Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 487–492

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of a Potent Wide-Spectrum Serotonin-, Norepinephrine-, Dopamine-Reuptake Inhibitor (SNDRI) and a Species-Selective Dopamine-Reuptake Inhibitor Based on the Gamma-Amino Alcohol Functional Group

Paul R. Carlier,*,[a] Michael M-C. Lo,[a] Priscilla C-K. Lo,[a] Elliott Richelson,*,[b] Masahiko Tatsumi,[b] Ian J. Reynolds,[c] Terre A. Sharma[c]

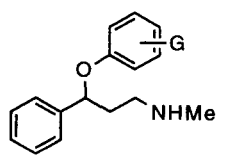
[a]*Department of Chemistry, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong*
[b]*Neuropsychopharmacology Research, Mayo Clinic Jacksonville Jacksonville, FL 32224, U. S. A.*
[c]*Department of Pharmacology, University of Pittsburgh Pittsburgh, PA 15621 U. S. A.*

Received 12 November 1997; accepted 22 January 1998

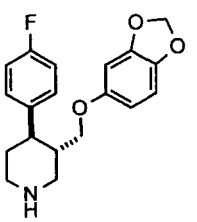
Abstract: A series of gamma-amino alcohols were synthesized and screened for reuptake inhibition and noncompetitive NMDA antagonism. Compound (±)-**3f** simultaneously and potently inhibits reuptake of 5-HT, NE, and DA, representing a potential wide-spectrum reuptake inhibitor antidepressant. In addition, comparative rat and human studies uncovered a species-selective DA reuptake inhibitor (±)-**2e**, K_D(hDAT)/K_D(rDAT) = 97.

The past 20 years have seen the development of selective serotonin (5-HT)-reuptake inhibitor (SSRI) antidepressants (e.g. fluoxetine, paroxetine, and sertaline) and selective norepinephrine (NE)-reuptake inhibitor antidepressants (e.g. tomoxetine and viloxazine) (Scheme 1). In addition, drugs which inhibit rat synaptosomal 5-HT- and NE-reuptake with similar potency (SNRI antidepressants, e.g. venlafaxine, nefazodone) have also been developed.[1] Examination of the structures of all these reuptake inhibiting antidepressants indicates that a number of them possess the gamma-amino ether or gamma-amino alcohol functional group (Scheme 1).

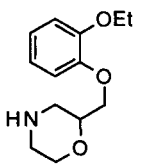
Scheme 1



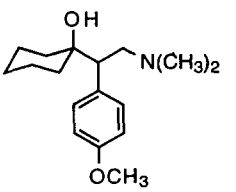
G = 4-CF_3: (±)-Fluoxetine
G = 2-Me: (±)-Tomoxetine



(-)-Paroxetine



(±)-Viloxazine



(±)-Venlafaxine

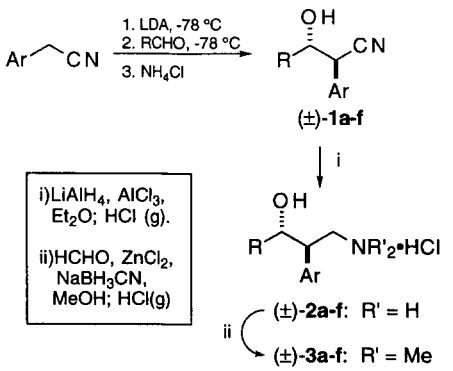
This realization led us to synthesize a series of structurally novel gamma-amino alcohols, and assay their ability to bind to the human transporters for 5-HT, NE, and dopamine (DA), and to inhibit rat synaptosomal uptake of these neurotransmitters. Key results described herein include the identification of a compound that potently and simultaneously inhibits binding at the human transporters for 5-HT, NE, *and* DA, and a compound that displays species-selectivity for inhibition of binding to the DA transporter.

The gamma-amino alcohol/ether unit contained in venlafaxine,[2] fluoxetine,[3] and tomoxetine[3] has been prepared by a sequence of nitrile aldol reaction and nitrile reduction. The single stereogenic center in each of these drugs is formed during the aldol step, by coupling one prochiral and one achiral component. Compounds (±)-**2a**-

0960-894X/98/$19.00
PII: S0960-894X(98)00062-6

f differ from these three antidepressants by possessing two stereogenic centers, one at C-2, and one at C-3. Their synthesis therefore requires coupling of two prochiral components, and depends on the *anti*-selective nitrile aldol methodology we have developed for preparation of beta-hydroxy nitriles (±)-**1a-f** (Table 1).[4,5]

Table 1. Synthesis of gamma-amino alcohols (±)-**2** and (±)-**3**.



aldol (±)-**1**	R	Ar	(±)-**2** yield (%)[a]	(±)-**3** yield (%)
a	Ph	Ph	84 (69)	96
b	*c*-C_6H_{11}	4-MeO-C_6H_4	93 (75)	96
c	Mes[b]	Ph	86	96
d	*t*-Bu	Ph	77 (75)	87
e	*t*-Bu	2-Naphth	91 (63)	98
f	Ph	2-Naphth	65	98

[a]Previously published yield in parenthesis.
[b]Mes = mesityl (1,3,5-$(Me)_3C_6H_2$-)

Reduction of (±)-**1a-f** using the combination of $LiAlH_4$ and $AlCl_3$ supresses retro-aldol reaction and provides gamma-amino alcohols (±)-**2a-f** in moderate to excellent yield (Table 1). Attempted dimethylation of 1°-amine (±)-**2b** according to the standard Eschweiler-Clarke protocol gave the corresponding tetrahydro-1,3-oxazine despite prolonged (16 hour) reflux. Successful transformation of 1° amines (±)-**2a-f** to the desired *N,N*-dimethyl derivatives (±)-**3a-f** was accomplished using a modified Eschweiler-Clarke protocol[6] (Table 1). The reaction proceeds under very mild conditions (6 hours, room temperature) and in excellent yields, giving products that require no further purification. All compounds were fully characterized by NMR (^1H, ^{13}C) and elemental analysis or high resolution mass spectroscopy.

To evaluate the potential antidepressant properties of these compounds, binding to the molecularly cloned human transporters for 5-HT, NET, and DA (hSERT, hNET, and hDAT respectively) was assayed according to the procedure of Richelson.[7] Reuptake inhibition performance of antidepressants has historically been evaluated on the basis of rat synaptosomal studies, and a recent study of 33 antidepressants revealed in general excellent correlation between rat synaptosomal pK_I and human transporter pK_D.[7] However, a few important exceptions relating to reuptake inhibition selectivity were uncovered. Most relevant to the current study is the finding that the SNRI profile displayed by venlafaxine and nefazodone in rat is not replicated in human (cf. entry 18 in Tables 2 and 3, entry 14 in Tables 2 and 3). Therefore, data at human transporters are greatly preferred.

Equilibrium dissociation constants K_D for binding of (±)-**2** and (±)-**3** to hSERT, hNET, and hDAT are given in Table 2.[8] Hill coefficients (n_H) for all of the compounds at each binding site were close to unity, suggesting that the binding of the drugs in the radioligand binding assay obeyed the law of mass action. As can be seen in Table 2, compounds (±)-**2e-f** and (±)-**3e** are noteworthy in that they bind to hSERT and hNET with K_D values in the nanomolar to 10 nanomolar range (entries 5,6,11,12). Thus unlike venlafaxine and nefazodone, these compounds are true SNRI antidepressant candidates. Compound (±)-**3f** is particularly remarkable, having affinities for all three transporters (hSERT, hNET, hDAT) in the nanomolar or ten nanomolar range (entry 12). Nefazodone has similar affinities at hSERT, hNET, and hDAT, but has low potency (entry 14). Furthermore, its blockade of $5\text{-}HT_{1A}$ and $5\text{-}HT_2$ receptors[9] reverses some of the desired effects of blocking reuptake of 5-HT.

Table 2. Equilibrium Dissociation Constants (K_D) for hSERT, hNET, and hDAT[a]

entry	compound	hSERT[b] K_D (nM)	hNET[c] K_D (nM)	hDAT[d] K_D(nM)
1	(±)-**2a**	3,050 ± 280	12,500 ± 260	24,000 ± 900
2	(±)-**2b**	76 ± 3	1,540 ± 90	4,310 ± 60
3	(±)-**2c**	118 ±3	22,500 ± 1,200	340 ± 30
4	(±)-**2d**	1,100 ± 40	47,900 ± 2,700	7,260 ± 40
5	(±)-**2e**	**6.1 ± 0.3**	**55 ± 1**	27,000 ± 2,000
6	(±)-**2f**	**6.2 ± 0.3**	**21 ± 1**	140 ± 20
7	(±)-**3a**	48 ± 5	2,250 ± 110	12,000 ± 1,800
8	(±)-**3b**	30 ± 0.9	1,800 ± 100	3,500 ± 200
9	(±)-**3c**	8.5 ± 0.2	35,800 ± 2,900	42,000 ± 4,000
10	(±)-**3d**	40 ± 2	3,430 ± 90	21,400 ± 2,000
11	(±)-**3e**	**1.2 ± 0.9**	**29.9 ± 0.4**	340 ± 10
12	(±)-**3f**	**5.59 ± 0.02**	**44 ± 2**	**70 ± 8**
13	(±)-fluoxetine[e]	0.81 ± 0.02	240 ± 10	3,600 ± 100
14	nefazodone[e]	200 ± 20	360 ± 40	360 ± 10
15	(-)-paroxetine[e]	0.13 ± 0.01	40 ± 2	490 ± 20
16	(1*S*)-sertraline[e]	0.29 ± 0.01	420 ± 20	25 ± 2
17	(±)-tomoxetine[e]	8.9 ± 0.3	2.03 ± 0.06	1,080 ± 50
18	(±)-venlafaxine[e]	8.9 ± 0.3	1,060 ± 40	9,300 ± 50
19	(±)-viloxazine[e]	17,300 ± 500	155 ± 8	> 100,000

[a]Uncertainty expressed as standard error of the mean.
[b]Competion binding between [^{3}H]imipramine and compounds at the hSERT.
[c]Competition binding between [^{3}H]nisoxetine and compounds at the hNET.
[d]Competition binding between [^{3}H]WIN35428 and compounds at the hDAT.
[e]Values for HCl salts reported in reference 7.

At present it is estimated that up to 30% of clinically diagnosed cases of depression are resistant to all forms of drug therapy.[10] To achieve an effective therapy for such patients, it is logical to look for drugs that possess reuptake inhibition profiles different from those currently available on the market. The exact role of dopamine in depressive illness is far from clear, but intervention in the DA system may hold promise for treatment of a subset of major depression,[11] such as patients with psychomotor retardation.[12] Pinder and Wieringa have commented that an agent which simultaneously inhibits reuptake of 5-HT, NE, and DA could be the ultimate reuptake-inhibiting antidepressant drug.[1] This type of selectivity profile (which we term "SNDRI") has been reported in *rat* for 3-aryl-1-indanamine,[13] 3-aryltropane,[14] and *N*-norcocaine[15] derivatives. The present study demonstrates an SNDRI profile for (±)-**3f** in *human*, although it remains to be seen whether the observed potencies derive from a single enantiomer, or are due to the combined effects of both enantiomers.

In view of the fact that noncompetitive antagonism of the *N*-methyl-D-aspartate (NMDA) receptor has been reported for another drug possessing the gamma-amino alcohol functional group (2-methyl-3,3-diphenyl-3-propanolamine, 2-MDP),[16] binding of (±)-**2** and (±)-**3** to the PCP site of NMDA receptor was studied according to Reynolds' protocol.[17] High affinity at this site would be indicative of potential neuroprotective properties, but would be undesirable in an antidepressant. IC_{50} values for noncompetitive inhibition of the NMDA receptor by (±)-**2** and (±)-**3** are given in Table 3. In general very low PCP site affinities were found, and affinities for tertiary amines (±)-**3a-f** were lower than the corresponding primary amines (±)-**2a-f** in every case.

Table 3. Noncompetitive Inhibition of the NMDA Receptor in Rat Brain Membranes and Inhibition of Reuptake (5-HT, NE, and DA) in Rat Brain Synaptosomes.[a]

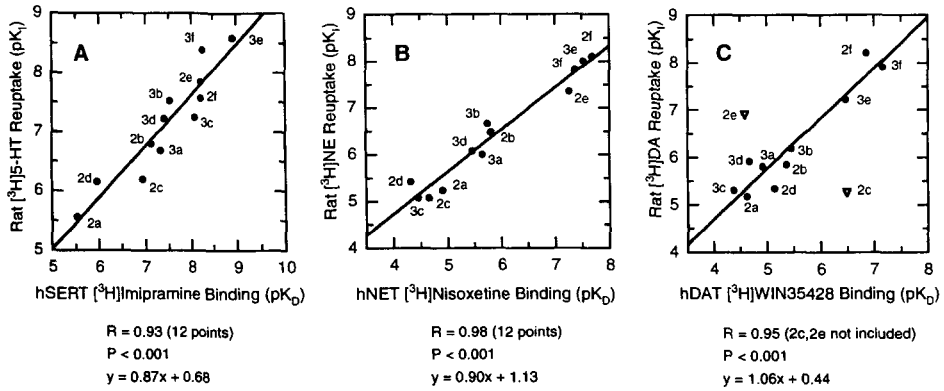
entry	compound	NMDA noncompetitive inhibition IC_{50} (nM)[b]	5-HT-reuptake K_I (nM)[c]	NE-reuptake K_I (nM)[d]	DA-reuptake K_I (nM)[e]
1	(±)-**2a**	5,600 ± 200	2,730 ± 250	5,780 ± 750	6,600 ± 1,000
2	(±)-**2b**	84,000 ± 5,000	164 ± 8	330 ± 25	1,460 ± 140
3	(±)-**2c**	63,000 ± 2,000	643 ± 78	8,600 ± 1,400	5,310 ± 240
4	(±)-**2d**	14,000 ± 200	700 ± 120	3,800 ± 350	4,570 ± 270
5	(±)-**2e**	3,000 ± 600	**14 ± 2**	**44 ± 8**	**120 ± 10**
6	(±)-**2f**	65,000 ± 7,000	**27 ± 3**	**7.7 ± 0.2**	**6.2 ± 0.2**
7	(±)-**3a**	47,000 ± 1,500	210 ± 7	990 ± 140	1,550 ± 300
8	(±)-**3b**	>200,000[f]	30 ± 5	220 ± 10	640 ± 60
9	(±)-**3c**	>200,000[f]	57 ± 5	8,500 ± 1,300	4,880 ± 250
10	(±)-**3d**	121,000 ± 27,000	60 ± 13	830 ± 90	1,200 ± 600
11	(±)-**3e**	21,000 ± 3,000	**2.6 ±0.3**	**10 ± 1**	**60 ± 10**
12	(±)-**3f**	143,000 ± 16,000	**4.1 ± 0.6**	**15 ± 2**	**12 ± 1**
13	(±)-fluoxetine[g]	nd	14 ± 3	143 ± 6	3,050 ± 70
14	nefazodone[g]	nd	137 ±4	570 ± 5	2,380 ± 80
15	(-)-paroxetine[g]	nd	0.73 ± 0.04	33 ± 2	1,700 ± 300
16	(1*S*)-sertraline[g]	nd	3.4 ± 0.4	220 ± 40	260 ± 4
17	(±)-tomoxetine[g]	nd	43 ± 2	0.7 ± 0.1	1,400 ± 200
18	(±)-venlafaxine[g]	nd	37 ± 2	138 ± 8	360 ± 53
19	(±)-viloxazine[h]	nd	16,500 ± 600	170 ± 20	48,000 ± 4,000

[a]Uncertainty expressed as standard error of the mean.
[b]Displacement of [^{3}H]MK-801 in rat brain membranes.
[c]Inhibition of [^{3}H]5-HT reuptake into rat frontal cortex synaptosomes
[d]Inhibition of [^{3}H]NE reuptake into rat hippocampal synaptosomes
[e]Inhibition of [^{3}H]DA reuptake into rat striatal synaptomes.
[f]Greater than 75% specific [^{3}H]MK-801 binding retained at the highest drug concentration tested (100 μM).
[g]Values for HCl salts reported in reference 18.
[h]Values for HCl salt reported in Richelson, E.; Pfenning, M. *Eur. J. Pharm.* **1984**, *104*, 277-286.

Thus even in the case of the most potent ligand tested ((±)-**2e**) the observed PCP site affinity (3 μM, entry 5) is unlikely to have any clinical relevance at dosages effective for inhibiting neurotransmitter reuptake.

Finally, to provide additional points of comparison with known antidepressants, and as a check on the human transporter studies, inhibition of reuptake of [^{3}H]5-HT, [^{3}H]NE, and [^{3}H]DA in rat synaptosomes by (±)-**2a-f** and (±)-**3a-f** was studied according to the published procedure of Bolden-Watson and Richelson (Table 3).[18] Visual comparison of rat K_I and human K_D data for 5-HT indicates good agreement; a plot of rat pK_I values versus human pK_D values further emphasizes this point (12 points, correlation = 0.93, $P < 0.001$, Figure 1A). The correlation between rat and human data for NE is even better (12 points, correlation = 0.98, $P < 0.001$, Figure 1B). However inspection of the DA data shows that acceptable correlation between rat and human can only be achieved if (±)-**2c** and (±)-**2e** are excluded from the analysis (10 points, correlation = 0.95, $P < 0.001$, Figure 1C). These outliers bear promise for significant species-selectivity, and indeed (±)-**2e** was found to exhibit $K_D/K_I = 225$. The species-selectivity of (±)-**2e** was confirmed by stably expressing the rat DAT and then

Figure 1. Comparison of Rat and Human Assay Data for (±)-**2** and (±)-**3**.



A)pK_I for 5-HT reuptake inhibition in rat frontal cortex synaptosomes vs pK_D for hSERT. B)pK_I for NE reuptake inhibition in rat hippocampal synaptosomes vs pK_D for hNET. C)pK_I for DA reuptake inhibition in rat striatal synaptosomes vs pK_D for hDAT.

determining K_D, under the identical conditions used for the human DAT. On the rat transporter (±)-**2e** exhibited K_D = 278 nM, therefore giving K_D(hDAT)/K_D(rDAT) = 97.[19] To our knowledge this degree of species-selectivity for the DAT is unprecedented.[20] (±)-**3e** or compounds like it may therefore prove useful for characterizing the structure and function of the DAT.

With pharmacology data for only 12 compounds it is not possible to state definitively which structural features elicit a given activity. In general the 2,3-disubstituted-3-amino alcohol functional group embodied by **2** and **3** conveys greater affinity for SERT than NET or DAT. Reasonable potency for 5-HT- and NE-reuptake inhibition was anticipated and motivated synthesis of these compounds. However, the significant DA-reuptake inhibition potencies exhibited by (±)-**2f** (hDAT K_D = 140 nM, rat DA-reuptake K_I = 6.2 nM) and (±)-**3f** (hDAT K_D = 70 nM, rat DA-reuptake K_I = 12 nM) were completely unexpected. To place these results in perspective, it should be noted that in *rat* the DA-reuptake inhibition potencies of (±)-**2f** and (±)-**3f** are comparable to those of drugs in the 3-phenyltropane (e. g. WIN35428 and β-CIT)[14] and GBR[21] series.

Comparison of compounds (±)-**2a-f** with (±)-**3a-f** reveals that *N,N*-dimethylation consistently increases potency at both rSERT and hSERT (Figure 1A). However, no correlation of potency with degree of *N*-methylation is seen for NET or DAT, either in rat or in human (Figures 1B & 1C). Finally, within this limited set of compounds it appears that placement of a 2-naphthyl group at C-2 (as in (±)-**2e-f**, (±)-**3e-f**) can increase affinity at all three transporters, both in rat and in human. Davies and coworkers have previously noted that incorporation of a 2-naphthyl ring into 3-aryltropane derivatives significantly increases the affinities for the 5-HT and DA transporters in rat.[22]

Further perturbation of the structures of these reuptake inhibitors (and preparation of pure enantiomers) is in progress, to optimize individual affinities for the human transporters, to achieve greater balance of these affinities in the hope of developing additional examples of SNDRIs, and to optimize species-selectivity for the dopamine transporter. These results will be reported in due course.

Acknowledgement. P. R. C. thanks the Research Grants Council (Hong Kong, HKUST203/93E and HKUST588/95P), E. R. thanks the Mayo Foundation for Medical Education and Research, and I. J. R. thanks the NIH (DA07490) for partial financial support of this work. We thank Dr. Yuan-Ping Pang (Mayo) for suggesting an NMDA PCP-site assay for (±)-**2** and (±)-**3**.

References and Notes

1. Pinder, R. M.; Wieringa, J. H. *Med. Res. Rev.* **1993**, *13*, 259-325.
2. Yardley, J. P.; Husbands, G. E. M.; Stack, G.; Butch, J.; Bicksler, J.; Moyer, J. A.; Muth, E. A.; Andree, T.; Fletcher, H.; James, M. N. G.; Sielecki, A. R. *J. Med. Chem.* **1990**, *33*, 2899-2905.
3. Koenig, T. M.; Mitchell, D. *Tetrahedron Lett.* **1994**, *35*, 1339-1342.
4. Carlier, P. R.; Lo, K. M.; Lo, M. M.-C.; Lo, P. C.-K.; Lo, C. W.-S. *J. Org. Chem.* **1997**, *62*, 6316-6321.
5. Carlier, P. R.; Lo, K.-M.; Lo, M. M.-C.; Williams, I. D. *J. Org. Chem.* **1995**, *60*, 7511-7517.
6. Kim, S.; Oh, C. H.; Ko, J. S.; Ahn, K. H.; Kim, Y. J. *J. Org. Chem.* **1985**, *50*, 1927.
7. Tatsumi, M.; Groshan, K.; Blakely, R. D.; Richelson, E. *Eur. J. Pharmacol.* **1997**, *340*, 249-258.
8. Data analysis was performed using the LIGAND program (Munson P. J.; Rodbard, D.. *Analyt. Biochem.* **1980**, *107*, 220-239) to provide values for the equilibrium dissociation constants (K_D). The program was modified to calculate the Hill Coefficient (n_H). Data are presented as geometric mean ± S.E.M. (De Lean A.; Hancock, A. A.; Lefkowitz, R. J. *Mol. Pharmacol.* **1982**, *21*, 5-16 and Fleming, W. W.; Westfall, D. P.; De La Lande, I. S.; Jellett, L. B. *J. Pharmacol. Exp. Ther.***1972**, *181*, 339-345) of at least 3 independent experiments. One-component models and two-component models were compared using the root mean square error of each fit and the F test.
9. Cusack, B.; Nelson, A.; Richelson, E. *Psychopharm.* **1994**, *114*, 559-565.
10. Broekkamp, C. L. E.; Leysen, D.; Peeters, B. W. M. M.; Pinder, R. M. *J. Med. Chem.* **1995**, *38*, 4615-4633.
11. Barchas, J. D.; Hamblin, M. W.; Malenka, R. C. In *Basic Neurochemistry*; 5th ed.; G. J. Siegel, B. W. Agranoff, R. W. Albers and P. B. Molinoff, Eds.; Raven Press: New York, 1994; pp 984-985.
12. Buckett, W. R. In *Experimental Approaches to Anxiety and Depression*; J. M. Elliott, D. J. Heal and C. A. Marsden, Eds.; John Wiley and Sons Ltd.: New York, 1992; pp 245-264.
13. Bøgesø, K. P.; Christensen, A. V.; Hyttel, J.; Liljefors, T. *J. Med. Chem.* **1985**, *28*, 1817-1828.
14. Carroll, F. I.; Kotian, P.; Dehgani, A.; Gray, J. L.; Kuzemo, M. A.; Parham, K. A.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1995**, *38*, 379-388.
15. Boja, J. W.; Kuhar, M. J.; Kopajtic, T.; Yang, E.; Abraham, P.; Lewin, A. H.; Caroll, F. I. *J. Med. Chem.* **1994**, *37*, 1220-1223.
16. Blake, J. C.; Davies, S. N.; Church, J.; Martin, D.; Lodge, D. *Pharmacol. Biochem. Behav.* **1986**, *24*, 23-25.
17. Reynolds, I. J. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 1001-1007.
18. Bolden-Watson, C.; Richelson, E. *Life Sci.* **1993**, *52*, 1023-1029.
19. The rDAT K_D value given is the mean of two independent experiments (276 nM, 281 nM, Boules, M.; Richelson, E., unpublished data).
20. Giros et al. has reported the [^{3}H]DA reuptake inhibition potency of of 24 drugs towards hDAT and rDAT permanently expressed in Ltk$^-$ cells. No significant species-selectivity was found: potency ratios in no case exceeded a factor of six. (Giros, B.; El Mestikawy, S.; Godinot, N.; Zheng, K.; Han, H.; Yang-Feng, T.; Caron, M. G. *Mol. Pharmacol.* **1992**, *42*, 383-390.)
21. Dutta, A. K.; Coffey, L. L.; Reith, M. E. A. *J. Med. Chem.* **1997**, *40*, 35-43.
22. Davies, H. M. L.; Saikali, E.; Huby, N. J. S.; Gilliatt, V. J.; Matasi, J. J.; Sexton, T.; Childers, S. R. *J. Med. Chem.* **1994**, *37*, 1262-1268.