SUPPORTING INFORMATION FOR:

Synthesis and Characterization of Iodinated Tetrahydroquinolines Targeting the G Protein-coupled Estrogen Receptor GPR30

*Chinnasamy Ramesh[1], Tapan K. Nayak[2,4], Ritwik Burai[1], Megan K. Dennis[2], Helen J. Hathaway[2,3], Larry A. Sklar[3,5], Eric R. Prossnitz[2,3], and Jeffrey B. Arterburn[1,3]**

[1] Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003

[2] Department of Cell Biology and Physiology, School of Medicine, University of New Mexico Health Science Center, Albuquerque, NM 87131

[3] UNM Cancer Center, University of New Mexico Health Science Center, Albuquerque, NM 87131

[4] College of Pharmacy, University of New Mexico Health Science Center, Albuquerque, NM 87131

[5] Department of Pathology, School of Medicine, University of New Mexico Health Science Center, Albuquerque, NM 87131

* Corresponding author. Email: jarterbu@nmsu.edu; Phone 575-646-2738; Fax 575-646-2649.

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Synthetic procedures and characterization data

2-[4-(6-Bromo-benzo[1,3]dioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-8-yl]-ethylamine (10). According to general procedure 6-bromopiperonal (0.229 g, 1 mmol), [2-(aminophenyl)-ethyl-carbamic acid *tert* butyl ester (0.236 g, 1 mmol), cyclopentadiene (0.330 g, 5 mmol) and $Sc(OTf)_3$(0.049 g, 0.1 mmol) were combined with a reaction time of 2 h. The volatiles were removed *in vacuo*. The residue was purified by silica gel column chromatography using ethyl acetate/hexanes (15:85) to provide the ^{t}Boc-protected amine as a colorless solid (0.490 g, 95%). The ^{t}Boc protecting group was removed using trifluoroacetic acid (2.5 mL) in dichloromethane (3 mL) for 1 h at room temperature. The reaction mixture was diluted with dichloromethane (15 mL) and washed with cold saturated $NaHCO_3$ (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by preparative column chromatography using methanol/methylene chloride (20:80) to provide **10** (0.234 g, 95%) as a white solid. Mp: 176-179˚C; IR (KBr, cm^{-1}) 3345, 2932, 1583, 1470, 1238. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.12 s, 1H), 7.02 (s, 1H), 6.89 (s, 1H), 6.81 (dd, *J* = 8.0, 1.8 Hz, 1H), 6.52 (d, *J* = 8.0 Hz, 1H), 5.98 (d, *J* =1.4 Hz, 1H), 5.97 (d, *J* = 1.4 Hz, 1H), 5.87-5.83 (m, 1H), 5.66-5.64 (m, 1H), 4.82 (d, *J* = 2.9 Hz, 1H), 4.04 (d, *J* = 8.8 Hz, 1H), 3.46 (bs, 1H), 3.25-3.00 (m, 3H), 2.84 (t, *J* = 6.8 Hz, 2H), 2.57-2.47 (m, 1H), 1.83-1.72 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 147.4, 147.2, 143.8, 134.5, 133.9,

130.3, 129.5, 129.2, 126.6, 126.2, 116.2, 113.0, 112.8, 108.0, 101.7, 56.7, 46.0, 42.9, 42.1, 37.6, 31.2; HPLC-MS: Elution with 60-90% CH_3CN in H_2O (gradient 1.5 % min^{-1}), exhibited a single peak at 3.92 min. ESI-MS *m/z* [ES+] calcd for $C_{21}H_{21}BrN_2O_2$ $[M+H]^+$ 413.08; found 413.02.

4-(6-Bromo-benzo[1,3]dioxol-5-yl)-8-tributylstannanyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline (15). According to the general procedure 6-bromopiperonal (0.007 g, 0.2 mmol), *p*-tributylstannyl aniline (0.012g, 0.03 mmol), cyclopentadiene (0.010 g, 0.15 mmol), and $Sc(OTf)_3$ (0.001g, 0.003 mmol) were combined with a reaction time of 4 h. The volatiles were removed *in vacuo*. The residue was purified by column chromatography using ethyl acetate/hexanes (10:90) to provide **15** (0.003g, 15%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.0 Hz, 1H) 7.20 (s, 1H), 7.10 (d, *J* = 8.0, 1H), 6.9 (d, *J* = 1.0 Hz, 1H), 7.04 (s, 1H), 5.99-5.98 (m, 2H), 5.86-5.84 (m, 1H), 5.69-5.64 (m, 1H), 4.95 (d, *J* = 3.3 Hz, 1H), 4.60 (bs, 1H), 4.16 (d, *J* = 8.6 Hz, 1H), 3.25-3.21 (m, 1H), 2.65-2.59 (m, 1H), 1.95-1.89 (m, 1H), 1.67-1.63 (m, 6H), 1.37-1.22 (m, 12H), 0.91 (t, *J* = 7.2 Hz, 9H).

4-Tributylstannanyl-phenylamine (16). *p*-Iodoaniline (0.066 g, 0.3 mmol), bistributyltin (0.203 g, 0.35 mmol) and *tetrakis*(triphenylphosphine)palladium (0.033 g, 0.03 mmol) were heated at 115 ^{0}C in dry toluene for 25 h under argon. The volatiles were removed by rotary evaporation and the crude product was purified by column chromatography using ethyl acetate/hexanes (6:94) to provide **16** (0.015g, 13%) as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.2 Hz, 2H), 6.68 (d, *J* = 8.2 Hz, 2H), 3.62 (s, 2H), 1.56-1.51 (m, 6H), 1.33-1.30 (m, 6H), 1.01-0.99 (m, 6H), 0.87 (t, *J* = 7.4 Hz, 9H).

3-Tributylstannanyl-phenylamine (17). *m*-Iodoaniline (0.219g, 1 mmol), bistributyltin (1.16g, 2 mmol) and *tetrakis*(triphenylphosphine)palladium (0.115g, 0.10 mmol) were heated at 100 ^{0}C in dry dioxane for 15 h under argon. The volatiles were removed by rotary evaporation and the crude product was purified by column chromatography using ethyl acetate/hexanes (6:94) to provide **17** (0.297 g, 68%) as a colorless liquid. IR (KBr, cm^{-1}) 3372, 2955, 2923, 1583, 1210; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14-7.09 (m, 1H), 6.87-6.82 (m, 1H), 6.79-6.76 (m, 1H), 6.64-6.60 (m, 1H), 3.57 (s, 2H), 1.58-1.48 (m, 6H), 1.38-1.26 (m, 6H), 1.04-0.99 (m, 6H), 0.88 (t, *J* = 7.4 Hz, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.7, 142.9, 128.5, 126.6, 122.9, 114.9, 29.0, 27.3, 23.6, 19.4.

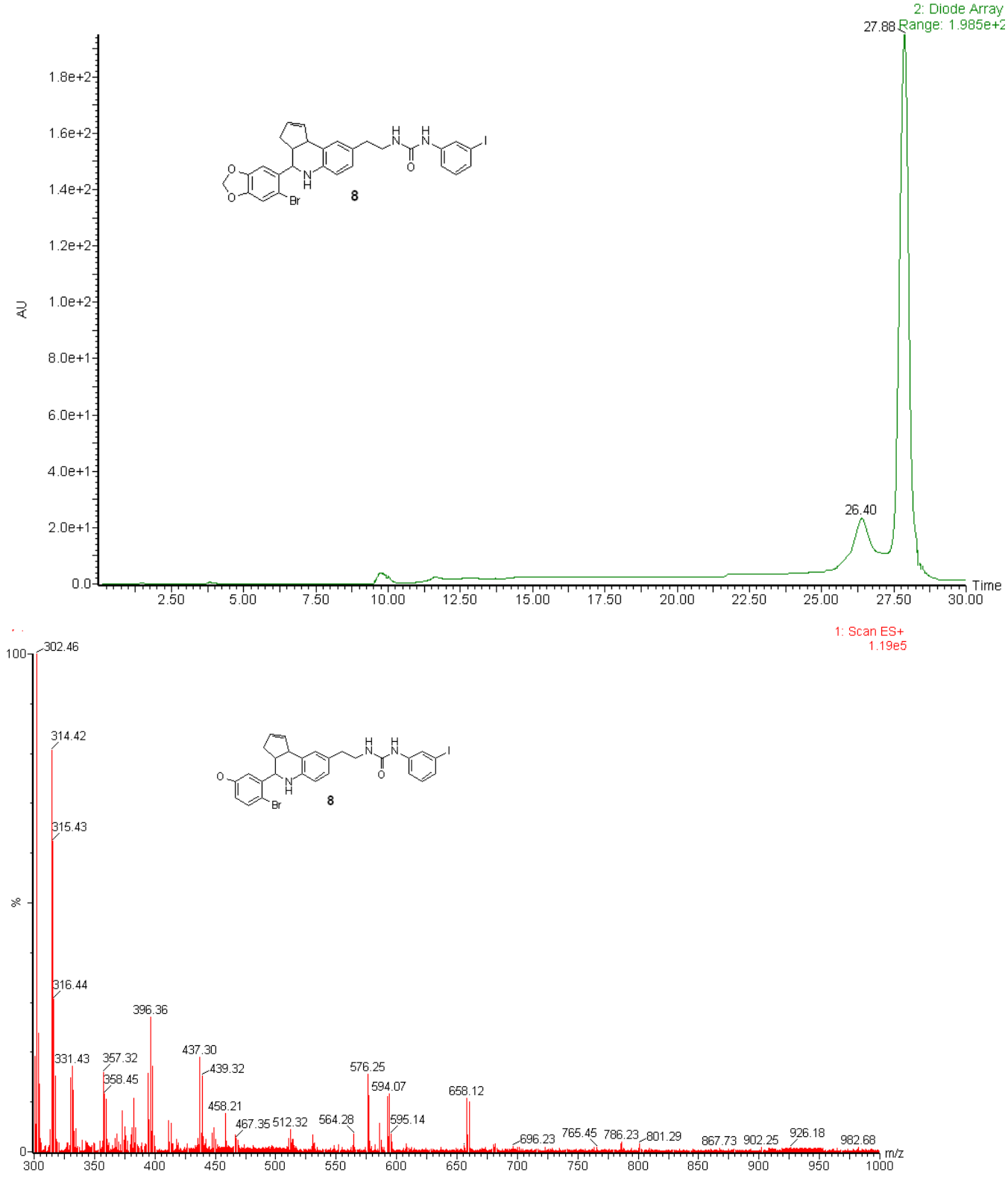
4-(6-Bromo-benzo[1,3]dioxol-5-yl)-7-tributylstannanyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline (18). According to the general procedure 6-bromopiperonal (0.046 g, 0.2 mmol), *m*-tributylstannyl aniline (0.086 g, 0.2 mmol), cyclopentadiene (0.066 g, 1 mmol), and $Sc(OTf)_3$ (0.098 g, 0.02 mmol) were combined with a reaction time of 2 h. The volatiles were removed in vacuo. The residue was purified using ethyl acetate/hexanes (10:90) to provide **18** (0.092 g, 65%) as a colorless liquid. IR (KBr, cm^{-1}) 3340, 2946, 1517, 1278, 1238; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (s, 1H), 7.03-7.01 (m, 2H), 6.84 (dd, *J* = 7.3, 1.0 Hz, 1H), 6.71 (s, 1H), 6.0-5.98 (m, 2H), 5.87-5.85 (m, 1H), 5.67-5.63 (m, 1H), 4.90 (d, *J* = 3.2 Hz, 1H), 4.10 (d, *J* = 8.5 Hz, 1H), 3.5 (s, 1H), 3.20-3.15 (m, 1H), 2.64-2.55 (m, 1H), 1.84-1.76 (m, 1H), 1.58-1.48 (m, 6H), 1.36-1.28 (m, 6H), 1.02-0.97 (m, 6H), 0.88 (t, *J* = 7.4 Hz, 9H).

4-Tributylstannanylbenzoic acid. *p*-Bromoethyl benzoate (0.229 g, 1 mmol), bistributyltin (1.16 g, 2 mmol) and *tetrakis*(triphenylphosphine)palladium (0.115 g, 0.10 mmol) were heated at 100 ^{0}C in dry toluene for 15 h under argon. The volatiles were removed by rotary evaporation and the residue

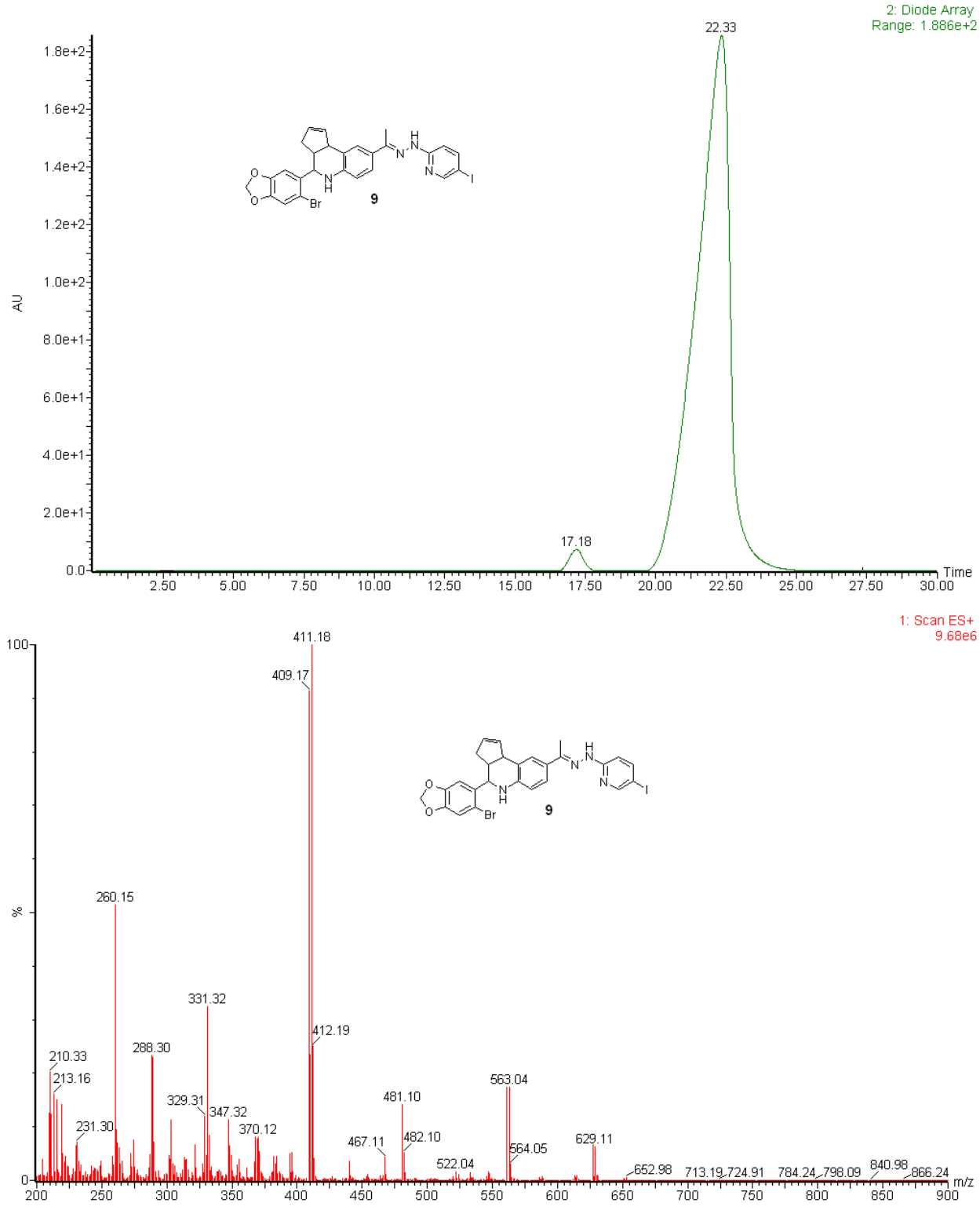
was purified by column chromatography using ethyl acetate/hexanes (5:95) to provide the ester as a colorless liquid. The ester was subjected to hydrolysis by lithium hydroxide (0.063 g. 1.5 mmol) in ethanol/water (0.95:0.05) to provide 4-tributylstannanylbenzoic acid (0.390 g, 95%). IR (KBr, cm^{-1}) 2956, 2922, 1742, 1211, 1152; ^1H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.2 Hz, 2H), 7.54 (d, *J* = 8.2 Hz, 2H), 4.37 (q, *J* = 7.2 Hz, 2H), 1.66-1.47 (m, 6H), 1.43-1.26 (m, 9H), 1.14-1.01 (m, 6H), 0.97-0.81 (t, *J* = 7.3 Hz, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 136.32, 130.1, 128.9, 128.2, 29.02, 27.32, 13.6, 9.6.

N-{2-[4-(6-Bromo-benzo[1,3]dioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta [c]quinolin-8-yl]-ethyl}-4-tributylstannanylbenzamide. The amine **10** (0.041 g, 0.1 mmol), 4-tributylstannylbenzoic acid (0.041 g, 0.1 mmol) and diisopropylethylamine (0.025 g, 0.2 mmol) were combined in dry DMF (0.75 mL), followed by the addition of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (0.051g, 0.1 mmol) and allowed to stir at ambient temperature for 20 h. The reaction mixture was poured into cold water (10 mL) and extracted with dichloromethane (40 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography using ethyl acetate/hexanes (20:80) to isolate the product as a white solid (0.057 g, 71%). mp: 94-96˚C; IR (KBr, cm^{-1}) 3350, 2925, 1638, 1503, 1240; ^1H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.17 (s, 1H), 7.03 (s, 1H), 6.92-6.84 (m, 2H), 6.59 (d, *J* = 7.9 Hz, 1H), 6.13 (t, *J* = 5.7 Hz, 1H), 6.00 (d, *J* = 1.3 Hz, 1H), 5.98 (d, *J* = 1.3 Hz, 1H), 5.84-5.77 (m, 1H), 5.67-5.62 (m, 1H), 4.88 (d, *J* = 2.9 Hz, 1H), 4.07 (d, *J* = 8.4 Hz, 1H), 3.78-3.55 (m, 3H), 3.22-3.12 (m, 1H), 2.80 (t, *J* = 6.7 Hz, 2H), 2.64-2.53 (m, 1H), 1.85-1.75 (m, 1H), 1.57-1.47 (m, 6H), 1.37-1.25 (m, 6H), 1.09-1.03 (m, 6H), 0.87 (t, *J* = 7.4 Hz, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.6, 147.5, 147.2, 147.1, 143.9, 136.5, 134.4,

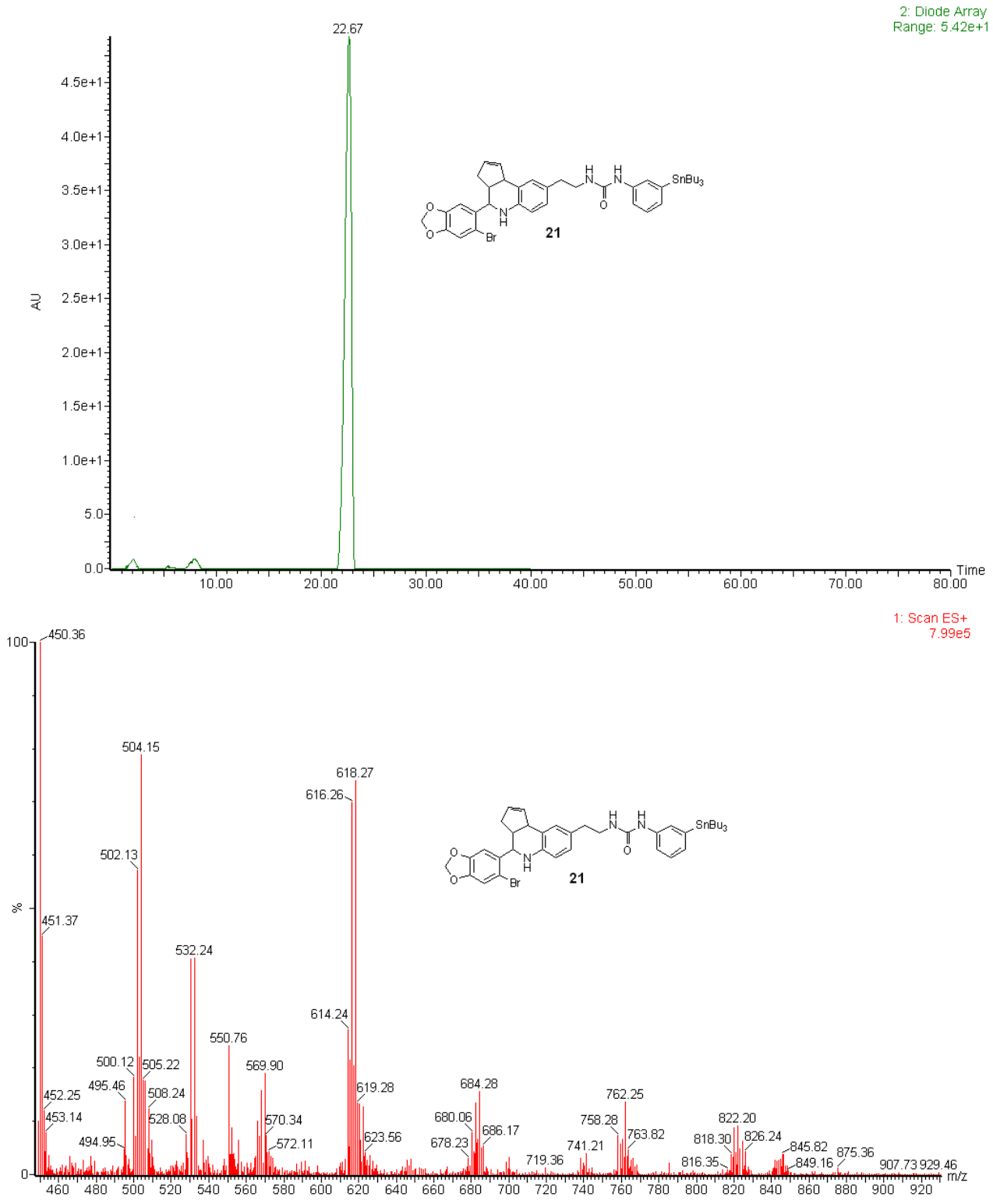
134.1, 133.9, 130.3, 129.5, 129.3, 126.6, 126.4, 125.8, 116.4, 113.0, 112.8, 108.0, 101.7, 56.7, 46.0, 42.1, 41.2, 34.9, 31.2, 29.0, 27.2, 13.6, 9.6; HPLC-MS: Elution with 93:7 CH_3CN/H_2O containing 0.01% formic acid, exhibited a single peak R_t = 24.93 min. ESI-MS *m/z* (ES+) calcd for $C_{40}H_{51}BrN_2O_3Sn$ $[M+H]^+$ 807.21; found 807.28.



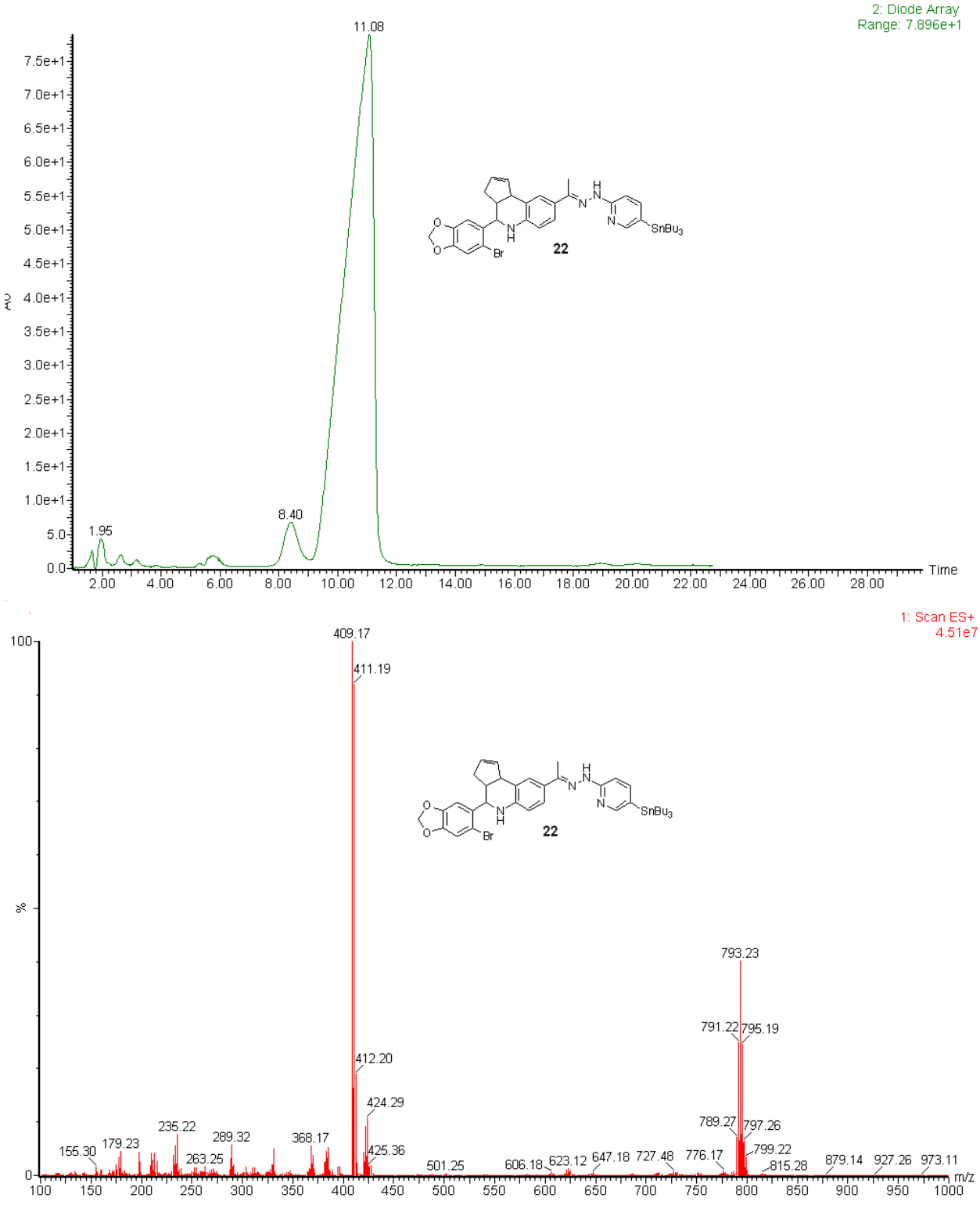
HPLC PDA (top) and ESI-MS (down) of **8**.



HPLC PDA (top) and ESI-MS (down) of **9**



HPLC PDA (top) and ESI-MS (down) of **21**



HPLC PDA (top) and ESI-MS (down) of **22**

Table 1. Binding and functional characterization of GPR30-targeted compounds

Compound	CLog P	ERα binding[1]	ERβ binding[1]	GPR30 binding (IC_{50})	GPR30 agonism (Calcium)[2]	GPR30 antagonism (Calcium)[3]	ERα agonism (PI3K)[4]	ERα antagonism (PI3K)[5]	GPR30 agonism (PI3K)[4]	GPR30 antagonism (PI3K)[5]
E2	3.37	78.2 ± 3.4 %	75.9 ± 6.2 %	3-6nM[6]	100 %	n/a	+++	-	+++	-
1	4.55	9.5 ± 4.1 %	11.2 ± 5.1 %	7.1nM (2.5-20nM)[7]	81.3 ± 2.4 %	n/a	-	-	+++	-
3	4.94	-9.6 ± 15.1 %	-4.6 ± 7.0 %	ND	89.2 ± 2.9 %	44.4 ± 2.4%	-	-	+++	-
4	5.82	11.5 ± 10.5 %	28.3 ± 3.9 %	ND	7.2 ± 0.8 %	62.8 ± 1.9 %	-	-	+/-	+/-
5	6.00	-4.1 ± 3.3 %	6.5 ± 2.7 %	ND	22.3 ± 3.1 %	41.8 ± 4.6 %	-	-	+/-	-
6	6.00	-2.9 ± 3.4 %	24.8 ± 2.0 %	1.7nM (0.6-4.5nM)	22.0 ± 7.7 %	49.3 ± 4.8 %	-	-	-	-
7	6.71	1.5 ± 5.2 %	25.9 ± 5.5 %	12.8nM (4.7-34nM)	24.5 ± 4.9 %	46.5 ± 2.8 %	-	-	++	+/-
8	7.00	6.8 ± 5.0 %	10.0 ± 3.0 %	8.4nM (3.4-20nM)	5.9 ± 3.0 %	80.1 ± 2.2 %	-	-	-	+++
9	6.35	30.5 ± 3.8 %	3.8 ± 6.7 %	1.7nM (0.7-4.4nM)	7.6 ± 3.7 %	67.7 ± 4.8 %	-	-	-	+

[1] denotes % inhibition of 10nM E2-Alexa binding by 10μM compound.

[2] calcium mobilization in response to 10 μM compound normalized to 200 nM E2 (100%).

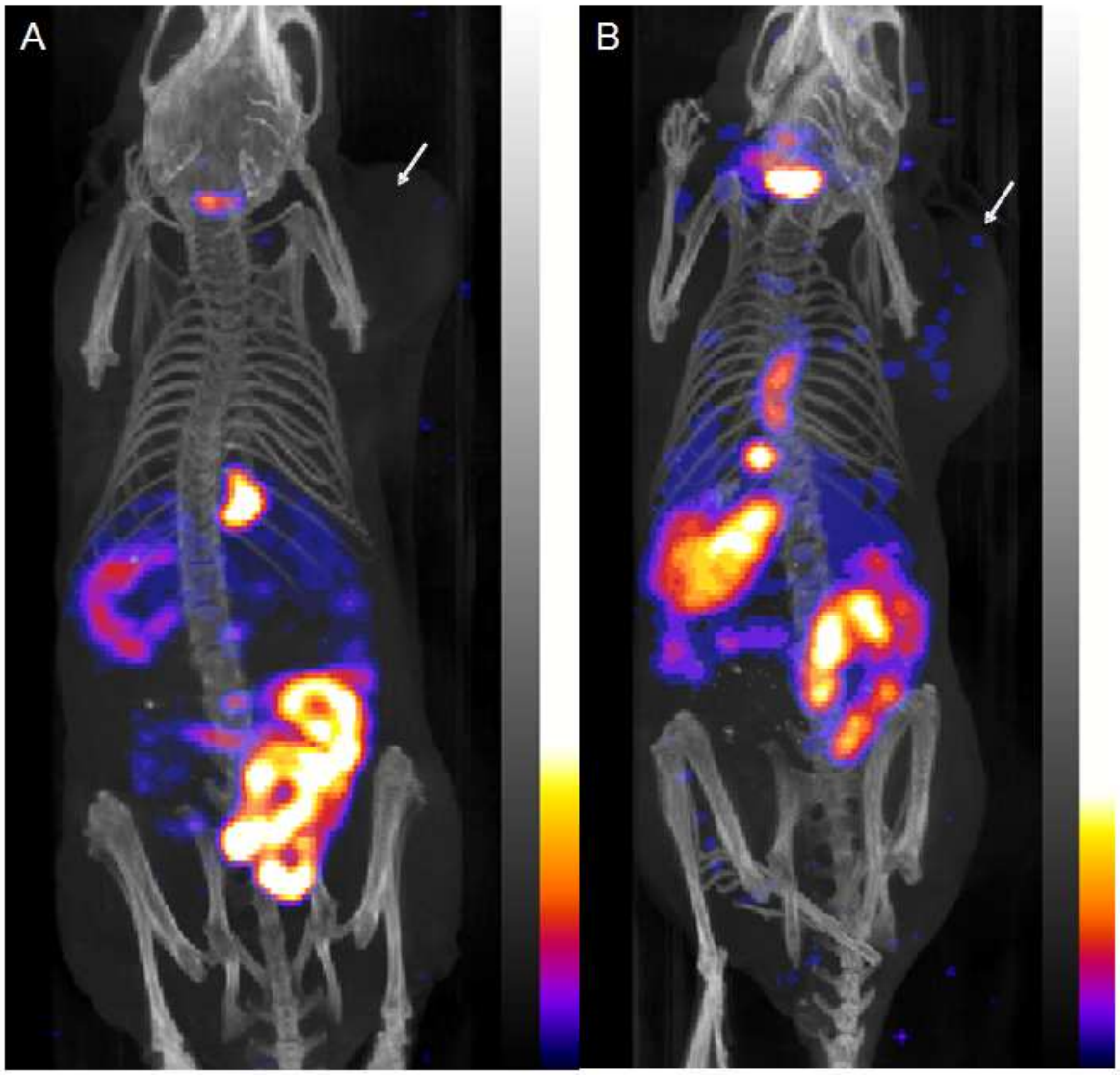
[3] denotes inhibition of 200 nM E2-induced calcium mobilization by 10μM compound.

[4] +++ denotes activation of PI3K by 10μM compound similar to activation induced by 10nM reference compound (E2), ++ and +/- denote decreased activation of PI3K (qualitative assay).

[5] +++ denotes complete antagonism of 10nM E2-induced PI3K activation by 10μM compound, + and +/- denote lesser degrees of antagonism of 10nM E2-mediated PI3K activation by 10μM compound.

[6] from refs. 5-6.

[7] values in parentheses represent 90% confidence intervals.



(A) Reconstructed co-registered maximum intensity projection (MIP) SPECT/CT image of radioiodinated **8***. **(B)** Reconstructed co-registered maximum intensity projection (MIP) SPECT/CT image of radioiodinated **9***. The whole body images was acquired 1 hr PI of radioiodinated GPR30-targeted agent via tail vein of an ovariectomized female athymic (NCr) nu/nu human endometrial Hec50 tumor bearing mice