Tuning the Selectivity of Monoamine Transporter Inhibitors by the Stereochemistry of the Nitrogen Lone Pair

Miles P. Smith,[†] Kenneth M. Johnson,[‡] Mei Zhang,[‡] Judith L. Flippen-Anderson,[§] and Alan P. Kozikowski*,[†]

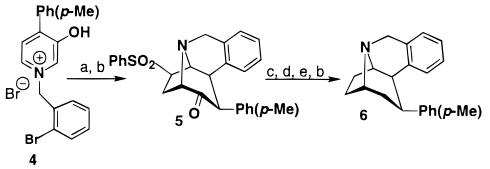
Institute for Cognitive and Computational Sciences
Georgetown University Medical Center
Washington, D.C. 20007-2197
Department of Pharmacology and Toxicology
University of Texas Medical Branch
Galveston, Texas 77555-1031
Laboratory for the Structure of Matter
Naval Research Laboratory, Washington, D.C. 20375

Received April 27, 1998

The specific reuptake of the monoamine neurotransmitters, dopamine (DA), serotonin (5-HT), and norepinephrine (NE), from the synaptic cleft is the primary physiological mechanism for the termination of monoaminergic neurotransmission. Blocking the uptake increases synaptic availability of the neurotransmitters, thereby potentiating the signal.[1] This has been exploited to develop treatments for a large number of neurological disorders. The selective dopamine transporter (DAT) antagonist, benztropine, is used clinically for the treatment of Parkinson's disease,[2] and methylphenidate (Ritalin) is used for attention deficit hyperactivity disorder.[3] Norepinephrine transporter (NET) reuptake inhibitors such as desipramine[4] exhibit effective antidepressant activity; however, they also exhibit a high incidence of adverse side effects.[5] The selective serotonin transporter (5-HTT) antagonists, fluoxetine (Prozac),[6] paroxetine,[7] and related agents[8] are used for the treatment of depression and related psychological disorders with significantly reduced adverse side effects.

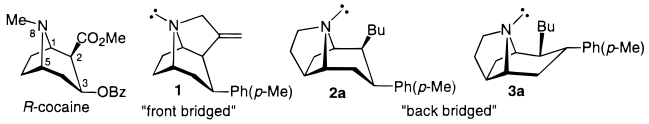
While a number of compounds showing high selectivity have been reported, little is known about factors influencing the selectivity for these agents at the specific monoamine transporter. To explore these factors, we have chosen cocaine[9] as a starting point. Cocaine is known to have multiple effects on the central nervous system, including potent inhibition of all three of the monoamine transporters. A growing body of evidence points to the ability of cocaine to bind to the DAT and to inhibit the reuptake of DA as being responsible for the reinforcing properties of this drug.[10] However, evidence suggests 5-HTT inhibition also plays a modulatory role in cocaine's reinforcing properties.[11] A number of highly potent cocaine analogues that exhibit varying degrees of selectivity for the DAT and information concerning their structure−activity relationships (SAR) have been reported.[12]

Scheme 1[a]



[a] Reagents and conditions: (a) phenyl vinyl sulfone, Et_3N, MeCN; (b) *n*-Bu_3SnH, AIBN, toluene; (c) DIBAL, CH_2Cl_2; (d) 6% Na(Hg), Na_2HPO_4, MeOH; (e) *n*-BuLi, THF then PhOC(S)Cl.

The precise details of the binding interactions between these compounds and the monoamine uptake transporters are still a matter of much discussion.[13] All of these compounds possess common structural features, i.e., a phenyl group and a basic amine. A pharmacophore model for the DAT based on conformational analysis of cocaine and methylphenidate has been proposed.[14] Little is known experimentally about the spatial requirements of the nitrogen lone pair of these cocaine analogues. The directionality of the nitrogen lone pair is, however, likely to be of some consequence to binding affinity.[15] We have recently reported the synthesis of a series of rigid cocaine analogues in which the conformation of the nitrogen lone pair is fixed by means of a tether to either the 3- or 2-carbon bridge of the tropane moiety



(**1**−**3**).[16] We now describe the synthesis of additional bridged cocaine analogues, and report on the spatial preferences of the DAT, 5-HTT, and NET for the nitrogen lone pair.

The tricyclic analogue **6**, bearing a benzo-tether to the 2β-position of the tropane moiety (front-bridged), was readily available by the versatile 1,3-dipolar cycloaddition of 3-oxidopyridinium betaines with electron-deficient olefins (Scheme 1). *N*-Alkylation of 3-hydroxy-4-(*p*-tolyl)pyridine[17] with 2-bromobenzyl bromide in THF afforded pyridinium bromide **4**. Using the tandem cycloaddition/radical cyclization methodology, the tricyclic ketone **5** was obtained, as a single isomer, in 40% yield for the three steps. The structure of **5** was assigned on the basis of NMR analysis. This intermediate was further converted to the desired bridged tropane **6** by a modification of the route previously reported for the synthesis of **1**.

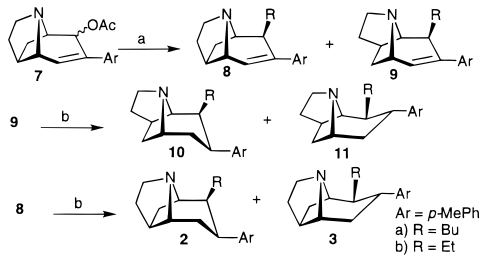
Tricycle **1** was prepared as described previously[16] and resolved by crystallization with (1*S*)-(+)-10-camphorsulfonic acid. The absolute configuration was unequivocally determined by crystallography.[18]

[†] Georgetown University Medical Center.
[‡] University of Texas Medical Branch.
[§] Naval Research Laboratory.

(1) Kitayama, S.; Dohi, T. *Jpn. J. Pharmacol.* **1996**, *72*, 195−208.
(2) Agoston, G. E.; Wu, J. H.; Izenwasser, S.; George, C.; Katz, J. L.; Kline, R. H.; Newman, A. H. *J. Med. Chem.* **1994**, *37*, 4329−4339.
(3) Klein, R. G. *Arch. Gen. Psychiatry* **1995**, *52*, 429−433.
(4) Thomas, D. R.; Nelson, D. R.; Johnson, A. M. *Psychopharmacology* **1987**, *93*, 193−200.
(5) Blackwell, B. *Drugs* **1981**, *21*, 201−219.
(6) Wong, D. T.; Bymaster, F. P.; Engleman, E. A. *Life Sci.* **1995**, *57*, 411−441.
(7) Dechant, K. L.; Clissold, S. P. *Drugs* **1991**, *41*, 225−253.
(8) Möller, H.-J.; Volz, H.-P. *Drugs* **1996**, *52*, 625−638.
(9) Reith, M. E. A.; Meisler, B.; Shershen, H.; Lajtha, A. *Biochem. Pharmacol.* **1986**, *35*, 1123−1129.
(10) Volkow, N. D.; Wang, G. J.; Fowler, J. S.; Logan, J.; Gatley, S. J.; Hitzemann, R.; Chen, A. D.; Dewey, S. L.; Pappas, N. *Nature* **1997**, *6627*, 830−833.
(11) Walsh, S. L.; Cunningham, K. A. *Psychopharmacology* **1997**, *130*, 41−58.
(12) (a) Kozikowski, A. P.; Araldi, G. L.; Boja, J.; Johnson, K. M.; Flippen-Anderson, J.; Saiah, E. *J. Med. Chem.* **1998**, *41*, 1962−1969. (b) Clarke, R. L.; Daum, S. J.; Gambino, A. J.; Aceto, M. D.; Pearl, J.; Levitt, M.; Cumiskey, W. R.; Bogado, E. F. *J. Med. Chem.* **1973**, *16*, 1260−1267.
(13) (a) Carroll, F. I.; Gao, Y.; Rahman, M. A.; Abraham, P.; Parham, K.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1991**, *34*, 2719−2725. (b) Carroll, F. I.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. In *Drug Design for Neuroscience;* Kozikowski, A. P., Ed.; Raven Press: New York, 1993; pp 149−166.
(14) Froimowitz, M. *J. Comput. Chem.* **1993**, *14*, 934−943.
(15) (a) Froimowitz, M.; Deutsch, H. M.; Shi, Q.; Wu, K.-M.; Glaser, R.; Adin, I.; George, C.; Schweri, M. M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1213−1218. (b) Kitayama, S.; Shimada, S.; Xu, H.; Markham, L.; Donovan, D. H.; Uhl, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7782−7785.
(16) Smith, M. P.; George, C.; Kozikowski, A. P. *Tetrahedron Lett.* **1998**, *39*, 197−200.
(17) Araldi, G. L.; Kozikowski, A. P.; Ball, R. B. *J. Org. Chem.* **1997**, *62*, 503−509.
(18) (−)-**1** as the (+)-CSA salt: monoclinic, *P*2(1), *R*(all data) = 0.0857. **11a** as the *p*-TsOH salt: triclinic, $P\bar{1}$, *R*(all data) = 0.0728. Additional crystallographic data have been deposited as Supporting Information.

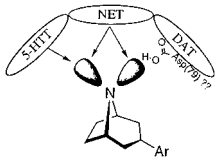
S0002-7863(98)01423-1 CCC: $15.00
Published on Web 08/22/1998

Scheme 2[a]



[a] Reagents and conditions: (a) CuCN, RMgBr, ether; (b) *p*-TsOH, 10% Pd/C, H_2, EtOAc.

Table 1. Activity at Monoamine Transporters, $K_i \pm$ SE (nM)[a]



	[^{3}H]Mazindol binding	[^{3}H]DA uptake	[^{3}H]5-HT uptake	[^{3}H]NE uptake	5-HT / DA
cocaine	375 ± 68	423 ± 147	155 ± 40	83.3 ± 1.5	0.37
(−)-**1**	54.3 ± 10.2	60.3 ± 0.4	1.76 ± 0.23	5.24 ± 0.07	0.029
(+)-**1**	79 ± 19	114 ± 28	1.48 ± 0.07	4.62 ± 0.31	0.013
(±)-**1**	61.7 ± 8.5	60.3 ± 0.4	2.32 ± 0.23	2.69 ± 0.12	0.038
6	6.86 ± 0.43	24.0 ± 1.3	1.77 ± 0.04	1.06 ± 0.03	0.074
3a	4.00 ± 0.07	2.23 ± 0.12	14.0 ± 0.6	2.99 ± 0.17	6.3
2a	17.2 ± 1.13	10.2 ± 1.4	78.9 ± 0.9	15.0 ± 0.4	7.8
3b	3.61 ± 0.43	11.3 ± 1.1	25.7 ± 4.3	4.43 ± 0.01	2.3
11a	149 ± 6	149 ± 2	810 ± 80	51.7 ± 12	5.5
10a	13.7 ± 0.8	14.2 ± 0.1	618 ± 87	3.84 ± 0.35	44

[a] All compounds exhibit spectral data in agreement with the assigned structures (see Supporting Data) and were tested and characterized as the toluenesulfonate salts. Data are the mean ± standard error of three experiments as described in ref 24, except an identical Krebs-Ringer-HEPES buffer was used for all assays (ref 25).

An intramolecular 1,3-dipolar cycloaddition was utilized to afford rigid cocaine analogues with the nitrogen tethered to either the 6- or 7-carbon of the 2-carbon bridge (back-bridged, Scheme 2). Toward this end, the previously reported 7-bridged analogue **9a** was subjected to hydrogenation in the presence of acid to afford a mixture of the chair- and boatlike isomers **10a** and **11a** (1:2 by GC/MS).[19] The structure of **11a** was confirmed by X-ray crystallography.[18] The corresponding cocaine analogue **11b** containing a 2β-ethyl substitutent was also prepared from the previously reported allylic acetate **7**, to allow for a more direct comparison of **1**, with an analogue containing a similar carbon count. Hydrogenation of **8b** afforded the boatlike cocaine analogue **3b**, with only a trace of **2b** (97:3 ratio of **3b** to **2b** by GC/MS). This structure was readily confirmed by comparison of its NMR spectral data with those previously reported for **3a**.

The rigidified compounds prepared in the present study as well as those reported previously were assayed for binding at the DAT as well as for the inhibition of monoamine reuptake at the DA, 5-HT, and NE transporters. The biological data for the rigid cocaine analogues are shown in Table 1. All of the compounds tested showed high binding affinity at the dopamine transporter with about 2.5- to 104-fold greater potency than cocaine. In the inhibition of monoamine reuptake, these rigid cocaine analogues are generally more potent than cocaine. This increased potency is most evident at the DAT, where the rigid analogues are 2.8- to 190-fold more active than cocaine. A somewhat smaller spread in activities was noted at the NET with a 1.6- to 78-fold increase in activity. The SAR of the activity at the 5-HT transporter is much more complicated. The most active compound (+)-**1** is about 100-fold more active while **10a** and **11a** are 4.0- and 5.2-fold less active than cocaine, respectively.

A comparison of the ratio of the reuptake inhibition at the 5-HTT and at the DAT for the rigidified analogues reveals a dramatic difference between the front-bridged (**1** and **6**) and back-bridged structures (**2a**, **3a**,**b**, **10a** and **11a**) with the former exhibiting up to 77-fold greater activity at the 5-HTT while the latter show up to 44-fold higher activity at the DAT. While the front-bridged analogues showed up to 25-fold selectivity for the NET over the DAT, the back-bridged analogues did not show such a dramatic reversal in NET/DAT selectivity as seen for the 5-HTT/DAT selectivity. An examination of the crystal structures for (−)-**1**, **3a**, and **11a** shows a strikingly good overlap of the C(1), N(8), C(5),[20] and the centroid of the phenyl substituent with a nitrogen-to-centroid distance of 5.55−5.62 Å. This is in close agreement with the value of 5.64 Å for the recently reported 2β-carbomethoxy-3α-(3,4-dichlorophenyl)tropane.[21] These data strongly suggest that the differences found in the binding are unlikely to be due to the slight changes in the conformation of the tropane skeleton arising from the introduction of the tether.

The noted selectivity is presumably the result of the stereochemistry of the nitrogen lone pair, with the 5-HTT exhibiting a strong preference for analogues with the nitrogen lone pair localized over the 2-carbon bridge of the tropane (front-bridged analogues). The DAT exhibits a slightly smaller preference for compounds with the lone pair located over the 3-carbon bridge (back-bridged analogues). Interestingly, the NET was significantly less influenced by the orientation of the nitrogen lone pair. On the basis of previous reports, it is quite surprising that the back-bridged analogues exhibit affinity for the DAT. All tropane analogues with substituents on the 2-carbon bridge reported previously exhibit substantially reduced activity at the DAT, presumably due to adverse steric interactions in this region.[22] Thus, the stereochemical preference of the DAT for an orientation of the lone pair over the 3-carbon bridge is likely to be underestimated by using the data for the back-bridged compounds of the present studies. From binding studies at the DAT, the (*R*)-enantiomer of cocaine has been found to be 150-fold more potent than the corresponding (*S*)-enantiomer.[23] In contrast, the binding potencies of (±)-**1** and (+)-**1** are only slightly decreased from that of the (−)-**1** (*R*-cocaine-like) enantiomer. This has recently been noted for other cocaine analogues.[22c]

The present results have important implications for design of selective agents for treatment of a wide range of neurological disorders, as well as for design of PET and SPECT imaging agents for use in diagnosis of neurodegenerative diseases.

Acknowledgment. We are indebted to the NIH, National Institute on Drug Abuse (DA10458, DA05867, and DA09045), and the Office of Naval Research for their financial support.

Supporting Information Available: Configurations of (−)-**1** and **11**, tables of crystal data, atomic coordinates, bond lengths and angles, anisotropic and isotropic displacement parameters, and hydrogen coordinates for (−)-**1** and **11**, and experimental details, and spectra of **1**, **6**, **3b**, **10a**, and **11a** (25 pages, print/PDF). See any current masthead page for ordering and Web access instructions.

JA981423S

(19) In the absence of *p*-toluenesulfonic acid, a ratio of 1:13 of **10a** to **11a** was obtained.

(20) Cocaine numbering.
(21) Meltzer, P. C.; Liang, A. Y.; Blundell, P.; Gonzalez, M. D.; Chen, Z.; George, C.; Madras, B. K. *J. Med. Chem.* **1997**, *40*, 2661−2673.
(22) (a) Simoni, D.; Stoelwinder, J.; Kozikowski, A. P.; Johnson, K. M.; Bergmann, J. S.; Ball, R. G. *J. Med. Chem.* **1993**, *36*, 3975−3977. (b) Prakash, K. R. C.; Araldi, G. L.; Smith, M. P.; Zhang, M.; Johnson, K. M.; Kozikowski, A. P. *Med. Chem. Res.* **1998**, *8*, 43−58. (c) Lomenzo, S. A.; Izenwasser, S.; Katz, J. L.; Terry, P. D.; Zhu, N.; Klein, C. L.; Trudell, M. L. *J. Med. Chem.* **1997**, *40*, 4406−4414.
(23) Carroll, F. I.; Lewin, A. H.; Abraham, P.; Parham, K.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1991**, *34*, 883−886.
(24) Yi, S.-J.; Johnson, K. M. *Neuropharmacology* **1991**, *29*, 475−486.
(25) Slusher, B. S.; Tiffany, C. W.; Olkowski, J. L.; Jackson, P. F. *Drug Alcohol Depend.* **1997**, *48*, 43−50.