Supporting Information

Haloarene derivatives of carbamazepine with reduced bioactivation liabilities: 2-monohalo and 2,8-dihalo derivatives

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Characterization data for compounds 7b-d and 6d-f.

2,8-Dichloro-10,11-dihydro-5H-dibenz[*b,f*]azepine (7b). This compound was obtained in 80% yield as a beige solid, mp. 114-115°C; ^{1}H NMR [400 MHz, $(CD_3)_2CO$] δ 7.93 (s, 1H, NH), 7.08-7.07 (d, 2H, *J*= 2.4 Hz, 1-H + 9-H), 7.06-7.04 (dd, 2H, *J*=8.4, 2.5Hz, 3-H + 7-H), 6.97-6.95 (d, 2H, *J*= 8.48 Hz, 4-H + 6-H) and 2.89 (s, 4H, CH_2CH_2); ^{13}C NMR [101 MHz, $(CD_3)_2CO$] δ 141.0, 130.1, 129.4, 125.4, 123.6, 120.9 and 32.5; $\nu_{max.}(cm^{-1})$ 3399.9 (m, NH), 1484 (s), 1330(m) and 802.2 (m); *m/z* (ES +ve mode) 263 ([M+H],$^{+}$ 100%), 265 (64%) and 266 (12%); HRMS: found, *m/z* 264.0341; $C_{14}H_{12}{}^{35}Cl_2N$ (MH^{+}) requires *m/z* 264.0347.

2-Bromo-10,11-dihydro-5*H*-dibenz[*b,f*]azepine (7c). This compound was obtained in 62% yield as a white powder, mp. 102-103°C (lit.[32] mp. 100-101°C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 - 7.17 (m, 1 H, 1-H), 7.01-7.11 (m, 3 H), 6.68 - 6.84 (m, 3 H), 5.96 (br s, 1 H, NH) and 3.00 - 3.10 (m, 4 H, CH_2CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.4, 141.6, 133.0, 130.7, 129.5, 128.6, 128.5, 126.9, 119.9, 119.4, 117.9, 111.2, 34.7 and 34.6; $\nu_{max.}(cm^{-1})$ 3386 (w, NH), 1484 (s), 1457 (m, Ar C=C stretch) and 802 (m); *m/z* (CI) 274 ([MH],$^{+}$ 100%) and 276 (97%).

2,8-Dibromo-10,11-dihydro-5H-dibenz[*b,f*]azepine (7d). This compound was obtained in 78% yield as pale blue needles, mp. 154-155°C (lit.[32] mp. 177-178°C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 - 7.18 (dd, J=9.1, 2.4 Hz, 2 H, 3-H + 7-H), 7.13 (d, J=2.4 Hz, 2 H, 1-H + 9-H), 6.60 (d, J=9.1 Hz, 2 H, 4-H + 6-H), 5.95 (br s, 1 H, NH) and 3.01 (s, 4 H, CH_2CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.0, 133.1, 130.4, 129.6, 119.6, 111.7 and 34.3; $\nu_{max.}(cm^{-1})$ 3402 (w, NH), 1485 (m), 1342 (m) and 802 (m); *m/z* (ES-ve mode) 350 ([M-H],$^{-}$ 51%), 351 (100%) and 353 (49%).

2,8-Dichloro-5*H*-dibenz[*b,f*]azepine (6d). This was similarly obtained as an orange solid in near quantitative yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (dd, *J*=8.4, 2.4 Hz, 2 H), 6.84 (d, *J*=2.5 Hz, 2 H), 6.42 (d, *J*=8.4 Hz, 2 H), 6.25 (s, 2 H) and 4.89 (br s, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.6, 132.0, 131.0, 130.1, 129.2, 128.3 and 120.4; HRMS: found, *m/z* 262.0186; $C_{14}H_{10}N^{35}Cl_2$ (MH^+) requires *m/z* 262.0190.

2-Bromo-5*H*-dibenz[*b,f*]azepine (6e). This could only be obtained as a mixture with a presumed positional isomer by the above method; it was converted to the carbamazepine analogue **5e** (see below) after final separation by preparative HPLC. Instead **6e** could be obtained in very low yield (5%), but high purity, via acid-catalysed rearrangement of 5-bromo-1-phenyl-1H-indole (see Results and Discussion: Chemistry),[35, 42] affording **6e** as an orange solid, mp. 152-154°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (dd, *J*=8.3, 2.3 Hz, 1 H), 7.03 (ddd, *J*=7.9, 5.9, 3.1 Hz, 2 H), 6.95 (d, *J*=2.3 Hz, 1 H), 6.82 - 6.86 (m, 1 H), 6.46 (d, *J*=7.6 Hz, 1 H), 6.35 (s, 1 H), 6.30 (d, *J*=12.0 Hz, 1 H), 6.17 (d, *J*=11.6 Hz, 1 H) and 4.89 (br s, 1 H); ^{13}C NMR (101 MHz,$CDCl_3$) δ 148.3, 147.4, 133.4, 132.8, 131.8, 131.7, 130.7, 130.6, 129.8, 129.3, 123.3, 120.7, 119.3 and 115.3; *m/z* (CI) 272 ([M+H],$^+$ 97%) and 274 (100%); HRMS: found, *m/z* 270.9991; $C_{14}H_{10}N^{79}Br$ (M^+) requires *m/z* 270.9997.

2,8-Dibromo-5H-dibenz[*b,f*]azepine (6f). This was obtained as an orange solid in near quantitative yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (dd, *J* = 2.3, 8.3 Hz, 2 H, 3-H and 7-H), 6.96 (d, *J* = 2.3 Hz, 2 H, 1-H and 9-H), 6.34 (d, *J* = 8.4 Hz, 2 H, 4-H and 6-H) and 6.21 (s, 2 H, 10-H and 11-H) and 4.88 (br s, 1 H, NH); *m/z* (EI-ve mode) 348 ([M-H]$^-$, 51%), 350 (100%) and 352 (49%); HRMS: found, *m/z* 347.9031; $C_{14}H_8N^{79}Br_2$ (M-H$^-$) requires *m/z* 347.9023.

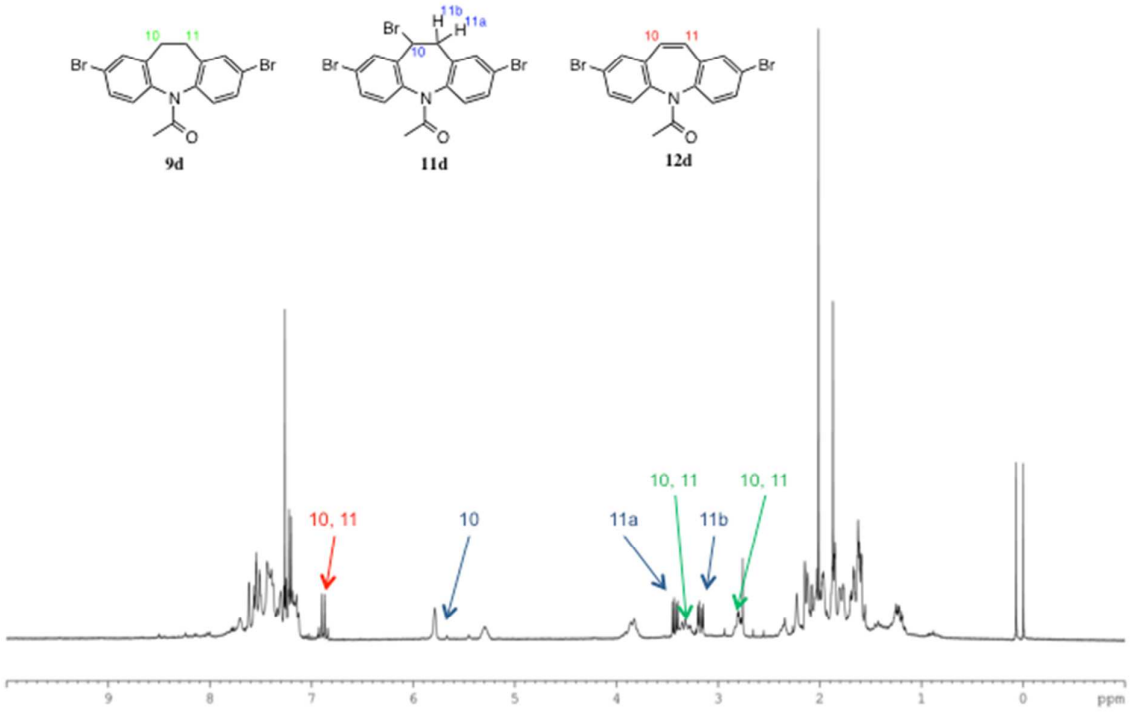

Figure S1. ^{1}H NMR spectrum of crude reaction mixture at 12 h during the radical bromination-elimination transformation of *N*-acetyl-2,8-dibromo-10,11-dihydro-5H-dibenz[*b,f*]azepine **9d** to **11d** and **12d**. At an intermediate stage, unreacted **9d**, **11d** and **12d** were all distinct. A characteristic ABX pattern was obtained from the C10 and C11 protons of 10-Br intermediate **11d**, with disappearance of the complex CH_2CH_2 multiplet (fluxional effects) of **9d**. Partial formation of the 10,11-double bond in **12d** at this stage was apparent from the appearance of an AB qt at δ 6.90. The radical bromination was continued until the -CH_2CH_2- signals of **9d** had disappeared. Treatment of the substantially pure **11d**, containing some **12d**, with excess KOH/EtOH afforded highly pure 2,8-dibromodibenz[*b,f*]azepine **6f**.

Mass chromatograms of monoxygenated metabolites of 1 and 5a-c in rat hepatocytes

Positive-ion electrospray mass chromatograms of the monoxygenated metabolites of **1** (**2-4**) and derivatives **5a** (2-fluoro), **5b** (2,8-difluoro) **and 5c** (2-chloro) were obtained by LC/MS. **5a** was the only derivative that underwent aryl hydroxylation – and subsequent complete *O*-sulfonation – by isolated male rat hepatocytes (Supplementary Table 2). C-2/C-8 difluorination and C-2 chlorination were the minimum halogen substitutions required to block aryl hydroxylation of **1**. With **5a-c**, the principal search criterion for an aryl hydroxyl metabolite, by analogy with fragmentations of authentic standards of 2-hydroxy **2** and 3-hydroxy **4**, was neutral loss of 43 amu ($[M+H-CONH]^+$) from a monoxygenated derivative of the parent in the absence of coincidental loss of 30 amu (CH_2O) characteristic of a 10,11-epoxide (Supplementary Tables 1-7). Metabolites were produced by 6-h incubations with 50 μM substrate.

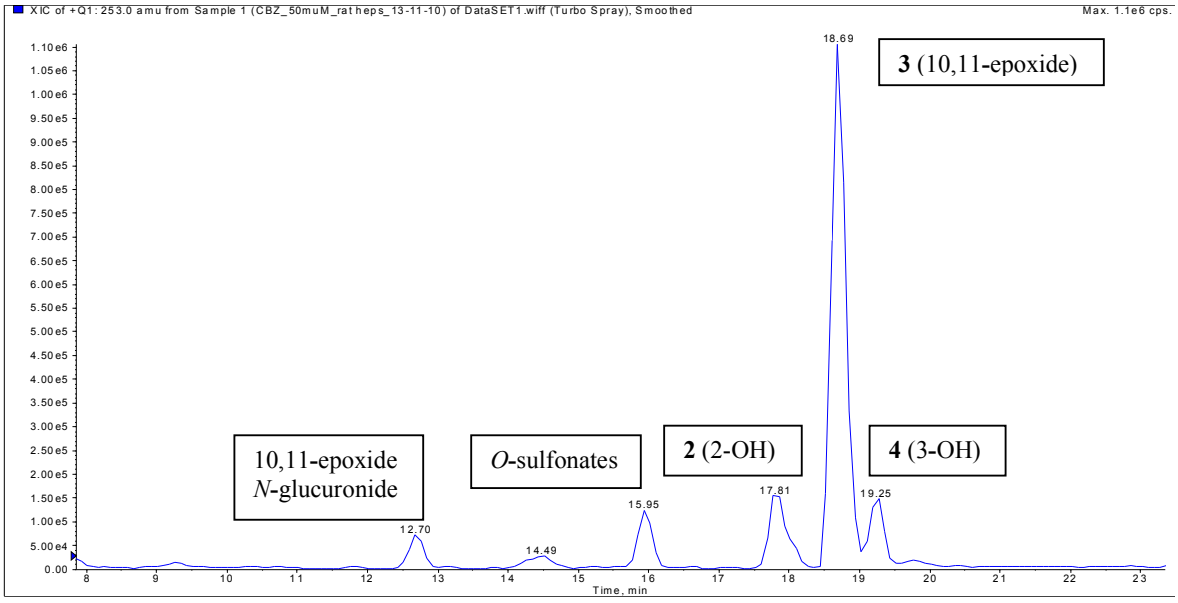

Figure S2. Mass chromatogram of the monoxygenated metabolites of **1** ($[M+H]^+$ at *m/z* 253; **2-4**). The additional peaks at 14.49 min and 15.95 min, attributed to *O*-sulfonates of **1**, were fragment ions ($[M+H-SO_3]^+$) of the minor and major *O*-sulfonates (regiochemistry undefined), respectively. The peak at 12.7 min attributed to the *N*-glucuronide of **3** was a fragment ion ($[M+H-176]^+$).

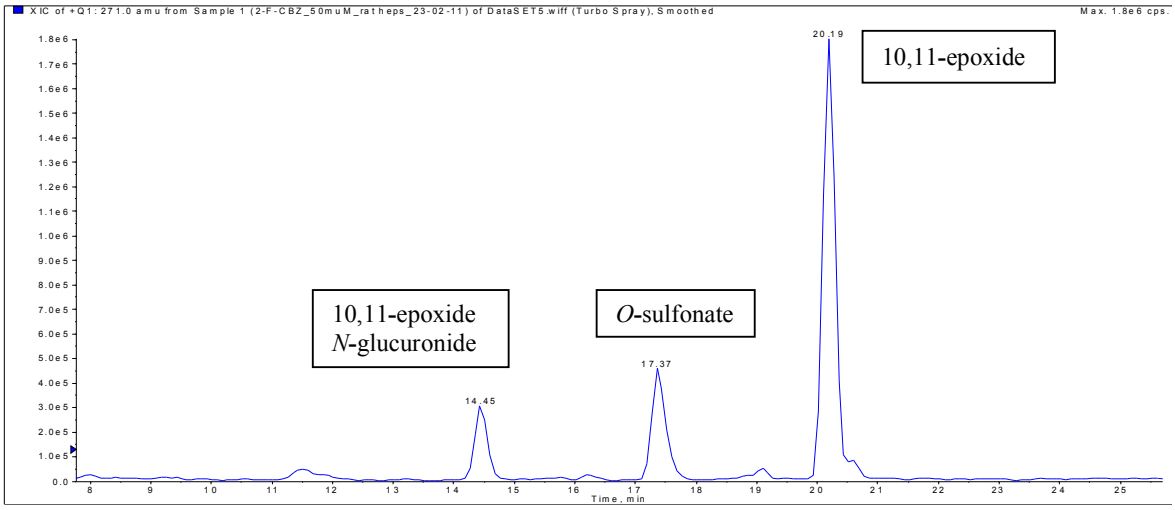

Figure S3. Mass chromatogram of the monoxygenated metabolites of **5a** ($[M+H]^+$ at *m/z* 271). The additional peaks at 14.45 min and 17.37 min, attributed to the *N*-glucuronide of the **5a** 10,11-epoxide and an *O*-sulfonate (regiochemistry unknown), respectively, were fragment ions: $[M+H-176]^+$ and $[M+H-SO_3]^+$, respectively.

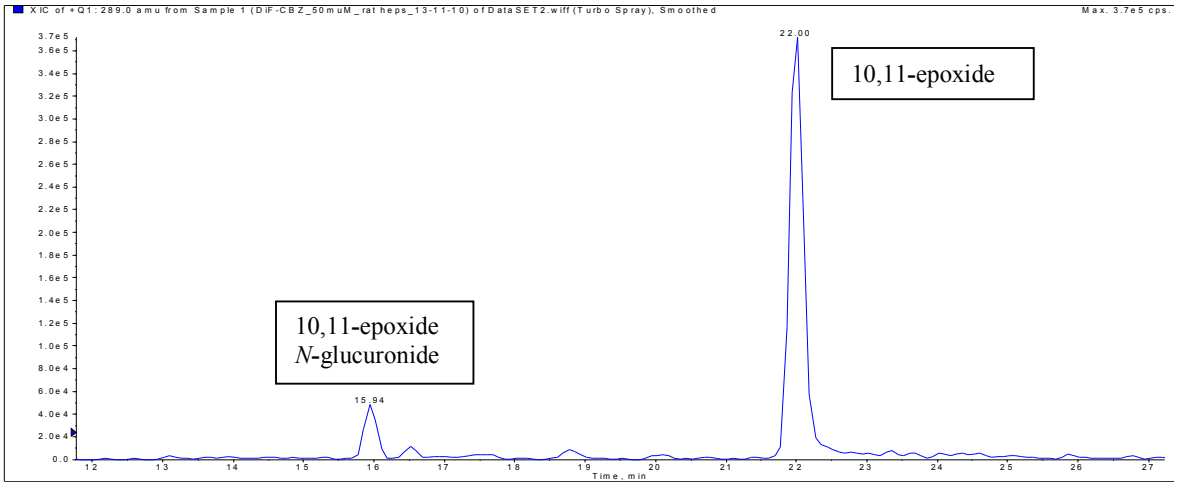

Figure S4. Mass chromatogram of the monoxygenated metabolites of **5b** ($[M+H]^+$ at *m/z* 289). The additional peak at 15.94 min attributed to the *N*-glucuronide of the **5b** 10,11-epoxide was a fragment ion ($[M+H-176]^+$).

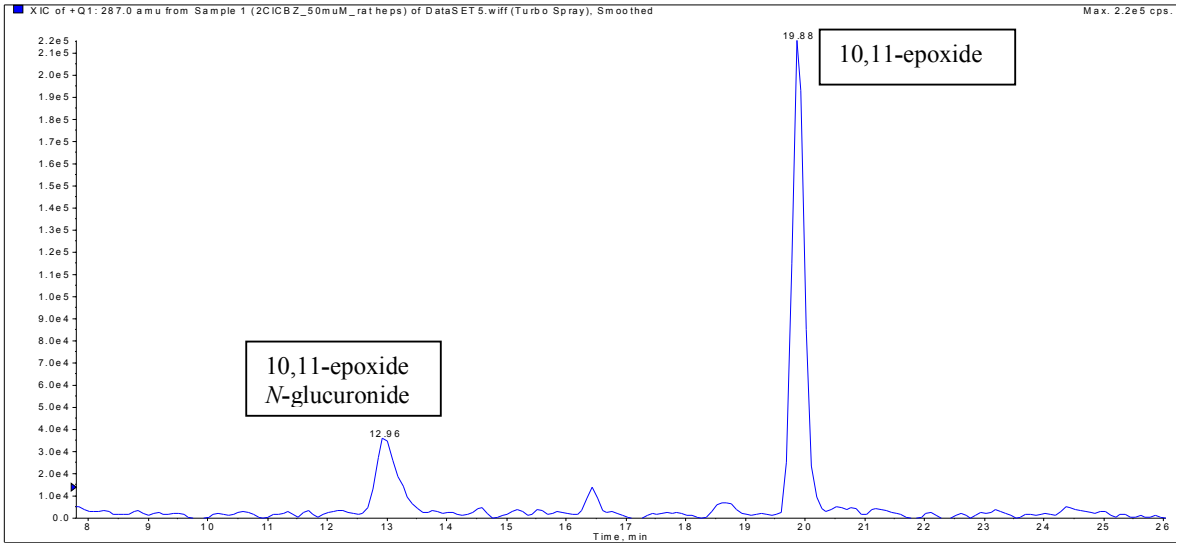

Figure S5. Mass chromatogram of the monoxygenated metabolites of **5c** ($[M+H]^+$ at *m/z* 287; ^{35}Cl isotope form). The additional peak at 12.96 min attributed to the *N*-glucuronide of the **5c** 10,11-epoxide was a fragment ion ($[M+H-176]^+$).

Mass chromatograms for GSH adducts of 1, 5a and 5b in rat hepatocytes

Positive-ion electrospray mass chromatograms for GSH adducts of **1** and derivatives **5a** (2-fluoro) and **5b** (2,8-difluoro) were obtained by LC/MS. Only **1** yielded an adduct as specified below.

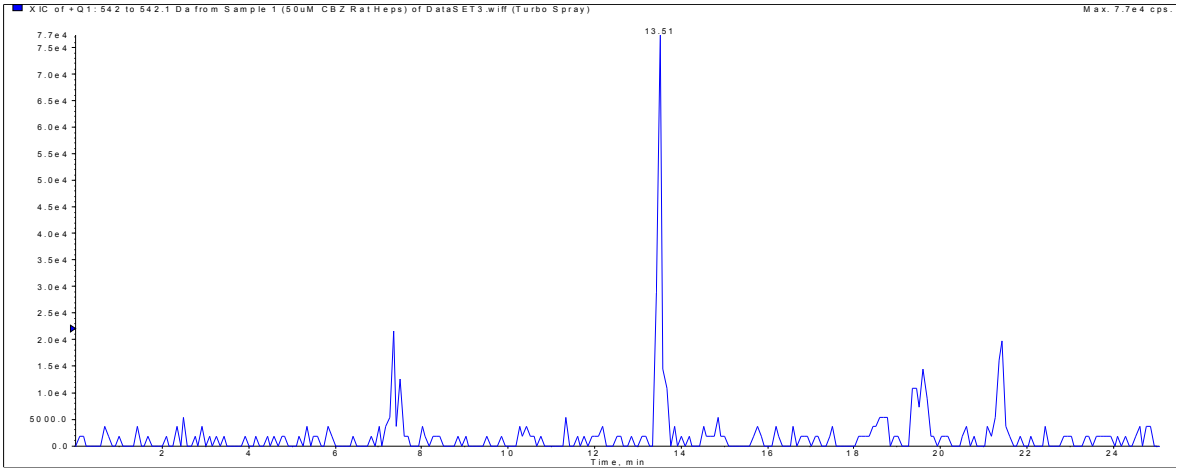

Figure S6. Mass chromatogram for the M-2H+GSH adduct of **1** ($[M+H]^+$ at *m/z* 542; peak at 13.51 min). Spectrum (Table S1) contained diagnostic fragments at m/z 467 ($[M+H\text{-glycine}]^+$) and m/z 413 ($[M+H\text{-pyroglutamate}]^+$).

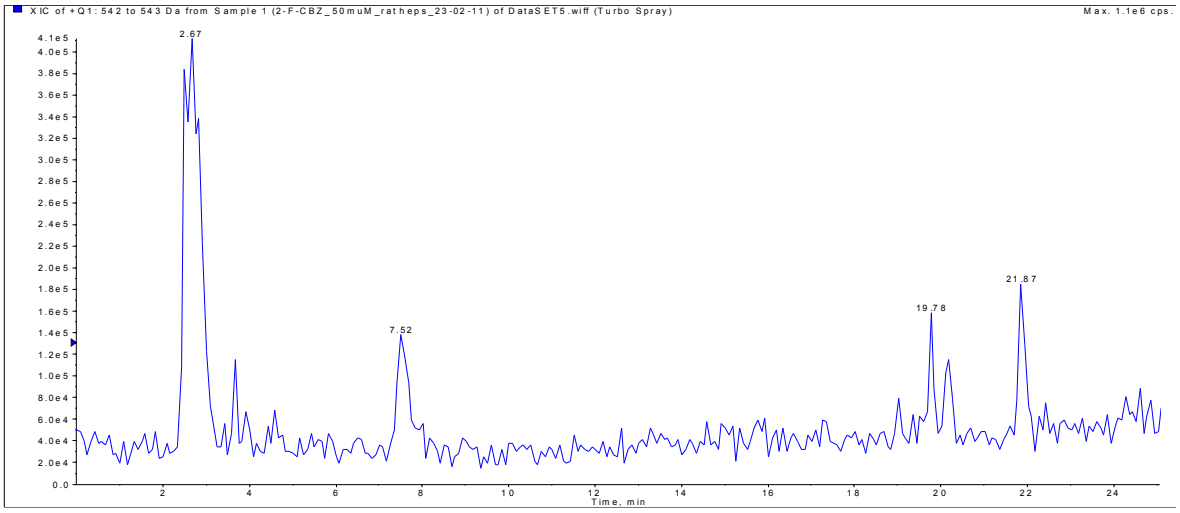

Figure S7. Mass chromatogram for the M-HX+GSH adduct of **5a** ($[M+H]^+$ at *m/z* 542).

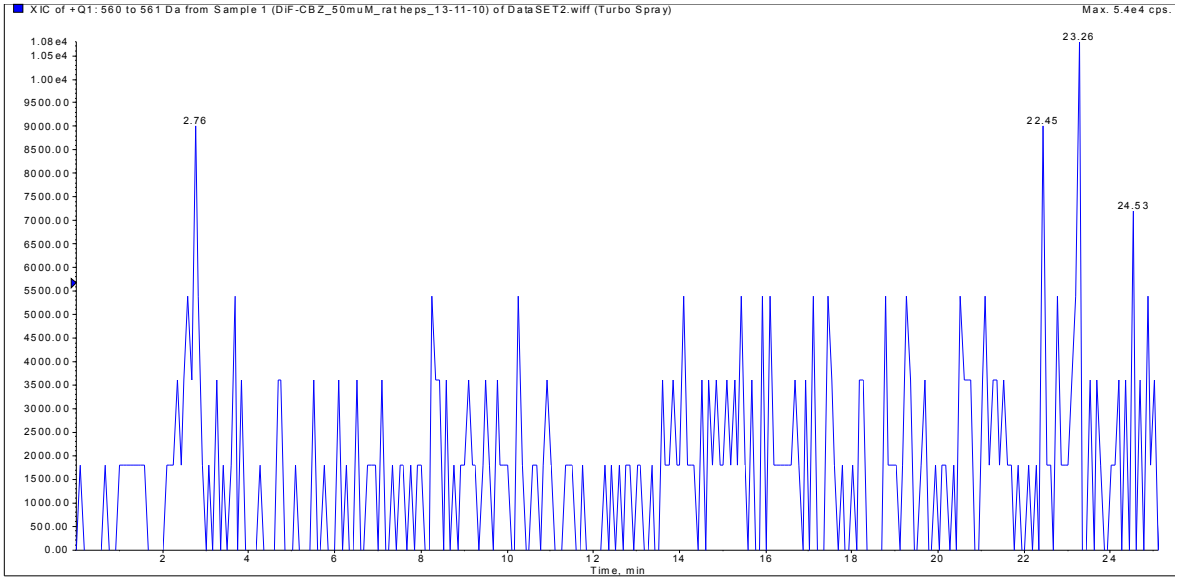

Figure S8. Mass chromatogram for the M-HX+GSH adduct of **5b** ($[M+H]^+$ at *m/z* 560).

Mass chromatograms of monoxygenated metabolites of 1 and 5b in human liver microsomes

Positive-ion electrospray mass chromatograms of the monoxygenated metabolites of **1** (**2-4**) and derivative **5b** (2,8-difluoro) were obtained by LC/MS. **5b** was refractory to arene oxidation. The only detected monoxygenated metabolite of **5b** yielded the neutral losses of 43 amu ([M+H-CONH]$^+$) and 30 amu (CH_2O) characteristic of a 10,11-epoxide. Metabolites were produced by 60-min incubations with 50 μM substrate.

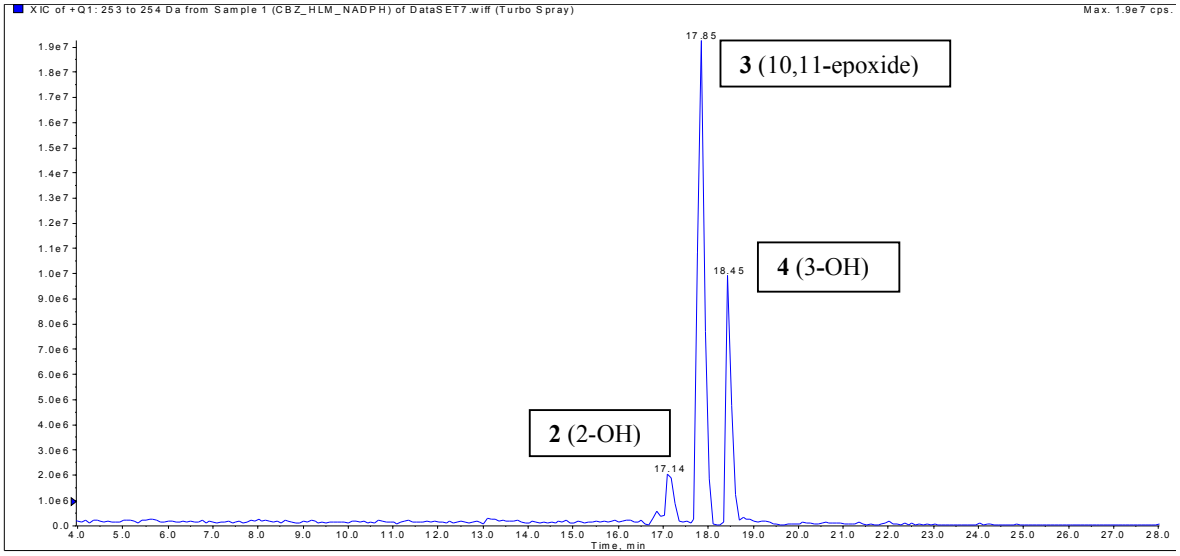

Figure S9. Mass chromatogram of the monoxygenated metabolites of **1** ([M+H]$^+$ at *m/z* 253; **2-4**).

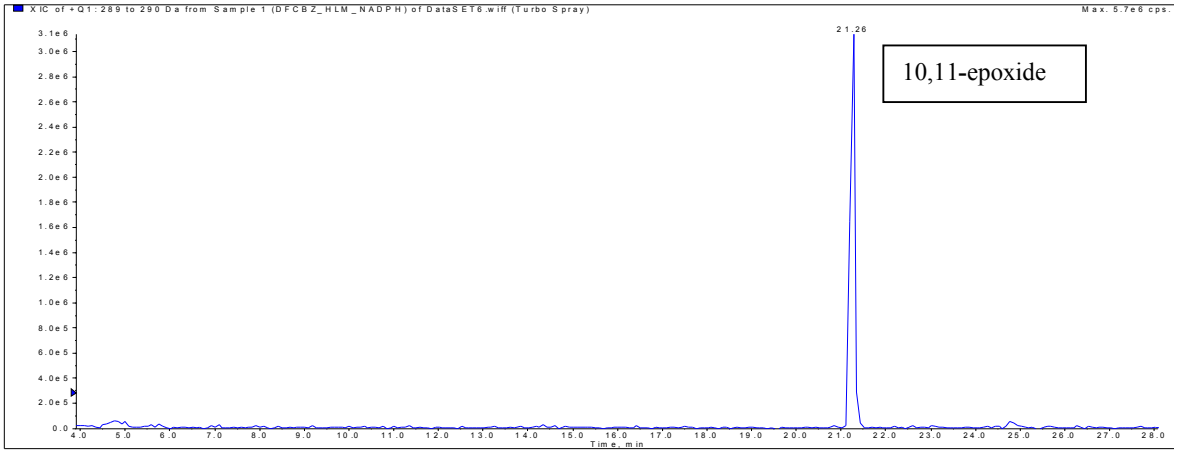

Figure S10. Mass chromatogram of the monoxygenated metabolite of **5b** ([M+H]$^+$ at *m/z* 289).

Mass spectra of metabolites

Positive-ion electrospray mass spectra of the metabolites of **1** and derivatives **5a-f** were obtained by LC/MS. The metabolites were produced by isolated male rat hepatocytes incubated with substrate (50 μM) for 6 h.

Table S1. Mass spectra of metabolites of **1**

Metabolite	**Mass spectrum (*m/z*)**
10,11-epoxide (**3**)	253 ($[M+H]^+$), 236 ($[M+H-NH_3]^+$), 210 ($[M+H-CONH]^+$), 180 ($[210-CH_2O]^+$)
2-hydroxyl (**2**)	253 ($[M+H]^+$), 210 ($[M+H-CONH]^+$)
3-hydroxyl (**4**)	253 ($[M+H]^+$), 210 ($[M+H-CONH]^+$)
N-glucuronide[a]	413 ($[M+H]^+$), 395 ($[M+H-H_2O]^+$), 377 ($[M+H-2H_2O]^+$), 303 ($[395-92]^+$), 279 ($[M+H-134]^+$), 237 ($[M+H-176]^+$), 220 ($[237-NH_3]^+$), 192 ($[237-CONH_3]^+$)
N-glucuronide 10,11-epoxide[a]	429 ($[M+H]^+$), 411 ($[M+H-H_2O]^+$), 295 ($[M+H-134]^+$), 253 ($[M+H-176]^+$), 236 ($[253-NH_3]^+$), 210 ($[253-CONH]^+$), 180 ($[210-CH_2O]^+$)
O-sulfonates[b]	333 ($[M+H]^+$), 253 ($[M+H-SO_3]^+$), 210 ($[253-CONH]^+$)
GSH adduct[c]	542 ($[M+H]^+$), 467 ($[M+H-glycine]^+$), 413 ($[M+H-pyroglutamate]^+$)

[a]Neutral losses of 92 amu and 134 amu, attributable to fissions within the glucuronic acid moiety, are characteristic of an *N*-glucuronide.[1]

[b]Two *O*-sulfonates of **1** (regiochemistry unknown) were resolved. They yielded identical mass spectra.

[c]Regiochemistry unknown.

Table S2. Mass spectra of metabolites of **5a**

Metabolite	Mass spectrum (*m/z*)
10,11-epoxide	271 ($[M+H]^+$), 254 ($[M+H-NH_3]^+$), 228 ($[M+H-CONH]^+$), 198 ($[228-CH_2O]^+$)
N-glucuronide	431 ($[M+H]^+$), 413 ($[M+H-H_2O]^+$), 395 ($[M+H-2H_2O]^+$), 321 ($[413-92]^+$), 297 ($[M+H-134]^+$), 255 ($[M+H-176]^+$), 238 ($[255-NH_3]^+$), 210 ($[255-CONH_3]^+$)
N-glucuronide 10,11-epoxide	447 ($[M+H]^+$), 429 ($[M+H-H_2O]^+$), 313 ($[M+H-134]^+$), 271 ($[M+H-176]^+$), 254 ($[271-NH_3]^+$), 228 ($[271-CONH]^+$), 198 ($[228-CH_2O]^+$)
O-sulfonate[a]	351 ($[M+H]^+$), 271 ($[M+H-SO_3]^+$), 228 ($[271-CONH]^+$)

[a]Regiochemistry of *O*-sulfonate unknown.

Table S3. Mass spectra of metabolites of **5b**

Metabolite	Mass spectrum (*m/z*)
10,11-epoxide	289 ($[M+H]^+$), 272 ($[M+H-NH_3]^+$), 246 ($[M+H-CONH]^+$), 216 ($[246-CH_2O]^+$)
N-glucuronide	449 ($[M+H]^+$), 431 ($[M+H-H_2O]^+$), 413 ($[M+H-2H_2O]^+$), 339 ($[431-92]^+$), 315 ($[M+H-134]^+$), 273 ($[M+H-176]^+$), 256 ($[273-NH_3]^+$), 228 ($[273-CONH_3]^+$)
N-glucuronide 10,11-epoxide	465 ($[M+H]^+$), 447 ($[M+H-H_2O]^+$), 331 ($[M+H-134]^+$), 289 ($[M+H-176]^+$), 272 ($[289-NH_3]^+$), 246 ($[289-CONH]^+$), 216 ($[246-CH_2O]^+$)

Table S4. Mass spectra of metabolites of **5c**

Metabolite	**Mass spectrum (*m/z*)[a]**
10,11-epoxide	287 ($[M+H]^+$), 270 ($[M+H-NH_3]^+$), 244 ($[M+H-CONH]^+$), 214 ($[244-CH_2O]^+$)
N-glucuronide	447 ($[M+H]^+$), 429 ($[M+H-H_2O]^+$), 411 ($[M+H-2H_2O]^+$), 337 ($[429-92]^+$), 313 ($[M+H-134]^+$), 271 ($[M+H-176]^+$), 254 ($[271-NH_3]^+$), 226 ($[271-CONH_3]^+$)
N-glucuronide 10,11-epoxide	463 ($[M+H]^+$), 445 ($[M+H-H_2O]^+$), 329 ($[M+H-134]^+$), 287 ($[M+H-176]^+$), 270 ($[287-NH_3]^+$), 244 ($[287-CONH]^+$), 214 ($[244-CH_2O]^+$)

[a]Values for ^{35}Cl isotope form.

Table S5. Mass spectra of metabolites of **5d**

Metabolite	**Mass spectrum (*m/z*)[a]**
10,11-epoxide	321 ($[M+H]^+$), 304 ($[M+H-NH_3]^+$), 278 ($[M+H-CONH]^+$), 248 ($[278-CH_2O]^+$)
N-glucuronide	481 ($[M+H]^+$), 463 ($[M+H-H_2O]^+$), 445 ($[M+H-2H_2O]^+$), 371 ($[463-92]^+$), 347 ($[M+H-134]^+$), 305 ($[M+H-176]^+$), 288 ($[305-NH_3]^+$), 260 ($[305-CONH_3]^+$)
N-glucuronide 10,11-epoxide	497 ($[M+H]^+$), 479 ($[M+H-H_2O]^+$), 363 ($[M+H-134]^+$), 321 ($[M+H-176]^+$), 304 ($[321-NH_3]^+$), 278 ($[321-CONH]^+$), 248 ($[278-CH_2O]^+$)

[a]Values for $^{35}Cl_2$ isotope form.

Table S6. Mass spectra of metabolites of **5e**

Metabolite	Mass spectrum (*m/z*)[a]
10,11-epoxide	331 ($[M+H]^+$), 314 ($[M+H-NH_3]^+$), 288 ($[M+H-CONH]^+$), 258 ($[288-CH_2O]^+$)
N-glucuronide	491 ($[M+H]^+$), 473 ($[M+H-H_2O]^+$), 455 ($[M+H-2H_2O]^+$), 381 ($[473-92]^+$), 357 ($[M+H-134]^+$), 315 ($[M+H-176]^+$), 298 ($[315-NH_3]^+$), 270 ($[315-CONH_3]^+$)
N-glucuronide 10,11-epoxide	507 ($[M+H]^+$), 489 ($[M+H-H_2O]^+$), 373 ($[M+H-134]^+$), 331 ($[M+H-176]^+$), 314 ($[331-NH_3]^+$), 288 ($[331-CONH]^+$), 258 ($[288-CH_2O]^+$)

[a]Values for ^{79}Br isotope form.

Table S7. Mass spectra of metabolites of **5f**

Metabolite	Mass spectrum (*m/z*)[a]
10,11-epoxide	411 ($[M+H]^+$), 394 ($[M+H-NH_3]^+$), 368 ($[M+H-CONH]^+$), 338 ($[368-CH_2O]^+$)
N-glucuronide	571 ($[M+H]^+$), 553 ($[M+H-H_2O]^+$), 535 ($[M+H-2H_2O]^+$), 461 ($[553-92]^+$), 437 ($[M+H-134]^+$), 395 ($[M+H-176]^+$), 378 ($[395-NH_3]^+$), 350 ($[395-CONH_3]^+$)
N-glucuronide 10,11-epoxide	587 ($[M+H]^+$), 569 ($[M+H-H_2O]^+$), 453 ($[M+H-134]^+$), 411 ($[M+H-176]^+$), 394 ($[411-NH_3]^+$), 368 ($[411-CONH]^+$), 338 ($[368-CH_2O]^+$)

[a]Values for ^{79}Br-^{81}Br isotope form.

Reference

1. Breyer-Pfaff, U.; Wachsmuth, H. Tertiary *N*-glucuronides of clozapine and its metabolite desmethylclozapine in patient urine. *Drug Metab*. *Dispos*. **2001**, *29*,1343-1348.