Metabolism of an Alkylated Polycyclic Aromatic Hydrocarbon 5-Methylchrysene in Human Hepatoma (HepG2) Cells

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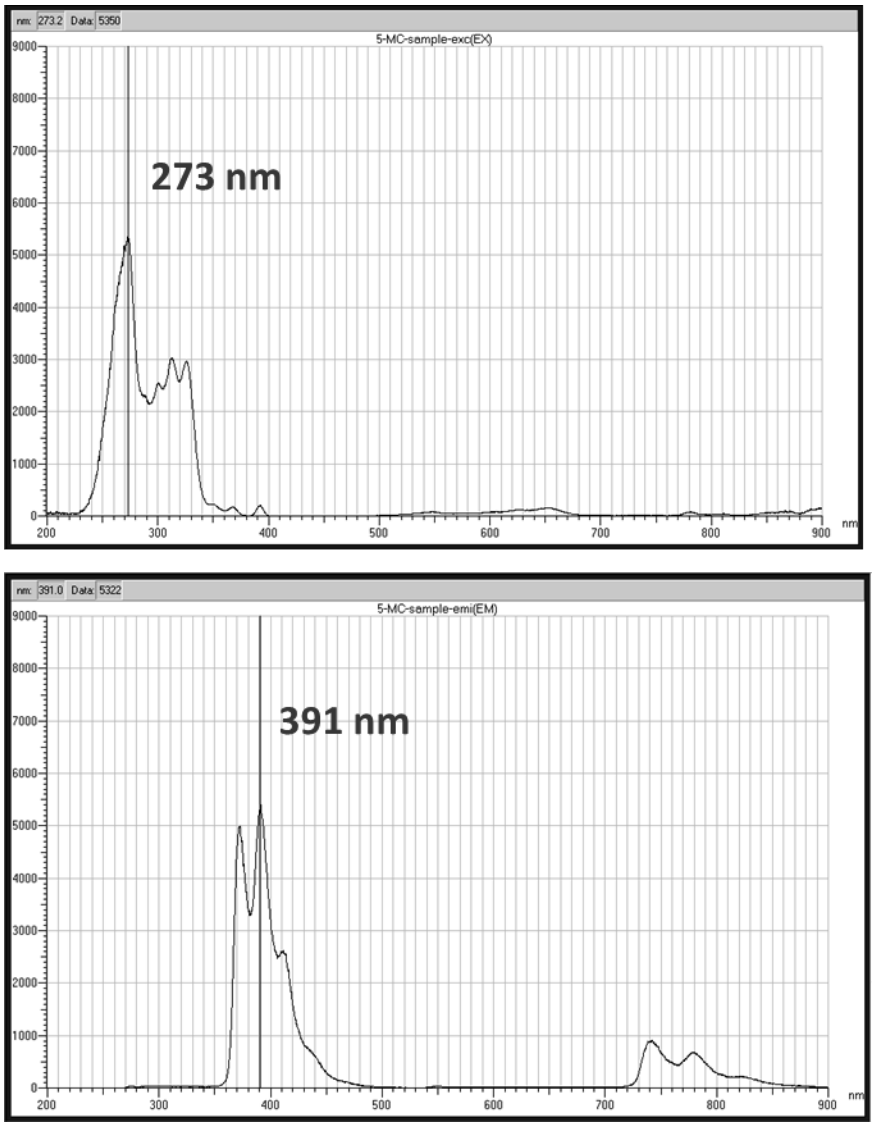

Figure S1. Excitation wavelength and emission wavelength spectra of 5-MC.

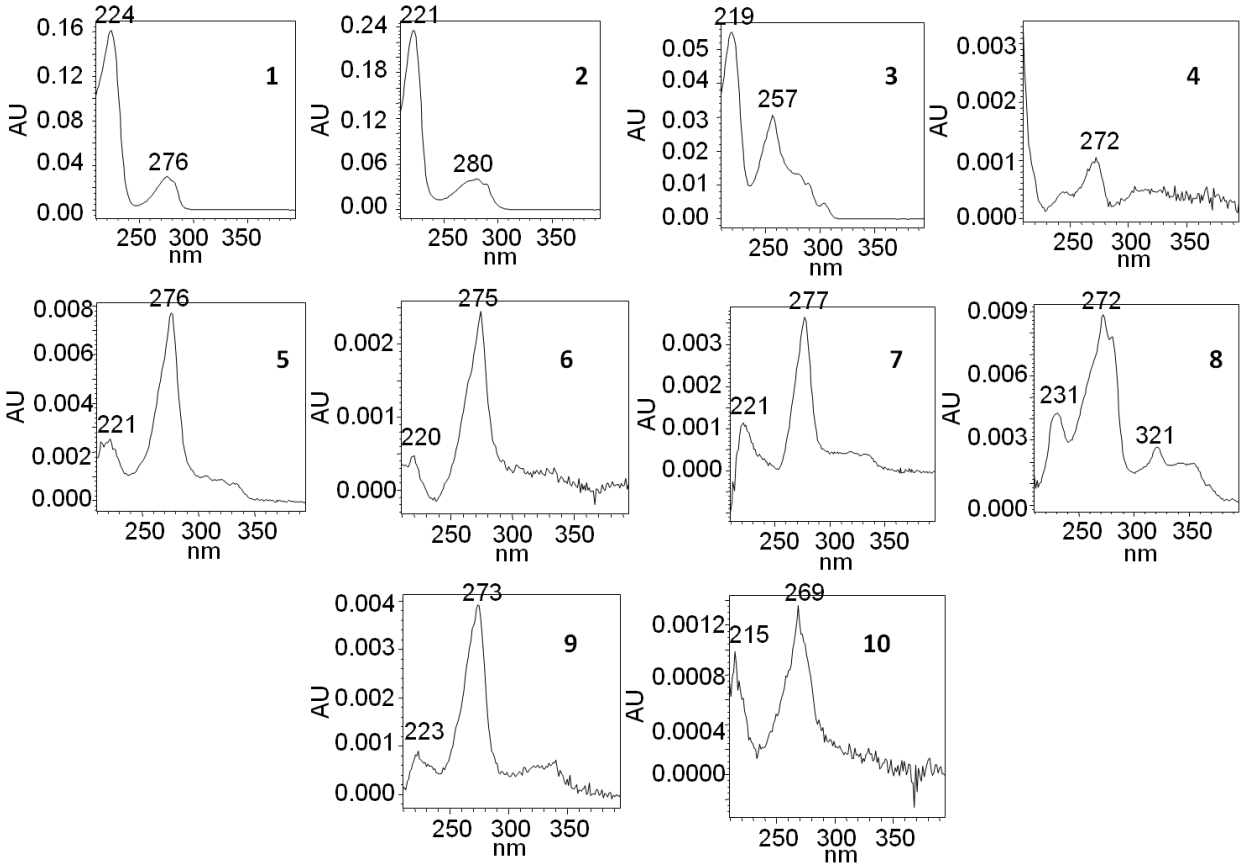

Figure S2. UV spectra of metabolites 1-10 in HepG2 cells.

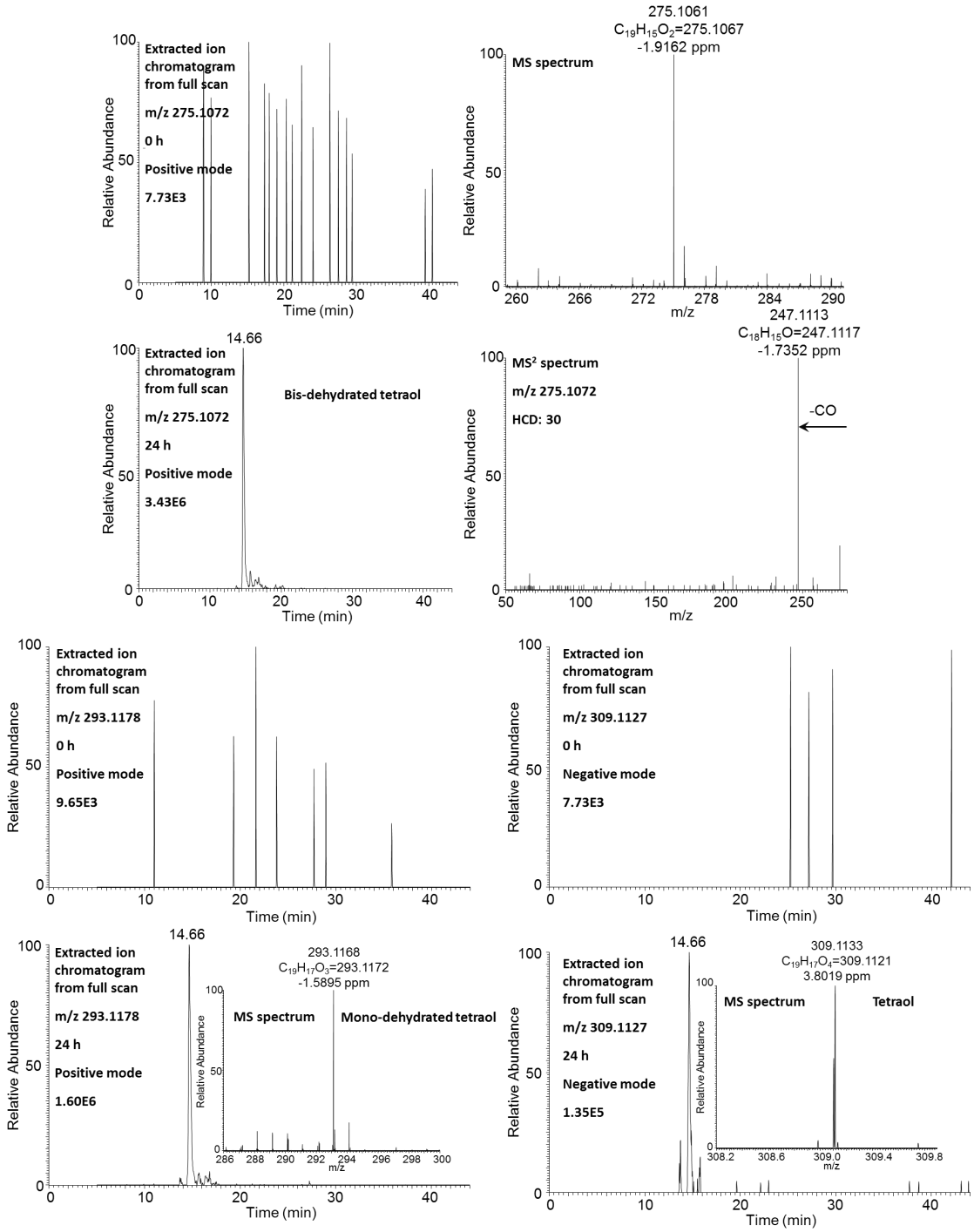

Figure S3. Extracted ion chromatograms of Orbitrap full scan for mono-dehydrated 5-MC-tetraol, bis-dehydrated 5-MC-tetraol, and 5-MC-tetraol detected in HepG2 cells.

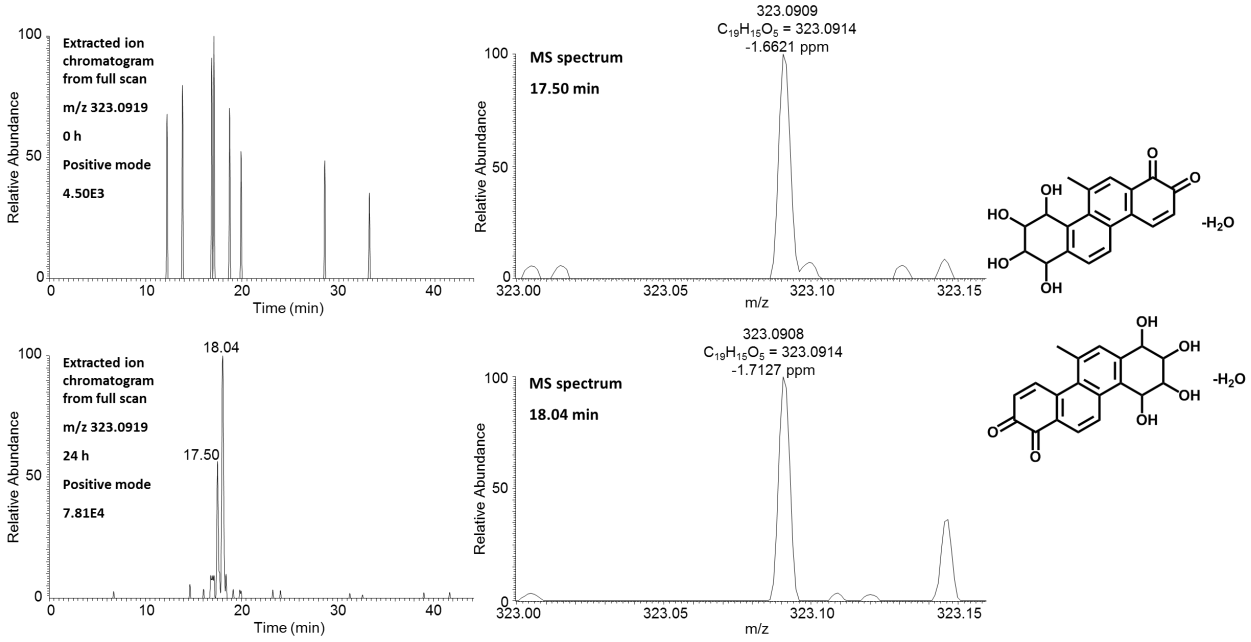

Figure S4. Extracted ion chromatograms of Orbitrap full scan for mono-dehydrated tetrahydroxy-5-MC-dione detected in HepG2 cells.

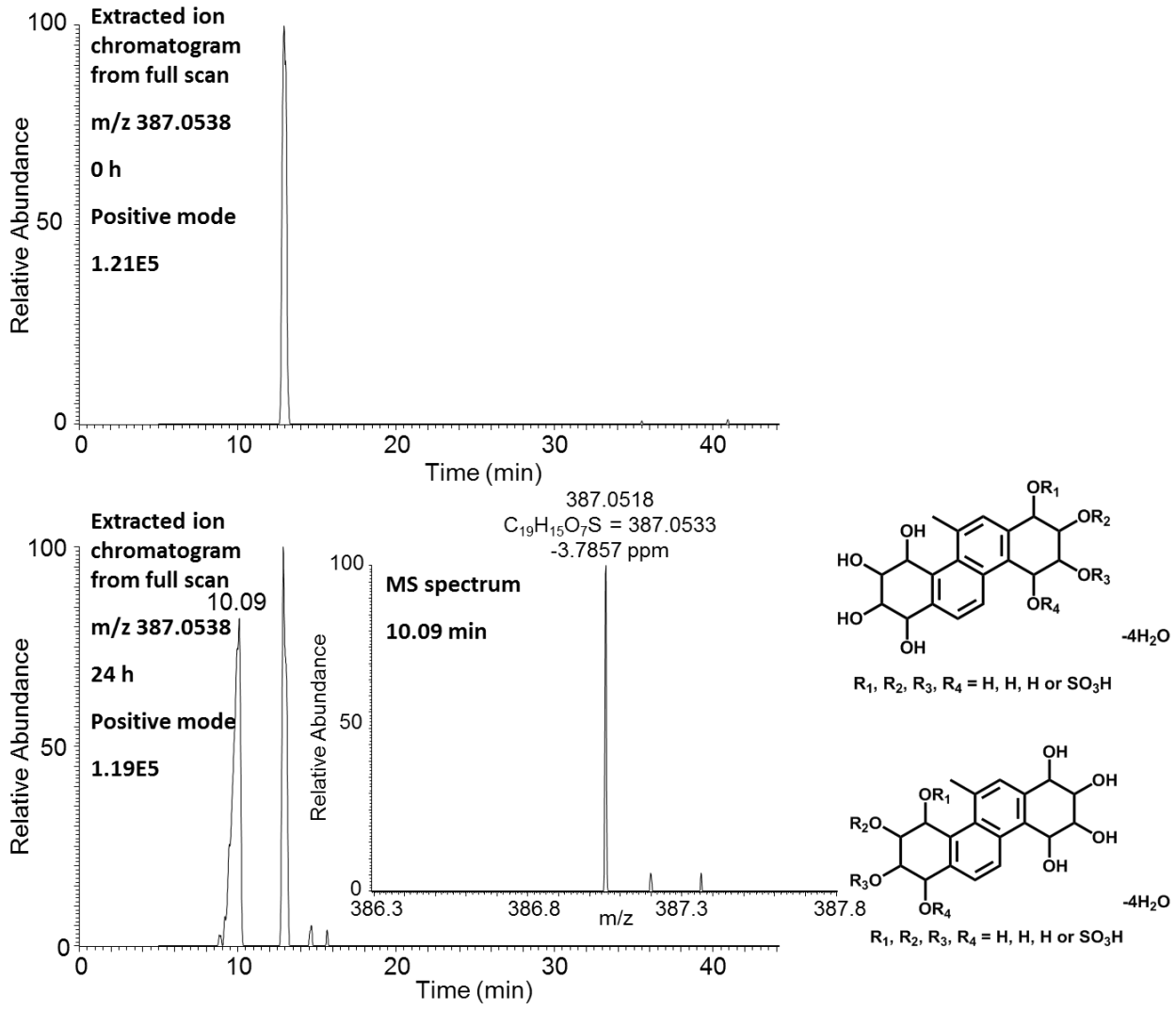

Figure S5. Extracted ion chromatograms of Orbitrap full scan for bis-dehydrated-tetraol plus O-monosulfonated-bis-dehydrated-tetraol detected in HepG2 cells.

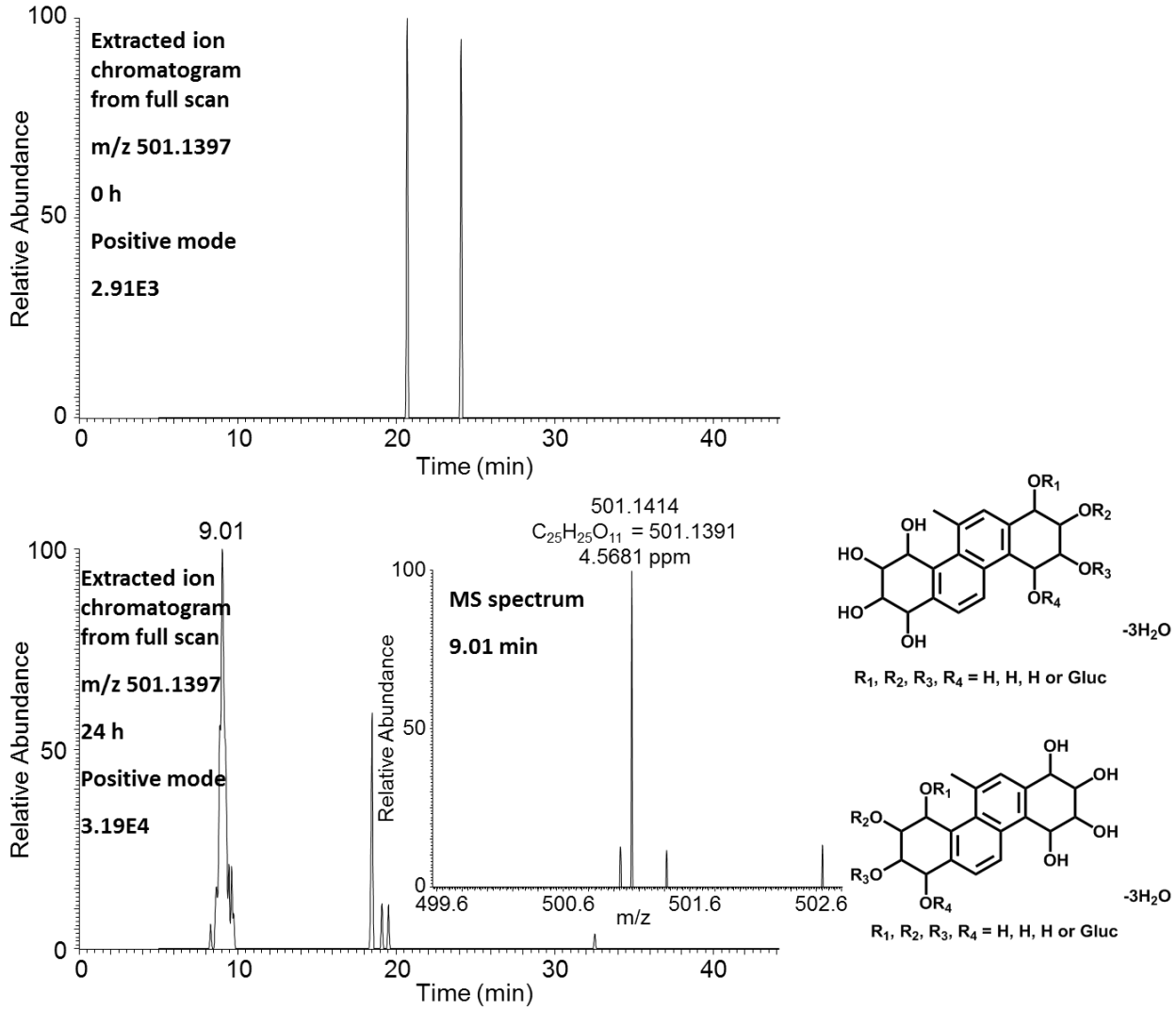

Figure S6. Extracted ion chromatograms of Orbitrap full scan for mono-dehydrated-tetraol plus O-monoglucuronosyl-bis-dehydrated-tetraol detected in HepG2 cells.

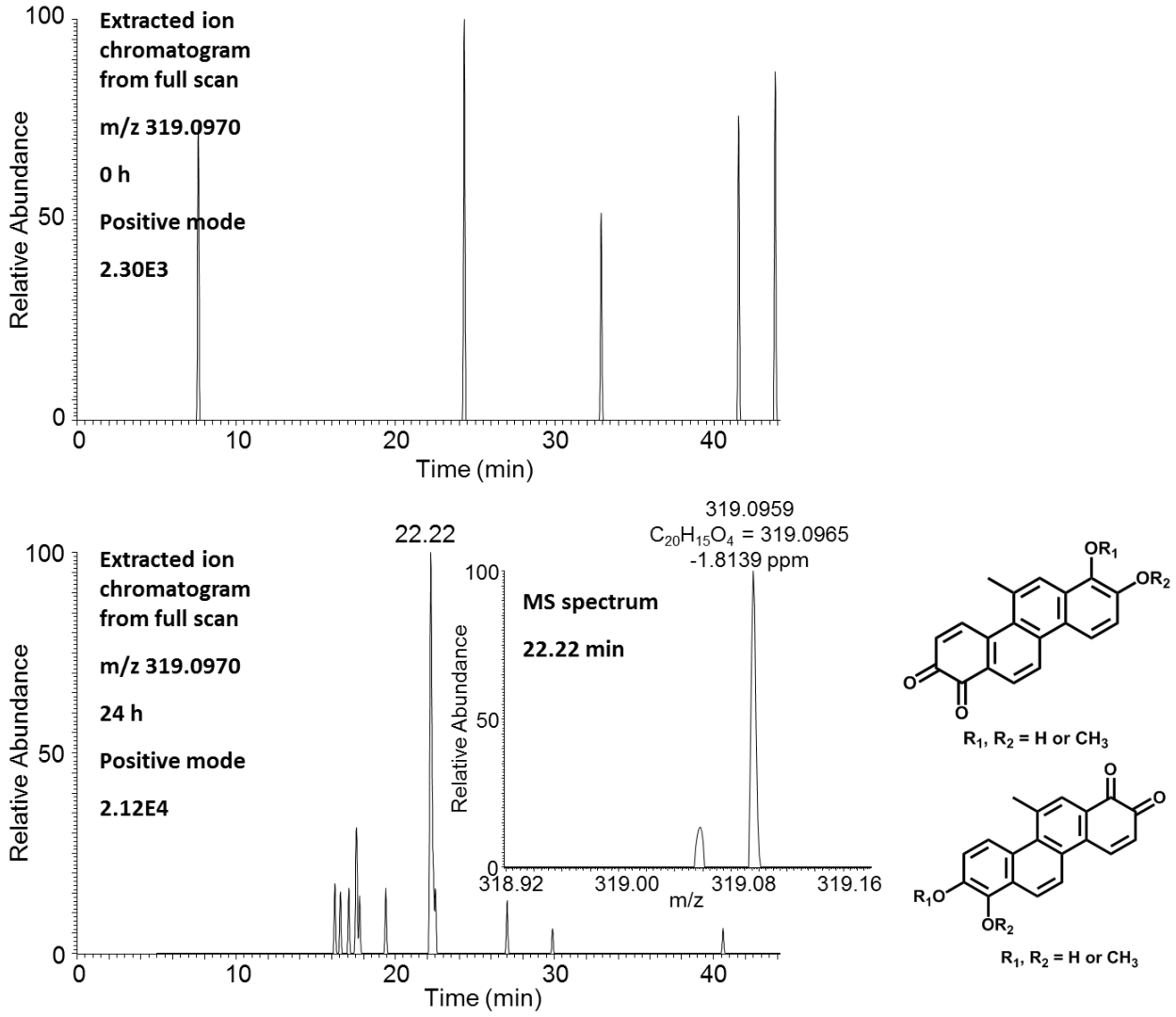

Figure S7. Extracted ion chromatograms of Orbitrap full scan for O-monomethyl-catechol-5-MC-ortho-quinone detected in HepG2 cells.

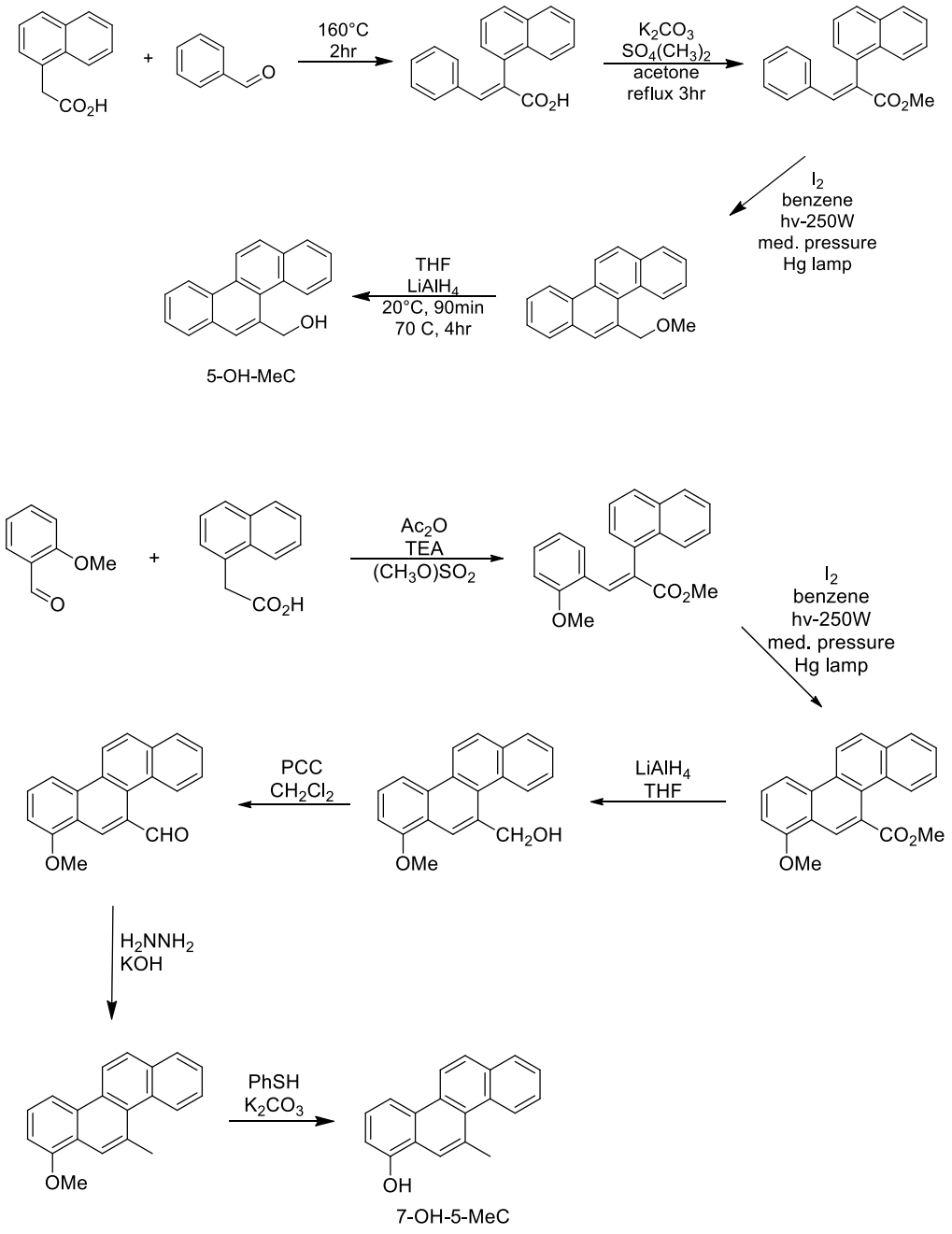

Figure S8. Synthetic routes and details of synthesis of 5-hydroxy-5-MC and 7-hydroxy-5-MC.

^{1}H NMR spectra were acquired on a Bruker Ultrashield 300, Advance III, 300 MHz.

Mass spectra were acquired on a Sciex 5800 Maldi TOF/TOF in negative mode.

5-Hydroxymethylchrysene (Figure S8)

5-Hydroxymethylchrysene was prepared using the procedure of Amin, S.; Hecht, S. S.; La Voie, E. and Hoffmann, D.[37] with the following modifications. The photolysis was done using a 450-W medium pressure mercury lamp for 4 ½ hr. 5-Hydroxymethylchrysene was purified by silica gel chromatography using hexane to 30% ethyl acetate / hexane as the eluent (27.3 % yield).

5-hydroxymethylchrysene: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.43 (s, 2H); 7.66 (m, 4H); 7.98 (m, 3H); 8.14 (s, 1H); 8.73 (dd, 1H, J = 0.57, 7.7 Hz); 8.73 (d, 1H, J = 9.2 Hz, 1H); 8.93 (dd, 1H, J = 0.96, 7.3 Hz). Maldi-MS^{-} m/z = 256; MW 258.3 g/mol.

7-Hydroxy-5-methylchrysene (Figure S8)

(E)-Methyl 3-(2-methoxyphenyl)-2-(naphthalen-1-yl)-acrylate [38]: 1-Naphthylacetic acid (10 g, 53.7 mmol) and 2-methoxybenzaldehyde (7.31 g, 53.7 mmol) were mixed in acetic anhydride (5.08 ml, 53.7 mmol). Triethylamine (5.24 ml, 37.6 mmol) was added and the mixture heated to 140°C for 21 hr. The mixture was cooled and diluted with water (75 mL), acidified with hydrochloric acid (29.1 ml, 354 mmol), and extracted with methylene chloride (2 x 75 mL). The organic layer was washed with water, dried over sodium sulfate and evaporated to dryness to give the crude acid (12.16 g) which was dissolved in acetone (Volume: 500 ml). Potassium carbonate (7.66 ml, 53.7 mmol) and dimethyl sulfate (16.88 g, 53.7 mmol) were added and the mixture was refluxed for 20 h. The potassium carbonate was filtered off and washed with

methylene chloride. The organic filtrate was washed with water, dried over sodium sulfate and evaporated to dryness. Silica gel chromatography (eluent: 20 / 80 methylene chloride / hexane) was done to purify (E)-methyl 3-(2-methoxyphenyl)-2-(naphthalen-1-yl)-acrylate (6.37 g, 20.01 mmol, 37.3 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.66 (s, 3H); 3.78 (s, 3H); 6.34 (tt, J = 0.51, 7.83 Hz, 1H); 6.44 (dd, J = 1.74, 6.06 Hz; 1H); 6.74 (dd, J = 0.81, 7.53 Hz, 1H); 7.03 (td, J = 1.74, 6.81Hz, 1H); 7.25 (dd, J = 1.26, 5.76 Hz, 1H); 7.39 (m , 3H); 7.8 (m, 3H); 8.51 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 52.1, 55.4, 110.3, 119.8, 123.3, 125, 125.6, 125.8, 126.2, 127.3, 128.1, 128.3, 129.7, 130.3, 130.4, 132.1, 133.5, 134.1, 136.8, 158.1, 168.6.

Methyl-7-methoxychrysene-5-carboxylate [38, 39]: (E)-Methyl-3-(2-methoxyphenyl)-2-(naphthalen-1-yl)-acrylate (6 g, 18.85 mmol) was dissolved in ether (Ratio: 4.00, Volume: 200 mL) and dichloromethane (Ratio: 1.000, Volume: 50 mL) and air was bubbled through the solution for 10 min. Iodine (0.431 g, 1.696 mmol) was added and the mixture was photolyzed with a 450 W medium pressure Hg lamp for 6 hr. Saturated sodium thiosulfate (50 mL) was added and the organic layer was dried over sodium sulfate and evaporated. Silica gel chromatography (eluent: 3 : 7 ethyl acetate : hexane) was done to purify methyl-7-methoxychrysene-5-carboxylate (2.78 g, 8.79 mmol, 46.6% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.98 (s, 3H); 4.03 (s, 3H); 6.98 (d, J = 7.71Hz, 1H); 7.6 (m, 3H); 7.96 (m, 2H); 8.20 (m, 1H); 8.25(dd, J = 0.45, 8.13 Hz, 1H); 8.61 (d, J = 9.18 Hz, 1H); 8.69 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 52.8, 55.9, 105.9, 115.5, 121.5, 122.3, 124.1, 125.8, 125.9, 126.7, 126.8, 128.0, 128.5, 128.6, 128.8, 129.5, 129.6, 132.7, 133.0, 156.6, 172.8.

(7-methoxychrysen-5-yl)-methanol [38]: Lithium aluminum hydride (0.557 g, 14.67 mmol) was suspended in anhydrous THF (Ratio: 2.000, Volume: 200 ml). Methyl-7-methoxychrysene-5-carboxylate (2.73 g, 8.63 mmol) was dissolved in anhydrous THF (Ratio: 1.000, Volume: 100 ml) and added dropwise. The mixture was stirred for 2hr and poured into water (300 mL) and extracted with ether (3 x 150 mL). The organic layer was washed with water, dried over sodium sulfate and evaporated to dryness to give (7-methoxychrysen-5-yl)-methanol (1.9 g, 6.59 mmol, 76% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.09 (s, 3H); 5.44 (s, 2H); 7.02 (d, J = 7.71 Hz, 1H); 7.66 (m, 3H); 8.0 (m, 2H); 8.32 (dd, J = 0.51, 8.07 Hz, 1H); 8.59 (s, 1H); 8.7 (d, J = 9.12 Hz, 1H); 9.03 (dd, J = 0.84, 7.5 Hz, 1H).

7-Methoxychrysene-5-carbaldehyde [38]: Pyridinium chlorochromate (2.7 g, 12.52 mmol) was dissolved in dichloromethane (Ratio: 2.52, Volume: 327 ml). (7-Methoxychrysen-5-yl)-methanol (1.9 g, 6.59 mmol) was dissolved in dichloromethane (Ratio: 1.000, Volume: 130 ml) and added dropwise over 10 min. The mixture was stirred for 3hr and then diluted with ether (Ratio: 1.538, Volume: 200 ml). The mixture was filtered through silica gel and evaporated. 7-Methoxychrysene-5-carbaldehyde (0.89 g, 3.11 mmol, 47.2 % yield) was recrystallized from methylene chloride / hexane. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.04 (s, 3H); 7.06 (d, J = 7.68 Hz, 1H); 7.8 (m, 3H); 8.06 (d, J = 8.34 Hz, 1H); 8.24 (m, 1H); 8.3 (d, J = 8.4 Hz, 1H); 8.67 (d, J = 8.82 Hz, 1H); 9.03 (s, 1H); 10.64 (s, 1H).

7-Methoxy-5-methylchrysene [38, 39]: 7-Methoxychrysene-5-carbaldehyde (0.88 g, 3.07 mmol) was dissolved in diethylene glycol (Ratio: 4.81, Volume: 154 ml). Hydrazine hydrate (6.13 ml, 126 mmol) and potassium hydroxide (0.69 g, 12.29 mmol) were added and the mixture was heated to reflux for 2hr. The mixture was cooled and hydrochloric acid (Ratio: 1.000, Volume:

32 ml) was added. The mixture was extracted with methylene chloride (3 x 75 mL). The organic layer was washed with water, dried over sodium sulfated and evaporated to dryness. Silica gel chromatography (eluent: 20 / 80 ethyl acetate / hexane) was done to purify 1-methoxy-11-methylchrysene which was further purified by recrystallization from ethanol (0.64 g, 2.350 mmol, 76 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.25 (s, 3H); 4.07 (s, 3H); 6.99 (d, J = 7.74 Hz, 1H): 7.55 (t, J = 8.46 Hz, 1H); 7.61 (m, 2H); 7.95 (d, J = 9 Hz, 1H); 7.99 (m, 1H); 8.30 (dd, J = 0.57, 7.98 Hz, 1 H); 8.32 (s, 1H); 8.71 (d, J = 7.62 Hz, 1H); 8.98 (m, 1H).

7-Hydroxy-5-methylchrysene [39, 40]: 7-Methoxy-5-methylchrysene (0.63 g, 2.313 mmol) was dissolved in 1-methyl-2-pyrrolidinone (Volume: 2.3 mL) and thiophenol (0.238 mL, 2.313 mmol) was added under a nitrogen atmosphere. Potassium carbonate (0.016 g, 0.116 mmol) was added and the mixture was heated to reflux for h. The solution was cooled to room temperature and made basic with 5% sodium hydroxide (23 mL). The aqueous layer was extracted with ether (3 x 15 mL). The aqueous layer was acidified with 6N hydrochloric acid and extracted with ether (3 x 15 mL). The combined organic layers were washed with brine, dried over sodium sulfate and evaporated. Silica gel chromatography (eluent: 60 / 40 methylene chloride / hexane) was done to purify 7-hydroxy-5-methylchrysene (0.33 g, 1.278 mmol, 55.2 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.23 (s, 3H); 5.99 (bs, 1H); 6.93 (d, J = 7.41 Hz, 1H): 7.44 (t, J = 8.28 Hz, 1H); 7.64 (m, 2H); 7.99 (m, 2H); 8.24 (s, 1H); 8.29 (d, J = 8.61 Hz, 1 H); 8.69 (d, J = 9.12 Hz, 1H); 8.97 (m, 1H). ^{13}C NMR (300 MHz, $CDCl_3$): 28.14, 110.03, 115.92, 122.18, 122.30, 123.88, 125.45, 125.93, 125.99, 127.61, 127.91, 128.65, 129.79, 129.81, 131.27, 131.72, 132.67, 133.55, 151.37. Maldi-MS^- m/z = 258.0; MW = 258.3 g/mol.

Supplementary References:

(37) Amin, S., Hecht, S. S., LaVoie, E., and Hoffmann, D. (1979) A study of chemical carcinogenesis. 19. Synthesis and mutagenicity of 5,11-dimethylchrysene and some methyl-oxidized derivatives of 5-methylchrysene. *J. Med. Chem. 22*, 1336-1340.

(38) Amin, S., Balanikas, G., Huie, K., and Hecht, S. S. (1988) Synthesis and tumor-initiating activities of dimethylchrysenes. *Chem. Res. Toxicol. 1*, 349-355.

(39) Amin, S., Hecht, S. S., and Hoffmann, D. (1981) Synthesis of angular ring methoxy-5-methylchrysenes and 5-methylchrysenols. *J. Org. Chem. 46*, 2394-2398.

(40) Chakraborti, A. K., Sharma, L., and Nayak, M. K. (2002) Demand-Based Thiolate Anion Generation under Virtually Neutral Conditions: Influence of Steric and Electronic Factors on Chemo- and Regioselective Cleavage of Aryl Alkyl Ethers. *J. Org. Chem. 67*, 6406-6414.