Supporting Information

Conversion of Amino Acids to Aryl/Heteroryl ethanol metabolites Using Human CYP2D6-Expressing Live Baker's Yeast

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Section S1: Plasmid maps of (A) extrachromosomal 2µ-plasmid that bears the *CYP2D6(2)* gene, (B) the two integrating plasmids that were used to create 1 and 2-copy *CYP2D6(2)* yeast strains.

(A)

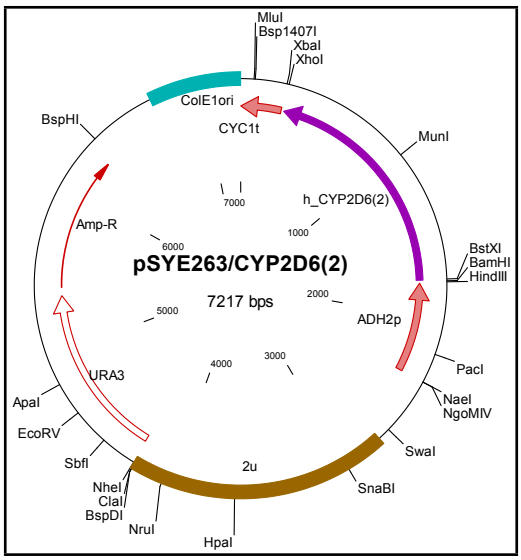

Figure 1S. Map of the 2µ-plasmid bearing the *CYP2D6(2)* gene (NCBI Accession No M20403), with restriction sites that occur in the plasmid only once. The *CYP2D6(2)* gene was placed downstream of the *ADH2* promoter [1]; the transcription termination signal used was from the yeast *CYC1* gene (SGD:S000003809). The plasmid was transformed into the yeast strain W303-1A (ATCC # 208352), as described earlier [1].

(B)

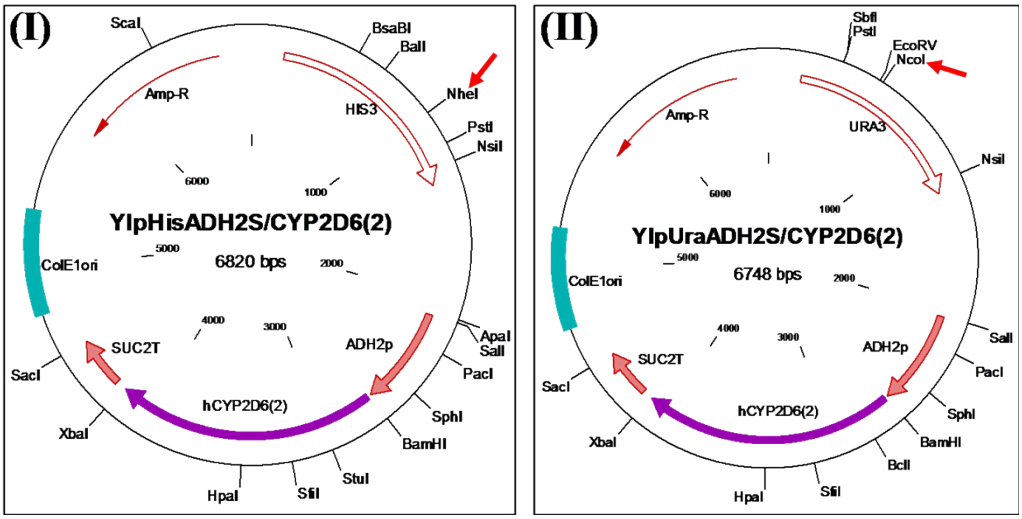

Figure 2S. Map of yeast integrating plasmids bearing the *CYP2D6(2)* gene (NCBI Accession No M20403), with restriction sites that occur in the plasmid only once. The *CYP2D6(2)* gene is downstream of the *ADH2* promoter and the transcription termination signal used was from the yeast *SUC2* gene [1]. The plasmids (I and II) were transformed into the yeast strain

W303-1A (ATCC # 208352), as described earlier, for genomic integration at the *HIS3*(using plasmid I) and *URA3*(using plasmid II) chromosomal lociof strain W303-1A [1]. The red arrows signify restriction sites that were used to linearize the plasmids for stepwise homologous recombination in yeast to create strains with 1-copy and 2-copies of *CYP2D6(2)* gene [1].

Section S2: Protein Sequence of CYP2D6(1); NCBI Accession No NM_000106

```
1  MGLEALVPLA VIVAIFLLLV DLMHRRQRWA ARYPPGPLPL PGLGNLLHVD FQNTPYCFDQ
61  LRRRFGDVFS LQLAWTPVVV LNGLAAVREA LVTHGEDTAD RPPVPITQIL GFGPRSQGVF
121  LARYGPAWRE QRRFSVSTLR NLGLGKKSLE QWVTEEAACL CAAFANHSGR PFRPNGLLDK
181  AVSNVIASLT CGRRFEYDDP RFLRLLDLAQ EGLKEESGFL REVLNAVPVL LHIPALAGKV
241  LRFQKAFLTQ LDELLTEHRM TWDPAQPPRD LTEAFLAEME KAKGNPESSF NDENLRIVVA
301  DLFSAGMVTT STTLAWGLLL MILHPDVQRR VQQEIDDVIG QVRRPEMGDQ AHMPYTTAVI
361  HEVQRFGDIV PLGVTHMTSR DIEVQGFRIP KGTTLITNLS SVLKDEAVWE KPFRFHPEHF
421  LDAQGHFVKP EAFLPFSAGR RACLGEPLAR MELFLFFTSL LQHFSFSVPT GQPRPSHHGV
481  FAFLVSPSPY ELCAVPR
```

Section 3: Protein Sequence of CYP2D6(2) which is also known as wild-type CYP2D6.; NCBI Accession No M20403[2]**.**

(A)

```
1  MGLEALVPLA VIVAIFLLLV DLMHRRQRWA ARYPPGPLPL PGLGNLLHVD FQNTPYCFDQ
61  LRRRFGDVFS LQLAWTPVVV LNGLAAVREA LVTHGEDTAD RPPVPITQIL GFGPRSQGVF
121  LARYGPAWRE QRRFSVSTLR NLGLGKKSLE QWVTEEAACL CAAFANHSGR PFRPNGLLDK
181  AVSNVIASLT CGRRFEYDDP RFLRLLDLAQ EGLKEESGFL REVLNAVPVL LHIPALAGKV
241  LRFQKAFLTQ LDELLTEHRM TWDPAQPPRD LTEAFLAEME KAKGNPESSF NDENLRIVVA
301  DLFSAGMVTT STTLAWGLLL MILHPDVQRR VQQEIDDVIG QVRRPEMGDQ AHMPYTTAVI
361  HEVQRFGDIV PLGMTHMTSR DIEVQGFRIP KGTTLITNLS SVLKDEAVWE KPFRFHPEHF
421  LDAQGHFVKP EAFLPFSAGR RACLGEPLAR MELFLFFTSL LQHFSFSVPT GQPRPSHHGV
481  FAFLVSPSPY ELCAVPR
```

(B)*Protein sequence in FASTA format used for molecular modelling*.

```
MGLEALVPLAVIVAIFLLLVDLMHRRQRWAARYPPGPLPLPGLGNLLHVDFQNTPYCFDQLRRRFGDVFSLQLAW
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QWVTEEAACLCAAFANHSGRPFRPNGLLDKAVSNVIASLTCGRRFEYDDPRFLRLLDLAQEGLKEESGFLREVLN
AVPVLLHIPALAGKVLRFQKAFLTQLDELLTEHRMTWDPAQPPRDLTEAFLAEMEKAKGNPESSFNDENLRIVVA
DLFSAGMVTTSTTLAWGLLLMILHPDVQRRVQQEIDDVIGQVRRPEMGDQAHMPYTTAVIHEVQRFGDIVPLGMT
HMTSRDIEVQGFRIPKGTTLITNLSSVLKDEAVWEKPFRFHPEHFLDAQGHFVKPEAFLPFSAGRRACLGEPLAR
MELFLFFTSLLQHFSFSVPTGQPRPSHHGVFAFLVSPSPYELCAVPR
```

Section S4: Protein Sequence of CYP2D6(C) [1].

```
1   MGLEALVPLA VIVAIFLLLV DLMHRRQRWA ARYSPGPLPL PGLGNLLHVD FQNTPYCFDQ
61   LRRRFGDVFS LQLAWTPVVV LNGLAAVREA LVTHGEDTAD RPPVPITQIL GFGPRSQGVF
121   LVRYGPAWRE QRRFSVSTLR NLGLGKKSLE QWVTEEAACL CAAFANHSGR PFRPNGLLDK
181   AVSNVIASLT CGRRFEYDDP RFLRLLDLAQ EGLKEESGFL REVLNAVPVL LHIPALAGKV
241   LRFQKAFLTQ LDELLTEHRM TWDPAQPPRD LTEAFLAEME KAKGNPESSF NDENLRIVVA
301   DLFSAGMVTT STTLAWGLLL MILHPDVQRR VQQEIDDVIG QVRRPEMGDQ AHMPYTTAVI
361   HEVQRFGDIV PLGVTHMTSR DIEVQGFRIP KGTTLITNLS SVLKDEAVWE KPFRFHPEHF
421   LDAQGHFVKP EAFLPFSAGR RACLGEPLAR MELFLFFTSL LQHFSFSVPT GQPRPSHHGV
481   FAFLVTPSPY ELCAVPR
```

Section S5: Alignment of Protein Sequences of CYP 2D6(1), 2D6(2) and 2D6(C).

The mis-matchesin the three protein sequences are highlighted in red.

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                        10        20        30        40        50        60
                         *         *         *         *         *         *
2D6(1)            MGLEALVPLAVIVAIFLLLVDLMHRRQRWAARYPPGPLPLPGLGNLLHVDFQNTPYCFDQ
2D6(2)            MGLEALVPLAVIVAIFLLLVDLMHRRQRWAARYPPGPLPLPGLGNLLHVDFQNTPYCFDQ
2D6(C)            MGLEALVPLAVIVAIFLLLVDLMHRRQRWAARYSPGPLPLPGLGNLLHVDFQNTPYCFDQ

                        70        80        90       100       110       120
                         *         *         *         *         *         *
2D6(1)            LRRRFGDVFSLQLAWTPVVVLNGLAAVREALVTHGEDTADRPPVPITQILGFGPRSQGVF
2D6(2)            LRRRFGDVFSLQLAWTPVVVLNGLAAVREALVTHGEDTADRPPVPITQILGFGPRSQGVF
2D6(C)            LRRRFGDVFSLQLAWTPVVVLNGLAAVREALVTHGEDTADRPPVPITQILGFGPRSQGVF

                       130       140       150       160       170       180
                         *         *         *         *         *         *
2D6(1)            LARYGPAWREQRRFSVSTLRNLGLGKKSLEQWVTEEAACLCAAFANHSGRPFRPNGLLDK
2D6(2)            LARYGPAWREQRRFSVSTLRNLGLGKKSLEQWVTEEAACLCAAFANHSGRPFRPNGLLDK
2D6(C)            LVRYGPAWREQRRFSVSTLRNLGLGKKSLEQWVTEEAACLCAAFANHSGRPFRPNGLLDK

                       190       200       210       220       230       240
                         *         *         *         *         *         *
2D6(1)            AVSNVIASLTCGRRFEYDDPRFLRLLDLAQEGLKEESGFLREVLNAVPVLLHIPALAGKV
2D6(2)            AVSNVIASLTCGRRFEYDDPRFLRLLDLAQEGLKEESGFLREVLNAVPVLLHIPALAGKV
2D6(C)            AVSNVIASLTCGRRFEYDDPRFLRLLDLAQEGLKEESGFLREVLNAVPVLLHIPALAGKV

                       250       260       270       280       290       300
                         *         *         *         *         *         *
2D6(1)            LRFQKAFLTQLDELLTEHRMTWDPAQPPRDLTEAFLAEMEKAKGNPESSFNDENLRIVVA
2D6(2)            LRFQKAFLTQLDELLTEHRMTWDPAQPPRDLTEAFLAEMEKAKGNPESSFNDENLRIVVA
2D6(C)            LRFQKAFLTQLDELLTEHRMTWDPAQPPRDLTEAFLAEMEKAKGNPESSFNDENLRIVVA

                       310       320       330       340       350       360
                         *         *         *         *         *         *
2D6(1)            DLFSAGMVTTSTTLAWGLLLMILHPDVQRRVQQEIDDVIGQVRRPEMGDQAHMPYTTAVI
2D6(2)            DLFSAGMVTTSTTLAWGLLLMILHPDVQRRVQQEIDDVIGQVRRPEMGDQAHMPYTTAVI
2D6(C)            DLFSAGMVTTSTTLAWGLLLMILHPDVQRRVQQEIDDVIGQVRRPEMGDQAHMPYTTAVI

                       370       380       390       400       410       420
                         *         *         *         *         *         *
2D6(1)            HEVQRFGDIVPLGVTHMTSRDIEVQGFRIPKGTTLITNLSSVLKDEAVWEKPFRFHPEHF
2D6(2)            HEVQRFGDIVPLGMTHMTSRDIEVQGFRIPKGTTLITNLSSVLKDEAVWEKPFRFHPEHF
2D6(C)            HEVQRFGDIVPLGVTHMTSRDIEVQGFRIPKGTTLITNLSSVLKDEAVWEKPFRFHPEHF

                       430       440       450       460       470       480
                         *         *         *         *         *         *
2D6(1)            LDAQGHFVKPEAFLPFSAGRRACLGEPLARMELFLFFTSLLQHFSFSVPTGQPRPSHHGV
2D6(2)            LDAQGHFVKPEAFLPFSAGRRACLGEPLARMELFLFFTSLLQHFSFSVPTGQPRPSHHGV
2D6(C)            LDAQGHFVKPEAFLPFSAGRRACLGEPLARMELFLFFTSLLQHFSFSVPTGQPRPSHHGV

                       490
                         *
2D6(1)            FAFLVSPSPYELCAVPR
2D6(2)            FAFLVSPSPYELCAVPR
2D6(C)            FAFLVTPSPYELCAVPR
```

Section S6:Plasmid maps of the two integrating plasmids that were used to create 1 and 2-copy *CYP2D6(1)* yeast strains.

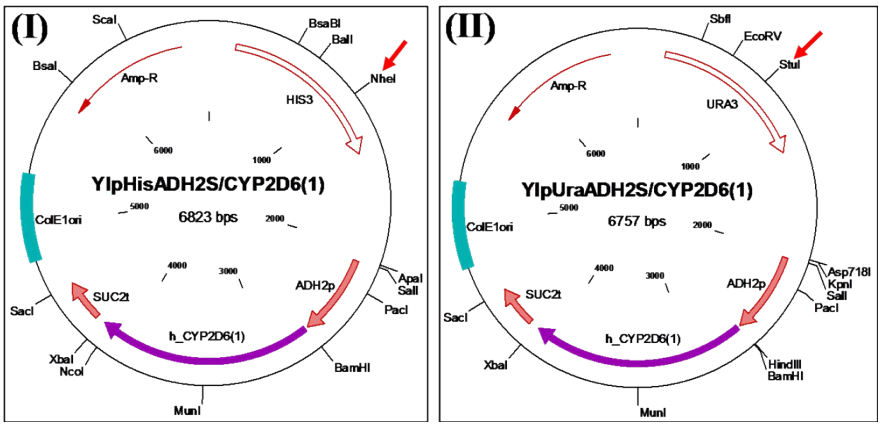


Figure 3S. Map of yeast integrating plasmids bearing the *CYP2D6(1)* gene (NCBI Accession No NM_000106), with restriction sites that occur in the plasmid only once. The *CYP2D6(1)* gene is downstream of the *ADH2* promoter and the transcription termination signal used was from the yeast *SUC2* gene [1]. The plasmids (I and II) were transformed into the yeast strain W303-1A (ATCC # 208352), as described earlier, for genomic integration at the *HIS3* (using plasmid I) and *URA3* (using plasmid II) chromosomal loci of strain W303-1A [1]. The red arrows signify restriction sites that were used to linearize the plasmids for stepwise homologous recombination in yeast to create strains with 1-copy and 2-copies of *CYP2D6(1)* gene [1].

Section S7:Plasmid maps of the two integrating plasmids that were used to create 1 and 2-copy *CYP2D6(C)* yeast strains.

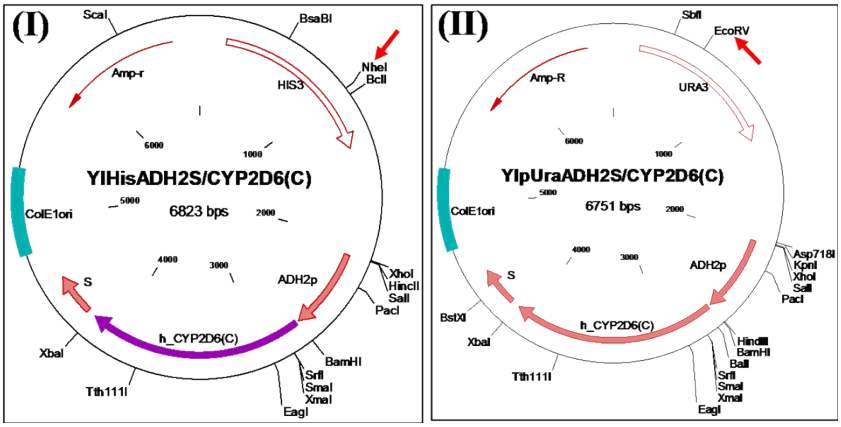


Figure 4S. Map of yeast integrating plasmids bearing the *CYP2D6(C)* gene [1], with restriction sites that occur in the plasmid only once. The *CYP2D6(C)* gene is downstream of the *ADH2* promoter and the transcription termination signal used was from the yeast *SUC2* gene [1]. The plasmids (I and II) were transformed into the yeast strain W303-1A (ATCC # 208352), as described earlier, for genomic integration at the *HIS3* (using plasmid I) and *URA3* (using plasmid II) chromosomal loci of strain W303-1A [1]. The red arrows signify restriction sites that were used to linearize the plasmids for stepwise homologous recombination in yeast to create strains with 1-copy and 2-copies of *CYP2D6(1)* gene [1].

SECTION S8. Growth and specific activity of CYP enzyme with respect to time

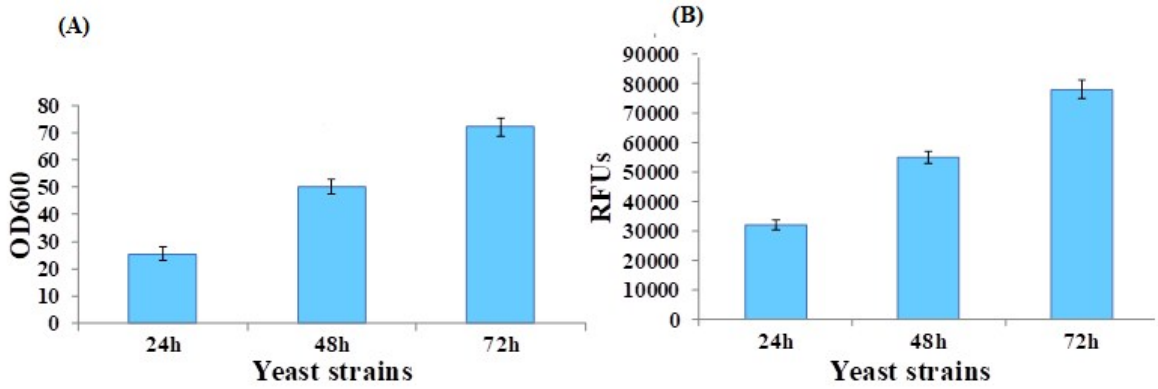


Figure 5S.(A) OD_{600} of yeast cells containing two integrated copies of Human CYP2D6(2) Gene Expression Cassette, (B) Activity of intracellular CYP2D6(2) enzyme expressed from 2-Integrated copies, over different time period, respectively.

As the volume of cells increases over time, there is an increase in the specific activity of CYPs produced within yeast cells,the specific activity of the CYP enzyme also increases in parallel. As an example, expression of CYP2D6(2) (CYP2D6-wt) enzyme was chosen. Optical density at 600 nm (OD_{600}) was measured per mL of YPD cell culture (A). At each time point (24, 48, 72h), enzyme activity was measured in 1x 10^5 recombinant cells using the fluorogenic substrate, EOMCC (B). After every 24h of growth, medium was discarded and replenished with fresh medium, mimicking fed-batch growth. 'RFUs' denote relative fluorescence units. The data represent mean ± S.D. of three independent experiments. 'wt' represents 'wild type'.

SECTION S9: Experimental Procedures

S9.1: Production of enzyme

Yeast strains (harbouring integrated copies of a CYP gene or an episomal plasmid bearing a CYP gene) from frozen stocks were revived in 500 mL Erlenmeyer baffled flasks containing 100 mL YPD medium (peptone 20 gL-1, yeast extract 10 gL-1, glucose 15 gL-1, pH 6.0). The flasks were shaken at 200 rpm for 24 h at 28°C. Three consecutive 400 mL YPD pre-cultures, starting with the 100 mL pre-culture inoculum, were then grown for production of high biomass in 2 L baffled flasks at 30°C for 18 h. The cells were harvested, after every 18 h, before inoculation into a new 2 L baffledflask containing 400 ml YPD medium. The process, with the recombinant yeast cells grown for a total time period of 54 h, yielded 400

mL of cells with a final OD_{600}, of ~80 (a total of ~1x 1011 cells). These cells were used for liberation of membrane-bound (microsomal) CYP proteins.

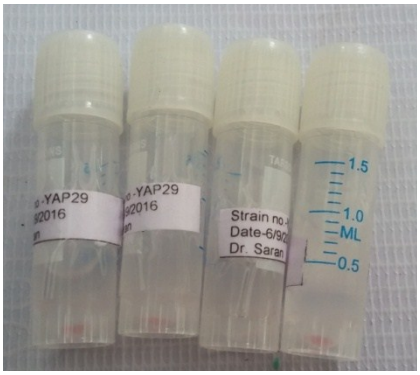


Figure 6S.CYP strains preserved at -80°C (glycerol stocks)

S9.2: Preparation of microsomal CYP enzymes

The cells, suspended in Buffer-A (4M sorbitol, 1M Tris-HCl pH 7.5, 0.5M EDTA pH 8.0, 0.1M AEBSF, 0.1M DTT), were lysed using a cell disruptor (Constant Systems Basic Z model with continuous processing head) at a pressure of 22.5 ksi. The cell lysates were collected from the cell disruptor and were centrifuged at 4,500g for 20 min at 4ºC using a Sorvall bench-top centrifuge to remove all cell debris and broken cell membranes. The resulting supernatants were then transferred into chilled 30 ml tubes and centrifuged at 35,000g for 15 min at 4°C in a high-speed centrifuge (Beckman Coulter Avanti J-20XP) to remove cell debris, nuclei, peroxisomes, lysosomes and mitochondria. Centrifugation was repeated twice. The supernatants, contained in a beaker, were diluted with ice cold Buffer-B (0.6M sorbitol, 60 mMTris-HCl pH 7.6, 1 mM EDTA pH 8, 0.4 mM AEBSF, 0.04 mM DTT). The beaker was stirred at 1-2 rpm at 4ºC. NaCl was added gently to give a final concentration of 0.125 M. Ice-cold 50% PEG solution was added drop-wise (1-2 drops per second) from a separating funnel, whilst gently stirring the mix, to precipitate the microsomal (ER-bound) CYP enzymes (20). Aliquots of the enzymes were frozen at -80ºC or were used directly for freeze-drying. Thawed enzymes from the -80ºC freezer or freeze-dried enzymes,

re-suspended in ultrapure water, were used to perform the comparative analyses described in the Sections that follow.

S9.3: Estimation of enzyme activity

The amounts (i.e. nanomoles) of functional CYP enzymes in the microsomes, obtained from 400 ml YPD cell culture, were ascertained via CO-difference spectroscopy (21). The assay is based on the fact that, when carbon monoxide (CO) reacts with the ferrous form of a functionally active CYP, a complex is formed that produces a spectrum with a wavelength maximum at ~450 nm. The cytochrome P450 (CYP) concentration present in the yeast microsomes (SacchroosmesTM) was calculated from the difference between the absorbance at 450 nm (peak) and 490 nm, using an extinction coefficient of 91 mM-1cm-1. When CO reacts with an inactive (i.e. misfolded, non-functional) CYP, the CO-inactive CYP complex absorbs at a wavelength maximum of 420 nm. The protein cytochrome b5 also absorbs at 420 nM. The cytochrome b5 peak is seen in the microsomal enzymes, Sacchrosomes, prepared from the YAB79 strain.

The amount of ER-bound CYP2D6(2) enzyme isolated from a litre of YPD cell culture was ~150 nanomoles (120 to 325 nmol/L of ER-bound human CYP enzymes are routinely isolated from yeast) [7]. The amounts obtained from a litre of yeast culture, grown in shake flasks, using this novel chromosomally-integrated system is far more than that obtained from 30 L yeast fermentors [3]. The reported yields from expression in bacterial cells (which have no intracellular ER membranes) of modified, truncated human *CYP* genes that allow secretion of CYP enzymes in cell culture supernatants, are < 100 nmol/L [4]. The yields from recombinant insect cells, which are not amenable to biotransformation reactions, are always very low compared to bacterial expression [5].

S9.4: Biotransformation experiment:

Three variant of Yeast 2D6 strains i.e. CYP 2D6(1), 2D6(2) and 2D6(C), each of which contain different copies of human CYP2D6 genes, downstream of the ADH2 promoter, integrated into chromosomal loci of the genome of the yeast strain W303-1a (ATCC 208352), were used for biotransformation. Initially all the strains of CYP 2D6 strains i.e. CYP 2D6(1), 2D6(2) and 2D6(C), were revived from glycerol stocks in 200 mL Erlenmeyer baffled flasks containing 50 mL YPD (Yeast, Peptone, Dextrose) medium with composition (gL-1): peptone 20; yeast extract 10; glucose 15.0, pH 6.0. The flasks were shaken at 200 rpm, at 28 °C. Pre-cultures were prepared (thrice) for all the three strains.

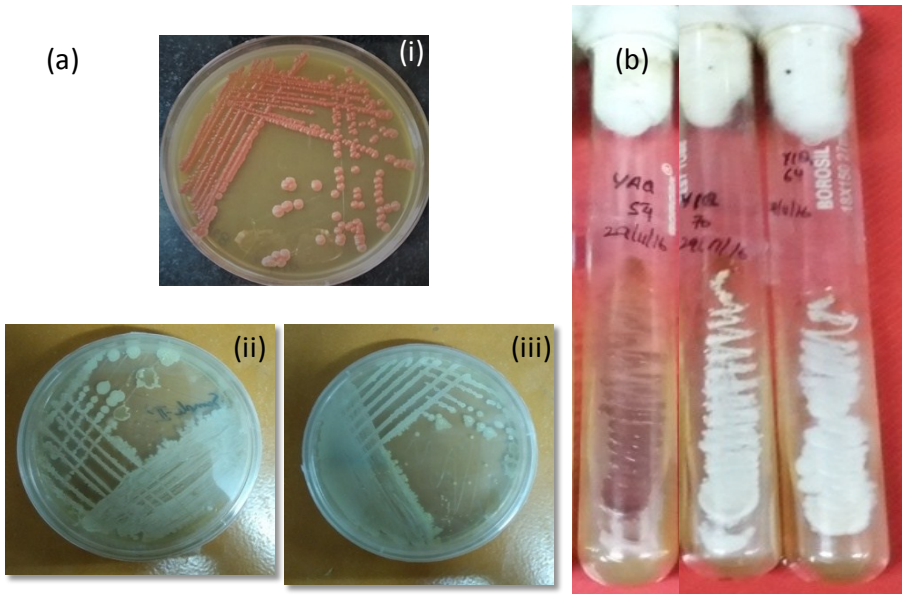


Figure7S: Three CYP variants (a) (i) CYP2D6(2), (ii) CYP2D6(1), (iii) CYP2D6(C) expressing cells grown in YPD medium in petriplates ; (b) test tubes

These consecutive YPD pre-cultures were grown for high biomass production, before addition of the substrate (Tryptophan) to cells grown in SD (Synthetic Defined) medium. Typically, a loopful of CYP-containing freshly grown yeast cells (three variant of CYP 2D6) were inoculated in a 500 ml Erlenmeyer baffledflask separately containing 100 ml YPD medium (pre-culture -1) at 30 °C for 24 h. The cells were harvested after 24 h and inoculated

into a new 500 ml baffledflask containing 100 ml YPD medium (pre-culture -2) at 30 °C for 18 h. The process was repeated three times for the cells to reach an OD600, of ~80 (pre-culture -3). The harvested cells, ~3.0 ml (OD600, ~80), were inoculated in 200 ml of minimal SD medium contained in a 1 L baffled flask. Composition of SD medium (gL-1): dextrose 1.0; dipotassium phosphate 7.0; monopotassium phosphate 2.0; sodium citrate 0.50; magnesium sulphate 0.10; ammonium sulphate 1.0, pH 6.0 ± 0.2 at 30 °C. Initially, reaction was carried out with 25 mg of tryptophan with all the strains of CYP 2D6. The tryptophan was dissolved (25 mg) in DMSO and further incubated in 50 mL of SD medium (keeping the DMSO concentration > 0.5%) for 60 h at 30°C, 200 rpm. After every 24 h, the medium was replenished with 1.5% w/v of glucose.

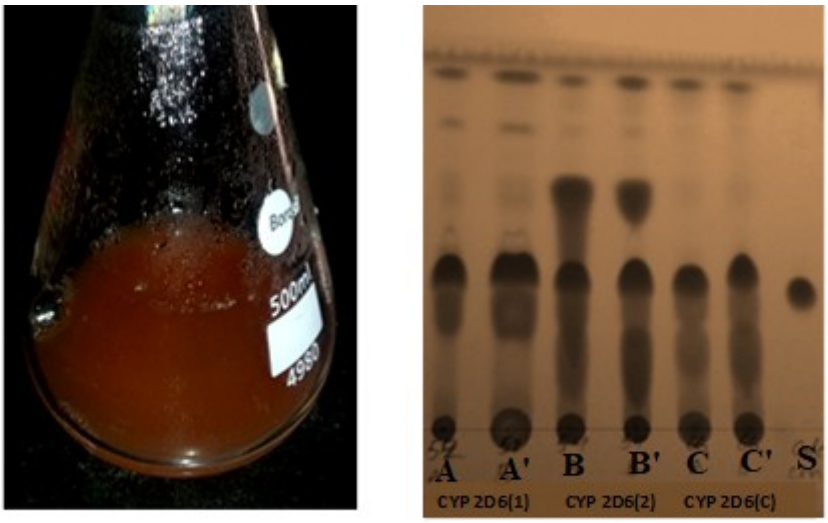


Figure 8S (a) Biotransformation reaction using CYP2D6(2) in SD medium in shake flasks using tryptophan as substrate, (b) TLC analysis after 24 and 36 h for product formation with all the three strains of CYP2D6 [TLC mobile phase : ethyl acetate and hexane ,50:50]

S9.5: Scale up and optimization of biotransformation reaction

Scale-up was performed as above; cells were at first cultivated in non-selective YPD media for 72 h, with fresh glucose (2%) being added every 24 h. Cells were re-suspended in selective SD minimal medium. 10, 50, 100, 250 and 500 mg of tryptophan were used as

substrate for separate biotransformation reactions. The tryptophan-containing cell culture media were replenished with glucose every 24 h to a final concentration of 1.5%. The reaction using 200 mg of tryptophan was observed to be the most efficient. With 250 and 500 mg, incomplete biotransformation occurred, probably because the small number of cells, used for growth in these specific experiments, was inhibited by the substrate. Based on these results, it was decided to optimize the time period for biotransformation. For this, we chose 100 mg of substrate with reaction time points of 24, 48, 72, 96, 120, 144 and 160 h. HPLC analyses of the reaction mixture after 24, 48, 72, 96, 120, 144 and 160 h incubations indicated that, at 144 h, there was nearly complete conversion (>80%) of tryptophan to the product.

S9.6: AntiDepressant Property Study:

1. ***Tryptophol and Tyrosolshows potential neuroprotective properties in cells.***

   Mouse neuroblastoma cells (N2a) were treated with various compounds at 10 μM concentration for 24 hrs followed by co-treatment of neurotoxic cortisone (400μM) for another 24hrs to observe the neuro-protective effect. It was observed that treatment of two compounds tryptophol and tyrosolwere able to protect the neurons from corticosterone induced toxicity, thereby displaying neuroprotective properties.

2. ***Tryptopholand Tyrosol elicit BDNF expression in vitro thereby acting as antidepressants.***

   To investigate the potential antidepressants properties of the tryptophol and tyrosol, BDNF expressions were estimated in cell culture supernatent collected after 4hrs of treatment. Differentiated human neuroblastoma cells (SHSY5Y) were pre incubated with compound for 2 hrs prior to the Corticostereone treatment. Addition of tryptophol, tyrosol both resulted in induction of BDNF neurotropic response supporting the fact that they might act as antidepressants (Figure 9S).

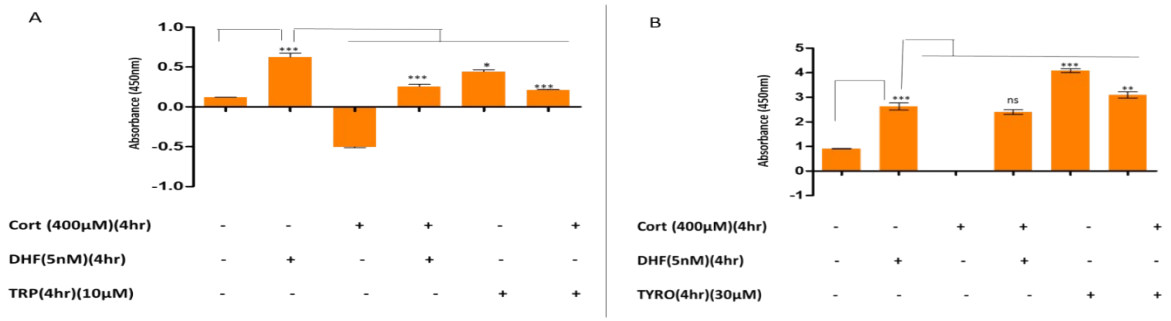


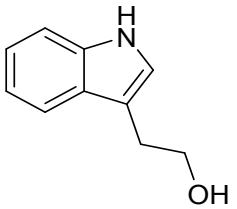
Figure 9S.Neurotrophic activity induces BDNF expression in vitro.
(A,B) TRP induces BDNF activation in differentiated neuroblastoma cell lines. Cells were subjected to differentiation 3 days ahead of treatment, pretreated with (10µM) TRP, followed by toxicity and subjected to quantitative analysis of BDNF using BDNF-ELISA kit. P value ≤ 0.05*, <0.01**., 0.001***

3. **Tryptophol and Tyrosol induce BDNF dependent activation of pTrkb along with its downstream signaling cascade.**

TRP and TYRO induces activation of prosurvival pathway in differentiated SHSY5Y cells thereby shutting off neuronal apoptosis. Addition of TRP and TYRO provoked activation of pro-survival AKT, ERK1/2 and TrKB signaling in differentiated neuroblastoma cell lines within 4 hrs of treatment at the treatment of 10µM. TRP and TYRO stimulated pTrKB activation pattern like that of BDNF thereby depicting potential antidepressant properties of these compounds.

- Mouse neuroblastoma cells (N2a) and human neuroblastoma cells (SHSY5Y) were obtained from ECACC through Sigma-Aldrich.

Section S10:Characterization data of Tryptophol (1a).

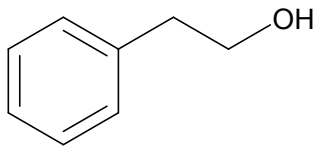


1a

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.26 (t, *J* = 7.5 Hz, 1H), 7.18 (t, *J* = 7.4 Hz, 1H), 6.96 (s, 1H), 3.92 (t, *J* = 6.4 Hz, 2H), 3.04 (t, *J* = 6.4 Hz, 2H), 1.36 (s, 1H).**^{13}C NMR (101 MHz, $CDCl_3$**δ 136.56, 130.14, 127.52,

122.73, 122.13, 119.43, 118.84, 115.63, 112.11, 111.41, 62.66, 28.73.HRMS (ESI+): m/z calcd. for $C_{10}H_{12}NO$ (M+H)+: 162.0919; found 162.0912

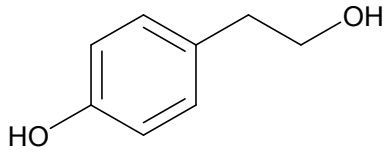
Section S11:Characterization data of 2-Phenylethanol (2a).



2a

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.32 (m, 1H), 7.30 – 7.24 (m, 1H), 3.89 (t, *J* = 6.6 Hz, 1H), 2.90 (t, *J* = 6.6 Hz, 1H).**^{13}C NMR (126 MHz, $CDCl_3$)**δ 138.52, 129.09, 128.62, 126.51, 63.70, 39.20. HRMS (ESI+): m/z calcd. for $C_8H_{11}O$ (M+H)+: 123.0810; found 123.0815

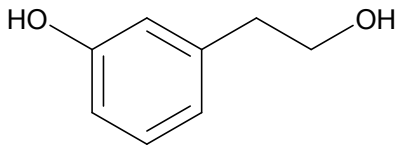
Section S12:Characterization data of Tyrosol (3a).



3a

^{1}H NMR (400 MHz, MeOD) δ 6.93 (d, *J* = 8.4 Hz, 1H), 6.66 – 6.55 (m, 1H), 3.59 (t, *J* = 7.2 Hz, 1H), 2.62 (t, *J* = 7.2 Hz, 1H).**^{13}C NMR (101 MHz, MeOD)** δ 155.35, 129.69, 129.49, 114.77, 63.20, 38.02. HRMS (ESI+): m/z calcd. for $C_8H_{11}O_2$ (M+H)+: 139.0759; found 139.0754

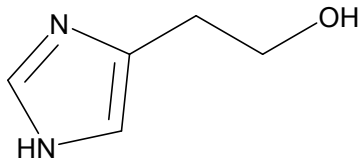
Section S13:Characterization data of m-Tyrosol (4a).



4a

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.19 (m, 1H), 6.81 (d, *J* = 7.6 Hz, 1H), 6.76 – 6.71 (m, 1H), 3.89 (t, *J* = 6.4 Hz, 1H), 2.84 (t, *J* = 6.3 Hz, 1H).**^{13}C NMR (101 MHz**, $CDCl_3$) δ 156.00, 140.25, 129.85, 121.22, 116.05, 113.59, 63.53, 38.93. HRMS (ESI+): m/z calcd. for $C_8H_{11}O_2$ (M+H)+: 139.0759; found 139.0753

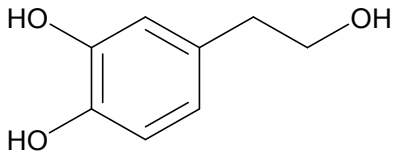
Section S14:Characterization data of Histaminol (5a).



5a

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 3.85 (t, *J* = 6.5 Hz, 1H), 3.78 – 3.71 (m, 2H), 2.83 (t, *J* = 6.5 Hz, 1H).**^{13}C NMR (101 MHz, $CDCl_3$)** δ 130.16, 115.48, 114.05, 63.82, 38.29. HRMS (ESI+): m/z calcd. for $C_5H_9N_2O$ (M+H)+: 113.0715; found 113.0718

Section S15:Characterization data of 3,4-dihydroxyphenyl ethanol(6a).

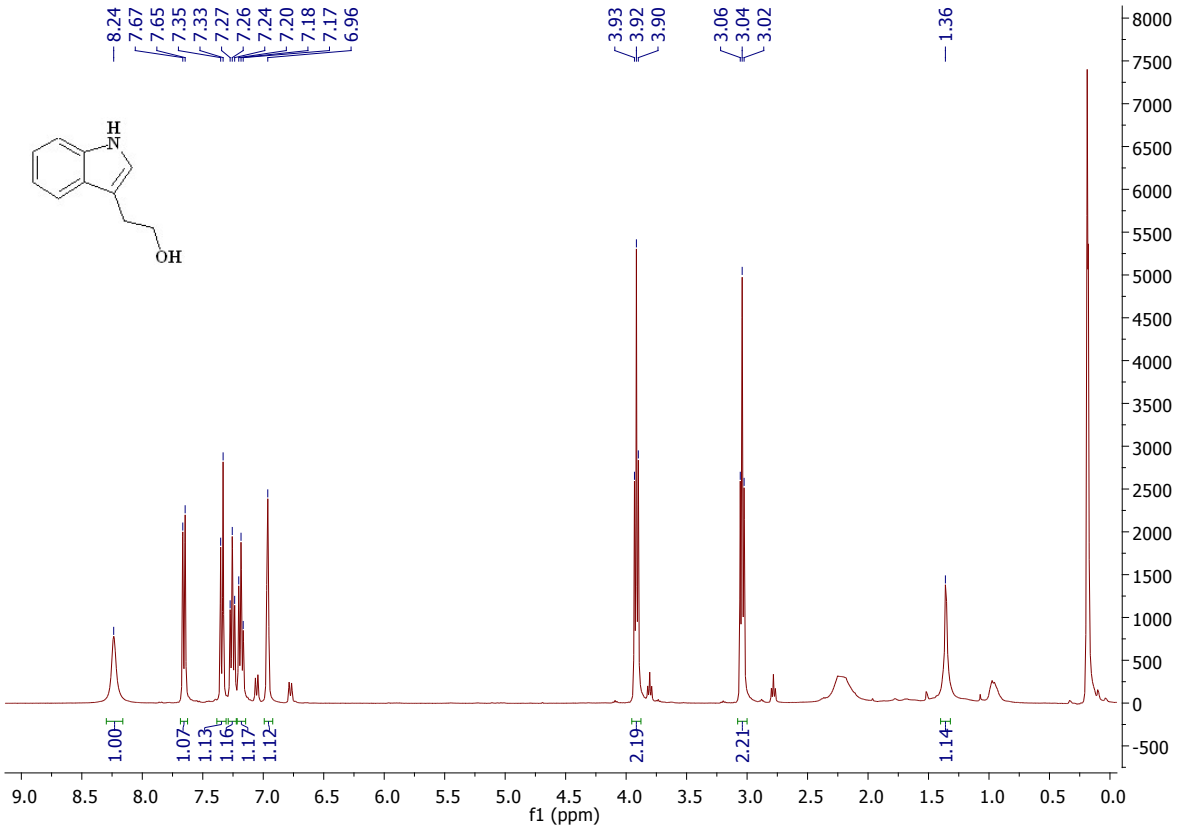


6a

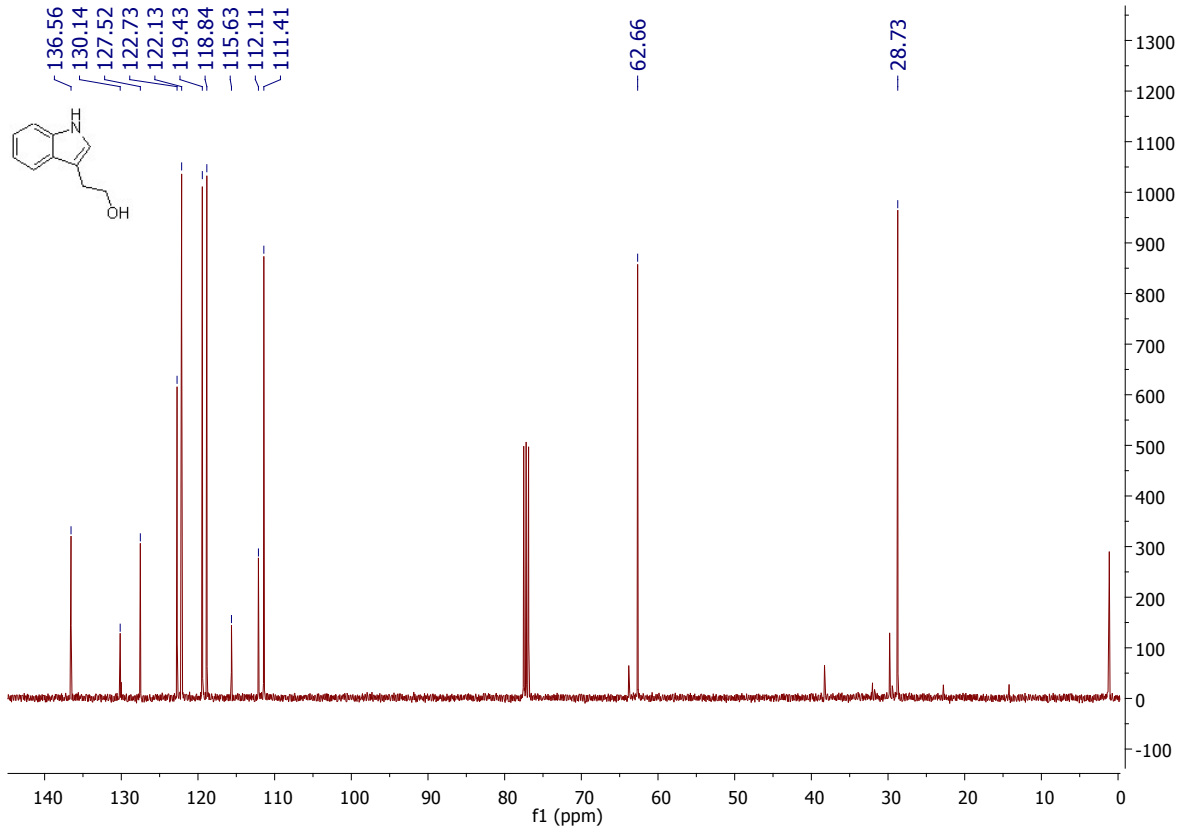
^{1}H NMR (400 MHz, MeOD) δ 6.60 (d, *J* = 1.0 Hz, 1H), 6.15 (d, *J* = 8.0 Hz, 1H), 6.10 (s, 1H), 6.01 (d, *J* = 7.8 Hz, 1H), 3.17 (t, *J* = 6.3 Hz, 1H), 2.11 (t, *J* = 6.2 Hz, 1H).**^{13}C NMR (126 MHz, $CDCl_3$)**δ 121.47, 116.08, 115.50, 63.74, 38.48. HRMS (ESI+): m/z calcd. for $C_8H_{11}O_3$ (M+H)+: 155.0708; found 155.0712

Section S16: NMR Spectraof isolated products

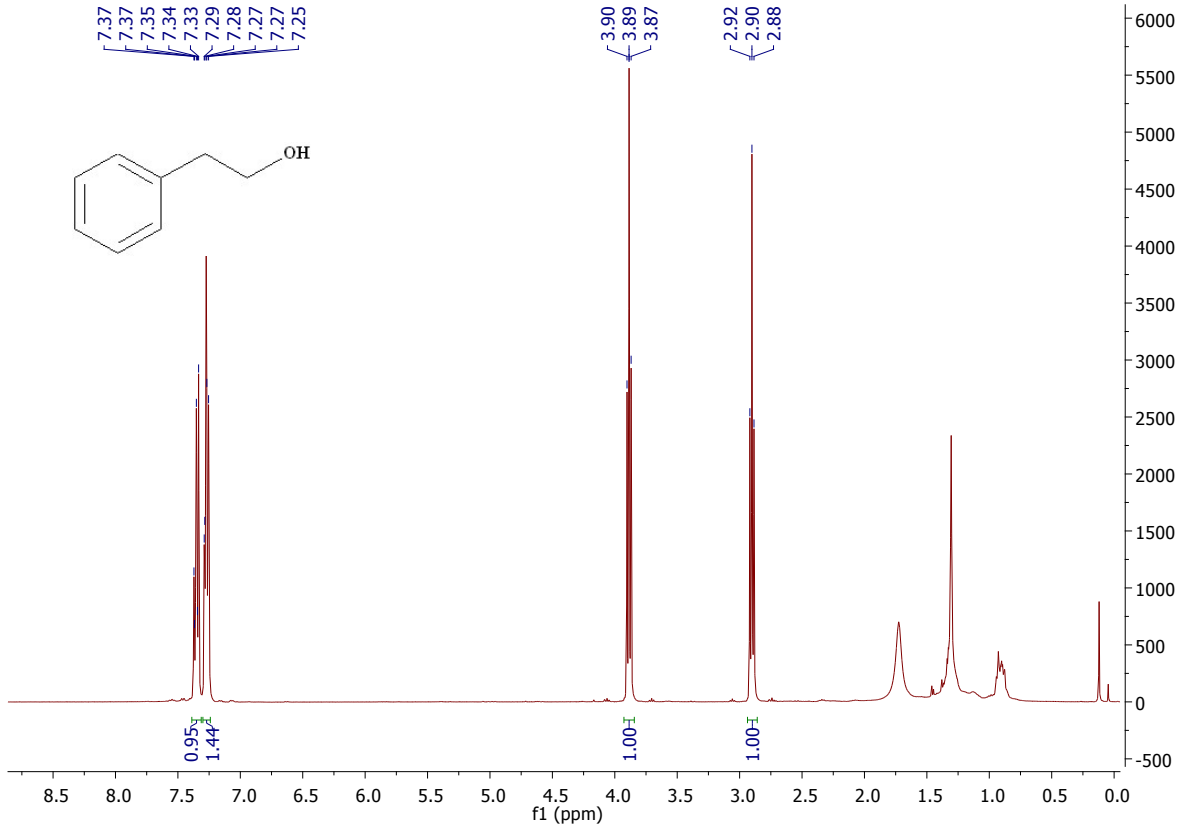
^{1}H NMR (400 MHz, CDCl3) of compound 1a



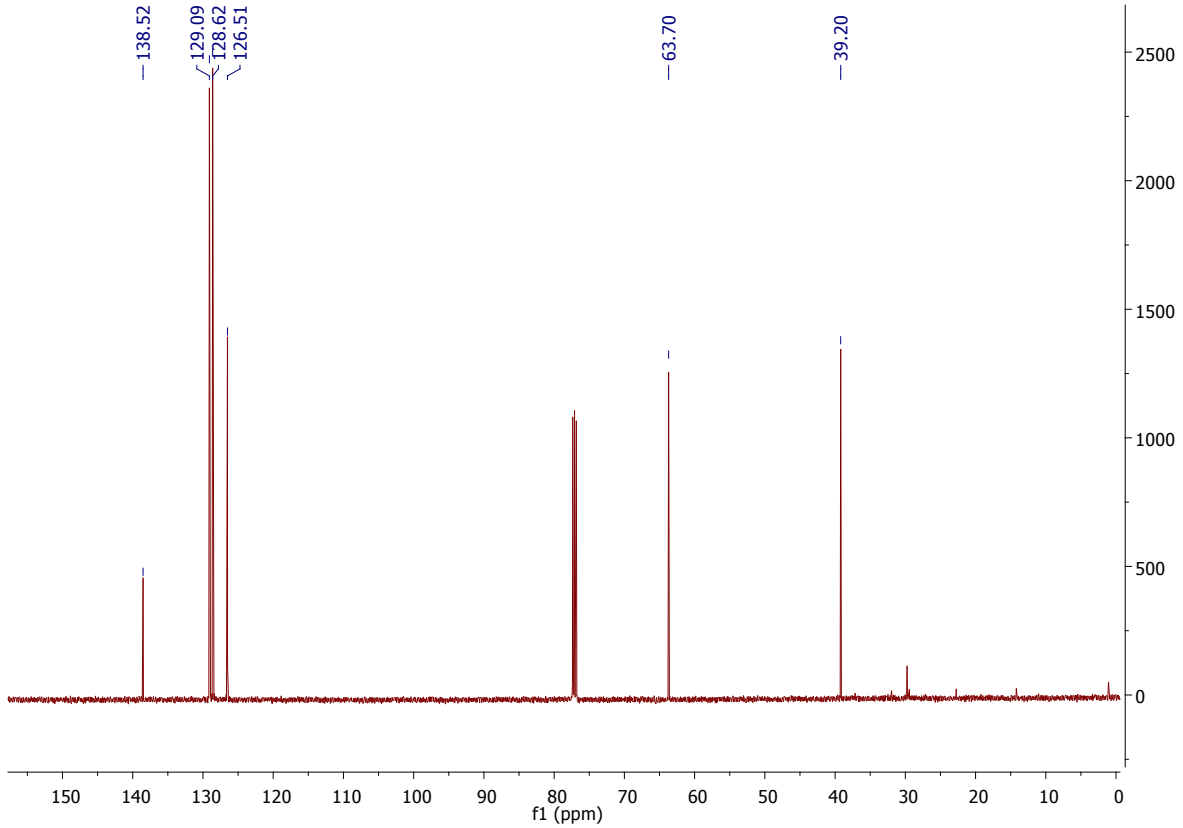
^{13}C NMR (101 MHz, CDCl3) of compound 1a



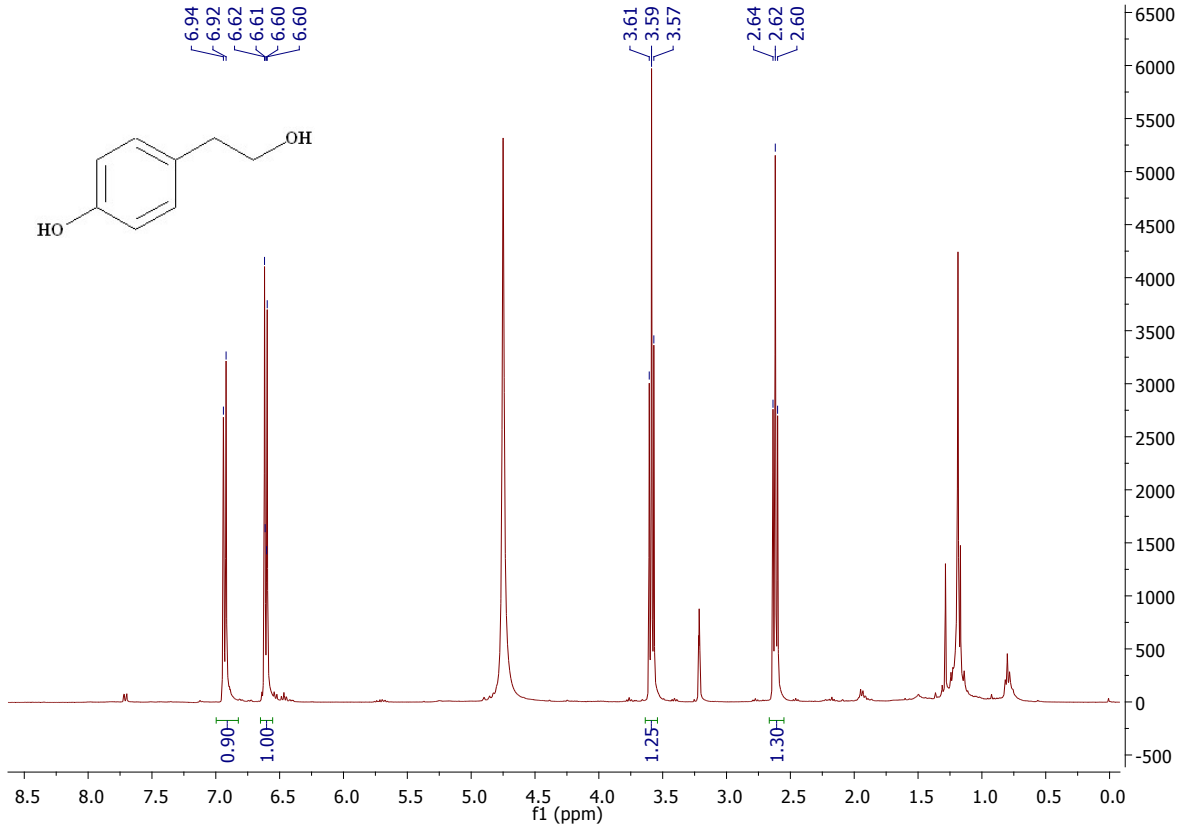
[1]H NMR (400 MHz, CDCl3) of compound 2a



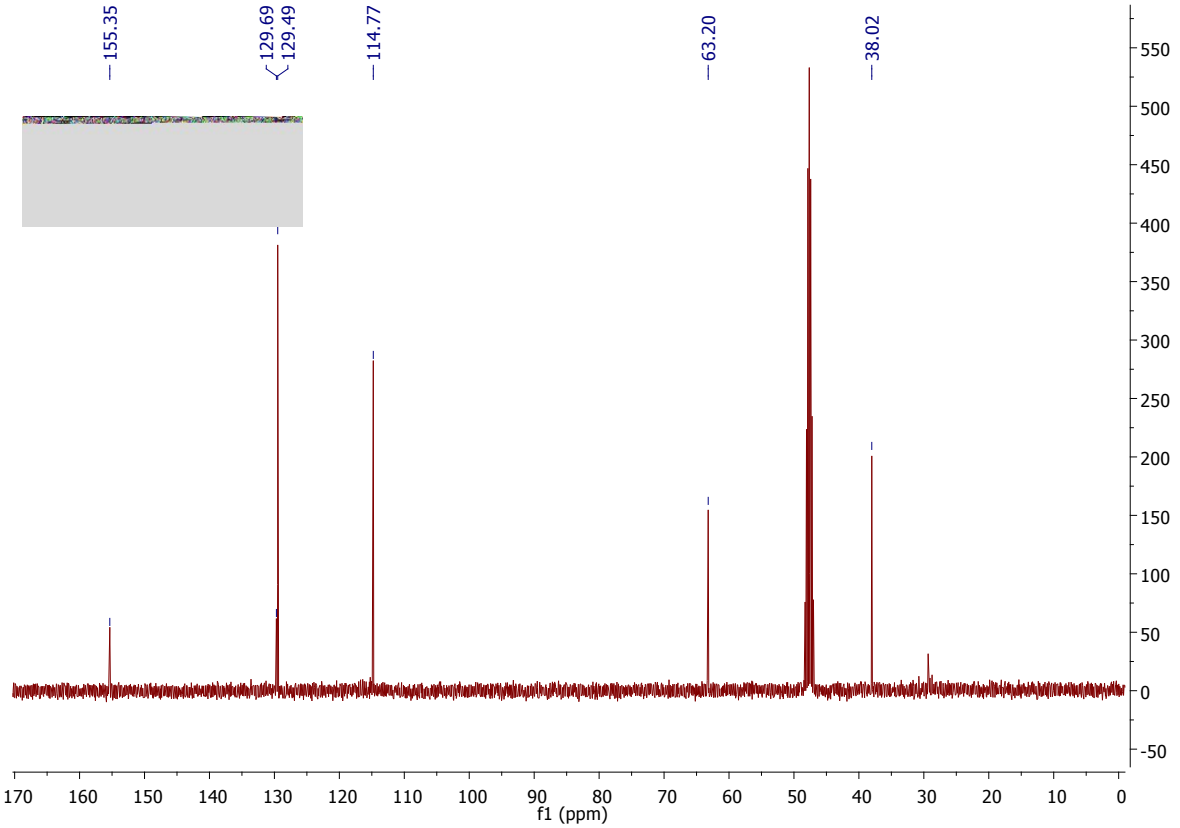
^{13}C NMR (101 MHz, CDCl3) of compound 2a



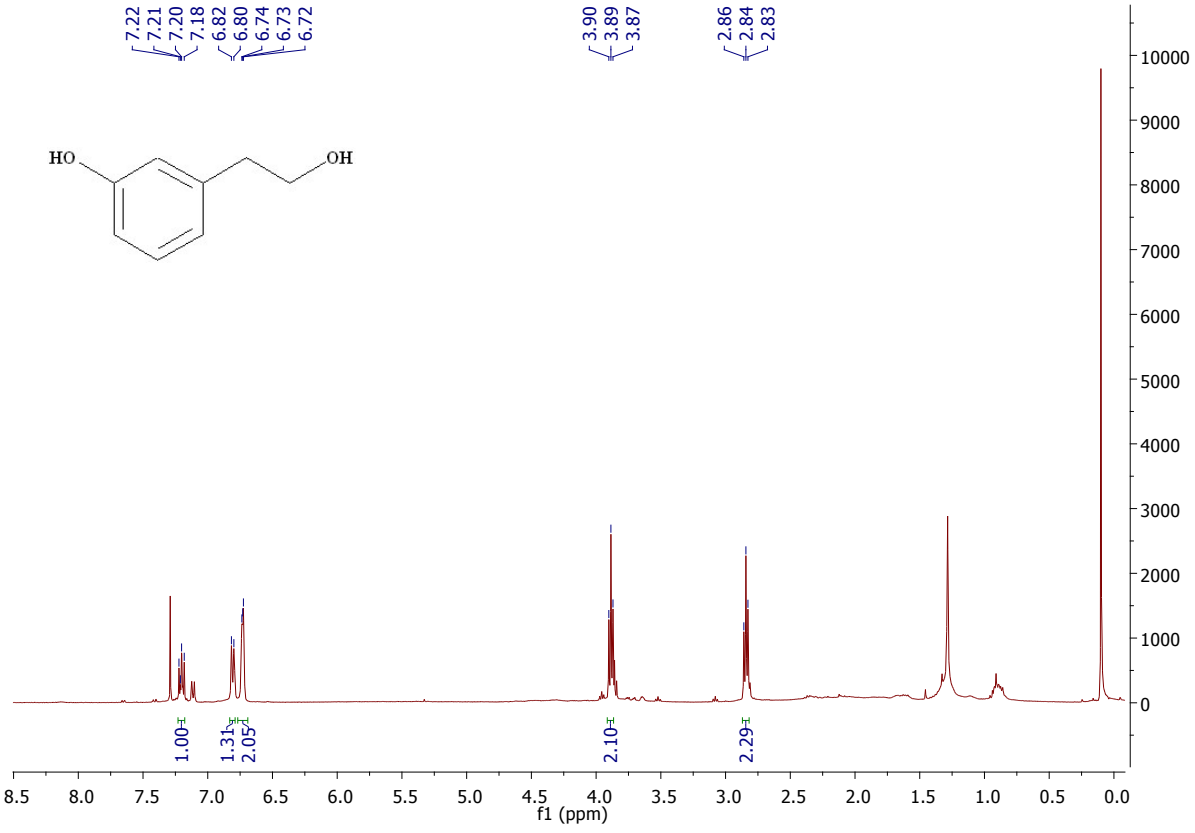
^{1}H NMR (400 MHz, CDCl3) of compound 3a



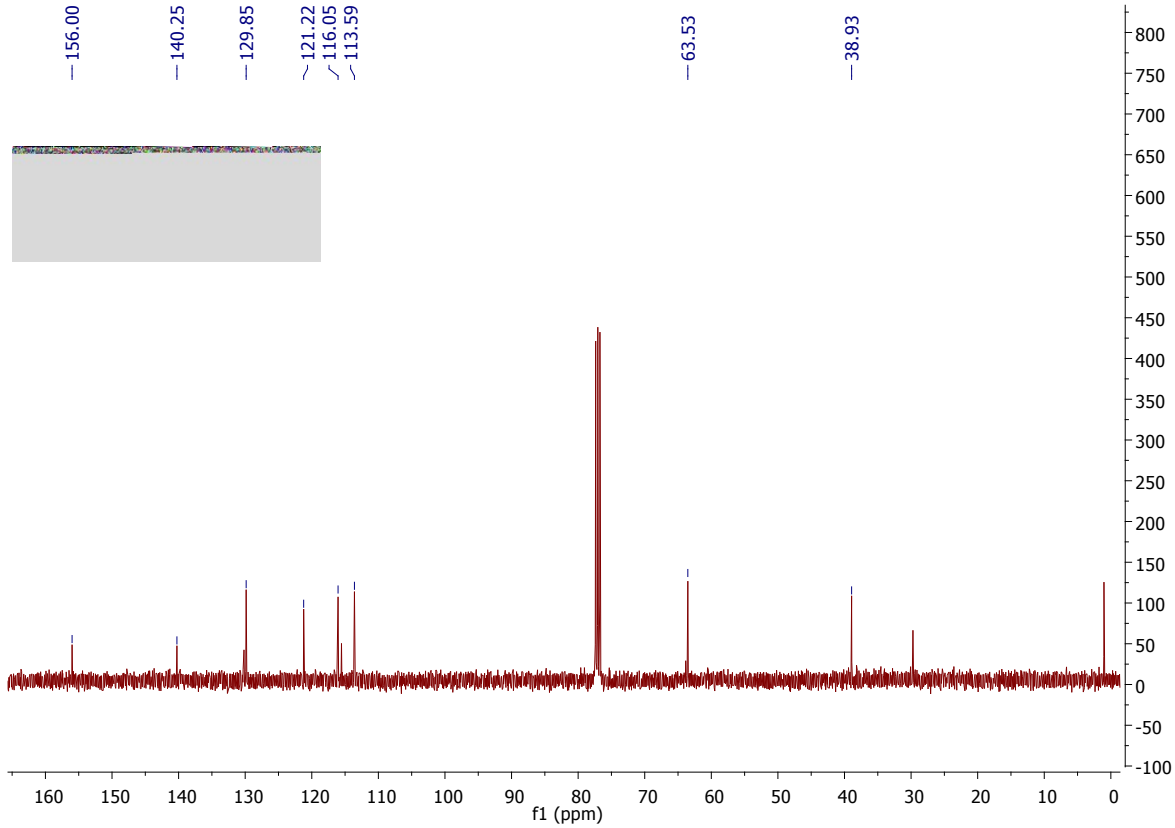
^{13}C NMR (101 MHz, CDCl3) of compound 3a



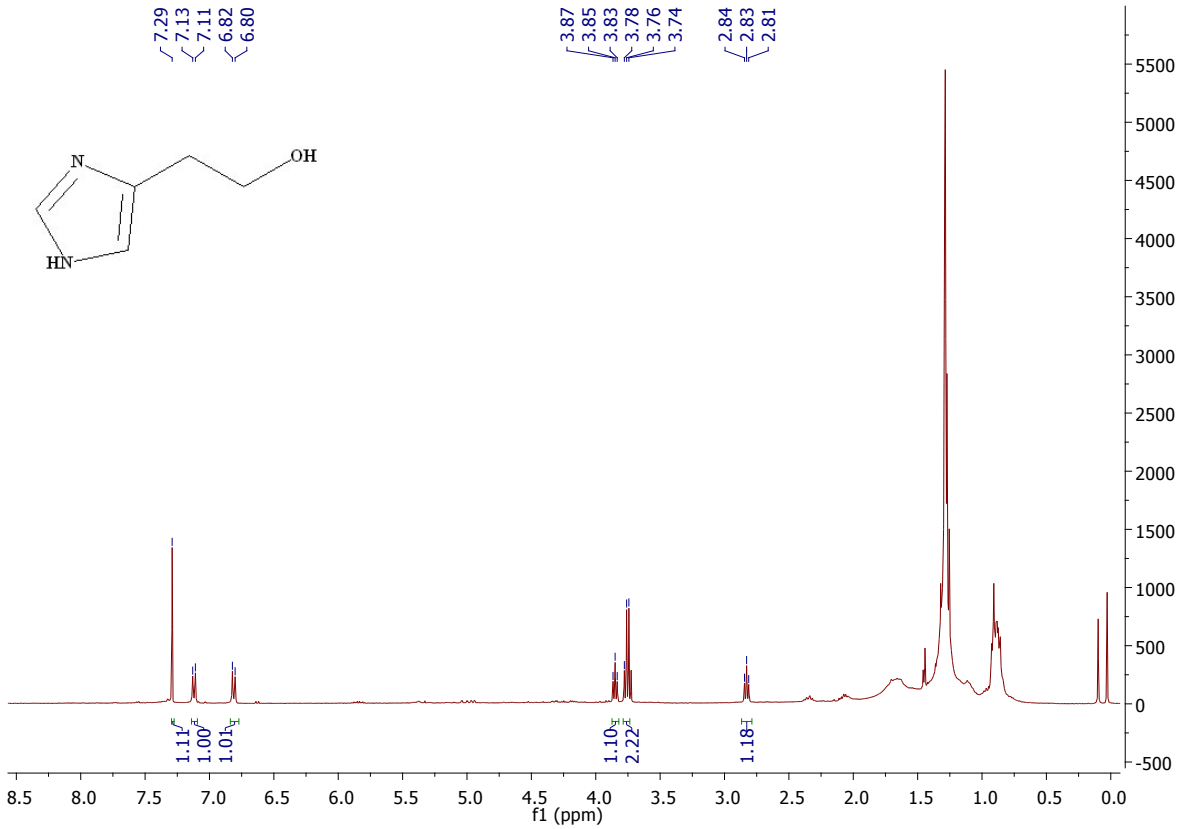
^{1}H NMR (400 MHz, CDCl3) of compound 4a



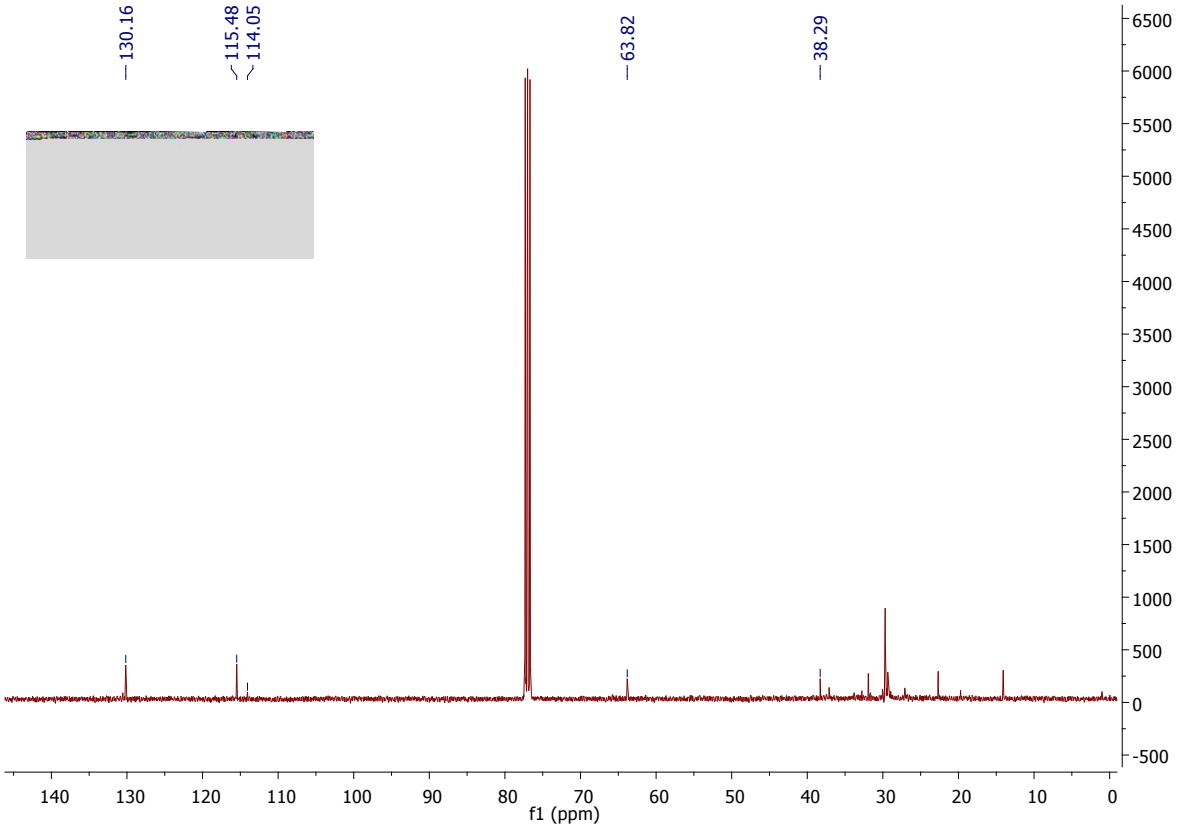
^{13}C NMR (101 MHz, CDCl3) of compound 4a



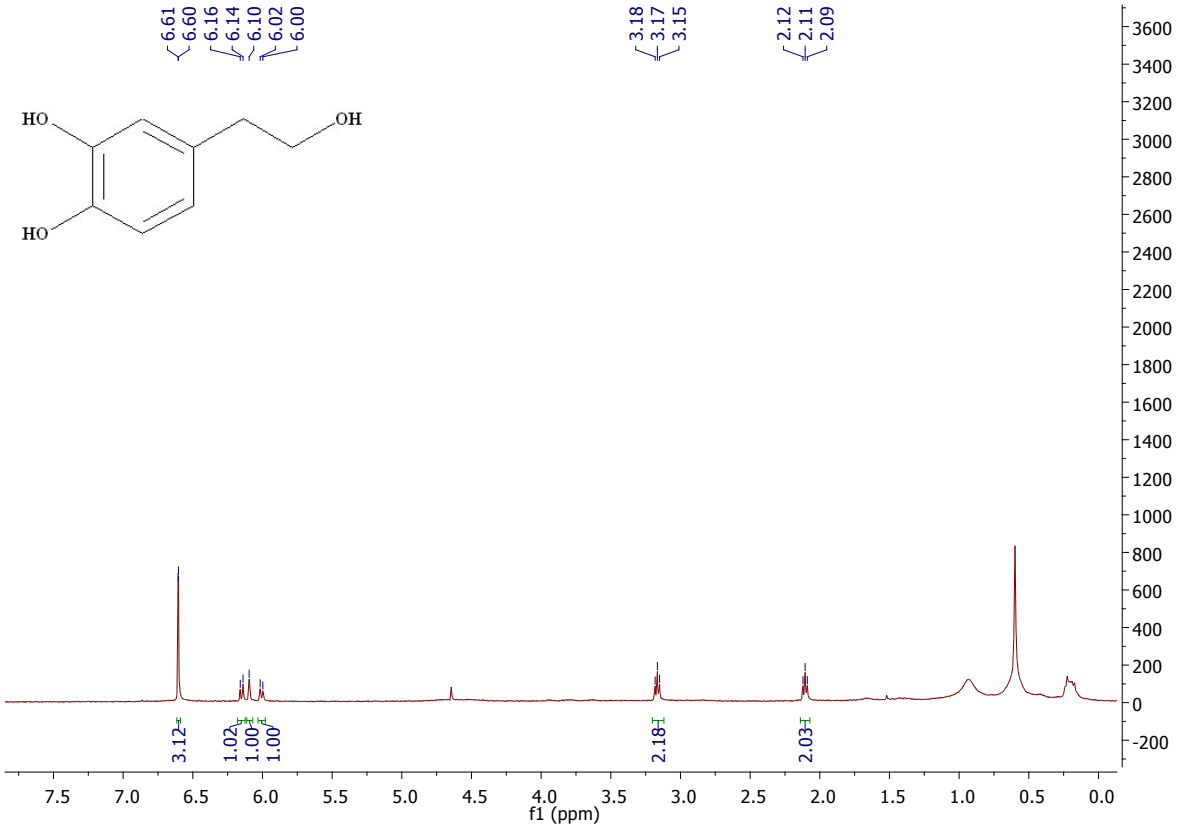
^{1}H NMR (400 MHz, CDCl3) of compound 5a



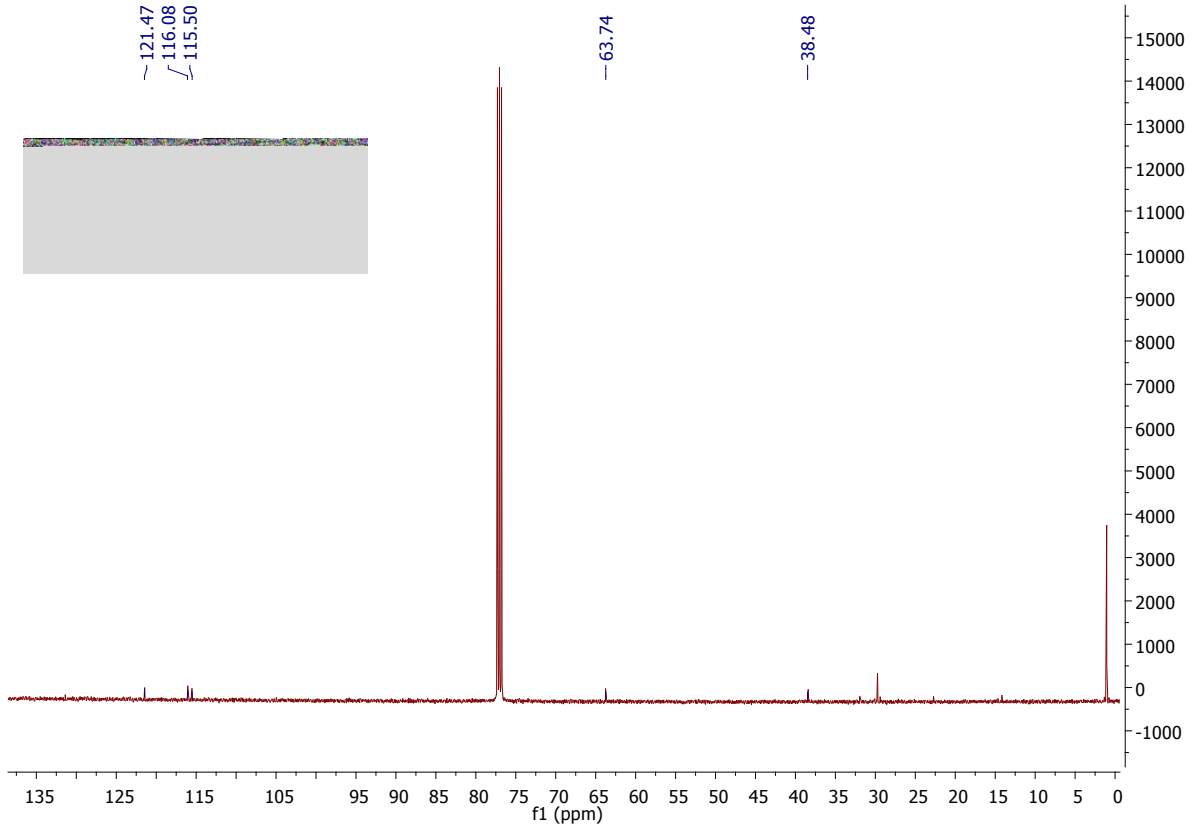
^{13}C NMR (101 MHz, CDCl3) of compound 5a



^{1}H NMR (400 MHz, CDCl3) of compound 6a



^{13}C NMR (101 MHz, CDCl3) of compound 6a



Section S18:References.

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[2] P.Rowland, F. E. Blaney, M. G. Smyth, J. J. Jones, V. R. Leydon, A. K. Oxbrow, C. J. Lewis, M. G. Tennant, S. Modi, D. S. Eggleston, R. J. Chenery, A. M. Bridges. *J. Bio.l Chem*. **2006,** 281, 7614-622.

[3] Y. Yabusaki, *Cytochrome P450 Protocols*, Totowa, New Jersey, Humana Press Incorporated, **1998**.

[4] C.M. Jenkins, I. Pikuleva, and M.R. Waterman, *Cytochrome P450 Protocols*, Totowa, New Jersey, Humana Press Incorporated, **1998**.

[5] S.R. Hood, G. Shah, and P. Jones, *Cytochrome P450 Protocols*, Totowa, New Jersey, Humana Press Incorporated, **1998**.