Supporting Information

Significance of Multiple Bioactivation Pathways for Meclofenamate as Revealed through Modeling and Reaction Kinetics

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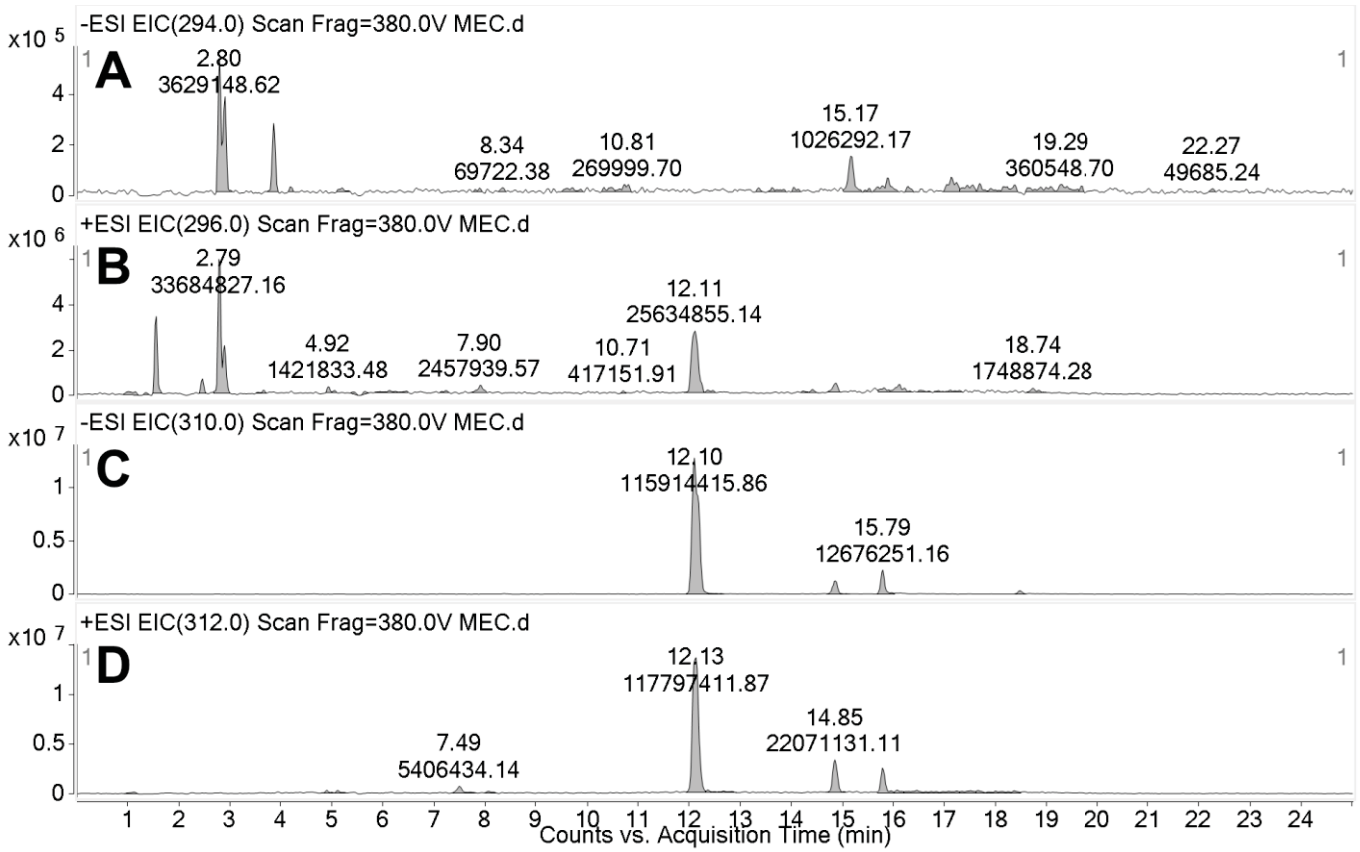
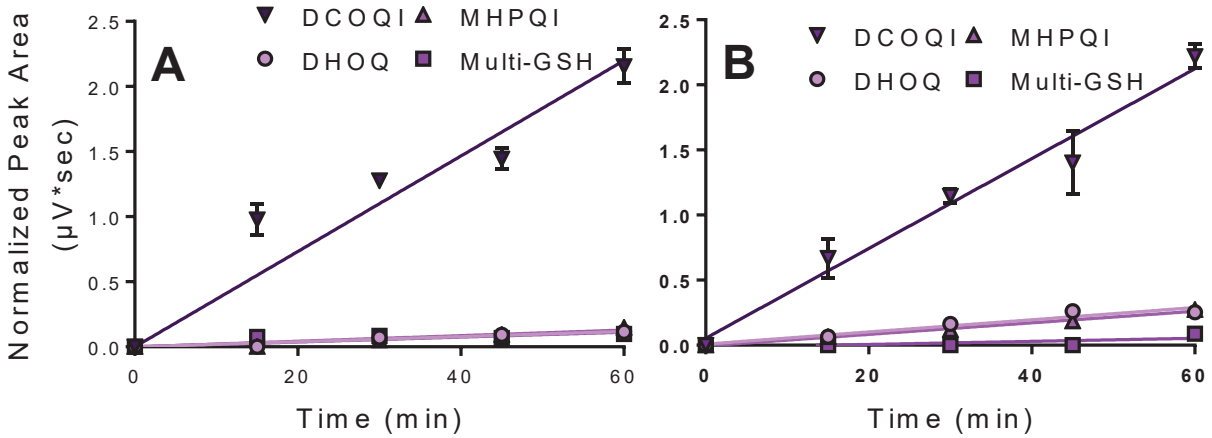
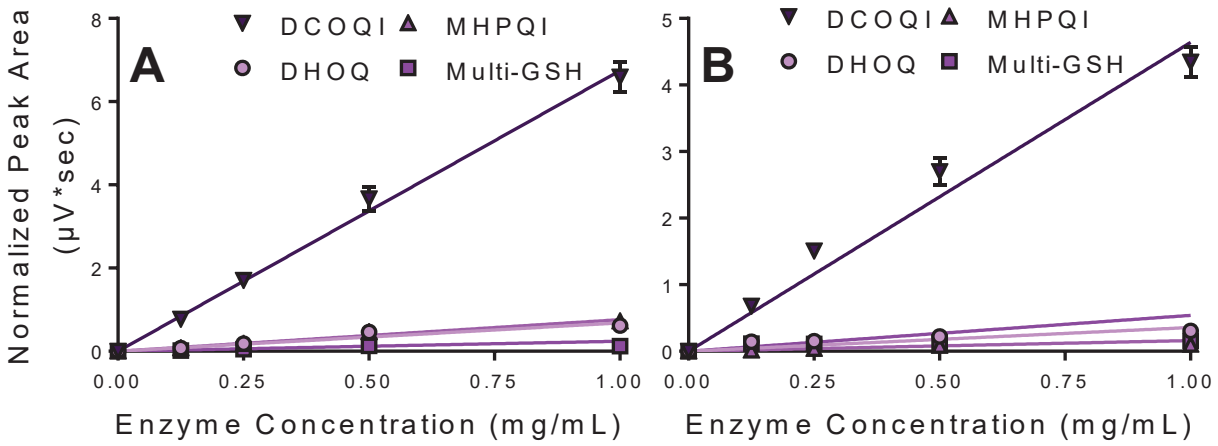


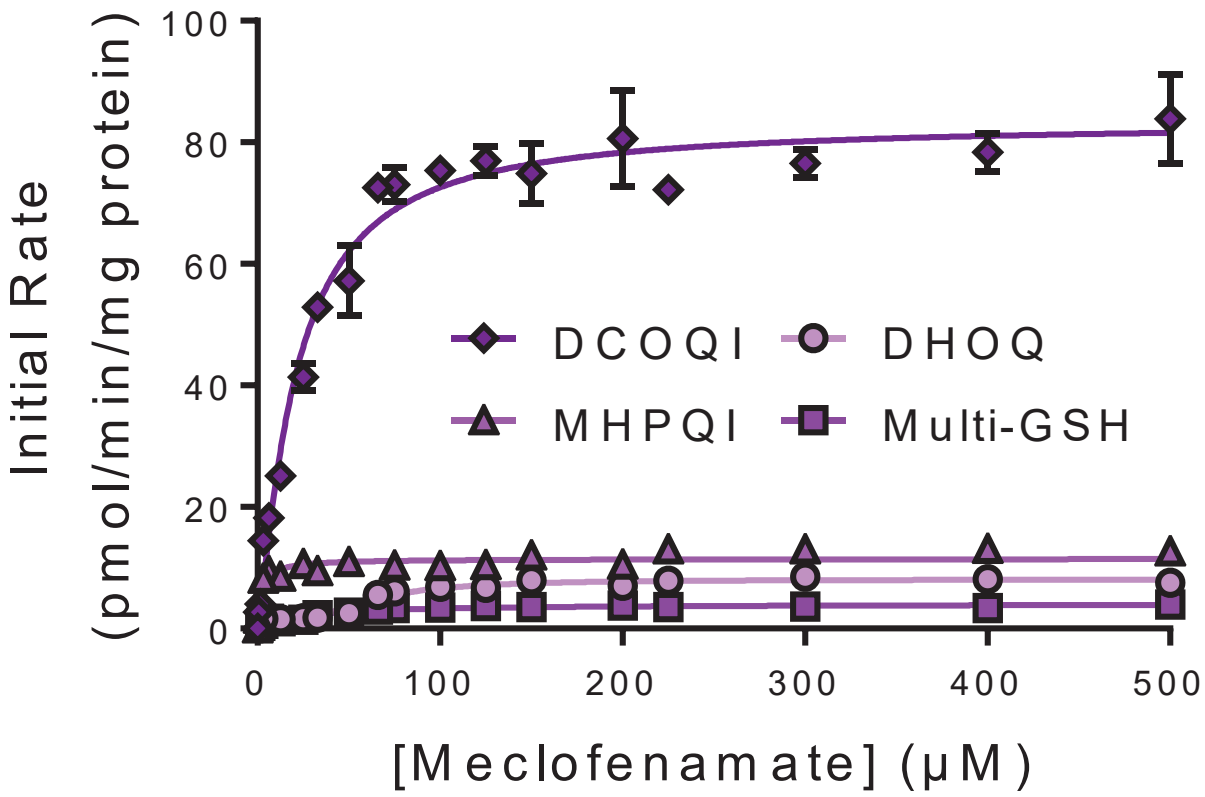
Supplemental Figure 1: Meclofenamate reactions were analyzed via parent mass ion scanning to identify parent masses for meclofenamate and suspected monohydroxylated metabolites of meclofenamate. **Panels A** and **B** target the meclofenamate parent mass (m/z 295.0) in negative (**Panel A**) and positive (**Panel B**) ion modes. **Panels C** and **D** target the meclofenamate monohydroxy metabolite parent mass (m/z 311.0) in negative (**Panel C**) and positive (**Panel D**) ion modes.



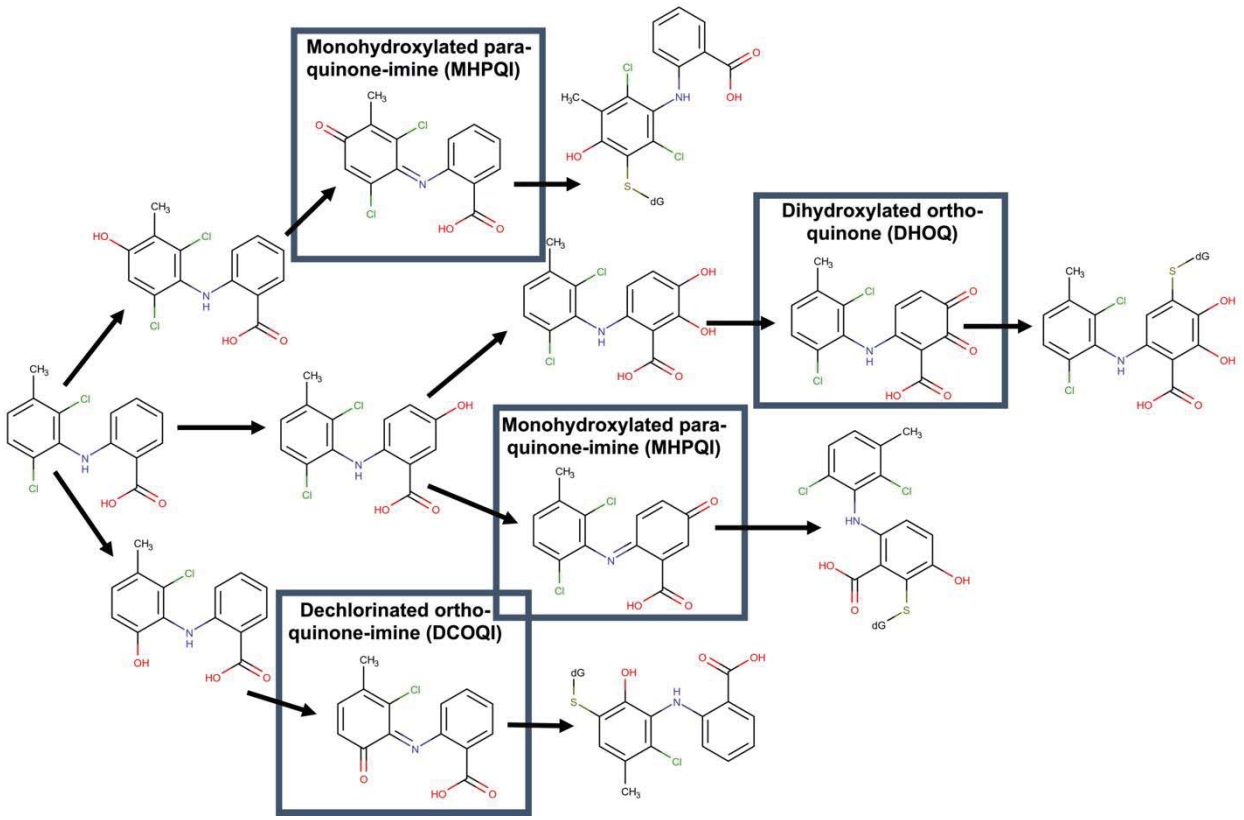
Supplemental Figure 2: Optimal reaction time points which adhered to the steady-state assumption as evidenced by metabolite formation linearity were determined for meclofenamate assessed at high and low substrate concentrations (500 µM, **Panel A**, and 50 µM, **Panel B**). Reaction times were varied from 15 min to 60 min. DHOQ, dihydroxy ortho-quinone; DCOQI, dechloro-ortho-quinone-imine; MHPQI, monohydroxy para-quinone-imine; Multi-GSH, meclofenamate adduct with multiple bound GSH molecules.



Supplemental Figure 3: Optimal reaction enzyme concentrations which adhered to the steady-state assumption as evidenced by metabolite formation linearity were determined for all seven substrates assessed at high and low substrate concentrations (500 µM, **Panel A**, and 50 µM, **Panel B**). Reaction concentrations of human liver microsomes 150 were varied from 0.25 mg/mL to 1.0 mg/mL. DHOQ, dihydroxy ortho-quinone; DCOQI, dechloro-ortho-quinone-imine; MHPQI, monohydroxy para-quinone-imine; Multi-GSH, meclofenamate adduct with multiple bound GSH molecules.



Supplemental Figure 4: Steady-state kinetics for meclofenamate metabolism shown up to 500 μM. Reaction conditions and data analysis were carried out as described in Materials and Methods. Each point is the average of three to six replicates. Corresponding constants are shown in **Tbl. 2**. Abbreviations are as follows: DHOQ, dihydroxy ortho-quinone; MHPQI, monohydroxy para-quinone-imine; DCOQI, dechloro-ortho-quinone-imine; Multi-GSH, suspected multiply glutathionylated metabolite.

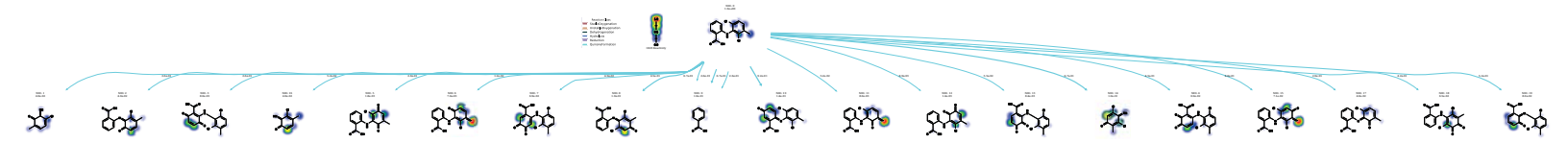


Supplemental Figure 5: Metabolic map for meclofenamate bioactivation. Meclofenamate underwent hydroxylation and subsequent bioactivation to quinones based on trapped dansyl glutathione adducts.

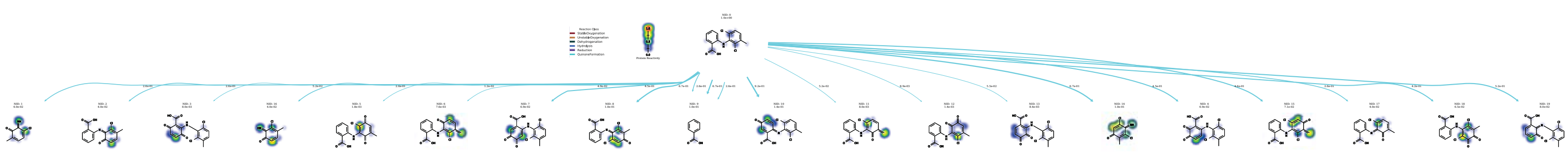
Mol.MolID.AtomOneID.AtomTwoID	Pair Predictions
M1.0	0.926213
M1.0.5.7	0.000578
M1.0.13.15	0.003771
M1.0.3.7	0.001205
M1.0.4.14	0.000292
M1.0.3.13	0.000737
M1.0.10.13	0.79394
M1.0.7.15	0.001932
M1.0.3.6	0.001428
M1.0.3.11	0.000798
M1.0.5.6	0.000864
M1.0.11.7	0.007516
M1.0.2.11	0.014054
M1.0.2.13	0.012137
M1.0.10.15	0.008133
M1.0.11.12	0.001438
M1.0.14.7	0.000314
M1.0.4.7	0.243394
M1.0.10.4	0.177404
M1.0.12.7	0.000809
M1.0.10.6	0.000878
M1.0.5.13	0.000393
M1.0.11.14	0.000407
M1.0.5.15	0.000417
M1.0.11.4	0.021354
M1.0.10.3	0.001898
M1.0.3.5	0.000492
M1.0.3.4	0.002394
M1.0.11.15	0.003045
M1.0.2.3	0.001891
M1.0.2.5	0.0007
M1.0.2.10	0.011793
M1.0.2.15	0.00358
M1.0.2.12	0.000483
M1.0.2.7	0.034053
M1.0.14.15	0.000489
M1.0.2.6	0.001023
M1.0.10.12	0.00091
M1.0.6.7	0.002327
M1.0.12.15	0.001149

M1.0.10.14	0.000345
M1.0.12.6	0.000455
M1.0.12.13	0.001575
M1.0.10.11	0.037857
M1.0.11.5	0.000442
M1.0.5.14	0.00032
M1.0.12.4	0.000632
M1.0.4.13	0.181478
M1.0.13.14	0.0007
M1.0.3.15	0.000768
M1.0.10.5	0.000998
M1.0.11.6	0.001073
M1.0.2.4	0.015536
M1.0.13.6	0.000961
M1.0.2.14	0.000216
M1.0.6.15	0.000972
M1.0.10.7	0.644791
M1.0.12.5	0.000587
M1.0.4.15	0.005074
M1.0.13.7	0.337229
M1.0.4.6	0.000994
M1.0.6.14	0.000208
M1.0.3.12	0.000768
M1.0.12.14	0.000296
M1.0.3.14	0.000363
M1.0.11.13	0.016163
M1.0.4.5	0.000915

Supplemental Figure 6: Numerical computational model outputs which provide whole-molecule and atom-by-atom scores for meclofenamate bioactivation into quinone-species metabolites using our XenoSite computational model. Meclofenamate is codified by the molecular ID (MolID) “M1.0”. Overall score for meclofenamate bioactivation is shown on the first row, with each subsequent row giving a score for quinone formation between two atoms. For example, row M1.0.5.7 assesses quinone formation likelihood between meclofenamate atoms 5 and 7.


Hydrogen Bond
Halogen Bond
Electrostatic Interaction
Pi-Pi Stacking
Hydrophobic
Repulsion
Unfavorable Interaction
Interaction Energy

Sup. Fig. 7: Graphical computational model outputs which show potential bioactivation pathways of meclofenamate into nineteen quinone-species metabolites with subsequent scoring for reactivity with glutathione. First, each bioactivation of meclofenamate into a metabolite is scored in terms of reaction likelihood and next, the propensity of each metabolite reacting with glutathione is scored. Larger numbers indicate a greater possibility for bioactivation into that specific molecule and subsequent adduction with glutathione.



Reaction Class
StableOxygenation
UnstableOxygenation
Dehydrogenation
Hydrolysis
Reduction
QuinoneFormation
1.0
0.5
0.0
Protein Reactivity
NID: 0
1.0e+00
NID: 1
4.0e-02
NID: 2
4.0e-02
NID: 3
8.0e-03
NID: 16
4.0e-02
NID: 5
1.8e-03
NID: 6
7.6e-03
NID: 7
6.9e-02
NID: 8
1.0e-01
NID: 9
1.0e-01
NID: 10
1.4e-01
NID: 11
NID: 12
NID: 13
NID: 14
NID: 4
NID: 15
NID: 17
NID: 18
NID: 19

Sup. Fig. 8: Graphical computational model outputs which show potential bioactivation pathways of meclofenamate into nineteen quinone-species metabolites with subsequent scoring for reactivity with endogenous proteins. First, each bioactivation of meclofenamate into a metabolite is scored in terms of reaction likelihood and next, the propensity of each metabolite reacting with endogenous proteins is scored. Larger numbers indicate a greater possibility for bioactivation into that specific molecule and subsequent adduction with proteins.