The Oxidation of Fenamate Compounds by Neutrophil Myeloperoxidase Produces Toxic Reactive Metabolites

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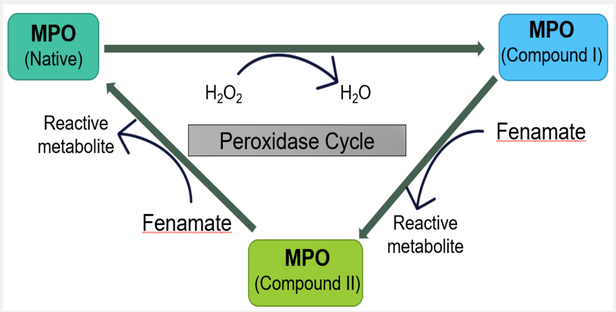
BACKGROUND

- **Fenamates** are a group of nonsteroidal anti-inflammatory drugs (or NSAIDs)
- Most common drugs prescribed globally in the treatment of pain and inflammation
- Undergo oxidation reactions to produce **reactive metabolites**, which may have **toxic effects**
- Toxicological concern due to their abilities to weaken cells and cause damage to proteins/DNA
- **Myeloperoxidase** (**MPO**) is an antibacterial heme protein that is involved in fighting infections
- Strong **oxidizing capacity** in the bioactivation of xenobiotics into toxic reactive metabolites



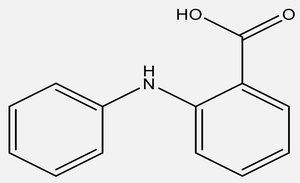
The aim of this study is to investigate the metabolism of fenamate compounds via the oxidation by MPO, and its ensuing toxicological effects.

HYPOTHESIS / OBJECTIVES

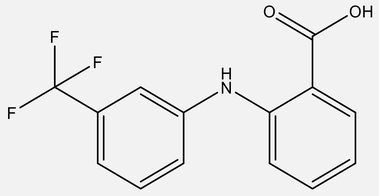
- The enzymatic activity of MPO is **required** for the oxidation of fenamate compounds, and therefore the production of reactive metabolites leading to toxic effects.
- Characterize the metabolites, determine cellular effects and responses, and understand the basis of metabolism and toxicity.

RESULTS

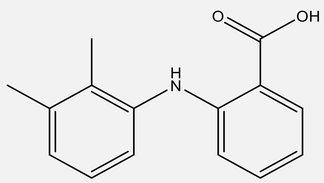
N-Phenylanthranilic Acid (NPA)



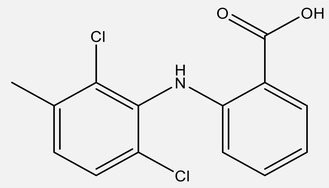
Flufenamic Acid (FFA)



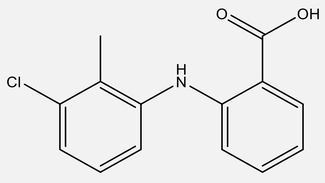
Mefenamic Acid (MFA)



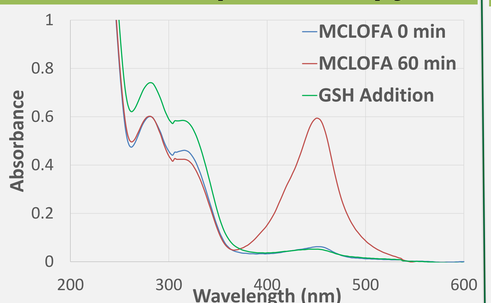
Meclofenamic Acid (MCLOFA)



Tolfenamic Acid (TFA)

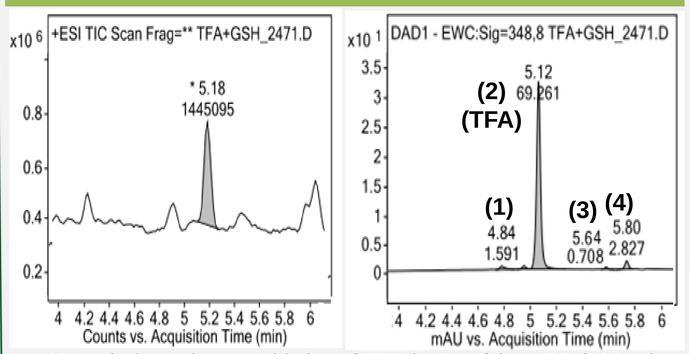


UV / Vis Spectroscopy



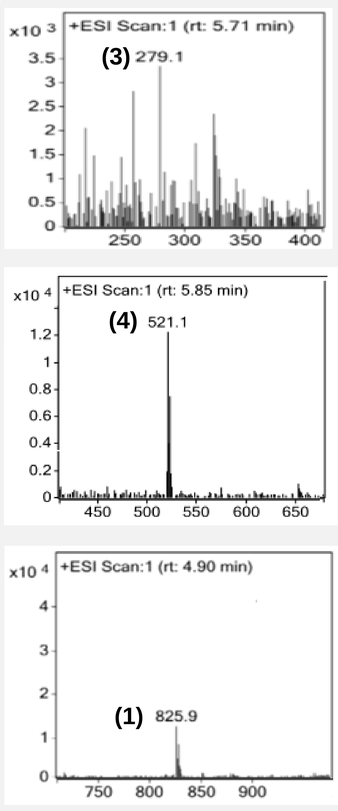
UV-Vis spectrophotometry studies on the peroxidation of **MCLOFA** (100 µM) by MPO (100nM) in PB (0.1 M at pH 7.4) and initiated with H_2O_2 (100 µM). Spectra was recorded every 30 seconds for a total of 60 minutes at $\lambda_{200\text{-}600}$ (orange). 500 µM GSH was added to the reaction mixture following the completion of the 60-minute time period (green).

LCMS Studies



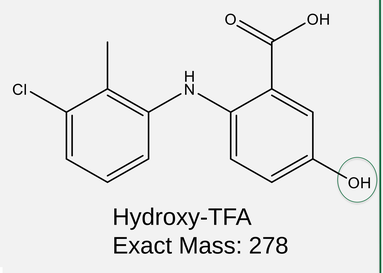
LCMS analysis on the peroxidation of **TFA** (100 µM) by MPO (100nM) in PB (0.1 M at pH 7.4) and initiated with H_2O_2 (100 µM). 500 µM GSH was added to the reaction mixture following the completion of the 60-minute time period. Hydroxy, dimer, and GSH-adduct metabolites were proposed based on the chromatogram peaks.

Chromatograms

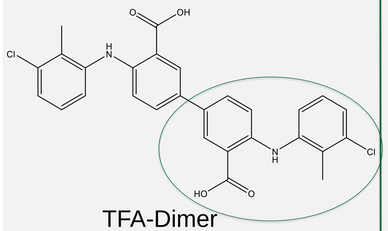


Proposed Structures

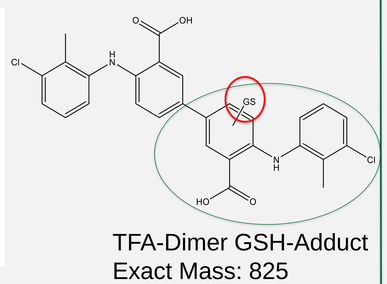
Hydroxy-TFA
Exact Mass: 278



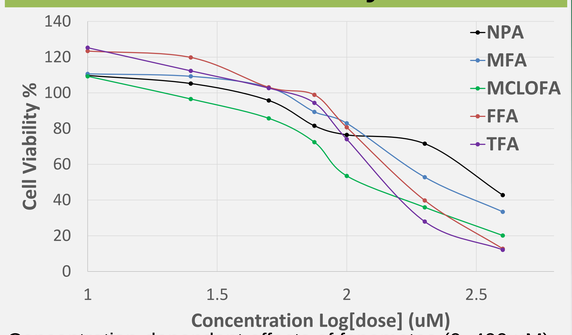
TFA-Dimer
Exact Mass: 520



TFA-Dimer GSH-Adduct
Exact Mass: 825

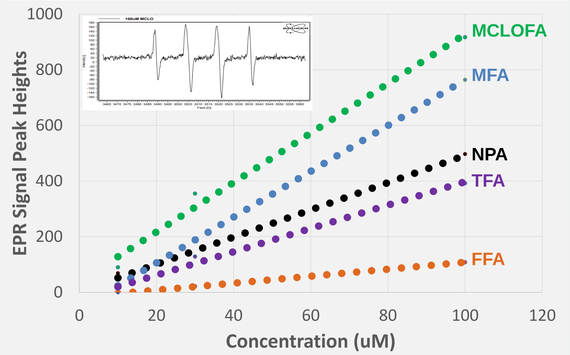


Cell Viability



Concentration-dependent effects of fenamates (0–400 uM) on HL-60 cells (1x10^6 cells/mL) incubated over 24 hours and assessed via CCK8 assay to evaluate cytotoxicity.

EPR Glutathionyl Radical (•SG) Signal



EPR Spectra of the peroxidation of fenamate compounds by myeloperoxidase. Reactions were prepared by mixing a fenamate compound (100 µM), MPO (100 nM), GSH (1 mM), and DMPO (100 mM) in phosphate buffer (0.1 M at pH 7.4) and initiated with H_2O_2 (100 µM).

CONCLUSION

- MPO catalyzed the oxidation of most fenamate compounds, but not NPA
- Hydroxy metabolites were observed for all fenamate compounds, quinoneimine and dimer products were observed for most fenamate compounds, and GSH adducts were only observed for NPA, MFA, and TFA
- All fenamate compounds produced glutathionyl radicals (•SG) in a linear concentration-dependent manner, with the highest signal response being MCLOFA
- All fenamate compounds demonstrated toxicity, with the most toxic compound being MCLOFA

SUMMARY

	NPA	FFA	MFA	MCLOFA	TFA
UV / Vis Spectral Changes	✗	✓	✓	✓	✓
LCMS GSH-Adducts	✓	✗	✓	✗	✓
Cell Viability (IC50 (µM))	187.7	144.7	139.9	94.9	122.3
EPR •SG Linearity	✓	✓	✓	✓	✓