

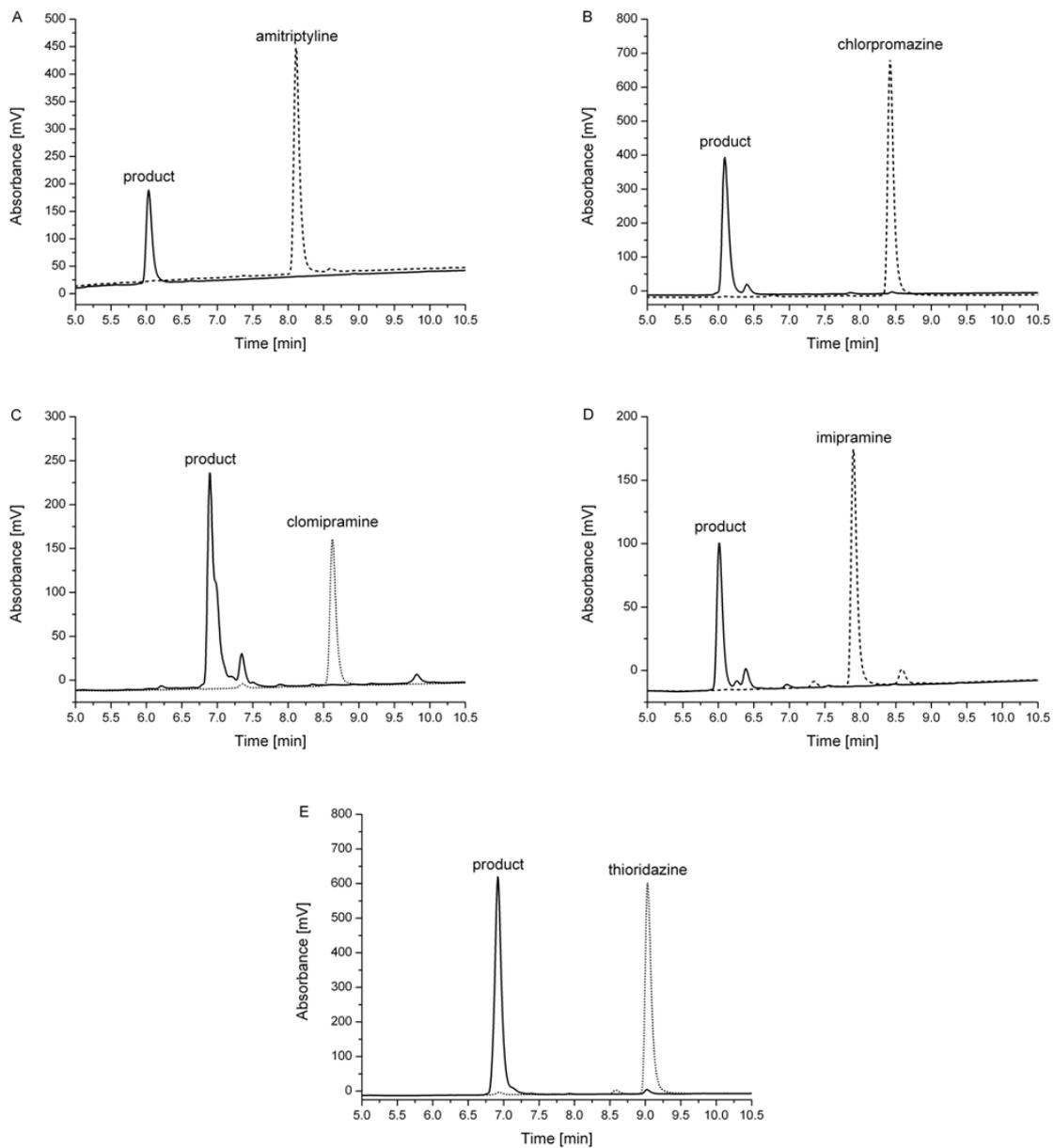
Drug metabolism and disposition

“Conversions of tricyclic Antidepressants and Antipsychotics with selected P450s from *Sorangium cellulosum* So ce56”

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1. Chromatograms of the purified products as well as the pure substrates



Supplemental Figure 1: Chromatograms of the purified products (solid lines) as well as the pure substrates (dashed lines): amitriptyline (A), chlorpromazine (B), clomipramine (C), imipramine (D) and thioridazine (E).

2. ¹H NMR, ¹³C NMR and GC-MS data

The EI spectra showed a complete fragmentation of the molecules and the relative intensities of the fragments are shown in brackets.

Conversion of amitriptyline by CYP264A1:

NMR-data:

¹H NMR (CDCl₃, 500 MHz): δ 2.12(s, 6H, H-15 and H-16), 2.21-2.35 (m, 4H, H-13 and H-14), 3.02 (dd, 1H, H-11), 3.59 (dd, 1H, H-11), 5.05 (d, 1H, H-10), 5.89 (t, 1H, H-12), 7.12-7.44 (m, 8H, H-1, H-2, H-3, H-4, H-6, H-7, H-8 and H-9); ¹³C NMR (CDCl₃, 125 MHz): δ 28.08 (C-13), 39.49 (C-11), 45.50 (C-15 and C-16), 59.60 (C-14), 70.27 (C-10), 126.82 (C-7), 127.89 (C-3), 128.12 (C-2), 128.52 (C-8), 128.72 (C-4), 130.26 (C-12), 130.79 (C-1), 131.46 (C-9), 133.99 (C-1a), 138.98 (C-9a), 140.40 (C-6a), 141.34 (C-4a), 142.98 (C-5).

EI mass spectra:

m/z 58.02 (100%), 41.98 (6%), 202.16 (5%), 215.32 (3%), 189.12 (3%), 165.13 (2%), 59.03 (2%), 42.59 (2%), 202.93 (2%), 217.21 (2%).

Conversion of chlorpromazine by CYP264A1:

¹H NMR (CDCl₃, 500 MHz): δ 1.98-2.08 (m, 2H, H-12), 2.29 (s, 6H, H14 and H15), 2.43 (dt, 2H, H-13), 4.29 (t, 2H, H-11), 7.19 (dd, 1H, H-3), 7.26 (dd, 1H, H-7), 7.50 (d, 1H, H-9), 7.59 (d, 1H, H-1), 7.61 (m, 1H, H-8), 7.84 (d, 1H, H-8), 7.91 (dd, 1H, H-6); ¹³C NMR (CDCl₃, 125 MHz): δ 24.62 (C-12), 45.73 (C-14 and C15), 46.07 (C-11), 56.53 (C-13), 115.98 (C-1), 116.10 (C-9), 121.95 (C-3), 122.28 (C-7), 131.71 (C-6), 132.84 (C-4), 133.04 (C-8), 137.90 (C-9a), 139.02 (C-1a), 142.28 (C-4a), 142.84 (C-6a).

EI mass spectra:

m/z 58.00 (100%), 245.99 (97%), 248.11 (33%), 42.02 (28%), 214.13 (22%), 233.08 (19%), 247.19 (19%), 83.89 (12%), 44.07 (9%), 232.24 (9%).

Conversion of clomipramine by CYP264A1:

¹H NMR (CDCl₃, 500 MHz): δ 1.67-1.74 (m, 2H, H-13), 2.10 (s, 6H, H-13 and H-14), 2.27 (t, 2H, H-14), 3.15 (dd, 1H, H-11), 3.41 (dd, 1H, H11), 3.75 (t, 2H, C-12), 4.96-5.09 (m, 1H, H-10), 6.89-7.39 (m, 7H, H-1, H-2, H-4, H-6, H-7, H-8, H-9); ¹³C NMR (CDCl₃, 125 MHz): δ 25.95 (C-13), 39.36 (C-11), 45.42 (C-15 and C-16), 48.74 (C-12), 57.49 (C-14), 69.96 (C-10), 118.86 (C-4), 121.02 (C-8), 121.94 (C-2), 123.94 (C-6), 127.12 (C-9), 130.54 (C-7), 132.15 (C-9a), 132.47 (C-1), 133.43 (C-1a), 147.65 (C-6a), 148.48 (C-4a).

EI mass spectra:

m/z 58.09 (100%), 84.78 (26%), 180.21 (20%), 85.32 (19%), 285.02 (18%), 57.45 (17%), 42.22 (15%), 226.92 (14%), 253.71 (14%), 83.90 (12%).

Conversion of imipramine by CYP264A1:

¹H NMR (CDCl₃, 500 MHz): δ 1.69-1.77 (m, 2H, H-11), 2.11 (s, 6H, H-15 and H-16), 2.29 (t, 2H, H-14), 3.19 (dd, 1H, H-11), 3.45 (dd, 1H, H-11), 3.73-3.84 (m, 2H, H-12), 5.06 (dd, 1H, H-10), 6.94- 7.21 (m, 8H, H-1, H-2, H-3, H-4, H-6,H-7, H-8, H-9); ¹³C NMR (CDCl₃, 125 MHz): δ 26.03 (C-13), 39.81 (C-11), 45.41 (C-15 and C-16), 48.57 (C-12), 57.63 (C-14), 70.48 (C-10), 118.90 (C-8), 120.54 (C-2), 122.25 (C-4), 123.33 (C-6), 126.89 (C-3), 128.04 (C-7), 130.57 (C-9), 130.69 (C-1), 131.97 (C-1a), 134.33 (C-9a), 146.76 (C-6a), 148.76 (C-4a).

El mass spectra:

m/z 58.00 (100%), 85.10 (29%), 180.09 (27%), 193.11 (27%), 42.02 (23%), 251.22 (19%), 194.14 (16%) 232.22 (15%), 206.23 (12%), 84.04 (12%)

Conversion of thioridazine by CYP267A1:

The use of the racemic mixture of (*R*)- and (*S*)-thioridazine as substrate leads to their respective enantiomeric products. As a consequence, the NMR-signals are duplicated. The enantiomeric signals are labeled as a and b.

¹H NMR (CDCl₃, 500 MHz): δ 1.33 (m, 2H, H-16a), 1.76 (m, 2H, H-16b), 1.52 (m, 2H, H-15a), 1.65 (m, 2H, H-15b), 1.93 (d, 2H, H-12a), 2.19 (d, 2H, H-12b), 2.11 (m, 2H, H-14a), 2.28 (m, 2H, H-14b), 2.20 (m, 2H, H-17a), 2.27 (s, 2H, H-19a and H-19b), 2.25 (m, 2H, H-13a and H-13b), 2.37 (s, 2H, H-19a and H-19b), 2.72(s, 2H, H-19a and H-19b) 2.91 (m, 2H, H-17b), 2.58 (s, 3H, H-21a), 2.59 (s, 3H, H-21b) 3.96 (m, 2H, H-11a), 4.05 (m, 2H, H-11b), 6.94 (d, 1H, H-4a), 6.98 (t, 1H, H-4b), 7.07 (dd, 1H, H-7a), 7.11 (dt, 1H, H-7b), 7.17 (d, 1H, H-3a), 7.22 (t, 2H, H-3b), 7.24 (d, 1H, H-1a), 7.26 (d, 1H, H-1b), 7.25 (m, 1H, H-9a), 7.28 (m, 1H, H-9b), 7.45 (m, 1H, H-6a), 7.63 (m, 1H, H-6b), 7.83 (m, 1H, H-8a), 7.93 (m, 1H, H-8b); ¹³C NMR (CDCl₃, 125 MHz): δ 15.37 (C-21), 23.85 (C-16a), 30.33 (C-16b), 25.07 (C-15a), 25.10 (C-15b), 29.13 (C-14a), 29.16 (C-14b), 29.61 (C-12a), 29.64 (C-12b), 44.04 (C-11a), 44.70 (C-11b), 56.66 (C-17a), 56.70 (C-17b), 61.95 (C-13a), 62.20 (C-13b), 109.76 (C-9a), 109.83 (C-9b), 115.95 (C-4a and C-4b), 117.25 (C-7a), 119.05 (C-7b), 124.44 (C-2a and C-2b), 127.62 (C-3a), 127.66 (C-3b), 127.85 (C-1a), 127.89 (C-1b), 129.14 (C-6a-a), 129.17 (C-6a-b), 144.35 (C-4a-a and C-4a-b), 144.94 (C-1a-a), 144.96 (C-1a-b), 145.55 (C-9a-a), 145.64 (C-9a-b).

El mass spectra:

m/z 98.08 (100%), 97.13 (14%), 244.94 (13%), 206.88 (12%), 42.03 (12%), 195.93 (11%), 223.07 (11%), 196.79 (10%), 211.20 (10%), 386.06 (9%)

3. Comparison of CYP264A1 and 267A1 with human CYP2D6

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CYP264A1      -----MSERVDIMTPAFRADPYTPYA 21
CYP267A1      -----MNSPDAPKPDAPPAANPAADADLDPFRLQSPETLANPYPVYA 42
CYP2D6        MGLEALVPLAVIVAI FLLLVDLMHRRQRWAARYPPGFLPLPGLGNLLHVDFQNTPYCFDQ 60

CYP264A1      AMRREAPVC-----QVDPGGMWAVSRYADVATVLRSP-----ERFSSQGF 61
CYP267A1      RLREQEAPVY-----FSAAYNGLWLI TRYDQVAAGFRDPRLSAKRSSAFVTKLF 89
CYP2D6        LRRRFQDVFSLQLAWTPVVVNLGLAAVREALVTHGEDTADRPPVPI TQILGFGPRSQGVF 120

CYP264A1      RAAWQPAWVGHNPLASSILAMDGPDHARLRGLVSRAFGAPAIARIEQ-----RARDLCER 116
CYP267A1      DEVQRLEPLRRNLASWALLDDPPEHTRIRSLINKAFVPRLVEGLRS-----RVETLVNE 144
CYP2D6        LARYGPAWREQRRFSVSTLRNLGLGKKSLEQWVTEEAACLCAAFANHSGRPFPRNGLLDK 180

CYP264A1      LAGRLD--GEVDFIAAAAAPLPAFVISELLGLDHALEPHFKRWMDDL SVT-PEPASA EH 173
CYP267A1      LLDVAVAPAGRMDVLRDLGLDLLPLLVIGEVLVGPAEDRHR LKGWSNALS GFLGAGRPTLEI 204
CYP2D6        AVSNVIASLTCGRREFYDDPRFLRLDLDAQEG LKEESGFLREVLNAV PVLHHPALAGKV 240

CYP264A1      AARVRATIAELDRYMADVIAARR-----RSPSDDL VSELARA-----GELLGDREI IDLLV 224
CYP267A1      AGGALSVAELEDYFRGVIAARR-----QSPGNDLLSQLILAE-EQGMILGEQELLSTCC 258
CYP2D6        LRFQKAFLTQLDELLETEHRMTWDPAQPPRDLTEAF LAEMEKAKGNPESSFN DENLRIVVA 300

CYP264A1      SILGGLETTHFLGSSMLLLAERPAELERLR-----ASPQLIPRFI 266
CYP267A1      MLLFGGHETTKNLIGNLLALLHRSEREARL-----ATPSLIGPAV 300
CYP2D6        DLFSAGMVTTSTTLAWGLLLMILHPDVQRRVQOEID DVIGQVRRPEMGDQAHPYTTAVI 360

CYP264A1      EEMRYDGPTQS-V PRLTTS DVALAGVTI PAGSLVLALVGSANRDEVRFTD PDRFD---- 321
CYP267A1      EELLRYDSPVQW-M SRVALDDI ELDGVRI PKGDRAFLVLGAANRDPAQFPDPDKLD---- 355
CYP2D6        HEVQRFGDIVPLGV THMSTRDIEVQGFRI PKGTTLITNLSSVLKDEAVWEKPF RFHPEHF 420

CYP264A1      -----LHRGQP-SLTFGHGAHFCLGAALARMEAKVALEVLVPRIGEVTRAPGEIPYNRTL 375
CYP267A1      -----FRRTDIRHISLGLGVHYCAGSALARVEAQAAI STFLRRFPDAELSPGPLTWRMNP 410
CYP2D6        LDAQGHFVKPEAFLPFSAGRRACLGEPLARME LFLFFTSLLQHFSFSVPTGQPRPSHHGV 480

CYP264A1      TVRGPVSLPLRFRPA---- 390
CYP267A1      GMRGVTALPIELGPQSSAS 429
CYP2D6        FAFLVSPSPYELCAVPR-- 497

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Supplemental Figure 2: Multiple sequence alignment of human CYP2D6 (AFX95842.1), and the *S. cellulorum* So ce56 CYP264A1 (YP_001616970.1) and CYP267A1 (YP_001611312.1). The amino acid sequences of CYP2D6, CYP264A1 and CYP267A1 were retrieved from EMBL database (<http://www.ebi.ac.uk/embl/>) and aligned by Clustal W2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). The absolutely conserved amino-acid residues are highlighted in grey, and the yellow highlight indicates the interacting residues during docking of imipramine as described (Handa et al., 2014).

Handa K, Nakagome I, Yamaotsu N, Gouda H, and Hirono S (2014) In Silico Study on the Inhibitory Interaction of Drugs with Wild-type CYP2D6.1 and the Natural Variant CYP2D6.17. *Drug Metabolism and Pharmacokinetics* 29:52-60.