Molecular Pharmacology

Supplemental Material

The molecular basis for selective serotonin re-uptake inhibition by the antidepressant agent fluoxetine (Prozac)

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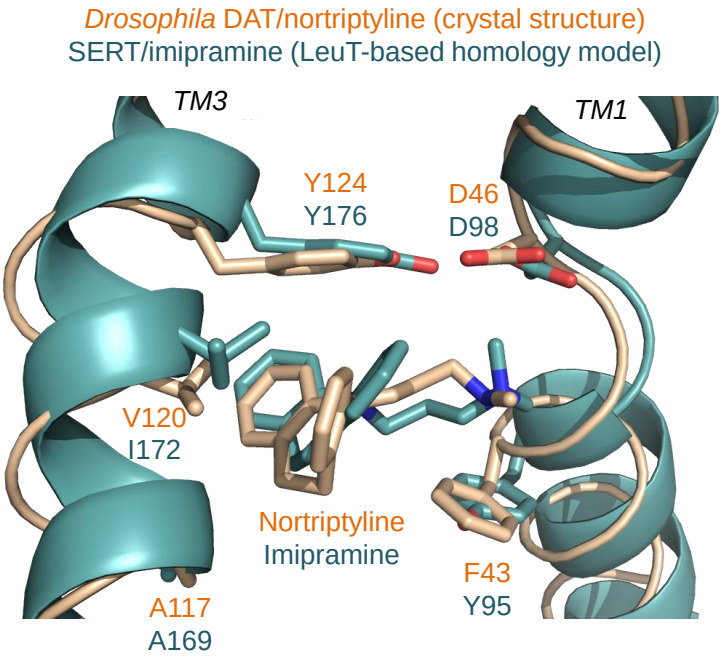
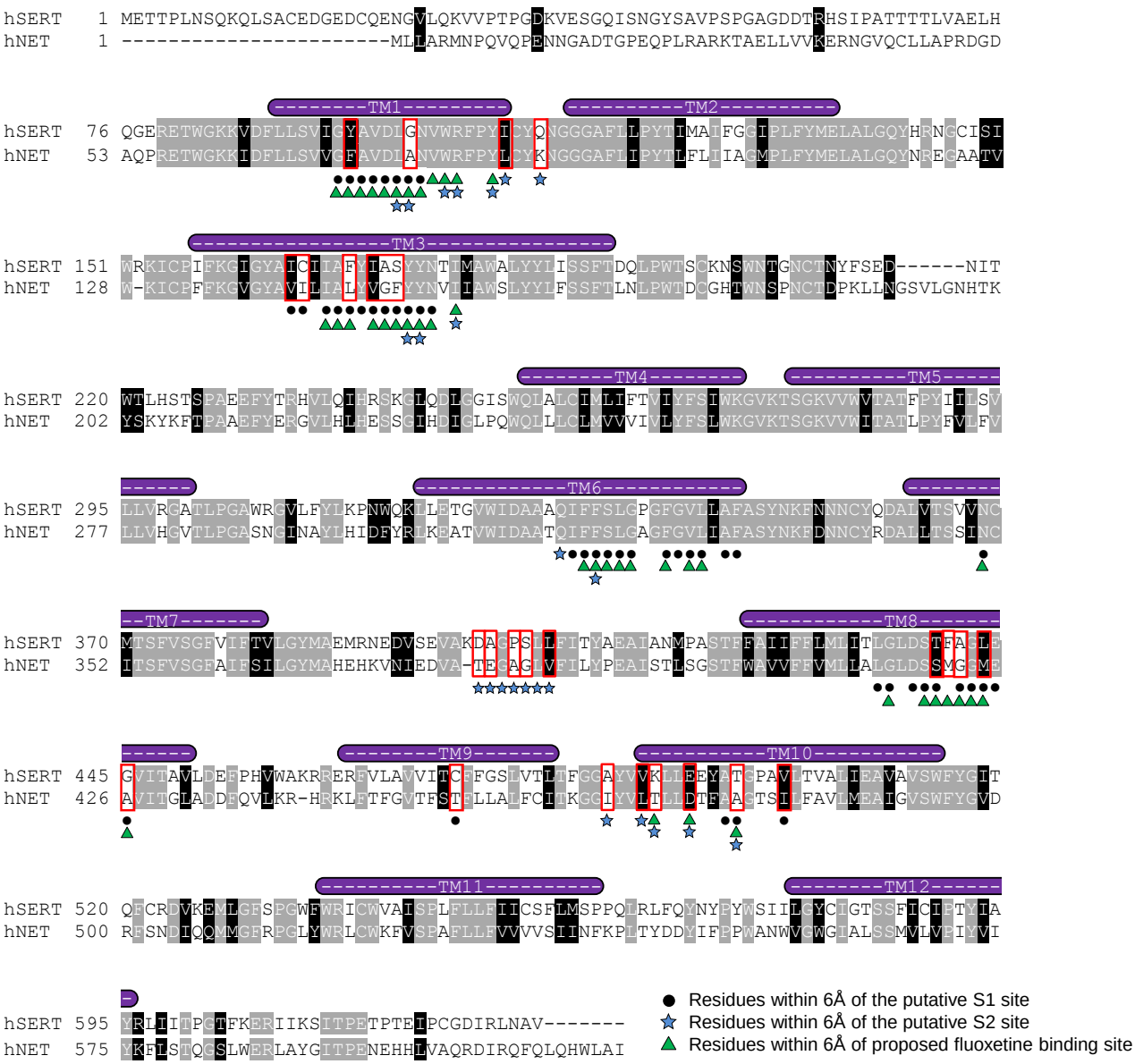
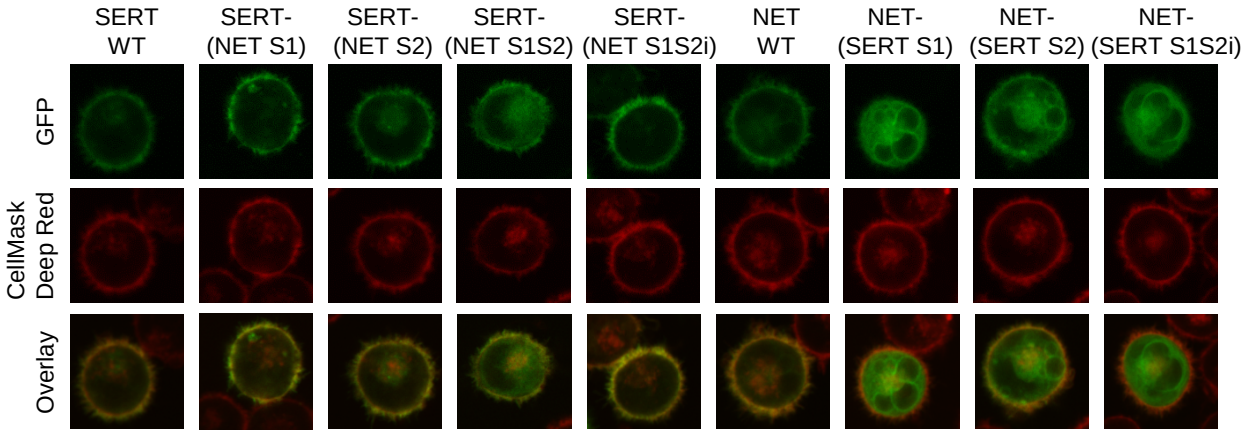


Supplemental Figure 1. Overlay of the crystal structure of *Drosophila* DAT in complex with the TCA nortriptyline (shown in wheat) (PDB ID 4M48) with a LeuT-based model of SERT in complex with the TCA imipramine (shown in teal) (Sinning et al., 2010). Selected residues in proximity of the inhibitor binding sites are shown as stick representations. The overlay illustrates that LeuT-based models are very predictive of inhibitor binding in eukaryotic transporters, and thus verifies the use of LeuT as structural template for human transporters.



Supplemental Figure 2. Amino acid sequence alignment of hSERT and hNET based on a previously published alignment of LeuT and *SLC6* transporters.(Beuming et al., 2006) Conserved residues are shown as white text on grey background, similar residues are shown as white text on black background and non-conserved residues are shown as black text on white background. Overall, 44% of the residues are conserved and 22% of the residues are similar in hSERT and hNET. The putative TM regions are depicted on top of the alignment. The filled black circles indicate residues that are located within 6Å of the putative S1 binding site [32 out of 47 residues (68%) are conserved], blue stars indicate residues that are located within 6Å of the putative S2 binding site [11 out of 24 residues (46%) are conserved] and green triangles indicate residues that are located within 6Å of the proposed binding site for fluoxetine in hSERT [28 out of 42 residues (67%) are conserved]. Non-conserved residues within these regions are highlighted in red boxes. The S1 site was defined as the proposed escitalopram binding site in hSERT (Andersen et al., 2010) and the S2 site was defined as the equivalent position in hSERT as the binding site for imipramine found in LeuT (PDB ID: 2Q72). The proposed fluoxetine binding site was defined from a representative pose of fluoxetine binding in SERT from the dominating SERT-Cluster 1 (see Fig. 2 and Fig. 3).



Supplemental Figure 3. Expression and cellular distribution of GFP-tagged versions of WT and mutant forms of SERT and NET where non-conserved SERT/NET residues within 6Å of putative binding regions have been interchanged (see also Supplemental Figure 1 and Supplemental Table 5). WT and mutant forms of GFP-tagged SERT and NET were transiently transfected into COS7 cells and the plasma membrane was visualized with CellMask Deep Red plasma membrane stain. Confocal laser scanning microscopy images show that all SERT mutants and NET-(SERT S2) displayed surface expression patterns comparable to WT transporters, whereas NET-(SERT S1) and NET-(SERT S1S2i) showed loss of cell-surface expression.

Supplemental Table 1. Impact of mutations on [^{3}H]5HT uptake kinetics and fluoxetine potency

Site	Mutant	K_m		Uptake activity	Fluoxetine K_i		Fold-change in K_i-value
		µM	*n*	*% of WT*	*nM*	*n*	
	WT	0.98 ± 0.09	30	100	110 ± 8	35	
S1	Y95A[a]	0.22 ± 0.04	3	45 ± 4	5,129 ± 243*	3	-46.3
S1	Y95V[a]	2.35 ± 0.31	4	7 ± 1	4,629 ± 1,283*	4	-41.8
S1	Y95W	2.16 ± 0.25	4	28 ± 6	13 ± 2*	8	8.4
S1	D98E[a]	0.22 ± 0.06	3	65 ± 8	1,300 ± 190*	6	-11.7
S1	I168A	0.93 ± 0.11	5	59 ± 4	25 ± 5*	4	4.4
S1	I168F	1.15 ± 0.07	5	41 ± 4	10 ± 1*	4	11.4
S1	A169D[a]	0.62 ± 0.06	3	89 ± 2	29 ± 7*	3	3.8
S1	I172A[a]	1.08 ± 0.34	5	63 ± 5	663 ± 64*	4	-6.0
S1	I172M[a]	0.77 ± 0.05	4	86 ± 3	8,786 ± 708*	7	-79.4
S1	I172Q[a]	3.12 ± 0.72	3	21 ± 3	2,002 ± 261*	4	-18.1
S1	N177A[a]	7.65 ± 0.42	3	42 ± 2	811 ± 209*	4	-7.3
S1	N177E[a]	1.55 ± 0.14	3	76 ± 1	124 ± 38	4	-1.1
S1	N177S[a]	4.25 ± 0.12	3	63 ± 1	2,708 ± 125*	3	-24.5
S1	F341A[a]	0.77 ± 0.18	3	51 ± 7	125 ± 24	4	-1.1
S1	F341I[a]	0.40 ± 0.11	3	44 ± 2	16 ± 2*	6	7.0
S1	F341L[a]	0.54 ± 0.1	3	52 ± 2	29 ± 5*	6	3.8
S1	F341Y[a]	0.08 ± 0.02	3	57 ± 8	524 ± 96*	4	-4.7
S1	V343N[a]	0.24 ± 0.01	3	20 ± 1	24 ± 7*	6	4.6
S1	V343S[a]	0.43 ± 0.304	3	87 ± 2	39 ± 6*	3	2.9
S1	S438T[a]	0.14 ± 0.02	3	20 ± 4	364 ± 60*	4	-3.3
S1/S2	R104A	*N.F.*					
S1/S2	Y175F[a]	0.24 ± 0.08	3	54 ± 5	34 ± 3*	6	3.2
S1/S2	Y175V	3.70 ± 0.61	5	49 ± 6	201 ± 45*	4	-1.8
S1/S2	Y176F[a]	0.88 ± 0.04	3	86 ± 5	94 ± 15	3	1.2
S1/S2	F335E[a]	0.29 ± 0.09	3	7 ± 1	45 ± 5*	3	2.5
S1/S2	F335H[a]	0.68 ± 0.18	4	13 ± 2	100 ± 14	4	1.1
S1/S2	F335Y[a]	0.62 ± 0.2	3	73 ± 7	148 ± 77	4	-1.3
S1/S2	F335W[a]	0.06 ± 0.01	3	63 ± 11	342 ± 64*	5	-3.1
S2	W103A	0.96 ± 0.09	5	15 ± 2	17 ± 5*	3	6.3
S2	W103E	*N.F.*					
S2	I179C	0.53 ± 0.11	3	19 ± 1	37 ± 4*	3	3.0
S2	I179H	*N.F.*					
S2	W182A	2.22 ± 0.26	5	86 ± 6	194 ± 40*	3	-1.8
S2	W182E	5.66 ± 3.79	5	112 ± 12	97 ± 35	3	1.1
S2	Y232A	0.19 ± 0.02	5	39 ± 4	82 ± 9	3	1.4
S2	Y232E	0.48 ± 0.08	5	48 ± 5	82 ± 16	4	1.3
S2	Y232F	1.33 ± 0.18	5	69 ± 7	160 ± 22	3	-1.4
S2	V236A	1.29 ± 0.05	5	67 ± 7	97 ± 16	3	1.1
S2	V236E	*N.F.*					
S2	V236F	0.42 ± 0.05	5	20 ± 2	31 ± 9*	4	3.6
S2	G338D	*N.F.*					
S2	G338F	*N.F.*					
S2	D400F[a]	0.18 ± 0.03	3	37 ± 4	67 ± 34	4	1.6
S2	D400K[a]	0.12 ± 0.03	3	21 ± 3	64 ± 17	4	1.7
S2	D400L[a]	0.60 ± 0.30	3	23 ± 3	94 ± 48	4	1.2
S2	A401E	0.55 ± 0.13	3	21 ± 2	55 ± 4	3	2.0
S2	G402H	*N.F.*					
S2	P403A	1.49 ± 0.08	5	85 ± 9	84 ± 29	4	1.3
S2	P403E	0.30 ± 0.04	5	17 ± 2	21 ± 8*	3	5.2
S2	P403F	0.67 ± 0.14	5	41 ± 5	53 ± 11*	4	2.1
S2	A486E	0.51 ± 0.09	5	59 ± 7	86 ± 13	6	1.3
S2	A486I	0.46 ± 0.06	5	60 ± 8	173 ± 29*	6	-1.6
S2	V489F[a]	0.63 ± 0.43	3	110 ± 9	189 ± 71*	5	-1.7
S2	K490D[a]	0.16 ± 0.02	4	61 ± 4	77 ± 36	4	1.4
S2	K490T[a]	0.27 ± 0.09	4	109 ± 4	153 ± 44	5	-1.4
S2	K490F[a]	0.17 ± 0.06	3	95 ± 5	232 ± 69*	5	-2.1
S2	E493A	1.50 ± 0.11	5	55 ± 8	99 ± 14	4	1.1
S2	E493F	*N.F.*					
S2	E493K	*N.F.*					

Uptake activity, substrate K_m values and fluoxetine K_i values were determined in a [^{3}H]5HT uptake assay. Results are presented as mean ± SEM from *n* indipendent experiments each performed in triplicate. The fold-changes in K_i values were calculated as K_i(WT)/K_i(mutant) or -K_i(mutant)/K_i(WT) for mutants increasing or decreasing, respectively, the potency of fluoxetine. *Asterisks* denote significantly different K_i value compared to WT ($p < 0.01$; Student's *t*-test). N.F. = non-functional.

[a] Values for K_m and uptake activity are taken from (Andersen et al., 2009, 2010, 2011) and are included for comparison.

Supplemental Table 2. Inhibitory potency of fluoxetine and analogues at selected S1 mutants in SERT

	Fluoxetine (**1**)		*R*-Fluoxetine (*R*-**1**)		*S*-Fluoxetine (*S*-**1**)		Nisoxetine (**2**)	
	nM	*n*	*nM*	*n*	*nM*	*n*	*nM*	*n*
SERT WT	110 ± 8	35	388 ± 38	3	332 ± 43	3	1,422 ± 162	61
Y95A	5,129 ± 243*	3	7,105 ± 277*	3	4,983 ± 692*	3	15,144 ± 2,059*	3
Y95W	13 ± 2*	8	*N.D.*		*N.D.*		356 ± 88	3
D98E	1,300 ± 190*	6	2,722 ± 457*	3	687 ± 62*	3	3,821 ± 558*	3
I172M	8,786 ± 708*	7	10,799 ± 1,148*	3	12,904 ± 1,495*	3	210,739 ± 36,687*	5
Y175F	34 ± 3*	6	55 ± 10*	3	68 ± 6*	3	255 ± 19	3
N177S	2,708 ± 125*	3	4,082 ± 759*	3	2,806 ± 176*	3	17,023 ± 4,456*	3

	N-Methylfluoxetine (**7**)		Des-CF_3-fluoxetine (**8**)		2-OCH_3-fluoxetine (**9**)		Homofluoxetine (**10**)	
	nM	*n*	*nM*	*n*	*nM*	*n*	*nM*	*n*
SERT WT	2,587 ± 343	7	3,793 ± 501	7	44 ± 4	21	1,026 ± 127	11
Y95A	16,963 ± 1,673*	4	6,134 ± 1,069*	4	3,307 ± 291*	5	4,615 ± 599*	3
Y95W	*N.D.*		*N.D.*		54 ± 6	4	42 ± 8*	4
D98E	2,766 ± 297	4	7,257 ± 1,069*	4	179 ± 34*	8	4,401 ± 487*	3
I172M	26,210 ± 3,315*	7	20,273 ± 3,571*	7	5,695 ± 569*	10	25,113 ± 1,881*	3
Y175F	446 ± 61*	8	583 ± 81*	4	15 ± 4*	4	350 ± 101*	4
N177S	15,801 ± 1,638*	8	6,040 ± 1,501	4	567 ± 123*	5	13,787 ± 3,071*	3

Inhibitor K_i values were determined in a [^{3}H]5HT uptake assay. Results are presented as mean ± SEM from *n* independent experiments each performed in triplicate. *Asterisks* denote significantly different inhibitor K_i value compared to WT ($p < 0.05$; Student's *t*-test). N.D. = not determined. K_i values for fluoxetine are taken from Supplemental Table 1, and are included for comparison.

Supplemental Table 3. Impact of mutations of non-conserved residues in the S1 site of SERT on uptake kinetics and inhibitor potency

Mutant	Residues mutated	K_m	Uptake activity	Fluoxetine K_i	Nisoxetine K_i
		µM	*% of WT*	*nM*	*nM*
SERT WT		0.98 ± 0.09	100	110 ± 8	1,422 ± 162
Y95F		0.35 ± 0.09	85 ± 5	387 ± 9*	3,447 ± 697*
G100A		0.57 ± 0.08	60 ± 3	75 ± 21	296 ± 88
I165V		0.73 ± 0.08	82 ± 2	261 ± 55	1,396 ± 304
C166I		0.87 ± 0.16	97 ± 2	315 ± 98*	1,678 ± 469
F170L		0.65 ± 0.08	83 ± 1	323 ± 69*	981 ± 204
I172V		0.73 ± 0.20	36 ± 3	162 ± 29	657 ± 97
A173G		0.26 ± 0.07	53 ± 11	189 ± 11	752 ± 25
S174F		0.63 ± 0.05	70 ± 3	274 ± 90	488 ± 103
T439S		0.75 ± 0.10	78 ± 6	822 ± 63*	1,659 ± 66
A441G		0.37 ± 0.09	72 ± 2	61 ± 20*	544 ± 172
L443M		0.49 ± 0.11	58 ± 5	231 ± 32	486 ± 79
G445A		0.73 ± 0.17	85 ± 3	295 ± 78*	1,662 ± 319
C473T		0.87 ± 0.24	74 ± 7	533 ± 63*	1,541 ± 171
T497A		1.03 ± 0.41	71 ± 5	138 ± 25	524 ± 143
V501I		2.87 ± 1.06	90 ± 3	128 ± 35	325 ± 114
S1	Y95A-G100A	0.67 ± 0.08	51 ± 3	183 ± 19	2,575 ± 359
S2	I172V-A173G-S174F	1.89 ± 0.50	78 ± 1	1,226 ± 220*	4,186 ± 237*
S3	G100A-I172V-A173G-S174F	1.49 ± 0.09	21 ± 3	708 ± 166*	1,272 ± 166
S4	G100A-L443M-G445A	1.50 ± 0.28	29 ± 5	324 ± 90*	1,472 ± 533
S5	L443M-G445A	2.62 ± 0.23	71 ± 4	240 ± 58	1,430 ± 302
S6	Y95A-G100A-L443M-G445A	1.12 ± 0.22	16 ± 4	111 ± 27	933 ± 269
S7	Y95A-G100A-I172V-A173G-S174F	0.85 ± 0.12	36 ± 5	544 ± 147*	3,110 ± 410*
S8	I172V-A173G-S174F-L443M-G445A	2.76 ± 0.38	57 ± 4	1,160 ± 227*	2,425 ± 334
S9	L443M-G445A-C473T	2.21 ± 0.34	90 ± 3	249 ± 82	766 ± 138
S10	Y95A-G100A-L443M-G445A-C473T	1.07 ± 0.05	54 ± 8	85 ± 16	1,332 ± 260
S11	Y95A-G100A-I172V-A173G-S174F-L443M-G445A-C473T	1.82 ± 0.41	31 ± 1	416 ± 17*	412 ± 212
S12	G100A-I172V	0.36 ± 0.11	19 ± 3	48 ± 11*	602 ± 229
S13	G100A-A173G	0.35 ± 0.12	13 ± 3	55 ± 7*	326 ± 78
S14	G100A-S174F	1.09 ± 0.33	31 ± 3	63 ± 18*	85 ± 15*
S15	I172V-L443M	0.41 ± 0.13	13 ± 2	65 ± 8	193 ± 41
S16	A173G-L443M	3.02 ± 0.94	18 ± 1	111 ± 29	75 ± 17*
S17	S174F-L443M	1.43 ± 0.38	53 ± 6	150 ± 22	132 ± 24*
S18	G100A-I172M-L443M	0.52 ± 0.20	15 ± 1	85 ± 45	561 ± 20
S19	G100A-A173G-L443M	0.77 ± 0.30	65 ± 4	79 ± 20	348 ± 114
S20	G100A-S174F-L443M	0.72 ± 0.27	47 ± 4	107 ± 22	876 ± 175
S21	G100A-I172M-A173G-S174F-L443M	1.21 ± 0.44	21 ± 2	208 ± 73	361 ± 57
S22	I172M-A173G-S174F-L443M	0.65 ± 0.29	39 ± 3	337 ± 51*	453 ± 109
S23	G100A-L443M	1.66 ± 0.97	10 ± 2	110 ± 73	169 ± 65
S24	A173G-S174F-L443M	0.70 ± 0.17	43 ± 5	129 ± 40	140 ± 31*
S25	G100A-A173G-S174F-L443M	3.70 ± 2.20	6 ± 1	55 ± 11	71 ± 20*
S26	G100A-A173G-S174F-A441G-L443M	*N.F.*			
S27	A173G-A441G-L443M	0.30 ± 0.16	56 ± 9	26 ± 5*	173 ± 64
S28	A173G-S174F-A441G-L443M	0.50 ± 0.14	40 ± 8	44 ± 11*	127 ± 23*
S29	S174F-A441G-L443M	2.32 ± 0.17	6 ± 1	20 ± 8*	60 ± 18*
S30	I172V-A173G-S174F-T439S	0.97 ± 0.48	33 ± 4	586 ± 200*	651 ± 200

Uptake activity, substrate K_m values and K_i values for fluoxetine and nisoxetine were determined in a [^{3}H]5HT uptake assay. Results are presented as mean ± SEM from 3 - 61 independent experiments each performed in triplicate. *Asterisks* denote significantly different inhibitor K_i value compared to WT ($p < 0.05$; Student's *t*-test). N.F. = non-functional. Values for substrate K_m and uptake activity for the 15 single mutants and multiple mutants S1-S10 are from (Andersen et al., 2011), and are included for comparison.

Supplemental Table 4. Impact of mutations of non-conserved residues in the S1 site of NET on uptake kinetics and inhibitor potency

Mutant	Residues mutated	K_m	Uptake activity	Fluoxetine K_i	Nisoxetine K_i
		µM	*% of WT*	*nM*	*nM*
NET WT		0.57 ± 0.09	100	2,993 ± 266	6 ± 1
F72Y		0.26 ± 0.16	28 ± 3	1,230 ± 213	1 ± 0.3
A77G		0.30 ± 0.04	46 ± 4	317 ± 97*	2 ± 1
V141I		0.47 ± 0.06	10 ± 1	318 ± 76*	1 ± 0.1
I142C		0.14 ± 0.01	52 ± 2	985 ± 507*	4 ± 1
L146F		0.11 ± 0.03	52 ± 3	683 ± 247*	2 ± 0.3
V148I		0.35 ± 0.05	18 ± 3	276 ± 114*	4 ± 2
G149A		0.39 ± 0.21	29 ± 4	689 ± 193*	19 ± 6*
F150S		0.37 ± 0.16	10 ± 2	3,515 ± 413	20 ± 6*
S420T		0.29 ± 0.08	16 ± 4	582 ± 93*	6 ± 2
G422A		0.08 ± 0.02	65 ± 9	442 ± 52*	2 ± 1
M424L		0.25 ± 0.16	43 ± 8	390 ± 33*	1 ± 0.4
A426G		0.07 ± 0.01	51 ± 3	627 ± 102*	3 ± 1
T453C		0.41 ± 0.15	23 ± 3	484 ± 83*	3 ± 1
T477A		1.08 ± 0.40	65 ± 5	2,363 ± 418	9 ± 1
I481V		0.84 ± 0.36	83 ± 3	1,077 ± 155*	13 ± 2*
N1	F72Y-A77G	5.71 ± 1.25	26 ± 2	746 ± 92*	3 ± 1
N2	V148I-G149A-F150S	*N.F.*			
N3	A77G-M424L-A426G	0.79 ± 0.24	60 ± 2	399 ± 83*	4 ± 1
N4	M424L-A426G	0.56 ± 0.26	68 ± 3	754 ± 161*	3 ± 0
N5	F72Y-A77G-M424L-A426G	1.50 ± 0.44	28 ± 8	412 ± 77*	6 ± 4
N6	M424L-A426G-T453C	0.60 ± 0.26	39 ± 5	299 ± 101*	4 ± 1
N7	F72Y-A77G-M424L-A426G-T453C	8.06 ± 1.98	35 ± 6	308 ± 112*	4 ± 0
N8	F72Y-M424L-A426G	0.21 ± 0.08	61 ± 4	1,324 ± 296*	2 ± 0
N9	A77G-M424L-A426G-T453C	0.78 ± 0.27	22 ± 7	217 ± 30*	4 ± 1
N10	V148I-S420T	*N.F.*			
N11	V141I-V148I-S420T	*N.F.*			
N12	V141I-T453C	*N.F.*			
N13	V148I-S420T-T453C	*N.F.*			
N14	V141I-S420T-T453C	*N.F.*			
N15	V141I-T453C	0.87 ± 0.38	12 ± 1	448 ± 25*	11 ± 3
N16	A77G-V141I	1.18 ± 0.07	7 ± 3		
N17	G149A-F150S-M424L	*N.F.*		367 ± 24*	4 ± 1

Uptake activity, substrate K_m values and K_i values for fluoxetine and nisoxetine were determined in a [^{3}H]DA uptake assay. Results are presented as mean ± SEM from 3 - 48 independent experiments each performed in triplicate. *Asterisks* denote significantly different inhibitor K_i value compared to WT ($p < 0.05$; Student's *t*-test). N.F. = non-functional. Values for substrate K_m and uptake activity for the 15 single mutants and multiple mutants N1-N8 are from (Andersen et al., 2011), and are included for comparison.

Supplemental Table 5. Binding affinity of fluoxetine and nisoxetine

Table S5. Binding affinity of fluoxetine and nisoxetine

Mutant	[^{125}I]β-CIT[a]			Fluoxetine K_i		Nisoxetine K_i	
	K_d, *nM*	B_{max}, *pmol/mg*	*n*	*nM*	*n*	*nM*	*n*
SERT WT	6 ± 2	6 ± 3	5	7 ± 2	7	167 ± 31	7
SERT-(NET S1)[b]	8 ± 3	4 ± 0.2	4	102 ± 16*	4	29 ± 4*	4
SERT-(NET S2)[c]	3 ± 1	11 ± 2	4	3 ± 0.1	3	73 ± 8	3
SERT-(NET S1S2)[d]	13 ± 1	2 ± 0.1	4	10 ± 4	4	15 ± 7*	4
SERT-(NET S1S2i)[e]	3 ± 1	11 ± 3	4	65 ± 5*	3	95 ± 17	3
NET WT	13 ± 0.2	10 ± 6	3	887 ± 115	5	4 ± 1	5
NET-(SERT S1)[f]	*no specific binding*						
NET-SERT S2)[g]	2 ± 0.1	3 ± 1	4	7,831 ± 2,644*	3	104 ± 4*	3
NET-(SERT S1S2i)[h]	*no specific binding*						

The binding affinities of fluoxetine and nisoxetine were determined in a [^{125}I]β-CIT compeition binding assay. Results are presented as mean ± SEM from *n* independent experiments each performed in dublicate. *Asterisks* denote significantly different inhibitor K_i value compared to WT ($p < 0.05$; Student's *t*-test).

[a] K_d and B_{max} values determined from a saturation binding assay.

[b] hSERT-Y95F-G100A-I165V-C166I-F170L-I172V-A173G-S174F-T439S-A441G-L443M-G445A-C473T-T497A-V501I.

[c] hSERT-G100A-I108L-Q111K-D400T-A401E-P403A-S404G-L406V-A486I-V489L-K490T-E493D-T497A.

[d] hSERT-Y95F-G100A-I108L-Q111K-I165V-C166I-F170L-I172V-A173G-S174F-D400T-A401E-P403A-S404G-L406V-T439S-A441G-L443M-G445A-C473T-A486I-V489L-K490T-E493D-T497A-V501I.

[e] hSERT-Y95F-G100A-F170L-I172V-A173G-S174F-T439S-F440M-A441G-L443M-G445A-K490T-E493D-T497A.

[f] hNET-F72Y-A77G-V141I-I142C-L146F-V148I-G149A-F150S-S420T-G422A-M424L-A426G-T453C-A477T-I481V.

[g] hNET-A77G-L85I-K88Q-T381D-E382A-A384P-G385S-V387L-I466A-L469V-T470K-D473E-A477T.

[h] hNET-F72Y-A77G-L146F-V148I-G149A-F150S-S420T-M421F-G422A-M424L-A426G-T470K-D473E-A477T.

SUPPLEMENTAL METHODS

Materials. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin, and penicillin-streptomycin was purchased from Invitrogen. All DNA restriction enzymes and T4 DNA ligase enzyme were from New England Biolabs. Cell culture dishes were from Sarstedt AG & Co, and white 96-well plates were from Nunc. [^{3}H]5HT (27–28 Ci/mmol), [^{3}H]DA (91–139 Ci/mmol), [^{125}I]*β*-CIT (2200 Ci/mmol), MicroScint-0 and -20 scintillation mixtures, and 96-well glass fibre filter plates were obtained from PerkinElmer Life Sciences. Polyethyleneimine, *R*- and *S*-fluoxetine (HCl salts), 5-HT (creatine sulfate complex) and DA (HCl salt) were purchased from Sigma-Aldrich, and *β*-CIT were purchased from ABX.

Cell culturing and expression of SERT and NET. Cell culturing and transfection was performed as described.(Andersen et al., 2011) Briefly, COS7 cells were cultured in DMEM media with 10% FBS, 100U/mL pencillin and 100µg/mL streptomycin at 37°C in a humidified 5% CO_2 environment. Cells were transfected using TransIT LT-1 DNA transfection reagent by following the protocol supplied by the manufacturer. Before transfection, confluent cells growing in monolayer were resuspended in DMEM at a concentration of $1.3x10^6$ cells pr. mL. Per 96-well plate, 7µg of DNA and 13µL of transfection reagent were mixed in 0.4mL of DMEM and incubated at 20°C for 20 min. Subsequently this mixture was added to the cell suspension and, immediately afterward, the cells were dispensed into white 96-well plates at 50% confluence.

Uptake assays. Uptake assays were performed 40-48 h after transfection. Cells were washed twice with PBSCM buffer (in mM: NaCl, 137; KCl, 2.7; Na_2HPO_4, 4.3; KH_2PO_4, 1.4; $CaCl_2$, 0.1; $MgCl_2$, 0.5) on an ELx50 automated microplate strip washer (BioTek Instruments) and left with PBSCM for 20 min prior to uptake experiments. In all NET assays, the PBSCM buffer was supplemented with 1 mM L-ascorbic acid.
In inhibition studies (IC_{50} determinations), cells were preincubated in 40µL of PBSCM per well with 0 or increasing concentrations of inhibitor for 30 min before addition of 10µL of PBSCM containing [^{3}H]5HT (SERT assays) or [^{3}H]DA (NET assays) giving a final substrate concentration of 50nM. Uptake was allowed to proceed at 20°C for 10 min before uptake was terminated as described below. For determination of K_M-values, cells were incubated with increasing concentration of cold substrate (5-HT in SERT assays and DA in NET assays) in PBSCM and 50 nM [^{3}H]5-HT (SERT assays) or 50 nM [^{3}H]DA (NET assays) at 20°C for 10 min. Uptake was terminated by washing three times with PBSCM (ELx50 automated microplate strip washer). The amount of accumulated radiolabelled substrate was determined by solubilising cells in scintillant cocktail (MicroScint-20) with counting of plates in a Packard TopCounter. Non-specific uptake was determined as uptake in non-transfected cells and total uptake was determined in the presence of PBSCM buffer alone. Assays were carried out in triplicate and repeated at least three times.

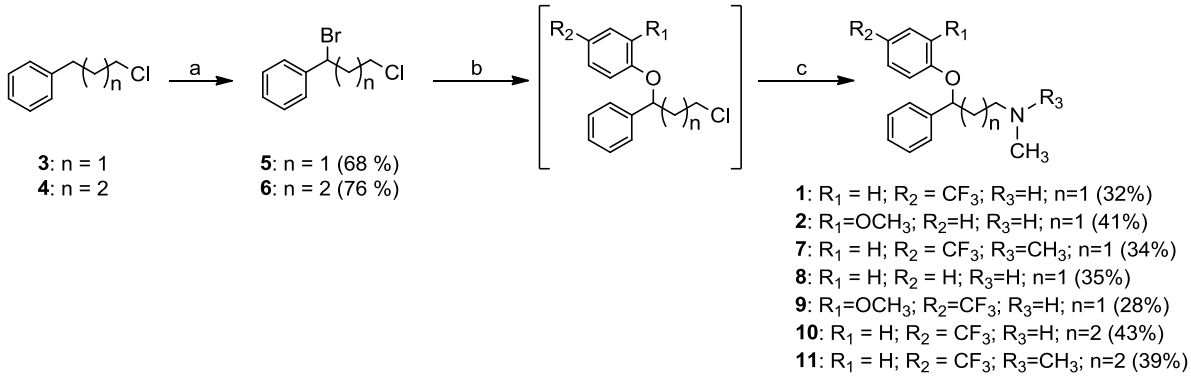
Cell membrane preparation and radioligand binding assay. Cell membranes of COS-7 cells were prepared as previously described.(Andersen et al., 2009) For saturation binding studies, increasing concentrations of [^{125}I]*β*-CIT diluted 1:20 with unlabelled *β*-CIT in PBSCM and 5-30 µg total membrane protein per sample were combined in 96-well plates and total volume adjusted to 200 µL PBSCM per sample. Binding proceeded for 2 h on ice with gentle rocking. Subsequently, membranes were transferred to

96-well glass fibre filter plates (Unifilter C, PerkinElmer) preincubated with 0.1% polyethyleneimine (30 µL pr well) using a Packard Bell cell harvester and washed four times with water. Non-specific binding was determined in parallel at membranes from non-transfected COS7 cells. Filter plates were dried and soaked in scintillant followed by counting in a Packard Topcounter. Saturation binding assays were carried out in duplicate and repeated at least three times. For competition binding assays, 5-30 µg total membrane protein was incubated with 0.25 nM [^{125}I]β-CIT in the presence of increasing concentrations of inhibitor in PBSCM using the same protocol as for saturation binding experiments. Competition binding assays were carried out in duplicate and repeated at least three times.

Data analysis. All data analysis was performed using Prism 6 software (GraphPad Inc, San Diego, CA, USA), and carried out as previously described.(Andersen et al., 2009) The inhibitory potency (K_i) for inhibitors were calculated from IC_{50} values determined in functional uptake assays using the equation: $K_i = IC_{50}/(1+([L]/K_M))$, where [L] is the concentration of [^{3}H]5-HT (SERT assays) or [^{3}H]DA (NET assays) and K_M is the Michaelis-Menten constant for the substrate. The binding affinity (K_i) for inhibitors were calculated from IC_{50} values determined in competition binding assays using the equation: $K_i = IC_{50}/(1+([L]/K_D))$, where [L] is the concentration of [^{125}I]β-CIT and K_D is the dissociation constant for β-CIT. K_i values were compared using Student's *t*-test unless otherwise indicated.

Synthesis of fluoxetine derivatives

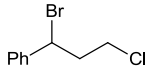
General procedures. All reagents were obtained from commercial sources and used without any further purification. Proton (^{1}H) NMR and carbon (^{13}C) NMR spectra were recorded on a Varian Mercury Plus (300 MHz) or a Bruker Avance (400 MHz) instrument. Chemical shifts (δ) are reported relative to tetramethylsilane (TMS) as an internal standard. The following abbreviations are used for the proton spectra multiplicities: s, singlet; br s, broad singlet; d, doublet; dd, double doublet, triplet; q, quartet; sep, septet m, multiplet. Coupling constants (*J*) are reported in hertz (Hz). Microanalyses were performed at the Microanalytical Laboratory at the Department of Chemistry, University of Copenhagen, Denmark. Gas chromatography-mass spectrometry (GC-MS) analysis was performed on a Shimadzu GCMS-QP5050A instrument.



Scheme S1. The synthesis of fluoxetine analogues was initiated from (3-chloropropyl)benzene (**3**) and (4-chlorobutyl)benzene (**4**). The chloroalkylbenzenes were brominated at the benzylic position using *N*-bromosuccinimide in the presence of a catalytic amount of benzoyl peroxide in chlorobenzene affording (1-bromo-3-chloropropyl)benzene (**5**) and (1-bromo-4-chlorobutyl)benzene (**6**), respectively (Silvestri et al., 2004). Phenolate, 2-methoxy-phenolate, 4-trifluoromethyl-phenolate and 2-methoxy-4-(trifluoromethyl)-phenolate were subsequently generated by reacting the corresponding phenols with sodium hydride and then reacted with **5** and **6** affording (3-chloro-1-aryloxypropyl)benzenes and (4-chloro-1-aryloxybutyl)benzenes, respectively. The favorable chemoselectivity of the phenolates towards the benzylic bromine over the terminal chlorine gave the desired products in 70-80% yield with purities above 85% (determined by GC-MS). The crude products were used directly in reactions with *N*-methyl- or *N*,*N*-dimethylamine affording fluoxetine (**1**), nisoxetine (**2**) and five analogues (**7**-**11**).

Reagents and conditions: (a) NBS, $(PhCO_2)_2$, PhCl, 85 ºC, 4 h; (b) ArONa, DMF, room temperature, 12 h; (c) *N*-Methyl- or *N*,*N*-dimethylamine, Bu_4NI, K_2CO_3, MeOH, 80 ºC, 24h (sealed tube). Isolated yields are shown in brackets.

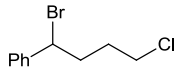
(1-Bromo-3-chloropropyl)benzene (5) (Silvestri et al., 2004).



A slurry of 1-chloro-3-phenylpropane (**3**) (14.3 mL, 100 mmol), *N*-bromosuccinimide (16.0 g, 90 mmol), and dibenzoyl peroxide (30 mg) in chlorobenzene (40 mL) was heated at 85 ˚C for 4 hours and then allowed to slowly cool at room temperature for one hour. Precipitated succinimide was filtered off and chlorobenzene

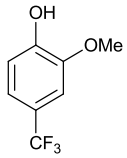
was removed on a rotary evaporator. Distillation at 84-85 ˚C/0.3 mbar afforded the benzylbrominated product **5** (14.3 g, 68%) as a colorless liquid. Anal. Calcd for $C_9H_{10}BrCl$: C, 46.29; H, 4.32. Found: C, 46.52; H, 4.39. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.40-2.52 (m, 1H), 2.64-2.75 (m, 1H), 3.52-3.60 (m, 1H), 3.66-3.75 (m, 1H), 5.18-5.23 (m, 1H), 7.27-7.42 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 42.20, 42.85, 51.55, 127.46, 128.82, 129.00, 141.00. GC-MS: EI (m/z, relative intensity): 234 (M^+, 1), 153 (47), 117 (16), 104 (10), 91 (100).

(1-Bromo-4-chlorobutyl)benzene (6).



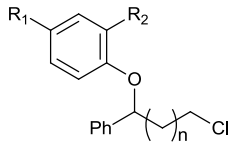
A slurry of 1-chloro-4-phenylpropane (**4**) (3.37 g, 20 mmol), *N*-bromosuccinimide (3.56 g, 20 mmol), and dibenzoyl peroxide (10 mg) in chlorobenzene (10 mL) was heated at 85 ˚C for 4 hours and then allowed to slowly cool at room temperature for one hour. Precipitated succinimide was filtered off and chlorobenzene was removed on a rotary evaporator. The product was separated by flash chromatography on silica gel 60F (20 g) by means of heptane. Evaporation of the solvent followed by bulb-to-bulb distillation (110 ˚C, 0.3 mbar) afforded the benzylbrominated product **6** (1.87 g, 76%) as a colorless liquid. Anal. Calcd for $C_{10}H_{12}BrCl$: C, 48.52; H, 4.89. Found: C, 48.38; H, 4.97. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73-1.85 (m, 1H), 1.92-2.08 (m, 1H), 2.23-2.46 (m, 2H), 3.53 (t, J = 6.3 Hz, 2H), 4.93 (t, J = 7.4 Hz, 1H), 7.26-7.39 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 31.30, 37.30, 44.24, 54.57, 125.89, 127.19, 128.80, 141.67. GC-MS: EI (m/z, relative intensity): 248 (M^+, 1), 167 (26), 131 (15), 117 (8), 104 (10), 91 (100).

2-Methoxy-4-(trifluoromethyl)phenol (12).



A solution of 2-methoxy-4-(trifluoromethyl)benzaldehyde (2.04 g, 10 mmol) and 3-chloroperbenzoic acid (70%, 2.47 g, 15 mmol) in dichloromethane (30 mL) was stirred for 12 hours at room temperature. At this stage GC-MS showed full conversion into the corresponding formyl ester and a white slurry had formed. A solution of *p*-toluenesulfonic acid monohydrate (1.90 g, 10 mmol) in methanol (30 mL) was added and the resulting colorless solution was stirred at room temperature for 2 hours in order to obtain complete trans-esterification. A saturated aqueous sodium thiosulfate solution (10 mL) was added and after evaporation on celite separation was performed by flash chromatography on silica gel 60F (20 g) by means of EtOAc/heptane (1:2). Evaporation of the solvent and drying in a vacuum oven (1 mbar, 40 ˚C, 2 hours) gave **12** (1.69 g, 88%) as a colorless liquid. Anal. Calcd for $C_8H_7F_3O_2$: C, 50.01; H, 3.67. Found: C, 49.69; H, 3.74. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.95 (s, 3H), 5.94 (br-s, 1H), 6.98 (d, J = 8.0 Hz, 1H), 7.07 (s, 1H), 7.18 (d, J = 8.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.24, 107.66, 114.40, 119.06, 122.48, 126.13, 146.42, 148.40. GC-MS: EI (m/z, relative intensity): 192 (M^+, 79), 177 (100), 149 (39).

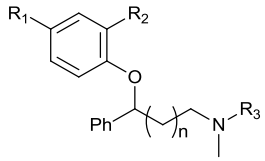
(3-chloro-1-aryloxypropyl)benzenes or (4-chloro-1-aryloxybutyl)benzenes.



R_1 = CF_3 or H
R_2 = OCH_3 or H
n = 1 or 2

To a solution of the appropriate phenol (1.1 mmol) in dry DMF (5 mL) cooled in an ice-bath under nitrogen was added sodium hydride (60% in mineral oil, 44 mg, 1.1 mmol) in one portion. The resulting slurry was treated in an ultrasound bath at room temperature for 30 minutes under nitrogen to give a clear yellow solution. Upon cooling in an ice bath (1-bromo-3-chloropropyl)benzene **5** or (1-bromo-4-chlorobutyl)benzene **6** (1 mmol) was added in a dropwise fashion during a ten minute period and stirring at room temperature was maintained for 12 hours. The resulting reaction mixtures were poured into ice (150 g) and extracted with Et_2O/heptane (1:2, 3 x 25 mL). The pooled extracts were washed with sodium hydroxide (4M, 2 x 10 mL), dried with magnesium sulfate, and concentrated *in vacuo* to afford (3-chloro-1-aryloxypropyl)benzenes or (4-chloro-1-aryloxybutyl)benzenes in 70 to 80 % yields as colorless liquids in purities above 85% with only negligible amounts of bis-substituted by-products according to GC-MS. These materials were used directly for synthesis of **1**, **2** and **7**-**11**.

***N*-Methyl or *N,N*-dimethyl 3-phenyl-3-aryloxy-1-propylamines or 4-phenyl-4-aryloxy-1-butylamines (1, 2, 7-11).**

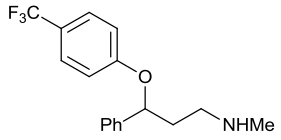


R_1 = CF_3 or H
R_2 = OCH_3 or H
R_3 = CH_3 or H
n = 1 or 2

General procedure: A slurry of the appropriate (3-chloro-1-aryloxypropyl)benzene or (4-chloro-1-aryloxybutyl)benzene (approx. 0.5 mmol), tetrabutylammonium iodide (0.037 g, 0.1 mmol) and potassium carbonate (0.27 g, 2 mmol) in a solution of *N*-methylamine or *N,N*-dimethylamine (2.0M in methanol, 4 mL, 8 mmol) was heated in a sealed tube for 24 hours. After cooling in an ice bath the white slurry was poured into water (30 mL) and extracted with ethyl acetate (3 x 25 mL). The combined organic phases were pooled, dried over sodium sulphate, filtered, and evaporated. The product was purified by flash chromatography through a short plug of silica by means of triethylamine-ethyl acetate (1:4) followed by evaporation and bulb-to-bulb distillation.

For the synthesis of compound **10** (*N*-methyl-4-phenyl-4-(4-(trifluoromethyl)phenoxy)-1-butylamine), a modified Hindsberg alkylation procedure was also applied (see below).

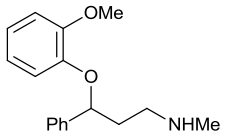
***N*-Methyl-3-phenyl-3-(4-(trifluoromethyl)phenoxy)-1-propylamine (1) (Molloy and Schmiegel).**



Bulb-to-bulb distillation (150 ˚C, 0.8 mbar) gave the amine **1** (0.10 g, 32 % from **5**) as a colorless liquid. Anal. Calcd for $C_{17}H_{18}F_3NO$: C, 66.01; H, 5.87; N, 4.53. Found: C, 66.22; H, 5.66; N, 4.38. ^{1}H NMR (300

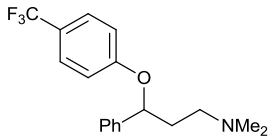
MHz, $CDCl_3$): δ 1.40 (br-s, 1H), 1.97-2.04 (m, 1H), 2.16-2.23 (m, 1H), 2.42 (s, 3H), 2.73 (t, J = 7.2 Hz, 2H), 5.26-5.31 (m, 1H), 6.88 (d, J = 8.5 Hz, 2H), 7.23-7.32 (m, 5H), 7.40 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 36.72, 38.96, 48.43, 78.72, 115.77, 123.12, 125.79, 126.11, 126.75, 127.83, 128.79, 141.05, 160.51. GC-MS: EI (m/z, relative intensity): 309 (M^+, 43), 251 (8), 232 (5), 205 (4), 183 (15), 59 (100).

***N*-Methyl-3-(2-methoxyphenoxy)-3-phenyl-1-propylamine (2) (Srebnik et al., 1988).**



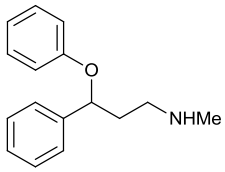
Bulb-to-bulb distillation (170 ˚C, 0.5 mbar) gave the amine **2** (0.11 g, 41 % from **5**) as a colorless liquid. Anal. Calcd for $C_{17}H_{21}NO_2$: C, 75.25; H, 7.80; N, 5.16. Found: C, 75.34; H, 5.24; N, 5.27. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.84 (br-s, 1H), 2.01-2.06 (m, 1H), 2.21-2.29 (m, 1H), 2.43 (s, 3H), 2.75-2.79 (m, 2H), 3.89 (s, 3H), 5.16-5.20 (m, 1H), 6.65-6.69 (m, 2H), 6.82-6.86 (m, 2H), 7.21-7.36 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 36.68, 38.70, 48.90, 56.07, 80.63, 111.96, 116.26, 120.66, 121.41, 125.96, 127.49, 128.49, 141.96, 147.58, 149.96. GC-MS: EI (m/z, relative intensity): 271 (M^+, 2), 167 (36), 148 (66), 124 (100).

***N,N*-Dimethyl-3-phenyl-3-(4-(trifluoromethyl)phenoxy)-1-propylamine (7) (Molloy and Schmiegel).**



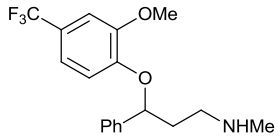
Bulb-to-bulb distillation (160 ˚C, 0.5 mbar) gave the amine **7** (0.092 g, 28% from **5**) as a colorless liquid. Anal. Calcd for $C_{18}H_{20}F_3NO$: C, 66.86; H, 6.23; N, 4.33. Found: C, 66.88; H, 6.31; N, 4.34. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.95-2.02 (m, 1H), 2.14-2.21 (m, 1H), 2.23 (s, 6H), 2.37-2.44 (m, 2H), 5.24-5.26 (m, 1H), 6.88 (d, J = 8.8 Hz, 2H), 7.23-7.33 (m, 5H), 7.40 (d, J = 8.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.01, 45.72, 55.91, 78.63, 115.84, 122.93, 125.90, 126.75, 127.33, 127.85, 128.79, 141.16, 160.64. GC-MS: EI (m/z, relative intensity): 323 (M^+, 2), 178 (6), 115 (4), 104 (4), 91 (6), 73 (11), 58 (100).

***N*-Methyl-3-phenoxy-3-phenyl-1-propylamine (8) (Castelli and Vailati).**



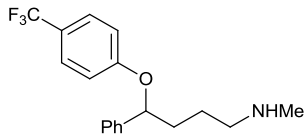
Bulb-to-bulb distillation (130 ˚C, 0.2 mbar) gave the amine **8** (0.083 g, 34 % from **5**) as a colorless liquid. Anal. Calcd for $C_{16}H_{19}NO$: C, 79.63; H, 7.94; N, 5.80. Found: C, 79.56; H, 7.96; N, 5.71. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.65 (br-s, 1H), 1.97-2.06 (m, 1H), 2.12-2.22 (m, 1H), 2.42 (s, 3H), 2.75 (t, J = 7.0 Hz, 2H), 5.21-5.26 (m, 1H), 6.83-6.88 (m, 3H), 7.15-7.30 (m, 3H), 7.32-7.37 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 36.64, 38.96, 48.63, 78.56, 116.01, 120.82, 125.96, 127.60, 128.72, 129.42, 142.04, 158.23. GC-MS: EI (m/z, relative intensity): 241 (M^+, 17), 209 (2), 148 (94), 45 (100).

***N*-Methyl-3-(2-methoxy-4-(trifluoromethyl)phenoxy)-3-phenyl-1-propylamine (9).**



Bulb-to-bulb distillation (160 ˚C, 0.5 mbar) gave the amine **9** (0.12 g, 35 % from **5**) as a colorless liquid. Anal. Calcd for $C_{18}H_{20}F_3NO_2$: C, 63.71; H, 5.94; N, 4.13. Found: C, 63.76; H, 6.04; N, 4.32. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.90 (br-s, 1H), 2.02-2.08 (m, 1H), 2.21-2.31 (m, 1H), 2.43 (s, 3H), 2.73-2.78 (m, 2H), 3.91 (s, 3H), 5.25-5.29 (m, 1H), 6.70 (d, J = 8.3 Hz, 1H), 6.96 (d, J = 8.3 Hz, 1H), 7.02 (s, 1H), 7.22-7.33 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 36.69, 38.71, 48.69, 56.26, 80.41, 108.56, 114.78, 118.10, 123.81, 125.81, 127.84, 128.63, 128.73, 141.11, 149.77, 150.91. GC-MS: EI (m/z, relative intensity): 235 (M^+, 20), 192 (74), 177 (72), 148 (100).

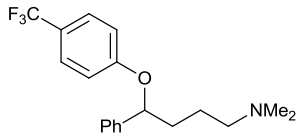
***N*-Methyl-4-phenyl-4-(4-(trifluoromethyl)phenoxy)-1-butylamine (10) (Seeman).**



Bulb-to-bulb distillation (180 ˚C, 0.5 mbar) gave the amine **10** (0.14 g, 43 % from **6**) as a colorless liquid.

Alternatively, compound **10** was also synthesized by using a modified Hindsberg alkylation procedure which gave the desired compound in higher yields compared to the Finkelstein conditions used above: To a solution of *N*-methyl-2-nitrobenzenesulfonamide (0.23 g, 0.6 mmol) and tetrabutylammonium iodide (0.037 g, 0.1 mmol) in DMF (2 mL) was added potassium *tert*-butoxide (1M in THF, 0.6 mL, 0.6 mmol) at 0 ˚C. After stirring for 5 minutes at room temperature 1-(4-chloro-1-phenylbutoxy)-4-(trifluoromethyl)benzene (0.16 g, 0.5 mmol) was added and heating at 90 ˚C was maintained for 24 hours under nitrogen. 1,8-Diazabicycloundec-7-ene (DBU, 0.30 mL, 1.2 mmol) and thiophenol (0.21 mL, 2 mmol) were added and stirring was maintained at room temperature for one hour. The orange reaction mixtuxe was poured into brine (30 mL) and extracted with ethyl acetate (3 x 20 mL). The pooled organic extracts were washed twice with brine (10 mL), dried over sodium sulfate, filtered and evaporated. Separation on silica by means of triethylamine-ethyl acetate (1:3), evaporation and bulb-to-bulb distillation (180 ˚C, 0.5 mbar) gave **10** (0.11 g, 68 %) as a light-yellow oil. Anal. Calcd for $C_{18}H_{20}F_3NO$: C, 66.86; H, 6.23; N, 4.33. Found: C, 66.85; H, 6.45; N, 4.26. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42-1.73 (m, 2H), 1.74-1.93 (m, 2H), 2.26 (s, 3H), 2.46 (t, J = 7.0 Hz, 2H), 5.01 (q, J = 6.3 Hz, 1H), 6.74 (d, J = 8.5 Hz, 2H), 7.06-7.17 (m, 5H), 7.26 (d, J = 8.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.05, 36.14, 36.71, 51.73, 80.26, 115.61, 122.45, 125.70, 126.56, 126.64, 127.62, 128.56, 140.98, 160.50. GC-MS: EI (m/z, relative intensity): 323 (M^+, 3), 178 (6), 162 (100).

***N,N*-Dimethyl-4-phenyl-4-(4-(trifluoromethyl)phenoxy)-1-butylamine (11).**



Bulb-to-bulb distillation (180 ˚C, 0.5 mbar) gave the amine **11** (0.13 g, 39 % from **6**) as a colorless liquid. Anal. Calcd for $C_{19}H_{22}F_3NO$: C, 67.64; H, 6.57; N, 4.15. Found: C, 67.54; H, 6.77; N, 12.33. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.59-1.74 (m, 2H), 1.88-2.06 (m, 2H), 2.21 (s, 6H), 2.30 (t, J = 7.2 Hz, 2H), 5.17 (q, J = 6.4 Hz, 1H), 6.89 (d, J = 8.3 Hz, 2H), 7.24-7.29 (m, 2H), 7.31-7.34 (m, 3H), 7.42 (d, J = 8.3 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.04, 36.63, 45.60, 59.50, 80.40, 115.74, 122.64, 125.84, 126.69, 126.74, 127.75, 128.69, 141.14, 160.65. GC-MS: EI (m/z, relative intensity): 337 (M^+, 1), 176 (3), 162 (8), 143 (5), 91 (6), 58 (100).

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