Supplementary Information

Synthesis of plasmodione metabolites and $^{13}$C-enriched plasmodione as chemical tools for drug metabolism investigation

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† Electronic Supplementary Information (ESI) available: $^1$H, $^{13}$C and $^{19}$F NMR spectrum, mass spectrum of compounds 1-17 and UHPLC-MS/MS spectrum of drug metabolites from mice urine. See DOI: 10.1039/x0xx00000x
Synthesis of 4-oxo-4-phenylbutanoic acid (1a/1b): Dihydrofuran-2,5-dione-2,3,4,5-13C4 (1 equiv., 500 mg, 4.81 mmol) was refluxed in sulfuryl chloride (24.2 mL) for 3 hours under argon atmosphere. Excess of sulfuryl chloride was removed under vacuum and the residue suspended in tetrachloroethane (20 mL) in an ice-water bath. Addition of benzene-1,2,3,4,5,6-13C6 (1 equiv., 404 mg, 0.43 mL, 6.71 mmol) was followed by portion-wise addition of fresh aluminium chloride (4.71 equiv., 3015 mg, 22.6 mmol) with stirring. The resulting mixture was stirred overnight at room temperature under argon atmosphere, gradually deepening in color. The reaction was poured into the mixture of ice (50 g) and concentrated HCl (5 mL, 37%), the solvent was removed under reduced pressure. The remaining mixture was extracted with ether (5×25 mL). The combined organic layers were washed with water (2×25 mL), brine, dried over MgSO4 and concentrated under reduced pressure to give a yellowish solid (887 mg, 98%). 4-oxo-4-phenylbutanoic acid (1a): 1H NMR (400 MHz, CDCl3): δ 7.99 (d, 2H, J = 7.2 Hz, phenylH), 7.58 (t, 1H, J = 7.6 Hz, phenylH), 7.47 (t, 2H, J = 7.6 Hz, phenylH), 3.32 (t, 2H, J = 6.6 Hz, CH2), 2.82 (t, 2H, J = 6.6 Hz, CH2) ppm (see Fig. S1, ESI†). 13C NMR (100 MHz, CDCl3): δ 197.9, 178.4, 136.5, 133.5, 128.8, 128.2, 33.3, 28.1 ppm (see Fig. S2, ESI†). HRMS (ESI) m/z: [M+H]+ calcd for C9H8O3: 179.0703; found 179.0702.

4-oxo-4-(phenyl-13C)butanoic acid 1,2,3,4,13C4 acid (1b): 1H NMR (400 MHz, CDCl3): δ 7.98 (dm, 2H, J = 164.0 Hz, phenylH), 7.56 (dt, 1H, J = 150.2 Hz, J = 7.7 Hz, phenylH), 7.47 (dt, 2H, J = 161.3 Hz, J = 7.9 Hz phenylH), 3.32 (dm, 2H, J = 126.8 Hz, J = 5.6 Hz, CH2), 2.82 (ddm, 2H, J = 129.3 Hz, J = 7.5 Hz, J = 3.5 Hz, CH2) ppm (see Fig. S1, ESI†). 13C NMR (100 MHz, CDCl3): δ 198.0 (dd, J = 52.5 Hz, J = 39.8 Hz), 177.7 (d, J = 56.3 Hz), 136.5 (qm, J = 49.5 Hz), 133.5 (tm, J = 50.3 Hz), 128.5 (td, J = 39.1 Hz, J = 3.5 Hz), 128.5 (dtt, J = 76.7 Hz, J = 53.3 Hz, J = 3.5 Hz), 33.4 (td, J = 40.0 Hz, J = 13.1 Hz), 28.0 (dd, J = 56.8 Hz, J = 38.3 Hz) ppm (see Fig. S2, ESI†). ESI-MS m/z: [M+H]+ calcd for 13C10H12O3: 189.15; found 189.10.

Synthesis of 4-phenylbutanoic acid (2a/2b): To a solution of 4-oxo-4-(phenyl-13C)butanoic-1,2,3,4,13C4 acid (1b (1 equiv., 800 mg, 4.25 mmol) in diethylene glycol (12.9 mL) was added hydrazine monohydrate (5.2 equiv., 1108 mg, 1.08 mL, 22.1 mmol). After stirring at room temperature for 30 min, KOH (4.57 equiv., 1282 mg, 19.4 mmol) was added and the reaction mixture was heated at 120°C for 1.5 hours. Then the reaction mixture was heated (T = 215°C) to distill low boiling material; when diethyle dione (3a/3b), ESI-MS m/z: [M+H]+ calcd for 13C10H12O2: 179.0703; found 179.0702.

Synthesis of 4,3-dihydronaphthalene-1(2H)-one (3a/3b): 4-(phenyl-13C)butanoic-1,2,3,4,13C4 acid (2b (1 equiv., 660 mg, 3.79 mmol) was added to methanesulfonic acid (41.3 equiv., 15036 mg, 10.15 mL, 156.5 mmol) and the mixture was heated at 90°C for 30 min under argon atmosphere. The reaction mixture was then poured into ice-water and extracted with ether (3×40 mL). The combined organic layers were washed with dilute sodium bicarbonate (20 mL), water (2×15 mL) and brine, dried over MgSO4 and concentrated under reduced pressure to give a yellowish liquid (554.7 mg, 94%). 3,4-dihydronaphthalene-1(2H)-one (3a): 1H NMR (400 MHz, CDCl3): δ 8.03 (d, 1H, J = 7.9 Hz, ArH), 7.47 (td, 1H, J = 7.6 Hz, J = 1.1 Hz, ArH), 7.31 (t, 1H, J = 7.7 Hz, ArH), 7.25 (m, 1H, ArH), 2.97 (t, 2H, J = 5.9 Hz, CH2), 2.66 (t, 2H, J = 6.6 Hz, CH2), 2.14 (tt, 2H, J = 5.9 Hz, J = 6.6 Hz, CH2) ppm (see Fig. S5, ESI†). 13C NMR (100 MHz, CDCl3): δ 198.7, 144.8, 133.7,132.9, 129.1, 127.5, 126.9, 39.5, 30.1, 23.6 ppm (see Fig. S5, ESI†). HRMS (ESI) m/z: [M+H]+ calcd for C9H8O2: 147.0804; found 147.0812. 3,4-dihydronaphthalene-1(2H)-one (3b): 1H NMR (400 MHz, CDCl3): δ 8.03 (d, 1H, J = 106.1 Hz, ArH), 7.47 (m, 1H, J = 157.4 Hz, ArH), 7.31 (m, 1H, J = 162.4 Hz, ArH), 7.25 (m, 1H, J = 158.3 Hz, ArH), 2.96 (d, 2H, J = 132.3 Hz, CH2), 2.66 (dd, 2H, J = 127.7 Hz, J = 5.7 Hz, CH2), 2.14 (d, 2H, J = 131.3 Hz, CH2) ppm (see Fig. S5, ESI†). 13C NMR (100 MHz, CDCl3): δ 198.5 (dd, J = 50.1 Hz, J = 41.0 Hz), 144.6 (dd, J = 102.7 Hz, J = 50.4 Hz), 133.3 (q, J = 51.9 Hz), 132.9 (dd, J = 140.3 Hz, J = 57.8 Hz), 128.9 (tq, J = 56.3 Hz, J = 3.0 Hz), 127.5 (m), 126.9 (m), 39.5 (dd, J = 42.1 Hz, J = 31.8 Hz, J = 12.2 Hz), 30.1 (dd, J = 39.8 Hz, J = 34.5 Hz), 23.6 (t, J = 33.2 Hz) ppm (see Fig. S5, ESI†). ESI-MS m/z: [M+H]+ calcd for 13C10H13O2: 157.16; found 157.12.
4-oxo-4-phenylbutanoic acid (1a/1b):

- $\text{a} = ^{12}\text{C}$
- $\text{b} = ^{13}\text{C}$

4-oxo-4-(phenyl-$^{13}\text{C}_6$)butanoic-1,2,3,4-$^{13}\text{C}_4$ acid

$^1\text{H NMR}$

Figure S1. $^1\text{H NMR}$ (400 MHz, CDCl$_3$) of 4-oxo-4-phenylbutanoic acid 1a and 4-oxo-4-(phenyl-$^{13}\text{C}_6$)butanoic-1,2,3,4-$^{13}\text{C}_4$ acid 1b. The black line represents 1a and the green line represents 1b.
$^{13}$C NMR:

Figure S2. $^{13}$C NMR (100 MHz, CDCl$_3$) of 4-oxo-4-phenylbutanoic acid 1a and 4-oxo-4-(phenyl-$^{13}$C$_6$)butanoic-1,2,3,4-$^{13}$C$_4$ acid 1b. The black line represents 1a and the green line represents 1b.
4-phenylbutanoic acid (2a/2b):

\[ \text{a} = ^{12}\text{C} \\
\text{b} = ^{13}\text{C} \]

4-(phenyl-\(^{13}\text{C}_6\)butanoic-1,2,3,4-\(^{13}\text{C}_4\) acid

\(^{1}H\) NMR

Figure S3. \(^{1}H\) NMR (400 MHz, CDCl\(_3\)) of 4-phenylbutanoic acid 2a and 4-(phenyl-\(^{13}\text{C}_6\))butanoic-1,2,3,4-\(^{13}\text{C}_4\) acid 2b. The black line represents 2a and the green line represents 2b.
Figure S4. $^{13}$C NMR (100 MHz, CDCl$_3$) of 4-phenylbutanoic acid 2a and 4-(phenyl-$^{13}$C$_6$)butanoic-1,2,3,4-$^{13}$C$_4$ acid 2b. The black line represents 2a and the green line represents 2b.
3,4-dihyronaphthalen-1(2H)-one (3a/3b):

\[ \text{a} = ^{12}\text{C} \]
\[ \text{b} = ^{13}\text{C} \]

\[ 3,4\text{-dihyronaphthalen-1(2H)-one} - ^{13}\text{C}_{10} \]

\[ ^{1}\text{H NMR} \]

Figure S5. \[^{1}\text{H NMR} \text{(400 MHz, CDCl}_3\text{)}\] of tetralone 3a and \[^{13}\text{C}_{10}\text{-enriched tetralone 3b}. \text{The black line represents 3a and the green line represents 3b.} \]
Figure S6. $^{13}$C NMR (100 MHz, CDCl$_3$) of tetralone 3a and $^{13}$C$_{10}$-enriched tetralone 3b. The black line represents 3a and the green line represents 3b.
1-bromo-4-iodobenzene (4a/4b):

$\text{Br}$

1-bromo-4-iodobenzene-1,2,3,4,5,6-$^{13}$C$_6$

$^1$H NMR

Figure S7. $^1$H NMR (400 MHz, CDCl$_3$) of 1-bromo-4-iodobenzene 4a and 1-bromo-4-iodobenzene-1,2,3,4,5,6-$^{13}$C$_6$ 4b. The black line represents 4a and the green line represents 4b.
$^{13}$C NMR

Figure S8. $^{13}$C NMR (100 MHz, CDCl$_3$) of 1-bromo-4-iodobenzene 4a and 1-bromo-4-iodobenzene-1,2,3,4,5,6-$^{13}$C$_6$ 4b. The black line represents 4a and the green line represents 4b.
4-(trifluoromethyl)benzaldehyde (6a/6b):

\[
\begin{align*}
\text{CF}_3 & \\
& \text{a}={^{12}\text{C}} \\
& \text{b}={^{13}\text{C}} \\
\end{align*}
\]

Figure S9. $^1\text{H NMR}$ (400 MHz, CDCl$_3$) of $p$-trifluoromethylbenzaldehyde 6a and $^{13}\text{C}_p$-$p$-trifluoromethylbenzaldehyde 6b. The black line represents 6a and the green line represents 6b.
Figure S10. $^{13}$C NMR (100 MHz, CDCl$_3$) of $p$-trifluoromethylbenzaldehyde 6a and $^{13}$C-$p$-trifluoromethylbenzaldehyde 6b. The black line represents 6a and the green line represents 6b.
Figure S11. $^{19}$F NMR (376 MHz, CDCl$_3$) of $p$-trifluoromethylbenzaldehyde 6a and $^{13}$C-$p$-trifluoromethylbenzaldehyde 6b. The black line represents 6a and the green line represents 6b.
2-(4-(trifluoromethyl)benzylidene)-3,4-dihyronaphthalen-1(2H)-one (7a/7b):

![Chemical structure](image)

2-(4-(trifluoromethyl)phenyl-1,2,3,4,5,6-\textsuperscript{13}C_{6})methylene-13\textsuperscript{13}C)-3,4-dihyronaphthalen-1(2H)-one-13\textsuperscript{13}C_{10}

\textsuperscript{1}H NMR

![NMR spectra](image)

Figure S12. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) of 2-(4-(trifluoromethyl)benzylidene)-3,4-dihyronaphthalen-1(2H)-one 7a and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6\textsuperscript{13}C_{6})methylene-\textsuperscript{13}C)-3,4-dihyronaphthalen-1(2H)-one-\textsuperscript{13}C_{10} 7b. The black line represents 7a and the green line represents 7b.
13C NMR

Figure S13. $^{13}$C NMR (100 MHz, CDCl$_3$) of 2-((4-(trifluoromethyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one 7a and 2-((4-(trifluoromethyl)phenyl)-1,2,3,4,5,6-^{13}C$_6$)-methylene-^{13}C)-3,4-dihydronaphthalen-1(2H)-one-^{13}C$_{10}$ 7b. The black line represents 7a and the green line represents 7b.
Figure S14. $^{19}$F NMR (376 MHz, CDCl$_3$) of 2-((4-(trifluoromethyl)benzylidene)-3,4-dihyronaphthalen-1(2H)-one 7a and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}$C$_6$)methylene-$^{13}$C)-3,4-dihyronaphthalen-1(2H)-one-$^{13}$C$_{10}$ 7b. The black line represents 7a and the green line represents 7b.
2-(4-(trifluoromethyl)benzyl)naphthalen-1-ol (8a/8b):

\[ \text{2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6,13C_6)methyl-13C)napthalen-1-ol-13C_{10}} \]

\( ^1H \) NMR

Figure S15. \(^1H\) NMR (400 MHz, CDCl\(_3\)) of 2-(4-(trifluoromethyl)benzyl)naphthalen-1-ol 8a and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-13C_6)methyl-13C)napthalen-1-ol-13C\(_{10}\) 8b. The black line represents 8a and the green line represents 8b.
$^{13}$C NMR

Figure S16. $^{13}$C NMR (100 MHz, CDCl$_3$) of 2-[(4-(trifluoromethyl)benzyl)naphthalen-1-ol $^8$a and 2-[(4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}$C$_6$)methyl-$^{13}$C]naphthalen-1-ol-$^{13}$C$_{10}$ $^8$b. The black line represents $^8$a and the green line represents $^8$b.
$^{19}$F NMR

Figure S17. $^{19}$F NMR (376 MHz, CDCl$_3$) of 2-((4-(trifluoromethyl)benzyl)naphthalen-1-ol $^{13}$C$_{10}$ 8a and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}$C$_6$)methyl-$^{13}$C)naphthalen-1-ol-$^{13}$C$_{10}$ 8b. The black line represents 8a and the green line represents 8b.
2-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione (9a/9b):

\[ \text{O} \quad \text{CF}_3 \]

\[ 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-}^{13}\text{C}_6)\text{methyl-}^{13}\text{C})\text{naphthalene-1,4-dione-}^{13}\text{C}_{10} \]

\(^1\text{H NMR} \]

Figure S18. \(^1\text{H NMR} (400 \text{ MHz, CDCl}_3)\) of 2-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione 9a and 2-((4-(trifluoromethyl)phenyl-1,2,3,4,5,6-}^{13}\text{C}_6)\text{methyl-}^{13}\text{C})\text{naphthalene-1,4-dione-}^{13}\text{C}_{10} 9b. The black line represents 9a and the green line represents 9b.
Figure S19. $^{13}$C NMR (100 MHz, CDCl$_3$) of 2-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione 9a and 2-(4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}$C$_6$)methyl-$^{13}$C)naphthalene-1,4-dione-1$^{13}$C$_6$ 9b. The black line represents 9a and the green line represents 9b.
$^{19}$F NMR

Figure S20. $^{19}$F NMR (376 MHz, CDCl$_3$) of 2-{4-(trifluoromethyl)benzyl}naphthalene-1,4-dione 9a and 2-{4-(trifluoromethyl)phenyl-1,2,3,4,5,6-$^{13}$C$_6$}methyl-$^{13}$C)naphthalene-1,4-dione-$^{13}$C$_{10}$ 9b. The black line represents 9a and the green line represents 9b.
2-(methyl-$^{13}$C)-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione (10a/10b):

$^1$H NMR

Figure S21. $^1$H NMR (400 MHz, CDCl$_3$) of plasmodione and $^{13}$C$_{16}$-plasmodione 10b and $^{13}$C$_{1}$-plasmodione 10a. The black line represents plasmodione, the red line represents 10b and the green line represents 10a.
Figure S22. $^{13}$C NMR (100 MHz, CDCl$_3$) of plasmodione and $^{13}$C$_{4p}$-plasmodione 10b and $^{13}$C$_{4}$-plasmodione 10a. The black line represents plasmodione, the red line represents 10b and the green line represents 10a.
Figure S23. $^{19}$F NMR (376 MHz, CDCl$_3$) of plasmodione and $^{13}$C$_{18}$-plasmodione 10b and $^{13}$C$_{1}$-plasmodione 10a. The black line represents plasmodione, the red line represents 10b and the green line represents 10a.
Figure S24. ESI-MS mass spectra of $^{13}$C$_{18}$-plasmodione 10b. Analyses were performed on an LTQ-Orbitrap Discovery instrument (R=30,000 at m/z 400).
2-methyl-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione-1-$^{13}$C (10c):

![Chemical Structure](image)

2-methyl-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione-1-$^{13}$C

Figure S25. $^1$H NMR (400 MHz, CDCl$_3$) of $^{13}$C$_1$-plasmodione 10c and its impurity. The black line represents $^{13}$C$_1$-plasmodione 10c contaminated by the $^{13}$C$_1$-epoxide as an impurity < 4% and the green line represents the pure epoxide 11.
Figure S26. $^{13}$C NMR (100 MHz, CDCl3) of $^{13}$C$_1$-plasmodione 10c and its impurity. The black line represents $^{13}$C$_1$-plasmodione 10c contaminated by the $^{13}$C$_1$-epoxide as an impurity < 4% and the green line represents the pure epoxide 11.
1a-methyl-7a-(4-(trifluoromethyl)benzyl)-1a,7a-dihydronaphtho[2,3-b]oxirene-2,7-dione (11):

\[
\begin{align*}
\text{CF}_3
\end{align*}
\]

\[
\begin{align*}
1a\text{-methyl-7a-(4-(trifluoromethyl)benzyl)}
\end{align*}
\]

\[
\begin{align*}
1a,7a\text{-dihydronaphtho[2,3-b]oxirene-2,7-dione}
\end{align*}
\]

\[\text{\textsuperscript{1}H NMR}\]

Figure S27. \[\text{\textsuperscript{1}H NMR (400 MHz, CDCl}_3\text{) of 1a-methyl-7a-(4-(trifluoromethyl)benzyl)}
\]

1a,7a-dihydronaphtho[2,3-b]oxirene-2,7-dione 11.
Figure S28. $^{13}$C NMR (100 MHz, CDCl$_3$) of 1a-methyl-7a-(4-(trifluoromethyl)benzyl)-1a,7a-dihyronaphtho[2,3-b]oxirene-2,7-dione 11.
Figure S29. $^{19}$F NMR (376 MHz, CDCl$_3$) of 1a-methyl-7a-(4-(trifluoromethyl)benzyl)-1a,7a-dihyronaphtho[2,3-b]oxirene-2,7-dione 11.
2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)-3-methylnaphthalene-1,4-dione (14):

\[
\text{HO} \quad \text{CF}_3
\]

\[\text{2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)-3-methylnaphthalene-1,4-dione}\]

\[\text{H NMR}\]

Figure S30. \(^1\text{H NMR}\) (400 MHz, CDCl\(_3\)) of 2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)-3-methylnaphthalene-1,4-dione 14.
Figure S31. $^{13}$C NMR (100 MHz, CDCl$_3$) of 2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)-3-methylnaphthalene-1,4-dione 14.
Figure S32. $^{19}$F NMR (376 MHz, CDCl$_3$) of 2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)-3-methylnaphthalene-1,4-dione 14.
(1,4-bis(methoxymethoxy)-3-methylnaphthalen-2-yl)(2-fluoro-4-(trifluoromethyl)phenyl)methanone (22):

(1,4-bis(methoxymethoxy)-3-methylnaphthalen-2-yl)(2-fluoro-4-(trifluoromethyl)phenyl)methanone

$^1$H NMR

Figure S33. $^1$H NMR (400 MHz, CDCl$_3$) of (1,4-bis(methoxymethoxy)-3-methylnaphthalen-2-yl)(2-fluoro-4-(trifluoromethyl)phenyl)methanone 22.
(2-fluoro-4-(trifluoromethyl)phenyl)(1-hydroxy-4-(methoxymethoxy)-3-methylnaphthalen-2-yl)methanone (23):

\[
\begin{align*}
&\text{OMOM} \\
&\text{CF}_3
\end{align*}
\]

(2-fluoro-4-(trifluoromethyl)phenyl)(1-hydroxy-4-(methoxymethoxy)-3-methylnaphthalen-2-yl)methanone

$^1$H NMR

Figure S34. $^1$H NMR (400 MHz, CDCl$_3$) of (2-fluoro-4-(trifluoromethyl)phenyl)(1-hydroxy-4-(methoxymethoxy)-3-methylnaphthalen-2-yl)methanone 23.
5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one (24):

\[
\text{MOMO} \quad \text{O} \quad \text{CF}_3
\]

5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one

\[\text{^1H NMR}\]

Figure S35. \(^1\text{H NMR (400 MHz, CDCl}_3\) of 5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one 24.\]
$^{13}$C NMR

Figure S36. $^{13}$C NMR (100 MHz, CDCl$_3$) of 5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one 24.
Figure S37. NMR spectrum of 5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one (24): zoom of $^{13}$C and DEPT spectrum.
Figure S38. $^{19}$F NMR (376 MHz, CDCl$_3$) of 5-(methoxymethoxy)-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one 24.
5-hydroxy-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one (15):

![Chemical structure of 5-hydroxy-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one](image)

**1H NMR**

Figure S39. $^1$H NMR (400 MHz, DMSO-$d_6$) of 5-hydroxy-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one 15.
Figure S40. $^1$H NMR (376 MHz, DMSO-$d_6$) of 5-hydroxy-6-methyl-10-(trifluoromethyl)-7H-benzo[c]xanthen-7-one 15.
2-(hydroxymethyl)-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione (17):

![Structure of the compound](image)

**1H NMR**

Figure S41. $^1$H NMR (400 MHz, CDCl$_3$) of 2-(hydroxymethyl)-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione 17.
Figure S42. $^{13}$C NMR (100 MHz, CDCl$_3$) of 2-(hydroxymethyl)-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione 17.
Figure S43. $^{19}$F NMR (376 MHz, CDCl$_3$) of 2-(hydroxymethyl)-3-(4-(trifluoromethyl)benzyl)naphthalene-1,4-dione 17.
MS data acquisition of drug metabolites

The experiment was performed by using an UHPLC coupling to a triple quadripole Shimadzu LC-MS 8030 operating in the negative ion mode at Plate-forme de Chimie Biologique Integrative de Strasbourg (TechMedLL, Patrick Gizzi, Strasbourg).

The urine samples were loaded and separated on kinetex 2.6µ C8 100A 50x2.1 mm maintained at 50°C, with a flow rate set at 500 µL/min. The gradient condition was described as follows: Mobile phases were water containing 0.05% formic acid (mobile phase A) and acetonitrile (ACN) (mobile phase B). The gradient conditions implied first a from 0 to 0.1 min equilibration at 40% B, then the proportion of B was raised to 100% from 0.6 to 0.9 min. The proportion of B was decreased to 40% from 0.92 to 2.3 min (total runtime: 2.3 min).

Figure S44. UHPLC spectrum of hydroxyl plasmodione metabolites. a) Naïve mouse 24 h post 1st injection of plasmodione. b) Naïve mouse 24 h post 2nd injection of plasmodione.
Figure S45. UHPLC spectrum of hydroxyl plasmodione metabolites. a) Infected mouse 1 day post 1st injection of plasmodione. b) Infected mouse 1 day post 2nd injection of plasmodione. c) Infected mouse 1 day post 3rd injection of plasmodione. d) Infected mouse 2 days post 3rd injection of plasmodione.

Aire sous le pic à 0.72 min
5a  12787
5b  14090
5c  13312
5d  39639
Figure S46. UHPLC/MS-MS spectrum of 6-hydroxyl plasmodione 16a which have incubated 0 or 1 hour with mice liver microsomes and 6-glucuronic plasmodione metabolites.
$t_R = 0.86 \text{ min} \quad \text{Area} = 247097$

$t_R = 0.86 \text{ min} \quad \text{Area} = 114673$
$t_R = 0.63 \text{ min} \quad \text{Area} = 130105$

Figure S47. UHPLC/MS-MS spectrum of 7-hydroxyl plasmodione 16b which have incubated 0 or 1 hour with mice liver microsomes and 7-glucuronic plasmodione metabolites.
Figure S48. UHPLC/MS-MS spectrum of mice urine: Naive mouse 24 h post 1st injection of plasmodione.

Figure S48. UHPLC/MS-MS spectrum of mice urine: Naive mouse 24 h post 1st injection of plasmodione.
Figure S49. UHPLC/MS-MS spectrum of mice urine: Naive mouse 24 h post 2nd injection of plasmodione.

I_{317.1} / I_{329} = 1.3

t_R = 0.86 min

Fragment not detected

t_R = 0.63 min
Figure S50. UHPLC/MS-MS spectrum of mice urine: Infected mouse 1 day post 3rd injection of plasmodione.
Infected mouse 2 days post 3rd injection of plasmodione

Figure S51. UHPLC/MS-MS spectrum of mice urine: Infected mouse 2 day post 3rd injection of plasmodione.