In Vitro and *In Silico* Analyses of the Inhibition of Human Aldehyde Oxidase by Bazedoxifene, Lasofoxifene, and Structural Analogues

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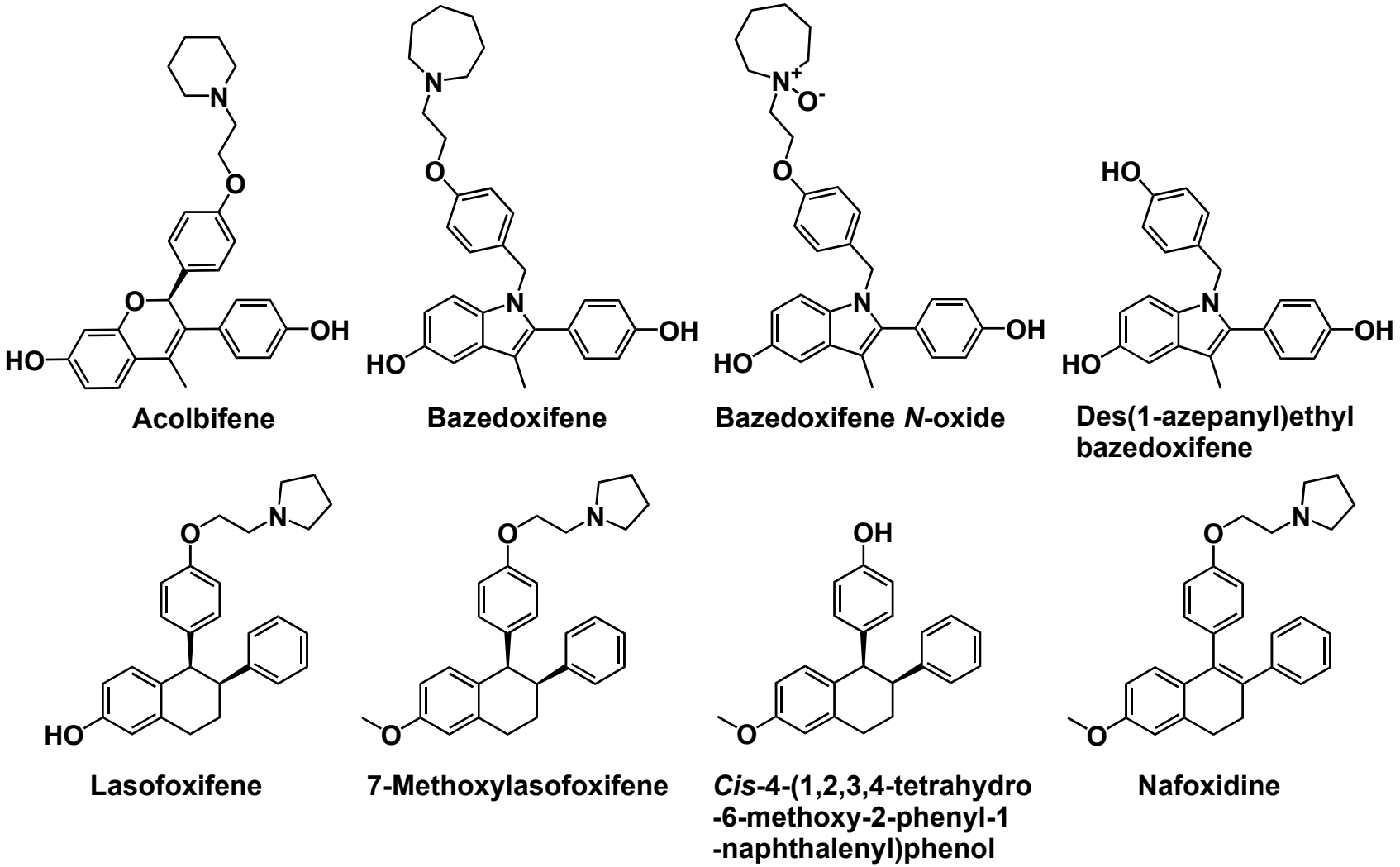
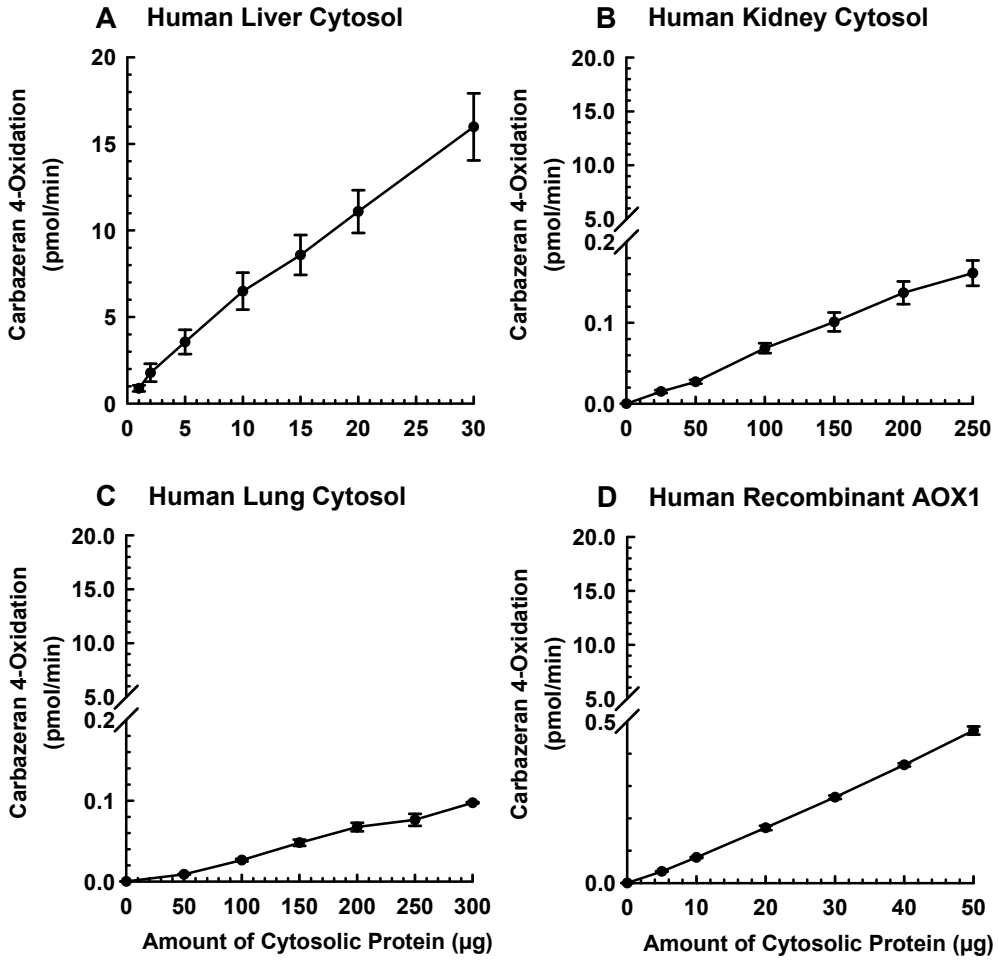
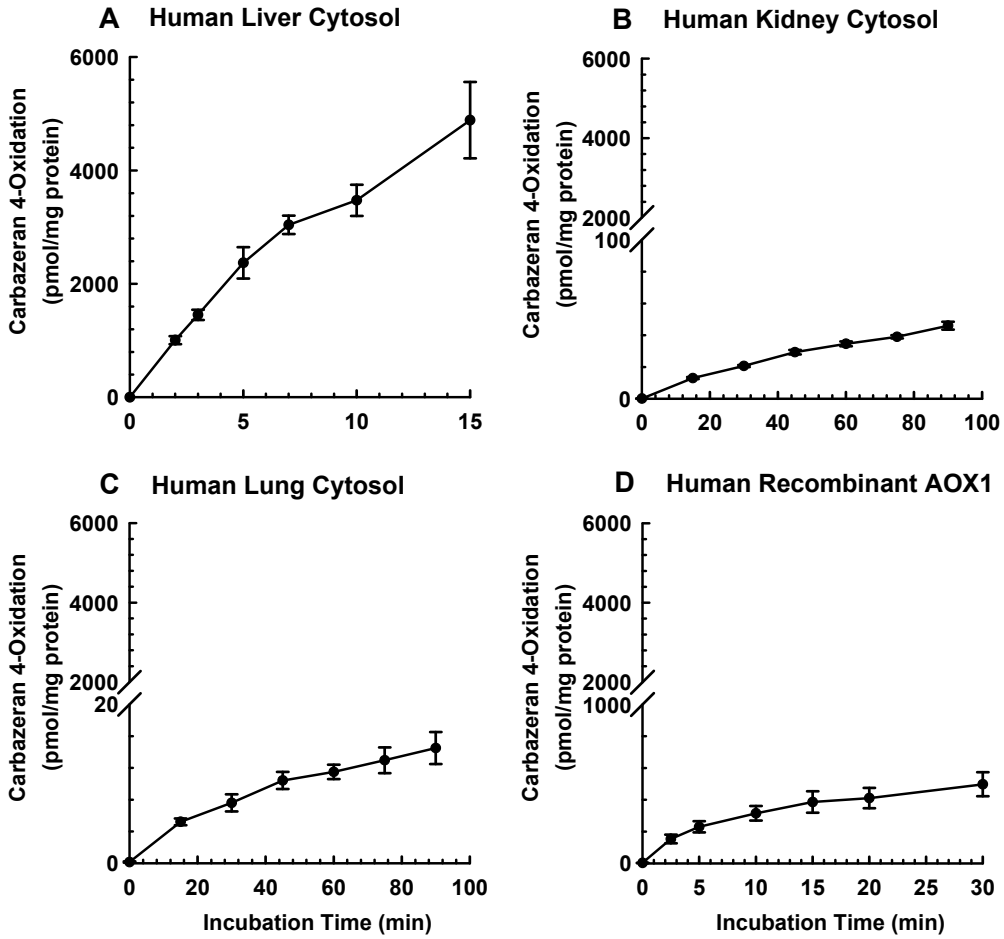


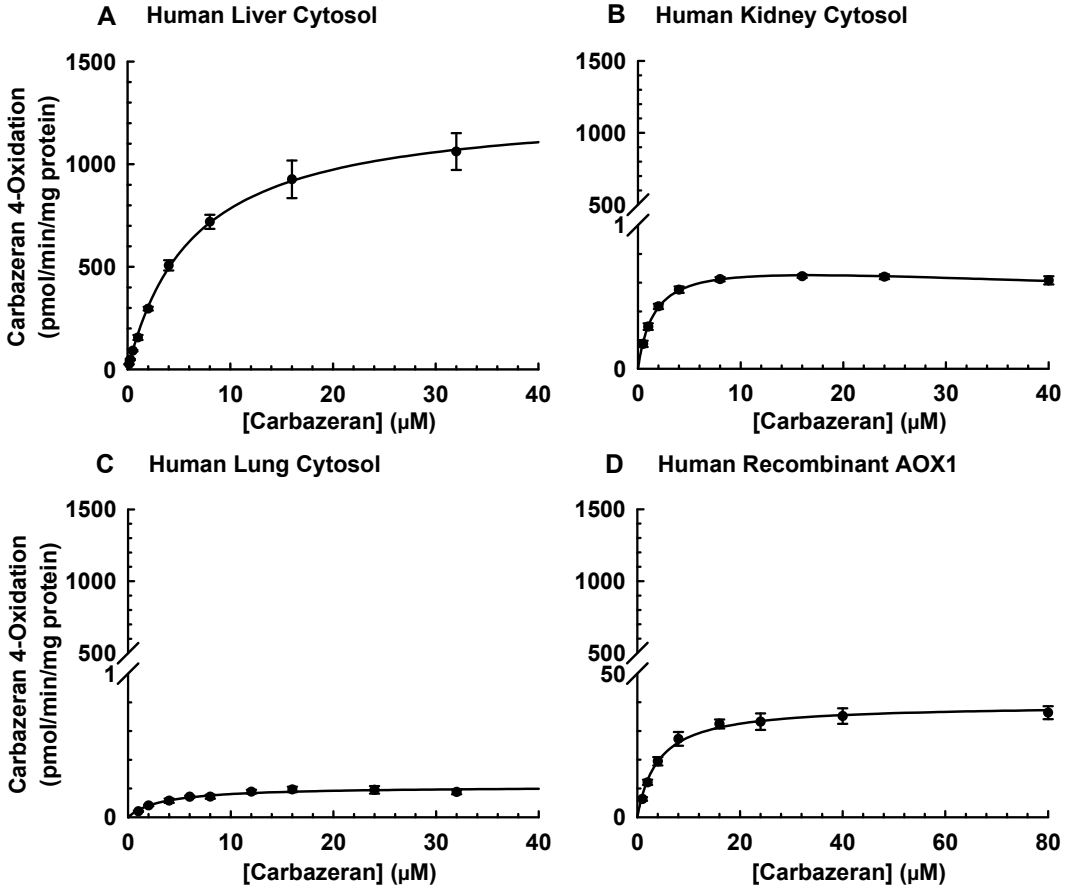
Supplemental Fig. S1. Chemical structures of acolbifene, bazedoxifene, lasofoxifene, and select structural analogues.



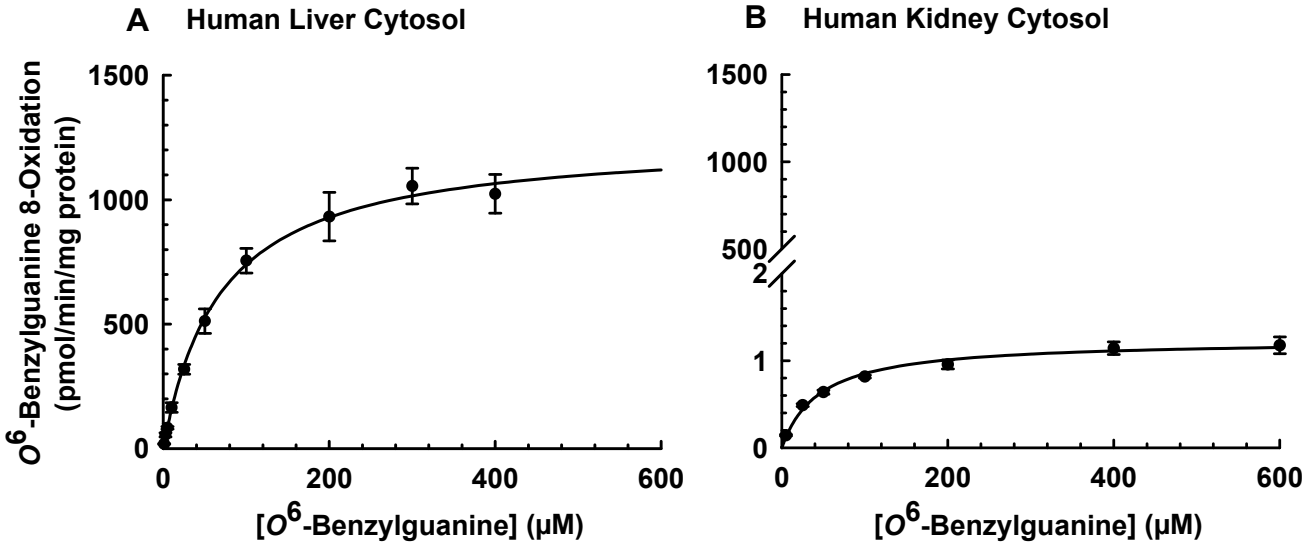
Supplemental Fig. S2. Carbazeran 4-oxidation catalyzed by human liver cytosol, kidney cytosol, lung cytosol, and recombinant AOX1 enzyme as a function of amount of cytosolic protein. (A) Varying amount of liver cytosol (1, 2, 5, 10, 15, 20, or 30 µg protein) was incubated with carbazeran (1 µM) at 37°C for 3 min. (B-D) Varying amount of kidney cytosol (0, 25, 50, 100, 150, 200, or 250 µg protein) (B), lung cytosol (0, 50, 100, 150, 200, 250, or 300 µg protein) (C), or recombinant AOX1 enzyme (0, 5, 10, 20, 30, 40, 50 µg protein) (D) was incubated with carbazeran (16 µM) at 37°C for 45 min. Data are expressed as mean ± S.E.M. of three independent experiments.



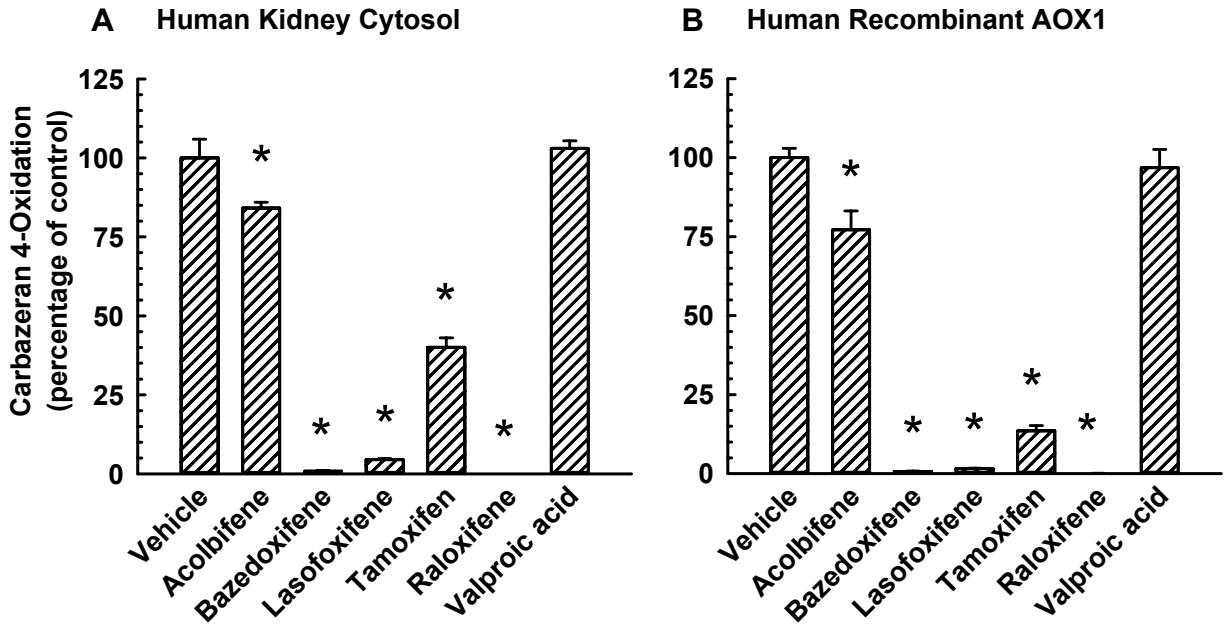
Supplemental Fig. S3. Carbazeran 4-oxidation catalyzed by human liver cytosol, kidney cytosol, lung cytosol, and recombinant AOX1 enzyme as a function of incubation time. (A) Liver cytosol (20 μg protein) was incubated with carbazeran (1 μM) at 37°C for 0, 2, 3, 5, 7, 10, or 15 min. (B-D) Kidney cytosol (200 μg protein) (B), lung cytosol (150 μg protein) (D), or recombinant AOX1 enzyme (30 μg protein) (D) was incubated with carbazeran (16 μM) at 37°C for 0, 15, 30, 45, 60, 75, or 90 min. Data are expressed as mean ± S.E.M. of three independent experiments.



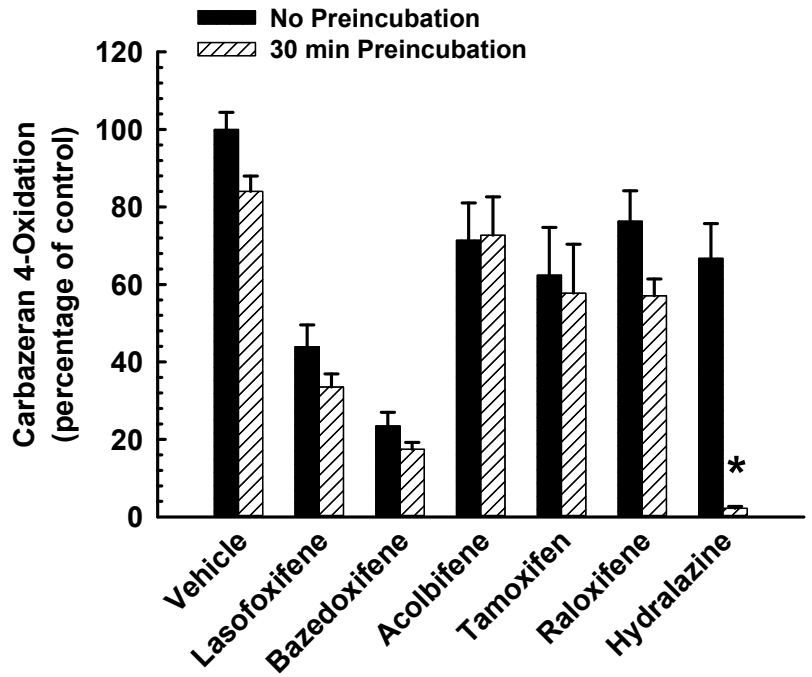
Supplemental Fig. S4. Carbazeran 4-oxidation catalyzed by human liver cytosol, kidney cytosol, lung cytosol, and recombinant AOX1 enzyme. (A) Pooled liver cytosol (20 μg protein) was incubated with varying concentrations of carbazeran (0.125, 0.25, 0.5, 1, 2, 4, 8, 16, or 32 μM) at 37°C for 5 min. (B) Pooled kidney cytosol (200 μg protein) was incubated with varying concentrations of carbazeran (0.5, 1, 2, 4, 8, 16, 24, or 40 μM) at 37°C for 75 min. (C) Pooled lung cytosol (150 μg protein) was incubated with carbazeran (1, 2, 4, 6, 8, 12, 16, 24, or 32 μM) at 37°C for 75 min. (D) Recombinant AOX1 enzyme (30 μg protein) was incubated with carbazeran (1, 2, 4, 8, 16, 24, 40, or 80 μM) at 37°C for 15 min. Data were analyzed by nonlinear least-squares regression and fitted into the Michaelis-Menten (A, C, D) or substrate inhibition model (B). Data are expressed as mean ± S.E.M. of three or four independent experiments conducted in duplicate.



Supplemental Fig. S5. O^6-Benzylguanine 8-oxidation catalyzed by human liver and kidney cytosol. (A) Pooled liver cytosol (20 μg protein) was incubated with varying concentrations of O^6-benzylguanine (1, 2.5, 5, 10, 25, 50, 100, 200, 300, or 400 μM) at 37°C for 5 min. (B) Pooled kidney cytosol (200 μg protein) was incubated with varying concentrations of O^6-benzylguanine (5, 25, 50, 100, 200, 400, or 600 μM) at 37°C for 75 min. Data are expressed as mean ± S.E.M. of three independent experiments.



Supplemental Fig. S6. Comparative effect of acolbifene, bazedoxifene, lasofoxifene, tamoxifen, and raloxifene on carbazeran 4-oxidation catalyzed by human kidney cytosol and recombinant AOX1 enzyme. (A) A SERM (25 μM), valproic acid (50 μM; negative control), or DMSO (1% v/v; vehicle) was co-incubated with carbazeran (2 μM) and pooled kidney cytosol (200 μg protein) at 37°C for 75 min. (B) A SERM (25 μM), valproic acid (50 μM; negative control), or DMSO (1% v/v; vehicle) was co-incubated with carbazeran (4 μM) and recombinant AOX1 enzyme (30 μg protein) at 37°C for 15 min. Data are expressed as percentage of activity in the vehicle-treated control group and expressed as mean ± S.E.M. of three independent experiments conducted in duplicate. *Significantly different from the vehicle-treated control group ($p < 0.05$). The rate of reaction in the vehicle-treated control group was 0.50 ± 0.03 pmol/min/mg protein (A) and 24 ± 0.7 pmol/min/mg protein (B).



Supplemental Fig. S7. Effect of preincubation of human liver cytosol with SERMs on carbazeran 4-oxidation. Human liver cytosol (100 μg protein) was preincubated with a SERM (10 μM lasofoxifene, 10 μM bazedoxifene, 10 μM acolbifene, 10 μM tamoxifen, 0.02 μM raloxifene, or 10 μM hydralazine), or vehicle (0.5% v/v DMSO) at 37°C for 0 or 30 min. An aliquot (10 μl) of the primary incubation mixture was incubated with carbazeran (3 μM) for 5 min. Data are expressed as percentage of activity in the vehicle-treated control group that was not subjected to preincubation (1164 ± 52 pmol/min/mg protein) and expressed as mean ± S.E.M. for three independent experiments.

SUPPLEMENTAL TABLE S1

Shown are the carbazeran 4-oxidation assay conditions in the AOX1 inhibition experiments.

Enzyme Source	Amount of Cytosolic Protein (μg)	Incubation Time (min)	Substrate (Carbazeran) Concentration (μM)
Human liver cytosol	20	5	3
Human kidney cytosol	200	75	2
Human lung cytosol	150	75	N/A
Human Recombinant AOX1	30	15	4

N/A, not applicable.

SUPPLEMENTAL TABLE S2

Human AOX1 protein content and enzyme kinetics of carbazeran 4-oxidation and O^6-benzylguanine 8-oxidation and catalyzed by human tissue cytosols or recombinant AOX1.

V_{max}, k_{cat}, apparent K_m, corrected K_m, and unbound intrinsic clearance ($Cl_{int,u}$) were calculated as described under *Materials and Methods*. Data are expressed as mean ± S.E.M. for three or four independent experiments conducted in duplicate.

Sample	AOX1 Protein Content (pmol/mg protein)	V_{max} (pmol/min/mg protein)	k_{cat} (min^{-1})	Apparent K_m (μM)	Corrected K_m (μM) [a]	$Cl_{int,u}$ (μl/ min/mg protein)	$Cl_{int,u}$ (μl/ min/pmol AOX1)
Carbazeran 4-Oxidation							
Liver cytosol	63.8 ± 4.5	1290 ± 138	20.2 ± 2.2	6.33 ± 0.66	5.93 ± 0.62	217 ± 5	3.41 ± 0.08
Kidney cytosol	21.0 ± 1.3	0.77 ± 0.03[b]	0.04 ± 0.001[b]	1.63 ± 0.30[b]	1.52 ± 0.28[b]	0.55 ± 0.07[b]	0.03 ± 0.003[b]
Lung cytosol	1.8 ± 0.1	0.22 ± 0.02[b]	0.12 ± 0.01[b]	3.30 ± 0.44[b]	3.09 ± 0.41[b]	0.07 ± 0.01[b]	0.04 ± 0.004[b]
Recombinant AOX1	n.d.	39.1 ± 2.6[b]	N/A	4.1 ± 0.08[b]	3.8 ± 0.08[b]	10.3 ± 0.9[b]	N/A
O^6-Benzylguanine 8-Oxidation							
Liver cytosol [c]	63.8 ± 4.5	1254 ± 102	19.7 ± 1.6	70 ± 8	71 ± 8	18 ± 2	0.28 ± 0.03
Kidney cytosol	21.0 ± 1.3	1.2 ± 0.1[b]	0.06 ± 0.005[b]	46 ± 5[b]	46 ± 5[b]	0.03 ± 0.001[b]	0.001 ± 0.000[b]

[a], f_u = 0.94 (carbazeran) or 1.01 (O^6-benzylguanine) was used in the calculations of correct K_m and $Cl_{int,u}$ (Xie et al., 2019).
[b], Significantly different from the human liver cytosol group ($p < 0.05$).
[c], Data from Xie et al., 2019.
Turnover number (k_{cat}) was calculated by dividing V_{max} by AOX1 protein concentration.
n.d., not determined.
LLOQ, lower limit of quantification. N/A, not applicable.