

1 **A tropane-based ibogaine analog rescues folding-deficient SERT and DAT**
2
3

4 Shreyas Bhat*¹, Daryl A. Guthrie*², Ameya Kasture³, Ali El-Kasaby¹, Jianjing Cao²,
5 Alessandro Bonifazi², Therese Ku², JoLynn B. Giancola², Thomas Hummel³, Michael
6 Freissmuth^{‡1} and Amy Hauck Newman^{‡2}

7
8 Affiliations: ¹Institute of Pharmacology and the Gaston H. Glock Research Laboratories for Exploratory Drug
9 Development, Center of Physiology and Pharmacology, Medical University of Vienna, Vienna,
10 Waehringerstrasse 13a, Vienna, Austria; ²Molecular Targets and Medications Discovery Branch, National
11 Institute on Drug Abuse, Intramural Research Program, Baltimore, MD 21224, U.S.A.; ³Department of
12 Neurobiology, University of Vienna, Austria.

13
14 *S. Bhat and D. Guthrie contributed equally to this paper.

15
16 [‡]Correspondence: Michael Freissmuth (michael.freissmuth@meduniwien.ac.at), Amy Hauck Newman
17 (anewman@intra.nida.nih.gov.)

18
19 **Abstract**

20 Missense mutations that give rise to protein misfolding are rare, but collectively, defective
21 protein folding diseases are consequential. Folding deficiencies are amenable to
22 pharmacological correction (pharmacochaperoning), but the underlying mechanisms remain
23 enigmatic. Ibogaine and its active metabolite noribogaine correct folding defects in the
24 dopamine transporter (DAT), but they rescue only a very limited number of folding-deficient
25 DAT mutants, which give rise to infantile Parkinsonism and dystonia. Herein, a series of
26 analogs was generated by reconfiguring the complex ibogaine ring system and exploring the
27 structural requirements for binding to wild type transporters, and for rescuing two equivalent
28 synthetic folding-deficient mutants, SERT-PG^{601,602}AA and DAT-PG^{584,585}AA. The most
29 active tropane-based analog (**9b**) was also an effective pharmacochaperone *in vivo*, in
30 *Drosophila* harboring DAT-PG^{584,585}AA and rescued six out of 13 disease-associated human
31 DAT mutants *in vitro*. Hence, a novel lead pharmacochaperone has been identified that
32 demonstrates medication development potential for patients harboring DAT mutants.

33
34
35

36 **Introduction**

37 Ibogaine, one of many alkaloids first isolated from the shrub *Tabernanthe iboga* in 1901
38 (*Dybowski and Landrin, 1901*), has three interesting pharmacological properties: (i) it is
39 hallucinogenic; presumably the reason why it has been used for centuries by West African
40 tribes in rites of passage (*Wasko et al., 2018; Corkery, 2018*). (ii) It has been reported to
41 mitigate substance use disorders (*Corkery, 2018; Brown and Alper, 2018; Noller et al., 2018*).
42 These actions have also been recapitulated in experimental paradigms, where animals are
43 given the opportunity to self-administer or express their preference for morphine and
44 psychostimulants (*Glick et al., 1991; Glick et al., 1994; Blackburn and Szumlinski, 1997*);
45 (iii) Ibogaine and its principal, active metabolite noribogaine (12-hydroxyibogamine; ref.
46 *Mash et al., 1995*) bind to the transporters for the monoamines serotonin (SERT), dopamine
47 (DAT) and norepinephrine (NET) (*Mash et al., 1995; Sweetnam et al., 1995*). Moreover,
48 ibogaine and noribogaine were the first compounds that were shown to rescue folding-
49 deficient versions of SERT (*El-Kasaby et al., 2010; El-Kasaby et al., 2014; Koban et al.,
50 2015*) and of DAT (*Kasture et al., 2016; Beerepoot et al., 2016; Asjad et al., 2017*). This
51 pharmacochaperoning action of ibogaine and noribogaine is of therapeutic interest because
52 folding-deficient mutants of DAT give rise to the dopamine transporter deficiency syndrome
53 or DTDS (*Kurian et al., 2011; Ng et al., 2014*). DTDS is a hyperkinetic movement disorder in
54 DAT deficient patients that progresses into Parkinsonism and dystonia. This disease manifests
55 generally in the first 6 months post birth (infantile) or occasionally during childhood,
56 adolescence or adulthood (juvenile). The cell surface levels of DAT variants associated with
57 DTDS are typically non-detectable (in infantile cases) or severely reduced (in juvenile cases)
58 due to their misfolding and subsequent retention within the endoplasmic reticulum (ER).
59 While the use of ibogaine or noribogaine as a medication for DTDS is warranted, its
60 hallucinogenic profile will likely preclude its therapeutic usefulness in this patient population
61 (*Wasko et al., 2018*).
62

63 The closely related monoamine transporters SERT (*SLC6A4*), DAT (*SLC6A3*) and NET
64 (*SLC6A2*) form a branch of the solute carrier-6 (SLC6) family of secondary active
65 transporters (*Kristensen et al., 2011*). They modulate monoaminergic neurotransmission by
66 retrieving their eponymous substrates from the synaptic cleft, which supports replenishing of
67 vesicular stores. SERT and - to a lesser extent - NET are the most important targets for
68 antidepressants, which act as inhibitors. For example, SSRIs (selective serotonin reuptake
69 inhibitors) are used to treat major depression, obsessive-compulsive disorders, and general

70 anxiety disorders. The therapeutic indication for DAT inhibition is more restricted (e.g.,
71 methylphenidate for attention-deficit-hyperactivity disorder or modafinil for sleep disorders).
72 However, DAT is a prominent target for illicit drugs (e.g., cocaine and amphetamines). This is
73 also true to some extent for SERT, which is the target of 3,4-methylene-
74 dioxymethamphetamine (MDMA, ecstasy) and its congeners. Thus, the chemical space for
75 these transporter targets has been explored (Sitte and Freissmuth, 2015). Of note, monoamine
76 transporter ligands can range from full substrates to typical inhibitors, as well as atypical
77 inhibitors, depending on chemical structure and transporter conformation (Schmitt *et al.*,
78 2013; Reith *et al.*, 2015; Bhat *et al.*, 2019). Typical inhibitors (e.g. cocaine, most
79 antidepressants) bind to and trap the transporter in the outward facing state thus precluding
80 any subsequent conformational transition required for entry of the protein into a transport
81 mode. In contrast, the substrate-bound transporter enters an occluded state. Substrate
82 translocation is initiated by opening of an inner gate, which releases the substrate together
83 with co-substrate ions (Na^+ and Cl^-) on the intracellular side (Sitte and Freissmuth, 2015).
84 Full substrates allow the transporter to undergo its transport cycle in a manner
85 indistinguishable from cognate neurotransmitter. Full substrates, which differ from
86 neurotransmitters in their cooperative interaction with the co-transported sodium, act as
87 amphetamine-like releasers by driving the transporter into an exchange mode (Hasenhuettl *et*
88 *al.*, 2019). Partial substrates/releasers support the transport cycle/exchange mode albeit less
89 efficiently than full substrates/releasers, because they bind tightly to conformational
90 intermediates and thus preclude rapid transitions (Bhat *et al.*, 2017). Atypical inhibitors trap
91 the transporter in conformations other than the outward facing state. Ibogaine is an atypical
92 inhibitor that binds the inward-facing state of SERT (Jacobs *et al.*, 2007; Bulling *et al.*, 2012;
93 Burtscher *et al.*, 2018; Coleman *et al.*, 2019) and, presumably, of DAT and NET. Several
94 arguments support the conjecture that the folding trajectory of monoamine transporters
95 proceeds through the inward facing state (Freissmuth *et al.*, 2017), thus providing a
96 mechanistic basis for rationalizing the pharmacochaperoning action of ibogaine (El-Kasaby *et*
97 *al.*, 2010; El-Kasaby *et al.*, 2014; Koban *et al.*, 2015; Kasture *et al.*, 2016; Beerepoot *et al.*,
98 2016; Asjad *et al.*, 2017) and of other compounds (Bhat *et al.*, 2017).

99 Ibogaine and its metabolite noribogaine are the most efficacious pharmacochaperones
100 for folding-deficient versions of SERT and DAT identified to date and provide templates to
101 generate promising new leads. In this study, we explored the chemistry of ibogaine to broaden
102 the efficacy profile for this drug in rescuing misfolded SERT and DAT mutants. Ibogaine has
103 a complex and rigid structure, which until recently has been largely unexplored (Kruegel *et*

104 *al.*, 2015, *Gassaway et al.*, 2016). Here, we generated a series of novel ibogaine analogs by
105 investigating the chemical space surrounding the parent molecule using a two-pronged
106 approach: 1) Deconstructing ibogaine by introducing flexible hydrocarbon linkers that
107 connect the indole ring to either a isoquinuclidine ring or a tropane ring and 2) Reconfiguring
108 and completely substituting the isoquinuclidine ring of ibogaine with the tropane ring system.
109 This allowed for defining structural determinants required for high-affinity binding to wild
110 type SERT and DAT and for pharmacochaperoning two synthetic folding-deficient mutants:
111 SERT-PG^{601,602}AA and the orthologous DAT-PG^{584,585}AA. Based on these experiments, we
112 identified a novel fluorinated tropane-based analog, which was more potent and more
113 efficacious than the parent compound in rescuing misfolded versions of SERT and DAT,
114 including disease-relevant DTDS mutants. Importantly, this compound was active *in vivo* and
115 restored sleep to flies harboring a misfolded DAT mutant.

116
117

Results

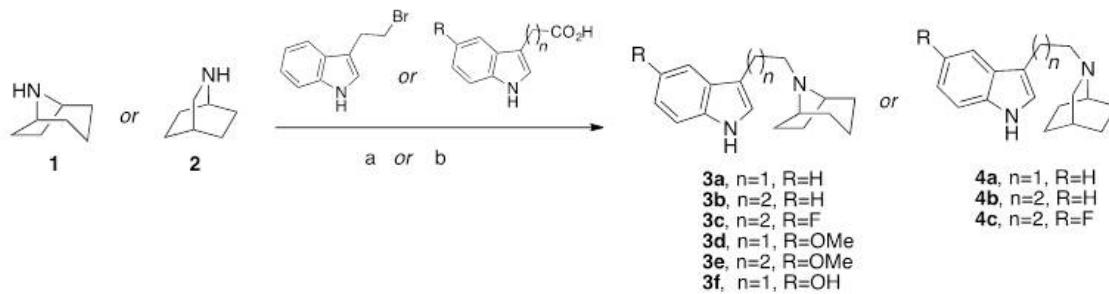
118

Synthesis

119
120
121
122
123
124
125
126
127
128
129
130
131
132
133

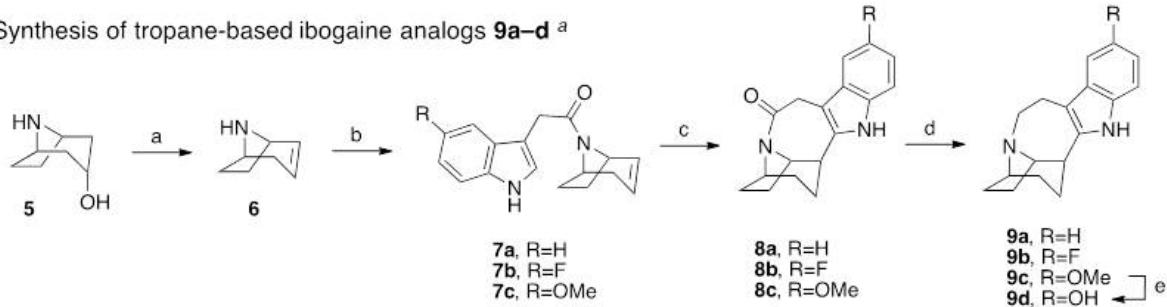
Ibogaine can be viewed as a serotonin analog, where the basic nitrogen is fixed in space by a fused bicyclic ring structure (*cf. Table 1*). Two strategic approaches were undertaken that yielded a number of unique products to the ibogaine series in relatively few synthetic steps. The first approach (**Scheme 1A**) involved a deconstructive strategy, whereby the indole ring was disconnected from the isoquinuclidine ring structure, thereby offering greater flexibility along with a comparable number of hydrocarbon atoms by either retaining the isoquinuclidine ring (**4a–c**) or replacing it with a tropane ring (**3a–f**). The second approach (**Scheme 1B**) aimed to reconfigure and completely substitute the fused isoquinuclidine ring of ibogaine with the tropane ring system conferring the novel intermediate amides (**8a–c**) and their reduced tertiary amine analogs (**9a–d**). Of note, the tropane ring is a hallmark structure in many classical monoamine transporter inhibitors (e.g., cocaine, benztrapine) and thus was envisioned to potentially retain binding affinities at DAT and SERT. In both approaches, the ethyl group of ibogaine was eliminated to save on synthetic effort. In addition, we surmised that the ethyl group was immaterial for binding affinity (see below).

A. Synthesis of deconstructed ibogaine analogs **3a-f and **4a-c** ^a**



^a Reagents and conditions: (a) 3-(2-bromoethyl)-1H-indole, **1** or **2**, K_2CO_3 , CH_3CN , reflux; (b) i. appropriate 3-indoleacetic acid or 3-indolepropanoic acid, CDI, THF, rt; ii. LAH, CH_2Cl_2 , rt.

B. Synthesis of tropane-based ibogaine analogs **9a-d ^a**



^a Reagents and conditions: (a) H_2SO_4 , 160 °C; (b) appropriate 3-indoleacetic acid, CDI, CH_2Cl_2 , rt; (c) i. $Pd(CH_3CN)_4BF_4$, CH_3CN , ii. $NaBH_4$, $EtOH$; (d) i. BMS, CH_2Cl_2 , reflux, ii. 3N HCl, reflux; (e) 3N HCl, reflux.

134 Scheme 1. Synthetic scheme of the ibogaine analogs

135 The synthetic schemes of the two approaches are shown in **Scheme 1**. 8-
136 Azabicyclo[3.2.1]octane (nortropine, **1**) or 2-azabicyclo[2.2.2]octane (**2**) was directly linked
137 at the *N*-position with various indole substituents via either nucleophilic substitution with 3-
138 (2-bromoethyl)-1H-indole or by tandem 1,1'-carbonyldiimidazole (CDI) coupling with an
139 appropriate 3-indoleacetic acid or 3-indolepropanoic acid followed by lithium aluminum
140 hydride (LAH) reduction to give the desired products (**3a-f**, **4a-c**) as free bases (**Scheme 1A**).
141 The second approach (**Scheme 1B**) started similarly following the dehydration of nortropine
142 (**5**) in sulfuric acid to give racemic nortropidene (**6**), which was linked with an appropriate 3-
143 indoleacetic acid substituent via CDI coupling. Next, similarly to Trost *et al.* (Trost *et*
144 *al.*, 1978) and Kruegel *et al.* (Kruegel *et al.*, 2015) in their syntheses of ibogamine,
145 electrophilic palladium promoted cyclization of racemic **7a-c**, followed by sodium
146 borohydride ($NaBH_4$) reduction, gave the racemic products **8a-c**. This key step produced the
147 corresponding amides in good yields (63-81% yields, see Experimental Methods in S. I. for
148 details), without necessitating chromatographic separation. Notably, Trost *et al.* (Trost *et*
149 *al.*, 1978) and Kruegal *et al.* (Kruegel *et al.*, 2015) reported much lower isolated yields of
150 ibogamine (19% and 33%, respectively) with this chemistry, by using the isoquinuclidine core
151
152

153 and corresponding amine instead. Finally, the amide reduction of racemic **8a–c** was
154 accomplished with borane dimethyl sulfide complex (BMS) to give racemic **9a–d**, where **9d**
155 was formed from the *O*-demethylation of **9c** following extended stirring at reflux, in 3N HCl.
156

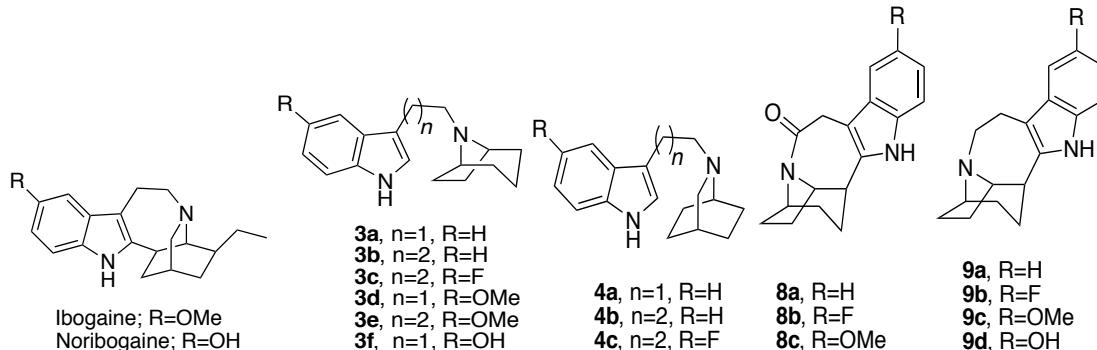
157 ***Structure activity-relationship of ibogaine analogs at SERT and DAT***

158 Ligand affinities at DAT and SERT can be determined by measuring their ability to displace a
159 radioligand from the transporter or to block substrate uptake. During substrate translocation,
160 transporters undergo a conformational cycle. Radioligands are high-affinity inhibitors, which
161 bind to the outward facing state. In addition, both the membrane potential and the asymmetric
162 ion distribution are absent in binding assays with membrane preparations. Hence, affinity
163 estimates for some ligands can differ substantially, if inhibition of substrate uptake and
164 displacement of substrate are assessed. Because of conformational trapping in the absence of
165 an ion gradient, these differences can reach several orders of magnitudes (Bhat *et al.*, 2017).
166 Accordingly, we determined the affinity of our novel analogs by measuring their ability to
167 both, displace a radioligand in membranes prepared from rat brain stem (**Supplementary Fig**
168 **1**, SERT) and rat striatum (**Supplementary Fig 2**, DAT) and inhibit cellular uptake in
169 transfected cells (**Supplementary Figs 3 and 4** for SERT and DAT, respectively).
170 Noribogaine is known to bind SERT with higher affinity, in both binding and uptake
171 inhibition assays, over the methoxylated parent compound, ibogaine, and our data confirm
172 this in **Table 1**. However, as previously reported (Mash *et al.*, 1995), ibogaine and
173 noribogaine have similar affinities for DAT (also seen in **Table 1**). It is evident from the data
174 in **Table 1** that the C-12 substitutions primarily determine affinity to SERT in all analogs
175 tested. Methoxy- (**3d**, **3e**, **9c**) or hydroxy-substitution (**3f**, **9d**) in this 12-position has little
176 effect on DAT affinity as compared to the unsubstituted analogs (**3a**, **3b**, **4a**, **4b**, **8a**, **9a**), but
177 the hydroxy-substituted analogs have higher affinity at SERT (**3f**, **9d**). Interestingly, when the
178 hydroxyl group is substituted with fluorine, compounds **3c**, **4c** and **9b** also exhibit similar
179 affinities at SERT to the hydroxy-analogs. Only **9b** showed submicromolar affinity for DAT,
180 suggesting a potentially pivotal role for fluorine substitution. In addition, compounds **9c** and
181 **9d** are ibogaine and noribogaine analogs, respectively, in which the 2-ethyl group has been
182 removed and the isoquinuclidine core has been replaced with a tropane ring. The affinities of
183 these compound pairs were similar at DAT and SERT (**9c**/ibogaine v. **9d**/noribogaine). These
184 data supported our prediction that the ethyl-group on the isoquinuclidine ring of the
185 ibogamine structure is dispensable and that the isoquinuclidine core can be replaced by
186 tropane. The rigidity imparted by the azepine-ring (compounds **9a–d**, ibogaine, noribogaine)

187 is a minor determinant of affinity. Indeed, the affinity of compounds **3c** and **9b**, for instance,
 188 were comparable.

189

190 **Table 1: Radioligand uptake and binding data at DAT and SERT^a**



191

	Uptake inhibition		Binding inhibition		ratio		Pharmacochaperoning			
	$IC_{50} \pm S.D.$		$K_i \pm S.D.$		IC_{50}/K_i		$EC_{50} \pm S.D.$	V_{max}	$EC_{50} \pm S.D.$	V_{max}
	WT-SERT	WT-DAT	WT-SERT	WT-DAT	WT-SERT	WT-DAT	μM	%	μM	%
Ibogaine	8.2 \pm 3.5	22.1 \pm 6.3	7.4 \pm 0.7	5.3 \pm 1.7	1.1	4.2	2.6 \pm 1.9	103 \pm 16	19.6 \pm 4.9	88 \pm 16
Noribogaine	1.2 \pm 0.5	15.5 \pm 7.8	0.6 \pm 0.1	4.0 \pm 1.8	2.1	3.9	4.6 \pm 1.2	100 \pm 8	17.1 \pm 6.1	100 \pm 13
3a	9.1 \pm 3.1	25.6 \pm 10.2	1.2 \pm 0.2	3.1 \pm 0.9	7.8	8.2	NC*	19 \pm 6*	NC*	15 \pm 10*
3b	6.5 \pm 5.2	24.4 \pm 17.9	1.85 \pm 0.3	1.7 \pm 0.1	3.5	14.4	12.9 \pm 6.3	60 \pm 17	NC*	15 \pm 8*
3c	0.5 \pm 0.1	9.09 \pm 1.64	0.19 \pm 0.03	4.4 \pm 1.4	2.4	2.1	2.7 \pm 0.78	57 \pm 13	17.1 \pm 5.6	47 \pm 18
3d	11.9 \pm 1.6	58.2 \pm 41.4	4.5 \pm 0.4	8.8 \pm 4.94	2.6	6.6	NC*	11 \pm 3*	NC*	20 \pm 15*
3e	21.6 \pm 17.4	179 \pm 94	13.0 \pm 2.7	10.2 \pm 3.8	1.7	17.6	NC*	38 \pm 19*	NC*	15 \pm 9*
3f	0.2 \pm 0.1	78.0 \pm 27.9	0.12 \pm 0.02	21.8 \pm 9.4	1.6	3.6	NC*	8 \pm 2*	NC*	13 \pm 10*
4a	4.6 \pm 2.63	6.5 \pm 2.1	0.81 \pm 0.03	1.9 \pm 0.2	5.7	3.5	NC*	25 \pm 6*	NC*	15 \pm 9*
4b	8.2 \pm 1.0	11.9 \pm 1.6	1.7 \pm 0.3	1.5 \pm 0.3	4.8	7.7	NC*	29 \pm 8*	NC*	10 \pm 6*
4c	0.3 \pm 0.2	11.8 \pm 9.1	0.13 \pm 0.34	2.6 \pm 0.7	2.5	4.5	0.7 \pm 0.3	68 \pm 16	NC*	24 \pm 8*
8a	ND	ND	>100	39.7 \pm 27.9	ND	ND	ND	ND	ND	ND
8b	ND	ND	>100	51.2 \pm 14.6	ND	ND	ND	ND	ND	ND
8c	ND	ND	>100	45.78 \pm 6.5	ND	ND	ND	ND	ND	ND
9a	6.9 \pm 3.9	16.7 \pm 11.2	4.4 \pm 0.2	3.6 \pm 0.2	1.6	4.6	3.05 \pm 2.3	94 \pm 23	18.9 \pm 2.9	51 \pm 21
9b	0.4 \pm 0.2	7.2 \pm 3.8	0.18 \pm 0.01	0.8 \pm 0.3	2.3	8.9	0.06 \pm 0.04	100 \pm 6	25.7 \pm 6.3	673 \pm 245
9c	29.9 \pm 16.4	10.8 \pm 9.3	7.3 \pm 0.5	3.1 \pm 0.3	4.1	3.5	2.1 \pm 0.8	81 \pm 20	19.2 \pm 8.7	53 \pm 13
9d	0.9 \pm 0.5	15.2 \pm 9.7	0.78 \pm 0.04	2.9 \pm 0.3	1.2	5.3	1.4 \pm 0.8	89 \pm 19	5.8 \pm 1.9	49 \pm 30

192 ^aEach IC_{50} , K_i , K_m and V_{max} value represent data from at least 3 independent experiments, each
 193 performed in triplicate. V_{max} refers to the maximum transport restored by noribogaine, which was set
 194 at 100% to account for inter-assay variation. Experimental procedures are described in detail in
 195 Methods. NC*: not calculable: a reliable EC_{50} could not be estimated, because functional rescue was
 196 too low; in this instance V_{max} * refers to the activity restored by 30 μM of the compound. ND: not
 197 determined.

198

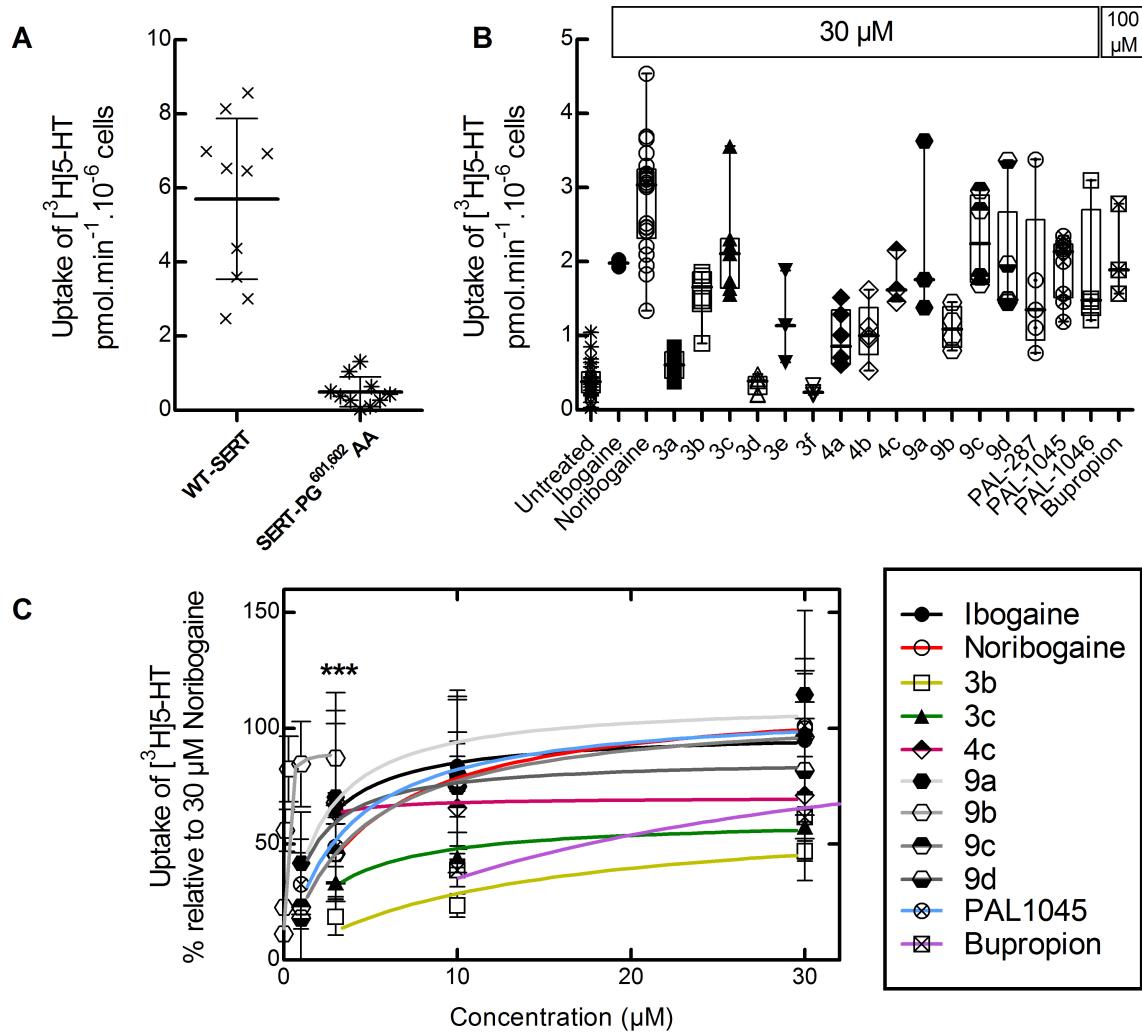
199 The amide analogs, in which the basic amine was neutralized, had substantially reduced
200 affinities (*cf.* compounds **8a–c** and **9a–c** in **Table 1**) demonstrating the necessity for a
201 protonatable nitrogen. In general, the affinities obtained from binding displacement were
202 higher than those estimated from uptake inhibition. Despite this difference, in the two affinity
203 estimates in SERT, the rank order of potency determined by uptake inhibition was reasonably
204 similar to that determined by binding: analogs with hydroxy- and fluorine substituents were
205 more potent in blocking substrate uptake by SERT than the methoxy- and unsubstituted
206 counterparts (**Table 1**).

207 In DAT, all fluorinated analogs (**3c**, **4c** and **9b**) also ranked among the most potent blockers
208 of DAT uptake (**Table 1**). However, affinity estimates from binding and uptake inhibition
209 differed in part substantially with IC_{50}/K_i ratios ranging from 2 to 14 (**Table 1**). Variations in
210 the ratio of IC_{50}/K_i (see **Table 1**) are indicative of differences in conformational trapping
211 (*Bhat et al.*, 2017) rather than of species differences (rat v. human transporters). More
212 importantly, conformational trapping is predictive of a pharmacochaperoning action (*Bhat et*
213 *al.*, 2017). Accordingly, we determined if some of these analogs were capable of rescuing
214 folding-deficient mutants of SERT and DAT.

215

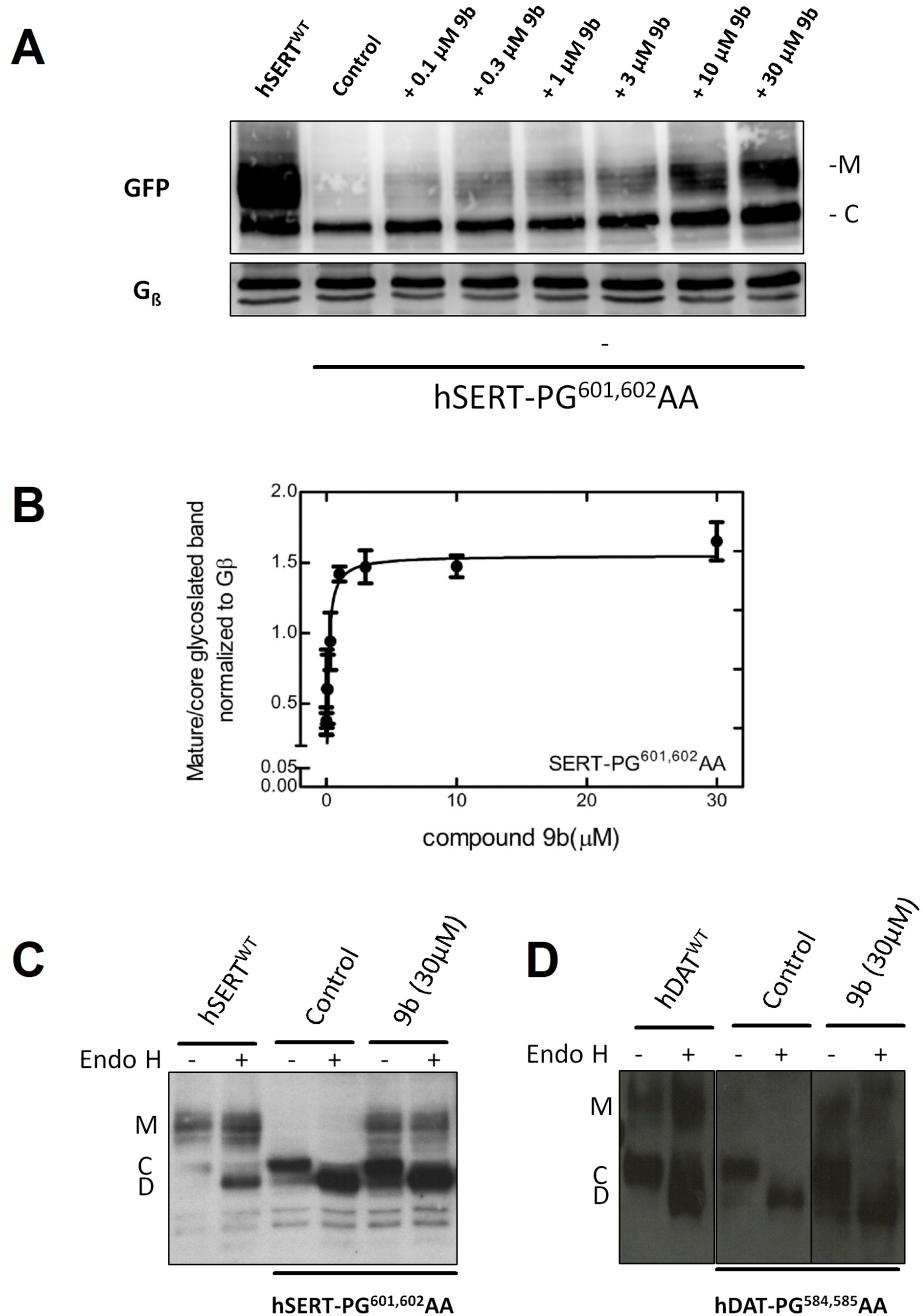
216 **Pharmacochaperoning action of the ibogaine analogs on SERT-PG^{601,602}AA and on** 217 **DAT-PG^{584,585}AA**

218 Several folding-deficient versions of SERT have been generated and characterized to
219 understand the actions of pharmacochaperones (*El-Kasaby et al.*, 2010; *El-Kasaby et al.*,
220 2014; *Koban et al.*, 2015). The mutant SERT-PG^{601,602}AA is stalled at an early stage of the
221 folding trajectory (*El-Kasaby et al.* 2014). Hence, based on its severe phenotype, we selected
222 this mutant to examine and compare the ability of our ibogaine analogs (i.e., compounds **3a–**
223 **3f**, **4a–4c**, **9a–9d**) with ibogaine and noribogaine to act as pharmacochaperones. Misfolded
224 transporters are retained in the ER and thus unavailable to accomplish their eponymous
225 actions, that is cellular uptake of substrate. Pretreatment of cells with a pharmacochaperone
226 restores folding of the protein and thus its subsequent delivery to the cell surface (*El-Kasaby*
227 *et al.*, 2010; *El-Kasaby et al.*, 2014; *Koban et al.*, 2015; *Kasture et al.*, 2016; *Beerepoot et al.*,
228 2016; *Asjad et al.*, 2017). This can be readily monitored by measuring cellular substrate
229 uptake. As shown in **Fig. 1A**, in cells expressing SERT-PG^{601,602}AA, there is negligible
230 uptake of [³H]5-HT (<<10%) when compared to those expressing wild type SERT.



231
232 **Figure 1. $[^3\text{H}]5\text{-HT}$ uptake in HEK293 cells expressing hSERT-PG 601,602 AA after pre-incubation with**
233 **ibogaine analogs. A.** Comparison of $[^3\text{H}]5\text{-HT}$ uptake by HEK293 cells transiently expressing wild type
234 (WT) hSERT or hSERT-PG 601,602 AA. Each symbols represents the result from an individual experiment
235 (done in triplicate); means \pm S.D. are also shown. **B.** hSERT-PG 601,602 AA expressing cells were incubated
236 in the presence of the indicated compounds (30 μM in all instances but bupropion, 100 μM). After 24
237 h, $[^3\text{H}]5\text{-HT}$ uptake was determined as outlined under “Methods”. Values from individual
238 experiments (done in triplicate) are shown as dots; box plots show the median and the interquartile
239 range. **C.** Concentration-response curves for pharmacochaperoning by the indicated compounds
240 (selected as positive hits from *panel B*). Rescued uptake was normalized to that achieved by 30 μM
241 noribogaine (=100%) to account for inter-experimental variations. ***, E_{max} for **9b** was achieved at 3
242 μM , uptake inhibition was observed after pre-incubation with higher concentrations. The solid lines
243 were drawn by fitting the data to the equation for a rectangular hyperbola (for EC_{50} and E_{max} of
244 mutant rescue see **Table 1**). Data were obtained in at least three independent experiments carried
245 out in triplicate. The error bars indicate S.D.

246 In cells, which were preincubated for 24 h in the presence of 30 μ M ibogaine, noribogaine
247 and other pharmacochaperones, i.e., the naphthyl-propanamine series (PAL-287, PAL-1045
248 and PAL-1046, *Rothman et al., 2012; Bhat et al., 2017*) and 100 μ M bupropion (*Beerepoot et*
249 *al., 2016*), substrate uptake supported by SERT-PG^{601,602}AA increased to levels, which
250 corresponded to 25-40% of transport activity of wild type SERT (**Fig. 1B**). Similarly, if the
251 cells were preincubated in the presence of 30 μ M of the new ibogaine analogs, appreciable
252 levels of transport were seen with compounds **3b**, **3c**, **4c**, **9a**, **9c** and **9d** (**Fig. 1B**). Additional
253 experiments (not shown) confirmed that compound **9b** was more effective at concentrations
254 <10 μ M than at 30 μ M. Hence, it was also included in the list of positive hits, which were
255 further investigated to determine their potency and their efficacy as pharmacochaperones. We
256 compared their action in individual transient transfections by normalizing their pharmaco-
257 chaperoning activity to the transport activity restored by 30 μ M noribogaine (**Fig. 1C**).
258 Known pharmacochaperones (PAL-1045, bupropion, ibogaine and noribogaine) were also
259 examined as reference compounds. All compounds belonging to the **9a–9d** series were as
260 effective as noribogaine, but compound **9b** had an EC₅₀ of ~60 nM (curve represented as ***
261 in **Fig. 1C**). The other fluoro-analog, **4c**, had an EC₅₀ of ~600 nM but was less efficacious
262 (E_{max} ~70% of that of noribogaine). Other compounds, including noribogaine (EC₅₀ = ~3 μ M),
263 rescued SERT-PG^{601,602}AA with EC₅₀-values in the low to mid μ M range (**Table 1**).
264 Our data suggests **9b** to be the most potent of all compounds tested in rescuing SERT-
265 PG^{601,602}AA. We visualized the glycosylation state of SERT-PG^{601,602}AA by immunoblotting
266 to confirm the pharmacochaperoning action of **9b** by an independent approach. During their
267 synthesis in the ER, membrane proteins undergo N-linked core glycosylation; this core
268 glycan, which can be removed by endoglycosidase H, is thus present on ER-retained
269 misfolded mutants. Rescued mutants are exported to the Golgi, where they acquire additional
270 sugar moieties. The resulting complex glycan structure is resistant to cleavage by
271 endoglycosidase H. The core glycosylated protein is homogeneous and smaller in size than
272 the mature glycosylated version, which is heterogeneous due to the stochastic nature of
273 complex glycosylation. Accordingly, in lysates prepared from transiently transfected cells,
274 wild type SERT was visualized by immunoblotting as a band migrating at 75 kDa and broad
275 smear coalescing from a collection of bands in the range of 90 to 110 kDa (left hand lane, **Fig.**
276 **2A**). The size of the lower band was reduced after cleavage by endoglycosidase H (*cf.* first
277 and second lane in **Fig. 2C**) confirming that it corresponded to the core glycosylated band
278 ("C"). In contrast, the migration of the collection of upper bands was insensitive to cleavage

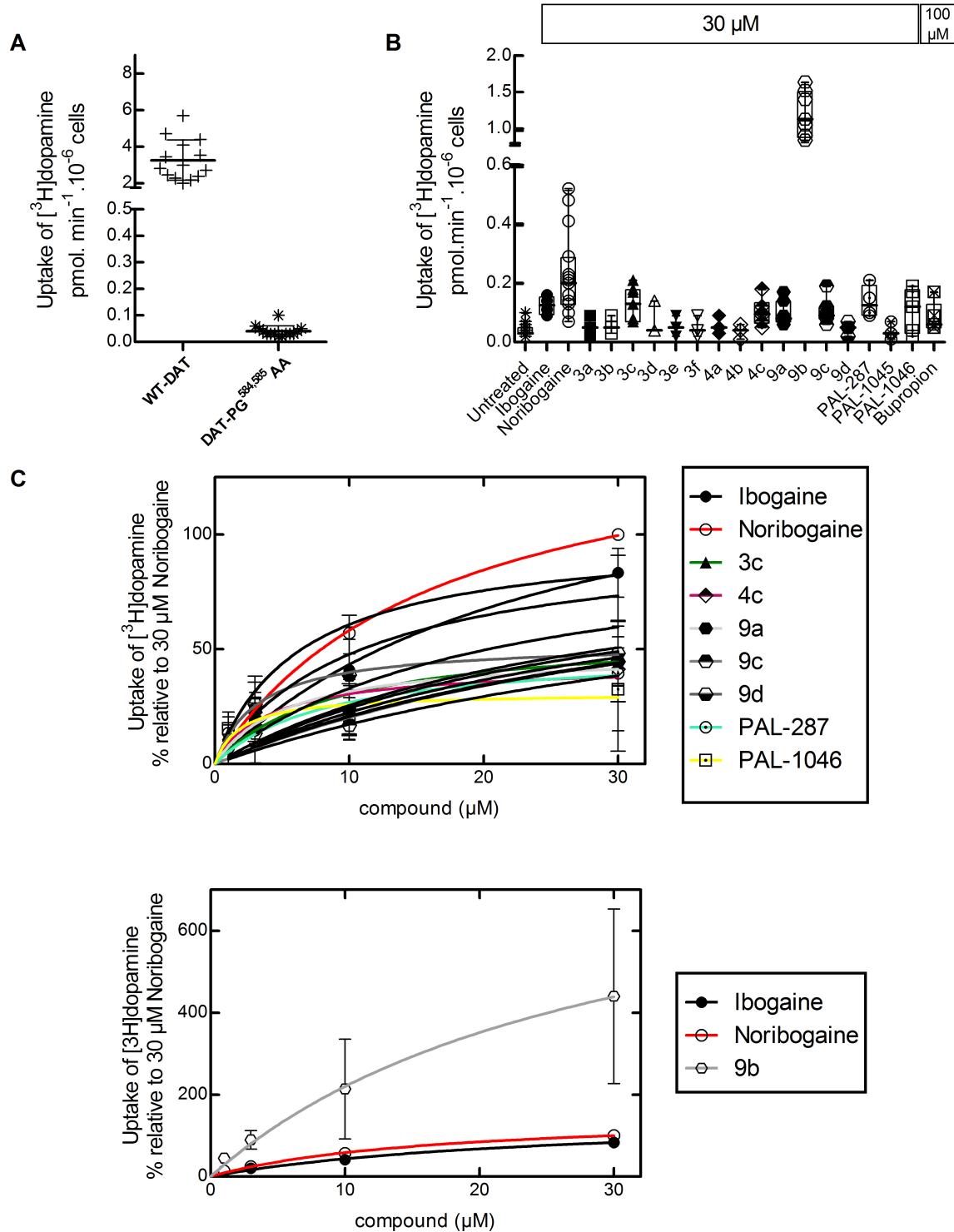


279
280 **Figure 2. Enhanced mature glycosylation of hSERT-PG^{601,602}AA or hDAT-PG^{584,585}AA in cells pre-incu-
281 bated with 9b. A.** HEK293 cells transiently expressing hSERT-PG^{601,602}AA were incubated in the
282 absence (negative control = lane 2) or presence of the indicated concentrations of **9b** for 24 h. Cells
283 expressing wild type (WT) hSERT were the positive control (first labeled as hSERT^{WT}). Membrane
284 proteins extracted from these cells were denatured, electrophoretically resolved and transferred
285 onto nitrocellulose membranes. The blots were incubated overnight at 4 °C with anti-GFP (top) or
286 anti-G β (bottom, loading control) antibodies. The immunoreactive bands were detected with using a
287 horseradish peroxidase-conjugated secondary antibody. **B.** Concentration-response curve generated
288 from three independent experiments carried out as in A. The ratio of mature (M) to core glycosylated

289 band (C) was quantified densitometrically, normalized to the density of G β (loading control) and
290 compared with the ratio observed in each blot for untreated control cells. The density ratios for
291 untreated control cells and those exposed to 30 μ M compound **9b** (30 μ M) were 0.38 \pm 0.09 and 1.65
292 \pm 0.14, respectively. The solid line was drawn by fitting the data to the equation for a rectangular
293 hyperbola. The EC₅₀ of rescue was 134 \pm 30 nM. **C.** and **D.** In separate experiments, lysates were
294 prepared from cells expressing the SERT-PG^{601,602}AA and DAT-PG^{584,585}AA mutants treated in the
295 absence or presence of 30 μ M **9b** and subjected to enzymatic digestion by endoglycosidase H (Endo
296 H). Endo H specifically cleaves core glycans (C) to generate lower molecular weight deglycosylated
297 fragments (D). Mature glycosylated bands (M) are resistant to the actions of Endo H.

298
299 by endoglycosidase H confirming that they had acquired the mature glycan (*cf.* bands "M" in
300 first and second lane in **Fig. 2C**). In contrast, lysates from cells expressing SERT-PG^{601,602}AA
301 contain predominantly the core glycosylated protein (second lane in **Fig. 2A** showing control
302 cells). As predicted from its ability to restore substrate uptake in pretreated cells (**Fig. 1B,C**),
303 **9b** induced the appearance of slowly migrating forms of SERT-PG^{601,602}AA in lysates
304 prepared after preincubation of the cells in a concentration-dependent manner (lanes 3 to 8 in
305 **Fig. 2A**). Plotting this concentration response revealed an EC₅₀ of 134 \pm 30 nM (**Fig. 2B**),
306 which is in excellent agreement with that calculated from restoration of substrate uptake (**Fig.**
307 **1C** and **Table 1**). We confirmed that the mature glycosylated species of the rescued mutant is
308 resistant to cleavage by endoglycosidase H (shown for lysates from cells compound **9b**-
309 pretreated cells in **Fig. 2C**).

310 We posited that the potency and efficacy of the ibogaine analogs in pharmacochaperoning
311 SERT and DAT would best be compared in the equivalent folding-deficient mutants. The
312 residues P⁶⁰¹ and G⁶⁰² in SERT are conserved in DAT at positions P⁵⁸⁴ and G⁵⁸⁵, respectively.
313 Thus, we created DAT-PG^{584,585}AA and verified that it was retained within the cell (not
314 shown), accumulated predominantly as core glycosylated species (*cf.* lanes 3 & 4 and lanes 1
315 & 2 in **Fig. 2D**) and only mediated very low level of substrate uptake (**Fig. 3A**). The folding
316 deficiency of this mutant is not only expected based on the homology of SERT and DAT but
317 also predicted from earlier work: mutation of glycine⁵⁸⁵ to an alanine in DAT suffices to
318 completely abrogate surface expression of the transporter (*Miranda et al., 2004*). As shown in
319 **Fig. 3B**, if cells transiently expressing DAT-PG^{584,585}AA were preincubated in the presence of
320 30 μ M noribogaine, there was a robust increase (~6 fold) in substrate uptake. Of the other
321 known pharmacochaperones, ibogaine, PAL-287 and PAL-1046 (but not PAL-1045) were
322 also active. Of the ibogaine analogs, compounds **3c**, **4c** (the fluorinated open-ring analogs)



323

324 **Figure 3. $[^3\text{H}]$ Dopamine uptake in HEK293 cells expressing the hDAT-PG^{584,585}AA after pre-
325 incubation with ibogaine analogs.** **A.** Comparison of $[^3\text{H}]$ DA uptake in HEK293 cells transiently
326 expressing wild type (WT) hDAT or hDAT-PG^{584,585}AA. Each symbols represents the result from an
327 individual experiment (done in triplicate); means \pm S.D. are also shown. **B.** hDAT-PG^{584,585}AA
328 expressing cells were incubated in the presence of the indicated compounds (30 μM in all instances

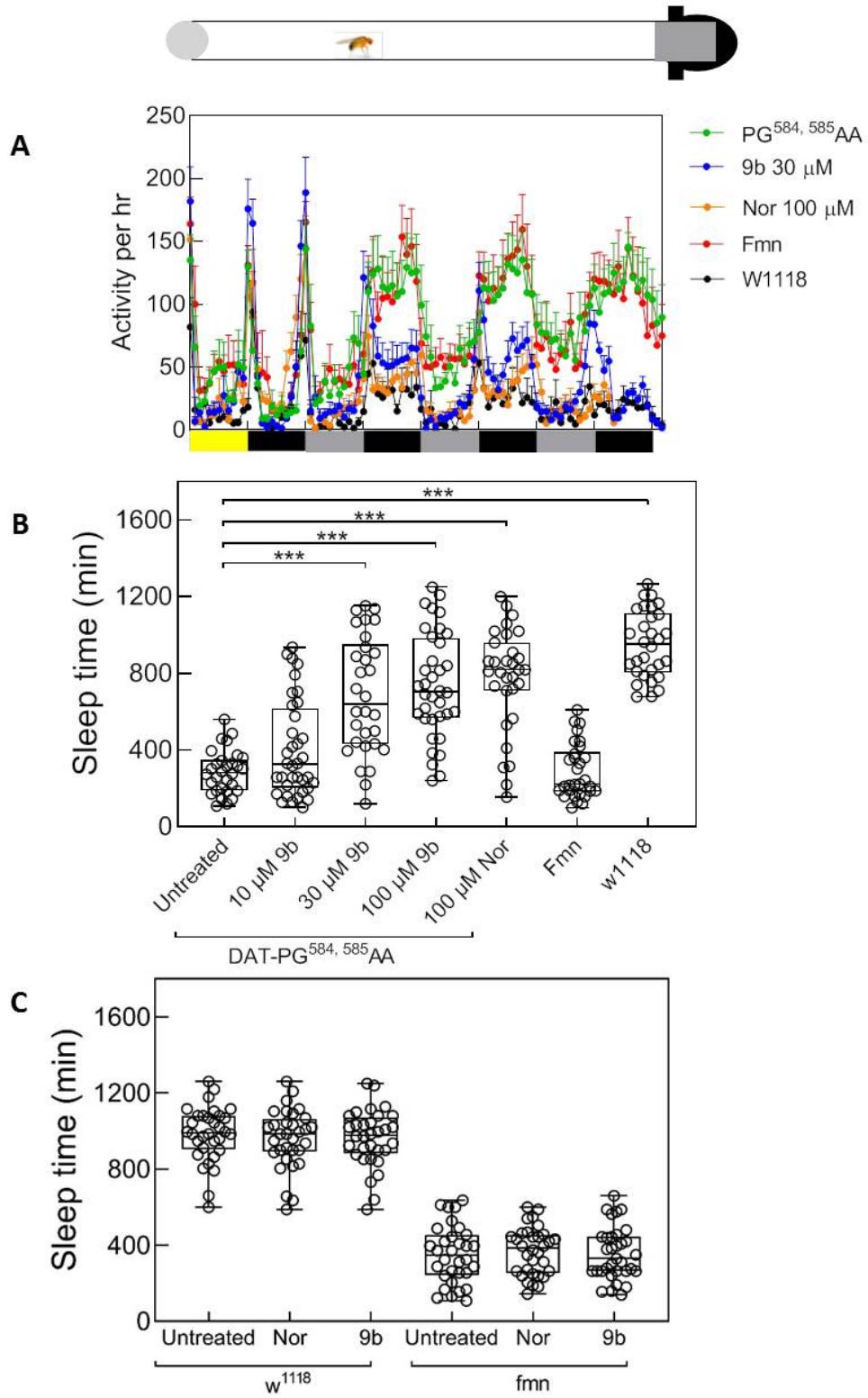
329 but bupropion, 100 μ M). After 24 h, , specific [3 H]dopamine uptake was determined as outlined
330 under “Methods”. Values from individual experiments (done in triplicate) are shown as dots; box
331 plots show the median and the interquartile range. **C. (Top)** Concentration-response curves for
332 pharmacochaperoning by the indicated compounds (selected as positive hits from *panel B*). Rescued
333 uptake was normalized to that achieved by 30 μ M noribogaine (=100%) to account for inter-
334 experimental variations. Rescue by **9b** was plotted separately because of its very high efficacy in
335 comparison to other analogs (*bottom*). The solid lines were drawn by fitting the data to the equation
336 for a rectangular hyperbola (for EC₅₀ and E_{max} of mutant rescue see **Table 1**). Data were obtained in at
337 least three independent experiments carried out in triplicate. The error bars indicate S.D.

338
339 and **9a**, **9c** and **9d** rescued the function of DAT-PG^{584,585}AA to a modest extent. In contrast,
340 compound **9b** was a very efficacious pharmacochaperone for DAT-PG^{584,585}AA mutant with
341 transport activity exceeding that restored by noribogaine by ~4-fold. Thus, after preincubation
342 in the presence of compound **9b**, cells expressing DAT-PG^{584,585}AA recovered up to ~40% of
343 the uptake velocity seen in cells expressing wild type DAT (*cf. Figs. 3A & 3B*) and mature
344 glycosylated protein accumulated to appreciable levels (lanes 5 and 6 in **Fig. 2D**). We
345 selected compounds **3c**, **4c**, **9a–9d**, PAL-287 and PAL-1046 as positive hits. Cells transiently
346 expressing DAT-PG^{584,585}AA were preincubated with increasing concentrations of these
347 compounds and substrate uptake was subsequently determined to obtain concentration-
348 response curves for their pharmacochaperoning activity. When compared to the reference
349 compounds ibogaine and noribogaine, compounds **3c**, **4c**, **9a**, **9c**, **9d**, PAL-287 and PAL-1046
350 were all less efficacious, and the EC₅₀-values of compounds **3c**, **4c**, **9a**, **9d** and PAL-1046
351 were lower than those of ibogaine and noribogaine (**Fig. 3C**, upper panel). As predicted from
352 the screening assays summarized in **Fig. 3B**, compound **9b** was substantially more efficacious
353 than noribogaine or ibogaine, but the EC₅₀-values of these three compounds were comparable
354 (**Fig. 3C**, lower panel; **Table 1**).
355

356 **Rescue of DAT-PG^{584,585}AA in flies by *in vivo* pharmacochaperoning with the ibogaine 357 analog **9b****

358 In drosophila, dopaminergic projections into the fan-shaped body are required to maintain a
359 wake/sleep cycle (*Liu et al., 2012; Pimentel et al., 2016*). In the absence of functional DAT,
360 flies are hyperactive and have abnormal sleep regulation. Accordingly, drosophilae, in which
361 the endogenous DAT gene is disrupted, are referred to as *fumin* (i.e., Japanese for sleepless)
362 flies (*Kume et al., 2005*). We previously verified that noribogaine was an effective

363 pharmacochaperone *in vivo* for some folding-deficient versions of DAT in flies: when
364 expressed in dopaminergic neurons of *fumin* flies, folding-deficient mutants of human DAT
365 were retained in the ER of the soma of PPL1 neurons but delivered to the axonal terminals of
366 the fan-shaped body, if flies were administered noribogaine; concomitantly, sleep was
367 restored (Kasture *et al.*, 2016; Asjad *et al.*, 2017). Accordingly, we tested compound **9b** to
368 determine if it was also effective as a pharmacochaperone *in vivo*. We generated *fumin* flies,
369 which harbored the human cDNA encoding DAT-PG^{584,585}AA under the control of GAL4
370 driven from a tyrosine hydroxylase promoter (Friggi-Grelin *et al.*, 2003). Adult flies were
371 individually placed in transparent tubes, which contained the food pellet supplemented with
372 designated concentrations of compound **9b** or of noribogaine, and allowed to recover for one
373 day; they then spent an additional day of a 12h light/12h dark cycle (marked as yellow and
374 black rectangle in **Fig. 4A**) to entrain their circadian rhythm. It is evident that, when
375 subsequently released into a dark-dark cycle (marked as gray and black rectangle in **Fig. 4A**),
376 these flies were as hyperactive as *fumin* flies (*cf.* green and red symbols in **Fig. 4A**). Their
377 hyperactivity was greatly reduced by treatment of either compound **9b** or noribogaine at a
378 concentration of 30 and 100 μ M, respectively, in the food pellet (*cf.* blue and orange symbols
379 in **Fig. 4A**). In fact, both compound **9b** and noribogaine reduced the locomotor activity to that
380 seen in the isogenic control line w1118. We quantified the effect of increasing doses of
381 compound **9b** (administered by raising its concentration in the food pellet from 10 to 100 μ M)
382 on sleep time (**Fig. 4B**): on average this increased in a dose-dependent manner by about 2.5-
383 fold, i.e. from 300 min in the absence of any pharmacochaperone in the food pellet to 750 min
384 with 100 μ M of compound **9b** in the food pellet. Sleep duration was comparable to that seen
385 after administration of feed containing 100 μ M noribogaine and approached that seen in
386 w1118 flies. This indicates that pharmacochaperoning by compound **9b** rescued DAT-
387 PG^{584,585}AA in amounts sufficient to restore clearance of dopamine from the synapse and to
388 thus reinstate normal dopaminergic transmission. Finally, the effect of compound **9b** (and of
389 noribogaine) was specific: sleep duration was neither affected by compound **9b** (or
390 noribogaine) in w1118 flies harboring an intact DAT nor in DAT-deficient *fumin* flies (**Fig.**
391 **4C**).



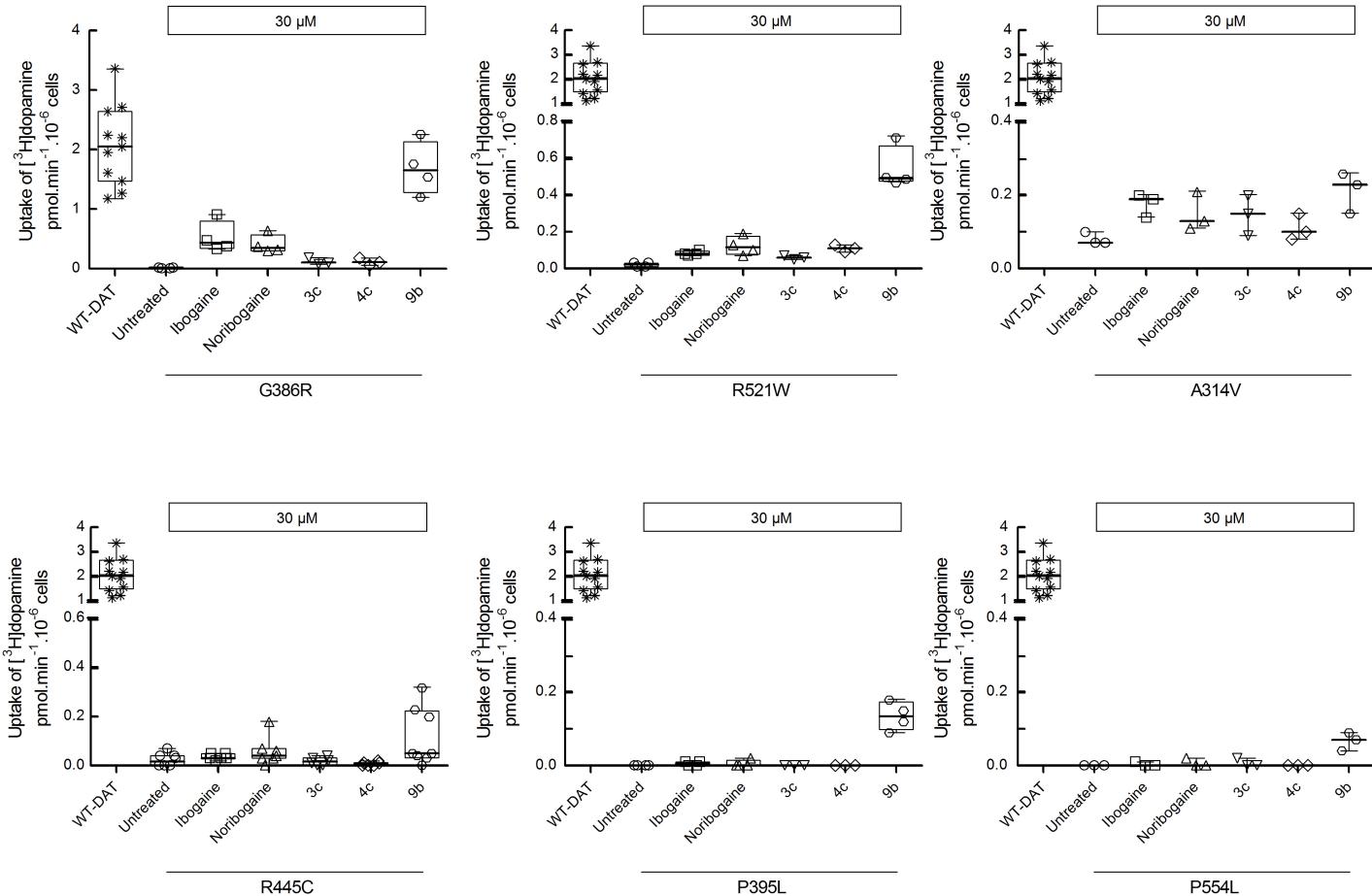
392

393 **Figure 4. Pharmacochaperones restore sleep in flies harboring DAT-PG^{584,585}AA.** **A.** Locomotor
394 activity (measured over 1 min interval and binned into 60 min intervals) was recorded from day 2 to
395 day 5 using a Drosophila activity monitor system (schematically represented above the graph, for
396 details see Methods). The data are means \pm SEM from three independent experiments, which were
397 each carried out in parallel with at least 10 flies/condition. The diurnal light cycles (light-dark on day

398 2, and dark-dark on days 3- 5) are outlined schematically below the graph. **B.** and **C**, sleep time of
399 treated DAT-PG^{584,585}AA mutant flies. Flies expressing hDAT-PG^{584,585}AA in the *fumin* background were
400 fed with food pellets containing the indicated concentrations of noribogaine and 9b. **C.** w1118 and
401 *fumin* flies were used as control and were given food containing 100 μ M noribogaine or 100 μ M 9b.
402 Locomotor activity on day 4 was used to quantify the sleep time using pySolo software. Empty circle
403 represents individual flies. Statistical significance of the observed differences was determined by
404 analysis of variance followed by Dunn's post-hoc test (***, $p < 0.001$).
405

406 **Pharmacochaperoning of folding-deficient DAT-mutants associated with human disease**
407 **by the ibogaine analog 9b**

408 The efficacy of compound **9b**, which was seen *in vivo*, is promising. We therefore examined
409 the ability of compound **9b** to rescue all disease-relevant mutants of DAT. When
410 heterologously expressed in HEK293 cells, measurable substrate influx was restored in six
411 mutants (Fig. 5); in four of these mutants, i.e. DAT-G³⁸⁶R, -R⁵²¹W, -P³⁹⁵L, and -P⁵⁵⁴L,
412 compound **9b** was more efficacious than the reference compounds ibogaine and noribogaine.
413 In contrast, preincubation in the presence of the corresponding deconstructed analogs **3c** and
414 **4c** invariably failed to restore any transport activity. Compound **9b** did not rescue the function
415 of DAT-R⁸⁵L, -V¹⁵⁸F, -L²²⁴P, -G³²⁷R, -L³⁶⁸Q, -Y⁴⁷⁰S and -P⁵²⁹L in any appreciable manner
416 (data not shown).



418 **Figure 5. Pharmacochaperoning of DTDS-associated DAT mutants by 9b.** HEK293 cells were
419 transiently transfected with plasmids encoding YFP-tagged wild-type hDAT or each of the 13 hDAT
420 mutants discovered in patients suffering from dopamine transporter deficiency syndrome (DTDS).
421 After 24 h, the cells were seeded onto 96-well plates for 24 h and were either incubated in the
422 absence (untreated) or presence of either of ibogaine, norbogaine or of the fluorinated ibogaine
423 analogs **3c**, **4c** and **9b** (all at 30 μM) for another 24 h. The cells were washed 4 times with Krebs-MES
424 buffer (pH 5.5) and once with Krebs-HEPES buffer (pH 7.4) to completely remove extracellular
425 reservoir of the compounds. Uptake of $[^3\text{H}]$ dopamine was subsequently measured as outlined under
426 "Material and Methods." The mutants with positive pharmacochaperoning effect by **9b** are
427 represented in this figure. The data are represented as the individual values from at least three
428 independent experiments carried out in triplicate for wild-type hDAT and the DAT mutants, and as
429 box plots with the median and the interquartile range; whiskers indicate the 95% confidence interval.
430 WT-DAT uptake values were used as a control for transfection efficiency. Compound **9b** failed to
431 restore activity in the other DAT mutants (i.e. DAT-R⁸⁵L, -V¹⁵⁸F, -L²²⁴P, -G³²⁷R, -L³⁶⁸Q, -Y⁴⁷⁰S, and -P⁵²⁹L).
432
433

434

435 **Discussion**

436 More than sixty point mutations have been identified, which give rise to human disease,
437 because they lead to misfolding of a member of the SLC6 transporter family (*Freissmuth et*
438 *al., 2018*). Folding defects can be overcome by pharmacochaperones, which reduce energy
439 barriers in the folding trajectory: stalled intermediates proceed to reach the native fold.
440 Pharmacochaperoning by ibogaine was serendipitously discovered, when studying ER export
441 of SERT (*El-Kasaby et al., 2010*). Ibogaine and its metabolite noribogaine effectively rescue
442 some disease-relevant DAT mutants, but with limited efficacy (*Beerepoot et al., 2016; Asjad*
443 *et al., 2017*). Progress is contingent on understanding the attributes, which account for the
444 pharmacochaperoning action of ibogaine. Here, we relied on an orthogonal approach by
445 interrogating the chemical space populated by variations of the ibogaine structure and by
446 probing their effect on the folding space of SERT and DAT, using two analogous mutations
447 and the disease-associated mutations of DAT. Our observations lead to the insight that affinity
448 for the wild type transporter (i.e. the native folded state) and pharmacochaperoning activity
449 are not tightly linked, as the structure-activity relationships differ. This conclusion is based on
450 the following lines of evidence: the affinity for SERT is governed predominantly by
451 substitution on the indole ring with structural rigidity playing a minor role (**Table 1**).
452 Accordingly, hydroxy- (**3f**, **9d**) and fluoro- (**3c**, **4c**, **9b**) analogs had higher affinities for SERT
453 than methoxy- and unsubstituted analogs, but **9d** had lower affinity for SERT than its more
454 flexible analogue **3f**.

455

456 The substitution on the indole ring is less important a determinant for DAT affinity. The most
457 important determinant for pharmacochaperoning efficacy is the structural rigidity imparted by
458 the azepine ring, which is retained by ibogaine, noribogaine and compounds of the **9a–9d**
459 series. In contrast, this rigidity is not required for supporting moderate to high-affinity binding
460 to SERT and DAT. The fluorinated compounds **3c** and **4c**, for instance, were as potent as **9b**
461 in inhibiting SERT, but they were substantially less efficacious and less potent in rescuing
462 SERT-PG^{601,602}AA. Similarly, these three analogs only differed modestly in their affinity for
463 DAT, but only compound **9b** was an efficacious pharmacochaperone for DAT-PG^{584,585}AA.
464 In fact, compound **9b** meets three important criteria to be considered a significant advance in
465 the pharmacochaperoning of DAT mutants: (i) the pharmacochaperoning efficacy of
466 compound **9b** exceeded that of the parent compound noribogaine. (ii) Compound **9b** had a
467 therapeutic window *in vivo*, i.e. there was an effective dose range, where it restored sleep in

468 drosophilae harboring DAT-PG^{584,585}AA. This effect was specifically linked to the
469 pharmacochaperoning action, because compound **9b** did not affect locomotion and sleep in
470 control flies (harboring endogenous DAT) or *fumin* flies lacking DAT. (iii) Compound **9b**
471 restored folding to disease-associated DAT mutants, which were unresponsive to noribogaine,
472 thus doubling the number of DAT mutants potentially amenable to folding correction.

473

474 The mechanistic basis underlying pharmacochaperoning is poorly understood. At least four
475 mechanisms are conceivable, i.e. (i) stabilization of the native state or (ii) of a folding
476 intermediate, (iii) prevention of aggregate formation or (iv) dissolution of aggregates
477 (Marinko *et al.*, 2019). Stabilization of the native state is posited to be the most common
478 mechanism of action for pharmacochaperones. In this instance, chaperoning efficacy is related
479 to binding affinity (Marinko *et al.*, 2019). Our observations are difficult to reconcile with
480 binding to the native state, because (i) the structure-activity relationships for binding to the
481 wild type transporters - i.e. the native state - differed substantially from for
482 pharmacochaperoning. (ii) The EC₅₀-values for rescuing DAT-PG^{584,585}AA and SERT-
483 PG^{601,602}AA differed by 400-fold. This difference was substantially larger than variation in
484 affinity for the native state of SERT and DAT. This indicates that compound **9b** has a high
485 and low affinity for the relevant folding intermediate(s) of SERT-PG^{601,602}AA and of DAT-
486 PG^{584,585}AA, respectively. (iii) The relative pharmacochaperoning efficacy of ibogaine,
487 noribogaine and compound **9b** was highly dependent on the nature of the DAT mutant: in
488 DAT-PG^{584,585}AA and in several disease-relevant DAT mutants (i.e., DAT-G³⁸⁶R, -R⁵²¹W, -
489 P³⁹⁵L, P⁵⁵⁴L) compound **9b** was substantially more efficacious than ibogaine and noribogaine,
490 but this was not the case in DAT-A³¹⁴V and DAT-R⁴⁴⁵C. This variation in relative efficacy is
491 again difficult to reconcile with stabilization of the native state. (iv) Circumstantial evidence
492 also argues that the other two proposed mechanisms of pharmacochaperoning - inhibition of
493 aggregation and disassembly of aggregates - do not apply: diseases arising from mutations in
494 SLC6 transporter can be transmitted in both a recessive and a dominant fashion. Because
495 SLC6 transporters are exported from the ER in an oligomeric form, folding-deficient mutants
496 can act in a dominant-negative manner and retain the wild type transporter (Chiba *et al.*,
497 2014; Lopez-Corcuera *et al.*, 2019). However, all disease-relevant human DAT mutants are
498 transmitted as recessive alleles (Kurian *et al.*, 2011; Ng *et al.*, 2014). Thus, their folding
499 trajectory is stalled at a stage, where they are complexed with and shielded by ER chaperones
500 such as calnexin, which precludes oligomerization (Korkhov *et al.*, 2008). Taken together,

501 these observations indicate that binding to folding intermediate(s) is the most likely
502 mechanism underlying the pharmacochaperoning action of compound **9b**.

503
504 Individual folding-deficient mutants of SERT and DAT are stalled at different points of their
505 folding trajectory and differ in their susceptibility to pharmacochaperoning (*El-Kasaby et al.*,
506 *2014; Koban et al., 2015; Beerepoot et al., 2016; Asjad et al., 2017; Freissmuth et al., 2017*).
507 Our approach relied on using the two analogous folding deficient mutants SERT-PG^{601,602}AA
508 and DAT-PG^{584,585}AA by assuming that these two proteins are stalled at related positions of
509 their folding trajectory. However, compound **9b** was more than 100-fold more potent in
510 rescuing SERT-PG^{601,602}AA than DAT-PG^{584,585}AA. A similar discrepancy was seen in the
511 naphthylamine series of pharmacochaperones (*Bhat et al., 2017*): PAL-287 was more
512 effective than PAL-1045 in rescuing DAT-PG^{584,585}AA, the reverse was true for SERT-
513 PG^{601,602}AA (cf. **Fig. 3** and *Bhat et al., 2017*). Taken together, these observations are again
514 consistent with the conjecture that the compounds act as pharmacochaperones by binding to
515 folding intermediates rather than by stabilizing the native state. The hypothetical model posits
516 that, in the folding trajectory of wild type SLC6 transporters, there is a large isoenergetic
517 conformational search space. Mutations convert this smooth surface into a rugged landscape.
518 Accordingly, they create multiple traps, which reside at different locations in the peripheral
519 ring of the champagne glass-like energy landscape (*Dill and Chan, 1997*). Some of these traps
520 can be overcome by pharmacochaperoning, but they differ - even for closely related folding-
521 deficient mutations - in their position in the conformational search space (*El-Kasaby et al.*,
522 *2014*). Hence, the folding intermediates in the vicinity of the trap(s) differ in their ability to
523 bind to and respond to a pharmacochaperoning ligand. General rules, which govern folding of
524 helical membrane proteins, have been inferred but the details are obscure (*Chiba et al., 2014*;
525 *Marinko et al., 2019*). Progress is hampered, because the nature of the folding intermediate(s)
526 is poorly understood. The affinity of compound **9b** for the folding intermediate(s) of SERT-
527 PG^{601,602}AA was estimated to be in the submicromolar range. Hence, compound **9b** may be
528 useful as a starting point to develop probes to address the folding trajectory of SERT-
529 PG^{601,602}AA and other misfolded SERT variants. We anticipate that the resulting insights will
530 also advance the search for additional pharmacochaperones. These are needed, because the
531 majority of disease-associated DAT mutants are still not remedied by the available
532 pharmacochaperones. Moreover, compound **9b** is a significant breakthrough, because it not
533 only expands the number of rescued DAT mutants but it also restores the functional activity
534 of DAT-G386R essentially to wild type levels. Thus, compound **9b** provides a proof-of-

535 principle that it is possible to fully correct the folding defect of a mutant by
536 pharmacochaperoning.

537

538

539 **References**

540 **Asjad HMM**, Kasture A, El-Kasaby A, Sackel M, Hummel T, Freissmuth M, Sucic S. 2017.
541 Pharmacochaperoning in a Drosophila model system rescues human dopamine transporter
542 variants associated with infantile/juvenile parkinsonism. *Journal of Biological Chemistry*
543 **292**:19250-19265. doi: 10.1074/jbc.M117.797092, PMID: 28972153

544 **Beerepoot P**, Lam VM, Salahpour A. 2016. Pharmacological chaperones of the dopamine
545 transporter rescue dopamine transporter deficiency syndrome mutations in heterologous cells.
546 *Journal of Biological Chemistry* **291**:22053-22062. doi: 10.1074/jbc.M116.749119, PMID:
547 27555326

548 **Bhat S**, Hasenhuettl PS, Kasture A, El-Kasaby A, Baumann MH, Blough BE, Sucic S,
549 Sandtner W, Freissmuth M. 2017. Conformational state interactions provide clues to the
550 pharmacochaperone potential of serotonin transporter partial substrates. *Journal of Biological
551 Chemistry* **292**:16773-16786. doi: 10.1074/jbc.M117.794081, PMID: 28842491

552 **Bhat S**, Newman AH, Freissmuth M. 2019. How to rescue misfolded SERT, DAT and NET:
553 targeting conformational intermediates with atypical inhibitors and partial releasers.
554 *Biochemical Society Transactions* **47**:861-874. doi: 10.1042/BST20180512, PMID: 31064865

555 **Blackburn J**, Szumlinski KK. 1997. Ibogaine effects on sweet preference and amphetamine
556 induced locomotion: implications for drug addiction. *Behavioural Brain Research* **89**:99-106.
557 doi: 10.1016/s0166-4328(97)00050-8, PMID: 9475618

558 **Brown TK**, Alper K. 2018. Treatment of opioid use disorder with ibogaine: detoxification
559 and drug use outcomes. *The American Journal of Drug and Alcohol Abuse* **44**:24-36. doi:
560 10.1080/00952990.2017.1320802, PMID: 28541119

561 **Bulling S**, Schicker K, Zhang YW, Steinkellner T, Stockner T, Gruber CW, Boehm S,
562 Freissmuth M, Rudnick G, Sitte HH, Sandtner W. 2012. The Mechanistic basis for
563 noncompetitive ibogaine inhibition of serotonin and dopamine Transporters. *Journal of
564 Biological Chemistry* **287**:18524-18534. doi: 10.1074/jbc.M112.343681, PMID: 22451652

565 **Burtscher V**, Hotka M, Li Y, Freissmuth M, Sandtner W. 2018. A label-free approach to
566 detect ligand binding to cell surface proteins in real time. *eLife* **7**:e34944. doi:
567 10.7554/eLife.34944, PMID: 29697048

568 **Chiba P**, Freissmuth M, Stockner T. 2014. Defining the blanks--pharmacochaperoning of
569 SLC6 transporters and ABC transporters. *Pharmacological Research* **83**:63-73. doi:
570 10.1016/j.phrs.2013.11.009, PMID: 24316454

571 **Coleman JA**, Yang D, Zhao Z, Wen PC, Yoshioka C, Tajkhorshid E, Gouaux E. 2019.
572 Serotonin transporter-ibogaine complexes illuminate mechanisms of inhibition and transport.
573 *Nature* **569**:141-145. doi: 10.1038/s41586-019-1135-1, PMID: 31019304

574 **Corkery JM**. 2018. Ibogaine as a treatment for substance misuse: Potential benefits and
575 practical dangers. *Progress in Brain Research* **242**:217-257. doi: 10.1016/bs.pbr.2018.08.005,
576 PMID: 30471681

577 **Dill KA**, Chan HS 1997. From Levinthal to pathways to funnels. *Nature Structural Biology* **4**:
578 10–19. doi: 10.1038/nsb0197-10, PMID: 8989315

579 **Dybowski J**, Landrin E. 1901. PLANT CHEMISTRY. Concerning Iboga, its excitement-
580 producing properties, its composition, and the new alkaloid it contains, ibogaine. *Comptes
581 rendus de l'Académie des Sciences* **133**:748.

582 **El-Kasaby A**, Just H, Malle E, Stolt-Bergner PC, Sitte HH, Freissmuth M, Kudlacek O. 2010.
583 Mutations in the carboxyl-terminal SEC24 binding motif of the serotonin transporter impair
584 folding of the transporter. *Journal of Biological Chemistry* **285**:39201-39210. doi:
585 10.1074/jbc.M110.118000, PMID: 20889976

586 **El-Kasaby A**, Koban F, Sitte HH, Freissmuth M, Sucic S. 2014. A cytosolic relay of heat
587 shock proteins HSP70-1A and HSP90 β monitors the folding trajectory of the serotonin
588 transporter. *Journal of Biological Chemistry* **289**:28987-29000. doi:
589 10.1074/jbc.M114.595090, PMID: 25202009

590 **Freissmuth M**, Stockner T, Sucic S. 2018. SLC6 Transporter Folding Diseases and
591 Pharmacochaperoning. *Handbook of Experimental Pharmacology* **245**: 249-270. doi:
592 10.1007/164_2017_71, PMID: 29086036

593 **Friggi-Grelin F**, Coulom H, Meller M, Gomez D, Hirsh J, Birman S. 2003. Targeted gene
594 expression in Drosophila dopaminergic cells using regulatory sequences from tyrosine
595 hydroxylase. *Journal of Neurobiology* **54**:618-627. doi: 10.1002/neu.10185, PMID: 12555273

596 **Gassaway MM**, Jacques TL, Kruegel AC, Karpowicz RJ Jr, Li X, Li S, Myer Y, Sames D.
597 2016. Deconstructing the Iboga Alkaloid Skeleton: Potentiation of FGF2-induced Glial Cell
598 Line-Derived Neurotrophic Factor Release by a Novel Compound. *ACS Chemical Biology*
599 **11**:77-87. doi: 10.1021/acscchembio.5b00678, PMID: 26517751

600 **Gilestro GF**, Cirelli C. 2009. pySolo: a complete suite for sleep analysis in Drosophila.
601 *Bioinformatics* **25**: 1466–1467. doi: 10.1093/bioinformatics/btp237, PMID: 19369499

602 **Glick SD**, Rossman K, Steindorf S, Maisonneuve IM, Carlson JN. 1991. Effects and after
603 effects of ibogaine on morphine self-administration in rats. *European Journal of*
604 *Pharmacology* **195**: 341-345. doi: 10.1016/0014-2999(91)90474-5, PMID: 1868880

605 **Glick SD**, Kuehne ME, Raucci J, Wilson TE, Larson D, Keller RW Jr, Carlson JN. 1994.
606 Effects of iboga alkaloids on morphine and cocaine self-administration in rats: relationship to
607 tremorigenic effects and to effects on dopamine release in nucleus accumbens and striatum.
608 *Brain Research* **657**:14-22. doi: 10.1016/0006-8993(94)90948-2, PMID: 7820611

609 **Hasenhuettl PS**, Bhat S, Freissmuth M, Sandtner W. 2019. Functional selectivity and partial
610 efficacy at the monoamine transporters: a unified model of allosteric modulation and
611 amphetamine-induced substrate release. *Molecular Pharmacology* **95**:303-312. doi:
612 10.1124/mol.118.114793, PMID: 30567955

613 **Hohenegger M**, Mitterauer T, Voss T, Nanoff C, Freissmuth M. 1996. Thiophosphorylation
614 of the G protein β -subunit in human platelet membranes: evidence against a direct phosphate
615 transfer reaction to $\text{G}\alpha$ -subunits. *Molecular Pharmacology* **49**:73-80. PMID: 8569715

616 **Jacobs MT**, Zhang YW, Campbell SD, Rudnick G. 2007. Ibogaine, a noncompetitive
617 inhibitor of serotonin transport, acts by stabilizing the cytoplasm-facing state of the
618 transporter. *Journal of Biological Chemistry* **282**:29441-29447. doi:
619 10.1074/jbc.M704456200, PMID: 17698848

620 **Kasture A**, El-Kasaby A, Szöllösi D, Asjad HMM, Grimm A, Stockner T, Freissmuth M,
621 Sucic S. 2016. Functional rescue of a misfolded *Drosophila melanogaster* dopamine
622 transporter mutant associated with a sleepless phenotype by pharmacological chaperones.
623 *Journal of Biological Chemistry* **291**:20876-20890. doi: 10.1074/jbc.M116.737551, PMID:
624 27481941

625 **Koban F**, El-Kasaby A, Häusler C, Stockner T, Simbrunner BM, Sitte, HH, Freissmuth M,
626 Sucic S. 2015. A salt bridge linking the first intracellular loop with the C terminus facilitates
627 the folding of the serotonin transporter. *Journal of Biological Chemistry* **290**:13263-13278.
628 doi: 10.1074/jbc.M115.641357, PMID: 25869136

629 **Korkhov VM**, Milan-Lobo L, Zuber B, Farhan H, Schmid JA, Freissmuth M, Sitte HH. 2008.
630 Peptide-based interactions with calnexin target misassembled membrane proteins into
631 endoplasmic reticulum-derived multilamellar bodies. *Journal of Molecular Biology* **378**:337-
632 352. doi: 10.1016/j.jmb.2008.02.056, PMID: 18367207

633 **Kristensen AS**, Andersen J, Jørgensen TN, Sørensen L, Eriksen J, Loland CJ, Strømgaard K,
634 Gether U. 2011. SLC6 neurotransmitter transporters: structure, function, and regulation.
635 *Pharmacological Review* **63**:585-640. doi: 10.1124/pr.108.000869, PMID: 21752877

636 **Kruegel AC**, Rakshit S, Li X, Sames D. 2015. Constructing Iboga alkaloids via C–H bond
637 functionalization: examination of the direct and catalytic union of heteroarenes and
638 isoquinuclidine alkenes. *The Journal of Organic Chemistry* **80**:2062-2071. doi:
639 10.1021/jo5018102, PMID: 25633249

640 **Kume K**, Kume S, Park SK, Hirsh J, Jackson FR. 2005. Dopamine is a regulator of arousal in
641 the fruit fly. *The Journal of Neuroscience* **25**:7377–7384. doi: 10.1523/JNEUROSCI.2048-
642 05.2005, PMID: 16093388

643 **Kurian MA**, Li Y, Zhen J, Meyer E, Hai N, Christen HJ, Hoffmann GF, Jardine P, von
644 Moers A, Mordekar SR, O'Callaghan F, Wassmer E, Wraige E, Dietrich C, Lewis T, Hyland
645 K, Heales S Jr, Sanger T, Gissen P, Assmann BE, Reith ME, Maher ER. 2011. Clinical and
646 molecular characterisation of hereditary dopamine transporter deficiency syndrome: an
647 observational cohort and experimental study. *The Lancet Neurology* **10**:54-62. doi:
648 10.1016/S1474-4422(10)70269-6, PMID: 21112253

649 **Liu Q**, Liu S, Kodama L, Driscoll MR, Wu MN. 2012. Two dopaminergic neurons signal to
650 the dorsal fan-shaped body to promote wakefulness in Drosophila. *Current Biology* **22**:2114-
651 2123. doi: 10.1016/j.cub.2012.09.008, PMID: 23022067

652 **López-Corcuera B**, Arribas-González E, Aragón C. 2019. Hyperekplexia-associated
653 mutations in the neuronal glycine transporter 2. *Neurochemistry International* **123**:95-100.
654 doi: 10.1016/j.neuint.2018.05.014, PMID: 29859229

655 **Marinko JT**, Huang H, Penn WD, Capra JA, Schlebach JP, Sanders CR 2019. Folding and
656 Misfolding of Human Membrane Proteins in Health and Disease: from single molecules to
657 Cellular proteostasis. *Chemical Reviews* **119**:5537-5606. doi: 10.1021/acs.chemrev.8b00532,
658 PMID: 30608666

659 **Mash DC**, Staley JK, Baumann MH, Rothman RB, Hearn WL. 1995. Identification of a
660 primary metabolite of ibogaine that targets serotonin transporters and elevates serotonin. *Life
661 Sciences* **157**:45-50. doi: 10.1016/0024-3205(95)00273-9, PMID: 7596224

662 **Miranda M**, Sorkina T, Grammatopoulos TN, Zawada WM, Sorkin A. 2004. Multiple
663 molecular determinants in the carboxyl terminus regulate dopamine transporter export from
664 endoplasmic reticulum. *Journal of Biological Chemistry* **279**:30760-30770. doi:
665 10.1074/jbc.M312774200, PMID: 15128747

666 **Ng J**, Zhen J, Meyer E, Erreger K, Li Y, Kakar N, Ahmad J, Thiele H, Kubisch C, Rider NL,
667 Morton DH, Strauss KA, Puffenberger EG, D'Agnano D, Anikster Y, Carducci C, Hyland K,
668 Rotstein M, Leuzzi V, Borck G, Reith ME, Kurian MA. 2014. Dopamine transporter

669 deficiency syndrome: phenotypic spectrum from infancy to adulthood. *Brain* **137**:1107-1119.
670 doi: 10.1093/brain/awu022, PMID: 24613933

671 **Noller GE**, Frampton CM, Yazar-Klosinski B. 2018. Ibogaine treatment outcomes for opioid
672 dependence from a twelve-month follow-up observational study. *The American Journal of*
673 *Drug and Alcohol Abuse* **44**:37-46. doi: 10.1080/00952990.2017.1310218, PMID: 28402682

674 **Pimentel D**, Donlea JM, Talbot CB, Song SM, Thurston AJF, Miesenböck G. 2016.
675 Operation of a homeostatic sleep switch. *Nature* **536**: 333–337. doi: 10.1038/nature19055,
676 PMID: 27487216

677 **Reith ME**, Blough BE, Hong WC, Jones KT, Schmitt KC, Baumann MH, Partilla JS,
678 Rothman RB, Katz JL. 2015. Behavioral, Biological, and Chemical Perspectives on Atypical
679 Agents Targeting the Dopamine Transporter. *Drug and Alcohol Dependence* **147**:1-19. doi:
680 10.1016/j.drugalcdep.2014.12.005, PMID: 25548026

681 **Rothman RB**, Partilla JS, Baumann MH, Lightfoot-Siordia C, Blough BE. 2012. Studies of
682 the biogenic amine transporters. 14. Identification of low-efficacy "partial" substrates for the
683 biogenic amine transporters. *The Journal of Pharmacology and Experimental Therapeutics*
684 **341**:251-62. doi: 10.1124/jpet.111.188946, PMID: 22271821

685 **Schmitt KC**, Rothman RB, Reith ME. 2013. Nonclassical pharmacology of the dopamine
686 transporter: atypical inhibitors, allosteric modulators and partial substrates. *The Journal of*
687 *Pharmacology and Experimental Therapeutics* **346**: 2-10. doi: 10.1124/jpet.111.191056,
688 PMID: 23568856

689 **Sitte HH**, Freissmuth M. 2015. Amphetamines, new psychoactive drugs and the monoamine
690 transporter cycle. *Trends in Pharmacological Sciences* **36**:41-50. doi:
691 10.1016/j.tips.2014.11.006, PMID: 25542076

692 **Sweetnam PM**, Lancaster J, Snowman A, Collins JL, Perschke S, Bauer C, Ferkany J. 1995.
693 Receptor binding profile suggests multiple mechanisms of action are responsible for
694 ibogaine's putative anti-addictive activity. *Psychopharmacology (Berl)* **1118**:369-376. doi:
695 10.1007/BF02245936, PMID: 7568622

696 **Trost BM**, Godleski SA, Genet JP. 1978. A total synthesis of racemic and optically active
697 ibogamine. Utilization and mechanism of a new silver ion assisted palladium catalyzed
698 cyclization. *Journal of the American Chemical Society* **100**:3930-3931.
699 doi.org/10.1021/ja00480a047

700 **Wasko MJ**, Witt-Enderby PA, Surratt CK. 2018. DARK classics in chemical neuroscience:
701 Ibogaine. *ACS Chemical Neuroscience*. **9**:2475-2483. doi: 10.1021/acschemneuro.8b00294,
702 PMID: 30216039

703

704

705

706

707

708

709

710 **Material and Methods**

711

712 **Cell culture and materials**

713 Cells were propagated Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10%
714 heat-inactivated fetal bovine serum (FBS), 100 $u\cdot100\text{ mL}^{-1}$ penicillin and 100 $u\cdot100\text{ mL}^{-1}$
715 streptomycin. Medium used in the maintenance of stable lines (see below) was, in addition,
716 supplemented with 50 $\mu\text{g ml}^{-1}$ geneticin (G418) for selection. HEK293 cells were transfected
717 by combining plasmid DNA with PEI (linear 25 kDa polyethylenimine; Santa Cruz, SC-
718 360988A) at a ratio of 1:3 (w:w) in serum-free DMEM. The plasmids encoding YFP-tagged
719 human wild type and mutant versions of SERT (El-Kasaby et al., 2014) and of DAT (Kasture
720 et al., 2016; Asjad et al., 2017) were previously described; YFP-tagged DAT-PG^{584,585}AA was
721 created by introducing the substitutions with created with the QuikChange Lightning Site-
722 Directed Mutagenesis Kit (Stratagene, La Jolla, CA), using wild type YFP-fagged DAT as the
723 template. The mutations were confirmed by automatic DNA sequencing (LGC Labor GmbH
724 Augsburg, Germany). For the pharmacochaperoning experiments, noribogaine was purchased
725 from Cfm Oskar Tropitzsch GmbH (Marktredwitz, Germany). [³H]5-HT (serotonin, 41.3
726 Ci/mmol), and [³H]dopamine (DA, 39.6 Ci/mmol) were purchased from PerkinElmer Life
727 Sciences. Scintillation mixture (Rotiszint® eco plus) was purchased from Carl Roth GmbH
728 (Karlsruhe, Germany). Cell culture media and antibiotics were obtained from Sigma and
729 Invitrogen, respectively. Anti-GFP antibody (rabbit, ab290) was from Abcam (Cambridge,
730 UK). An antibody raised against an N-terminal peptide of the G protein β subunit was used to
731 verify comparable loading of lanes (Hohenegger et al., 1996). Horseradish peroxidase-linked
732 anti-rabbit IgG1 antibody was purchased from Amersham Biosciences. All other chemicals
733 were of analytical grade.

734

735 **Radioligand Binding Studies**

736 For DAT binding assays, frozen striata, previously dissected from freshly harvested male
737 Sprague-Dawley rat brains (supplied on ice by Bioreclamation, Hicksville, NY), were
738 homogenized in 20 volumes (w/v) of ice-cold modified sucrose phosphate buffer (0.32 M
739 sucrose, 7.74 mM Na₂HPO₄, and 2.26 mM NaH₂PO₄, pH adjusted to 7.4) using a Brinkman
740 Polytron (Setting 6 for 20 s) and centrifuged at 48,400 x g for 10 min at 4°C. The resulting
741 pellet was washed by resuspension in buffer, the suspension was centrifuged again, and the
742 final pellet resuspended in ice-cold buffer to a concentration of 20 mg/mL (original wet
743 weight/volume, OWW/V). Experiments were conducted in 96-well polypropylene plates

744 containing 50 μ L of various concentrations of the inhibitor, diluted using 30% DMSO
745 vehicle, 300 μ L of sucrose phosphate buffer, 50 μ L of [3 H]WIN 35,428 (final concentration
746 1.5 nM; K_D = 28.2 nM; PerkinElmer Life Sciences, Waltham, MA), and 100 μ L of tissue (2.0
747 mg/well OWW). All compound dilutions were tested in triplicate and the competition
748 reactions started with the addition of tissue, and the plates were incubated for 120 min at 0-
749 4°C. Nonspecific binding was determined using 10 μ M indatraline.

750 For SERT binding assays, frozen brain stem tissue, previously dissected from freshly
751 harvested male Sprague-Dawley rat brains (supplied on ice by Bioreclamation, Hicksville,
752 NY), was homogenized in 20 volumes (w/v) of 50 mM Tris buffer (120 mM NaCl and 5 mM
753 KCl, adjusted to pH 7.4) at 25°C using a Brinkman Polytron (at setting 6 for 20 s) and
754 centrifuged at 48,400 \times g for 10 min at 4°C. The resulting pellet was resuspended in buffer,
755 the suspension was centrifuged, and the final pellet suspended in buffer again to a
756 concentration of 20 mg/mL (OWW/V). Experiments were conducted in 96-well
757 polypropylene plates containing 50 μ L of various concentrations of the inhibitor, diluted
758 using 30% DMSO vehicle, 300 μ L of Tris buffer, 50 μ L of [3 H]citalopram (final
759 concentration 1.5 nM; K_d = 6.91 nM; PerkinElmer Life Sciences, Waltham, MA), and 100 μ L
760 of tissue (2.0 mg/well OWW). All compound dilutions were tested in triplicate and the
761 competition reactions started with the addition of tissue, and the plates were incubated for 60
762 min at rt. Nonspecific binding was determined using 10 μ M fluoxetine.

763 For all binding assays, incubations were terminated by rapid filtration through Perkin Elmer
764 Uni-Filter-96 GF/B presoaked in either 0.3% (SERT) or 0.05% (DAT) polyethylenimine,
765 using a Brandel 96-well harvester manifold or Brandel R48 filtering manifold (Brandel
766 Instruments, Gaithersburg, MD). The filters were washed a total of 3 times with 3 mL (3 \times 1
767 mL/well or 3 \times 1 mL/tube) of ice cold binding buffer. Perkin Elmer MicroScint 20
768 Scintillation Cocktail (65 μ L) was added to each filter well. Radioactivity was counted in a
769 Perkin Elmer MicroBeta Microplate Counter. IC₅₀ values for each compound were
770 determined from inhibition curves and K_i values were calculated using the Cheng-Prusoff
771 equation. When a complete inhibition could not be achieved at the highest tested
772 concentrations, K_i values were estimated by extrapolation after constraining the bottom of the
773 dose-response curves (= 0% residual specific binding) in the non-linear regression analysis.
774 These analyses were performed using GraphPad Prism version 8.00 for Macintosh (GraphPad
775 Software, San Diego, CA). K_d values for the radioligands were determined via separate
776 homologous competitive binding or radioligand binding saturation experiments. K_i values

777 were determined from at least 3 independent experiments performed in triplicate and are
778 reported as means \pm S.D.

779

780 **[³H]5-HT and [³H]dopamine uptake assays**

781 For uptake inhibition assays, HEK293 cells stably expressing either wild-type human YFP-
782 tagged hSERT or YFP-tagged hDAT were seeded on poly-D-lysine-coated 96-well plates at a
783 density of \sim 20,000 cells/well. After 24 h, the medium in each well was aspirated and the cells
784 were washed once with Krebs-HEPES buffer (10 mM HEPES.NaOH, pH 7.4, 120 mM NaCl,
785 3 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, and 2 mM glucose). Cells were pre-incubated in
786 buffer containing logarithmically spaced concentrations (0.003–300 μ M) of ibogaine analogs
787 for 10 minutes. Subsequently the reaction was started by addition of substrate (0.4 μ M of
788 either [³H]5-HT or [³H]dopamine) at constant concentrations of ibogaine analogs for 1
789 minute. The reaction was terminated by aspirating the reaction medium followed by a wash
790 with ice-cold buffer. The cells were lysed with 1% SDS and the released radioactivity was
791 quantified by liquid scintillation counting.

792 For uptake assays determining functional rescue of mutant transporters, HEK293 cells were
793 transfected with either YFP-tagged SERT-PG^{601,602}AA or YFP-tagged DAT-PG^{584,585}AA
794 plasmids. Transfected cells were seeded on poly-D-lysine-coated 96-well plates at a density of
795 \sim 60-80,000 cells/well either in the absence or presence of increasing concentrations (0.1 –
796 100 μ M) of the ibogaine analogs. After 24 h, the cells were washed four times with Krebs-
797 MES buffer (10 mM 2-(N-morpholino)ethanesulfonic acid, pH 5.5, 120 mM NaCl, 3 mM
798 KCl, 2 mM CaCl₂, 2 mM MgCl₂, and 2 mM glucose) in a 10 min interval and once with
799 Krebs-HEPES (pH 7.4) buffer. The cells were subsequently incubated with 0.2 μ M of [³H]5-
800 HT for 1 minute or [³H]dopamine for 5 min. and processed as outlined above.

801

802 **Immunoblotting after pharmacochaperoning of SERT-PG^{601,602}AA or DAT-PG^{584,585}AA**

803 HEK293 cells were transiently transfected with plasmids encoding either wild type SERT,
804 SERT-PG^{601,602}AA, wild type DAT or DAT-PG^{584,585}AA. Approximately 1.5 – 2 \times 10⁶ of
805 these transfected cells were seeded either in 6-well plates or 6 cm dishes in the presence of 30
806 μ M of the individual candidate hits identified by uptake assays. After 24 h, cells were washed
807 thrice with ice-cold phosphate-buffered saline, detached by mechanical scraping, and
808 harvested by centrifugation at 1000 \times g for 5 min. The cell pellet was lysed in a buffer
809 containing Tris·HCl, pH 8.0, 150 mM NaCl, 1% dodecyl maltoside, 1 mM EDTA, and
810 protease inhibitors (CompleteTM, Roche Applied Science). This soluble protein lysate was

811 separated from the detergent-insoluble material by centrifugation (16,000 \times g for 15 min at 4
812 °C). An aliquot of this lysate (20 μ g) was mixed with 1% SDS and 20 mM DTT containing
813 sample buffer, denatured at 45 °C for 30 min, and resolved in denaturing polyacrylamide gels.
814 After protein transfer onto nitrocellulose membranes, the blots were probed with an antibody
815 against GFP (rabbit, ab290) at a 1:3000 dilution overnight. This immunoreactivity was
816 detected using a horseradish peroxidase conjugated secondary antibody (1:5000, Amersham
817 ECL Prime Western Blotting Detection Reagent). In separate experiments, lysates were
818 prepared from cells treated in the absence or presence of 30 μ M DG4-69; these were
819 incubated in the presence and absence of endoglycosidase H (New England Biolabs) (16 =
820 Asjad et al., 2017) and aliquots (20 μ g) were then resolved electrophoretically as described
821 above. Densitometric analyses of individual blots were done using ImageJ.
822

823 **Fly genetics, treatment and locomotion assay**

824 The transgenic UAS reporter line for YFP-tagged hDAT-PG^{584, 585}AA was generated using
825 pUASg-attB vector (gift from Drs. Bischof and Basler, University of Zürich). The sequenced
826 construct was injected into embryos from ZH-86Fb flies (Bloomington stock no 24749).
827 Positive transformants were selected and crossed with balancer flies (Bloomington stock no.
828 3704). Fumin flies (fmn or DAT-KO mutant flies) was a generous gift from Dr. Kume,
829 Nagoya City University, Japan. Tyrosine hydroxylase Gal4 (TH-Gal4, Bloomington stock no.
830 8848) was used to drive the expression of hDAT-PG^{584, 585}AA in dopaminergic neurons.
831 Isogenized fmn and w^{1118} flies were used as control. The genotypes of flies used in Fig. 7a and
832 b were w^{1118} ; fmn(w ; roo{ δ DAT^{fmn}}); TH-Gal4/UAS-hDAT- PG^{584, 585}AA (PG^{584, 585}AA),
833 w^{1118} / y ; fmn(w ; roo{ δ DAT^{fmn}}); +/+ (Fmn) and w^{1118} . All flies were kept at 25 °C in a 12-h
834 light/12-h dark cycle, and all crosses were performed at 25 °C. As described previously (14 =
835 Kasture et al., 2016), locomotion assay was performed on three-to-five-day old male flies
836 using *Drosophila* activity monitor system (DAM2, Trikinetics, Waltham, MA). Briefly,
837 individual flies were housed in 5-mm-diameter glass tubes carrying food pellet supplemented
838 with specified concentrations of noribogaine and **9b**. Flies were entrained for 12h:12h day:
839 night rhythm for first two days and locomotion activity was studied on second day in
840 subsequent 12h; 12h dark: dark phase. Locomotion activity was measured in 1 min bins and
841 pySolo software (Gilestro and Cirelli, 2009) was used to quantify sleep time.
842
843
844
845

846 **Supporting Information**
847 **Synthesis**

848 All chemicals and solvents were purchased from chemical suppliers unless otherwise stated
849 and used without further purification. ^1H and ^{13}C NMR spectra were acquired using a Varian
850 Mercury Plus 400 spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts are
851 reported in parts-per-million (ppm) and referenced according to deuterated solvent for ^1H
852 NMR spectra (CDCl_3 , 7.26; D_2O , 4.79 or DMSO-d_6 , 2.50) and ^{13}C NMR spectra (CDCl_3 , 77.2
853 or DMSO-d_6 , 39.52). Gas chromatography-mass spectrometry (GC/MS) data were acquired
854 (where obtainable) using an Agilent Technologies (Santa Clara, CA) 7890B GC equipped
855 with an HP-5MS column (cross-linked 5% PH ME siloxane, 30 m \times 0.25 mm i.d. \times 0.25 μm
856 film thickness) and a 5977B mass-selective ion detector in electron-impact mode. Ultrapure
857 grade helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injection port and
858 transfer line temperatures were 250 and 280 °C, respectively, and the oven temperature
859 gradient used was as follows: the initial temperature (70 °C) was held for 1 min and then
860 increased to 300 °C at 20 °C/min over 11.5 min, and finally maintained at 300 °C for 4 min.
861 All column chromatography was performed using a Teledyne Isco CombiFlash RF flash
862 chromatography system. Combustion analyses were performed by Atlantic Microlab, Inc.
863 (Norcross, GA) and agree with $\pm 0.4\%$ of calculated values. HRMS (mass error within 5 ppm)
864 and MS/MS fragmentation analysis were performed on a LTQ-Orbitrap Velos (Thermo-
865 Scientific, San Jose, CA) coupled with an ESI source in positive ion mode to confirm the
866 assigned structures and regiochemistry. All melting points were determined on an OptiMelt
867 automated melting point system and are uncorrected. On the basis of NMR and combustion
868 data, all final compounds are $>95\%$ pure.

869
870 *3-(2-(8-azabicyclo[3.2.1]octan-8-yl)ethyl)-1H-indole (3a).* 8-Azabicyclo[3.2.1]octane (**1**)
871 (222.4 mg, 2 mmol), 3-(2-bromoethyl)-1H-indole (448.2 mg, 2 mmol), K_2CO_3 (1.1 g, 8
872 mmol) and acetonitrile (24 mL) were added in a sealed bottle (100 mL). The reaction mixture
873 was stirred at 100 °C overnight and filtered. The filtrate was evaporated and purified by flash
874 column chromatography ($\text{DCM}/\text{MeOH}/\text{NH}_4\text{OH} = 95 : 5 : 0.5$) to give the product (470 mg,
875 92% yield) as a yellow oil. The free base was converted to the HCl salt and recrystallized
876 from methanol to give a white solid. Mp 247-248 °C; GC/MS (EI) m/z 254 (M^+); ^1H NMR
877 (400 MHz, CDCl_3) δ 8.05 (s, 1H), 7.62-7.63 (m, 1H), 7.34-7.36 (m, 1H), 7.02-7.19 (m, 3H),
878 3.34 (m, 2H), 2.95-2.99 (m, 2H), 2.68-2.72 (m, 2H), 1.94-1.99 (m, 2H), 1.76-1.85 (m, 2H),

879 1.35-1.64 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 136.2, 127.6, 121.9, 121.4, 119.2, 118.9,
880 114.9, 111.1, 59.6, 53.4, 30.7, 26.5, 25.0, 16.7, ; Anal. ($\text{C}_{17}\text{H}_{22}\text{N}_2 \cdot \text{HCl}$) C, H, N.

881 *3-(3-(8-azabicyclo[3.2.1]octan-8-yl)propyl)-1H-indole (3b).* 3-(1H-indol-3-yl)propanoic
882 acid (378.4 mg, 2 mmol) was dissolved in THF (20 mL), CDI (1 equiv) was added and stirred
883 for 2 h at rt followed by adding 8-azabicyclo[3.2.1]octane (**1**) (222.4 mg, 2 mmol) in THF
884 (13 mL). The reaction mixture was stirred overnight at rt. The solvent was removed *in vacuo*
885 and residue was diluted with CHCl_3 (50 mL) and washed with saturated aq Na_2CO_3 solution
886 (2 x 30 mL). The organic layer was dried with MgSO_4 and concentrated *in vacuo*. The crude
887 product was purified by column chromatography (DCM/MeOH/NH₄OH = 97 : 3 : 0.5) to give
888 the amide as yellow oil. The amide was dissolved in anhydrous THF (5.6 mL) and added
889 dropwise to the suspension of LAH (110 mg, 2.84 mmol) in THF (2 mL) at 0 °C. The reaction
890 mixture was allowed to warm to rt and stirred for 3 h. H_2O (0.3 mL) was added
891 carefully at 0 °C, followed by the addition of 0.5 mL of aq NaOH (2 M). The resulting
892 mixture was filtered, and the filtrate was dried (K_2CO_3) filtered and the solvent was removed
893 *in vacuo*. The residue was purified by column chromatography (DCM/MeOH/NH₄OH = 95 :
894 5 : 0.5) to give the product (250 mg, 47% yield over two steps) as a yellow oil. The free base
895 was converted to the HCl salt and recrystallized from methanol to give a tan foam; GC/MS
896 (EI) *m/z* 268 (M^+); ^1H NMR (400 MHz, CDCl_3) δ 7.98 (s, 1H), 7.60-7.62 (m, 1H), 7.33-7.35
897 (m, 1H), 6.99-7.19 (m, 3H), 3.20 (m, 2H), 2.76-2.80 (m, 2H), 2.42-2.46 (m, 2H), 1.87-1.95
898 (m, 4H), 1.41-1.78 (m, 6H), 1.26-1.33 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 136.3, 127.6,
899 121.8, 121.0, 119.0, 116.7, 111.0, 59.4, 52.3, 30.7, 29.2, 26.4, 23.1, 16.8; Anal. ($\text{C}_{18}\text{H}_{24}\text{N}_2 \cdot$
900 $\text{HCl} \cdot 0.5\text{H}_2\text{O} \cdot 0.25\text{i-PrOH}$) C, H, N.

901 *3-(2-(8-azabicyclo[3.2.1]octan-8-yl)ethyl)-5-fluoro-1H-indole (3c).* Compound **3c** was
902 prepared as described for **3b** using 8-azabicyclo[3.2.1]octane (**1**) (111.2 mg, 1 mmol) and 3-
903 (5-fluoro-1H-indol-3-yl)propanoic acid (207.2 mg, 1 mmol) to give the product (250 mg, 28%
904 yield over two steps) as a yellow oil. GC/MS (EI) *m/z* 286 (M^+); ^1H NMR (400 MHz, CDCl_3)
905 δ 8.58 (s, 1H), 7.21-7.24 (m, 2H), 6.89-6.96 (m, 2H), 3.22-3.24 (m, 2H), 2.70-2.74 (m, 2H)
906 2.43-2.47 (m, 2H), 1.73-1.94 (m, 6H), 1.30-1.58 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ
907 158.7, 156.4, 132.8, 128.1, 128.0, 123.0, 116.7, 116.6, 111.6, 111.5, 110.1, 110.0, 103.9,
908 103.6, 59.5, 52.2, 30.6, 29.1, 26.4, 23.0, 16.7; Anal. ($\text{C}_{18}\text{H}_{23}\text{FN}_2 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

909 *3-(2-(8-azabicyclo[3.2.1]octan-8-yl)ethyl)-5-methoxy-1H-indole (3d).* Compound **3d** was
910 prepared as described for **3b** using 8-azabicyclo[3.2.1]octane (**1**) (222.4 mg, 2 mmol) and 2-
911 (5-methoxy-1H-indol-3-yl)acetic acid (410.0 mg, 2 mmol) to give the product (380 mg, 49%
912 yield over two steps) as a brown oil. The free base was converted to the HCl salt and

913 recrystallized from methanol to give a tan foam; GC/MS (EI) m/z 284 (M^+); 1 H NMR (400
914 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.22-7.24 (m, 1H), 7.07 (s, 1H), 7.00 (s, 1H), 6.83-6.86 (m, 1H),
915 3.86 (s, 3H), 3.34-3.35 (m, 2H), 2.91-2.95 (m, 2H), 2.67-2.70 (m, 2H), 1.95-1.99 (m, 2H),
916 1.78-1.85 (m, 2H), 1.46-1.61 (m, 6H); 13 C NMR (100 MHz, $CDCl_3$) δ 153.9, 131.3, 128.0,
917 122.2, 114.6, 112.1, 111.8, 100.8, 59.6, 55.9, 53.3, 30.7, 26.5, 25.0, 16.7; Anal. ($C_{18}H_{24}N_2O \cdot$
918 $HCl \cdot H_2O$) C, H, N.

919 *3-(3-(8-azabicyclo[3.2.1]octan-8-yl)propyl)-5-methoxy-1H-indole (3e)*. Compound **3e** was
920 prepared as described in for **3b** using 8-azabicyclo[3.2.1]octane (**1**) (222.4 mg, 2 mmol) and
921 3-(5-methoxy-1H-indol-3-yl)propanoic acid (438.0 mg, 2 mmol) to give the product (380 mg,
922 79% yield over two steps) as a tan oil; GC/MS (EI) m/z 298 (M^+); 1 H NMR (400 MHz,
923 $CDCl_3$) δ 8.07 (s, 1H), 7.21-7.24 (m, 1H), 7.04 (s, 1H), 6.95 (s, 1H), 6.83-6.94 (m, 1H), 3.86
924 (s, 3H), 3.21-3.23 (m, 2H), 2.73-2.76 (m, 2H), 2.44-2.48 (m, 2H), 1.87-1.94 (m, 4H), 1.72-
925 1.80 (m, 2H), 1.43-1.59 (m, 4H), 1.30-1.34 (m, 2H); 13 C NMR (100 MHz, $CDCl_3$) δ 153.8,
926 131.5, 128.0, 121.9, 116.4, 112.0, 111.7, 100.9, 59.4, 56.0, 52.3, 30.7, 29.1, 26.4, 23.1, 16.7;
927 Anal. ($C_{19}H_{26}N_2O \cdot 0.5H_2O$) C, H, N.

928 *3-(2-(8-azabicyclo[3.2.1]octan-8-yl)ethyl)-1H-indol-5-ol (3f)*. Compound **3f** was prepared as
929 described for **3b** using 8-azabicyclo[3.2.1]octane (**1**) (222.4 mg, 2 mmol) and 2-(5-hydroxy-
930 1H-indol-3-yl)acetic acid (382.4 mg, 2 mmol) to give the product (158 mg, 29% yield over
931 two steps) as a tan oil; GC/MS (EI) m/z 270 (M^+); 1 H NMR (400 MHz, $CDCl_3$) δ 8.12 (s,
932 1H), 7.11-7.13 (m, 1H), 6.87-6.88 (m, 2H), 6.75-6.78 (m, 1H), 3.34-3.35 (m, 2H), 2.91-2.95
933 (m, 2H), 2.68-2.72 (m, 2H), 1.84-1.95 (m, 4H), 1.45-1.58 (m, 4H), 1.30-1.33 (m, 2H); 13 C
934 NMR (100 MHz, $CDCl_3$) δ 150.3, 131.2, 128.1, 122.5, 113.3, 112.8, 111.9, 103.6, 59.5, 52.9,
935 50.6, 29.8, 26.3, 24.2, 16.4; Anal. ($C_{17}H_{22}N_2O \cdot 0.75H_2O$) C, H, N

936 *2-(2-(1H-indol-3-yl)ethyl)-2-azabicyclo[2.2.2]octane (4a)*. Compound **4a** was prepared as
937 described for **3a** using 2-azabicyclo[2.2.2]octane (**2**) (222.4 mg, 2 mmol) and 3-(2-
938 bromoethyl)-1H-indole (448.2 mg, 2 mmol) to give the product (370 mg, 73% yield) as a
939 yellow oil. The free base was converted to the HCl salt and recrystallized from methanol to
940 give a tan solid. Mp 251-253 °C; GC/MS (EI) m/z 254 (M^+); 1 H NMR (400 MHz, $CDCl_3$) δ
941 8.00 (s, 1H), 7.63-7.65 (m, 1H), 7.34-7.36 (m, 1H), 7.04-7.20 (m, 3H), 2.93-2.97 (m, 2H),
942 2.81-2.89 (m, 4H), 2.66-2.67 (m, 1H), 1.96-2.02 (m, 2H) 1.46-1.72 (m, 7H); 13 C NMR (100
943 MHz, $CDCl_3$) δ 136.2, 127.6, 121.9, 121.4, 119.1, 119.0, 115.0, 111.0, 57.6, 56.8, 49.6, 25.8,
944 25.0, 24.4, 24.3; Anal. ($C_{17}H_{22}N_2 \cdot HCl \cdot 0.25H_2O$) C, H, N.

945 *2-(3-(1H-indol-3-yl)propyl)-2-azabicyclo[2.2.2]octane (4b)*. Compound **4b** was prepared as
946 described for **3b** using 2-azabicyclo[2.2.2]octane (**2**) (180 mg, 1.6 mmol) and 3-(1H-indol-3-

947 yl)propanoic acid (302.7 mg, 1.6 mmol) to give the product (350 mg, 82% yield over two
948 steps) as a yellow oil. The free base was converted to the HCl salt and recrystallized from
949 methanol to give a tan foam; GC/MS (EI) m/z 268 (M^+); 1 H NMR (400 MHz, $CDCl_3$) δ 8.00
950 (s, 1H), 7.61-7.63 (m, 1H), 7.33-7.36 (m, 1H), 7.08-7.20 (m, 2H), 6.98 (m, 1H), 13 C NMR
951 (100 MHz, $CDCl_3$) δ 136.2, 127.6, 121.9, 121.4, 119.1, 119.0, 115.0, 111.0, 57.6, 56.8, 49.6,
952 25.8, 25.0, 24.4, 24.3 ; Anal. ($C_{18}H_{24}N_2 \cdot HCl \cdot 0.5H_2O$) C, H, N.

953 *3-(2-(8-azabicyclo[3.2.1]octan-8-yl)ethyl)-5-fluoro-1H-indole (4c)*. Compound **4c** was
954 prepared as for **3b** using 2-azabicyclo[2.2.2]octane (**2**) (180.0 mg, 1.62 mmol) and 3-(5-
955 fluoro-1H-indol-3-yl)propanoic acid (335.4 mg, 1.62 mmol) to give the product (140 mg,
956 30% yield over two steps) as a yellow oil. GC/MS (EI) m/z 286 (M^+); 1 H NMR (400 MHz,
957 $CDCl_3$) δ 8.51 (s, 1H), 7.20-7.26 (m, 2H), 6.88-7.00 (m, 2H), 2.59-3.67 (m, 7H), 1.90-1.96
958 (m, 4H) 1.44-1.68 (m, 7H); 13 C NMR (100 MHz, $CDCl_3$) δ 158.8, 156.4, 132.8, 128.0, 127.9,
959 123.2, 116.4, 116.3, 111.6, 110.2, 109.9, 103.8, 103.6, 62.7, 56.3, 56.1, 49.6, 30.0, 27.9, 25.5,
960 24.6, 23.9, 22.8; Anal. ($C_{18}H_{23}FN_2 \cdot 0.5H_2O$) C, H, N.

961 *Nortropidene (6)*. Nortropine (**5**; 10.0 g, 78.6 mmol) was added portion wise to sulfuric acid
962 (10 mL) cooled in an ice bath, then stirred at 160 °C for 4 h. Once cooled to rt, the reaction
963 mixture was diluted with 50 mL H_2O , 50 mL NaOH (12.5 M), and extracted with Et_2O (3x 75
964 mL). The combined organic phases were washed with brine (50 mL), dried over $MgSO_4$, and
965 concentrated *in vacuo* to give the title compound as a light orange oil (4.0 g, 47% yield). The
966 free base proved unstable overtime. Thus, it was converted to the HCl salt for long term
967 storage by dissolving in EtOH/conc. HCl, concentrated *in vacuo*, and triturated with
968 DCM/ Et_2O to give a white solid. 1 H NMR (400 MHz, D_2O) δ 6.01 (m, 1H), 5.81 (m, 1H),
969 4.22 (m, 2H), 2.81 (d, J = 24 Hz, 1H), 2.27 (m, 3H), 2.13 (m, 1H), 1.96 (m, 1H). HRMS:
970 found m/z = 110.0964 (MH^+), calcd for $C_7H_{12}N$ (MH^+).

971 *1-(8-azabicyclo[3.2.1]oct-2-en-8-yl)-2-(1H-indol-3-yl)ethan-1-one (7a)*. To a solution of
972 indole-3-acetic acid (1.75 g, 10 mmol) in THF (50 mL) was added CDI (1.95 g, 12 mmol),
973 and the reaction stirred at rt for 2 h. A solution of **6** (1.09 g, 10 mmol) in THF (1 mL) was
974 added to the reaction mixture and stirring continued overnight. The reaction mixture was
975 concentrated, diluted with EtOAc (100 mL), and successively washed with 1N HCl (2x 50
976 mL), sat. $NaHCO_3$ (1x 50 mL), and brine (1x 50 mL). The extract was dried over $MgSO_4$,
977 concentrated *in vacuo*, re-dissolved in minimal DCM and precipitated with hexane to give the
978 title compound as an off-white powder and mixture of two diastereomers (A:B, 0.45:0.55)
979 (2.08 g, 78% yield). Diastereomer A: 1 H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.61 (d, J =
980 8 Hz, 1H), 7.33 (d, J = 8 Hz, 1H), 7.12 (m, 3H), 5.99 (m, 1H), 5.47 (m, 1H), 4.89 (m, 1H),

981 4.33 (m, 1H), 3.79 (m, 2H), 2.43 (d, $J = 20$ Hz, 1H), 2.08 (m, 1H), 1.87 (m, 3H), 1.68 (m,
982 1H). Diastereomer B: ^1H NMR (400 MHz, CDCl_3) δ 8.28 (s, 1H), 7.61 (d, $J = 8$ Hz, 1H),
983 7.33 (d, $J = 8$ Hz, 1H), 7.12 (m, 3H), 5.81 (m, 1H), 5.47 (m, 1H), 4.79 (m, 1H), 4.33 (m, 1H),
984 3.79 (m, 2H), 2.82 (d, $J = 20$ Hz, 1H), 2.08 (m, 1H), 1.87 (m, 3H), 1.68 (m, 1H). HRMS:
985 found m/z = 267.1494 (MH^+), calcd for $\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}$ (MH^+).

986 *1-(8-azabicyclo[3.2.1]oct-2-en-8-yl)-2-(5-fluoro-1H-indol-3-yl)ethan-1-one (7b).* To a
987 solution of 5-fluoro-indole-3-acetic acid (0.976 g, 5.05 mmol) in THF (25 mL) was added
988 CDI (0.973 g, 6 mmol), and the reaction stirred at rt for 2 h. The HCl salt of **6** (0.874 g, 6
989 mmol) was added as a solid, followed by *N,N*-diisopropylethylamine (1.05 mL, 6 mmol) and
990 stirring continued overnight. The reaction mixture was diluted with EtOAc (100 mL), and
991 successively washed with 1N HCl (3x 50 mL), sat. NaHCO_3 (1x 50 mL), and brine (1x 50
992 mL). The product was purified by column chromatography (100% DCM to
993 DCM/MeOH/NH₄OH = 90 : 10 : 1) to give the title compound as a light peach-colored solid
994 and mixture of two diastereomers (A:B, 0.4:0.6) (1.174 g, 82% yield). Diastereomer A: ^1H
995 NMR (400 MHz, CDCl_3) δ 8.16 (s, 1H), 7.25 (m, 2H), 7.14 (d, $J = 8$ Hz, 1H), 6.92 (t, $J = 8$
996 Hz, 1H), 6.01 (m, 1H), 5.49 (m, 1H), 4.87 (m, 1H), 4.34 (m, 1H), 3.72 (m, 2H), 2.46 (d, $J =$
997 20 Hz, 1H), 2.12 (m, 1H), 1.90 (m, 3H), 1.72 (m, 1H). Diastereomer B: ^1H NMR (400 MHz,
998 CDCl_3) δ 8.16 (s, 1H), 7.25 (m, 2H), 7.14 (d, $J = 8$ Hz, 1H), 6.92 (t, $J = 8$ Hz, 1H), 5.84 (m,
999 1H), 5.49 (m, 1H), 4.79 (m, 1H), 4.34 (m, 1H), 3.72 (m, 2H), 2.83 (d, $J = 20$ Hz, 1H), 2.12
1000 (m, 1H), 1.90 (m, 3H), 1.72 (m, 1H). HRMS: found m/z = 285.1402 (MH^+), calcd for
1001 $\text{C}_{17}\text{H}_{18}\text{N}_2\text{OF}$ (MH^+).

1002 *1-(8-azabicyclo[3.2.1]oct-2-en-8-yl)-2-(5-methoxy-1H-indol-3-yl)ethan-1-one (7c).* To a
1003 solution of 5- methoxy-indole-3-acetic acid (0.371 g, 1.81 mmol) in THF (10 mL) was added
1004 CDI (0.352 g, 2.17 mmol), and the reaction stirred at rt for 2 h. A solution of **6** (0.236 g, 2.17
1005 mmol) in THF (1 mL) was added to the reaction mixture and stirring continued overnight.
1006 The reaction mixture was diluted with EtOAc (40 mL), and successively washed with 1N HCl
1007 (3x 25 mL), sat. NaHCO_3 (1x 25 mL), and brine (1x 25 mL). The extract was dried over
1008 MgSO_4 , concentrated *in vacuo*, and the residue was purified by column chromatography
1009 (100% DCM to DCM/MeOH/NH₄OH = 90 : 10 : 1) to give the title compound as an off-white
1010 solid and mixture of two diastereomers (A:B, 0.4:0.6) (0.412 g, 77% yield). Diastereomer A:
1011 ^1H NMR (400 MHz, CDCl_3) δ 8.09 (s, 1H), 7.24 (m, 1H), 7.06 (m, 2H), 6.85 (m 1H), 6.00
1012 (m, 1H), 5.49 (m, 1H), 4.88 (m, 1H), 4.33 (m, 1H), 3.86 (s, 3H), 3.75 (m, 2H), 2.43 (d, $J = 20$
1013 Hz, 1H), 2.11 (m, 1H), 1.88 (m, 3H), 1.66 (m, 1H). Diastereomer B: ^1H NMR (400 MHz,
1014 CDCl_3) δ 8.06 (s, 1H), 7.24 (m, 1H), 7.06 (m, 2H), 6.85 (m 1H), 5.81 (m, 1H), 5.49 (m, 1H),

1015 4.80 (m, 1H), 4.33 (m, 1H), 3.86 (s, 3H), 3.75 (m, 2H), 2.83 (d, $J = 20$ Hz, 1H), 2.11 (m, 1H),
1016 1.88 (m, 3H), 1.66 (m, 1H). HRMS: found m/z = 297.1601 (MH⁺), calcd for C₁₈H₂₁N₂O₂
1017 (MH⁺).

1018 (*3R,4R,12S,12aR*)-*2,3,6,11,12,12a-hexahydro-3,12-ethanopyrrolo[1',2':1,2]azepino[4,5-*
1019 *b]indol-5(1H)-one (8a)*. A 25 mL Schlenk tube in an argon atmosphere was charged with **7a**
1020 (0.266 g, 1.00 mmol) and Pd(CH₃CN)₄(BF₄)₂ (0.577 g, 1.30 mmol). Anhyd acetonitrile (10
1021 mL) was added resulting in a dark red solution, which was stirred at rt overnight, maintaining
1022 its appearance throughout the time of reaction. The flask was equipped with a deflated
1023 balloon, converted to static argon, and a solution of NaBH₄ (0.113 g, 3 mmol) in 4 mL EtOH
1024 was added dropwise over 10 min, precipitating Pd(0) black and filling the balloon with H₂
1025 gas. The reaction continued to stir for 1 h, where most of the H₂ was consumed and the
1026 product precipitated along with the Pd(0) black. The reaction was diluted with 50 mL 20%
1027 MeOH/DCM, vacuum filtered (Pd(0)), and the filtrate successively washed with 1N HCl (1x
1028 20 mL) and brine (1x 20 mL), dried over MgSO₄ and concentrated *in vacuo*. The product was
1029 separated from byproducts by taking up in minimal DCM, diluted with Et₂O, and filtered to
1030 give the title compound as an off-white solid (0.169 g, 63% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 10.92 (s, 1H), 7.47 (d, $J = 8$ Hz, 1H), 7.25 (d, $J = 8$ Hz, 1H), 7.03 (t, $J = 8$ Hz,
1031 1H), 6.97 (t, $J = 8$ Hz, 1H), 4.57 (d, $J = 8$ Hz, 1H), 4.27 (d, $J = 8$ Hz, 1H), 3.92 (d, $J = 16$ Hz,
1032 1H), 3.41 (d, $J = 16$ Hz, 1H), 2.98 (d, $J = 8$ Hz, 1H), 2.24 (m, 2H), 2.00 (m, 2H), 1.80 (m,
1033 2H), 1.53 (m, 1H), 1.21 (m, 1H). ¹³C NMR (100 MHz, DMSO-d₆) δ 171.35, 136.57, 134.78,
1034 127.28, 120.74, 118.39, 117.32, 110.62, 103.88, 55.91, 53.03, 38.29, 32.58, 28.69, 26.92,
1035 25.78, 22.69. HRMS: found m/z = 267.1483 (MH⁺), calcd for C₁₇H₁₉N₂O (MH⁺).

1036 (*3R,4R,12S,12aR*)-*8-fluoro-2,3,6,11,12,12a-hexahydro-3,12-*
1037 *ethanopyrrolo[1',2':1,2]azepino[4,5-b]indol-5(1H)-one (8b)*. Compound **8b** was prepared as
1038 described for **8a** using **7b** (0.142 g, 0.50 mmol) and Pd(CH₃CN)₄(BF₄)₂ (0.289 g, 0.65 mmol)
1039 to give the title compound (0.990 g, 70% yield) as a white solid. ¹H NMR (400 MHz, DMSO-
1040 d₆) δ 11.04 (s, 1H), 7.26 (m, 2H), 6.86 (m, 1H), 4.56 (d, $J = 8$ Hz, 1H), 4.27 (d, $J = 8$ Hz, 1H),
1041 3.90 (d, $J = 16$ Hz, 1H), 3.38 (d, $J = 16$ Hz, 1H), 2.98 (d, $J = 8$ Hz, 1H), 2.23 (m, 2H), 1.99
1042 (m, 2H), 1.79 (m, 2H), 1.51 (m, 1H), 1.18 (m, 1H). ¹³C NMR (100 MHz, DMSO-d₆) δ 171.19,
1043 156.88 (d, $J_{c,f} = 230$ Hz), 138.83, 131.39, 127.61 (d, $J_{c,f} = 10$ Hz), 111.46 (d, $J_{c,f} = 9$ Hz),
1044 108.63, (d, $J_{c,f} = 25$ Hz), 104.38, (d, $J_{c,f} = 4$ Hz), 102.34, (d, $J_{c,f} = 23$ Hz), 55.79, 53.02,
1045 38.36, 32.57, 28.64, 26.93, 25.78, 22.70. HRMS: found m/z = 285.1397 (MH⁺), calcd for
1046 C₁₇H₁₈N₂OF (MH⁺).

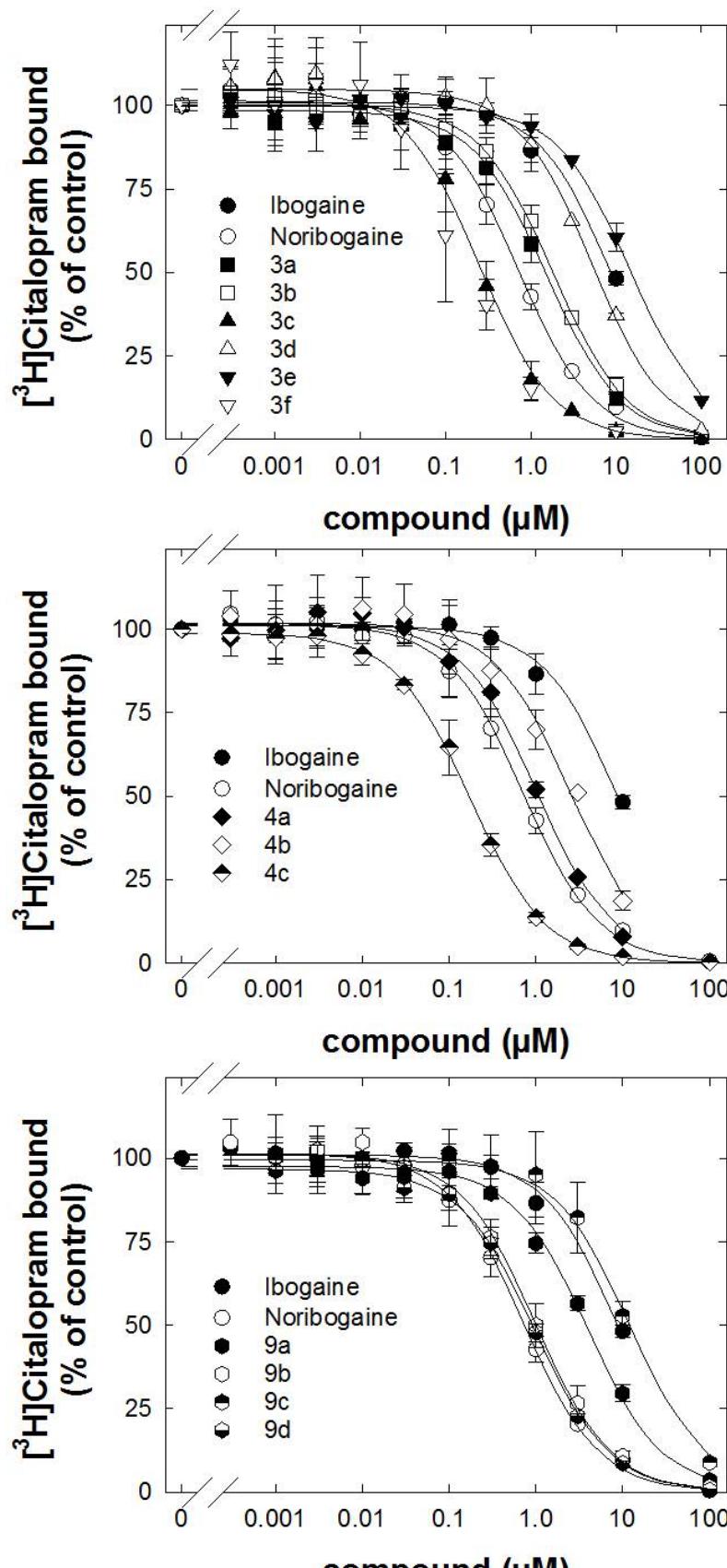
1048 *(3R,4R,12S,12aR)-8-methoxy-2,3,6,11,12,12a-hexahydro-3,12-*
1049 *ethanopyrrolo[1',2':1,2]azepino[4,5-b]indol-5(1H)-one (8c)*. Compound **8c** was prepared as
1050 for **8a** using **7c** (0.260 g, 0.88 mmol) and Pd(CH₃CN)₄(BF₄)₂ (0.506 g, 1.14 mmol) to give the
1051 title compound (0.210 g, 81% yield) as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ 10.74
1052 (s, 1H), 7.13 (d, *J* = 8 Hz, 1H), 6.99 (s, 1H), 6.67 (d, *J* = 8 Hz, 1H), 4.56 (d, *J* = 8 Hz, 1H),
1053 4.27 (m, 1H), 3.89 (d, *J* = 16 Hz, 1H), 3.76 (s, 3H), 3.41 (d, *J* = 16 Hz, 1H), 2.95 (m, 1H),
1054 2.25 (m, 2H), 2.00 (m, 2H), 1.78 (m, 2H), 1.52 (m, 1H), 1.21 (m, 1H). ¹³C NMR (100 MHz,
1055 DMSO-d₆) δ 171.40, 153.18, 137.25, 129.81, 127.62, 111.26, 110.73, 103.78, 99.48, 55.91,
1056 55.37, 52.99, 38.37, 32.64, 28.69, 26.92, 25.79, 22.78. HRMS: found m/z = 297.1586 (MH⁺),
1057 calcd for C₁₈H₂₁N₂O₂ (MH⁺).

1058 *(3R,4R,12S,12aR)-1,2,3,5,6,11,12,12a-octahydro-3,12-ethanopyrrolo[1',2':1,2]azepino[4,5-*
1059 *b]indole (9a)*. To a suspension of **8a** (0.030 g, 0.11 mmol) in 3 mL THF at rt was added BMS
1060 (0.20 mL, 2.1 mmol) and the reaction was stirred at reflux for 1 h. Once cooled to rt, the
1061 reaction was slowly quenched with MeOH and concentrated *in vacuo*. The reaction was then
1062 taken up in 3 M HCl (5 mL) and stirred at reflux overnight to ensure hydrolysis of the boron
1063 complex with the product. The reaction was basified with 1 M NaOH and extracted with
1064 DCM (3 x 10 mL). The combined organic phases were washed with brine, dried over MgSO₄,
1065 and concentrated *in vacuo*. The residue was purified by column chromatography (100% DCM
1066 to DCM/MeOH/NH₄OH = 90 : 10 : 1) to give the title compound as a white solid (0.019 g,
1067 68% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.70 (s, 1H), 7.50 (m, 1H), 7.26 (m, 1H), 7.12 (m,
1068 2H), 3.91 (d, *J* = 4 Hz, 1H), 3.49 (m, 1H), 3.38 (m, 2H), 3.17 (m, 1H), 3.03 (m, 1H), 2.80 (d,
1069 *J* = 8 Hz, 1H), 2.29 (m, 1H), 2.09 (m, 3H), 1.75 (m, 3H), 1.08 (dd, *J* = 8, 16 Hz, 1H). ¹³C
1070 NMR (100 MHz, DMSO-d₆) δ 139.23, 134.91, 128.27, 120.04, 117.92, 117.17, 111.39,
1071 110.30, 56.25, 56.00, 47.98, 38.39, 29.18, 28.29, 23.88, 23.51, 21.82. HRMS: found m/z =
1072 253.1698 (MH⁺), calcd for C₁₇H₂₁N₂ (MH⁺).

1073 *(3R,4R,12S,12aR)-8-fluoro-1,2,3,5,6,11,12,12a-octahydro-3,12-*
1074 *ethanopyrrolo[1',2':1,2]azepino[4,5-b]indole (9b)*. Compound **9b** was prepared as described
1075 for **9a** using **8b** (0.028 g, 0.10 mmol) and BMS (0.20 mL, 2.1 mmol) to give the title
1076 compound as a white solid (0.019 g, 70% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.64 (s, 1H),
1077 7.15 (m, 2H), 6.86 (m, 1H), 3.88 (d, *J* = 8 Hz, 1H), 3.48 (m, 1H), 3.38 (m, 2H), 3.09 (m, 1H),
1078 2.97 (m, 1H), 2.77 (d, *J* = 8 Hz, 1H), 2.29 (m, 1H), 2.10 (m, 3H), 1.74 (m, 3H), 1.09 (dd, *J* =
1079 8, 16 Hz, 1H). ¹³C NMR (100 MHz, DMSO-d₆) δ 156.74 (d, *J*_{c,f} = 229 Hz), 141.23, 131.56,
1080 128.46 (d, *J*_{c,f} = 10 Hz), 111.77 (d, *J*_{c,f} = 5 Hz), 111.08 (d, *J*_{c,f} = 9 Hz), 107.88 (d, *J*_{c,f} = 25

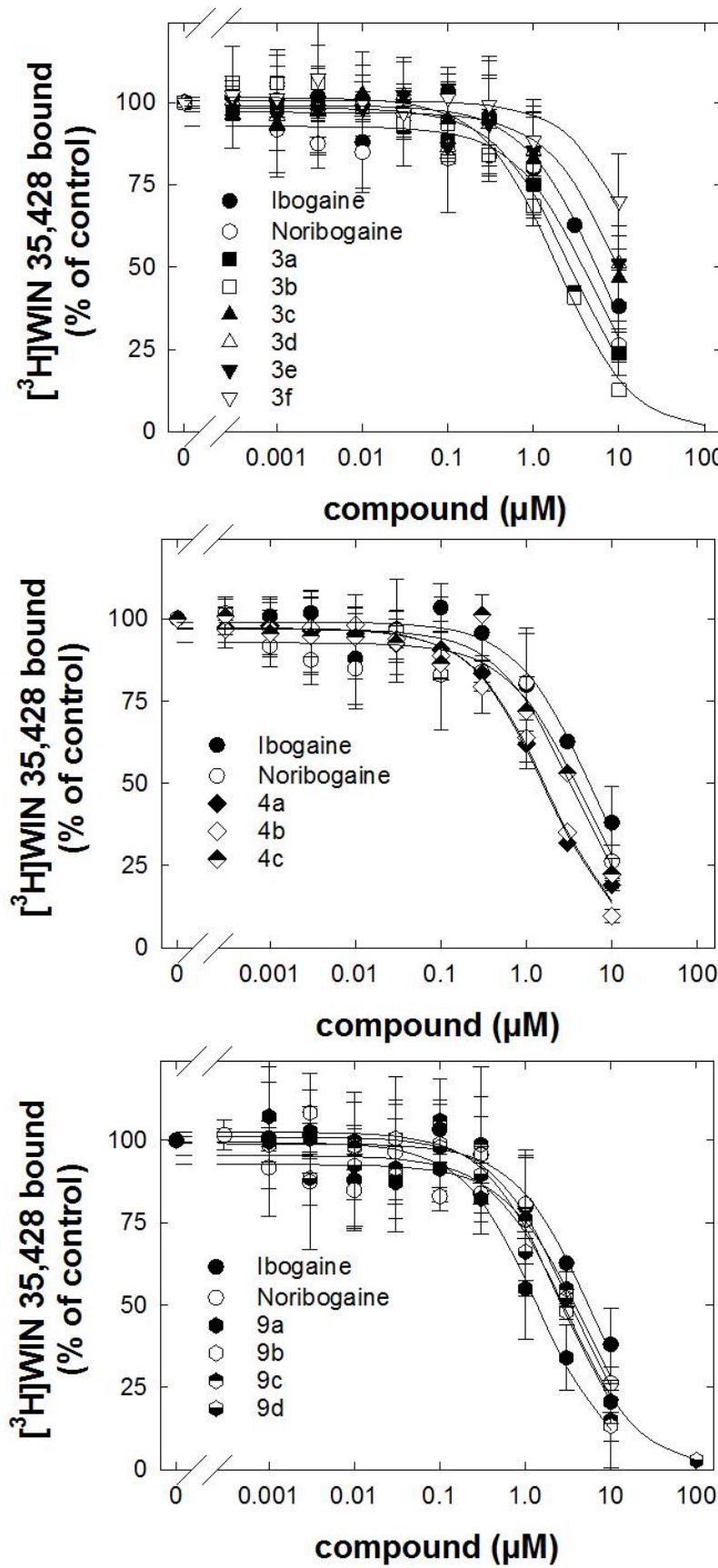
1081 Hz), 102.11 (d, $J_{c,f}$ = 23 Hz), 56.36, 56.22, 47.89, 38.24, 28.85, 27.96, 23.54, 23.20, 21.72.
1082 HRMS: found m/z = 271.1604 (MH⁺), calcd for C₁₇H₂₀N₂F (MH⁺).
1083 *(3R,4R,12S,12aR)-8-methoxy-1,2,3,5,6,11,12,12a-octahydro-3,12*
1084 *ethanopyrrolo[1',2':1,2]azepino[4,5-b]indole (9c)*. Compound **9c** was prepared as described
1085 for **9a** using **8c** (0.444 g, 1.5 mmol) and BMS (1.50 mL, 15.8 mmol) in THF (40 mL) without
1086 the 3 M HCl reflux step to give the title compound as a white solid (0.365 g, 86% yield). ¹H
1087 NMR (400 MHz, CDCl₃) δ 7.50 (s, 1H), 7.16 (d, J = 8 Hz, 1H), 6.96 (s, 1H), 6.79 (d, J = 8
1088 Hz, 1H), 3.89 (m, 1H), 3.86 (s, 3H), 3.44 (m, 1H), 3.39 (m, 2H), 3.13 (m, 1H), 2.98 (m, 1H),
1089 2.77 (m, 1H), 2.28 (m, 1H), 2.08 (m, 3H), 1.75 (m, 3H), 1.08 (m, 1H). HRMS: found m/z =
1090 283.1804 (MH⁺), calcd for C₁₈H₂₃N₂O (MH⁺).
1091 *(3R,4R,12S,12aR)-1,2,3,5,6,11,12,12a-octahydro-3,12-ethanopyrrolo[1',2':1,2]azepino[4,5-*
1092 *b]indol-8-ol (9d)*. Compound **9d** was prepared as described for **9a** using **8c** (0.450 g, 1.5
1093 mmol) and BMS (1.50 mL, 15.8 mmol) in THF (40 mL) with the 3 M HCl reflux step (12
1094 mL, overnight) to give the title compound as a tan solid (0.078 g, 19% yield). ¹H NMR (400
1095 MHz, CDCl₃) δ 7.49 (s, 1H), 7.11 (d, J = 8 Hz, 1H), 6.90 (s, 1H), 6.69 (d, J = 8 Hz, 1H), 3.88
1096 (d, J = 8 Hz, 1H), 3.47 (m, 1H), 3.38 (m, 2H), 3.06 (m, 1H), 2.96 (m, 1H), 2.77 (d, J = 8 Hz,
1097 1H), 2.28 (m, 1H), 2.10 (m, 3H), 1.75 (m, 3H), 1.08 (dd, J = 8, 16 Hz, 1H). HRMS: found
1098 m/z = 269.1654 (MH⁺), calcd for C₁₇H₂₁N₂O (MH⁺).
1099

1100 **Supplementary figures**



1101

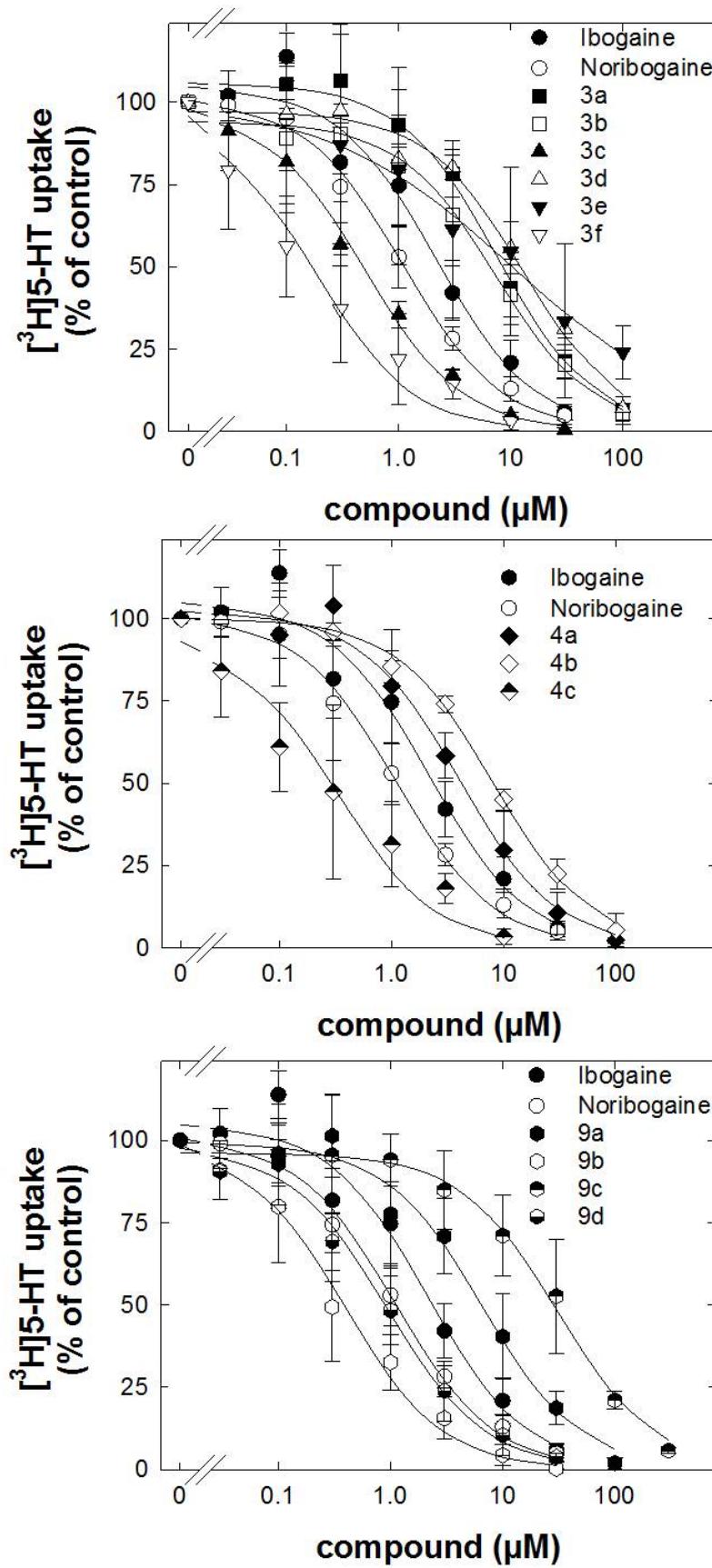
1102 **Supplementary Figure 1. Inhibition by ibogaine analogs of [³H]citalopram binding to rat**
1103 **SERT.** Brain stem membranes were dissected and prepared from male Sprague–Dawley rat
1104 brains (see Methods). Membranes binding was conducted in 96-well polypropylene plates
1105 containing 50 μ L of various concentrations of the inhibitor, diluted using 30% DMSO vehicle,
1106 300 μ L of Tris buffer (SERT), 50 μ L of [³H]citalopram solution (final concentration 1.5 nM) and
1107 100 μ L of tissue (2.0 mg/well original wet weight) for 60 min at room temperature (SERT).
1108 Nonspecific binding was determined using 10 μ M fluoxetine, which was <10% of specific
1109 binding. Data are represented as the means \pm S.D. (error bars) from at least three independent
1110 experiments, each performed in triplicate. Specific binding (between 3000 and 5000 cpm) was
1111 set to 100% to normalize for inter-assay variation. The solid curves were drawn by fitting the
1112 data to the equation for a monophasic inhibition. K_i -values were calculated from the IC₅₀ values
1113 using the Cheng-Prusoff equation (see **Table 1**).
1114



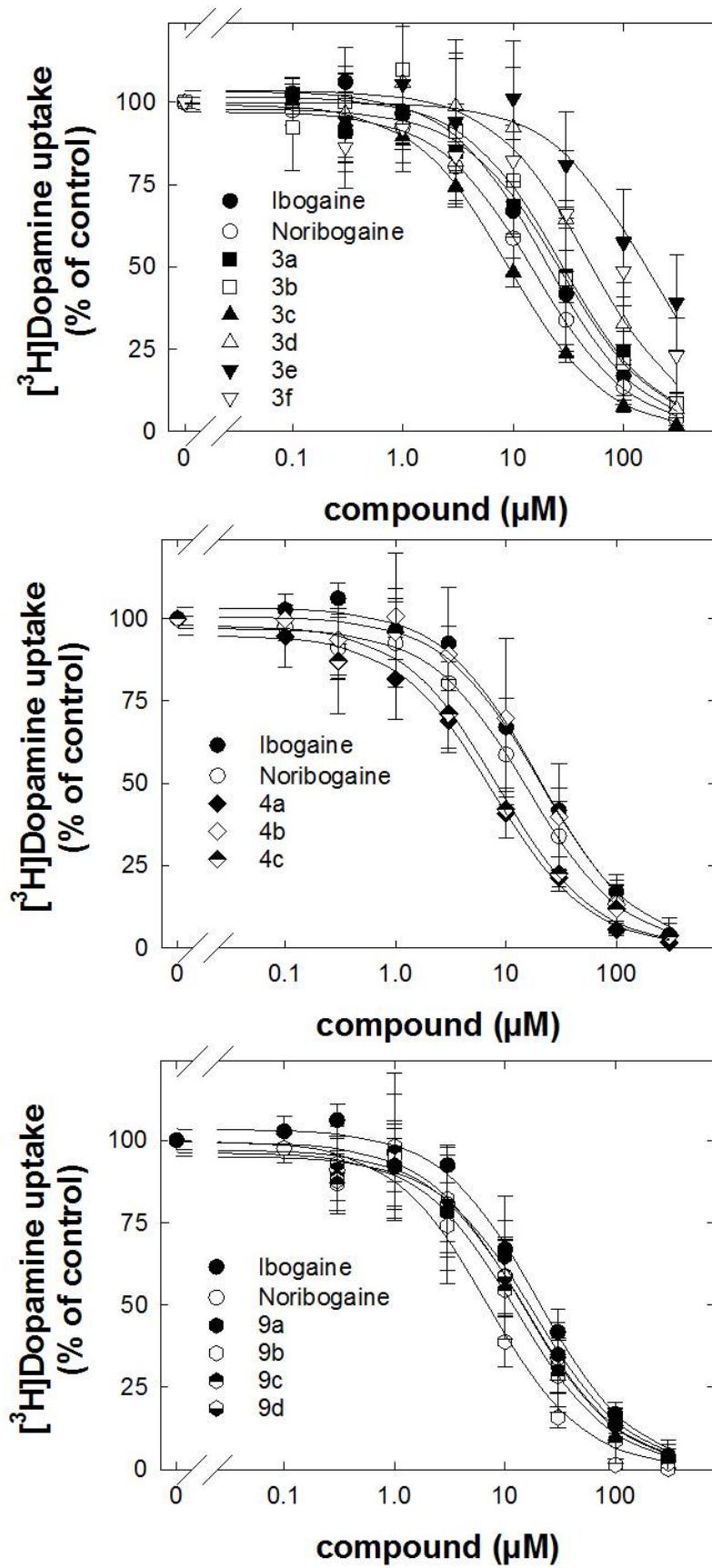
1115

1116

1117 **Supplementary Figure 2. Inhibition by ibogaine analogs of [³H]WIN 35,428 binding to rat DAT.**
1118 Striatum membranes (for DAT) were dissected and prepared from male Sprague-Dawley rat brains
1119 (see Methods). Membranes binding was conducted in 96-well polypropylene plates containing 50 μ L
1120 of various concentrations of the inhibitor, diluted using 30% DMSO vehicle, 300 μ L of sucrose
1121 phosphate buffer, 50 μ L of [³H]-WIN35,428 solution (final concentration 1.5 nM) and 100 μ L of tissue
1122 (2.0 mg/well original wet weight) for 120 min at 0-4 °C. Nonspecific binding was determined using 10
1123 μ M indatraline, which was <10% of specific binding. Data are represented as the means \pm S.D. (error
1124 bars) from at least three independent experiments, each performed in triplicate. Specific binding
1125 (between 3000 and 5000 cpm) was set to 100% to normalize for inter-assay variation. The solid
1126 curves were drawn by fitting the data to the equation for a monophasic inhibition. Ki-values were
1127 calculated from the IC₅₀ values using the Cheng-Prusoff equation (see Table 1).
1128



1130 **Supplementary Figure 3. Inhibition by ibogaine analogs of [³H]5-HT uptake by hSERT.** HEK293 cells
1131 stably expressing wild-type YFP-hSERT were seeded onto 96-well plates for 24 h. Cells were
1132 incubated with logarithmically spaced concentrations (0.003–300 μ M) of ibogaine analogs for 10
1133 minutes and subsequently with the same concentration of the ibogaine analogs with 0.4 μ M [³H]5-HT
1134 for 1 minute. Non-specific uptake was defined as cellular accumulation of radioactivity in the
1135 presence of 30 μ M paroxetine; this was <10% of total uptake. Specific uptake is the difference
1136 between total and non-specific uptake. Data are the means \pm S.D. from three independent
1137 experiments done in triplicates. Specific uptake for SERT was $4.46 \pm 1.47 \text{ pmol} \cdot \text{min}^{-1} \cdot 10^{-6}$ cells and was
1138 set to 100% to normalize for inter-assay variation. The solid curves were drawn by fitting the data to
1139 the equation for a monophasic inhibition. The IC₅₀-values are reported in **Table 1**.
1140



1142 **Supplementary Figure 4. Inhibition by ibogaine analogs of [³H]5-DA uptake by hDAT.** HEK293 cells
1143 stably expressing YFP-hDAT were seeded onto 96-well plates for 24 h. Cells were incubated with
1144 logarithmically spaced concentrations (0.003–300 μ M) of ibogaine analogs for 10 minutes and
1145 subsequently with the same concentration of the ibogaine analogs with 0.4 μ M of [³H]DA for 1
1146 minute. Non-specific uptake was defined as cellular accumulation of radioactivity in the presence of
1147 30 μ M mazindol; this was <10% of total uptake. Specific uptake is the difference between total and
1148 non-specific uptake. Data are the means \pm S.D. from three independent experiments done in
1149 triplicates. Specific uptake for DAT was 7.5 ± 2.1 pmol·min⁻¹10⁻⁶ cells, respectively, and was set to
1150 100% to normalize for inter-assay variation. The solid curves were drawn by fitting the data to the
1151 equation for a monophasic inhibition. The IC₅₀-values are reported in **Table 1**.
1152
1153