SUPPLEMENTAL MATERIAL

Natural Products Inhibition of CYP2B6 Activity and Methadone Metabolism

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Supplemental Figure 1
Supplemental Figure 2
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Supplemental Figure 3
Supplemental Figure Legends

**Supplemental Figure 1.** Screening of kavalactones (1, 2, 4-6), flavokavain A (35), flavokavain B (22), gambogic acid (7), euphorbiasteroid (8), daphnoretin (9) and cardiotonic steroids (10-13) for CYP2B6 inhibition using the ETFMC assay. All assays were performed at fixed inhibitor concentration (10 µM), and fixed substrate concentration (50 µM 7-ETFMC). Results are the mean ± SD of triplicate determinations.

**Supplemental Figure 2.** Screening for CYP2B6 inhibition using the ETFMC assay. Substrate concentration was fixed (50 µM 7-ETFMC), and three inhibitor concentrations were evaluated (0.1, 1, 10 µM). A. Screening of 29 chalcones (14-34, 36-43). Results are single determinations. The boxes and arrows in red show the three compounds (14, 32, 17) that displayed concentration-dependent CYP2B6 inhibition. B. Evaluation of the three chalcones, 2,2'-dihydroxychalcone (14), 3,4,2',5'-tetramethoxychalcone (32), and 4-dimethylamino-2',4',6'-trimethoxychalcone (37) identified as inhibitory in the primary screen (Figure 3A). Results are the means ± SD of triplicate determinations.

**Supplemental Figure 3.** Pre-incubation compared with co-incubation for the seven compounds subjected to methadone screening. In the pre-incubation test, the inhibitors were incubated with CYP2B6/POR/b5 and NADPH regenerating system at 37° for 30 min, followed by addition of methadone to initiate the reaction. In the co-incubation test, the 30-min incubation step was skipped. Results are the mean ± SD of triplicate determinations.