aluminum chloride (4.79g, 35.9 mmol) was added and the mixture cooled in ice bath. Acetyl chloride (2.93 mL, 41.2 mmol) in 55 mL anhydrous carbon disulfide under argon was added dropwise to the stirring solution. The mixture was refluxed for 4 hrs, poured over 100 mL ice water and the layers were mixed, allowed to come to room temperature and then separated. The aqueous layer was further extracted with chloroform (3 x 100 mL). The combined organic layers were washed with 100mL 5% sodium bicarbonate, dried over sodium sulfate, filtered and evaporated to give methyl 6-acetyldehydroabietate (4.46g, 12.51 mmol, 94 % yield) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$): 1.16 (d, J = 7.5 Hz, 3H); 1.22 (s, 3H); 1.23 (d, 7.6 Hz, 3H); 1.28 (s, 3H); 1.41-1.57 (m, 2H); 1.64-1.84 (m, 5H); 2.20 (dd, J = 2.2, 10.4 Hz, 1H); 2.31 (m, 1H); 2.55 (s, 3H); 2.90 (dd, J = 4.3, 4.7 Hz, 2H); 3.47 (septet, J = 6.8 Hz, 1H); 3.67 (s, 3H); 7.04 (s, 1H); 7.40 (s, 1H).

Methyl 6-O-acetyldehydroabietate:[13,14] Methyl 6-Acetyldehydroabietate (4.41g, 12.37 mmol) was dissolved in 35 mL 1,2-dichloroethane in a 100 mL RB flask fitted with a reflux condenser and magnetic stir bar, and stirred at room temperature. The mixture was cooled to 0 °C and mCPBA (5.55g, 32.2 mmol) and p-toluenesulfonic acid (471mg, 2.474 mmol) were added. The mixture was refluxed for 4 hrs and cooled to room temperature. The organic solution was extracted with 50 mL 1M sodium bicarbonate followed by 50 mL water. The organic layer was dried over sodium sulfate, filtered and evaporated to give a crude product which was purified by silica gel chromatography using 100% hexane to 30% ethyl acetate / hexane as eluant gradient to give methyl 6-O-acetyldehydroabietate (1.69g, 4.54 mmol, 36.7 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): 1.16 (d, J = 7.5 Hz, 3H); 1.22 (s, 3H); 1.23 (d, 7.6 Hz, 3H); 1.28 (s, 3H); 1.38-1.57 (m, 2H); 1.60-1.84 (m, 5H); 2.20 (dd, J = 2.2, 10.4 Hz, 1H); 2.31 (m, 1H); 2.55 (s, 3H); 2.8 (dd, J = 4.35, 4.6 Hz, 2H); 3.12 (quintet, J = 6.8 Hz, 1H); 3.66 (s, 3H); 6.62 (s, 1H); 6.82 (s, 1H).

Methyl 6-hydroxy-dehydroabietate:[13,14] Methyl 6-O-acetyldehydroabietate (1.69g, 4.54 mmol) was dissolved in 9 mL methanol and 36 mL water in a 100mL RB flask containing a magnetic stir bar. Sodium bicarbonate (3.05 g, 36.3 mmol) was added and the mixture was stirred at room temperature for 4 hrs. Most of the methanol was removed by evaporation and the resulting aqueous layer extracted with ethyl acetate (3 x 60 mL), dried over sodium sulfate, filtered and evaporated to give methyl 6-hydroxy-dehydroabietate (1.21 g, 3.66 mmol, 81 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.19 (s, 3H); 1.21 (d, J = 4.6 Hz, 3H); 1.23 (d, J = 4.6 Hz, 3H); 1.26 (s, 3H); 1.26-1.35 (m, 2H); 1.61-1.81 (m, 5H); 2.16 - 2.29 (m, 2H); 2.8 (dd, J = 4.4, 4.7 Hz, 2H); 3.12 (septet, J = 6.9 Hz, 1H); 3.66 (s, 3H); 6.63 (s, 1H); 6.82 (s, 1H).

6-Hydroxyretene:[13,14] Under a stream of nitrogen, methyl 6-hydroxydehydroabietate (450mg, 1.36 mmol) was mixed with selenium (480 mg, 6.13 mmol) in a 10mL RB flask fitted with a reflux condenser containing a magnetic stir bar and then heated at 280-285 °C for 19 hrs followed by heating at 335°C for 4 hrs. The product was cooled to room temperature and extracted with 50 mL ether, washed with 50 mL 5% sodium hydroxide, dried over sodium sulfate, filtered and evaporated. The solid was triturated with hexane, filtered and dried. The crude product was purified on a silica gel column using 100% hexane to 60% ethyl acetate / hexane as the eluant gradient to give 6-hydroxyretene (120mg, 0.48 mmol, 35.2% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): 1.39 (d, J = 6.9 Hz, 6H); 2.73 (s, 3H); 3.39 (heptet, J = 6.9 Hz, 1H); 5.14 (bs, 1H); 7.39 (d, J = 7.0 Hz, 1H); 7.48 (t, J = 7.2 Hz, 1H); 7.69 (s, 1H); 7.7 (d, J = 9.3 Hz, 1H); 7.78 (d, J = 9.0 Hz, 1H); 7.94 (s, 1H); 8.37 (d, J = 8.1 Hz, 1H). Maldi MS^+ = 250.1; MW = 250.335 g/moL.

REFERENCES

(1) Campbell, W. P. and Todd, D. (1940) Acetylretene and 6-retenol. *J. Amer. Chem. Soc. 62*, 1287-1292.
(2) Minabe, M., Cho, B. P. and Harvey, R. G. (1989) Polycyclic fluoranthene hydrocarbons. Part 4. Electrophilic substitution of polycyclic fluoranthene hydrocarbons. *J. Amer. Chem. Soc. 111*, 3809-3812.
(3) Cheng, C. C., Huang, X., Shipps, G. W. Jr., Wang, Y-S., Wyss, D. F., Soucy, K. A., Jiang, C-K., Agrawal, S., Ferrari, E., He, Z., and Huang, H-C. (2010) Pyridine carboxamide potent palm site inhibitors of HCV NS5B polymerase. *ACS Med. Chem. Lett. 1*, 466-471.
(4) Soderberg, B. C. and Shriver, J. A. (1997) Palladium-catalyzed synthesis of indoles by reductive N-heteroannulation of 2-nitrostyrenes. *J. Org. Chem*. *62*, 5838-5845.
(5) Nandi, D., Ghosh, D., Chen, S.-J., Kuo, B.-C., Wand, N. W., and Lee, H. M. (2013) One-step synthesis of isocoumarins and 3-benzylidenephthalides via ligandless Pd-catalyzed oxidative coupling of benzoic acids and vinylarenes. *J Org Chem 78*, 3445-3451.
(6) Wassmundt, F.W. and Kiesman, W. F. (1995) Soluble catalysts for improved Pschorr cyclizations. *J. Org. Chem*. *60*, 196-201.
(7) Amin, S., Hecht, S. S., LaVoie, E., and Hoffmann, D. (1979) Synthesis and mutagenicity of 5,11-dimethylchrysene and some methyl-oxidized derivatives of 5-methylchrysene. *J. Med. Chem. 22,* 1336-1340

(8) Gonzalez, M. A., Correa-Royero, J., Agudelo, L., Mesa, A., and Betancur-Galvis, L. (2009) Synthesis and biological evaluation of abietic acid derivatives. *Eur. J. Med. Chem. 44*, 2468-2472.
(9) Gonzalez, M. A., Perez-Guaita, D., Correa-Royero, J., Zapata, B., Agudelo, L., Mesa-Arango, A., and Betancur-Galvis, L. (2010) Synthesis and biological evaluation of dehydroabietic acid derivatives. *Eur. J. Med. Chem. 45*, 811-6.
(10) Silvestre, A.J.D., Monteiro, M.C., Silva, A.M.S., Cavaleiro, J.A.S., Felix, V.M.S., Ferreira, P., and Drew, M. G. (1998) New methyl dehydroabietate derivatives. Synthesis and structural characterization. *Monatshefte fuer Chemie 129*, 1183-1197.
(11) Burnell, R. H. and Cote, C. (1988) Substitution of the isopropyl residue of dehydroabietane derivatives. A retro-Friedel-Crafts reaction. *Syn Comm 18*, 1753-1756.
(12) Tolmacheva, I. A., Tarantin, A. V., Boteva, A. A., Anikina, L. V., Vikharev, Y. B., Grishko, V. V., and Tolstikov, A. G. (2006) Synthesis and biological activity of nitrogen-containing derivatives of methyl dehydroabietate. *Pharm Chem J 40*, 489-493.
(13) Cui, Y-M., Yasutomi, E., Otani, Y., Yoshinage, T., Ido, K., Sawada, K., and Ohwada, T. (2008) Novel BK channel openers containing dehydroabietic acid skeleton: structure-activity relationship for peripheral substituents on ring C. *Bioorg Med Chem Lett 18*, 5201-5205.
(14) Alvarez-Mazaneda, E., Chahboun, R., Cabrera, E., Alvarez, E., Alvarez-Manzaneda, R., Lachkar, M., and Messouri, I. (2007) First synthesis of picealactone C. A new route toward taxodione-related terpenoids from abietic acid. *Tetrahedron Lett. 48*, 989-992.