Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2151–2154

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Bivalent Biogenic Amine Reuptake Inhibitors

Keith Fandrick,[a] Xianqi Feng,[a] Aaron Janowsky,[b] Robert Johnson[b] and John R. Cashman[a,*]

[a]*Human BioMolecular Research Institute, 5310 Eastgate Mall, San Diego, CA 92121, USA*
[b]*Veterans Administration Hospital, Portland, OR 97201, USA*

Received 24 January 2003; accepted 4 April 2003

Abstract—A series of aryltropane-based bivalent ligands was prepared and investigated for binding potency and for their ability to inhibit reuptake of human dopamine, serotonin and norepinephrine transporters. The bivalent ligand **4**, comprised of linking an aryltropane by an octamethylene spacer showed high efficacy for the human dopamine transporter and had a discrimination ratio of 130.

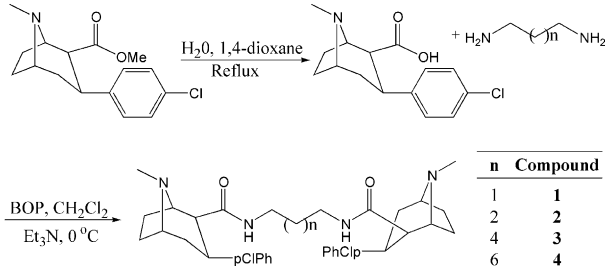
Cocaine abuse is a significant medical problem in the United States. The number of annual cocaine-related emergency room visits is approximately 100,000.[1] Fatal symptoms related to cocaine toxicity include cardiac arrest, stroke, grand mal seizures and drug-induced psychosis with elevated body temperatures.[2,3] Currently, there is no medication available to treat cocaine addiction. Significant progress with the development of small molecules directed at antagonizing the pharmacological effects of cocaine have been reported.[4–8] Recently, piperidine-based bivalent ligands have been synthesized and found to have significant affinity for the 5-hydroxytryptamine transporter (SERT) and dopamine transporter (DAT).[9] For example, two (+)-*trans*-piperidine units linked by a pentamethylene spacer showed the greatest DAT and SERT potency and decreased cocaine's locomotor effects in mice. In another study, a conformationally constrained tricyclic tropane analogue showed very high affinity against the SERT.[10] In another study the synthesis of isomers of ethylene glycol bis(tropane-3-carboxylate) was reported but no biological data were available.[11]

Using the structure of an aryltropane as a lead, we synthesized bivalent ligands by varying the length of the linking chain connecting the two aryltropane monomer units through amide linkages. The goal of this work was to identify the length of the spacer that provided greatest binding potency and reuptake inhibition efficacy against the human DAT, human SERT or human norepinephrine transporter (NET).

Results and Discussion

The aryltropane bivalent ligands were prepared by combining 2ß-carboxy-3ß-(*para*-chlorophenyl) *N*-methyl tropane[12] in dichloromethane to a solution containing 1.3 equiv of benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexafluoro phosphate (BOP reagent), 1.1 equiv Et_3N in CH_2Cl_2 at 0 °C.[13] The reaction was stirred for 30 min and 1.0 equiv of the appropriate diaminoalkane hydrochloride was added and allowed to warm to room temperature and stirred overnight (Fig. 1).

For the target compounds **1–4**, all were formed in quantitative yield. Compounds **1–4** gave a single apparent product on the basis of NMR, MS, and silica gel TLC.



n	Compound
1	**1**
2	**2**
4	**3**
6	**4**

Figure 1. Synthesis of 1,*n* di-3β-(4′-chlorophenyl) tropane 2β-carboxamide linker molecules.

*Corresponding author. Tel.: +1-858-458-9305; fax: +1-858-458-9311; e-mail: jcashman@hbri.org

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00386-X

Each compound was examined for potency of inhibition of binding radiolabeled RTI-55 to the human DAT, human SERT and human NET.[14] As shown in Table 1, compared with cocaine, compound **4** (comprised of two aryltropane units linked by an octamethylene spacer), possessed significant binding affinity for hDAT, hSERT but not for the hNET. Except for compound **1**, for hDAT, hSERT and hNET, binding affinity generally increased as the chain length separating the aryltropane units increased to eight. It is possible that compound **1**, with a trimethylene unit afforded a compound that only recognized one binding domain.

For inhibition of hDAT and hNET neurotransmitter uptake, **1** was most efficacious (Table 2). For hSERT, the most efficacious reuptake inhibitor was compound **4**. From the data presented for **4**, apparently a secondary site of the hSERT that is quite distinct from that of the hDAT or hNET is located at least eight methylene units away and may contribute to uptake inhibition (Table 2).

For compound **4**, the selectivity of either binding potency or reuptake efficacy for hDAT, hSERT or hNET was relatively low. Thus, the selectivity ratios, K_i(hDAT)/K_i(hSERT), K_i(hNET)/K_i(hDAT) and K_i(hNET)/K_i(hSERT) for compound **4** were 0.85, 6.6, and 5.6, respectively. Likewise, the binding potency for compound **3** was quite modest and the K_i(hDAT)/K_i(hSERT), K_i(hNET)/K_i(hDAT) and K_i(hNET)/K_i(hSERT) were 0.18, 45.6, and 8.3, respectively.

Generally, compounds that showed low potency in binding were also poorly efficacious in reuptake inhibition. However, there were some notable exceptions. Compounds **2**, **3**, and **4** showed relatively weak hDAT uptake inhibition activity and exhibited the highest uptake-to-binding discrimination ratios against the hDAT in the current series. Discrimination ratios are calculated constants that indicate in a general sense if the transporter binding site and uptake site overlap. A large discrimination ratio suggests that the overlap of sites is not complete. The discrimination ratio for compound **4** (i.e., 130) is among the higher discrimination ratios currently reported in the literature (Table 3).

In summary, synthesis of aryltropane-based bivalent ligands has led to compounds with high discrimination ratios. The results suggest that certain bivalent aryltropane analogues bind to different domains on the hDAT and this has previously been supported by evidence from structural biology[15,16] and recombinant hDAT chimera studies.[17,18]

Table 1. Inhibition of radioligand binding in HEK-hDAT, HEK-hSERT and HEK-hNET cells by Bis 2β-carboxamido 3β-(*p*-chlorophenyl) *N*-methyl tropanes[a]

Compd	HEK hDAT		HEK hSERT		HEK hNET	
	K_i (nM)	SEM	K_i (nM)	SEM	K_i (nM)	SEM
Cocaine	272	±58	601	±132	830	±147
RTI-31	1.1[b]	±0.1	44.5	±1.3	37	±2.1
1	65.1	±8.1	357	±70.6	2970	±332
2	21.7	±6.8	3135.7	±945	2068	±580
3	18.4	±3.5	102.3	±16.9	330	±75.9
4	6.7	±1.4	7.9	±2.1	830	±147

[a]Drug inhibition of [^{125}I]-RTI-55 binding in HEK-hDAT, HEK-hSERT or HEK-hNET cell membranes. Values represent the mean±SEM for three to four experiments.
[b]Values from ref 12.

Table 2. Inhibition of radiolabelled neurotransmitter uptake in HEK-hDAT, HEK-hSERT and HEK-hNET cells by Bis 2β-carboxamido 3β-(*p*-chlorophenyl) *N*-methyl tropanes[a]

Compd	HEK hDAT		HEK hSERT		HEK hNET	
	IC_{50} (nM)	SEM	IC_{50} (nM)	SEM	IC_{50} (nM)	SEM
Cocaine	267	±47	318	±57	385	±40
RTI-31	1.7	±0.1	19	±2.7	8.2	±1.6
1	52.2	±2.9	441	±54	266	±130
2	1667	±184	176	±23	2258	±383
3	1401	±240	225	±104	1846	±377
4	871	±316	99	±37	487	±131

[a]Inhibition of [^{3}H]-DA, [^{3}H]-5HT or [^{3}H]-NE in the presence of HEK-hDAT, HEK-hSERT or HEK-hNET cells, respectively. Values are the mean±SEM for three to four experiments.

Table 3. Discrimination ratios of uptake inhibition to binding affinity

Compd	hDAT IC_{50}/K_i[a]	hSERT IC_{50}/K_i	hNET IC_{50}/K_i
Cocaine	1.0	0.53	0.46
RTI-31	1.5	0.43	0.22
1	0.80	1.17	0.09
2	76.8	0.06	1.09
3	76.3	2.20	5.59
4	130	12.53	10.99

[a]The IC_{50} value (Table 2) for uptake inhibition was divided by the K_i value (Table 1) for binding to obtain the discrimination ratio listed.

Acknowledgements

We are grateful to the National Institute on Drug Abuse for the financial support of this work (Grant Number DA11547).

References and Notes

1. Das, G. *J. Clin. Pharmacol.* **1993**, *33*, 296.
2. Rich, J. A.; Singer, D. E. *Ann. Emerg. Med.* **1991**, *20*, 616.
3. Warner, E. A. *Ann. Intern. Med.* **1993**, *119*, 226.
4. Carroll, F. I.; Lewin, A. H.; Kuhar, M. J. In *Neurotransmitter Transporters: Structure and Function*; Reith, M. E. A., Ed.; Human: Totowa, 1997; p 263.
5. Smith, M. P.; Hoepping, A.; Johnson, K. M.; Trzcinska, M.; Kozikowsi, A. P. *Drug Discov. Today* **1999**, *4*, 322.
6. Carroll, F. I.; Howell, L. L.; Kuhar, M. J. *J. Med. Chem.* **1999**, *42*, 1.
7. Newman, A. H. *Med. Chem. Res.* **1998**, *8*, 1.
8. Meltzer, P. C.; Blundell, P.; Madras, B. K. *Med. Chem. Res.* **1998**, *8*, 12.
9. Tamiz, A. P.; Bandyopadhyay, B. C.; Zhang, J.; Flippen-

Anderson, J. L.; Zhang, M.; Wang, C. Z.; Johnson, K. M.; Tella, S.; Kozikowski, A. P. *J. Med. Chem.* **2001**, *44*, 1615.
10. Zhang, A.; Zhou, G.; Hoepping, A.; Mukhopadhyaya, J.; Johnson, K. M.; Shang, M.; Kozikowski, A. P. *J. Med. Chem.* **2002**, *45*, 1930.
11. Cheng, J.; Moore, Z.; Stevens, E. D.; Trudell, M. L. *J. Org. Chem.* **2002**, *67*, 5433.
12. Carroll, F. I.; Kotian, P.; Dehghani, A.; Gray, J. L.; Kuzemko, M. A.; Parham, K. A.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1995**, *38*, 379. The synthesis of 3β-(4′-chlorophenyl)tropane-2β-carboxylic acid was done as follows. To an oven-dried flask was added 5 mL of deionized water, 5 mL of 1,4 dioxane, and 354 mg of 2β-carbomethoxy 3β-(*p*-chlorophenyl) *N*-methyl tropane (i.e., RTI 31) (Fig. 1).[12] The flask was fitted with a condenser and refluxed for 10 h. The solvent was removed by reduced pressure to afford a thick oil. The product was purified by silica gel chromatography (R_f=0.25, MeOH/CH_2Cl_2, 10:90, v:v) to afford 289 mg (87%) of 3β-(4′-chlorophenyl)tropane-2β-carboxylic acid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.76 (dt, J=3.4 and 7.9 Hz, 1H), 1.93–1.97 (m, 2H), 2.27–2.31 (m, 1H), 2.52 (s, 3H), 2.60 (dt, J=2.9 and 13.5 Hz, 1H), 2.66–2.68 (m, 1H), 3.14 (dt, J=6.7 and 13.1 Hz, 1H), 3.59–3.63 (m, 1H), 7.16 (d, J=8.3 Hz, 2H), 7.25–7.27 (m, 2H). ESMS m/z 280 $(MH)^+$ positive mode, m/z 278 $(M-H)^-$ negative mode.
13. The synthesis of the title compounds was done as follows: 1,3-di-(3β-(*p*-chlorophenyl)tropane-2β-carboxamide)-propane (**1**). To an oven-dried vial was placed 82 mg (1.4 equiv) of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexaflourophosphate (BOP reagent), and 200 μmol of Et_3N in 2 mL of CH_2Cl_2. The mixture was cooled to 0 °C and stirred for 20 min. Thirty-seven mg (1.0 equiv) of 2β-carboxy 3β-(*p*-chlorophenyl) *N*-methyl tropane in 3 mL CH_2Cl_2 was added to the mixture. The reaction was stirred at 0 °C for 10 min. Five mg (1.2 equiv) of 1,3 diaminopropane in 2 mL of CH_2Cl_2 was added to the reaction dropwise and the reaction was allowed to warm to room temperature and stirred overnight. The reaction was stopped by addition of 5 mL water. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried with Na_2SO_4 and reduced to a thick liquid. The product was isolated by preparative TLC (R_f 0.26, CH_2Cl_2/CH_3OH/NH_4OH, 80:20:1) to afford 37.4 mg of 1,4-bis (2β-carboxamido 3β-(*p*-chlorophenyl) *N*-methyl tropane) propane; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25 (m, 2H), 1.88–2.08 (m, 4H), 2.27 (m, 8H), 2.55–2.75 (m, 6H), 3.08 (m, 2H), 3.21 (m, 2H), 3.48 (s, 6H), 7.08–7.18 (m, 6H), 7.51 (m, 1H), 7.77 (m, 1H). ESMS m/z 597 $(MH)^+$, and 619 $(M+Na)^+$ positive mode, m/z 594 $(M-H)^-$ and m/z 631 $(M+Cl)^-$ negative mode.

1,4-di-(3β-(*p*-Chlorophenyl)tropane-2β-carboxamide) butane (**2**). To an oven dried vial was placed 60 mg (1.3 equiv) of BOP reagent and 120 μmol of Et_3N in 1.3 mL of CH_2Cl_2. The vial was cooled to 0 °C and stirred for 20 min. Fifty mg (1.67 equiv) of 2β-carboxy 3β-(*p*-chlorophenyl) *N*-methyl tropane was added to the mixture. The mixture was stirred at 0 °C for 30 min. Six mg (1.29 equiv) of 1,4-diaminobutane in 2 mL of CH_2Cl_2 was added to the reaction dropwise. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was stopped by addition of 8 mL of water. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried with Na_2SO_4 and reduced to a thick liquid. The product was isolated by preparative TLC (R_f 0.34, CH_2Cl_2/CH_3OH/NH_4OH, 80:20:1, v:v).

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (m, 4H), 1.24 (m, 10H), 1.67–1.96 (m, 6H), 2.04–2.65 (m, 14H), 3.01 (m, 4H), 3.21 (m, 2H), 3.48–3.71 (m, 4H), 7.16–7.27 (m, 8H), ESMS m/z 611 $(M+1)^+$ positive mode, m/z 645 $(M+Cl)^-$ negative mode. 1,6-di-(3β-(*p*-Chlorophenyl)tropane-2β-carboxamide)-hexane (**3**). To an oven dried vial was placed 52.1 mg (1.1 equiv) of BOP reagent and 200 μmol of Et_3N in 4 mL of CH_2Cl_2. The mixture was cooled to 0 °C and stirred for 20 min. Thirty three mg (1.1 equiv) of 2β-carboxy 3β-(*p*-chlorophenyl) *N*-methyl tropane in 2 mL of CH_2Cl_2 was added to the reaction. The reaction was stirred at 0 °C for 30 min. Ten mg (1.0 equiv) of 1,6-diaminohexane dihydrochloride was added to the reaction and the mixture was allowed to warm to room temperature and stirred overnight. The reaction was stopped by addition of 8 mL of water. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried with Na_2SO_4 and reduced to a thick liquid. The product was isolated by preparative TLC (R_f 0.15 CH_2Cl_2/MeOH/NH_4OH, 80:20:1, v:v) to afford 34 mg (quantitative yield) of 1,6-bis (2β-carboxamido 3β-(*p*-chlorophenyl) *N*-methyl tropane) hexane; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.52–1.41 (m, 8H), 1.75–1.79 (m, 8H), 2.16–2.34 (m, 12H), 3.11–3.15 (m, 6H), 3.42–3.47 (m, 6H), 7.12–7.22 (m, 8H), electrospray MS m/z 639 (M+1) positive mode, m/z 673 $(M+Cl^-)$ negative mode. 1,8-di [2β-Carboxamido 3β-(*p*-chlorophenyl) *N*-methyl tropane] octane (**4**). To an oven dried vial was placed 57.7 mg (1.2 equiv) of BOP reagent and 200 μmol of Et_3N in 4 mL of CH_2Cl_2. The mixture was cooled to 0 °C and stirred for 20 min. Thirty-three mg (1.1 equiv) of 2β-carboxy 3β-(*p*-chlorophenyl) *N*-methyl tropane in 2 mL of CH_2Cl_2 was added to the mixture. The reaction was stirred for 30 min at 0 °C. Twelve mg (1.0 equiv) of 1,8-diaminooctane dihydrochloride was added to the reaction. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was stopped by addition of 8 mL of water. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried with Na_2SO_4 and reduced to a thick liquid. The product was isolated by preparative TLC (R_f 0.10 CH_2Cl_2/MeOH/NH_4OH, 80:20:1, v:v) to afford 36 mg (quantitative yield) of 1,8-bis [2β-carboxamido 3β-(*p*-chlorophenyl) *N*-methyl tropane] octane; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25–1.28 (m, 14H), 1.70–1.79 (m, 8H), 2.16–2.34 (m, 12H), 3.09–3.16 (m, 6H), 3.41 (m, 4H), 7.12 (m, 4H), 7.22 (m, 4H), electrospray MS m/z 667 (M+1) positive mode, m/z 701 $(M+Cl^-)$ negative mode.
14. Eshleman, A. J.; Neve, R. L.; Janowsky, A.; Neve, K. A. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 276. In vitro binding experiments were done as follows. Cells containing the human DAT, SERT or NET cDNA were prepared and membranes were obtained as previously described.[14] Each assay contained approximately 50 μg of protein, 25 μL of test compound prepared in assay buffer and 0.1% DMSO and 25 μL of [^{125}I]RTI-55 (40–80 pM final concentration). The assay buffer contained Krebs-HEPES, pH 7.4 (122 mM NaCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 μM pargyline, 100 μM tropolone, 0.2% glucose and 0.02% ascorbic acid buffered with 25 mM HEPES) in a final volume of 250 μL. Assays were run for 90 min at 25 °C. Binding was terminated by filtration over a GF/C filter using a Tomtec 96-well cell harvester and radioactivity remaining on the filter was determined using a Wallac beta-plate reader. Specific binding was defined as the difference in binding observed in the presence and absence of 5 μM mazindol (or 5 μM imipramine for human SERT). Two or three independent competition experiments were conducted with duplicate determinations. GraphPad Prism was used to analyze the data with IC_{50} values converted to K_i values using the Cheng–Prusoff equation.

For uptake inhibition assays, Krebs–HEPES and test compound were added to cells as above.[13] The assay was initiated by the addition of the tritiated neurotransmitter (20 nM final concentration). Specific uptake was defined as the difference in uptake observed in the presence and absence of 5 μM mazindol or 5 μM imipramine (i.e., human SERT). Filtration of the cells through a Whatman GF/C filter presoaked with 0.05% polyethylenimine was used to stop uptake after 10 min. The

IC_{50} values were calculated as described above. Triplicate curves made up of six drug concentrations each were made for each determination and the results were expressed at IC_{50} values ± SEM.

15. Vaughan, R. A.; Kuhar, M. J. *J. Biol. Chem.* **1996**, *271*, 21672.
16. Vaughan, R. A.; Agoston, G. E.; Lever, J. R.; Newman, A. H. *J. Neurosci.* **1999**, *19*, 630.
17. Buck, K. J.; Amara, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12584.
18. Syringas, M.; Janin, F.; Mezghanni, S.; Giros, B.; Costentin, J.; Bonnett, J. *J. Mol. Pharmacol.* **2000**, *58*, 1404.