SUPPLEMENTAL FIGURES:

Supplemental Figure 1. Chromatograms and MS data for metoclopramide and observed metabolites. Data shown are representative of a minimum of four separate analyses for each CYP.

Supplemental Figure 2. AutoDock modeling of CYP2D6 lowest energy binding conformations with metoclopramide (shown in black ball and stick). Helix I is shown. The heme is shown in dark grey. (A) Docking of metoclopramide with the phenyl ring pointing toward heme iron consistent with metabolites M4a, M4c, and M10. (B) Docking of metoclopramide with the diethyl-amine pointing toward the heme iron consistent with metabolites M3 and M4b. The CYP2D6 model from PDB 2F9Q (no ligand bound model) was used for simulations shown (Rowland et al., 2006). To mimic the structure of CYP Compound I, a water was added to the model at 1.7 Å above the heme iron using COOT prior to simulation.

Supplemental Figure 3. Dixon Analysis of CYP2D6 Inhibition by Metoclopramide. Bufuralol was used as the reporter substrate at 5 (●), 10 (◆), 50 (■), and 100 (▲) μM final concentrations. Dixon analysis shows competitive inhibition of CYP2D6 by metoclopramide with K_i = 13.8 μM. Data shown are representative of two separate experiments.

Supplemental Figure 4. Partition Ratio Examination. (A) CYP2D6 was incubated with varying concentrations of metoclopramide for 60 minutes to allow for complete

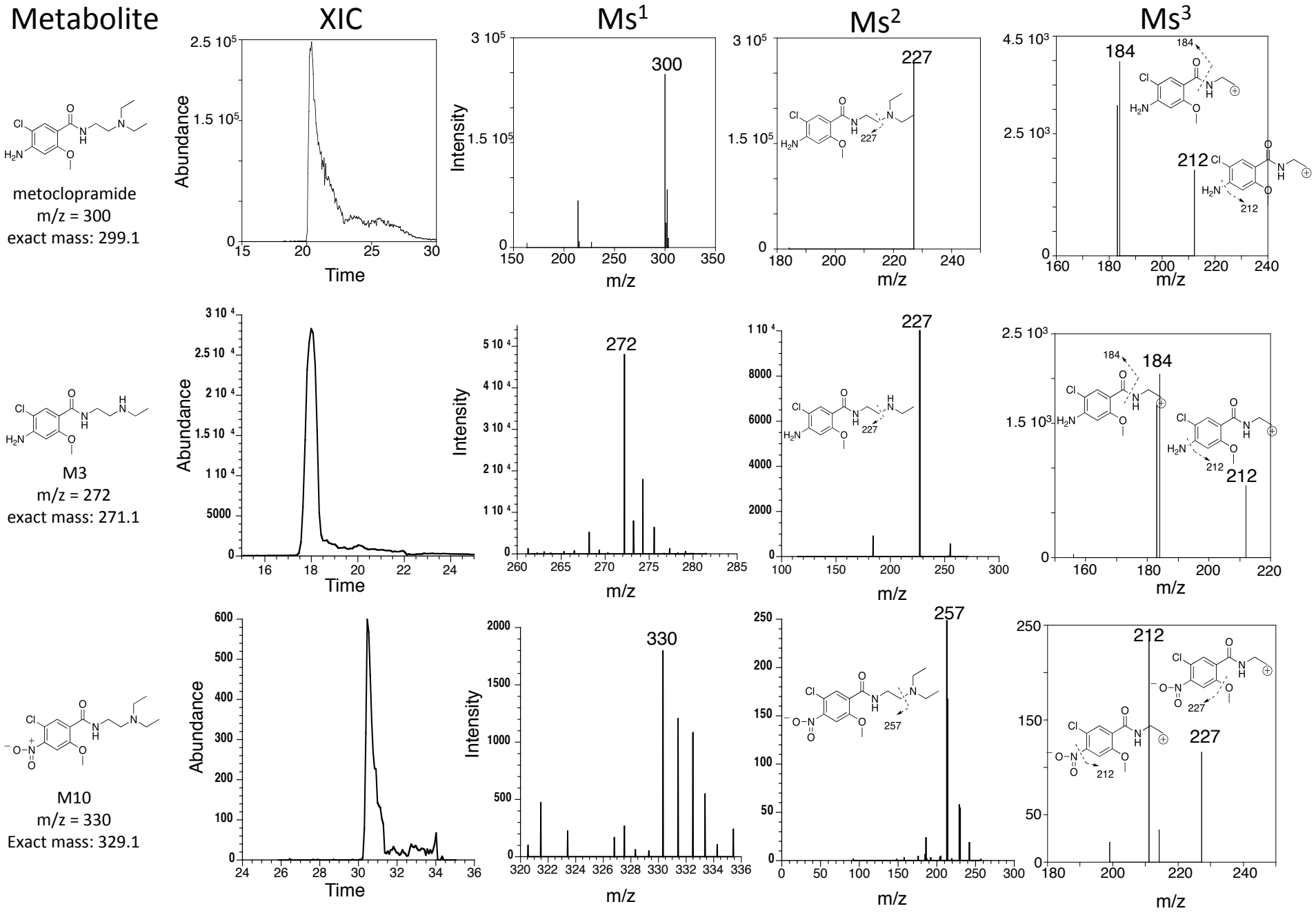
inactivation before dilution in reporter assays with bufuralol as described in the Materials and Methods. However, inactivation was not observed and partition ratio could not be determined. Data shown are representative of five separate trials of this experiment. (B) CYP2D6 was incubated with varying concentrations of dextromethorphan for 60 minutes before dilution in reporter assays with bufuralol as described in the Methods.

Supplemental Figure 5. Substrate Addition Assay for Determination of Metoclopramide (A) Time-dependent and (B) Concentration-dependent Inhibition of CYP2D6 in pooled HLMs. Time-dependent assays contained 25 μM metoclopramide in the inactivation reaction while concentration-dependent assays were carried out for 20 min with dextromethorphan as the reporter substrate all as described previously by Desta et al. (Desta et al., 2002). Control reactions without metoclopramide are shown as solid circles (●). Reactions containing metoclopramide are shown as open squares (□). Data shown are representative of two separate experiments.

Supplemental Figure 6. Substrate Addition Assay for Determination of Metoclopramide (A) Time-dependent and (B) Concentration-dependent Inhibition of CYP2D6. Time-dependent assays contained 25 μM metoclopramide in the inactivation reaction while concentration-dependent assays were carried out for 60 min. Bufuralol was used as the reporter substrate. Control reactions without metoclopramide are shown as solid circles (●). Reactions containing metoclopramide are shown as open squares (□). Data shown are representative of three separate experiments averaged with error bars given.

Supplemental Figure 7. Substrate Addition Assay for Determination of Metoclopramide Concentration-dependent Inhibition of CYP2D6 with NADPH in the Control Reaction. Concentration-dependent assays were carried out for 40 min. Bufuralol was used as the reporter substrate. The control reaction with 0 μM metoclopramide contained 1 mM NADPH.

Supplemental Figure 8. *N*-deethylated metoclopramide did not inhibit CYP2D6 in dilution assays over a range of concentrations. Data shown are from a single experiment.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M10
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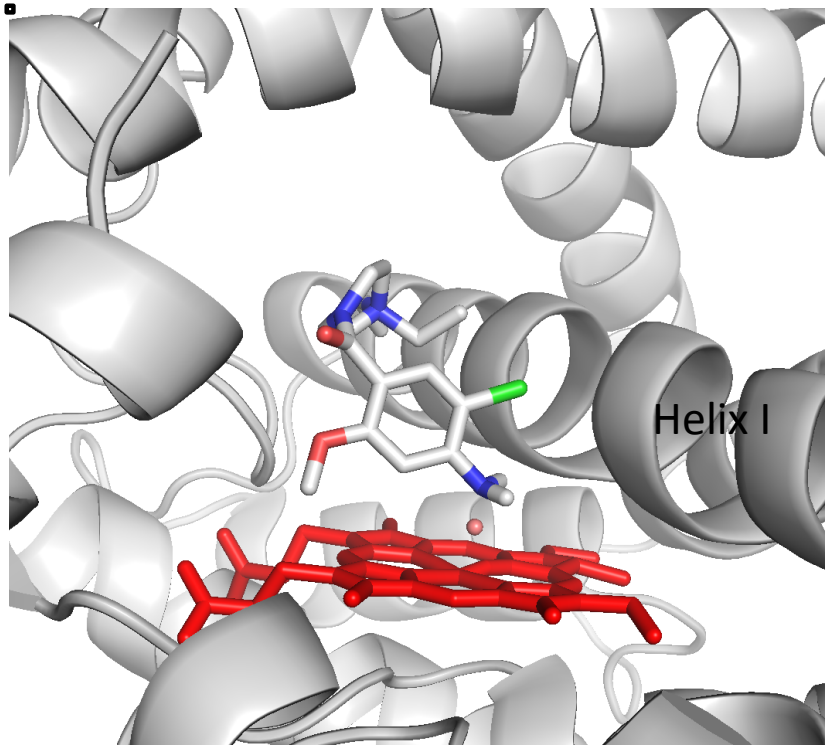
Abundance
Intensity
Time
m/z
300
227
184
212
272
330
257

Supplemental Figure 2

A



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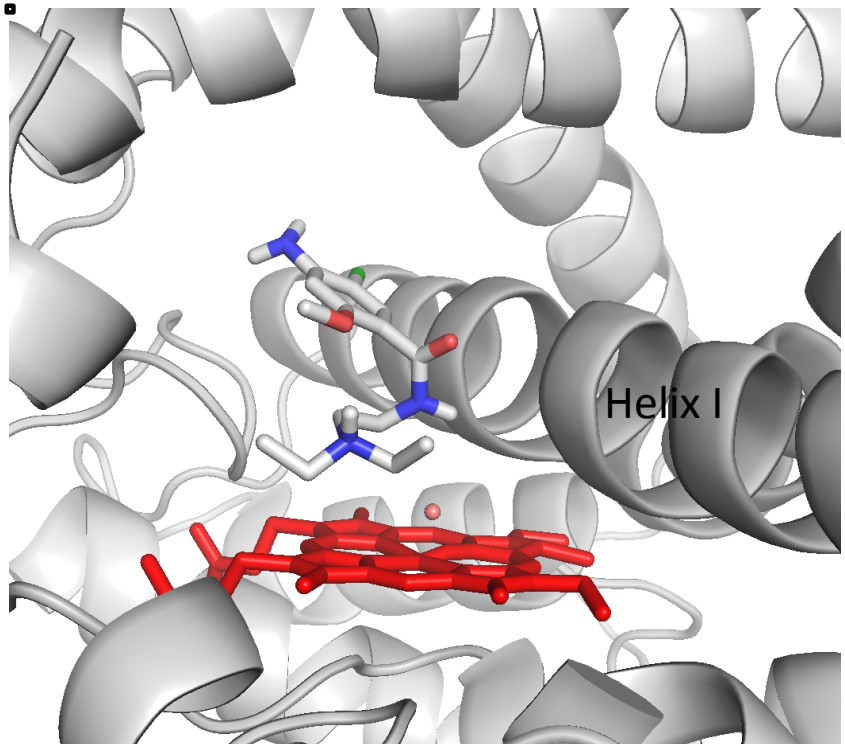
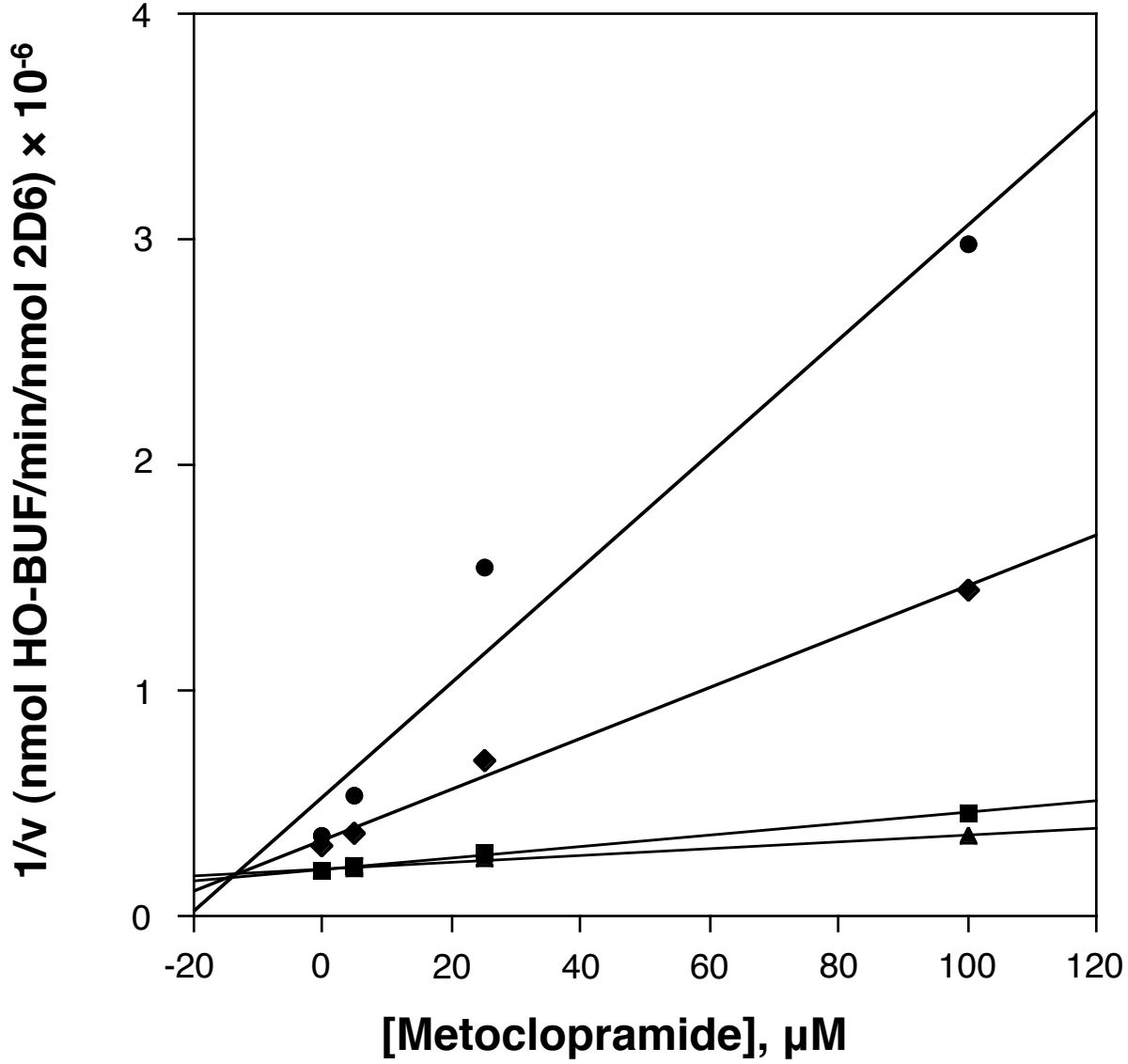
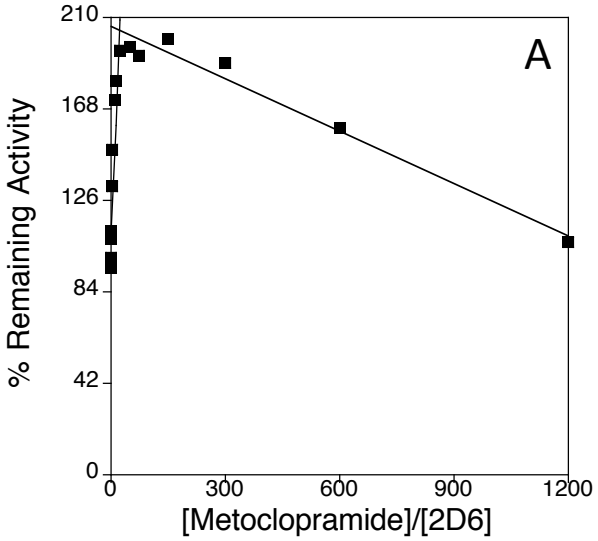
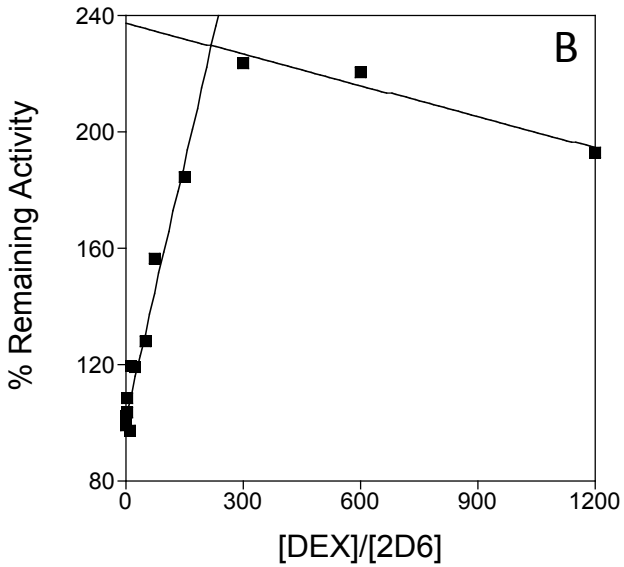


Supplemental Figure 3

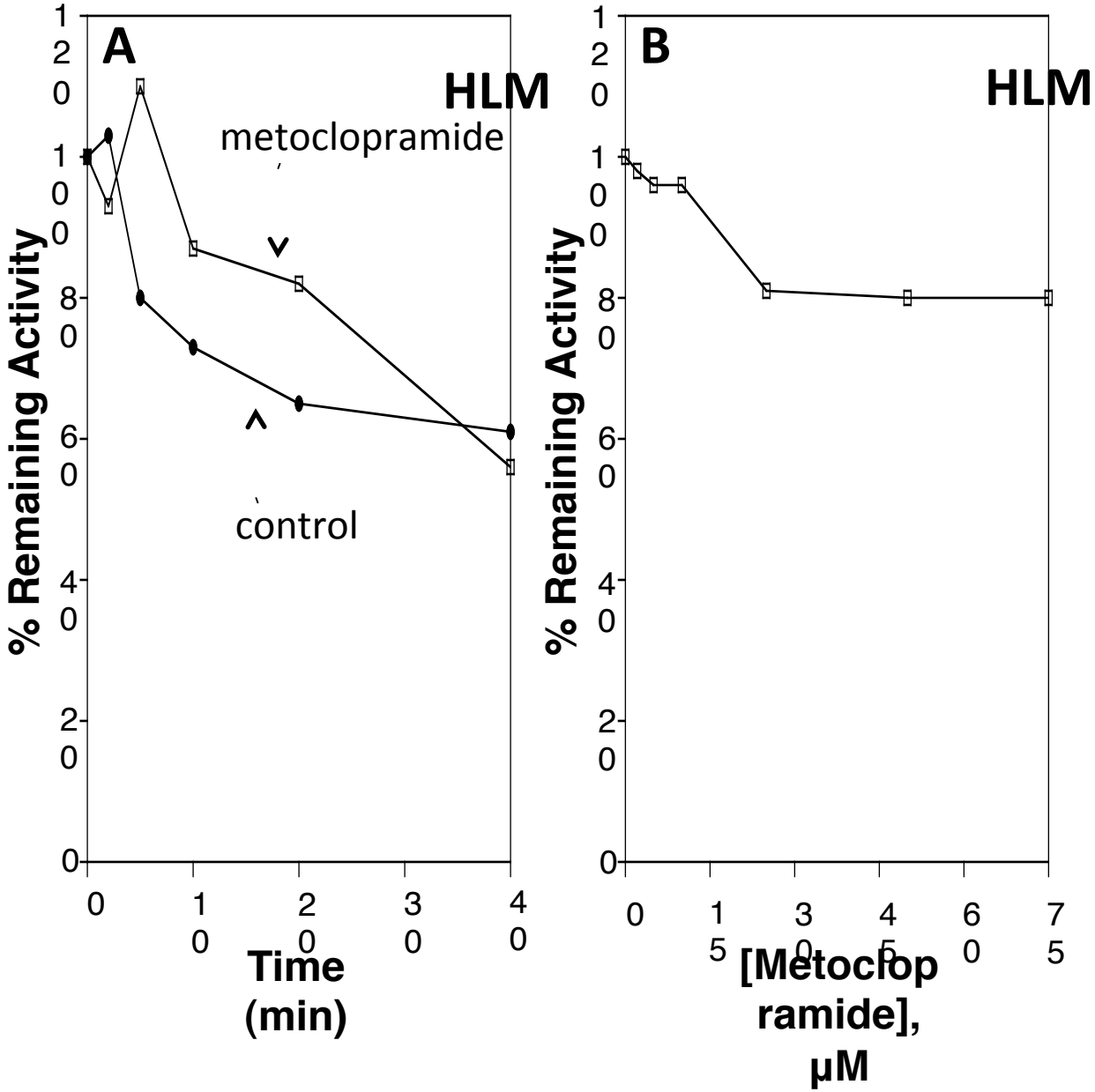


Supplemental Figure 4

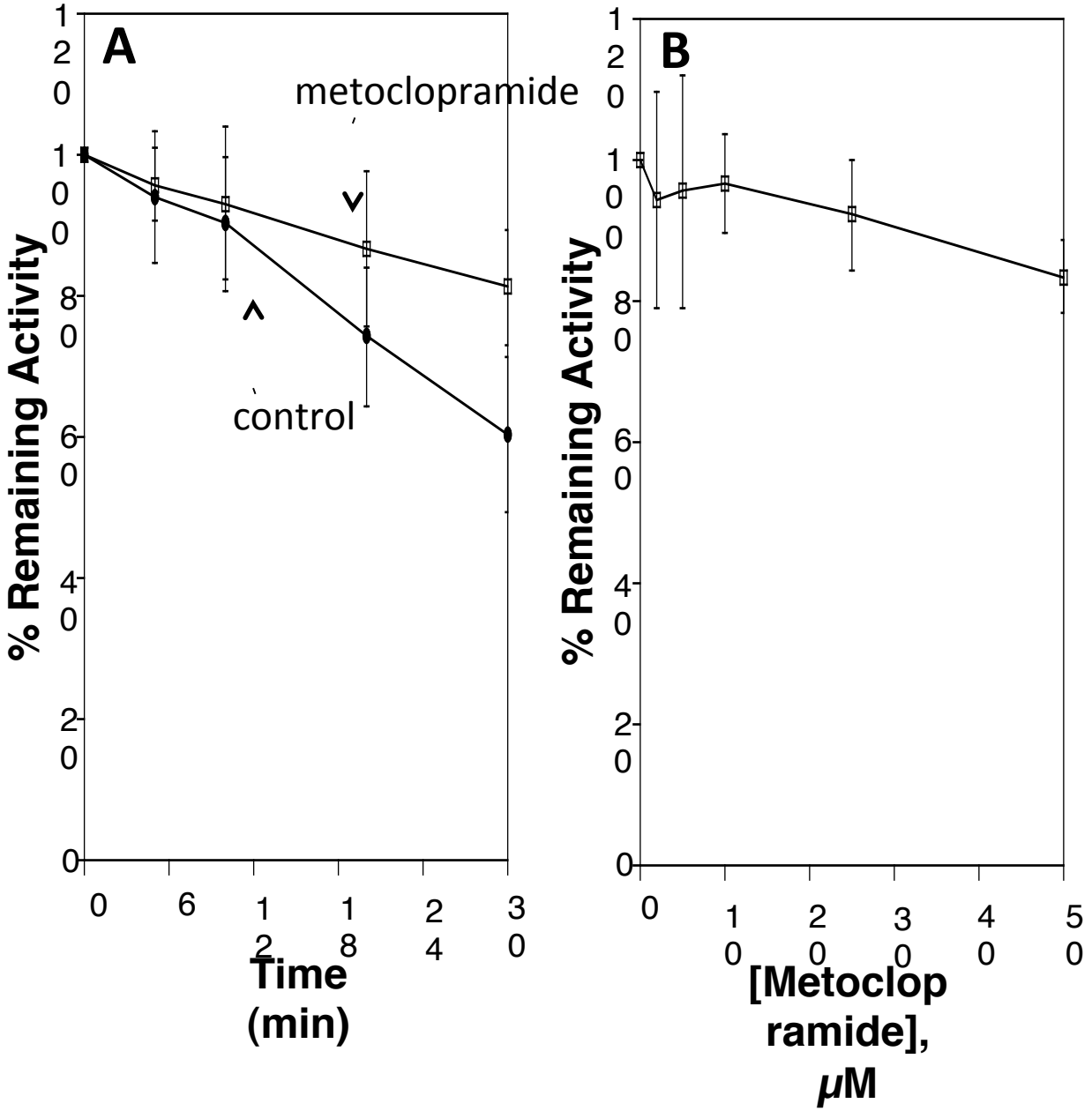




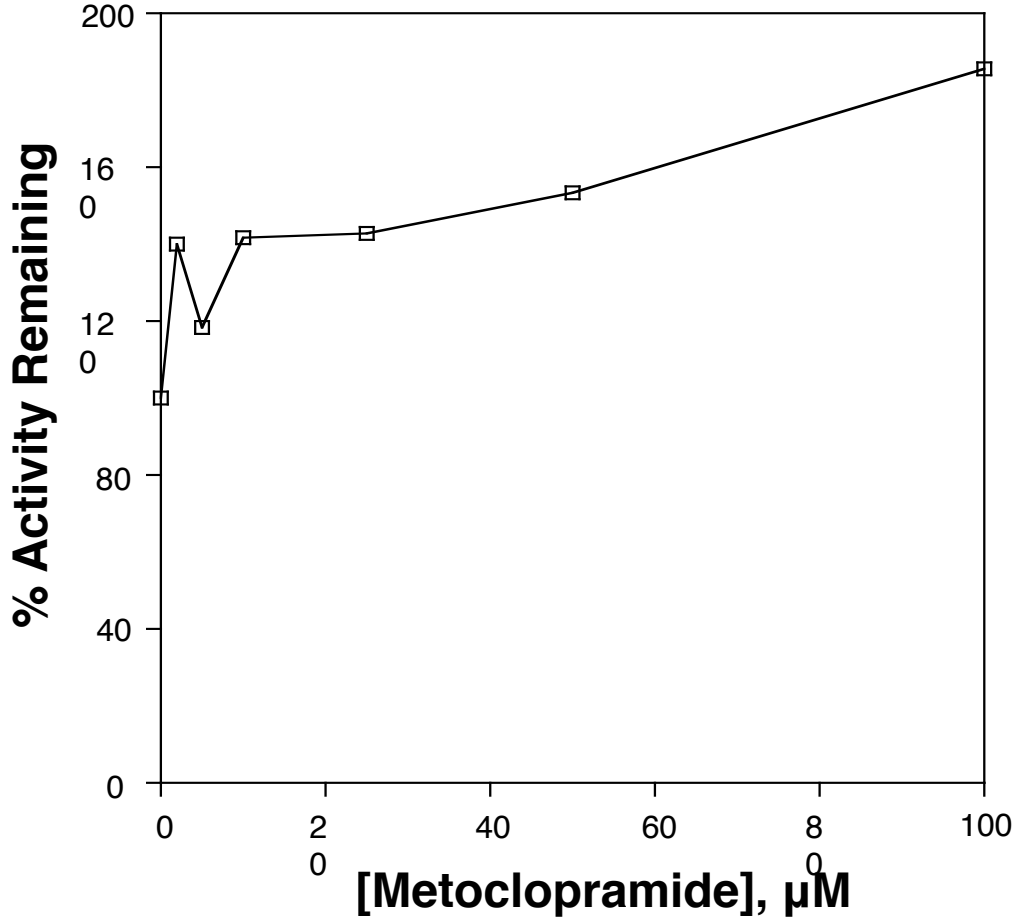
Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7



Supplemental Figure 8