

Supplementary Materials: Biooxidation of Ciguatoxins Leads to Species-Specific Toxin Profiles

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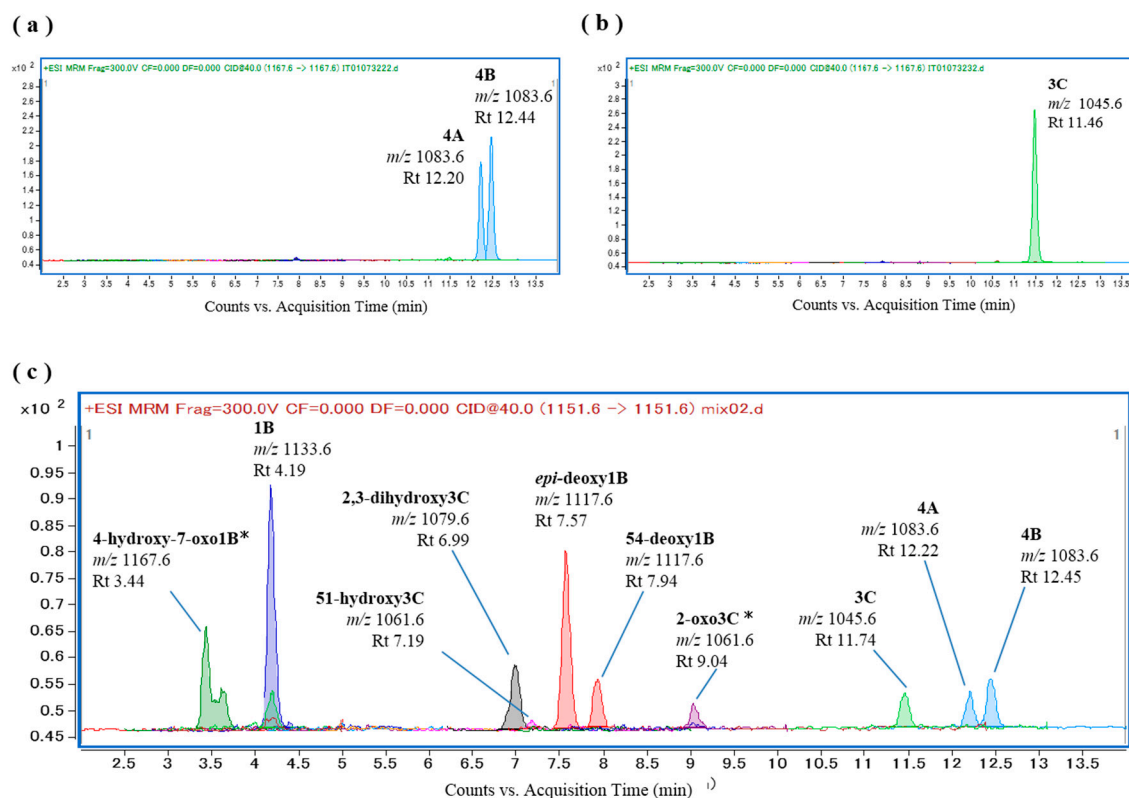


Figure S1. Chromatograms, retention times (Rts), and m/z values for the ciguatoxins (CTXs) that were used as substrates for *in vitro* oxidation. (a) CTX4A/4B; (b) CTX3C; and (c) reference toxins for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. 4A: CTX4A; 4B: CTX4B; 3C: CTX3C; 4-hydroxy-7-oxo-1B: 4-hydroxy-7-oxo-CTX1B; 1B: CTX1B; 2,3-dihydroxy3C: 2,3-dihydroxyCTX3C; 51-hydroxy3C: 51-hydroxyCTX3C; epi-deoxy1B: 52-epi-54-deoxyCTX1B; deoxy1B: 54-deoxyCTX1B; 2-oxo3C: 2-oxo-CTX3C.

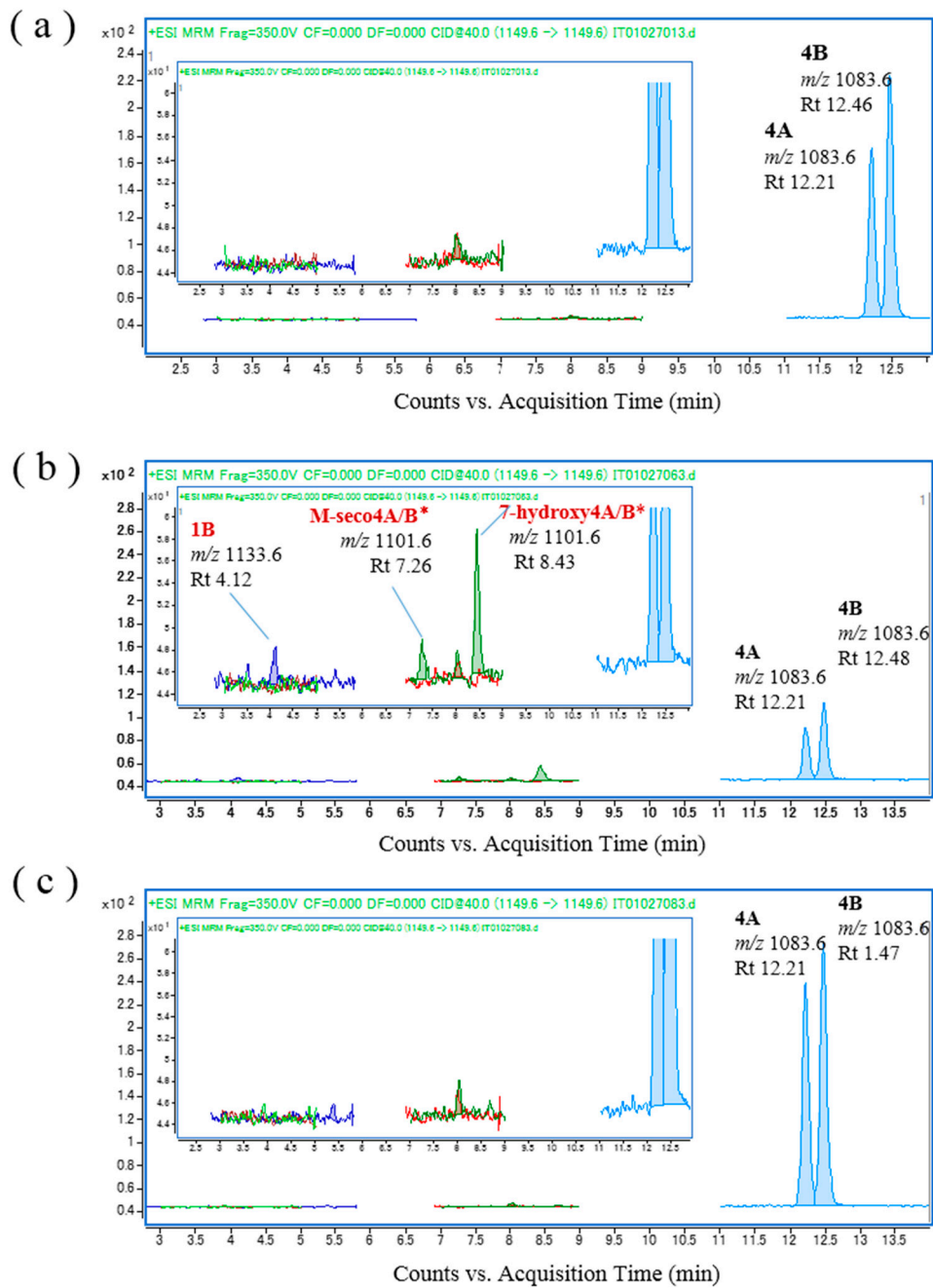


Figure S2. Chromatograms of the *in vitro* oxidation products of ciguatoxin-4A/4B (CTX4A/4B) following incubation with recombinant human CYP3A4 (rhCYP3A4). Products are shown (a) at 0 min, (b) at 60 min, and (c) at 60 min without rhCYP3A4. The inset in each panel shows a magnified view of the chromatogram. 4A: CTX4A; 4B: CTX4B; 1B: CTX1B; M-seco4A/4B: M-secoCTX4A/4B; 7-hydroxy4A/4B: 7-hydroxyCTX4A/4B.

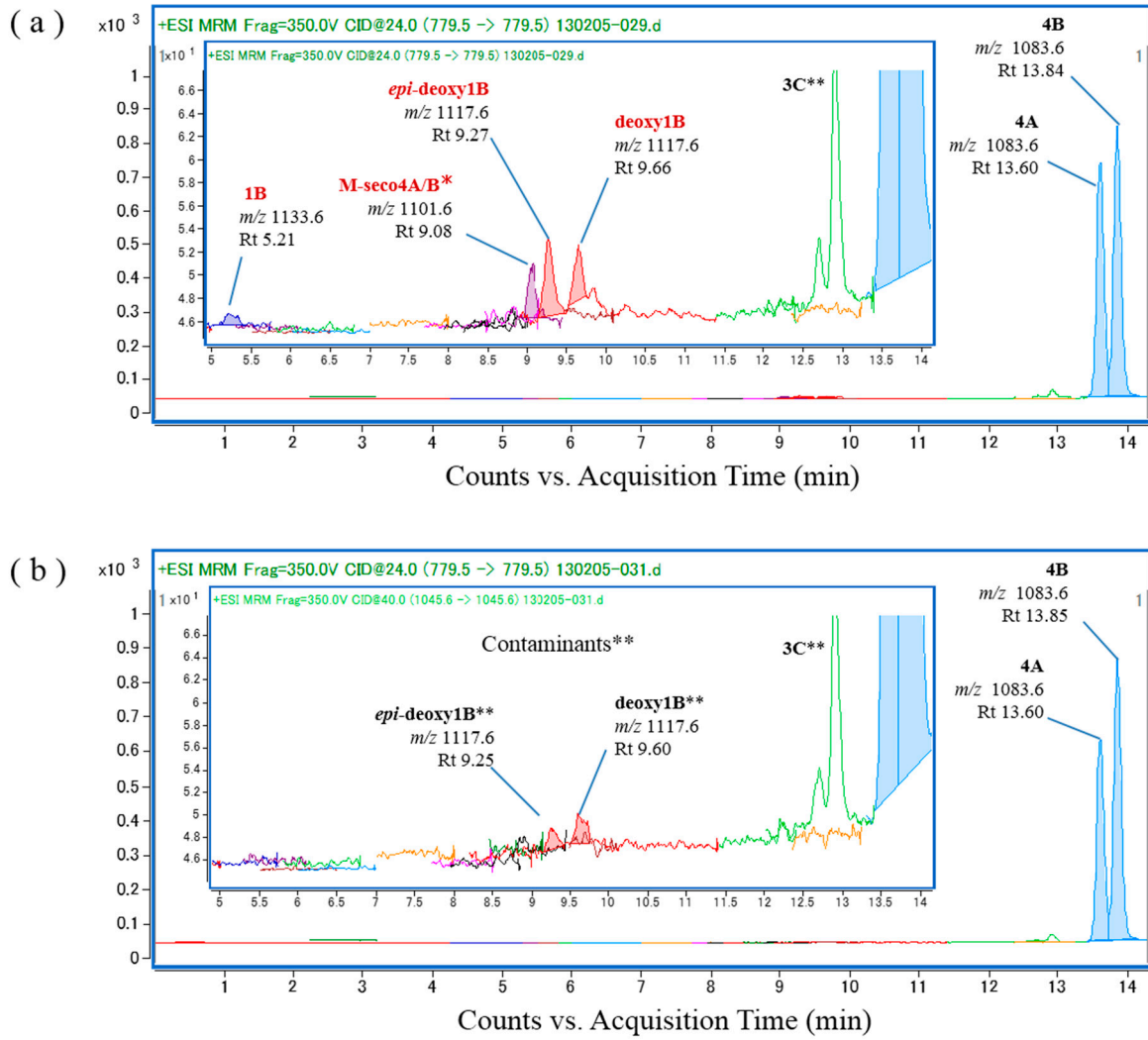


Figure S3. Chromatograms of the *in vitro* oxidation products of ciguatoxin-4A/4B (CTX4A/4B) following incubation with human liver microsomes. (a) Untreated microsomes and (b) heat-treated microsomes at 90 °C for 5 min. The inset in each panel shows a magnified view of the chromatogram. ** The 4A/4B substrate contained trace amounts of CTX3C, epi-deoxy1B, and deoxy1B (m/z 1117). 1B: CTX1B; M-seco4A/4B: M-secoCTX4A/4B; epi-deoxy1B: 52-epi-54-deoxyCTX1B; deoxy1B: 54-deoxyCTX1B; 3C: CTX3C; 4A: CTX4A; 4B: CTX4B.

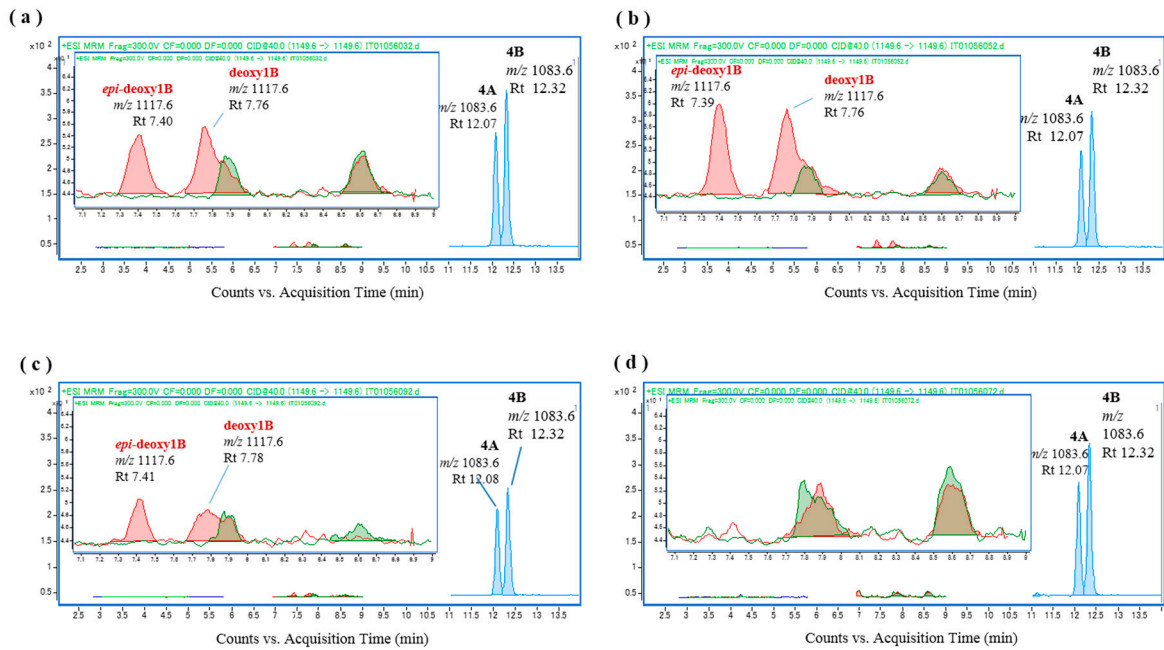


Figure S4. Chromatograms of the *in vitro* oxidation products of ciguatoxin-4A/4B (CTX4A/4B) following incubation with the S9 fractions from four different *Lutjanus bohar* specimens. (a) Specimen of 1.5 kg body weight (BW) and 45 cm total length (TL); (b) specimen of 1.1 kg BW and 42 cm TL; (c) specimen of 3.8 kg BW and 62 cm TL; and (d) specimen of 0.5 kg BW and 41 cm TL. The inset in each panel shows a magnified view of the chromatogram. epi-deoxy1B: 52-epi-54-deoxyCTX1B; deoxy1B: 54-deoxyCTX1B; 4A: CTX4A; 4B: CTX4B.

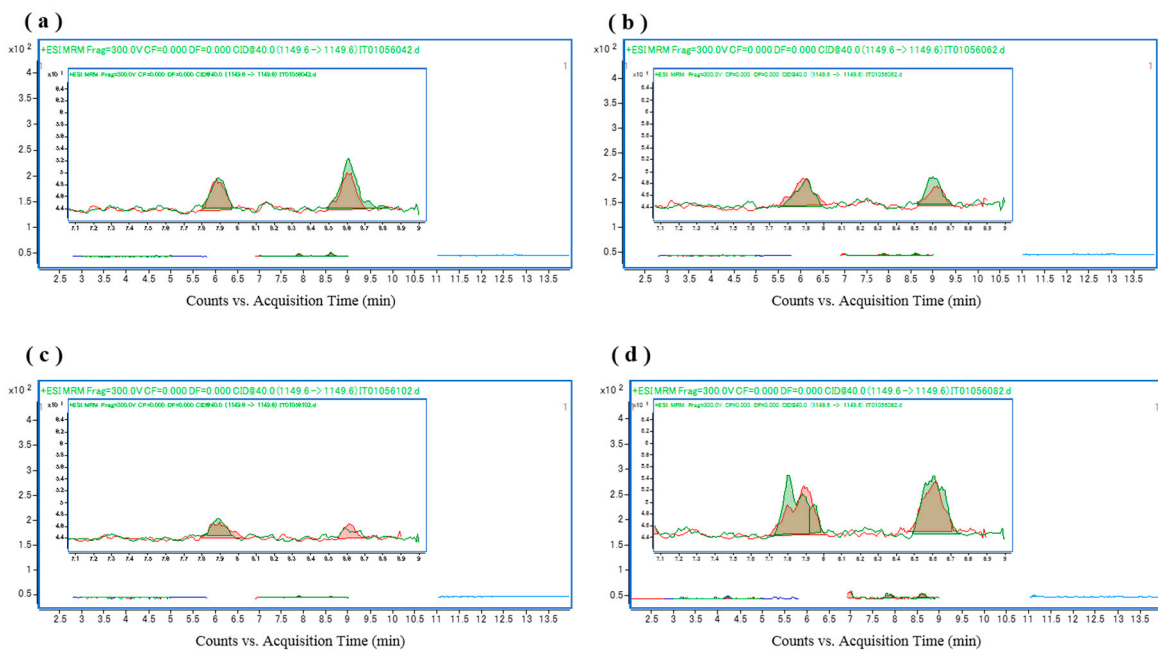


Figure S5. Chromatograms of the S9 fractions of four different *Lutjanus bohar* specimens. (a) Specimen of 1.5 kg body weight (BW) and 45 cm total length (TL); (b) specimen of 1.1 kg BW and 42 cm TL; (c) specimen of 3.8 kg BW and 62 cm TL; and (d) specimen of 0.5 kg BW and 41 cm TL. The inset in each panel shows a magnified view of the chromatogram.

Table S1. Body size of fish samples used in this study

Sample No.	Species	Sample size	
		Body weight (kg)	Body length (cm)
1		3.80	63.0
2		1.50	45.0
3	<i>Lutjanus bohar</i>	1.10	42.0
4		3.80	62.0
5		0.50	31.0
6	<i>Lutjanus monostigma</i>	0.50	30.0
7		1.70	47.0
8	<i>Oplegnathus punctatus</i>	1.10	30.0
9		2.00	50.0
10		0.88	37.0
11		0.82	38.0
12	<i>Lutjanus gibbus</i>	0.72	36.0
13		0.86	38.5
14		0.84	38.0
15		0.42	29.0
16	<i>Lutjanus fulviflamma</i>	0.36	27.5
17		0.37	26.0
18		0.42	28.0