Supporting Information for

CYP3A Mediates an Unusual C(sp^2)-C(sp^3) Bond Cleavage via *Ipso*-Addition of Oxygen in Drug Metabolism

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I. Supplementary Material Information.

Pexidartinib was purchased from Cayman Chemical (Ann Arbor, Michigan). Ketoconazole (KCZ), $^{18}O_2$, $H_2{}^{18}O$, methyl 6-aminonicotinate, formic acid, 6-(Trifluoromethyl)pyridine-3-methanol, NADPH (β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate), methoxyamine, hydrogen peroxide, sodium azide, bovine liver catalase, and reduced glutathione were obtained from Millipore Sigma (St. Louis, MO). PLX-647, FLT3-IN-2, and PLX-5622 were obtained from MedChemExpress LLC (Monmouth Junction, NJ). Human liver microsomes (HLM, 20 mg protein/ml; Lot No. 1910096) and recombinant human P450s (EasyCYP Bactosomes) were purchased from XenoTech (Lenexa, KS). All the recombinant human P450s with P450 Concentration: 1.0 nmol/ml and Protein Concentration: 10.0 mg/ml. The Cytochrome c Reductase Activity of each EasyCyp is listed as below:

EasyCyp	Batch Number:	Cytochrome c Reductase Activity (nmol/min/mg protein)
CYP1A2	C1A2R014C	137
CYP2A6	C2A6R011A	385
CYP2B6	C2B6R058A	564
CYP2C8	C2C8R008C	298
CYP2C9	C2C9HR032	903
CYP2C19	C2C19R024B	568
CYP2D6	C2D6R028C	592
CYP2E1	C2E1R023C	186
CYP3A4	C3A4R055A	903
CYP3A5	C3A5R007	1808

All solvents for liquid chromatography and mass spectrometry were of the highest grade commercially available.

II. Methods and data analysis

General procedures for drug metabolism of PEX in HLMs and recombinant human P450s enzymes. Incubations were conducted in 1× phosphate-buffered saline (1× PBS, pH 7.4) containing 30 μM PEX, M3 or M7 and 0.1 mg HLM or 1 pmol of each cDNA-expressed P450 enzyme (control, CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5) in a final volume of 95 μl. After a 5 min pre-incubation at 37 °C, 5 μl of 20 mM NADPH was added (final concentration 1.0 mM) and incubation was continued for 40 min with gentle shaking. Incubations without NADPH were used as controls. Ketoconazole (KCZ, a CYP3A inhibitor) at a final concentration of 2.0 μM was used to determine the role of CYP3As in the formation of metabolites M24-M31 in HLM. Reactions were terminated by adding 100 μl of ice-cold acetonitrile and vortexing for 30 seconds, and then centrifuged at rcf 15,000 for 15 min. Each supernatant was transferred to an auto sampler vial, and 3.0 μl was injected on to UHPLC-Q Exactive MS system for analysis. Incubations were performed in triplicate. $^{18}O_2$ and $H_2{}^{18}O$ were employed to determine the origin of oxygen in metabolites from C-C bond cleavage by enriching incubation system with $^{18}O_2$ and $H_2{}^{18}O$.

Testing contributions of hydrogen peroxide to PEX metabolism.
Incubations were conducted in 1× PBS (pH 7.4) containing 30 μM PEX, with no enzyme or 0.1 mg HLM or 1 pmol of CYP3A4 in a final volume of 95 μl. After a 5 min pre-incubation at 37 °C, 5 μl of 100 mM hydrogen peroxide (final concentration 5 mM) or 20 mM NADPH (final concentration 1.0 mM) was added. The incubations were continued at 37 °C for 40 min. To inhibit the microsomal associated catalase activity in HLM reactions with added hydrogen peroxide, 1 mM of sodium azide was added. To block the contributions of NADPH-dependent hydrogen peroxide production to PEX metabolite formation in the presence of NADPH, bovine liver catalase (final concentration 1000 U/mL) was added to the incubation reactions. Reactions were terminated by adding 100 μl of ice-cold acetonitrile and vortexing for 30 seconds, and then centrifuged at rcf 15,000 for 15 min. Each supernatant was transferred to an auto sampler vial, and 3.0 μl was injected on to UHPLC-Q Exactive MS system for analysis.

Procedures for time-course experiments in HLMs or with recombinant human CYP3A. For time-course experiments, incubations were conducted in 1× phosphate-buffered saline (1× PBS,

pH 7.4) containing 30 µM PEX (or M3, or M7) and 0.3 mg HLM or 3 pmol of each cDNA-expressed P450 enzyme (CYP3A4 or 3A5) in a final volume of 270 µl. After a 5 min pre-incubation at 37 °C, 30 µl of 20 mM NADPH was added (final concentration 1.0 mM) and the 50 µl of incubation mixtures were collected at 0, 15, 30, 60, and 90 min. Reactions were terminated by adding 50 µl of ice-cold acetonitrile and vortexing for 30 seconds, and then centrifuged at rcf 15,000 for 15 min. Each supernatant was transferred to an auto sampler vial, and 3.0 µl was injected on to UHPLC-Q Exactive MS system for analysis. The quantification of each metabolite was performed based on the corresponding standard curve.

UHPLC-Q Exactive Orbitrap MS analyses. Drug and its metabolites were resolved, identified and quantified (relatively) using UHPLC coupled with Q Exactive Orbitrap MS (Thermo Fisher Scientific, San Jose, CA). The setup included a 100 mm x 2.1 mm column (BEH C-18, 1.7 µm, Waters, MA) maintained at a temperature of 40 °C. The flow rate of was set at 0.3 mL/min and a gradient from 2% to 95% aqueous acetonitrile containing 0.1% formic acid was used over a 15-min run. The Q Exactive MS was operated in positive mode with electrospray ionization. Ultra-pure nitrogen was applied as the sheath (45 arbitrary unit), auxiliary (10 arbitrary unit), sweep (1.0 arbitrary unit) and the collision gas. The capillary gas temperature was set at 350 °C and the capillary voltage was set at 4.3 kV. The MS data were acquired in profile mode within the range of 80 to 1200 Da and lock mass at *m/z* 371.1012 was utilized as the reference ion during acquisition. The MS/MS of metabolites was performed in targeted mode with an isolation width of 2 *m/z* and ramp collision energy was set at 15, 20, and 35 eV.

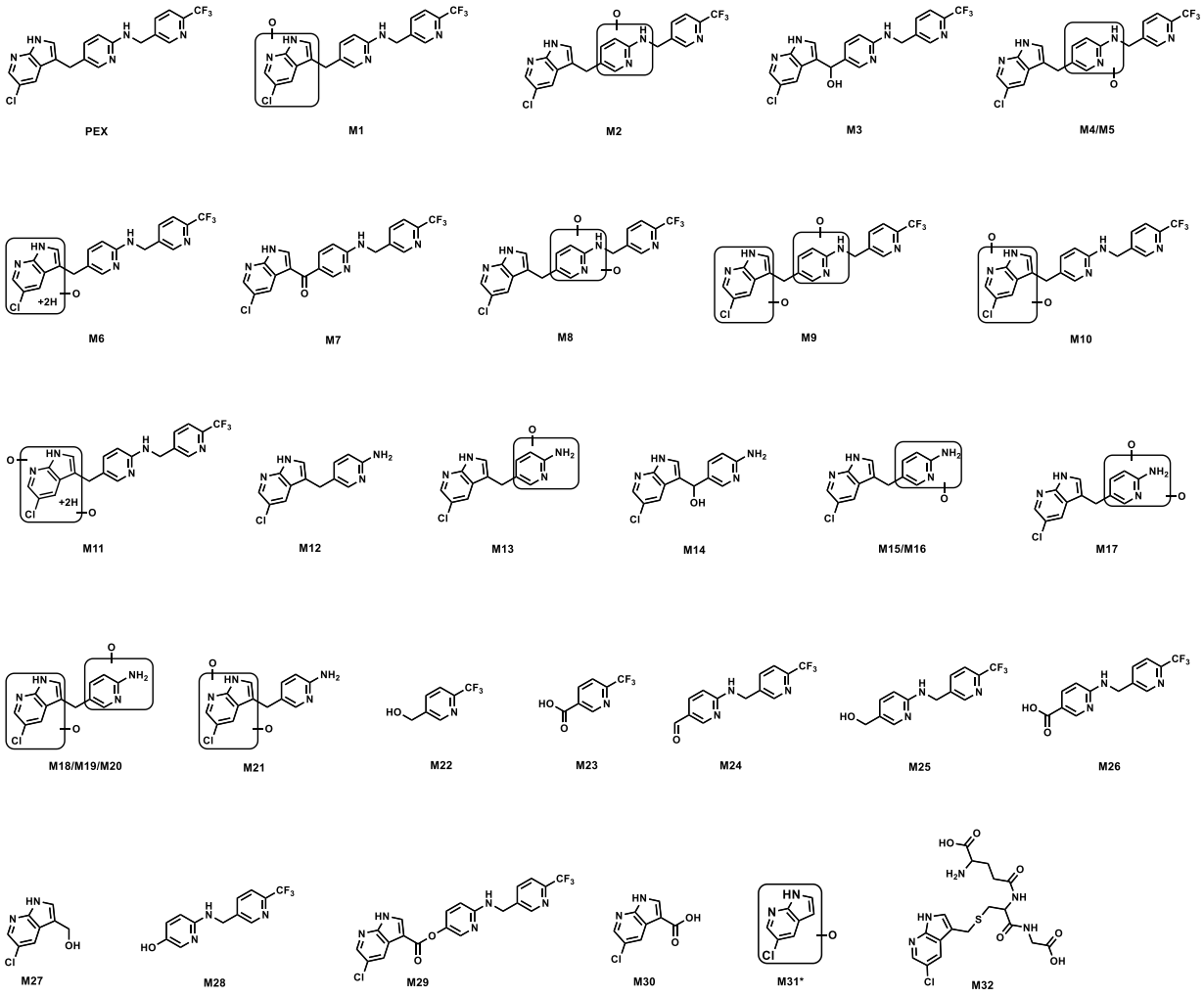
Molecular docking of CYP3A with PEX. The protein structures used in docking experiments were publicly available X-ray crystal structures for CYP3A4 (PDB: 6MA7) and CYP3A5 (PDB: 6MJM) that were processed using the Schrödinger Suite Release 2022-1 and prepared using the Protein Preparation Wizard[49]. Default settings were used except where indicated. PEX was prepared with the LigPrep program (Schrödinger, LLC, New York, NY, 2023). This entails obtaining accurate 3D-structures of the ligands in their correctly assigned protonation states and tautomeric forms at a target pH of 7.0 ± 2.0 by the program Epik. The prepared ligands were docked into the ligand binding site using the Glide program in the extra precision (XP) mode[50].

The docking poses were visualized and analyzed using PyMOL Molecular Graphics System (Version 2.0 Schrödinger, LLC).

Data analysis. Mass chromatograms and mass spectra were acquired and processed using the Xcalibur software (Thermo Fisher Scientific, San Jose, CA) in profile formats from *m/z* 80 to 1200. The acquired data were processed by Compound Discoverer 3.3 software (Thermo Fisher Scientific, San Jose, CA) to generate a multivariate data matrix. Data matrices were exported into SIMCA14 (Umetrics, Kinnelon, NJ) for multivariate data analysis. OPLS-DA was conducted on Pareto-scaled data [51]. Statistical analysis was conducted using two-tailed Student's independent *t*-test. Experimental data are presented as mean ± s.e.m.

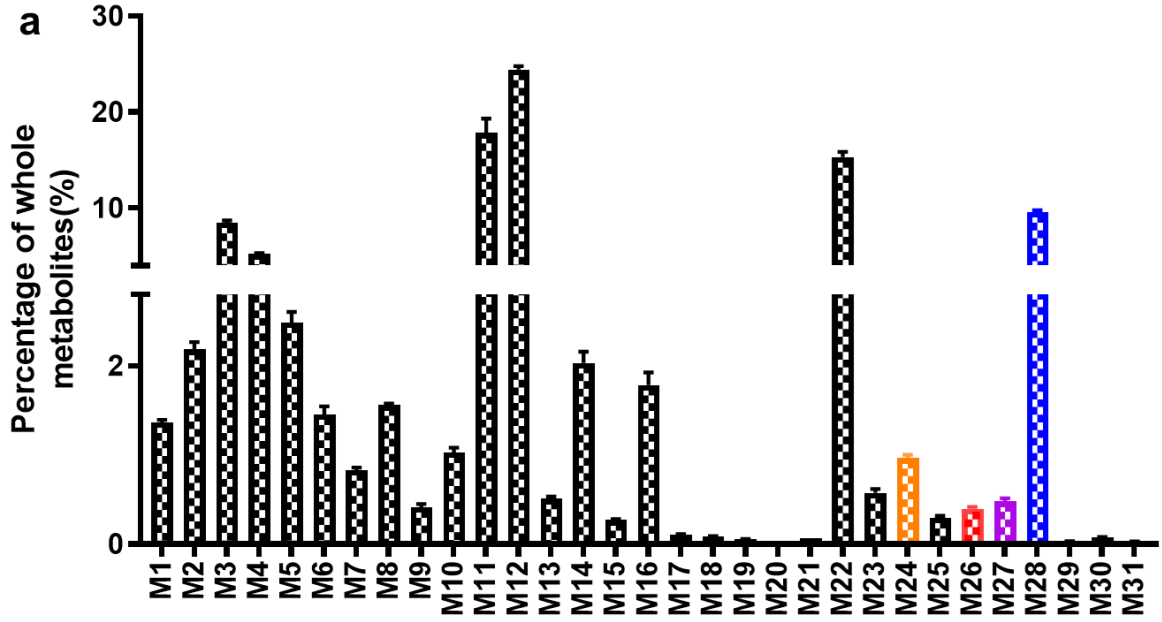
III. Supplementary Figures

Figure S1. Summary of inferred structures of PEX metabolites and adducts.

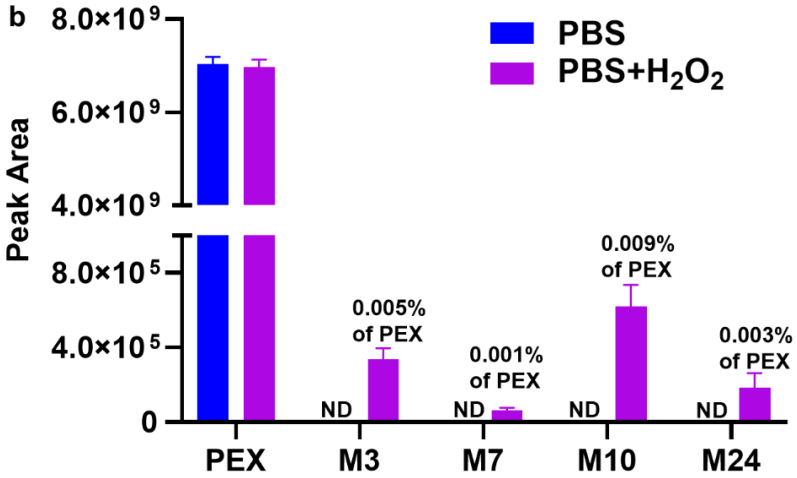


Incubations were conducted in 1× phosphate-buffered saline (1 × PBS, pH 7.4) containing 30 μM PEX, 0.1 mg HLM in a final volume of 95 μl. After 5 min of pre-incubation at 37 °C, the reaction was initiated by adding 5 μl of 20 mM NADPH (final concentration 1.0 mM) and continued for 40 min with gentle shaking. Samples were analyzed by UHPLC-Q Exactive MS system for analysis. Incubations were performed in triplicate. All structures were determined based on the exact mass (mass error less than 5 ppm) and MS/MS fragments, except M31*. The structures of metabolites M3, M7, M12 and M24-M30 were verified by standard compounds. *, M31 was identified only based on the exact mass and predicted formula as its low abundance precluded obtaining high quality MS/MS spectra.

Figure S2. Relative abundance of ions of PEX metabolites in HLM and formation of PEX metabolites in H_2O_2 with/without enzyme.

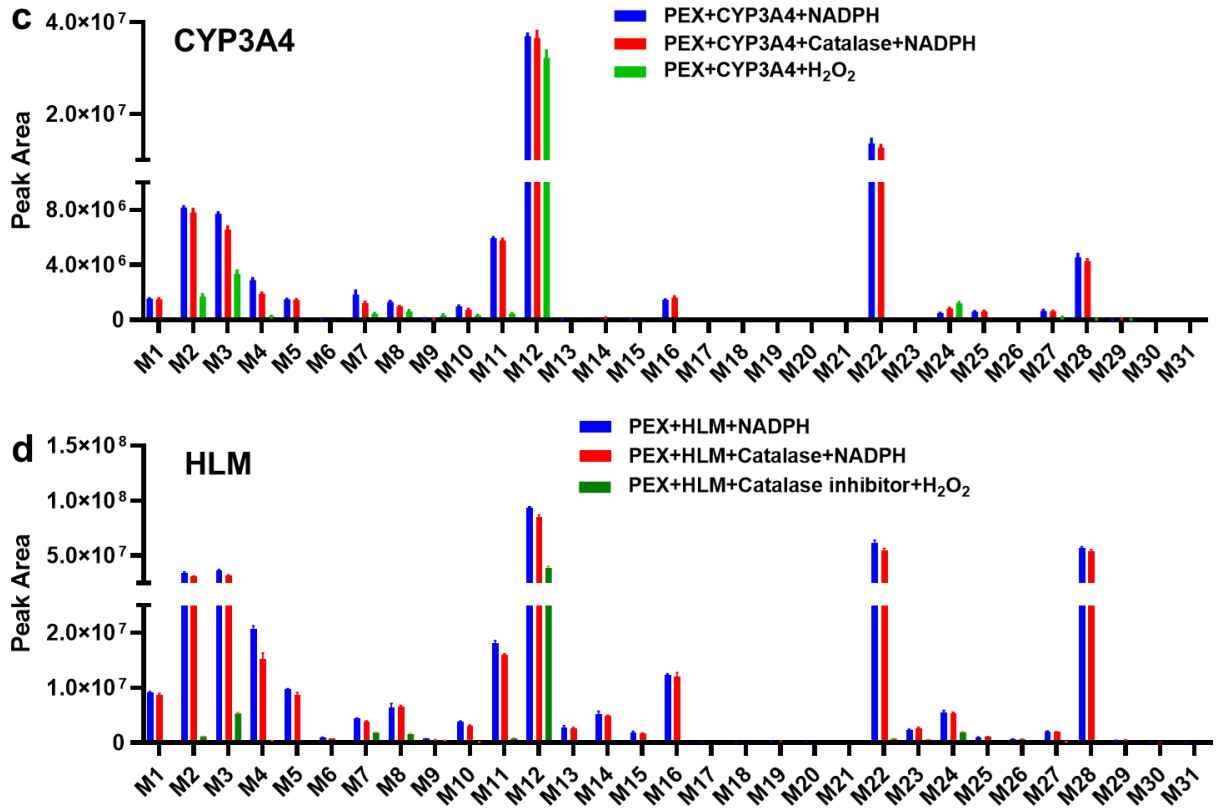


Incubations were as described in **Fig. S1**. Samples were analyzed by UHPLC-Q Exactive MS system. The relative quantification was conducted based on the peak area. The overall abundance of metabolite ions was set as 100% in each sample. Data are expressed as mean ± s.e.m (n = 3).



Incubations were conducted in 1× PBS (pH 7.4) containing 30 μM PEX in a final volume of 95 μl. After a 5 min pre-incubation at 37 °C, 5 μl of PBS (or PBS with 100 mM hydrogen peroxide, final concentration 5 mM) was added. The incubations were continued at 37 °C for 40 min.

Reactions were terminated by adding 100 µl of ice-cold acetonitrile and vortexing for 30 seconds, and then centrifuged at rcf 15,000 for 15 min. Each supernatant was transferred to an auto sampler vial, and 3.0 µl was injected on to UHPLC-Q Exactive MS system for analysis. Data are expressed as mean ± s.e.m (n = 3).

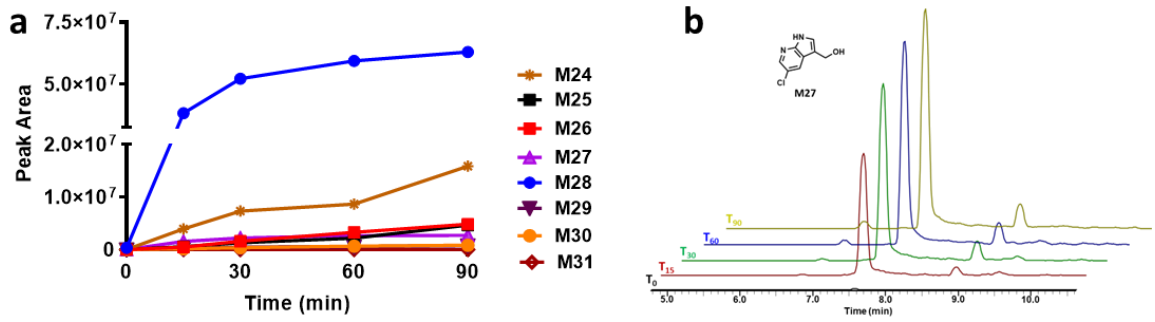


Incubations were conducted in 1× PBS (pH 7.4) containing 30 µM PEX, 0.1 mg HLM or 1 pmol of CYP3A4 in a final volume of 95 µl. After a 5 min pre-incubation at 37 °C, 5 µl of 100 mM hydrogen peroxide (final concentration 5 mM) or 20 mM NADPH (final concentration 1.0 mM) was added. The incubations were continued at 37 °C for 40 min. To inhibit the HLM-associated catalase activity in reactions with added H_2O_2, 1 mM sodium azide was added together with HLM. To block the contributions of NADPH-dependent hydrogen peroxide production to PEX metabolite formation, bovine liver catalase (final concentration 1000 U/mL) was added. Reactions were terminated by adding 100 µl of ice-cold acetonitrile and vortexing for 30 seconds, and then centrifuged at rcf 15,000 for 15 min. Each supernatant was transferred to an auto sampler vial, and 3.0 µl was injected on to UHPLC-Q Exactive MS system for analysis.

Data are expressed as mean ± s.e.m (n = 3). M24 is generated more effectively by CYP3A4 using H_2O_2 than using NADPH, and M12 is generated almost as effectively using H_2O_2 as NADPH, which strongly suggests that these products are generated by the Compound I state of CYP3A4. CYP3A4 can also use H_2O_2 to produce significant amounts of M2, M3, and M7-M11; these products are likely also produced in part by Compound I.

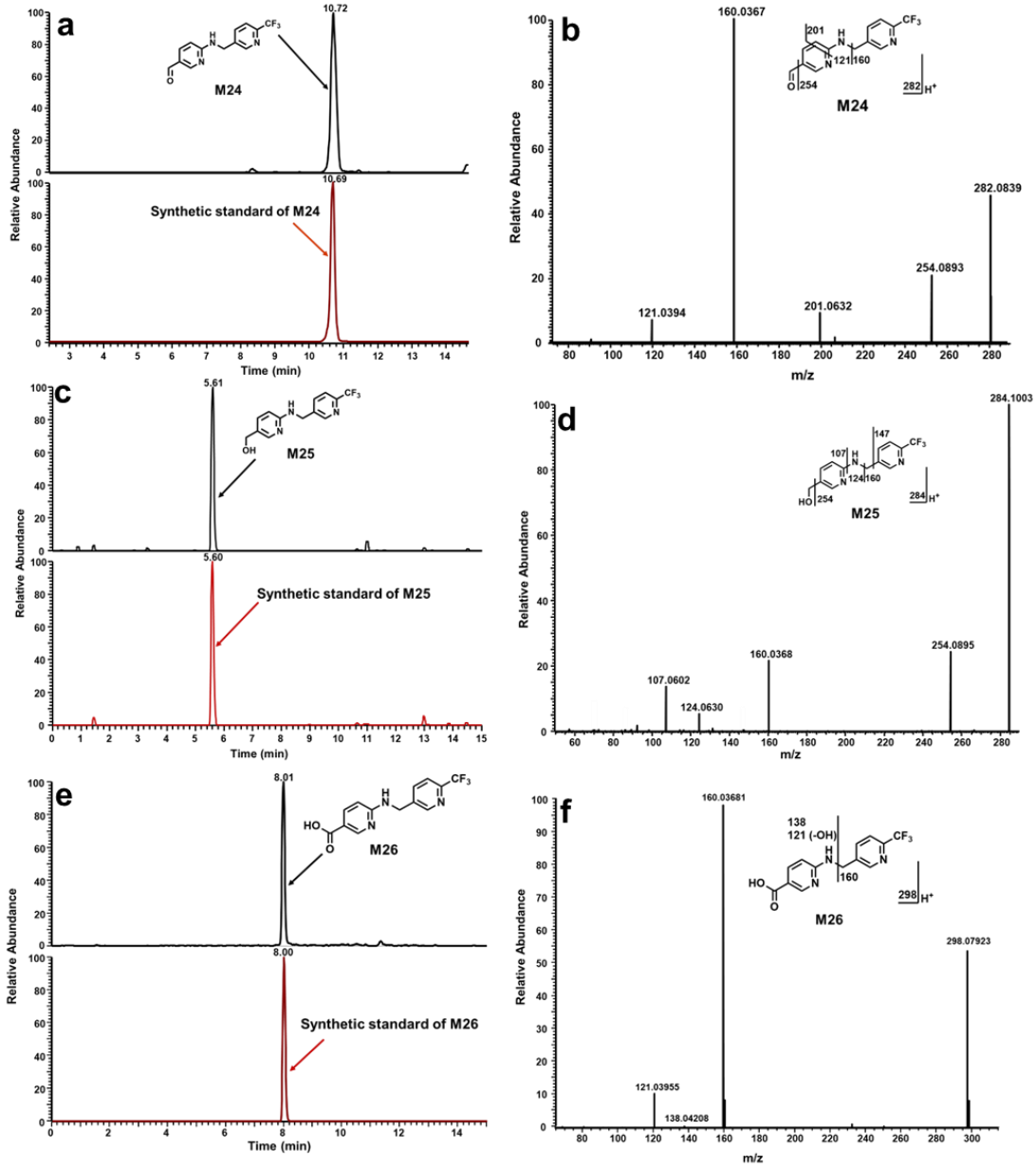
Reference: M. R. Anari, P. D. Josephy, T. Henry, and P. J. O'Brien, *Chem. Res. Toxicol*. **1997,** *10,* 582-588.

Figure S3. Time-dependent production of M24-M31 in HLM and chromatograms for M27.



Incubations were conducted in 1× PBS (pH 7.4) containing 30 µM PEX and 0.3 mg HLM or 3 pmol of each cDNA-expressed P450 enzyme (CYP3A4 or 3A5) in a final volume of 270 µl. After a 5 min pre-incubation at 37 °C, 30 µl of 20 mM NADPH was added (final concentration 1.0 mM) and 50 µl of incubation mixtures were collected at 0, 15, 30, 60, and 90 min. Reactions were terminated by adding 50 µl of ice-cold acetonitrile and vortexing for 30 seconds, and then centrifuged at rcf 15,000 for 15 min. Each supernatant was transferred to an auto sampler vial, and 3.0 µl was injected on to UHPLC-Q Exactive MS system for analysis. **a**. Time-dependent production of M24-M31 in HLM. **b**. Time-point chromatograms for M27. Data points indicate the mean ± s.e.m. (n =3).

Figure S4. Identification of PEX metabolites M24, M25 and M26



Incubation conditions are as described in Fig. **S1**. Structural elucidations were based on exact mass (mass errors less than 5 ppm), MS/MS fragmentations and standard compounds. MS/MS was performed with collision energy ramping from 10, 20, 35 arbitrary unit. The major fragmental ions are interpreted in the inlaid structural diagrams. For time-course experiments, incubations were conducted in 1× PBS (pH 7.4) containing 30 μM PEX and 0.3 mg HLM in a final volume of 270 μl. NADPH was added to initiate the reactions (final concentration 1.0 mM)

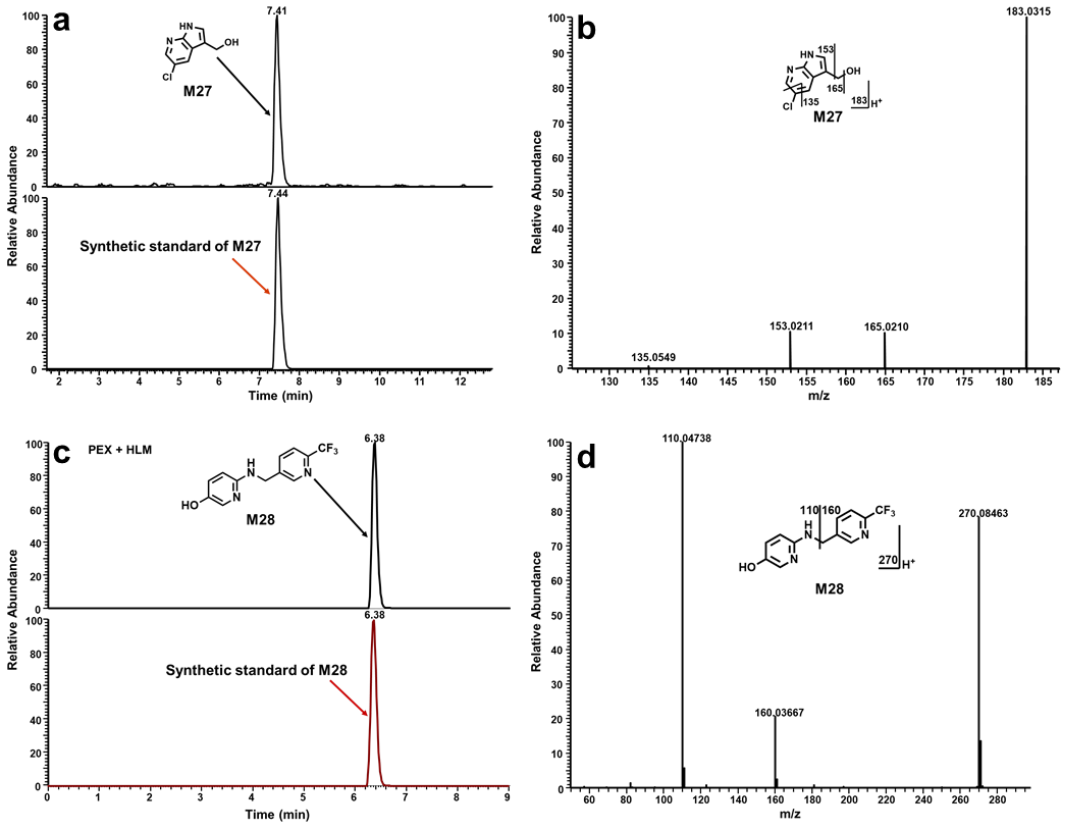
and 50 µl of the incubation mixtures were collected at 0, 15, 30, 60, and 90 min. The details were described in the method section. **a.** Chromatograms of metabolite M24. **b.** MS/MS of M24. **c.** Chromatogram of M25. **d.** MS/MS of M25. MS/MS was performed with collision energy ramping from 10, 20, 35 eV. **e.** Chromatograms of metabolite M26. **f.** MS/MS of M26.

Aldehyde M24 eluted at 10.7 min (Table **S1** and Fig. **S4a**) and was detected as a protonated molecule at *m/z* 282.0848. MS/MS of M24 yielded major fragmental ions at *m/z* 254, 160, 201, and 121, which are interpreted in the inlaid structural diagram (Fig. **S4b**).

Alcohol M25 eluted at 5.61 min and was detected as a protonated molecule at m/z 284.1003 (Table **S1**, Figs. **S4c** and **S4d**). MS/MS of M25 produced major fragmental ions at m/z 254, 160, 124 and 107 (Fig. **S4d**). The M25 fragmental ions are interpreted in the inlaid structural diagram (Fig. **S4d**).

Aminonicotinic acid M26 was eluted at 8.01 min (Table **S1** and Fig. **S4e**) and was detected as a protonated molecule at *m/z* 298.0792. MS/MS of M26 produced major fragmental ions at *m/z* 160, 138, and 121, which are interpreted in the inlaid structural diagram (Fig. **S4f**).

Figure S5. Identification of PEX metabolites M27 and M28

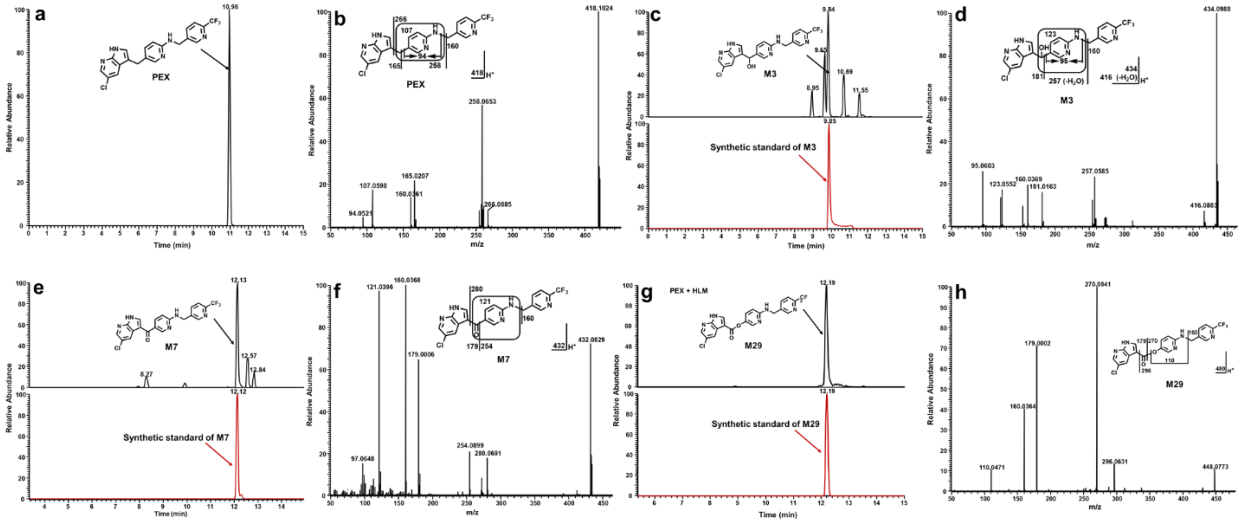


Incubation conditions are as described in Fig. **S1**. Structural elucidations were based on exact mass (mass errors less than 5 ppm), MS/MS fragmentations and standard compound. MS/MS was performed with collision energy ramping from 10, 20, 35 eV. The major fragmental ions are interpreted in the inlaid structural diagrams. **a.** Chromatograms of metabolite M27. **b.** MS/MS of M27. **c.** Chromatograms of metabolite M28. **d.** MS/MS of M28.

Pyrrolopyridin-3-yl-methanol M27 eluted at 7.41 min (Table **S1** and Fig. **S5a**) and was detected as a protonated molecule at *m/z* 183.0321. MS/MS of M27 produced major fragmental ions at *m/z* 165, 153, and 135, which are interpreted in the inlaid structural diagram (Fig. **S5b**).

Aminopyridinol M28 eluted at 6.38 min (Table **S1** and Fig. **S5c**) and was detected as a protonated molecule at *m/z* 270.0846. MS/MS of M28 produced major fragmental ions at *m/z* 160 and 110, which are interpreted in the inlaid structural diagram (Fig. **S5d**).

Figure S6. Identification of PEX metabolites M3, M7 and M29.



Incubation conditions are as described in **Fig. S1**. Structural elucidations were based on exact mass (mass errors less than 5 ppm), MS/MS fragmentations and standard compounds. MS/MS was performed with collision energy ramping from 10, 20, 35 eV. The major fragmental ions are interpreted in the inlaid structural diagrams. **a.** Chromatogram of PEX. **b.** MS/MS of PEX. **c.** Chromatograms of metabolite M3. **d.** MS/MS of M3. **e.** Chromatograms of M7. **f.** MS/MS of M7. **g.** Chromatograms of M29. **h.** MS/MS of M29.

M3 eluted at 9.84 min and was detected as a protonated molecule at m/z 434.0991, 16 Da higher than parent PEX (Table **S1**, Figs. **S6c** and **S6d**). MS/MS of M3 produces major fragmental ions at m/z 416 (loss of H_2O), 257, 181, 160, 123, and 95. Comparison of the fragmental ions at m/z 181 and 123 of M3 with those at m/z 165 and 107 for PEX (Figs. **S6a** and **S6b**) indicates that the oxidation occurs on the CH_2 position (Fig. **S6d**).

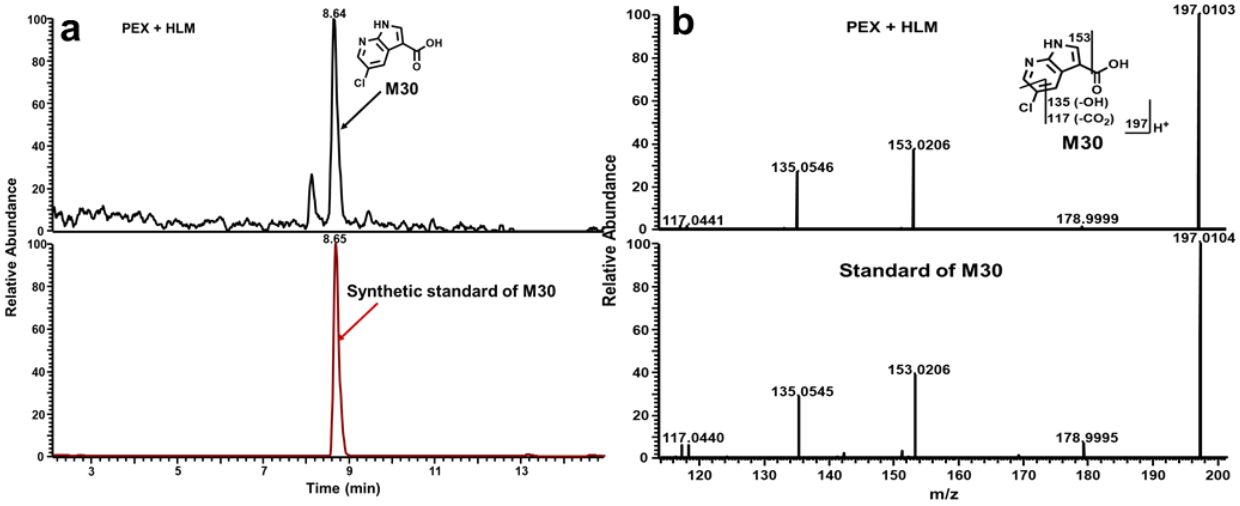
M7 eluted at 12.13 min and was detected as a protonated molecule at m/z 432.0837 (Table **S1**, Figs. **S6e** and **S6f**). MS/MS of M7 produced major fragmental ions at m/z 280, 254, 179, 160, and 121 (Fig. **S6f**). The fragmental ions at m/z 280 and 179 compared with those at m/z 282 and 181 for M3 indicates the further oxidation of M3 to form a ketone (Fig. **S6d**). The M7 fragmental ions are interpreted in the inlaid structural diagram (Fig. **S6f**).

Ester M29 eluted at 12.19 min and was detected as a protonated molecule at m/z 448.0773 (Table **S1**, Figs. **S6g** and **S6h**). MS/MS of M29 produces major fragmental ions at m/z 294, 270,

179, 160, and 110. The M7 fragmental ions are interpreted in the inlaid structural diagram (Fig. **S6h**).

The structures of M3, M7 and M29 were confirmed by comparative LC-MS/MS of their standard compounds.

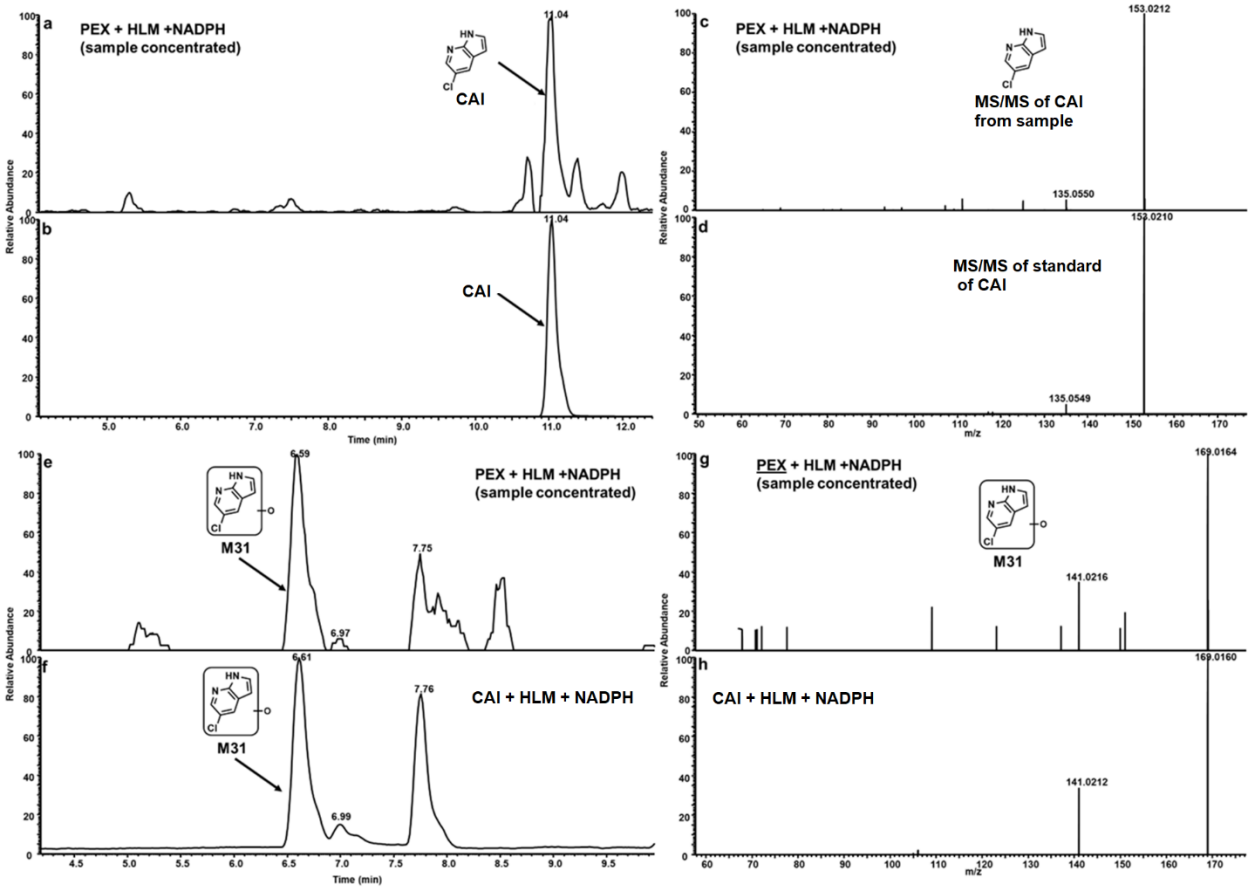
Figure S7. Identification of PEX metabolite M30



Incubation conditions are as described in Fig. **S1**. Structural elucidations were based on exact mass (mass errors less than 5 ppm), MS/MS fragmentations and standard compound. MS/MS was performed with collision energy ramping from 10, 20, 35 eV. The major fragmental ions are interpreted in the inlaid structural diagrams. **a.** Chromatograms of M30. **b.** MS/MS of M30.

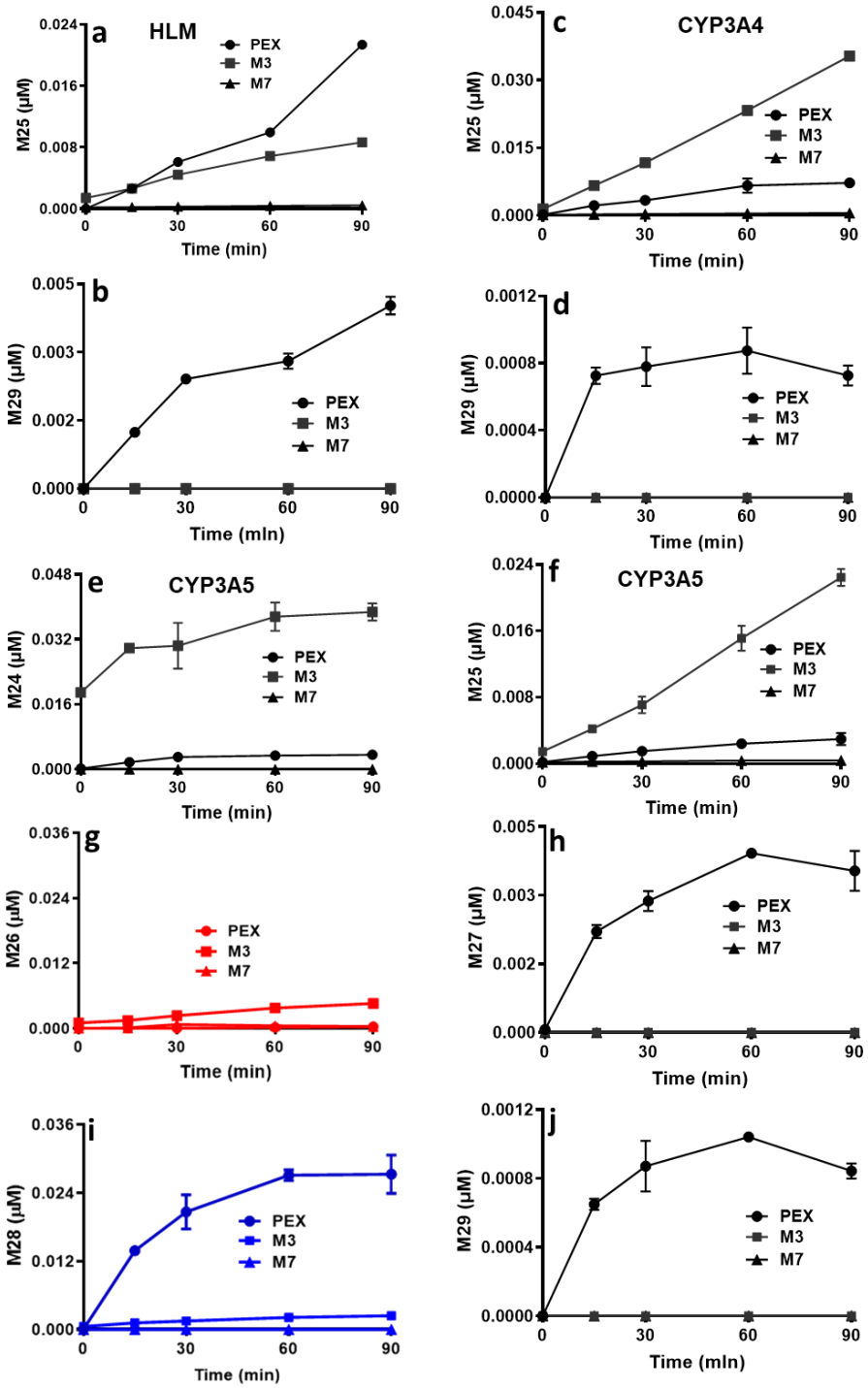
Acid M30 eluted at 8.64 min (Table **S1** and Fig. **S7a**) and was detected as a protonated molecule at *m/z* 197.0103. MS/MS of M30 yielded major fragmental ions at *m/z* 153, and 135, which are interpreted in the inlaid structural diagram (Fig. **S7b**). The structure was confirmed by comparative LC-MS/MS of its standard compound.

Figure S8. Identification of 5-chloro-7-azaindole (CAI) and M31



Incubation conditions of PEX or 5-chloro-7-azaindole (CAI, final concentration at 30 μM) in HLM were as described in Fig. **S1**. The PEX/HLM samples were concentrated before LC-MS analysis. **a.** and **b.** Extracted chromatograms of CAI from HLM-treated PEX and the standard, respectively. **c.** and **d.** MS/MS of CAI. **e.** and **f.** Extracted ion chromatograms at the mass of M31 for HLM-treated PEX or HLM-treated CAI, respectively. **g.** and **h.** MS/MS of M31.

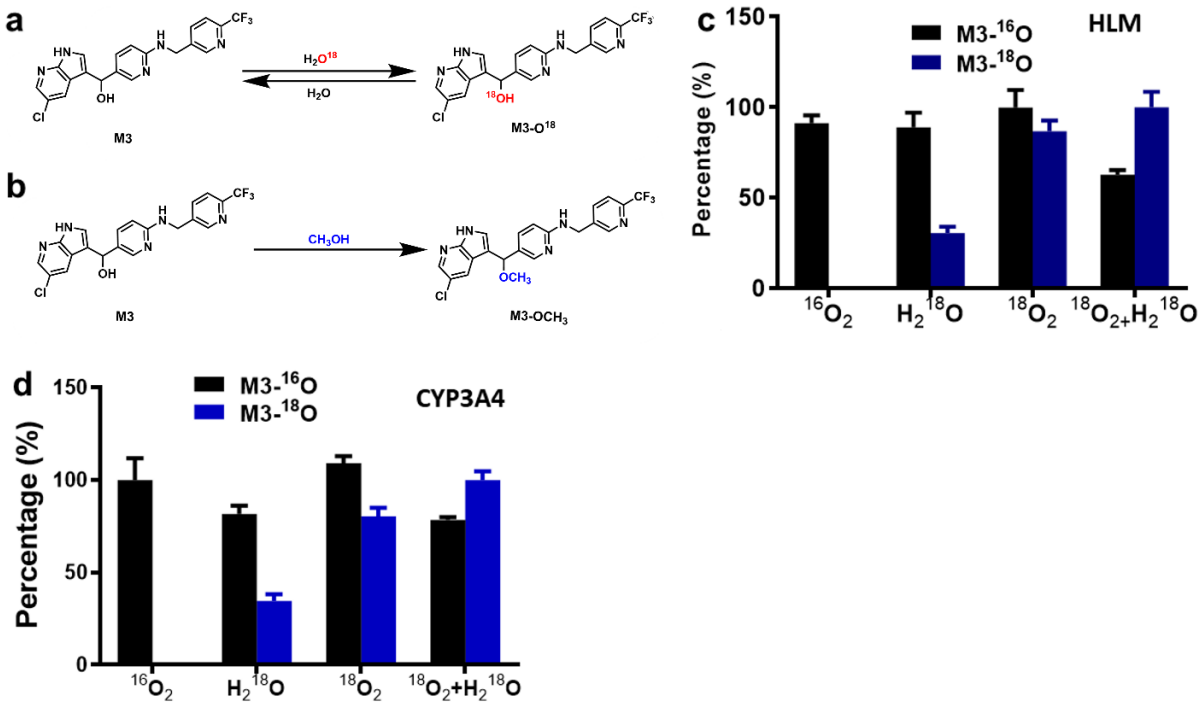
Figure S9. Production of M24-M29 using PEX, M3 or M7 as substrates.



The time-dependent experiments were performed as described in Fig. **S3**. **a,b** Time-course formation of M25 and M29 in HLM. **c,d** Time-course formation of M25 and M29 in CYP3A4.

e-j Time-course formation of M24-M29 in CYP3A5. Data points indicate the mean ± s.e.m. (n = 3). The production of M25 exhibits linearity in HLM, CYP3A4 and CYP3A5. M26 shows linearity in CYP3A5 and M29 is linear in HLM. The time-dependent inhibition may occur for the non-linear formation of M29 in CYP3A4/5 as well as M24, M27-M28 in CYP3A5.

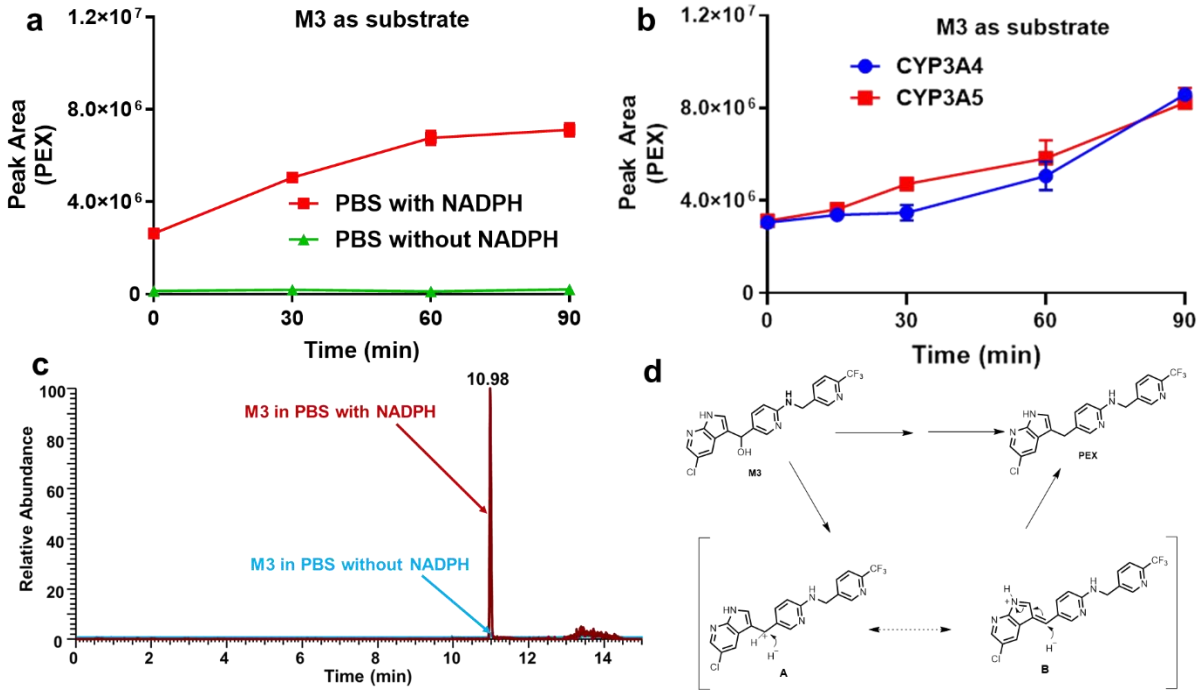
Figure S10. Stability study of alcohol M3 and ^{18}O incorporation in HLM or CYP3A4



For the $H_2{}^{18}O$ and $H_2{}^{18}O$ + $^{18}O_2$ groups, the 10 × phosphate-buffered saline (10× PBS, pH 7.4, Bio Rad, Hercules, CA) was diluted to 1× PBS with $H_2{}^{18}O$ (Millipore Sigma, St. Louis, MO). Incubations were conducted in 1× PBS with $H_2{}^{18}O$ containing 30 μM PEX and 0.1 mg HLM in a final volume of 98 μl. After a 5 min pre-incubation at 37 °C, 2 μL of 50 mM NADPH (dissolved in pure H_2O) was added (final concentration 1.0 mM) and incubation was continued at 37 °C for 40 min with gentle shaking. For the $^{18}O_2$ and $H_2{}^{18}O$ + $^{18}O_2$ groups, the Eppendorf tubes and solutions are enriched with $^{18}O_2$ (Millipore Sigma, St. Louis, MO) before and after adding NADPH. Incubations with normal water and air were used as controls. Reactions were terminated by adding 100 μl of ice-cold acetonitrile and vortexing for 30 seconds, and then centrifuged at rcf 15,000 for 15 min at 4 °C. Each supernatant was transferred to an auto sampler vial, and 3 μL was injected on to UHPLC-Q Exactive MS system for analysis. Incubations of all groups were performed in triplicate. The peaks of the respective ^{16}O, ^{18}O, 2×^{18}O products were extracted by their exact mass (error 5 ppm), and the peak area was normalized by the largest one (set as 100%) in any of 4 groups (control, $H_2{}^{18}O$, $^{18}O_2$ or $H_2{}^{18}O$ + $^{18}O_2$). Structural elucidations were performed based on accurate mass (mass errors less than 5 ppm). **a.** Exchange of -^{18}OH

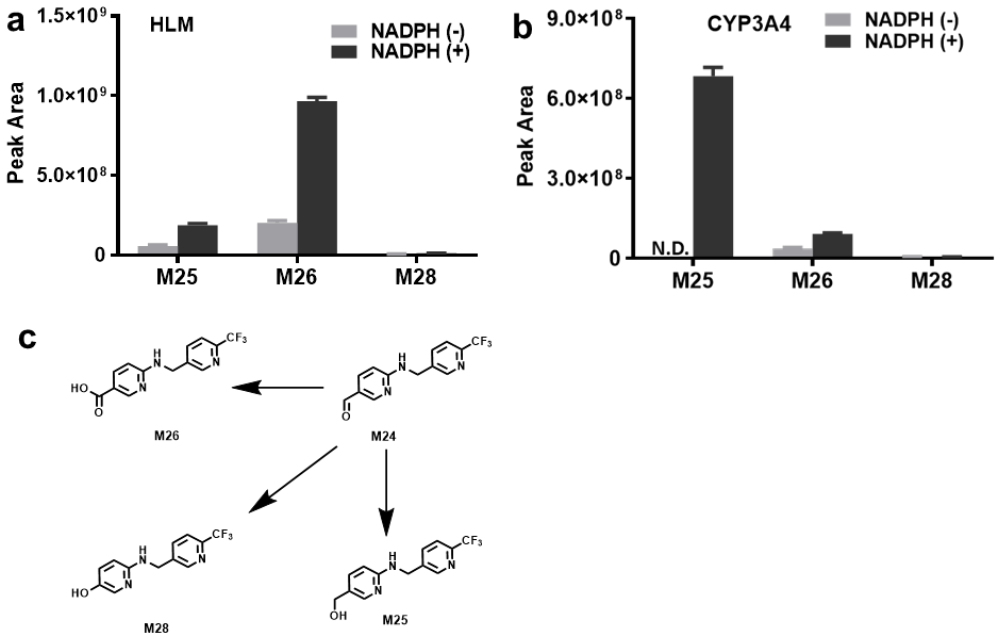
with -^{16}OH in M3. **b.** Exchange of -OH in M3 with -OCH_3 of solvent CH_3OH. **c.** Formation of M3-^{16}O and M3-^{18}O in HLM using PEX as a substrate. **d.** Formation of M3-^{16}O and M3- ^{18}O PEX in CYP3A4 using PEX as a substrate.

Figure S11. NADPH-dependent Production of PEX from M3 and proposed mechanism.



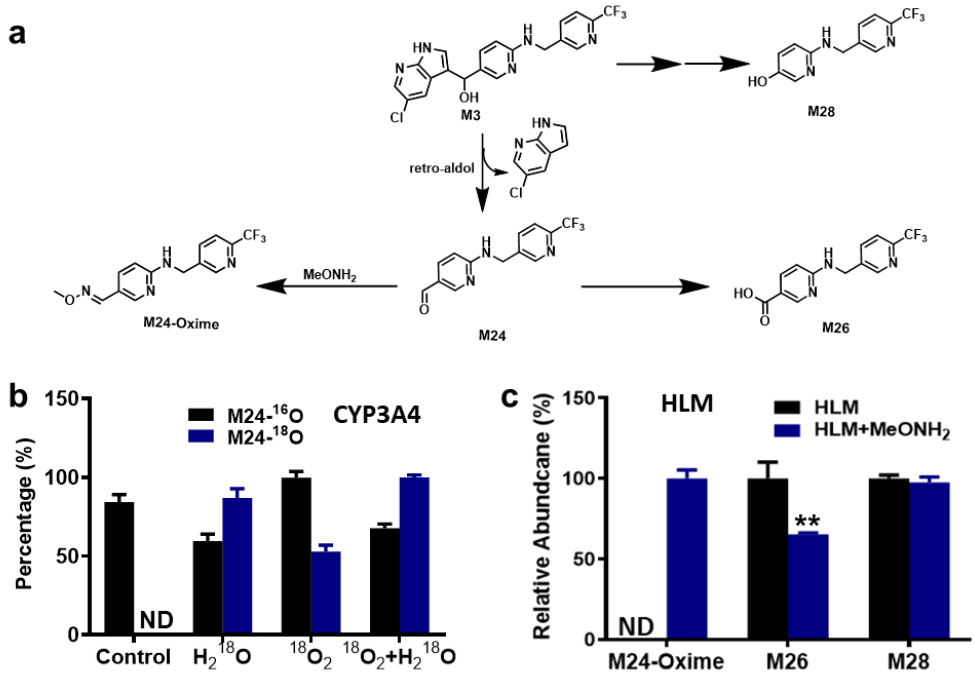
Production of PEX in PBS with/without NADPH. Incubations were conducted in 1× PBS (pH 7.4) containing 30 µM M3 with or without NADPH (final concentration 1.0 mM) in a final volume of 300 µl. 50 µl of incubation mixtures were collected at 0, 30, 60 and 90 min. Reactions were terminated by adding 50 µl of ice-cold acetonitrile and vortexing for 30 seconds, incubated at 95 °C for 5 min to force the degradation of NADPH and then centrifuged at rcf 15,000 for 15 min. Each supernatant was transferred to an auto sampler vial, and 3.0 µl was injected on to UHPLC-Q Exactive MS system for analysis. **Production of PEX in CYP3A with NADPH.** Incubation conditions are as described in **Fig. S4. a.** Formation of PEX from M3 in PBS with/without NADPH. **b.** Formation of PEX from M3 in CYP3A with NADPH. **c.** Representative chromatograms of PEX formed from M3 in PBS with/without NADPH. **d.** Proposed mechanism of the inter-conversation of M3 and PEX. M3 can add a proton and lose water to form carbocation **A** and the resonance stabilized **B**. $^{-}$H donated by NADPH can react with A or B to form PEX. Data points indicate the mean ± s.e.m. (n = 3).

Figure S12. Metabolic fate of aldehyde M24 in HLM or CYP3A4.



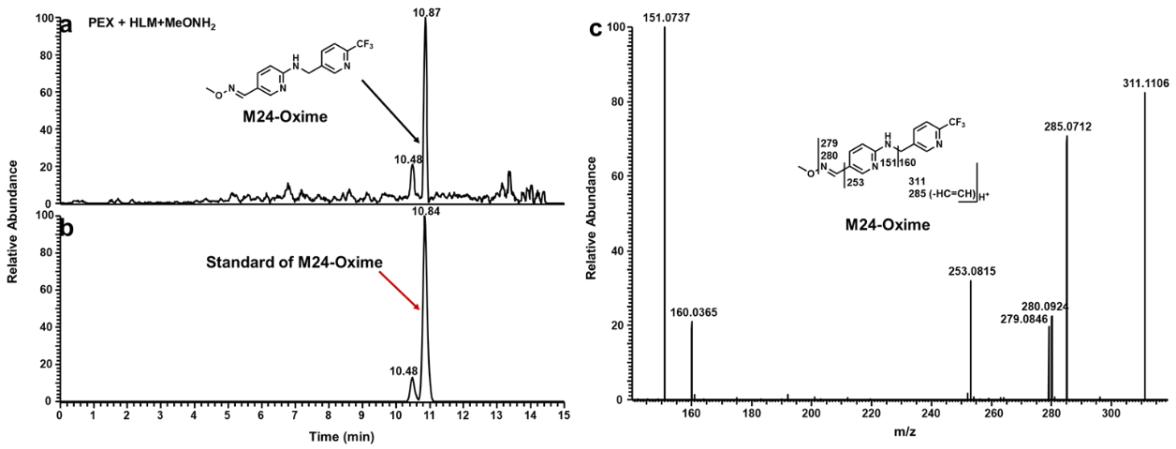
Incubations were conducted in 1 × PBS (pH 7.4), containing 30 μM aldehyde M24, 0.1 mg HLM or 1 pmol of CYP3A4, in a final volume of 95 μl. After 5 min of pre-incubation at 37 ˚C, the reaction was initiated by adding 5 μl of 20 mM NADPH (final concentration 1.0 mM) and continued for 40 min with gentle shaking. Incubations without NADPH were used as controls. Reactions were terminated by adding 100 μl of ice-cold acetonitrile and vortexing for 30 seconds, and then centrifuged at rcf 15,000 for 15 min at 4 °C. The relative abundance of metabolites was presented by the peak area. **a.** The formation of M25, M26, and M28 in HLM. **b.** The formation of M25, M26, and M28 by CYP3A4. **c.** Metabolic map of M24 in HLM and CYP3A4.

Figure S13. Metabolic fate of alcohol M3 in HLM or with CYP3A4.



Incubation conditions enriched with $H_2{}^{18}O$ and/or $^{18}O_2$ and sample analysis were as described in Fig. **S10**. The peak area was normalized by the largest one (set as 100%) in any of 4 groups (control, $H_2{}^{18}O$, $^{18}O_2$, or $H_2{}^{18}O$ + $^{18}O_2$). For aldehyde M24 trapping, incubations were conducted in 1 × PBS (pH 7.4) containing 30 µM PEX, 0.1 mg HLM, 2.5 mM methoxyamine in a final volume of 95 µl. After 5 min of pre-incubation at 37 °C, the reaction was initiated by adding 5 µl of 20 mM NADPH (final concentration 1.0 mM) and continued for 40 min with gentle shaking. Incubations without methoxyamine were used as controls. Reactions were terminated by adding 100 µl of ice-cold acetonitrile and vortexing for 30 seconds, and then centrifuged at rcf 15,000 for 15 min at 4 °C. The relative abundances of the metabolite were calculated based on the peak area. The overall abundance of metabolites M26 and M28 were set up as 100% in control groups and M24-Oxime was set as 100% in the group with methoxyamine. **a.** Trapping aldehyde M24 with methoxyamine. **b.** Formation of M24-^{16}O and M24-^{18}O from PEX by CYP3A4. **c.** Trapping M24 as the oxime using methoxyamine decreases the formation of acid M26 but has no significant effect on the formation of M28. The data are expressed as mean ± s.e.m (n = 3). **p < 0.01. M24-oxime is identified in Fig. **S14**.

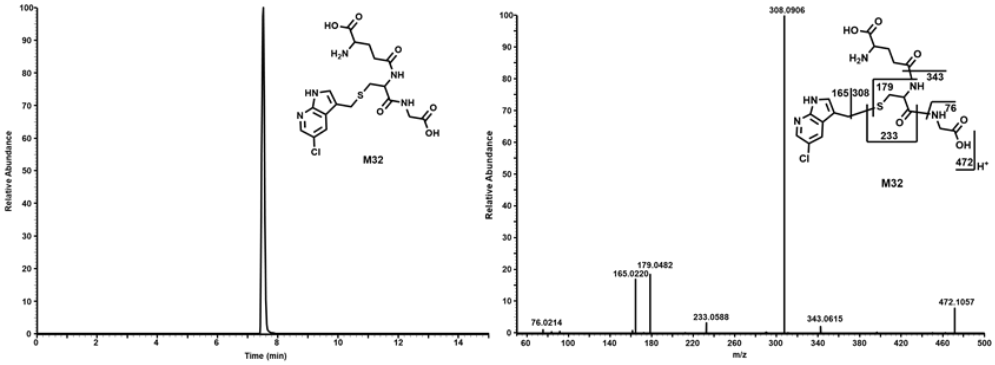
Figure S14. Identification of M24-Oxime



Incubation conditions with methoxyamine were as described in Fig. **S13**. Structural elucidations were performed based on accurate mass (mass errors less than 5 ppm), MS/MS fragmentations and standard compound. MS/MS was performed with collision energy ramping from 10, 20, 35 eV. The major fragmental ions are interpreted in the inlaid structural diagrams. Extracted ion chromatograms for M24-oxime from HLM (**a**) or for the synthetic standard (**b**). **c.** MS/MS of M24-Oxime. Synthesis of the M24-Oxime standard was performed by mixing the aldehyde M24 (final 10 µM) and methoxyamine (final 10 µM) in methanol/water (v/v 1:1).

M24-oxime eluted at 10.87 min and was detected as a protonated molecule at m/z 311.1106 (Figs. **S14a, b** and **c**). MS/MS of M24-oxime produced major fragmental ions at m/z 285, 280, 279, 253, 160 and 151 (Fig. **S14c**). The fragmental ions are interpreted in the inlaid structural diagram (Fig. **S14c**).

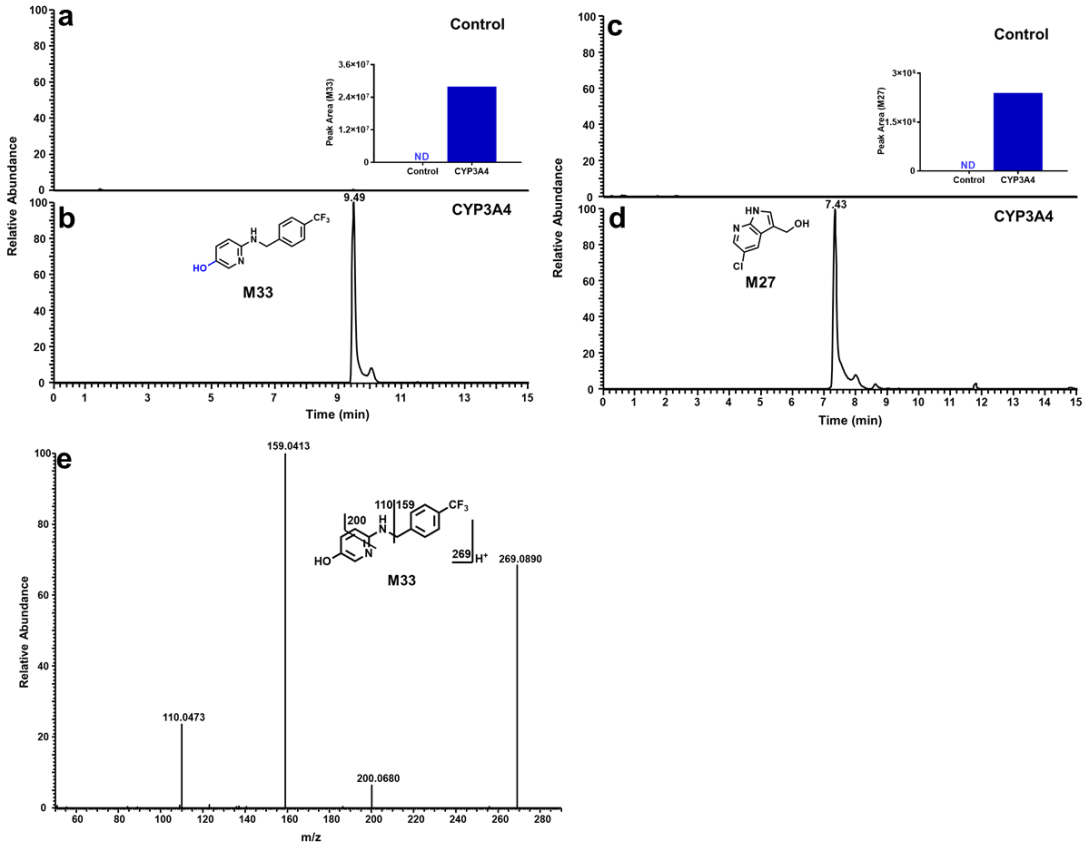
Figure S15. Identification of M32



Incubations were conducted in 1× PBS (pH 7.4) containing 30 μM PEX, 0.1 mg HLM, 2.5 mM GSH in a final volume of 95 μl. After a 5 min pre-incubation at 37 °C, 5 μl of 20 mM NADPH was added (final concentration 1.0 mM) and incubation was continued for 40 min with gentle shaking. Reactions were terminated by adding 100 μl of ice-cold acetonitrile. Samples were analyzed using UHPLC-Q Exactive MS. Incubations were performed in duplicate.

M32 eluted at 7.56 min and was detected as a protonated molecule at m/z 472.1057 (Table **S1** and Fig. **S15a**). MS/MS of M32 produced major fragmental ions at m/z 343, 308, 233, 179, and 165 (Fig. **S15b**). The fragmental ions are interpreted in the inlaid structural diagram (Fig. **S15b**).

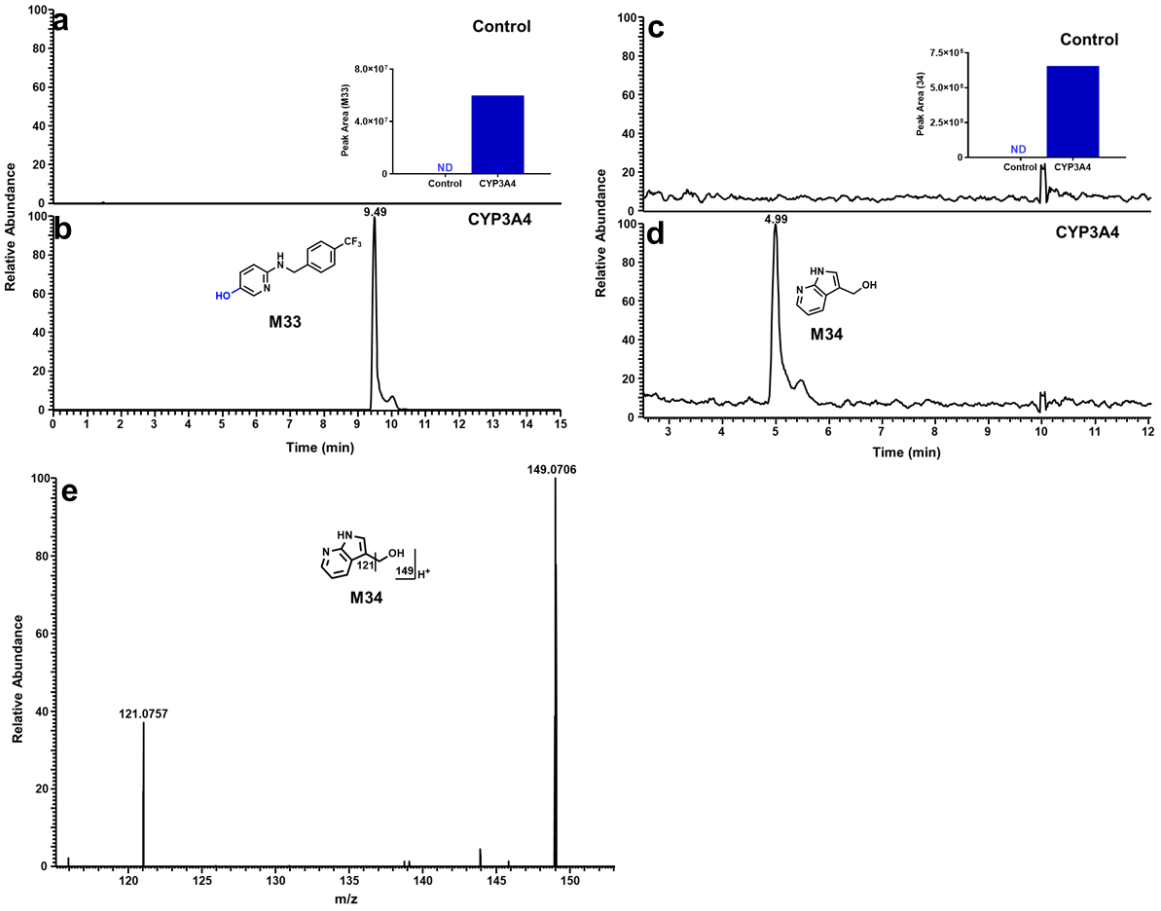
Figure S16. Identification of M33 generated from FLT3-IN-2 by CYP3A4.



Incubations were conducted in 1× PBS (pH 7.4) containing 30 µM FLT3-IN-2 and 1 pmol of CYP3A4, in a final volume of 95 µl. After 5 min of pre-incubation at 37 °C, the reaction was initiated by adding 5 µl of 20 mM NADPH (final concentration 1.0 mM) and continued for 40 min with gentle shaking. Incubations without NADPH were used as controls. Reactions were terminated by adding 100 µl of ice-cold acetonitrile and vortexing for 30 seconds, and then centrifuged at rcf 15,000 for 15 min at 4 °C. The relative abundance of metabolites was based on the peak area. The experiment was performed in duplicates and the data were expressed as a mean (inlaid in the figures). **a.** and **b.** The formation of M33 without and with NADPH. **c.** and **d.** The formation of M27 in CYP3A4 without and with NADPH. **e.** MS/MS of M33. MS/MS was performed with collision energy ramping from 10, 20, 35 eV. ND, not detected.

M33 eluted at 9.49 min and was detected as a protonated molecule at m/z 269.0890 (Figs. **S16a** and **S16b**). MS/MS of M33 produced major fragmental ions at m/z 200, 159, and 110 (Fig. **S16e**). The fragmental ions are interpreted in the inlaid structural diagram (Fig. **S16e**).

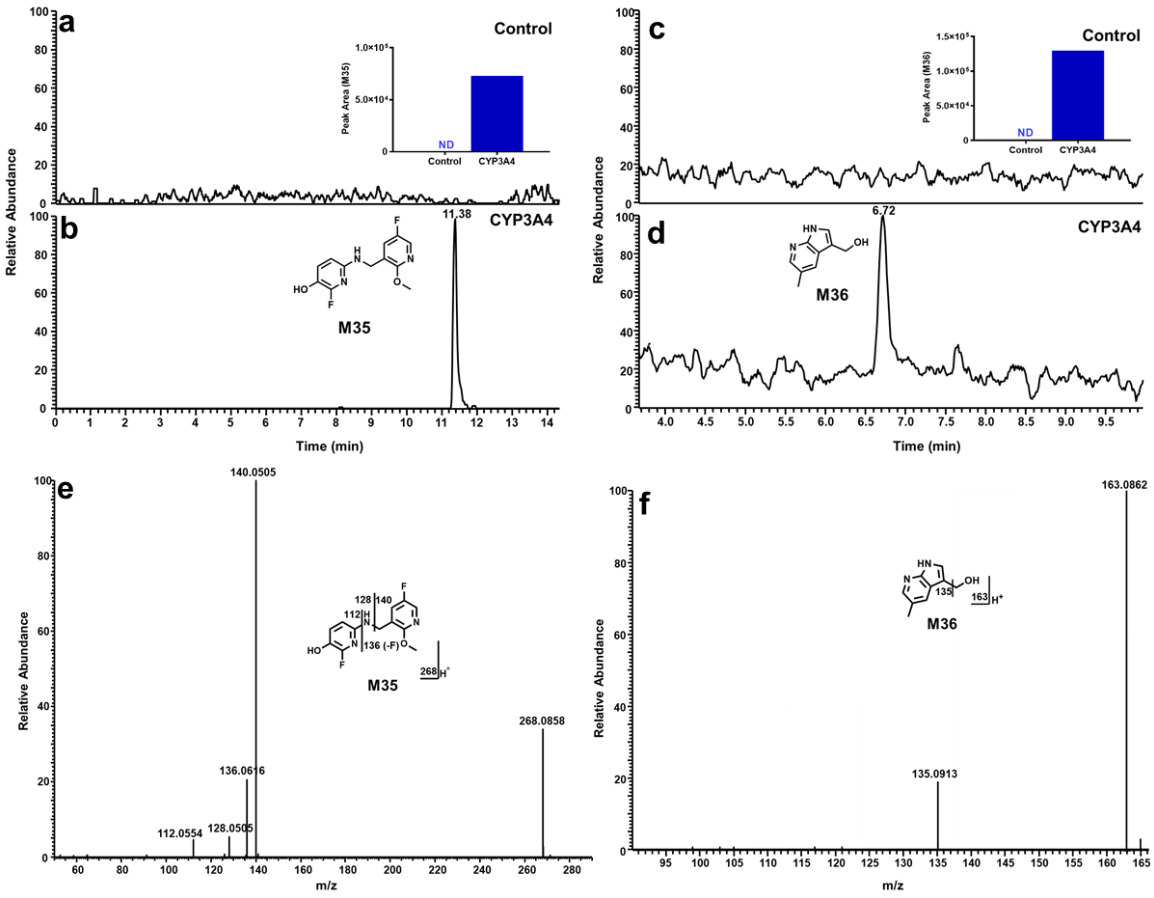
Figure S17. Identification of M33 and M34 generated from PLX-647 by CYP3A4.



The incubation conditions of PLX-647 in CYP3A4 were as described in Fig. **S16**. The relative abundance of metabolites was based on the peak area. The experiment was performed in duplicates and the data were expressed as a mean (inlaid in the Figures). **a.** and **b.** The formation of M33 without and with NADPH. **c.** and **d.** The formation of M34 without and with NADPH. **e.** MS/MS of M34. MS/MS was performed with collision energy ramping from 10, 20, 35 eV.

M34 eluted at 4.99 min and was detected as a protonated molecule at m/z 149.0706 (Fig. **S17d**). MS/MS of M34 produced major fragmental ions at m/z 121 (Fig. **S17e**). The fragmental ions are interpreted in the inlaid structural diagram (Fig. **S17e**)

Figure S18. Identification of M35 and M36 generated from PLX-5622 by CYP3A4.



The incubation conditions of PLX-5622 in CYP3A4 were as described in Fig. **S16**. The relative abundance of metabolites was based on the peak area. The experiment was performed in duplicate and the data were expressed as a mean (inlaid in the top panels). **a.** and **b.** The formation of M35 without and with NADPH. **c.** and **d.** The formation of M36 without and with NADPH. **e.** MS/MS of M35. **f.** MS/MS of M36. MS/MS was performed with collision energy ramping from 10, 20, 35 eV.

M35 eluted at 11.38 min and was detected as a protonated molecule at m/z 268.0858 (Figs. **S18b** and **S18e**). MS/MS of M35 produced major fragmental ions at m/z 112, 128, 136 and 140 (S. Fig. **18e**). The fragmental ions are interpreted in the inlaid structural diagram (Fig. **S18e**).

M36 eluted at 6.72 min and was detected as a protonated molecule at m/z 268.0853 (Figs. **S18d** and **S18f**). MS/MS of M35 produced major fragmental ions at m/z 135 and 163 (Fig. **S18f**). The fragmental ions are interpreted in the inlaid structural diagram (Fig. **S18f**).

IV. Supplementary Tables

Table S1. Summary of PEX metabolites detected in HLM.

RT (min)	Observed *m/z* $[M+H]^+$	Calculated *m/z* $[M+H]^+$	Mass error (ppm)	Predicted molecular formula	Identification	Metabolite ID	Source
10.96	418.1038	418.1036	0.48	$C_{20}H_{15}ClF_3N_5$	Pexidartinib	PEX	HLM
8.98	434.0991	434.0990	0.23	$C_{20}H_{15}ClF_3N_5$	PEX+O	M1	HLM
9.68	434.0991	434.0990	0.23	$C_{20}H_{15}ClF_3N_5O$	PEX+O	M2	HLM
9.84	434.0990	434.0990	0.00	$C_{20}H_{15}ClF_3N_5O$	PEX+O	M3	HLM
10.73	434.0991	434.0990	0.23	$C_{20}H_{15}ClF_3N_5O$	PEX+O	M4	HLM
11.57	434.0991	434.0990	0.23	$C_{20}H_{15}ClF_3N_5O$	PEX+O	M5	HLM
8.25	436.1151	436.1149	0.46	$C_{20}H_{17}ClF_3N_5O$	PEX+O+2H	M6	HLM
12.13	432.0837	432.0837	0.00	$C_{20}H_{13}ClF_3N_5$	PEX+O-2H	M7	HLM
8.99	450.0940	450.0940	0.00	$C_{20}H_{15}ClF_3N_5O_2$	PEX+2O	M8	HLM
10.59	450.0940	450.0940	0.00	$C_{20}H_{15}ClF_3N_5O_2$	PEX+2O	M9	HLM
10.97	450.0942	450.0940	0.44	$C_{20}H_{15}ClF_3N_5O_2$	PEX+2O	M10	HLM
8.60	452.1096	452.1097	-0.22	$C_{20}H_{17}ClF_3N_5O_2$	PEX+2O+2H	M11	HLM
7.63	259.0745	259.0744	0.39	$C_{13}H_{11}ClN_4$	Aniline	M12	HLM
4.75	275.0692	275.0693	-0.36	$C_{13}H_{11}ClN_4O$	Aniline+O	M13	HLM
5.28	275.0693	275.0693	0.00	$C_{13}H_{11}ClN_4O$	Aniline+O	M14	HLM
7.11	275.0693	275.0693	0.00	$C_{13}H_{11}ClN_4O$	Aniline+O	M15	HLM
7.34	275.0692	275.0693	-0.36	$C_{13}H_{11}ClN_4O$	Aniline+O	M16	HLM
3.85	291.0643	291.0641	0.69	$C_{13}H_{11}ClN_4O_2$	Aniline+2O	M17	HLM
4.64	291.0643	291.0641	0.69	$C_{13}H_{11}ClN_4O_2$	Aniline+2O	M18	HLM
5.17	291.0642	291.0641	0.34	$C_{13}H_{11}ClN_4O_2$	Aniline+2O	M19	HLM
6.00	291.0643	291.0641	0.69	$C_{13}H_{11}ClN_4O_2$	Aniline+2O	M20	HLM
7.57	291.0643	291.0641	0.69	$C_{13}H_{11}ClN_4O_2$	Aniline+2O	M21	HLM
7.26	178.0475	178.0474	0.56	$C_7H_6F_3NO$	Alcohol	M22	HLM
9.08	192.0267	192.0268	-0.52	$C_7H_4F_3NO_2$	Acid	M23	HLM
10.72	282.0848	282.0849	-0.35	$C_{13}H_{10}F_3N_3O$	Aldehyde	M24	HLM
5.61	284.1005	284.1005	0.0	$C_{13}H_{12}F_3N_3O$	Alcohol	M25	HLM
8.01	298.0797	298.0800	-1.01	$C_{13}H_{10}F_3N_3O_2$	Acid	M26	HLM
7.41	183.0321	183.0320	0.55	$C_8H_7ClN_2O$	Alcohol	M27	HLM

6.38	270.0848	270.0849	-0.37	$C_{12}H_{10}F_3N_3O$	Phenol	M28	HLM
12.19	448.0785	448.0783	0.45	$C_{20}H_{13}ClF_3N_5O_2$	Ester	M29	HLM
8.64	197.0113	197.0112	0.51	$C_8H_5ClN_2O_2$	Acid	M30	HLM
6.59	169.0164	169.0163	0.59	$C_7H_5ClN_2O$	Alcohol	M31	HLM
7.56	472.1055	472.1052	0.63	$C_{18}H_{22}ClN_5O_6S$	GSH-adduct	M32	HLM+GSH

PEX, pexidartinib; +O, monooxygenation; +2O, dioxygenation; +O-2H, monooxygenation + dehydrogenation; +O+2H, monooxygenation + hydrogenation; +2O+2H, dioxygenation + dihydrogenation; HLM, human liver microsomes; GSH, glutathione; CPP, 5-chloropyrrolopyridine.

Table S2. P450s involved in the formation of PEX metabolites (as a percentage of the most abundant ion count).

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	45.7	93.7	0.0	0.0	0.0	0.0	0.0	0.0
CYP1A2	6.7	1.0	2.6	0.0	0.7	0.5	19.1	4.1	42.9	93.2	1.4	7.6	4.6	2.5	0.0	1.1
CYP2A6	0.5	0.0	10.2	0.0	0.0	0.0	60.5	0.0	38.6	89.6	0.0	0.0	0.0	5.1	0.0	0.1
CYP2B6	0.8	0.0	0.7	0.0	0.0	0.0	3.7	0.7	37.1	90.3	0.0	0.0	0.0	0.5	0.0	0.0
CYP2C8	0.3	0.0	0.0	1.4	0.3	0.0	0.0	0.0	36.8	94.4	0.0	0.0	0.0	0.0	0.0	0.2
CYP2C9	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	37.4	93.2	0.0	0.0	0.0	0.0	0.0	0.0
CYP2C19	0.0	1.3	2.3	2.4	0.6	0.0	1.5	0.0	46.4	100.0	0.5	0.0	0.0	0.0	0.0	0.0
CYP2D6	100.0	81.4	15.5	4.3	3.6	7.7	11.9	53.9	40.5	91.5	13.2	3.5	3.2	1.0	0.0	2.5
CYP2E1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	34.9	93.1	0.0	0.0	0.5	0.0	0.0	0.0
CYP3A4	33.6	72.4	68.6	82.3	36.3	74.2	100.0	100.0	100.0	86.5	99.0	24.6	47.8	64.9	19.1	27.1
CYP3A5	33.6	100.0	100.0	100.0	100.0	100.0	41.8	87.7	91.0	88.1	100.0	100.0	100.0	100.0	100.0	100.0

	M17	M18	M19	M20	M21	M22	M23	M24	M25	M26	M27	M28	M29	M30	M31	M32
Control	0.0	0.0	0.0	0.0	11.4	1.2	0.0	9.2	21.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP1A2	0.0	0.0	0.0	0.0	19.4	6.3	0.0	17.7	21.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP2A6	0.0	0.0	0.0	0.0	11.5	1.4	0.0	44.1	37.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP2B6	0.0	0.0	0.0	0.0	11.1	1.2	4.5	9.6	11.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP2C8	0.0	0.0	0.0	0.0	12.1	1.1	7.4	11.9	16.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP2C9	0.0	0.0	0.0	0.0	11.9	0.9	5.7	7.9	15.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP2C19	0.0	0.0	0.0	0.0	12.9	1.2	10.2	11.3	16.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP2D6	0.0	0.0	0.0	0.0	15.8	2.5	11.5	25.5	20.2	0.0	1.5	2.1	0.0	0.0	0.0	0.0
CYP2E1	0.0	0.0	0.0	0.0	10.4	1.1	9.6	11.4	14.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP3A4	92.9	29.5	41.9	100.0	37.1	30.4	50.9	100.0	100.0	0.0	100.0	100.0	58.8	0.0	0.0	100.0
CYP3A5	100.0	100.0	100.0	37.2	100.0	100.0	100.0	75.9	48.0	0.0	34.9	38.5	100.0	0.0	0.0	46.0

Table S3. P450 production of aldehyde M24 from alcohol M3 (present in percentage).

	M24
Control	49.2
CYP1A2	49.6
CYP2A6	52.1
CYP2A13	63.1
CYP2B6	56.7
CYP2C8	55.9
CYP2C9	49.7
CYP2C19	54.8
CYP2D6	60.1
CYP2E1	52.2
CYP3A4	95.8
CYP3A5	100.0

M24 is also detected in the M3 aqueous stock solution after sitting at room temperature.

Table S4. P450 production of acid M30 from alcohol M27 (present in percentage).

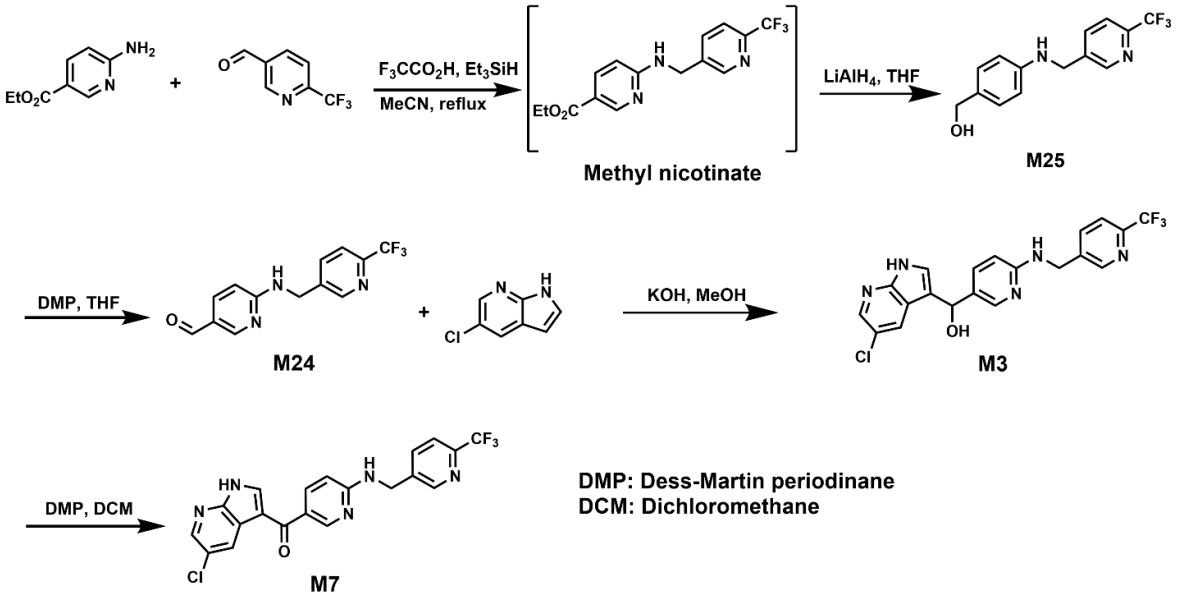
	M30
Control	0.2
CYP1A2	100.0
CYP2A6	12.3
CYP2B6	0.4
CYP2C8	1.0
CYP2C9	2.6
CYP2C19	19.2
CYP2D6	12.0
CYP2E1	0.4
CYP3A4	5.8
CYP3A5	1.6

Incubations were conducted in 1× PBS (pH 7.4) containing 30 μM PEX, M3 or M27, and 1 pmol of each cDNA-expressed P450 enzyme (control, CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5) in a final volume of 95 μl. After a 5 min pre-incubation at 37 °C, 5 μl of 20 mM NADPH was added (final concentration 1.0 mM) and incubation was continued for 40 min with gentle shaking. Reactions were terminated by adding 100 μl of ice-cold acetonitrile and vortexing for 30 seconds. Samples was injected on to UHPLC-Q Exactive MS system for

analysis. The largest peak area of each metabolite from CYP enzymes was set as 100%. All data are expressed as mean (n = 2).

V. Supplementary Schemes

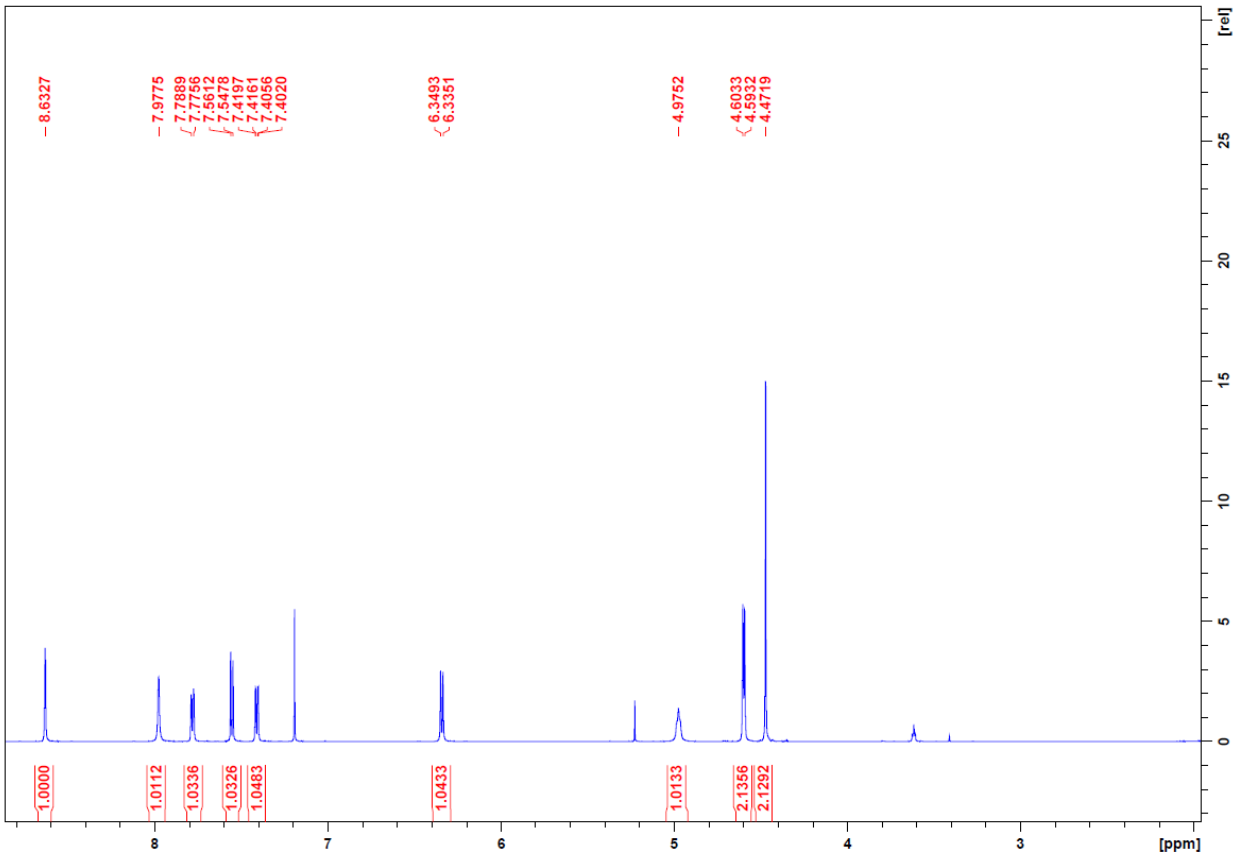
Scheme S1. Synthesis of M3, M7, M24, and M25



Synthesis of methyl 6-(((6-(trifluoromethyl)pyridin-3-yl)methyl)amino)nicotinate: To methyl 6-aminonicotinate (46 mg, 0.3 mmol) in acetonitrile (1.5 ml), 6-trifluoromethyl-pyridine-3-carbaldehyde (55 mg, 0.31 mmol), trifluoroacetic acid (115 µl, 1.5 mmol) and triethylsilane (240 µl, 1.5 mmol) were added. The reaction mixture was refluxed for 4 hours. The reaction was concentrated, poured into aqueous potassium carbonate, and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated and purified with silica gel column chromatography to give methyl nicotinate as a semi-solid (75 mg, 80%).

Synthesis of (4-(((6-(trifluoromethyl)pyridin-3-yl)methyl)amino)phenyl)methanol (M25): To a solution of ethyl nicotinate (130 mg, 0.4 mmol) in dry THF (3 ml) was added $LiAlH_4$ solution (1 M in THF) at 0 ºC. After stirring at room temperature for 2 hours, the reaction solution was carefully quenched at 0 °C with MeOH and HCl (1 M) until pH = 9. The mixture solution was extracted with CH_2Cl_2 (DCM) 3 times and the combined organic layers were concentrated and dried under high vacuum to give the crude alcohol product, which was purified by chromatography. The yield is 80%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.63 (s, 1H), 7.98 (s, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 6.34 (d, J = 8.4 Hz, 1H), 4.98 (s, 1H), 4.59 (d, J = 6.0 Hz, 2H), 4.47 (s, 2H).

^{1}H NMR spectrum of M25:

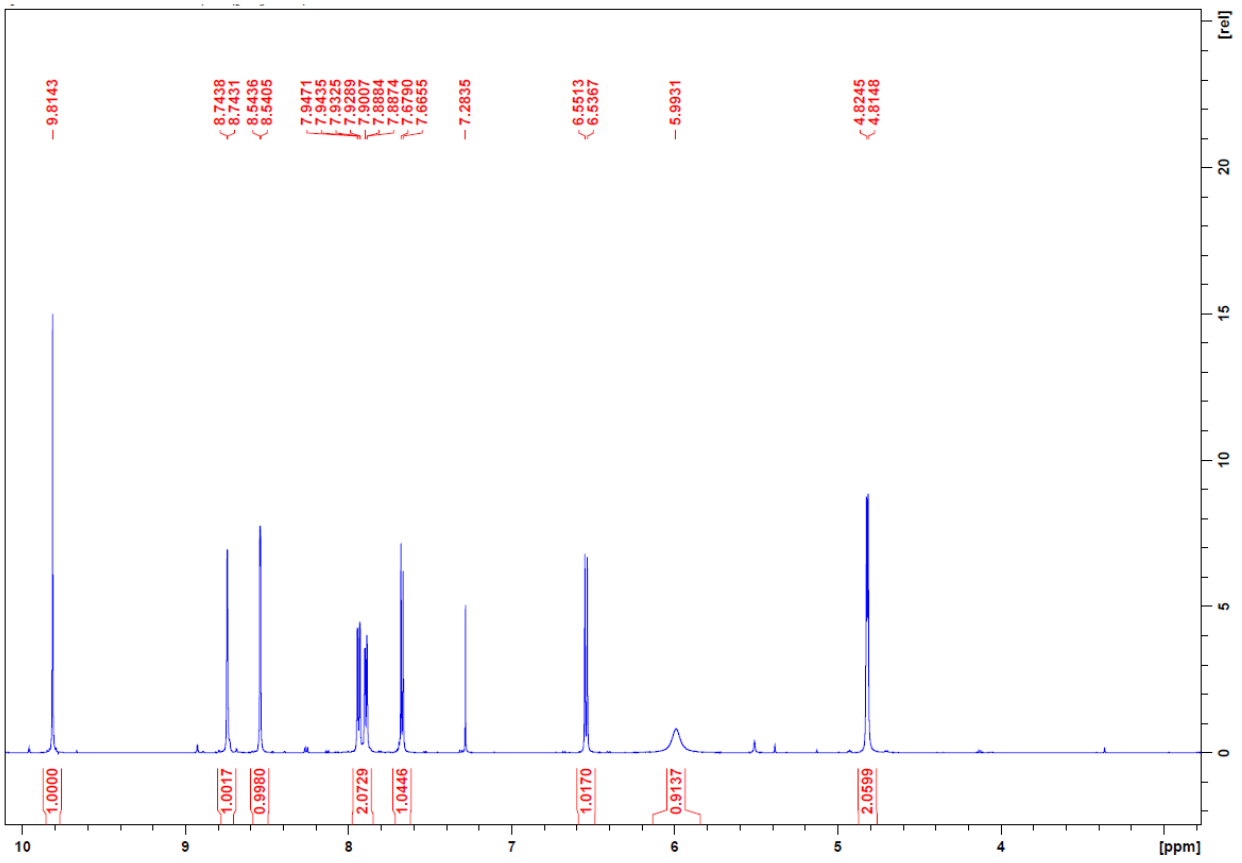


Synthesis of 6-(((6-(trifluoromethyl)pyridin-3-yl)methyl)amino)nicotinaldehyde (M24)

M25 (85 mg, 0.3 mmol) was dissolved in DCM (3 ml), and Dess–Martin periodinane (168 mg, 0.4 mmol) was added at room temperature. After stirring for 2 hours, the reaction was quenched by saturated $Na_2S_2O_3$ and $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted 2 times. The combined organic layers were concentrated and the crude product was separated by chromatography to give the desired product M24 (63 mg, 75% yield).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 9.81 (s, 1H), 8.74 (d, J = 0.4 Hz, 1H), 8.54 (d, J = 1.8 Hz, 1H), 7.94 (d, J = 3.0 Hz, 1H), 7.89 (d, J = 3.0 Hz, 1H), 7.67(d, J = 8.4 Hz, 1H), 6.54 (d, J = 9.0 Hz, 1H), 5.99 (s, 1H), 4.82 (d, J = 6.0 Hz, 2H).

^{1}H NMR spectrum of M24

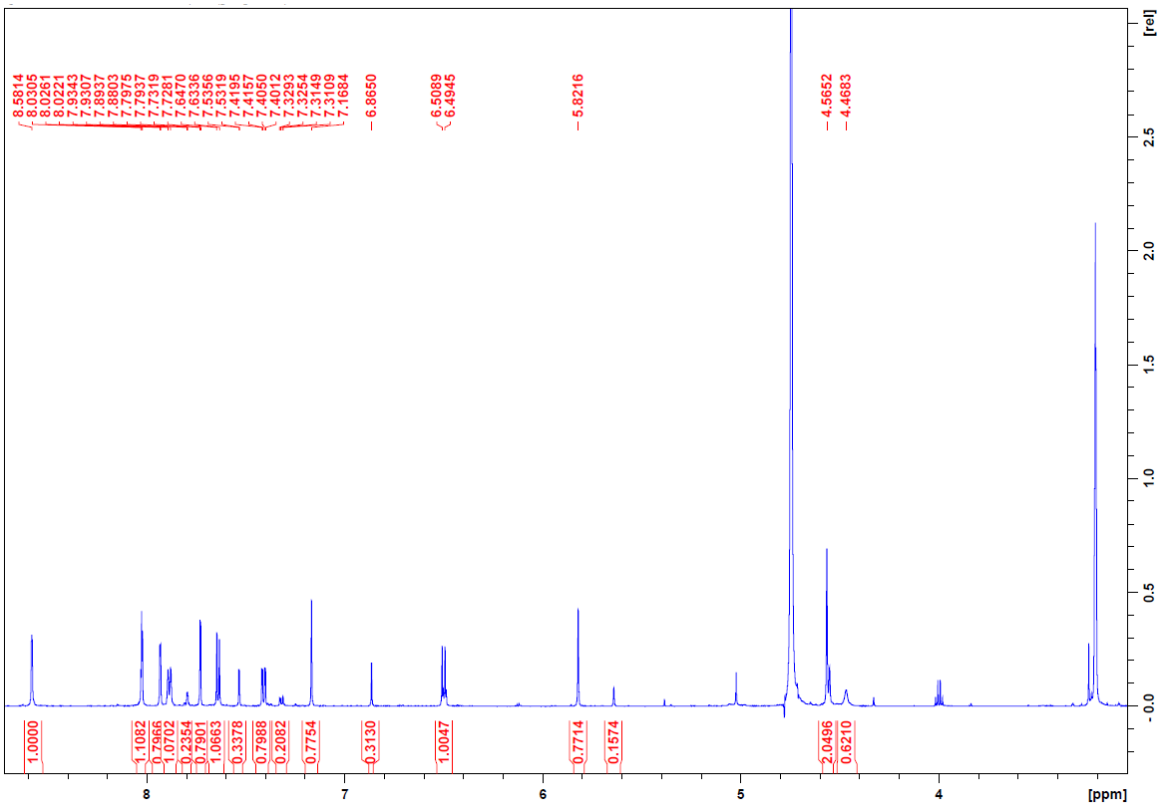


Synthesis of (5-chloro-1H-pyrrolo[2,3-b]pyridin-3-yl)(6-(((6-(trifluoromethyl)pyridin-3-yl)methyl)amino)pyridin-3-yl)methanol (M3):

To a solution of 5-chloro-1H-pyrrolo[2,3-b]pyridine (30 mg, 0.2 mmol) in dry MeOH (3 ml) was added KOH (34 mg, 0.6 mmol) at 0 ºC, then after 30 min of stirring at room temperature, a solution of **M24** (84 mg, 0.3 mmol) in dry MeOH (1 ml) was added slowly into the reaction solution. After reacting overnight, the solution was quenched by adding 1M HCl until pH = 9. After concentration and chromatography separation, the desired compound **M3** (26 mg, 30%) was obtained.

^{1}H NMR (600 MHz, CD_3OD): δ = 8.58 (s, 1H), 8.03 (s, 1H), 7.93 (s, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.73 (s, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.42 (d, J = 6.0 Hz, 1H), 7.17 (s, 1H), 6.50 (d, J = 8.4 Hz, 1H), 5.82 (s, 1H), 4.57 (s, 2H), 4.47 (s, 1H).

^{1}H NMR spectrum of M3

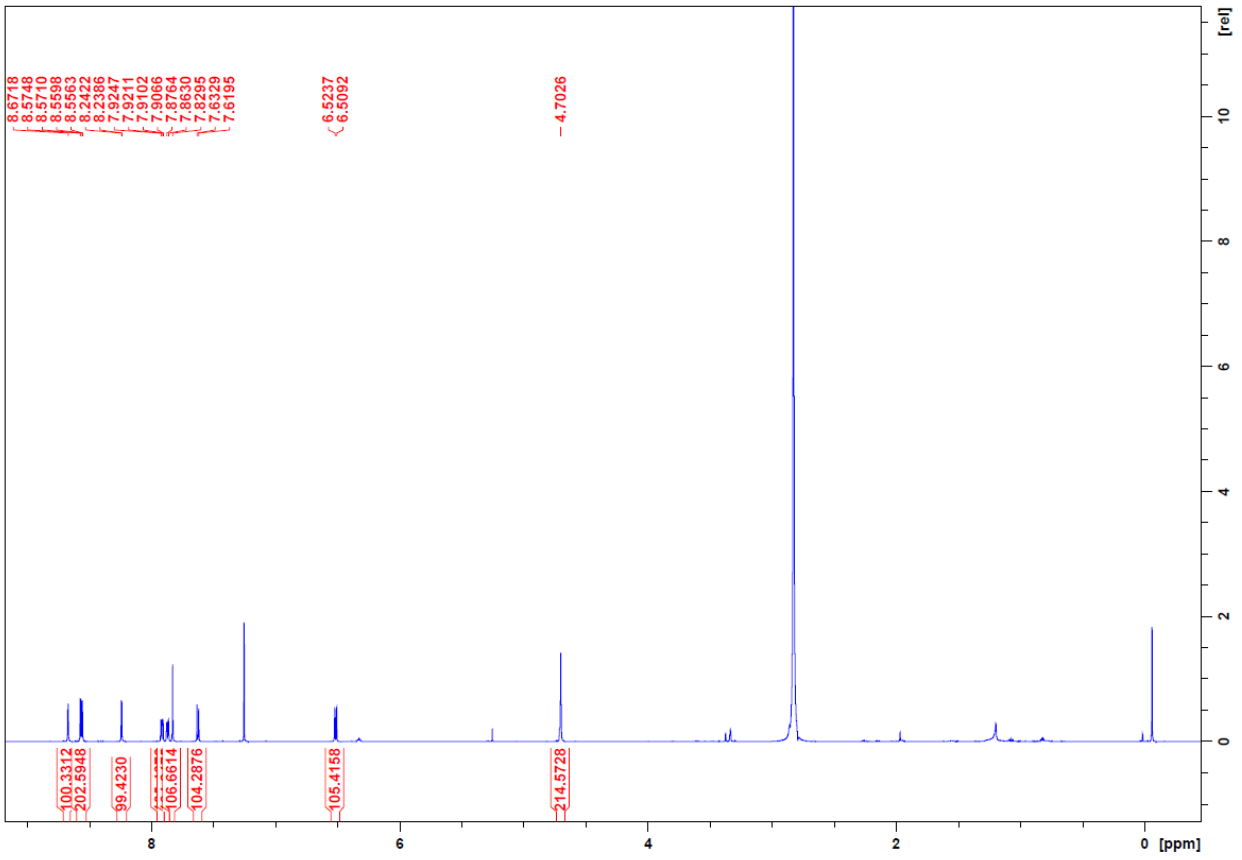


Synthesis of (5-chloro-1H-pyrrolo[2,3-b]pyridin-3-yl)(6-(((6-(trifluoromethyl)pyridin-3-yl)methyl)amino)pyridin-3-yl)methanone (M7):

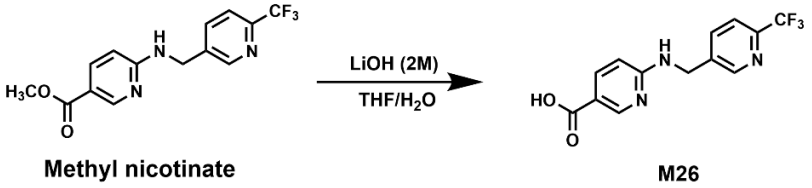
To a solution of **M3** (21 mg, 0.05 mmol) in DCM (0.5ml) was added Dess–Martin periodinane (42 mg, 0.1 mmol). After 1 hour, the reaction was quenched by saturated $Na_2S_2O_3$ and $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted 2 times. The combined organic layers were concentrated and the crude product was separated by chromatography to give the desired product **M7** (15 mg, 75% yield).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.67 (s, 1H), 8.57 (d, J = 6.0 Hz, 1H), 8.24 (s, 1H), 7.92 (d, J = 6.6 Hz, 1H), 7.88 (d, J = 6.6 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 6.51(d, J = 8.4 Hz, 1H), 4.70 (s, 2H).

^{1}H NMR spectrum of M7



Scheme S2. Synthesis of M26

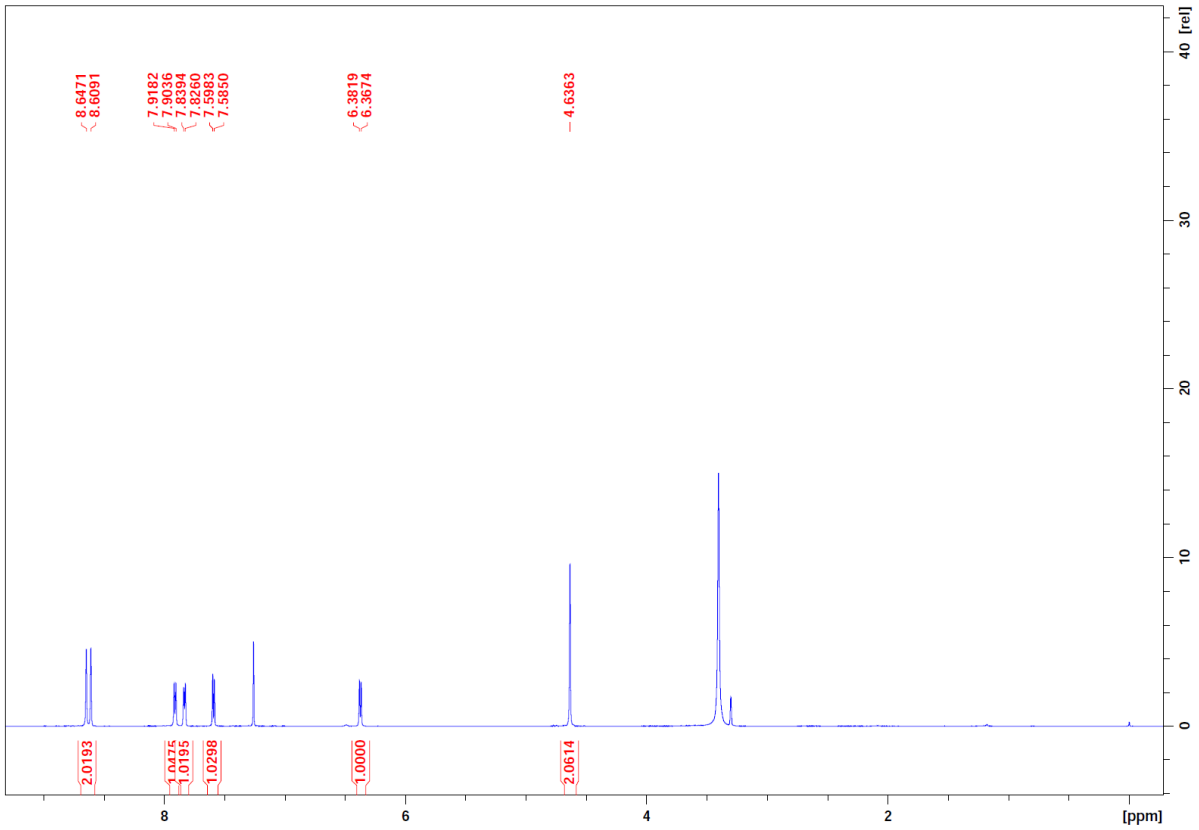


Methyl nicotinate M26

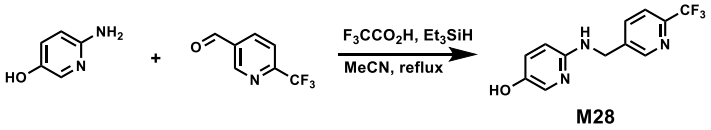
Synthesis of 6-(((6-(trifluoromethyl)pyridin-3-yl)methyl)amino)nicotinic acid (M26): To a solution of methyl 6-(((6-(trifluoromethyl)pyridin-3-yl)methyl)amino)nicotinate (31 mg), 0.1 mmol) in 1 ml THF/H_2O (4:1), was added LiOH (2 M in H_2O, 0.15 ml) dropwise at room temperature. After 2 hours, the solution was acidified to pH = 5, and reverse chromatography was used to separate the product (white solid, 24 mg, 80% yield).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.65 (s, 1H), 8.61 (s, 1H), 7.90 (d, J = 8.7 Hz, 1H), 7.83 (d, J= 8.1 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 6.37 (d, J = 8.7 Hz, 1H), 4.64 (s, 2H).

^{1}H NMR Spectrum of M26:



Scheme S3. Synthesis of M28

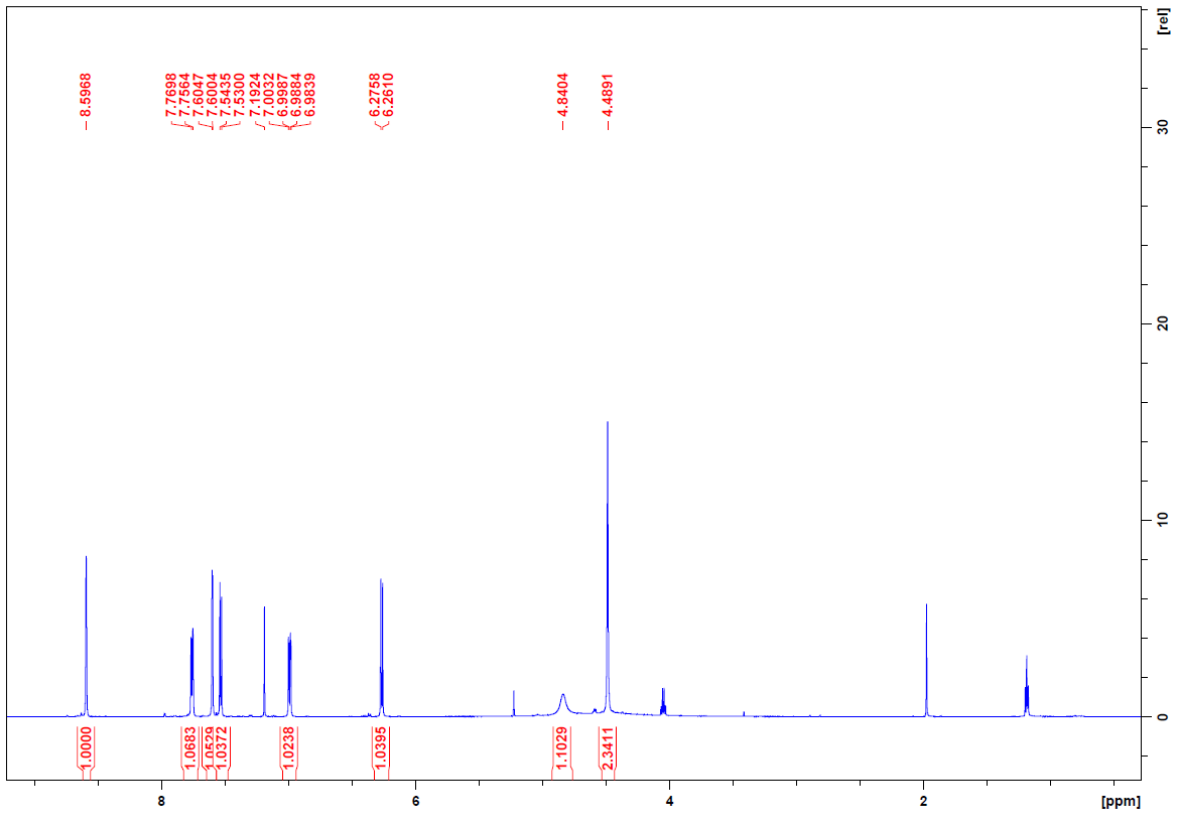


M28

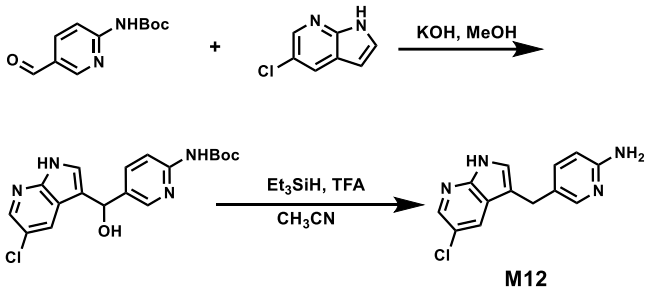
Synthesis of 6-(((6-(trifluoromethyl)pyridin-3-yl)methyl)amino)pyridin-3-ol (M28): To 6-aminopyridin-3-ol (33 mg, 0.3 mmol) in acetonitrile (1.5 mL), 6-trifluoromethyl-pyridine-3-carbaldehyde (55 mg, 0.31 mmol), trifluoroacetic acid (115 µL, 1.5 mmol) and triethylsilane (240 µL, 1.5 mmol) were added. The reaction was heated to reflux for 4 hours. The reaction was concentrated, poured into aqueous potassium carbonate, and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, filtered, concentrated and purified with silica gel column chromatography to give a semi-solid product M28 (63 mg, 78% yield). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.60 (s, 1H), 7.76 (d, J = 8.1H z, 1H), 7.60 (s, d, J = 2.7 Hz, 1H 1H), 7.53 (d, J = 8.1 Hz, 1H), 6.99 (dd, J = 8.9, 2.7 Hz, 1H), 6.26 (d, J = 8.9 Hz, 1H), 4.84 (s, 1H), 4.49 (s, 2H).

Ref. 35. Zhang, C., et al. Compounds modulating c-fms and/or c-kit activity and uses therefor, PCT Int. Appl., 2008064265, **2008**

^{1}H NMR spectrum of M28:



Scheme S4. Synthesis of M12



DMP: Dess-Martin periodinane

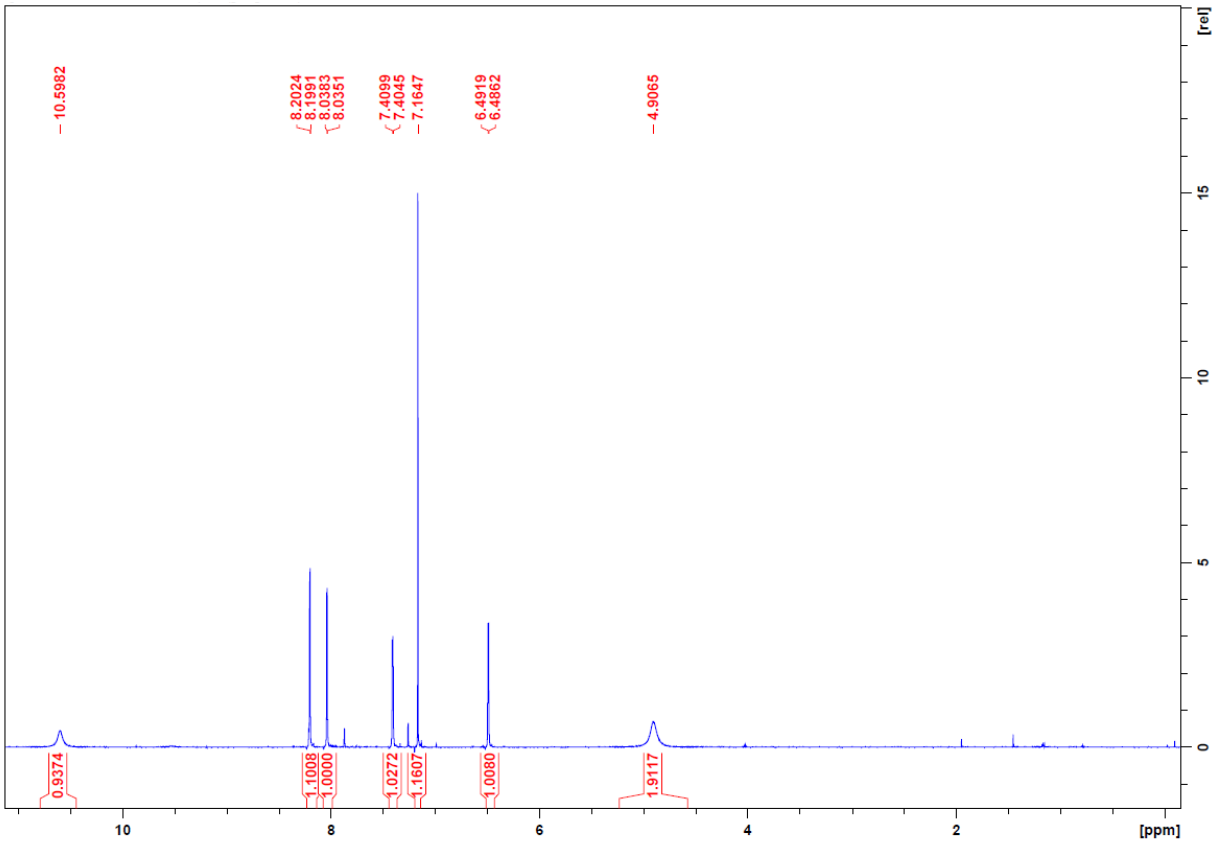
Synthesis of 5-((5-chloro-1H-pyrrolo[2,3-b]pyridin-3-yl)methyl)pyridin-2-amine (M12):

To a solution of 5-chloro-1H-pyrrolo[2,3-b]pyridine (30 mg, 0.2 mmol) in dry MeOH (3 ml) was added KOH (34 mg, 0.6 mmol) at 0 °C, then after 30 mins of stirring at room temperature, a solution of tert-butyl (5-formylpyridin-2-yl)carbamate (66 mg, 0.3 mmol) in dry MeOH (1 ml) was added slowly into the reaction solution. After reacting overnight, the solution was quenched by adding 1M HCl until pH = 9. After concentration and chromatography separation, the N-Boc protected alcohol (26 mg, 35% yield) was obtained. Subsequently, to the solution of the

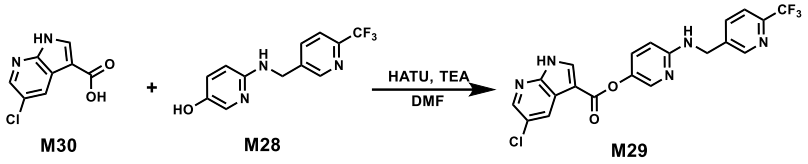
secondary alcohol (19 mg, 0.038mmol) in CH_3CN (1.2 ml), TFA (102 µL) and triethylsilane (219 µl) were added slowly. The reaction solution was refluxed for 3 hours, and after cooled to room temperature the solution was concentrated and separated by chromatography to yield the desired product **M12** (11 mg, 60% yield).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 10.60 (s, 1H), 8.20 (s, 1H), 8.04 (s, 1H), 7.41 (s, 1H), 7.16 (s, 1H), 6.49 (s, 1H), 4.91 (s, 2H).

^{1}H NMR spectrum of M12:



Scheme S5. Synthesis of ester M29

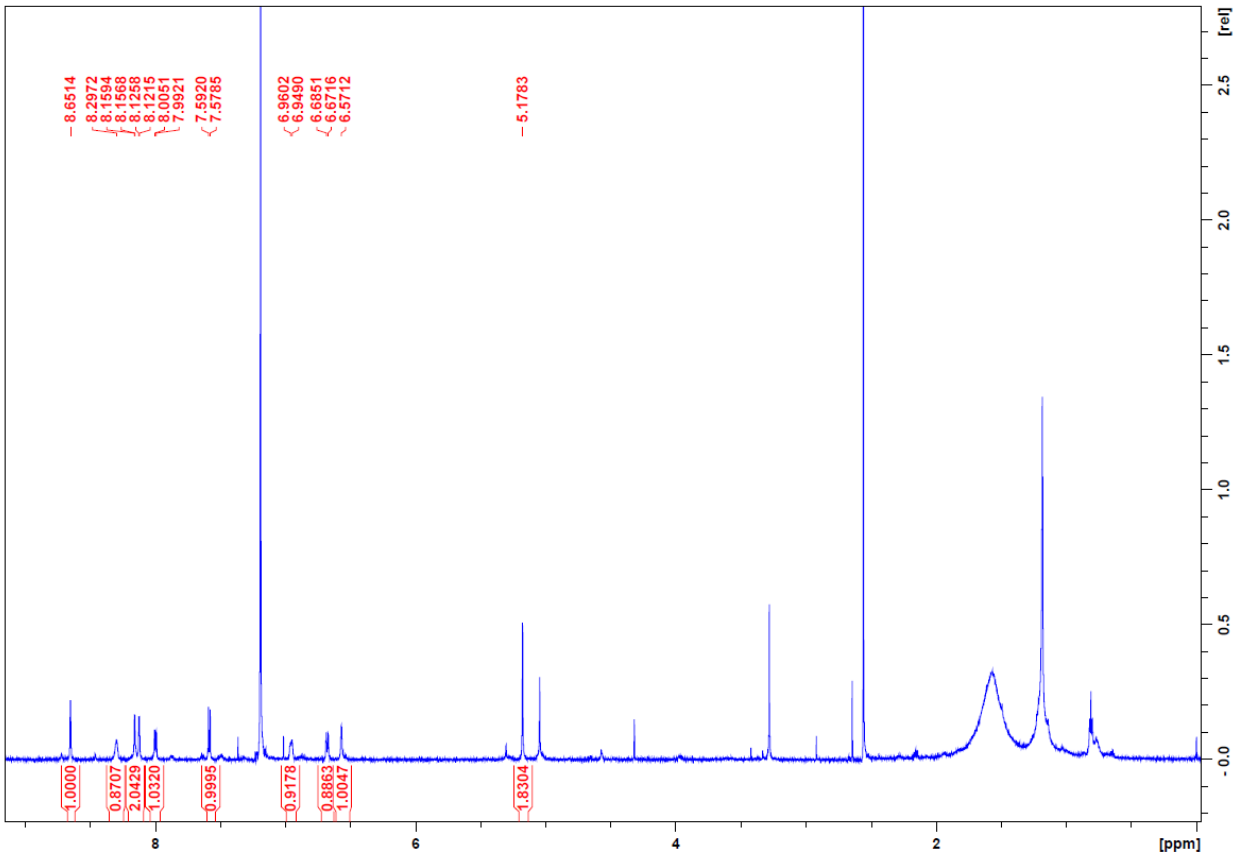


Synthesis of 6-(((6-(trifluoromethyl)pyridin-3-yl)methyl)amino)pyridin-3-yl 5-chloro-1H-pyrrolo[2,3-b]pyridine-3-carboxylate (M29)

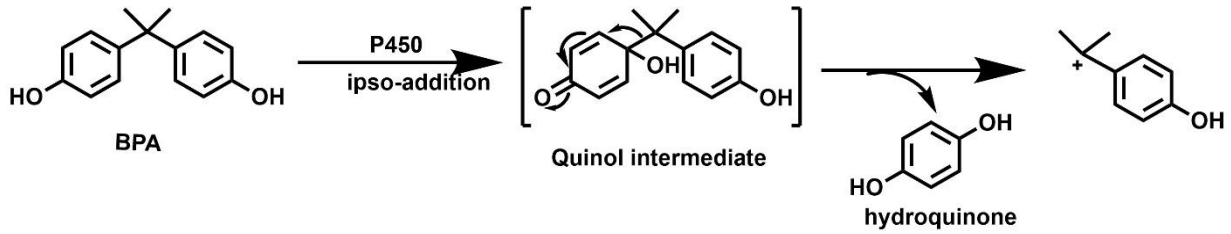
To a solution of 5-chloro-1H-pyrrolo[2,3-b]pyridine-3-carboxylic acid M30 (10 mg, 0.05 mmol) was added HATU (31 mg, 0.08 mmol). After 5 minutes of stirring at room temperature, 6-(((6-(trifluoromethyl)pyridin-3-yl)methyl)amino)pyridin-3-ol M28 (22 mg, 0.08 mmol) and trimethylamine (15 µl, 0.1 mmol) were added sequentially. After overnight, the reaction solution was quenched with saturated $NaHCO_3$, and then extracted with EtOAc three times. The combined organic phases were washed by brine. The organic layer was concentrated and separated by chromatography to give the desired product (11 mg, 50% yield).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.65 (s, 1H), 8.30 (s, 1H), 8.15 (d, J = 1.6 Hz, 1H), 8.12 (d, J = 2.6 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.58 (d, J = 8.1 Hz, 1H), 6.95 (d, J = 6.7 Hz, 1H), 6.68 (d, J = 8.1 Hz, 1H), 6.57 (s, 1H), 5.17 (s, 2H).

^{1}H NMR spectrum of M29:



Scheme S6. P450-mediated C(sp^2)-C(sp^3) bond cleavage in bisphenol A (BPA).



Ref. 31. Nakamura S., et al. Ipso substitution of bisphenol A catalyzed by microsomal cytochrome P450 and enhancement of estrogenic activity. *Toxicology Letters*, **2011**, *203*, 92-95.