Synthesis of 11-Thialinoleic Acid and 14-Thialinoleic Acid, Inhibitors of Soybean and Human Lipoxygenases

Electronic Supplementary Information

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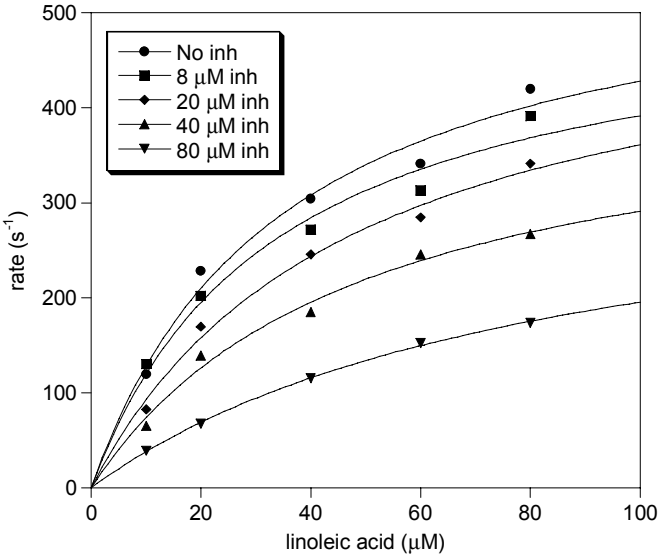
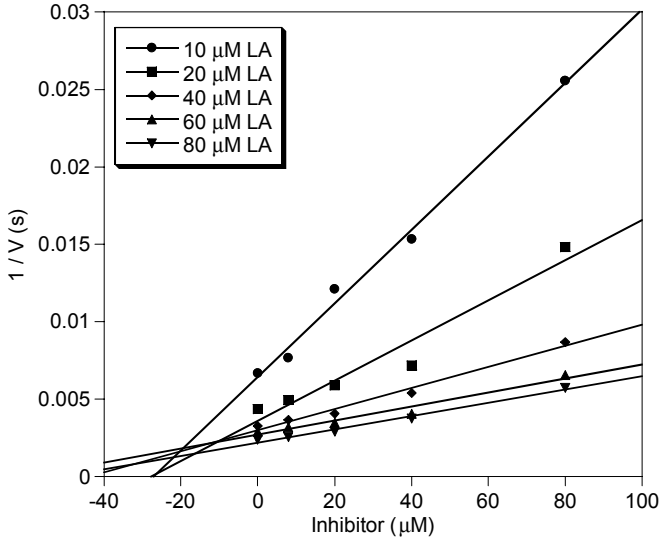
I - Kinetic inhibition plots with thialinoleic acids

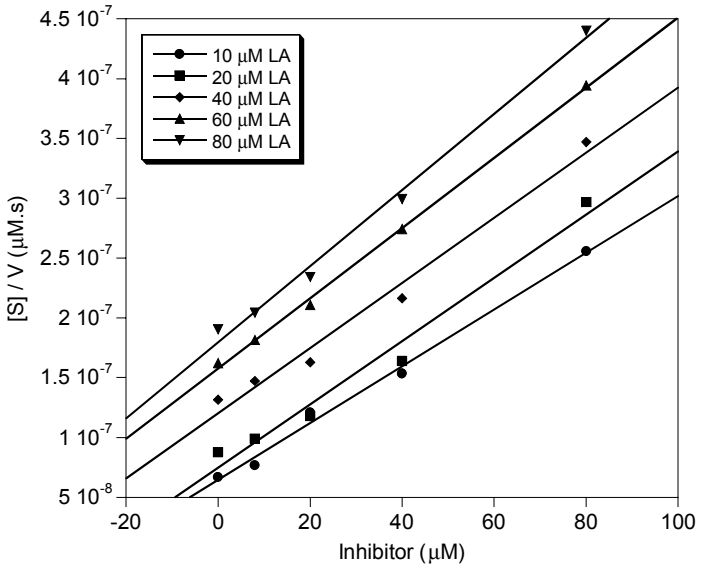
A. Soybean lipoxygenase-1, linoleic acid and 11-thialinoleic acid at pH 10



Supplementary Figure 1. Michaelis-Menten fits for the reaction of sLO-1 and linoleic acid (LA) in the presence of 11-thialinoleic acid (11-thiaLA) at pH 10.



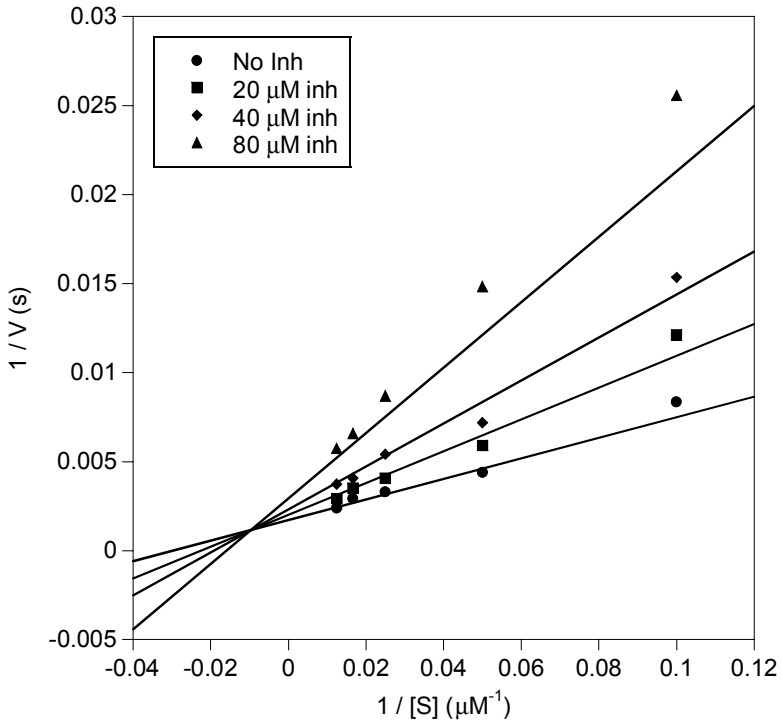
Supplementary Figure 2. Dixon plot showing the inhibition of sLO-1 and LA in the presence of 11-thiaLA.



Supplementary Figure 3. Cornish-Bowden plot of the same data as in Supplementary Figure 2 showing the competitive inhibition of sLO-1 and LA in the presence of 11-thiaLA. Although not perfectly parallel, the lines are much closer to the pattern expected for competitive inhibition than non- or uncompetitive inhibition.

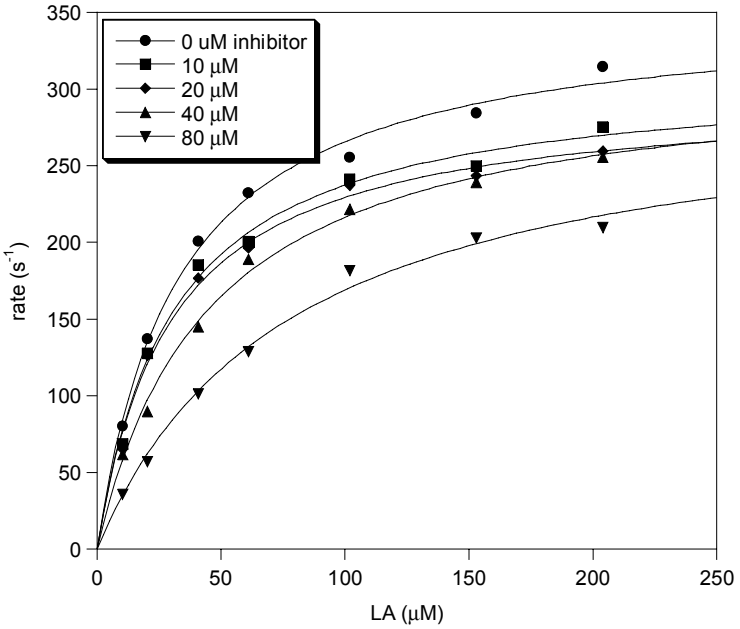
Supplementary Table 1. Error analysis of fits to different inhibition models for the reaction of sLO-1 and LA in the presence of 11-thiaLA at pH 10. The values were obtained using an algorithm developed by Cleland.[1]

Mechanism	K_m (μM)	k_{cat} (s^{-1})	K_{is} (μM)	K_{ii} (μM)	Sigma	variance
Competitive	28.5 ± 4.4	486 ± 31	22.3 ± 3.0	-	17.9	320
Noncompetitive	33.6 ± 5.5	513 ± 39	36.5 ± 11.3	115 ± 60	16.6	276
Uncompetitive	56.3 ± 11.2	528 ± 80	-	31.4 ± 5.2	24.0	576

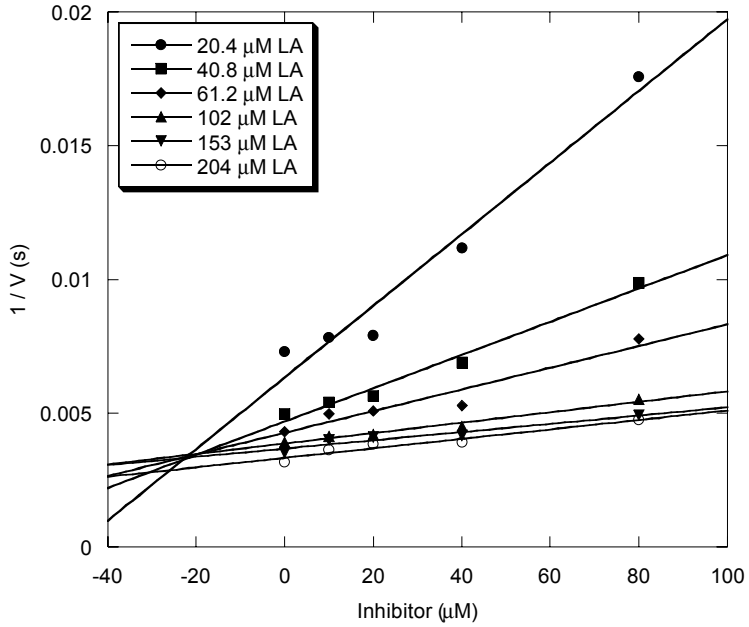


Supplementary Figure 4. Double reciprocal plot showing a fit to the noncompetitive model for the inhibition of the reaction of sLO-1 with LA by 11-thiaLA at pH 10.

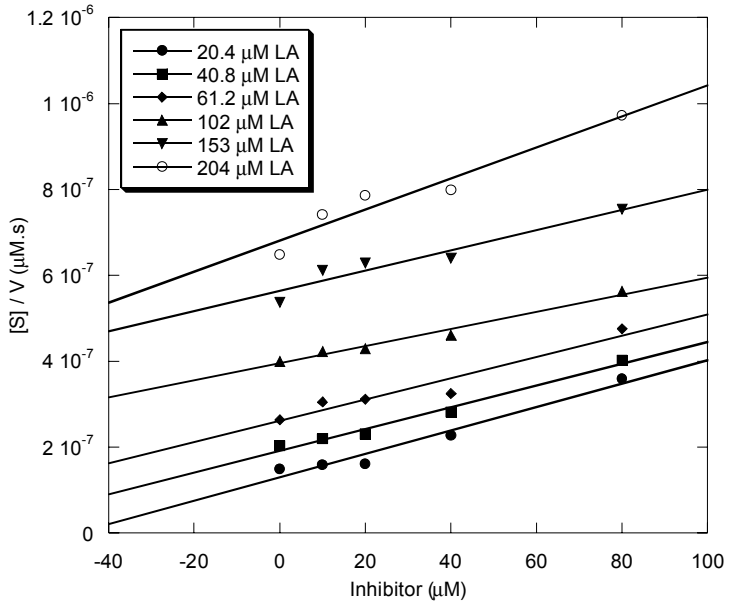
B. Soybean lipoxygenase-1, linoleic acid and 14-thialinoleic acid at pH 10



Supplementary Figure 5. Michaelis-Menten fits for the reaction of sLO-1 and linoleic acid (LA) in the presence of 14-thialinoleic acid (14-thiaLA) at pH 10.



Supplementary Figure 6. Dixon plot showing the inhibition of sLO-1 and LA in the presence of 14-thiaLA.

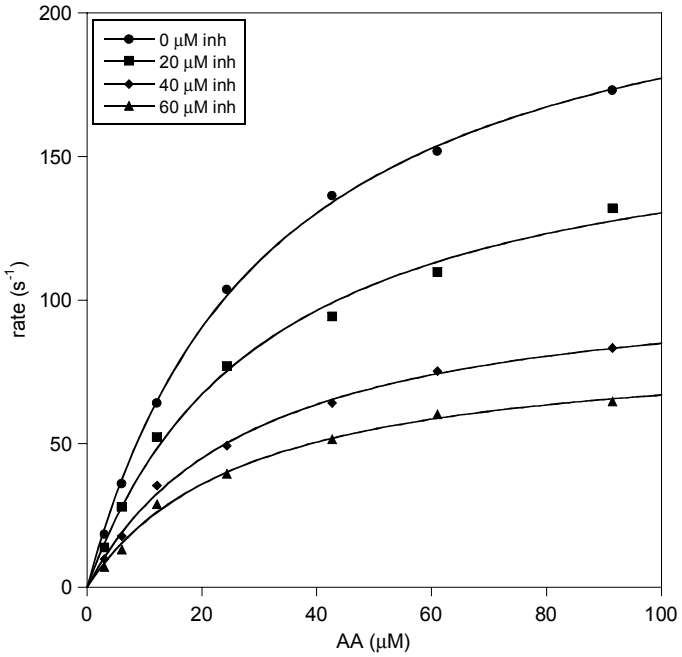


Supplementary Figure 7. Cornish-Bowden plot of the same data as Supplementary Figure 6 showing the competitive inhibition of sLO-1 and LA in the presence of 14-thiaLA. Although not perfectly parallel, the lines are closer to the pattern expected for competitive inhibition than non- or uncompetitive inhibition.

Supplementary Table 2. Error analysis of inhibition mode fits for the reaction of sLO-1 and LA in the presence of 14-thialinoleic acid at pH 10.

Mechanism	K_m (μM)	k_{cat} (s^{-1})	K_{is} (μM)	K_{ii} (μM)	Sigma	variance
Competitive	24.7 ± 2.0	321 ± 6	34.5 ± 4.1	-	10.4	108
Noncompetitive	28.0 ± 2.5	333 ± 8	49 ± 10	437 ± 180	9.6	92
Uncompetitive	43.2 ± 4.4	371 ± 15	-	117 ± 17	16.1	259

C. Soybean lipoxygenase-1, arachidonic acid and 11-thialinoleic acid, pH 7.5

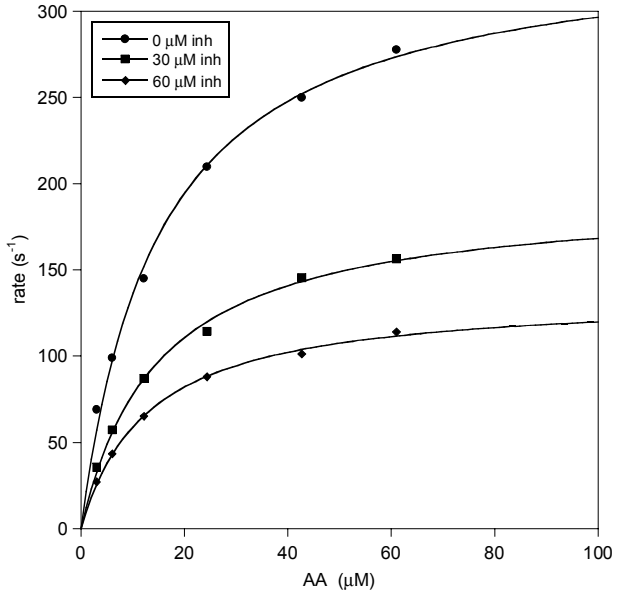


Supplementary Figure 8. Michaelis-Menten fits for the reaction of sLO-1 and AA in the presence of 11-thiaLA at pH 7.5.

Supplementary Table 3. Error analysis of inhibition mode fits for the reaction of sLO-1 and AA in the presence of 11-thiaLA at pH 7.5.

Mechanism	K_m (μM)	k_{cat} (s^{-1})	K_{is} (μM)	K_{ii} (μM)	Sigma	variance
Competitive	23.8 ± 4.7	210 ± 15	13.1 ± 2.2	-	9.4	88
Noncompetitive	31.7 ± 3.5	237 ± 11	45.6 ± 11.1	38.2 ± 5.4	4.8	23
Uncompetitive	45.3 ± 6.5	272 ± 19	-	22.2 ± 2.3	7.3	53

D. Soybean lipoxygenase-1, arachidonic acid and 11-thialinoleic acid, pH 10

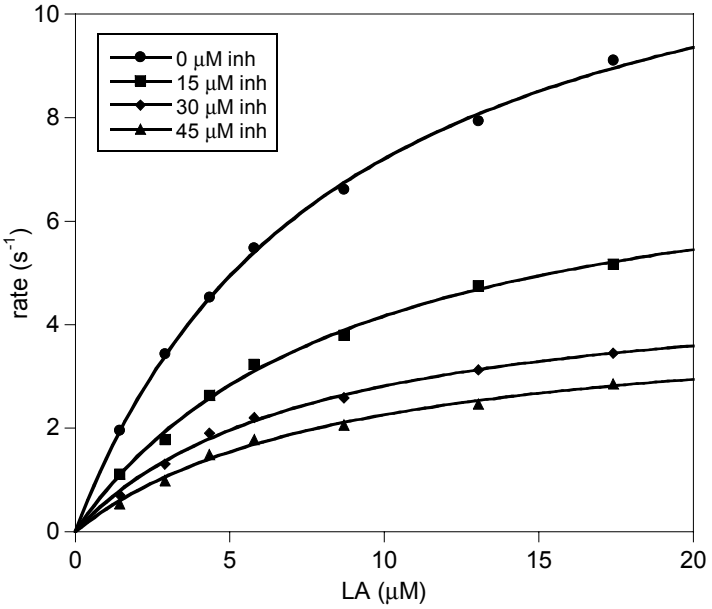


Supplementary Figure 9. Michaelis-Menten rate equation fits plot for the reaction of sLO-1 and AA in the presence of 11-thiaLA at pH 10.

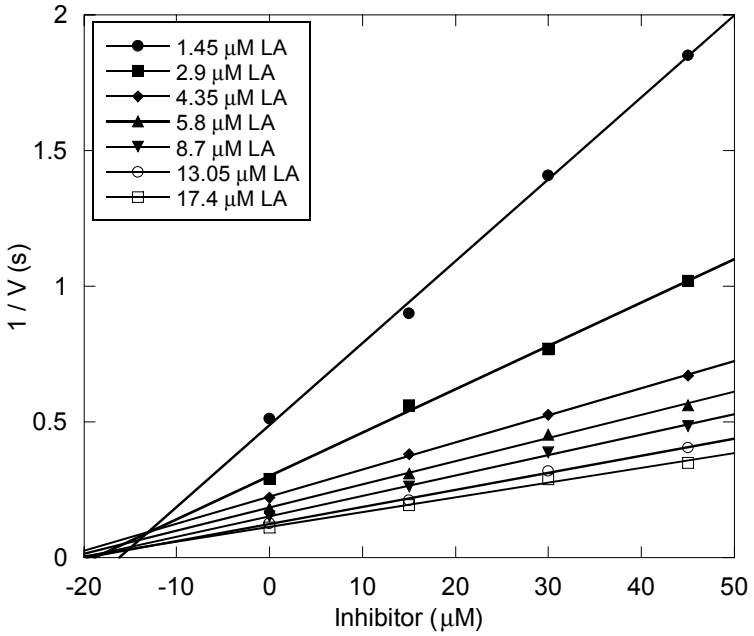
Supplementary Table 4. Error analysis of inhibition mode fits for the reaction of sLO-1 and AA in the presence of 11-thiaLA at pH 10.

Mechanism	K_m (μM)	k_{cat} (s^{-1})	K_{is} (μM)	K_{ii} (μM)	Sigma	variance
Competitive	12.2 ± 3.0	314 ± 25	10.3 ± 2.2	-	18.0	324
Noncompetitive	15.1 ± 1.5	340 ± 12	48.5 ± 12.9	36.0 ± 4.0	6.9	48
Uncompetitive	19.2 ± 2.6	366 ± 20	-	24.6 ± 2.4	10.9	119

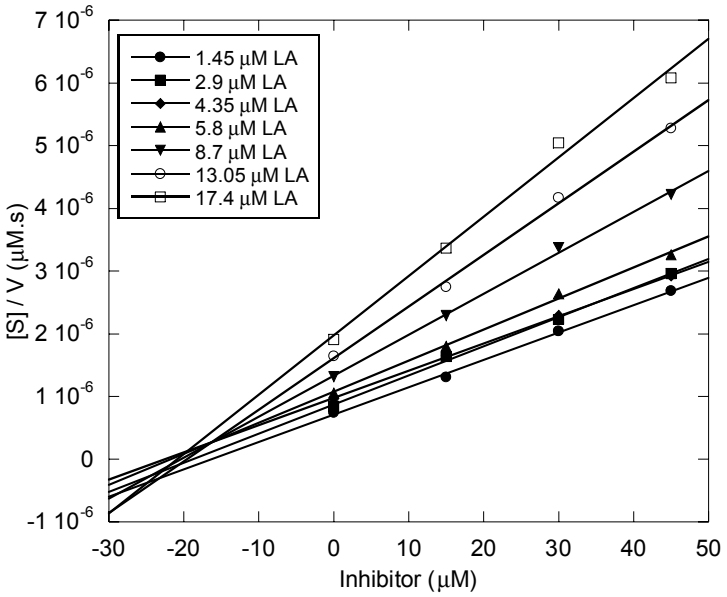
E. Human 15-lipoxygenase-1, linoleic acid and 11-thialinoleic acid at pH 7.5



Supplementary Figure 10. Michaelis-Menten fits for the reaction of 15-hLO-1 and LA in the presence of 11-thiaLA at pH 7.5.



Supplementary Figure 11. Dixon plot showing the inhibition of 15-hLO-1 and LA in the presence of 11-thiaLA.

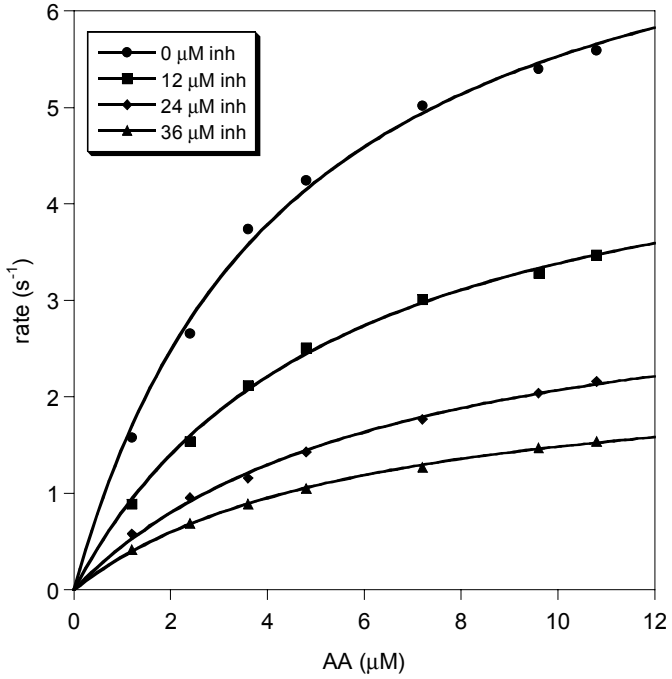


Supplementary Figure 12. Cornish-Bowden plot of the same data as in Supplementary Figure 11 showing the non-competitive inhibition of 15-hLO-1 and LA in the presence of 11-thiaLA.

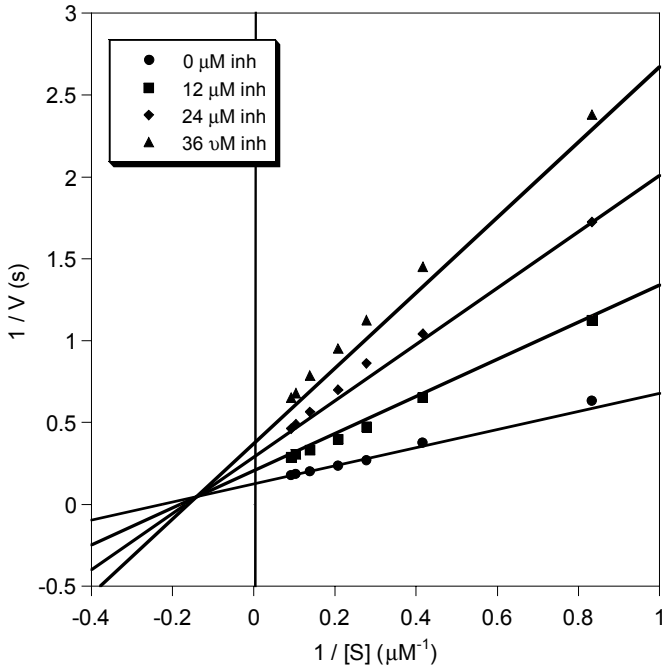
Supplementary Table 5. Error analysis of inhibition mode fits for the reaction of 15-hLO-1 and LA in the presence of 11-thiaLA at pH 7.5.

Mechanism	K_m (μM)	k_{cat} (s^{-1})	K_{is} (μM)	K_{ii} (μM)	Sigma	variance
Competitive	6.9 ± 1.0	12.1 ± 0.8	7.9 ± 0.9	-	0.35	0.123
Noncompetitive	8.6 ± 0.4	13.4 ± 0.3	20.1 ± 1.6	20.0 ± 1.4	0.10	0.010
Uncompetitive	12.7 ± 2.1	16.1 ± 1.5	-	9.0 ± 1.0	0.37	0.137

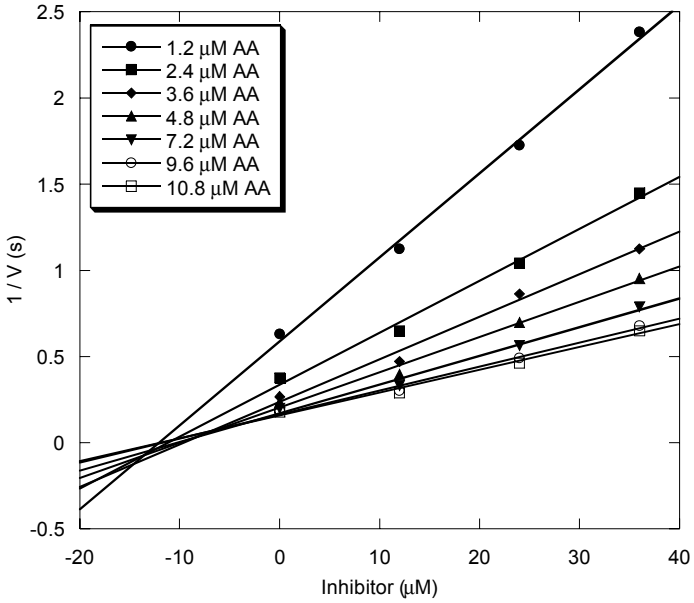
F. Human 15-lipoxygenase-1, arachidonic acid and 11-thialinoleic acid at pH 7.5



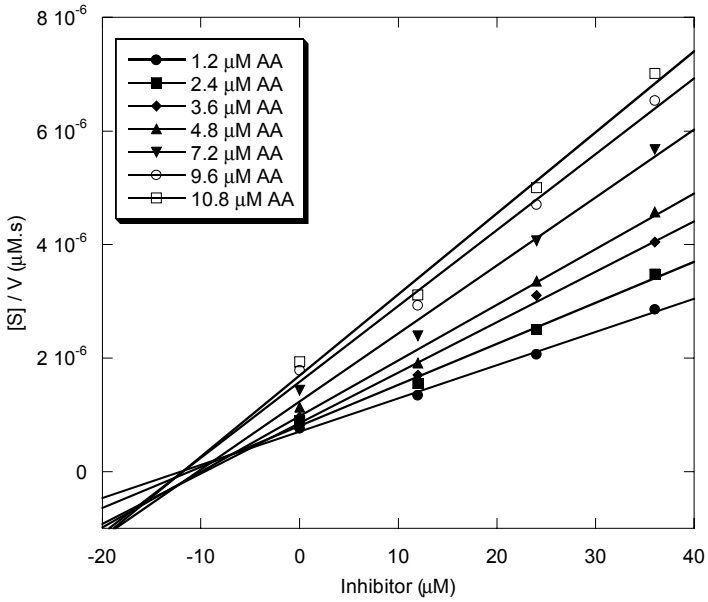
Supplementary Figure 13. Michaelis-Menten fits for the reaction of 15-hLO-1 and arachidonic acid (AA) in the presence of 11-thiaLA at pH 7.5.



Supplementary Figure 14. Double reciprocal plot showing the noncompetitive inhibition of 11-thiaLA in the reaction of 15-hLO-1 with AA at pH 7.5.



Supplementary Figure 15. Dixon plot showing the inhibition of 15-hLO-1 and AA in the presence of 11-thiaLA.

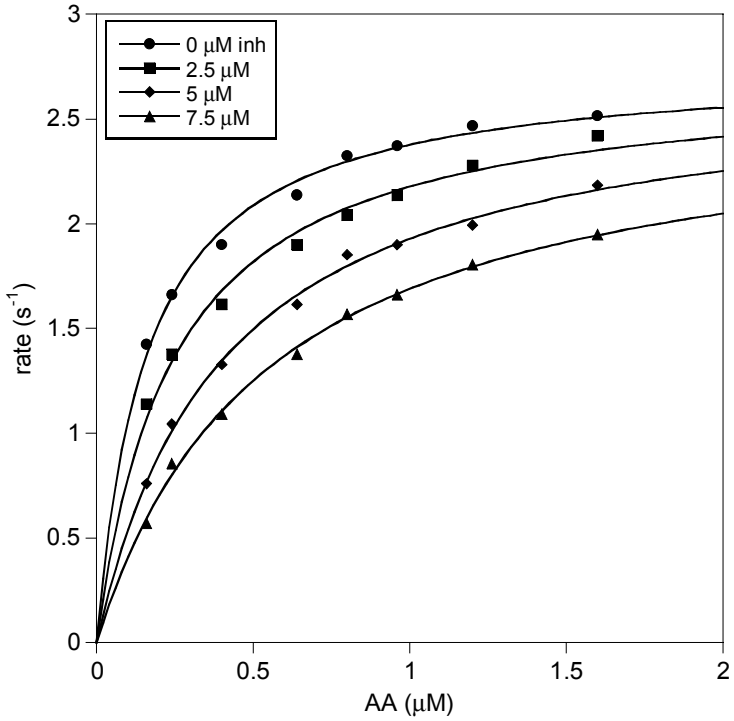


Supplementary Figure 16. Cornish-Bowden plot of the same data as in Supplementary Figure 15 showing the non-competitive inhibition of 15-hLO-1 and AA in the presence of 11-thiaLA.

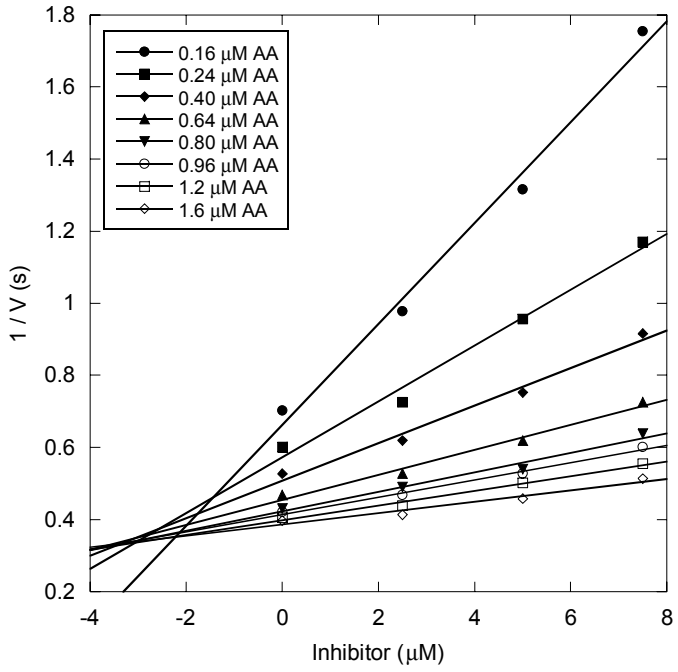
Supplementary Table 6. Error analysis of inhibition mode fits for the reaction of 15-hLO-1 and AA in the presence of 11-thiaLA at pH 7.5.

Mechanism	K_m (μM)	k_{cat} (s^{-1})	K_{is} (μM)	K_{ii} (μM)	Sigma	variance
Competitive	3.9 ± 0.6	7.6 ± 0.5	5.2 ± 0.6	-	0.15	0.023
Noncompetitive	4.4 ± 0.5	8.1 ± 0.4	11.4 ± 2.0	18.1 ± 3.1	0.08	0.006
Uncompetitive	6.5 ± 1.3	9.4 ± 1.0	-	7.5 ± 1.0	0.30	0.090

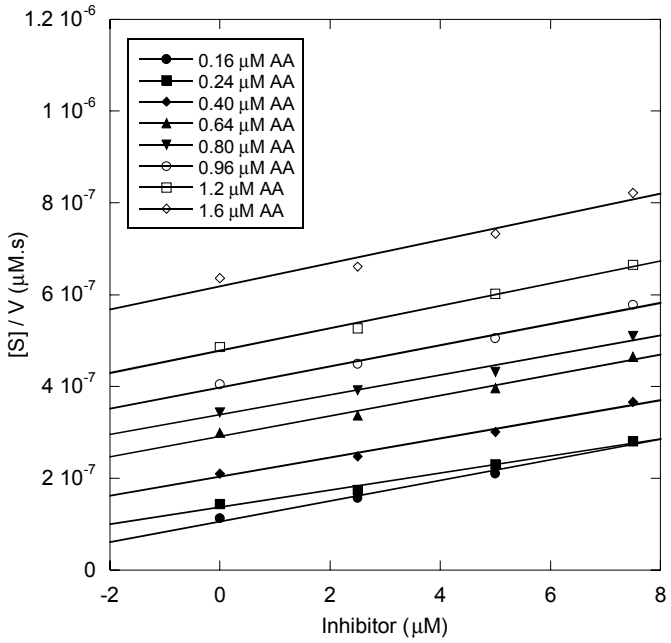
G. Human 12-lipoxygenase, arachidonic acid and 11-thialinoleic acid at pH 7.5



Supplementary Figure 17. Michaelis-Menten fits for the reaction of 12-hLO and AA in the presence of 11-thiaLA at pH 7.5.



Supplementary Figure 18. Dixon plot showing the inhibition of 12-hLO and AA in the presence of 11-thiaLA.

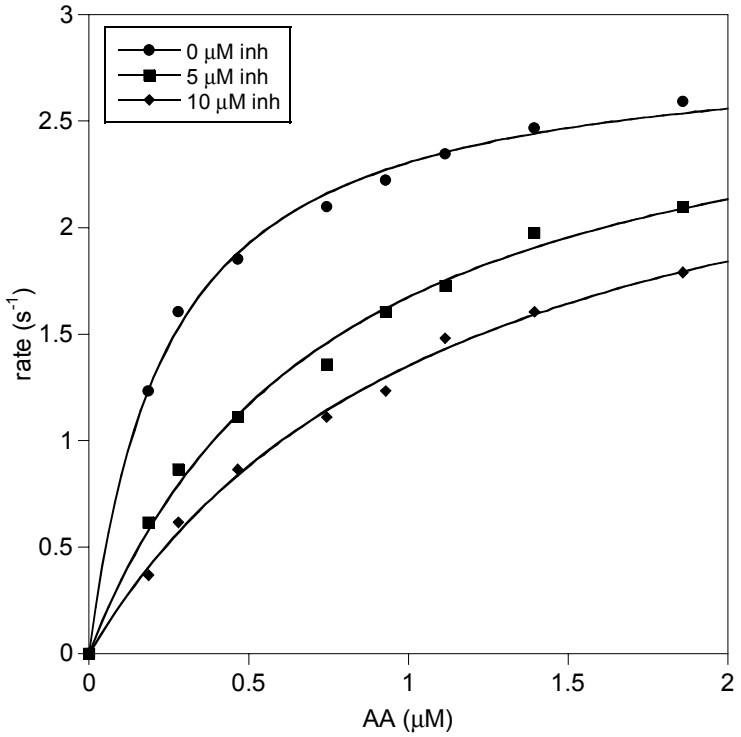


Supplementary Figure 19. Cornish-Bowden plot of the same data as in Supplementary Figure 18 showing the competitive inhibition of 12-hLO and AA in the presence of 11-thiaLA.

Supplementary Table 7. Error analysis of inhibition mode fits for the reaction of 12-hLO and AA in the presence of 11-thiaLA.

Mechanism	K_m (µM)	k_{cat} (s^{-1})	K_{is} (µM)	K_{ii} (µM)	Sigma	variance
Competitive	0.27 ± 0.03	3.76 ± 0.08	2.6 ± 0.2	-	0.10	0.010
Noncompetitive	0.29 ± 0.03	3.84 ± 0.09	3.2 ± 0.4	77.8 ± 35.2	0.10	0.010
Uncompetitive	0.59 ± 0.12	4.55 ± 0.35	-	10.4 ± 1.5	0.27	0.073

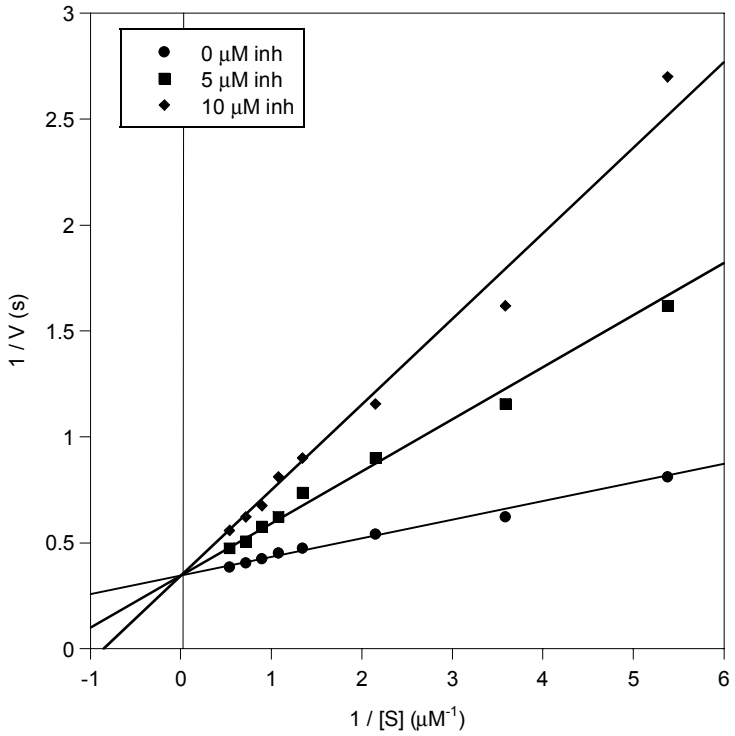
H. Human 12-lipoxygenase, arachidonic acid, 13-HPODE and 11-thialinoleic acid at pH 7.5



Supplementary Figure 20. Michaelis-Menten fits for the reaction of 12-hLO and arachidonic acid in the presence of 11-thiaLA and 13-HPODE (16 μM) at pH 7.5.

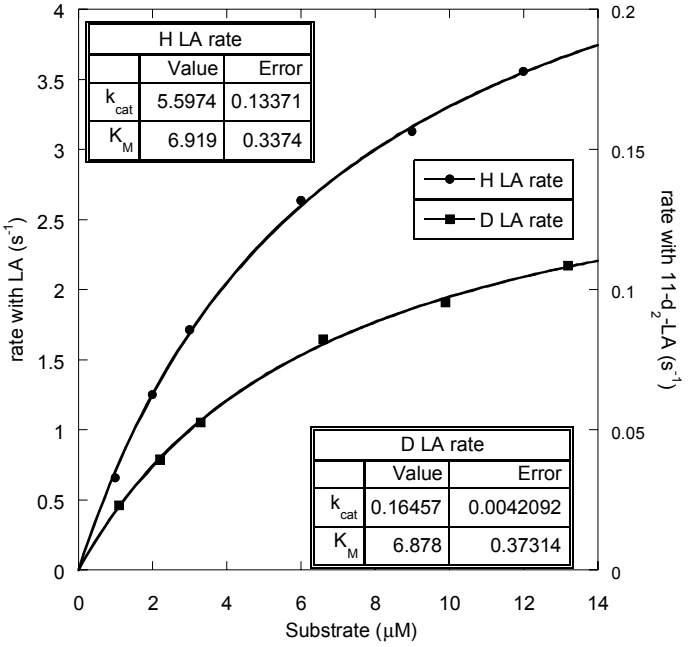
Supplementary Table 8. Error analysis of inhibition mode fits for the reaction of 12-hLO and AA in the presence of 11-thiaLA and 13-HPODE.

Mechanism	K_m (μM)	k_{cat} (s^{-1})	K_{is} (μM)	K_{ii} (μM)	Sigma	variance
Competitive	0.25 ± 0.02	2.89 ± 0.05	2.8 ± 0.2	-	0.051	0.0026
Noncompetitive	0.25 ± 0.02	2.88 ± 0.06	2.7 ± 0.3	-278 ± 450	0.051	0.0026
Uncompetitive	0.54 ± 0.12	3.52 ± 0.32	-	9.4 ± 1.7	0.210	0.0441

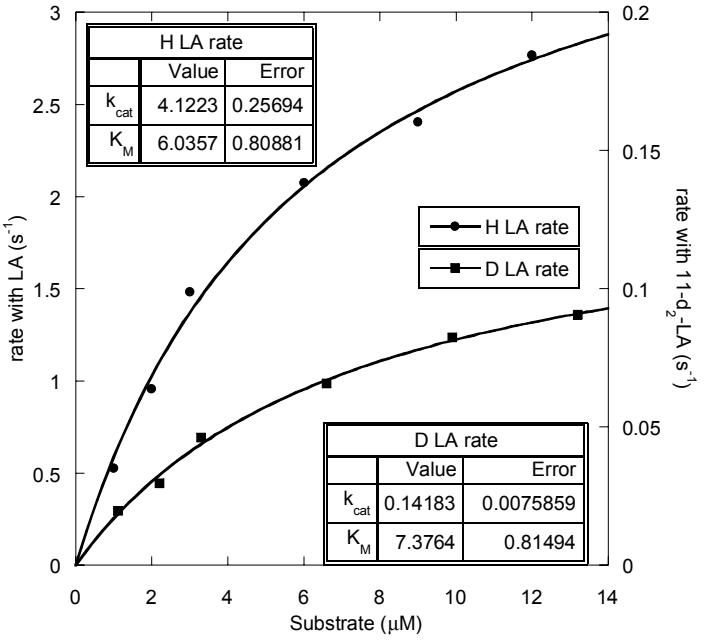


Supplementary Figure 21. Double reciprocal plot showing the competitive inhibition of 11-thiaLA in the reaction of human 12-lipoxygenase with arachidonic acid and 13-HPODE (16 μM).

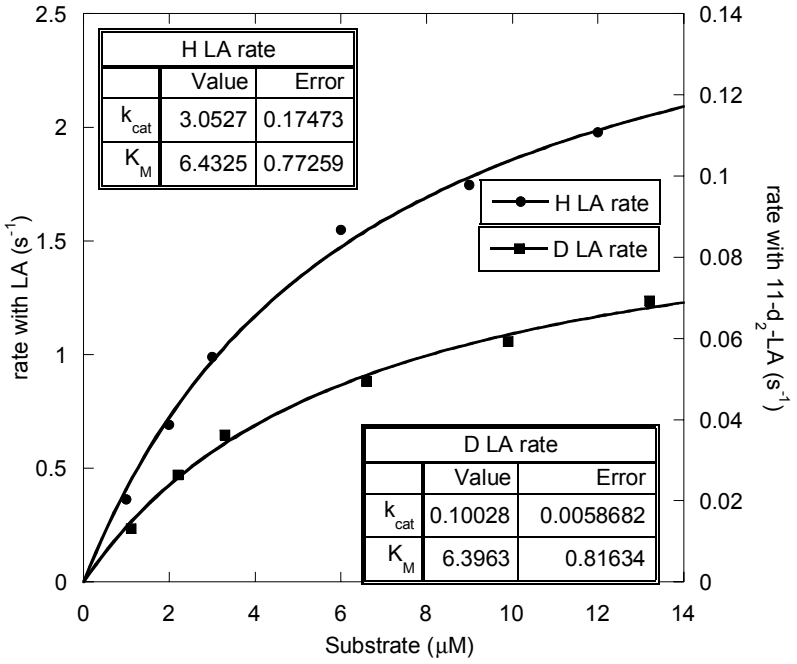
II – KIE measurement to determine if ternary enzyme-substrate-inhibitor complex is active



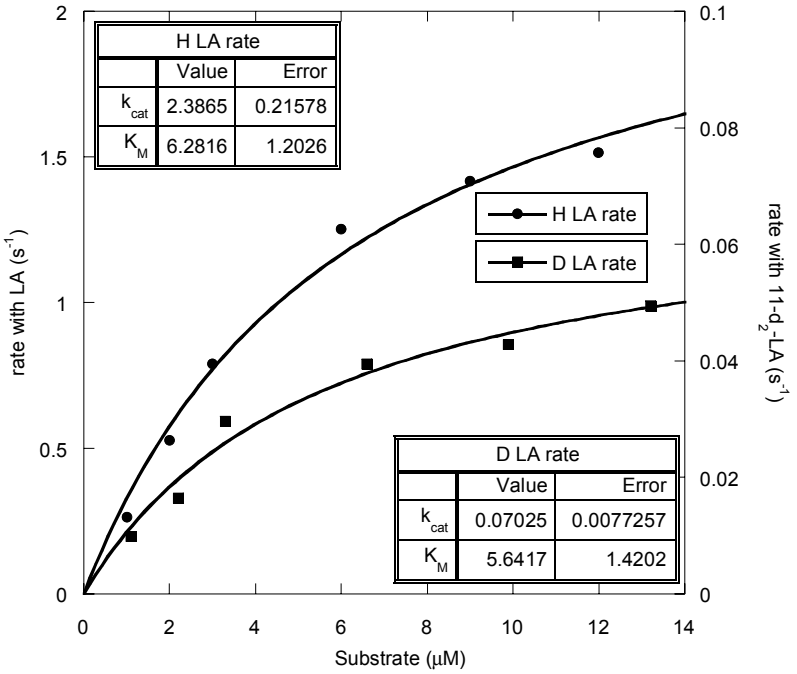
Supplementary Figure 22. Comparison of rates of 15-hLO-1 with linoleic acid and 11-d_2-linoleic acid in the presence of no 11-thiaLA at pH 7.5.



Supplementary Figure 23. Comparison of rates of 15-hLO-1 with linoleic acid and 11-d_2-linoleic acid in the presence of 15 μM 11-thiaLA at pH 7.5.

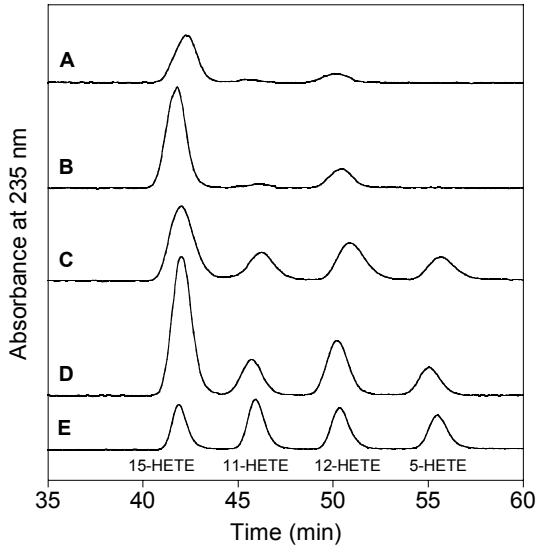


Supplementary Figure 24. Comparison of rates of 15-hLO-1 with linoleic acid and 11-d_2-linoleic acid in the presence of 30 μM 11-thiaLA at pH 7.5.

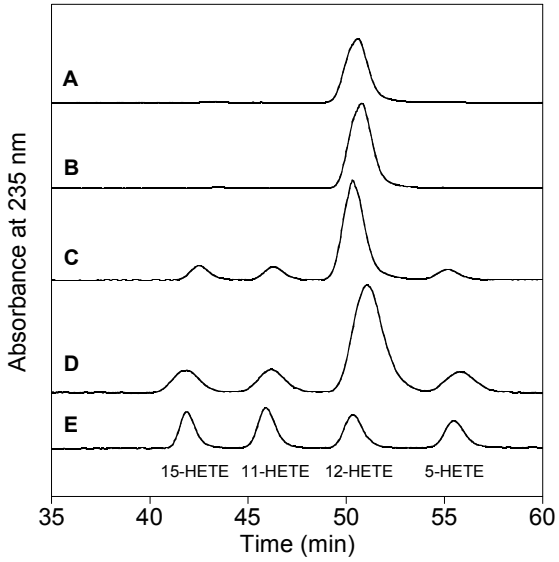


Supplementary Figure 25. Comparison of rates of 15-hLO-1 with linoleic acid and 11-d_2-linoleic acid in the presence of 45 μM 11-thiaLA at pH 7.5.

III –Product distribution in the presence of inhibitor



Supplementary Figure 26. Product distribution for the reaction of 15-hLO-1 with arachidonic acid in the presence and absence of 11-thialinoleic acid. Chromatograms shown are from injections of (A) reduced reaction mixture without inhibitor, (B) reduced reaction mixture with inhibitor, (C) reduced reaction mixture without inhibitor spiked with HETE standards, (D) reduced reaction mixture with inhibitor added spiked with HETE standards and (E) 5-, 11-, 12-, and 15-HETE standards.



Supplementary Figure 27. Product distribution for the reaction of 12-hLO with arachidonic acid in the presence and absence of 11-thialinoleic acid. Chromatograms shown are from injections of (A) reduced reaction mixture without inhibitor, (B) reduced reaction mixture with inhibitor, (C) reduced reaction mixture without inhibitor spiked with HETE standards, (D) reduced reaction mixture with inhibitor added spiked with HETE standards and (E) 5-, 11-, 12-, and 15-HETE standards.

1. W. W. Cleland, *Methods in Enzymology*, 1979, **63**, 103-138.