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High dopamine transporter selectivity can be displayed by remarkably simple non-nitrogen containing inhibitors

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Abstract—A series of 2-(3,4-dichlorophenyl)-cyclopent-1-enyl carboxylic acid esters and amides were prepared and tested for binding to the DAT, SERT, and NET. The achiral compounds were easily attained and found to inhibit DAT binding with K_i-values ranging from 0.095 to 0.00003 mM. Among the compounds tested 2-(3,4-dichlorophenyl)-cyclopent-1-enyl carboxylic acid 2-methylphenyl ester was found to be highly selective with SERT/DAT > 7000; NET/DAT > 1700, K_i = 60 nM.

The monoamine neurotransmitters, dopamine (DA), serotonin (SER), and norepinephrine (NE) control a variety of functions in the central nervous system. Associated to these monoamines, the dopamine, serotonin, and norepinephrine transporters (DAT, SERT, and NET, respectively) play an important role in determining duration and magnitude of the signals from neurons releasing these neurotransmitters. The transporter proteins are for this reason key targets for, for example, antidepressant drugs and recreational drugs of abuse. Thus, being able to chemotherapeutically adjust the levels of, for example, SER using selective SERT-uptake inhibitors, has led to an increased quality of life for many patients suffering from depression.[1] On the other hand the monoamine transporters are key players in mediating the action of cocaine and amphetamines. Psychostimulants enhance monoaminergic signalling by inhibiting their respective transporters thereby increasing extracellular levels of monoamines. In particular, several lines of evidence have provided support for a role of the DAT as a primary site for cocaine reward. Structure–activity studies document good correlations between psychostimulant properties in reward tests and in their abilities to block DAT.[2]

Many selective binders of monoamine transporters have to date been prepared. More specifically have DAT selective binders been oriented towards finding a small molecule that would antagonise the effect of cocaine.[2]

For some types of ligands, synthesis have required expensive homochiral starting materials and synthetic routes have been lengthy and in some cases demanding. Based on some recent promising results in our group on simplifying the phenyl tropane skeleton[3] we wondered whether it would possible to further increase affinity and selectivity without adding considerable complexity to a potential ligand. In this communication we report several easily attainable DAT selective achiral ligands with sub-micromolar affinity see Table 1.

Meltzer and co-workers have previously shown that replacement of the tropane nitrogen with C or O only causes a slight drop in affinity and is therefore not crucial for binding.[4–6] In a recent study of non-nitrogen containing truncated phenyl tropane analogues we found several cycloalkene ligands that showed moderate monoamine transporter binding and, possibly because of the lack of an endocyclic N-atom, good selectivity for DAT/NET-binding over SERT binding (Fig. 1).[3] Ring size (5, 6, or 7) did not seem to largely influence affinity to DAT and 3,4-dichlorophenyl was found persistently to provide the best results (Fig. 1). Furthermore cyclopentenes had a slightly superior DAT/SERT

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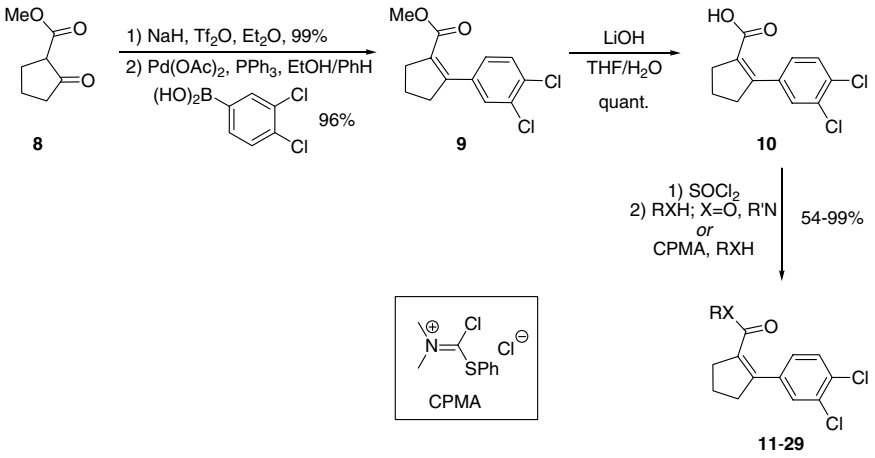


Scheme 1. Chemical synthesis of cyclopentene ligands.

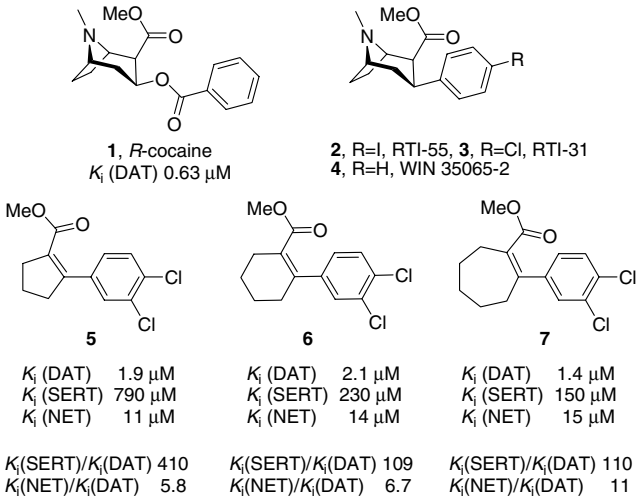


Figure 1. Cocaine **1**, and phenyl tropanes **2**–**4**. Truncated non-nitrogen containing monoamine transporter binders from earlier studies (**5**–**7**).Values are for binding to human transporters, Ref. 3.

selectivity when compared to the analogous cyclohexene and cycloheptene (Fig. 1).[3]

Marked increases in DAT selectivity by changing the methyl ester function to other esters[7,8] or amides[8] in phenyl tropanes of the type **2**–**4** without greatly affecting affinity have previously been reported. We speculated whether the same approach would result in selective DAT binders based on our cycloalkene scaffold. Contrary to our previous study[3] we consequently decided to vary the carboxyl substituents but kept the ring-size and aryl-substituent constant.

For the synthesis of the various esters and amides the carboxylic acid precursor was prepared by hydrolysis of the corresponding methyl ester in quantitative yield.[9] The ester was efficiently prepared by a Suzuki cross coupling as described in previous work.[3]

Ester and amide coupling reactions were carried out either by direct coupling promoted by CPMA[10] or by using a two step procedure involving the acid chloride as the intermediate. Synthetic efficiency of the two different protocols was found to be similar giving yields ranging from 54–99%[9] (Scheme 1).

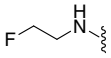
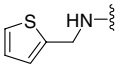
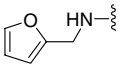
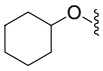
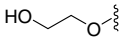
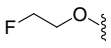
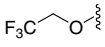
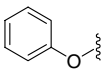
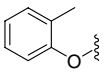
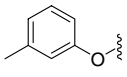
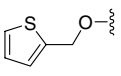
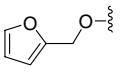
The new compounds (**11**–**29**, Table 1) were screened for activity against hDAT, hSERT, and hNET in a RTI-55 (**2**, Fig. 1) competition assay[11] and the results displayed in Table 1.

With regards to SERT binding a hydroxyethyl ester (**22**) together with a furan-2-yl methyl ester (**29**) and amide (**19**) are relatively well tolerated as a 15- to 30-fold increase in affinity compared to the parent methyl ester. From these results it appears that having an O-atom as a hydrogen bond acceptor spaced three atoms from the carbonyl function is able to improve binding. It should be noted that larger substituents like cyclohexyl- and phenyl esters **20**, **25**–**27** are poorly accommodated by SERT but not necessarily significantly worse than methyl ester **5** due to inaccuracy in precisely determining binding constants in the high micromolar range (Table 1).

No compounds tested showed a substantial improvement of NET binding compared to the parent methyl ester **5**. Linear substituents like hydroxyethyl and fluoroethyl esters (**22** and **23**) and fluoroethyl amide (**15**) were found to give similar binding constants to NET as methyl ester **5**. Less flexible, cyclic acyl substituents like morpholinyl (**11**), pyrrodinyl (**12**), cyclohexyl (**20**) and 2-methylphenyl (**27**) have the lowest NET affinity of the compounds tested with a decrease of 27-, 37-, 12-, and 9-fold, respectively, compared to the parent methyl ester (**5**).

It seems slightly beneficial for NET binding to have a hydrogen bond donor next to the carbonyl function as thiophene-2-ylmethyl amide **18** shows a 2-fold stronger binding than the corresponding ester **28**. The analogous furan-2-ylmethyl amide **19** furthermore binds 5 times stronger than the ester **29**. This is in line with hydrogen bond donation not being possible for Weinreb amide **16** but for its nor-*N*-methyl analogue **17**, which shows 27-fold stronger binding towards NET.

Table 1. Binding constants for cyclopentene ligands to monoamine transporters

Compound	Structure, RX	hSERT		hDAT		hNET		hSERT/hDAT	hNET/hDAT
		K_i/μM	SEM	K_i/μM	SEM	K_i/μM	SEM		
5[3]	CH_3O	790	270	1.9	0.3	11	11	410	6
11		>500	—	11	0.6	300	170	>45	27
12		520	220	58	60	410	230	9.0	7.0
13		300	40	94	16	210	140	3.2	2.2
14		>1000	—	9	2	50	14	>110	5.6
15		>500	—	0.07	0.06	8.0	7.7	>7100	110
16		540	130	6.4	7.0	250	130	84	39
17		>500	—	0.83	1.0	9.2	7.0	>600	11
18		110	210	0.14	0.18	18	30	790	130
19		24	37	0.03	0.06	12	14	800	400
20		>1000	—	12	4	130	120	>83	11
21		500	160	2.6	1.6	26	13	190	10
22		50	70	0.3	0.7	9	15	170	30
23		390	520	0.5	1.1	5.8	9.2	780	12
24		>1000	—	3.4	1.7	170	140	290	50
25		>500	—	0.17	0.07	85	42	>2900	500
26		>500	—	0.22	0.09	33	17	>2300	150
27		>1000	—	0.06	0.04	>100	—	>17000	>1700
28		910	980	0.4	0.2	42	26	2300	110
29		24	—	1.5	1	61	53	16	41

High DAT affinity was the goal of this study and this was achieved by replacing the methyl ester functional group by other side-chains. More than a factor 60 in binding affinity was gained by replacing the methyl ester with a furan-2-ylmethyl amide (**19**) which has a moderately good DAT selectivity.

Substitution of the methyl ester with an *N*-methoxy amide (**17**) function gives slightly stronger binding to DAT (1.9 μM vs 0.83 μM), whereas Weinreb amide **16** is approximately 3 times less potent than the parent **5**. Otherwise common for compounds showing enhanced affinity for DAT is the presence of either an aromatic (**25**–**27**), heteroaromatic (**18**, **19**, **28**,**29**), or hydro/fluoroethyl (**15**, **22**, **23**) ester or amide. Of these most potent ligands the 2-methylphenyl ester (**27**, K_i 60 nM) shows an outstanding selectivity being more than 17,000-fold more selective for DAT than SERT, and more than 1,700-fold more selective for DAT than NET. Interestingly, we found the order of binding/DAT selectivity among the aromatic esters to be 3-Me–Ph > Ph > 2-Me–Ph, which is different from the profile Ph > 2-Me–Ph > 3-Me–Ph displayed by the analogues ester of the *p*-chloro phenyl tropane (**3**, Fig. 1).[8] These cyclopentene ligands (**25**–**27**), furthermore, become more than 10-fold more potent by having an aryl ester, whilst for the same change in the *p*-chloro substituted phenyl tropane (**3**, Fig. 1) a drop in affinity has been observed.[8]

Changing from a methyl ester to a morpholinyl amide (**11**) causes a slight drop in affinity towards the DAT which has also been found in the *p*-chloro substituted phenyl tropane.[8] It was, however, not possible with great accuracy to establish the SERT binding, which in the phenyl tropane series was found to be very low and therefore provide a selectivity better than 30,000 in favour of DAT binding.

Finally, with respect to DAT/SERT- and DAT/NET selectivity we found fluoroethyl- and furan-2ylmethyl amides (**15** and **19**) to be an order of magnitude more DAT selective than the corresponding esters (**23** and **29**).

In this work we have shown that readily prepared achiral cyclopentene ligands provide strong DAT binding with excellent selectivity over SERT and NET, which is crucial in the development of possible cocaine antagonists that can be used in the treatment of cocaine addiction. We regard molecules synthesised in this study as outstanding leads for further examination. Especially do compounds like the 3-methylphenyl ester (**27**) and the 2-fluoroethyl amide (**15**) appeal to the authors for further investigation, which is currently on-going in our laboratory.

Experimental: Compound **10**: The methyl ester (450 mg, 1.66 mmol) and LiOH (477 mg, 19.9 mmol) were dissolved in THF/H_2O (1:1, 20 mL). The reaction mixture was stirred overnight at 60 °C before it was acidified with HCl (aq, dilute) and diluted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and concentrated under vacuum to obtain the carboxylic acid as a solid in quantitative yield.^{1}H NMR ($CDCl_3$) δ_H 7.41 (d, 1H, *J* 2.1 Hz), 7.39 (d, 1H, *J* 8.4 Hz), 7.17 (dd, 1H, $J_1$2.1 Hz, J_2 = 8.4 Hz), 2.84 (m, 4H); 2.00 (qv, 2H, *J*7.6 Hz).^{13}C NMR ($CDCl_3$) δ_C 170.5, 154.4, 136.8, 132.0, 132.2, 132.1, 130.0, 129.9, 129.8, 41.0, 35.0, 21.9.

HRMS(ES+): calcd for $C_{12}H_{10}Cl_2O_2 + Na^+$: 278.9955, found: 278.9955.

Synthesis of amides and esters from carboxylic acid using CPMA was conducted as described in ref. 10 or by making the acid chloride as described below:

2-(3,4-Dichloro-phenyl)-cyclopent-1-enecarboxylic acid chloride: The acid (478 mg, 1.86 mmol) was dissolved in thionyl chloride (2.5 mL). The reaction mixture was stirred for 15 min. The thionyl chloride was removed under reduce pressure to give the acid chloride in quantitative yield.

A solution of alcohol or amine (1.2 equiv) in CH_2Cl_2 (0.5 mL) was then added dropwise at 0 °C to a solution of acid chloride (1 equiv) in CH_2Cl_2 (1.25 mL), after DIEA (1.2 equiv) was added. The reaction mixture was stirred at rt overnight and then concentrated under vacuum.

Compound **11**. Purification by flash chromatography (EtOAc/pentane, 1:3 → 1:2) afforded **11** (39 mg) in 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.41 (d, 1H, *J* 2.0 Hz), 7.36 (d, 1H, *J* 8.4 Hz), 7.17 (dd, 1H, *J* 2.4 Hz, *J* 8.4 Hz), 3.64–3.57 (m, 4H), 3.23–3.16 (m, 4H), 2.81–2.74 (m, 4H), 2.04 (qv, 2H, *J* 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 168.8, 137.6, 135.8, 134.5, 132.7, 131.8, 130.5, 128.7, 126.2, 46.5, 41.6, 37.2, 36.0, 22.4; HRMS(ES+): calcd for $C_{16}H_{17}Cl_2NO_2 + Na^+$ 348.0534, found: 348.0533.

Compound **12**. Purification by flash chromatography (EtOAc/pentane 2:5 → 2:3) afforded **12** (51 mg) in 87% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.39 (d, 1H, *J* 2.0 Hz), 7.32 (d, 1H, *J* 8.4 Hz), 7.19 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz), 3.47 (t, 2H, *J* 6.8 Hz), 3.01 (t, 2H, *J* 6.6 Hz), 2.78 (t, 4H, *J* 7.4 Hz), 2.02 (qv, 2H, *J* 7.4 Hz), 1.79 (qv, 2H, *J* 6.8 Hz), 1.70 (qv, 2H, *J* 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 168.7, 136.7, 136.3, 136.2, 132.6, 131.5, 130.4, 128.4, 126.0, 46.8, 45.4, 36.5, 35.9, 25.8, 24.3, 22.4. HRMS(ES+): calcd for $C_{16}H_{17}Cl_2NO$ + Na 332.0585, found: 332.0585.

Compound **13**. Purification by flash chromatography (EtOAc/pentane 2:7) afforded **13** (53 mg) in 89% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.43 (d, 1H, *J* 2.0 Hz), 7.31 (d, 1H, *J* 8.4 Hz), 7.20 (dd, 1H, *J* 2.4 Hz, *J* 8.4 Hz), 3.42 (q, 2H, *J* 7.2 Hz), 3.08 (q, 2H, *J*7.2 Hz), 2.80–2.75 (m, 4H), 2.03 (qv, 2H, *J* 7.6 Hz), 1.12 (t, 3H, *J* 7.2 Hz), 0.88 (t, 3H, *J* 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 169.7, 136.1, 136.0, 135.6, 132.5, 131.3, 130.2, 128.7, 126.2, 42.4, 38.5, 37.5, 35.8, 22.4, 14.3, 12.4. HRMS(ES+): calcd for $C_{16}H_{19}Cl_2NO_2$ + Na 334.0741, found: 334.0748.

Compound **14**. Purification by flash chromatography (EtOAc/pentane 2:7) afforded **14** (52 mg) in 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.41 (d, 1H, *J* 2.0 Hz), 7.38 (d, 1H, *J* 8.4 Hz), 7.17 (dd, 1H, *J* 2.4 Hz, *J* 8.4 Hz), 5.47 (bd, 1H, *J* 6.8 Hz), 4.43–4.37 (m, 1H), 2.81–2.76 (m, 4H), 2.29–2.23 (m, 2H), 2.00 (qv, 2H, *J* 7.6 Hz), 1.68–1.62 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 168.4, 141.4, 136.3, 136.1, 132.5, 131.9, 130.3, 129.6, 127.2, 44.5, 38.3, 36.2, 30.9, 22.1, 15.2. HRMS(ES+): calcd for $C_{16}H_{17}Cl_2NO$ + H 310.0765, found: 310.0764.

Compound **15**. Purification by flash chromatography (EtOAc/pentane, 2:5 → 2:3) afforded **15** (45 mg) in 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.42 (d, 1H, *J* 2.0 Hz), 7.40 (d, 1H, *J* 8.4 Hz), 7.18 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz), 5.71 (bs, 1H), 4.48 (t, 1H, *J* 4.8 Hz, CH_2F), 4.36 (t, 1H, *J* 4.8 Hz), 3.57 (q, 1H, *J* 5.2 Hz), 3.50 (q, 1H, *J* 5.2 Hz), 2.85–2.78 (m, 4H), 2.02 (qv, 2H, *J* 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 167.3, 143.1, 136.3, 135.1, 132.6, 132.1, 130.4, 128.5, 127.1, 82.5 (d, J_{CF} 166.5 Hz)), 39.8 (d, J_{CF} 19.1 Hz), 38.7, 36.0, 22.1. ^{19}F NMR (377 MHz, $CDCl_3$) δ_F 6.22 (t, *J* 28 Hz). HRMS(ES+): calcd for $C_{14}H_{14}Cl_2FNO$+Na 324.0334, found: 324.0347.

Compound **16**. Purification by flash chromatography (EtOAc/pentane 2:7) afforded **16** (54 mg) in 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 50 °C) δ_H 7.45 (d, 1H, *J*2.0 Hz), 7.35 (d, 1H, *J* 8.4 Hz), 7.24 (dd, 1H, *J* 2.4 Hz, *J* 8.4 Hz), 3.52 (s, 3H), 3.12 (s, 3H), 2.81 (t, 4H, *J* 7.4 Hz), 2.05 (qv, 2H, *J* 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, 50 °C) δ_C 170.2, 138.4, 136.4, 135.0, 132.7, 131.7, 130.4, 128.9, 126.4, 61.5, 37.0, 36.5, 33.4, 22.7. HRMS(ES+): calcd for $C_{14}H_{15}Cl_2NO_2$ + Na 322.0378, found: 322.0374.

Compound **17**. Purification by flash chromatography (EtOAc/pentane, 2:5 → 2:3) afforded **17** (39 mg) in 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.28 (bs, 1H), 7.41 (d, 1 H, *J* 1.6 Hz), 7.38 (d, 1H, *J* 8.4 Hz), 7.18 (bd, 1H, *J* 8.4 Hz), 3.66 (s, 3H), 2.78 (t, 4H, *J* 7.6 Hz), 2.01 (qv, 2H, *J* 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.5, 144.0, 135.8, 132.7, 132.5, 132.3, 130.5, 129.4, 127.0, 64.2, 38.2, 36.1, 22.2. HRMS(ES+): calcd for $C_{13}H_{13}Cl_2NO_2$ + Na 308.0221, found: 308.0218.

Compound **18**. Purification by flash chromatography (EtOAc/pentane 2:7) afforded **18** (59 mg) in 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.36 (d, 1H, *J* 2.0 Hz), 7.24 (d, 1H, *J* 8.4 Hz), 7.19 (dd, 1H, *J* 1.2 Hz, *J* 5.2 Hz), 7.09 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz), 6.90 (dd, 1H, *J* 3.4 Hz, *J* 5.2 Hz), 6.86 (dd, 1H, *J* 1.2 Hz, *J* 3.4 Hz), 5.77 (bs, 1H), 4.54 (d, 2H, *J* 5.6 Hz), 2.84–2.74 (m, 4H), 1.99 (qv, 2H, *J* 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.7, 142.7, 140.2, 136.2, 135.4, 132.6, 131.9, 130.3, 129.4, 127.2, 126.9, 126.3, 125.4, 38.6, 38.1, 36.1, 22.1. HRMS(ES+): calcd for $C_{17}H_{15}Cl_2NOS$ + Na 374.0149, found: 374.0157.

Compound **19**. Purification by flash chromatography (EtOAc/pentane 2:7) afforded **19** (60 mg) in 94% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.30 (d, 1H, *J* 2.0 Hz), 7.22–7.19 (m, 2H), 7.02 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz), 6.22 (dd, 1H, *J* 1.6 Hz, *J* 3.2 Hz), 6.07 (d, 1H, *J* 3.2 Hz), 5.59 (bs, 1H), 4.32 (d, 2H, *J* 5.6 Hz), 2.78–2.68 (m, 4H), 1.93 (qv, 2H, *J*7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.8, 150.7, 142.6, 136.2, 135.4, 132.6, 131.9, 142.3, 130.3, 129.4, 127.1, 110.5, 107.7, 38.6, 36.2, 36.0, 22.1. HRMS(ES+): calcd for $C_{17}H_{15}Cl_2NO_2$ + Na 358.0377, found: 358.0383.

Compound **20**. Purification by flash chromatography (EtOAc/pentane 1:60) afforded **20** (32 mg) in 54% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.42 (d, 1H, *J* 2.0 Hz), 7.38 (d, 1H, *J* 8.0 Hz), 7.16 (dd, 1H, *J* 2.0 Hz, *J* 8.0 Hz), 4.79–4.75 (m, 1H), 2.84–2.78 (m, 4H), 1.98 (qv, 2H, *J* 7.6 Hz), 1.75 (m, 2H), 1.56 (m, 2H), 1.50–1.46 (m, 1H), 1.36–1.19 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.3, 149.7, 137.4, 131.9, 131.7, 131.6, 130.0, 129.8, 127.3, 72.7, 40.2, 35.3, 31.5, 25.5, 23.6, 21.9. HRMS(ES+): calcd for $C_{18}H_{20}Cl_2O_2$ + Na 361.0738, found: 361.0734.

Compound **21**. Purification by flash chromatography (EtOAc/pentane 1:30) afforded **21** (31 mg) in 55% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.41 (d, 1H, *J* 2.0 Hz), 7.38 (d, 1H, *J* 8.4 Hz), 7.15 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz), 4.98 (qv, 1H, *J* 6.2 Hz), 2.82–2.78 (m, 4H), 1.98 (qv, 2H, *J* 7.6 Hz), 1.13 (d, 6H, *J* 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.4, 149.6, 137.3, 131.8, 131.7, 131.6, 130.1, 129.7, 127.4, 67.8, 40.1, 35.3, 21.9, 21.8. HRMS(ES+): calcd for $C_{15}H_{16}Cl_2O_2$ + Na 321.0425, found: 321.0419.

Compound **22**. Purification by flash chromatography (EtOAc/pentane 1:2) afforded **22** (84 mg) in 54% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.43 (d, 1H, *J* 2.0 Hz), 7.40 (d, 1H, *J* 8.4 Hz), 7.17 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz), 4.18–4.16 (m, 2H), 3.72–3.69 (m, 2H), 2.86–2.80 (m, 4H), 2.00 (qv, 2H, *J* 7.6 Hz), 1.68 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.8, 151.7, 137.2, 132.0, 131.9, 130.6, 129.9, 129.8, 127.3, 66.0, 61.1, 40.4, 35.1, 21.9. HRMS(ES+): calcd for $C_{14}H_{14}Cl_2O_3$ + Na 323.0218, found: 323.0220.

Compound **23**. Purification by flash chromatography (ether/pentane 1:4) afforded **23** (36 mg) in 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.43 (d, 1H, *J* 1.6 Hz), 7.40 (d, 1H, *J* 8.0 Hz), 7.18 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz), 4.56 (t, 1H, *J* 4.0 Hz), 4.44 (t, 1H, *J* 4.0 Hz), 4.33 (t, 1H, *J* 4.0 Hz), 4.26 (t, 1H, *J* 4.0 Hz), 2.88–2.81 (m, 4H), 2.00 (qv, 2H, *J*7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.2, 152.2, 137.0, 132.0, 130.3, 129.7, 127.5, 81.2 (d, J_{CF} 170.3 Hz), 63.2 (d, J_{CF} 19.8 Hz), 40.4, 35.1, 21.9. ^{19}F NMR (377 MHz, $CDCl_3$) δ_F 5.98 (t, *J* 29.6 Hz). HRMS(ES+): calcd for $C_{14}H_{13}Cl_2FO_2$ + -Na 325.0174, found: 325.0204.

Compound **24**. Purification by flash chromatography (EtOAc/pentane 1:60) afforded **24** (60 mg) in 98% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.41 (d, 1H, *J* 2.0 Hz), 7.41 (d, 1H, *J* 8.4 Hz), 7.16 (dd, 1H, *J* 2.0 Hz, *J* 8.0 Hz), 4.42 (q, 2H, *J* 8.4 Hz), 2.89–2.83 (m, 4H), 2.03 (qv, 2H, *J* 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 163.4, 154.8,

136.6, 132.3, 132.2, 128.9, 130.0, 129.7, 127.3, 123.0 (d, J_{CF} 275.6 Hz), 60.2 (d, J_{CF} 36.4 Hz), 40.8, 34.9, 21.9; ^{19}F NMR (377 MHz, $CDCl_3$) δ_F −74.1; HRMS(ES+): calcd for $C_{14}H_{11}Cl_2F_3O_2$ + Na 360.9986, found: 360.9970.

Compound **25**. Purification by flash chromatography (EtOAc/pentane 1:60) afforded **25** (58 mg) in 96% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.43 (d, 1H, *J* 1.6 Hz), 7.32 (d, 1H, *J* 8.4 Hz), 7.26 (t, 2H, *J* 7.8 Hz), 7.18 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz), 7.11 (t, 1H, *J* 7.4 Hz), 6.94 (d, 2H, *J* 8.0 Hz), 2.93–2.88 (m, 2H), 2.84–2.79 (m, 2H), 1.99 (qv, 2H, *J* 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 163.8, 153.4, 150.6, 136.8, 132.2, 132.1, 130.1, 129.9, 129.5, 127.5, 125.8, 121.5, 40.5, 35.3, 22.0. HRMS(ES+): calcd for $C_{18}H_{14}Cl_2O_2$ + Na 355.0269, found: 355.0271.

Compound **26**. Purification by flash chromatography (EtOAc/pentane, 1:60) afforded **26** (62 mg) in 99% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.45 (d, 1H, *J* 2.0 Hz), 7.32 (d, 1H, *J* 8.0 Hz), 7.28 (dd, 1H, *J* 2.0 Hz, *J* 8.0 Hz), 7.21 (bd, 1H, *J* 7.6 Hz, 7.18 (bd, 1H, *J* 7.6 Hz), 7.12 (t, 1H, *J* 7.2 Hz), 6.98 (d, 1H, *J* 8.0 Hz), 3.05–3.00 (m, 2H), 2.94–2.89 (m, 2H), 2.12 (s, 3H), 2.09 (qv, 2H, *J* 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 163.6, 153.5, 149.2, 136.8, 132.2, 132.1, 130.1, 130.0, 131.2, 130.0, 129.9, 127.5, 126.9, 40.6, 35.4, 21.9, 16.4. HRMS(ES+): calcd for $C_{19}H_{16}Cl_2O_2$ + Na 369.0425, found: 369.0424.

Compound **27**. Purification by flash chromatography (EtOAc/pentane 1:60) afforded **27** (62 mg) in 99% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, 1H, *J* 1.6 Hz), 7.40 (d, 1H, *J* 8.0 Hz), 7.26 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz), 7.21 (d, 1H, *J* 7.6 Hz), 7.01 (d, 1H, *J* 7.6 Hz), 6.84–6.81 (m, 2H), 3.01–2.96 (m, 2H), 2.92–2.87 (m, 2H), 2.33 (s, 3H), 2.07 (qv, 2H, *J* 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 164.1, 153.5, 150.6, 139.8, 136.9, 132.3, 132.2, 130.3, 130.1, 129.3, 127.6, 126.8, 122.3, 118.6, 40.6, 35.4, 22.1, 21.5. HRMS(ES+): calcd for $C_{19}H_{16}Cl_2O_2$ + Na 369.0425, found: 369.0423.

Compound **28**. Purification by flash chromatography (EtOAc/pentane 1:30) afforded **28** (46 mg) in 81% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.37 (d, 1H, *J* 2.0 Hz), 7.31–7.29 (m, 2H), 7.09 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz), 7.00 (bd, 1H, *J* 3.6 Hz), 6.96 (dd, 1H, *J* 3.6 Hz, *J* 5.2 Hz), 5.23 (s, 2H), 2.87–2.78 (m, 4H), 1.99 (qv, 2H, *J* 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.2, 151.6, 137.7, 137.0, 132.0, 131.8, 129.8, 129.7, 128.2, 127.4, 126.9, 126.8, 60.4, 40.3, 35.1, 21.9. HRMS(ES+): calcd for $C_{17}H_{14}Cl_2O_2S$ + Na 374.9989, found: 374.9992.

Compound **29**. Purification by flash chromatography (EtOAc/pentane 1:60) afforded **29** (33 mg) in 54% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.38 (dd, 1H, *J* 0.8 Hz, *J* 2.0 Hz), 7.36 (d, 1H, *J* 2.0 Hz), 7.30 (d, 1H, *J* 8.4 Hz), 7.08 (dd, 1H, *J* 2.0 Hz, *J* 8.4 Hz), 6.33 (dd, 1H, *J* 1.8 Hz, *J* 3.4 Hz), 6.31 (d, 1H, *J* 3.2 Hz), 5.03 (s, 2H), 2.85–2.77 (m, 4H), 1.98 (qv, 2H, *J* 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.2, 151.8, 149.4, 137.0, 132.0, 131.8, 130.6, 143.3, 129.8, 129.7, 127.4, 110.7, 110.6, 57.9, 40.2, 35.1, 22.0. HRMS(ES+): calcd for $C_{17}H_{14}Cl_2O_3$ + Na 359.0218, found: 359.0217.

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11. Cell lines stably expressing hDAT were established by transfecting COS-1 cells with hSERT, hDAT and hNET inserted in the pIRES vector (BD Biosciences Clontech) also carrying a Blasticidin resistance gene. Cells were cultured in DMEM (BioWhitaker) supplemented with 10% FCS (Gibco Life Technologies), 1% penicillin/streptomycin (BioWhitaker) and 10 μg/mL of Blasticidin (Cayla) selection of transfected cells. After 14 days of selection, blasticidin was adjusted to 2 μg/mL in the culture medium and the cells were subcultured under this selection regime and grown at 37 °C, 5% CO_2 and 95% humidity. Membrane preparations for the binding assay were produced by scraping the stably transfected cells from cell culture dishes (Nunc), pelleting the cells in ice-cold PBSCM by centrifugation and homogenizing the cells in ice-cold harvest buffer I (150 mM NaCl, 50 mM Tris, 20 mM EDTA) using an Ultra-Turrax (Janke and Kunkel AG) for 60 s. The membrane was pelleted by centrifugation at 12,000*g* for 10 min at 4 °C and washed in ice-cold Harvest buffer I. The membranes were pelleted again and, finally, resuspended in PBSCM using Ultraturrax briefly. Membrane preparations were aliquoted into 2 mL portions and stored at −80 °C until use. The concentration of total protein in the membrane preparation was determined with the MicroBCA kit (Pierce). A concentration of 5 μg/well of membrane preparation was used with the chosen concentration of drug of interest in combination with 0.1–0.25 nM ^{125}I-RTI-55. Membrane and ligands were incubated for 1 h at 20 °C using a Filtermate cell harvester (Packard), membranes were captured on GF/B 96-well filterplates (Packard) presoaked with 0.5% polyethylene-

imine (Merck) and washed thrice with ice-cold water. The filter in each well was dissolved in 40 μL Microscint 20 and scintillation counts were determined with a Packard Topcounter. Precise concentration of radioligand was quantified by liquid scintillation counting on a Packard Tri-Carb. For data analysis counts from the Packard Topcounter were fitted to a sigmoidal dose–response curve using the built-in nonlinear regression tool in the Graphpad Prism 3 software. From at least three independent experiments, the resulting IC_{50} values were transformed into K_i values using the equation described in Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.