SUPPLEMENTARY METHODS

Metabolite and parent drug quantitation

For dextromethorphan and dextrorphan, calibration standards were prepared over the range of 0.004 to 1 μM; dextrorphan-d3 (0.5 ng/μl) was used as an internal standard. Ions m/z 258.1 and 261.1 were monitored for dextrorphan and dextrorphan-d3, respectively. Mobile phase was 0.1% formic acid in water (A) and acetonitrile (B) using the following gradient: 0 min 20% B, 1 min 20% B, 5 min 40% B, 6 min 20% B, and 9 min 20% B.

For metoprolol and α-hydroxymetoprolol, calibration standards were prepared over the range of 0.003 to 0.176 μM; carvedilol-d4 (1 ng/μl) was used as an internal standard. Ions m/z 284.1 and 411.1 were monitored for α-hydroxyl-metoprolol and carvedilol-d4, respectively. Mobile phase was 10 mM ammonium formate, pH 4, in water (A) and acetonitrile (B) using the following gradient: 0 min 15% B, 3.5 min 60% B, 4.5 min 90% B, 5 min 90% B, 5.1 min 15% B, and 9.5 min 15% B.

Synthesis of CYP2D6 probe, ticlopidine 5'-carboxypropargyl amide (tic-ABP1P)

Bulk solvents were obtained from Fisher Scientific (Fair Lawn, NJ). All other chemicals and anhydrous solvents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Flash chromatography was performed with a Combiflash® Rf purification system (Teledyne Isco, Lincoln, NE). Proton NMR spectra were taken on an Agilent DD2 500 MHz spectrometer at 25°C. Coupling constants (*J*) are reported in Hertz (Hz) and peak multiplicities are denoted by the following abbreviations: s=singlet, d=doublet, t=triplet, dd=doublet of doublets, m=multiplet, b=broad. Chemical shift values (δ) are reported in ppm and are referenced to the residual solvent signals (δ=7.25 for chloroform and δ=3.30 for methanol). High resolution mass spectroscopy (HRMS) was performed on a Thermo Fisher LTQ Orbitrap equipped with an ESI probe.

Ticlopidine 5'-Methyl Carboxylate

Yield=81% after purification by flash chromatography (0-10% ethyl acetate in hexanes). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.21 (bs, 1H), 7.88 (bd, *J*=8.32 Hz, 1H), 7.45 (d, *J*=8.32 Hz, 1H), 7.09 (d, *J*=5.12 Hz, 1H), 6.72 (d, *J*=5.12 Hz, 1H), 3.91 (s, 3H), 3.86 (bs, 2H), 3.67 (bs, 2H), 3.0-2.8 (m, 4H). HRMS ESI$^+$ calculated for $C_{16}H_{17}ClNSO_2$ (M+H): 322.0669, found: 322.0673.

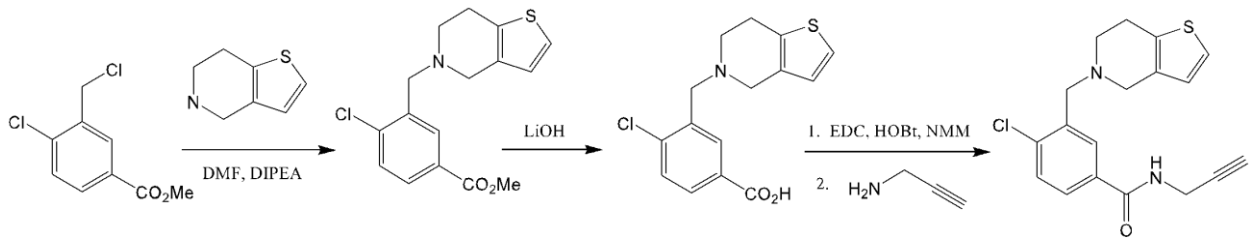
Ticlopidine 5'-Carboxylic Acid

The product acid was obtained as its hydrochloride salt in quantitative yield and was used in the next reaction step without further purification. ^{1}H NMR (500 MHz, CD_3OD): δ 8.34 (d, *J*=2.09 Hz, 1H), 8.09 (dd, *J*=2.09, 8.41 Hz, 1H), 7.69 (d, *J*=8.41 Hz, 1H), 7.35 (d, *J*=5.23 Hz, 1H), 6.86 (d, *J*=5.23 Hz, 1H), 4.60 (s, 2H), 4.31 (bs, 2H), 3.63 (t, *J*=6.15 Hz, 2H), 3.19 (t, *J*=6.15 Hz, 2H). HRMS ESI$^+$ calculated for $C_{16}H_{17}ClNSO_2$ (M+H): 308.0512, found: 308.0517.

Ticlopidine 5'-Carboxypropargyl Amide (tic-ABP1P)

Yield=70% after purification by flash chromatography (0-20% ethyl acetate in hexanes). ^{1}H NMR (500 MHz, CD_3OD): δ 8.01 (d, *J*=2.26 Hz, 1H), 7.70 (dd, *J*=2.26, 8.33 Hz, 1H), 7.49 (d, *J*=8.33 Hz, 1H), 7.12 (d, *J*=5.14 Hz, 1H), 6.70 (d, *J*=5.14 Hz, 1H), 4.13 (d, *J*=2.53, 2H), 3.85 (s, 2H), 3.61 (s, 2H), 2.90-2.83 (m, 4H), 2.59 (t, *J*=2.53, 1H). HRMS ESI$^+$ calculated for $C_{16}H_{17}ClNSO_2$ (M+H): 345.0828, found: 345.0831.

Reaction scheme



Construction of yeast expression plasmid: p41K*GAL1-CYP2D6*-HA

S. cerevisiae codon-optimized *CYP2D6* sequence (Uniprot: P10635) was synthesized by a commercial supplier (Integrated DNA Technologies, Coralville, IA) and cloned into a low-copy p41K*GAL1* plasmid.[45] To create *CYP2D6* variants, point mutations were engineered with KAPA HiFi DNA Polymerase (Kapa Biosystems, Wilmington, MA) using a standard site-directed mutagenesis method. The final *CYP2D6* plasmid constructs and protein expression levels were verified by Sanger sequencing and Western blot, respectively.

CYP2D6 expression in yeast

CYP2D6 variant proteins were expressed in a humanized cytochrome P450 *S. cerevisiae* strain as described previously[45] with the following modifications. Yeast strain S288C derivative (*MATα HAP1*+ *ura3*Δ*0*::p*GPD-MYC*-b5-URA3 *HO*::p41K*GAL1-POR*-FLAG-*TRP1 leu2*Δ*1 his3*Δ*1 trp1*Δ*63 pep4*Δ*0 prb1*Δ*0*) was transformed with p41K*GAL1-CYP2D6*-HA using a standard lithium acetate procedure[46] and was propagated on liquid media supplemented with 200 μg/ml G418 to maintain the plasmid. At an OD_{600} of 0.025, cells were pelleted, and resuspended in fresh media containing 2% (w/v) galactose to induce the over-expression of CYP2D6-HA. Cells were grown at 30°C and collected after 7 doublings for Western blotting and functional assay.

Functional activity of CYP2D6 variant proteins in yeast

Isogenic yeast strains were generated for the induction of three control (wildtype, C443H, and P34S) and seven coding (P8S, S168A, D198N, P267L, V338M, A449D, and R474Q) *CYP2D6* variant genes. Each expression construct contained only a single variant. P34S confers decreased function of CYP2D6.[14] C443H is a synthetic nonfunctional allele resulting in a catalytically inactive control.[48–50] CYP2D6 enzymatic activity was probed using a click chemistry compatible ticlopidine 5'-carboxypropargyl amide activity-based protein profiling probe (tic-ABP1P) that have specificity for CYP2D6 activity with minimum reactivity towards other yeast proteins. Yeast cells induced with

CYP2D6 variants were collected at OD_{600} of 1, pelleted and saponin-permeabilized cells were pre-incubated with 2 mM NADPH (Sigma-Aldrich) for 20 min at 30°C. Next, cells were exposed to tic-ABP1P (20 μM) for 17 h in a rotator in the dark at 37°C to form CYP2D6 activity-dependent P450-probe adducts. To append a fluorophore reporter (Alexa Fluor™ 488 picolyl azide) via a copper-catalyzed azide-alkyne cycloaddition reaction,[47] the Click-iT™ kit (Sigma-Aldrich) was used. PBS washed cells were incubated with a Click-iT™ Plus reaction cocktail according to the manufacturer's instructions. Cells were washed in PBS 5 times prior to flow cytometry analysis. For each strain, a total of 20,000 events was collected using the flow cytometer BD™ LSRII with the FITC channel (BD Bioscience, San Jose, CA). A standard yeast gate was applied to all cytometry data, and fluorescence data was analyzed using FlowJo (Ashland, OR). The geometric mean fluorescence (GMF) of each strain was normalized to the GMF of the wildtype CYP2D6 control strain, and ratios of normalized GMF of variants to normalized GMF of wildtype CYP2D6 were calculated and converted to percentage.

CYP2D6 activity was also confirmed using a standard fluorogenic CYP2D6 substrate, Vivid™ 7-ethoxymethoxy-3-cyanocoumarin. Yeast cells induced with *CYP2D6* variants were collected at OD_{600} of 2 and potassium phosphate buffer resuspended cells were pre-incubated with 2 mM NADPH (Sigma-Aldrich) for 20 min at 30°C. Next, 100 μl of cells containing 20 μM of Vivid™ substrate were dispensed into each well of a black 96-well plate and incubated for an additional 20 min at 37°C. Fluorescence unit readings were collected over ~2 hours in a plate shaker using 408 nm and 450 nm excitation and emission wavelengths, respectively. The relative fluorescence unit (RFU) of each strain was normalized to the RFU of control wells containing buffer and substrate alone.