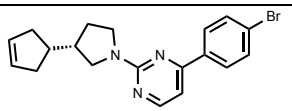
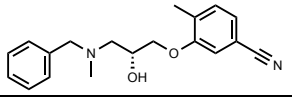
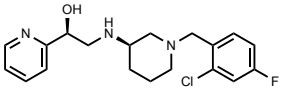
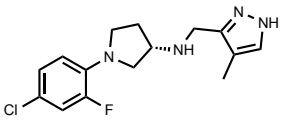
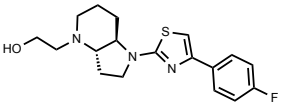
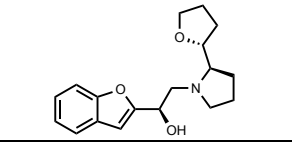
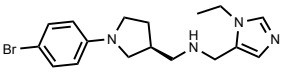
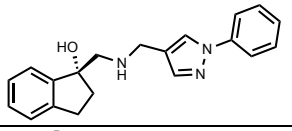
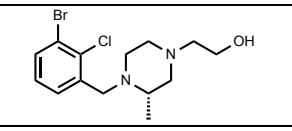
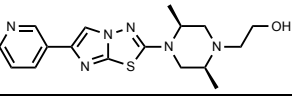
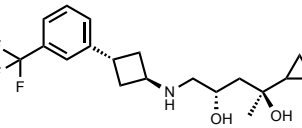
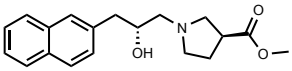
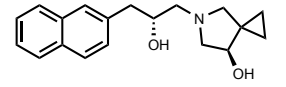
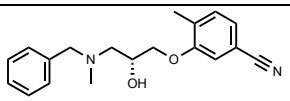
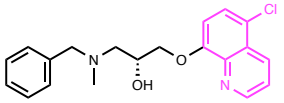
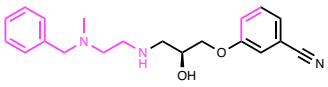
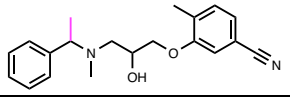
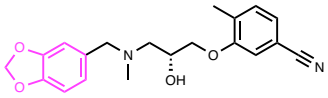
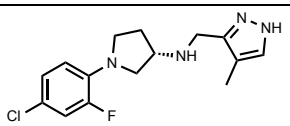
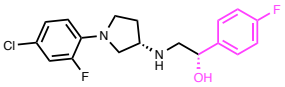
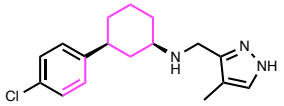
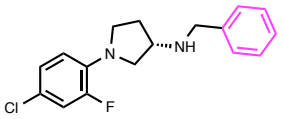
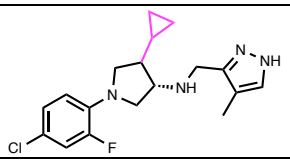
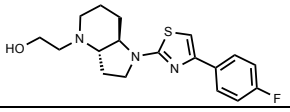
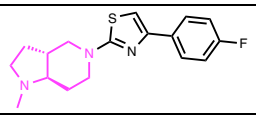
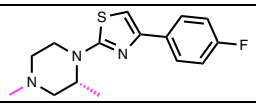
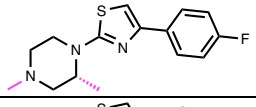
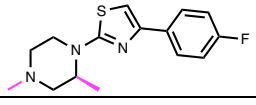
Table S1. Active docking hits that inhibited ≥ 50% of [^{3}H]5-HT transport by SERT, related to Figure 1 and STAR methods.

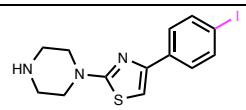
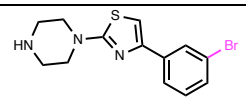
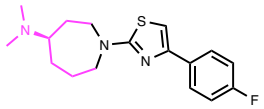
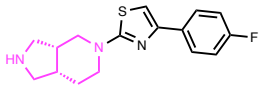
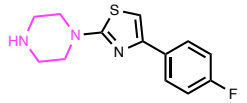
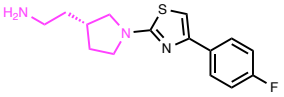
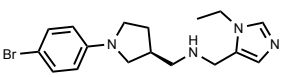
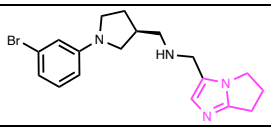
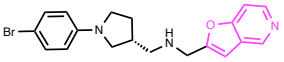
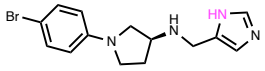
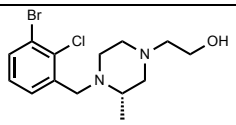
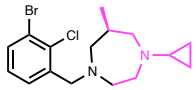
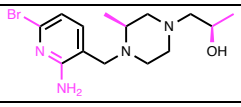
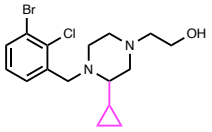
ZINC-ID	Chemical Structure	% Inhibition	SEM	n
ZINC000623997601		63	4	3
ZINC000305339642		94	1	3
ZINC000853098204		50	7	3
ZINC000623756919		82	2	4
ZINC000897222313		81	2	4
ZINC000294308141		56	13	4
ZINC000417864931		92	1	3
ZINC000269761690		54	2	3
ZINC000411862305		87	1	4
ZINC000580533739		54	4	3
ZINC000634713432		64	4	3
ZINC000279319971		60	5	3
ZINC000348114651		54	4	3

Each of the successfully synthesized compounds selected from the virtual screen were tested at a concentration of

30 μM for inhibition of 5-HT transport into cells expressing WT SERT. Shown are the 13 compounds that inhibited transport ≥ 50%.

Table S2. Active analogs of top five hits tested at SERT, related to Figure 1 and STAR methods.

ZINC-ID/Supplier code	Comment	Chemical Structure	n	Ki, µM	*SEM
ZINC000305339642	**Initial hit**		**5**	**0.028**	**0.006**
ZINC000677296032	analog of ZINC000305339642		1	0.141	0.021
ZINC000387330406	analog of ZINC000305339642		5	0.173	0.033
Z4135312238	analog of ZINC000305339642		3	0.040	0.008
Z4135312239	analog of ZINC000305339642		4	0.027	0.005
ZINC000623756919	**Initial hit**		**3**	**1.63**	**0.02**
ZINC000863582102	analog of ZINC000623756919		1	0.14	0.02
ZINC000449730871	analog of ZINC000623756919		1	0.71	0.11
Z4145515674	analog of ZINC000623756919		1	2.50	0.39
Z4167665784	analog of ZINC000623756919		1	2.21	0.33
ZINC000897222313	**Initial hit**		**3**	**1.47**	**0.07**
ZINC000866205875	analog of ZINC000897222313		4	0.057	0.030
ZINC000443438219 Racemic mix -8221	analog of ZINC000897222313		5	0.016	0.002
ZINC000443438**8219**	R-isomer of ZINC000443438219		3	0.0030	0.0004
ZINC000443438221	S-isomer of ZINC000443438219		3	0.17	0.06

ZINC000236204386	analog of ZINC000443438219		3	0.21	0.07
ZINC000044494100	analog of ZINC000443438219		5	0.015	0.006
ZINC000277296162	analog of ZINC000897222313		3	0.026	0.014
ZINC000420267969	analog of ZINC000897222313		3	0.048	0.015
ZINC000006658090	analog of ZINC000897222313		3	0.014	0.001
ZINC000900380018	analog of ZINC000897222313		2	0.50	0.19
ZINC000417864931	**Initial hit**		**2**	**1.16**	**0.14**
ZINC000657329003	analog of ZINC000417864931		3	0.27	0.18
ZINC000651685066	analog of ZINC000417864931		2	0.99	0.03
ZINC000894225026	analog of ZINC000417864931		2	0.40	0.13
ZINC000411862305	**Initial hit**		**2**	**1.22**	**0.06**
ZINC001170690351	analog of ZINC000411862305		7	0.11	0.04
ZINC001332938263	analog of ZINC000411862305		3	0.27	0.18
Z4135312264	analog of ZINC000411862305		2	0.28	0.19

Affinities were measured as the half-maximal concentration required to displace [^{125}I]β-CIT from membranes from cells expressing WT SERT. IC_{50} values were determined by analyzing displacement curves using Origin (Originlab). *SEM values were calculated from multiple experiments using Origin, except for cases when only one

measurement was made, and then, the value shown for SEM was the uncertainty reported by Origin for the determination of IC_{50} from the curve fit.

Table S3. Cryo-EM data collection, refinement and validation statistics, related to Figure 4 and Figure S3.

	SERT-Fab15B8 '8090-bound (EMDB: EMD-26160) (PDB: 7TXT)
Data collection	
Microscope	Thermo Scientific Krios G3i
Detector	Gatan K3 with Gatan BioQuantum Energy filter
Voltage (kV)	300
Magnification	105,000
Defocus range (μm)	-1.0 to -2.1
Pixel size (Å)	0.81 (physical)
Total exposure ($e^-/Å^2$)	50
Frame exposure ($e^-/Å^2$/frame)	0.833
Images, number of	5,010
Frames/image, number of	60
Initial particles, number of	3,313,742
Final particles, number of	187,696
Symmetry imposed	C1
Map sharpening *B* factor ($Å^2$)	-100
Map resolution (Å)	3.0
FSC threshold	0.143
Refinement and validation	
Initial model used (PDB code)	6VRH
Model resolution (Å)	3.1
FSC threshold	0.5
Model composition	
Chains	4
Non-hydrogen atoms	6093
Protein residues	772
Water	N/A
Ligands	1
B factors ($Å^2$)	
Protein	43
Ligand	20
R.m.s. deviations	
Bond length (Å)	0.007 (1)
Bond angles (°)	0.941 (0)
Validation	
MolProbity score	1.56
Clash score	8.18
EMRinger score	4.50

Rotamer outliers (%)	0.00
Ramachandran plot	
Favored (%)	97.39
Allowed (%)	2.61
Disallowed (%)	0.00

Table S4. Sucrose preference and total fluid volume consumed by the vehicle and fluoxetine cohort on day -2 of learned helplessness conditioning, related to Figure 6.

Sucrose-Water Pairing[a]	**Mean**	**SEM**[c]	**N**[c]
Veh[c]	0.041 Preference	±0.0258	19
Flx[c]	0.184 Preference	±0.0464	10
Total Fluid Consumption[b]			
Veh[c]	2.365 mL	±0.063	19
Flx[c]	2.290 mL	±0.072	10

[a][t(27)=2.913, P=0.007]; two-tailed test.
[b][t(27)=0.737, P=0.467]; two-tailed test.
[c]Abbreviations: SEM, standard error of the mean; N, number of mice; Veh, vehicle; Flx, fluoxetine.

Table S5. Shock escape and latency to escape by the vehicle and fluoxetine cohort on days -9 and -2 of learned helplessness conditioning, related to Figure 6 and S6.

# Escapes[a]		Mean	SEM[c]	N[c]
Day -9	Veh[c]	2.21	±0.575	19
	Flx[c]	3.00	±1.054	10
Day -2	Veh[c]	1.89	±0.350	19
	Flx[c]	3.700	±1.126	10
Shock Latency[b]				
Day -9	Veh[c]	17.56 s	±0.755	19
	Flx[c]	15.95 s	±1.528	10
Day -2	Veh[c]	18.14 s	±0.436	19
	Flx[c]	14.83 s	±1.788	10

[a]RMANOVA: days [F(1,27)=0.110, P=0.743]; day x treatment [F(1,27)=0.766, P=0.389]; treatment [F91,27)=2.382, p=0.134].

[b]RMANOVA: days [F(1,27)=0.108, 0.744]; day x treatment [F(1,27)=1.101, p=0.303]; treatment [F91,27)=4.077, P=0.054]. For treatment, none of the Bonferroni *post-hoc* tests were significant.

[c]Abbreviations: SEM, standard error of the mean; N, number of mice; Veh, vehicle; Flx, fluoxetine.

Table S6. Statistics for the behavioral studies, related to Figures 5, 6, S3-S5, and Supplemental Figures S5-S7.

Figure	Statistical Model	Variable	Degrees of Freedom	F-statistic	*P*-value	# Mice per Group
Figure 5	Three-way RMANOVA[a]	Days	3,450	32.940	<0.001	8-12
		Days x Gene[a]	3,450	8.047	<0.001	
		Days x Treat[a]	24,450	2.161	0.001	
		Days x Gene x Treat[a]	24.450	2.276	<0.001	
		Treat[a]	8,150	3.373	0.001	
		Gene x Treat[a]	8.150	4.115	<0.001	
Figure 6A	None	Experimental Design	None	None	None	N.A.
Figure 6B	Two-way RMANOVA	Days	1,36	30.469	<0.001	10
		Days x Treat[a]	3,36	5.534	0.003	
		Treat[a]	3,36	8.083	<0.001	
Figure 6C	Two-way RMANOVA	Days	1,36	79.751	<0.001	10
		Days x Treat[a]	3,36	3.673	0.021	
		Treat[a]	3,36	28.221	<0.001	
Supplemental.pdf Figure S3A	Two-way RMANOVA	Days	1,36	23.286	<0.001	10
		Days x Treat[a]	3,36	8.700	<0.001	
		Treat[a]	3,36	0.982	n.s.	
Supplemental.pdf Figure S3B	Two-way RMANOVA	Days	8,208	4.714	<0.001	9-10
		Days x Treat[a]	16,208	1.851	0.027	
		Treat[a]	2,26	1.324	n.s.	
Supplemental.pdf Figure S3C	Two-way RMANOVA	Days	8,208	0.544	n.s.	9-10
		Days x Treat[a]	16,208	0.589	n.s.	
		Treat[a]	2,26	5.546	0.010	
Supplemental.pdf Figure S3D	Two-way RMANOVA	Days	7,182	31.228	<0.001	9-10
		Days x Treat[a]	14,182	8.350	<0.001	
		Treat[a]	2,26	1.099	n.s.	
Supplemental.pdf Figure S4A	Two-way RMANOVA	Time	1,36	118.728	<0.001	10
		Time x Treat[a]	3,36	0.148	n.s.	
		Treat[a]	3,36	1.132	n.s.	
Supplemental.pdf Figure S4B	Two-way RMANOVA	Time	1,26	96.849	<0.001	9-10
		Time x Treat[a]	2,26	1.670	n.s.	
		Treat[a]	2,26	4.458	0.022	
	ANCOVA	Treat[a]	2,25	2.948	n.s.	
Supplemental.pdf Figure S5A	One-way ANOVA	Treat[a]	3,36	9.819	<0.001	10
Supplemental.pdf Figure S5B	One-way ANOVA	Treat[a]	2,28	34.563	<0.001	9-10
	ANCOVA	Treat[a]	2,25	21.958	<0.001	
Supplemental.pdf Figure S5C	One-way ANOVA	Treat[a]	3,36	5.403	0.004	10
Supplemental.pdf Figure S5D	One-way ANOVA	Treat[a]	2,28	3.136	n.s.	9-10

	ANCOVA	Treat[a]	2,25	1.508	n.s.	
Supplemental.pdf Figure S5E	One-way ANOVA	Treat[a]	3,36	3.893	0.017	10
Supplemental.pdf Figure S5F	One-way ANOVA	Treat[a]	2,28	61.312	<0.001	9-10
Figure S5A	Two-way RMANOVA	Days	1,36	36.013	<0.001	10
		Days x Treat[a]	3,36	2.759	0.053	
		Treat[a]	3,36	5.778	0.002	
Figure S5B	Two-way RMANOVA	Days	1,36	17.449	<0.001	10
		Days x Treat[a]	3,36	3.308	0.031	
		Treat[a]	3,36	3.838	0.002	
Figure S5C	Three-way RMANOVA (Nested)	Days	1,36	0.772	n.s.	10
		Days x Treat[a]	3,36	0.414	n.s.	
		Inten[a]	3,108	52.962	<0.001	
		Inten x Treat[a]	9,108	0.914	n.s.	
		Days x Inten[a]	3,108	9.132	<0.001	
		Days x Inten x Treat[a]	9,108	0.729	n.s.	
		Treat[a]	3,36	1.120	n.s.	
Figure S6A	Two-way RMANOVA	Days	3,78	2.229	n.s.	9-10
		Days x Treat[a]	6,78	1,841	n.s.	
		Treat[a]	2,26	14.064	<0.001	
Figure S6B	Two-way RMANOVA	Days	3,78	1.892	n.s.	9-10
		Days x Treat[a]	6,78	1.127	n.s.	
		Treat[a]	2,26	10.093	<0.001	
Figure S6C	Three-way RMANOVA (Nested)	Days	1,26	0.606	n.s.	9-10
		Days x Treat[a]	2,26	2.688	n.s.	
		Inten[a]	3,78	24.635	<0.001	
		Inten x Treat[a]	6,78	0.880	n.s.	
		Days x Inten[a]	3,78	0.676	n.s.	
		Days x Inten x Treat[a]	6,78	1.758	n.s.	
		Treat[a]	2,26	0.126	n.s.	
Figure S7A	One-way ANOVA	Treat[a]	3,36	2.087	0.119	10
Figure S7B	One-way ANOVA	Treat[a]	2,26	10.687	<0.001	9-10
	ANCOVA	Treat[a]	2,25	5.945	0.008	
Figure S7C	One-way ANOVA	Treat[a]	3,36	5.624	0.003	10
Figure S7D	One-way ANOVA	Treat[a]	2,26	12.959	<0.001	9-10
	ANCOVA	Treat[a]	2,25	4.001	0.031	
Figure S7E	One-way ANOVA	Treat[a]	3,36	0.084	0.968	10
Figure S7F	One-way ANOVA	Treat[a]	2,26	23.015	<0.001	9-10

[a]Abbreviations: all statistical analyses were run as two-tailed tests; ANOVA, analysis of variance; RMANOVA, repeated measures ANOVA; ANCOVA, analyses of covariance; Gene, genotype; Treat, treatment; Inten, intensity; n.s., not significant; N.A., not applicable.