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Synthesis and biological evaluation of new N-substituted 4-(arylmethoxy)piperidines as dopamine transporter inhibitors

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Design. Computer models were created in DS ViewerPro 6.0 (Discovery Studio, Accelrys, San Diego, CA). Final model of 4-(diphenylmetoxy)piperidine (DPP) was cleaned by the option of “clean structure” which used a fast, Dreiding-like force field to quickly optimize the geometry of all or selected structures in the 3D Window. The elements, bond orders, number of bonds, and valences are taken into consideration when the terms of the energy equation are calculated. Cocaine’s structure was taken from the file 1QYG.pdb. The alignment of DPP was made along the along the piperidine cycle and ether bond of cocaine structure in agreement of company's guidelines. RMS was calculated as a difference in the atoms coordinates (x,y,z) for only common atoms of piperidine cycle, ether bond and aromatic ring.

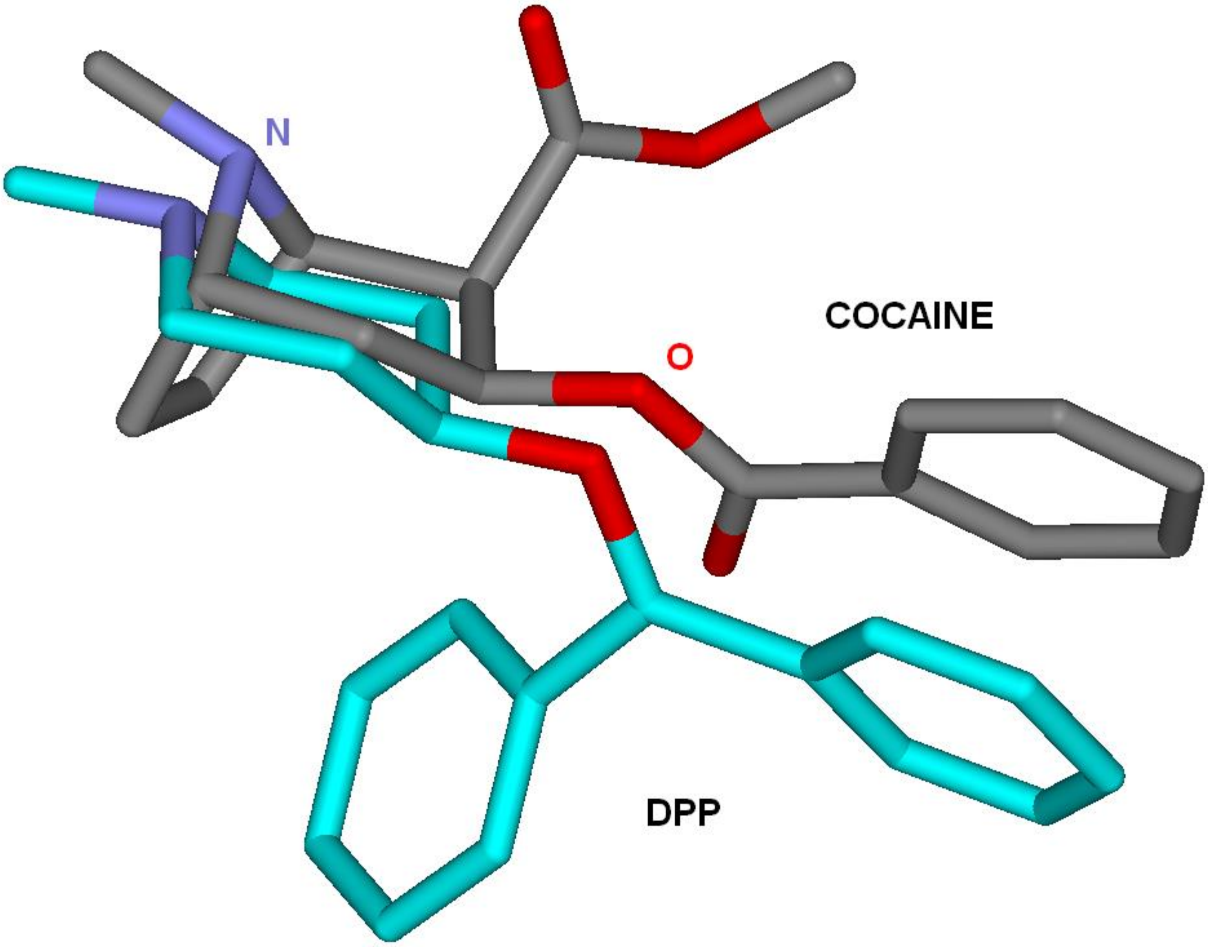
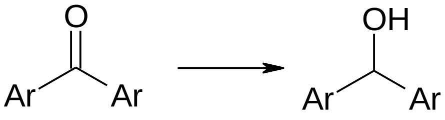


Figure S1 The alignment of 4-(diphenylmethoxy)piperidine (DPP) along the cocaine structure (1Q72.pdb).

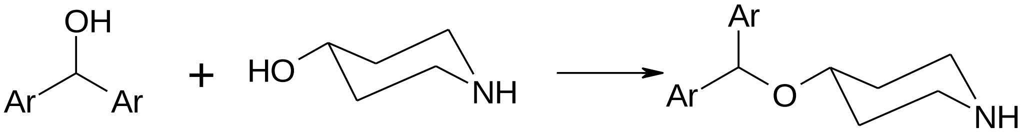
Chemistry

Varian Anaspect EN360 NMR spectrometer was used. The ^{1}H NMR (300 MHz, $CDCl_3$) spectra of *N*-methylpiperidines showed typical signals: 1.30–1.35 (m, 2H, H-2,6 ax), 1.85–1.90 (m, 2H, H-2,6 eq.), 2.00–2.05 (m, 2H, H-3,5 ax), 2.40–2.45 (s, 3H, N-CH3), 2.90–3.00 (m, 2H, H-3,5 eq.), 3.50–3.55 (m, 1H, H-4 ax), 5.35– 5.45 (s, 1H, Ar-CH-Ar), 7.20–7.60 (m, aromatic C–H).
In some cases, Varian Anaspect spectrometer (400 MHz, ^{1}H NMR, DMSO-d_6) was used.

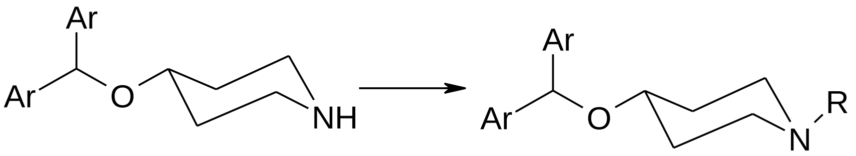
An Agilent 1100 HPLC system featured a binary pump and diode array detector (DAD). The column was a 2x100 mm C18(2) Luna, 3 µm, (Phenomenex, Torrance, CA), with a flow of 0.25 ml min^{-1}. Mobile phase A: 5% MeOH in water with 5 mM ammonium formate; mobile phase B: MeOH with 5 mM ammonium formate. The gradient ran from 50% A (initially kept for 1 min) to 100% B within 5 min, and the latter was kept for 10 min. The DAD was scanned from 190 – 400 nm. The HPLC was also interfaced to a Micromass Quattro Ultima triple stage mass spectrometer (Waters, Milford, MA) operated in the positive electrospray ionization mode. The instrument scanned *m/*z 80 – 800. An Agilent 5973B GC-MS system was used for analysis of the solutions. A DBWAX-15W-0.5 column was used for separation with a temperature gradient from 200 to 235 °C. Electron impact mass spectra were generated from *m/z* 10 – 400. For all compounds the mass spectra contained weak $(M+H)^+$ ions characteristic of piperidines and the typical isotope patterns for one or more halogen atoms in the structures. Characteristic ions that occurred for compounds **1,2,4, 6-9, 12,13** were m/z 99 (methylpiperidine fragment) as the base peak and m/z 114 (methylpiperidinol fragment) at approximately 50% R.A. in each mass spectrum. Significant diarylmethane fragments also occurred for all compounds except for **16**. For compounds with very weak $(M+H)^+$ ions in the EI mass spectrum, electrospray ionization mass spectra from a methanol solution were taken to confirm the molecular weight as the $(M+H)^+$ ion. The relative response of all compounds was from 95.8% up to 99.3%.



Benzhydrols (general procedure). Sodium borohydride (0.01 mol, 2 eq) were added to a solution benzophenone (0.01 mol, 1 eq) in 2-propanol (20 ml). The mixture was refluxed for 30 min and evaporated in vacuum. The product was extracted with ice water solution of 0.5% HCl and methyl *tert*-butyl ether. The organic extract was dried by passing through silica gel – Na_2SO_4 pad and evaporated in vacuum. The product was pure (98%, LC-MS data) and was used in the next step.

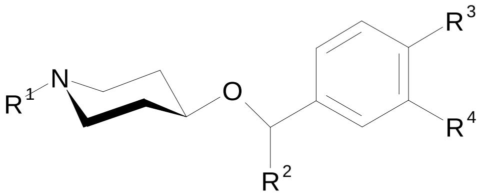


4-(Diarylmethoxy)piperidines (general procedure). *p*-Toluenesulfonic acid (12.5 mmol, 1.25 eq) were added to the toluene-DMF (25:1) solution (50 ml) of benzhydrole (10 mmol, 1 eq) and piperidin-4-ol (10.5 mmol, 1.05 eq). The mixture was refluxed with a Dean–Stark trap for 4 h (TLC monitoring). The mixture was evaporated in vacuum. The residue was treated with 5% K_2CO_3 solution in ice water, and the product was extracted with ethyl acetate. The organic extract was dried by passing through silica gel - Na_2SO_4 pad and evaporated in vacuum. The residue was purified by flash chromatography with silica gel to afford product with 95% or higher purity (LC-MS data) suitable for using in the next step.



***N*-Alkyl-4-(diarylmethoxy)piperidines (general procedure)**. Alkyl halide (5 mmol, 1 eq) was added to a mixture of NH-piperidine (5 mmol, 1 eq), K_2CO_3 (10 mmol, 2 eq) and dry DMF (25 ml), and the mixture was stirred overnight at room temperature. The mixture was diluted with water, the product was extracted with methyl *tert*-butyl ether or ethyl acetate, and the extract was evaporated *in vacuo*. Flash-chromatography on silica gel afforded the final product whose purity was determined by GC-MS or LC-MS (Table S1). Some compounds were converted into salts with oxalic acid in acetone, however, but this did not improve their solubility in water.

Table S1 Purity and some properties of 4-(diarylmethoxy)piperidines **1-15**.



#	ID	R^1	R^2	R^3	R^4	Molecular Formula	GC-MS, %	FW calculated	FW found (M+H)$^+$	Oxalate m.p., °C
1	DPP[a]	Me	Ph	H	H	$C_{19}H_{23}NO$	97.5	281.1779	282.16	203-5[b]
2	WSS2	Me	4-FC_6H_4	F	H	$C_{19}H_{21}F_2NO$	95.9	317.1591	318.12	
3	WSS10	Bu	4-FC_6H_4	Cl	H	$C_{22}H_{27}Cl_2NO$	96.8	391.1469	392.13	
4	WSS11	Me	4-FC_6H_4	Cl	H	$C_{19}H_{21}Cl_2NO$	97.1	349.1000	350.08	
5	WSS3	4-$FC_6H_4CH_2$	4-FC_6H_4	Cl	H	$C_{25}H_{24}Cl_2FNO$	99.0	443.1218	444.10	
6	WSS1	Me	Ph	Cl	H	$C_{19}H_{22}ClNO$	97.4	315.1389	316.12 (318.02 30%)	84-86
7	WSS8	Me	Me	F	H	$C_{14}H_{20}FNO$	98.2[c]	237.1528	238.15	
8	WSS4	Me	Ph	F	H	$C_{19}H_{22}FNO$	95.8	299.1685	300.08	
9	WSS5	Me	Ph	H	Me	$C_{20}H_{25}NO$	97.8	295.1936	296.16	142-144
10	WSS12	Bu	4-FC_6H_4	F	H	$C_{22}H_{27}F_2NO$	98.5	359.2060	360.17	
11	WSS13	i-Pr	4-FC_6H_4	F	H	$C_{21}H_{27}F_2NO$	95.4	347.2060	348.15	199-202
12	WSS14	Me	Ph	Cl	Cl	$C_{19}H_{21}Cl_2NO$	99.3	349.1000	350.09	
13	WSS15	Me	Ph	Me	H	$C_{20}H_{25}NO$	99.1*	295.1936	296.16	139-141
14	WSS16	H	4-FC_6H_4	F	H	$C_{18}H_{19}F_2NO$	94.4*	303.1434	304.39	178-180
15	WSS17	CH_2CN	4-FC_6H_4	F	H	$C_{20}H_{20}F_2N_2O$	95.1	342.1543	343.10	157-159

[a]DPP was purchased from Aldrich. [b]For the hydrochloride. [c]LC-MS data.

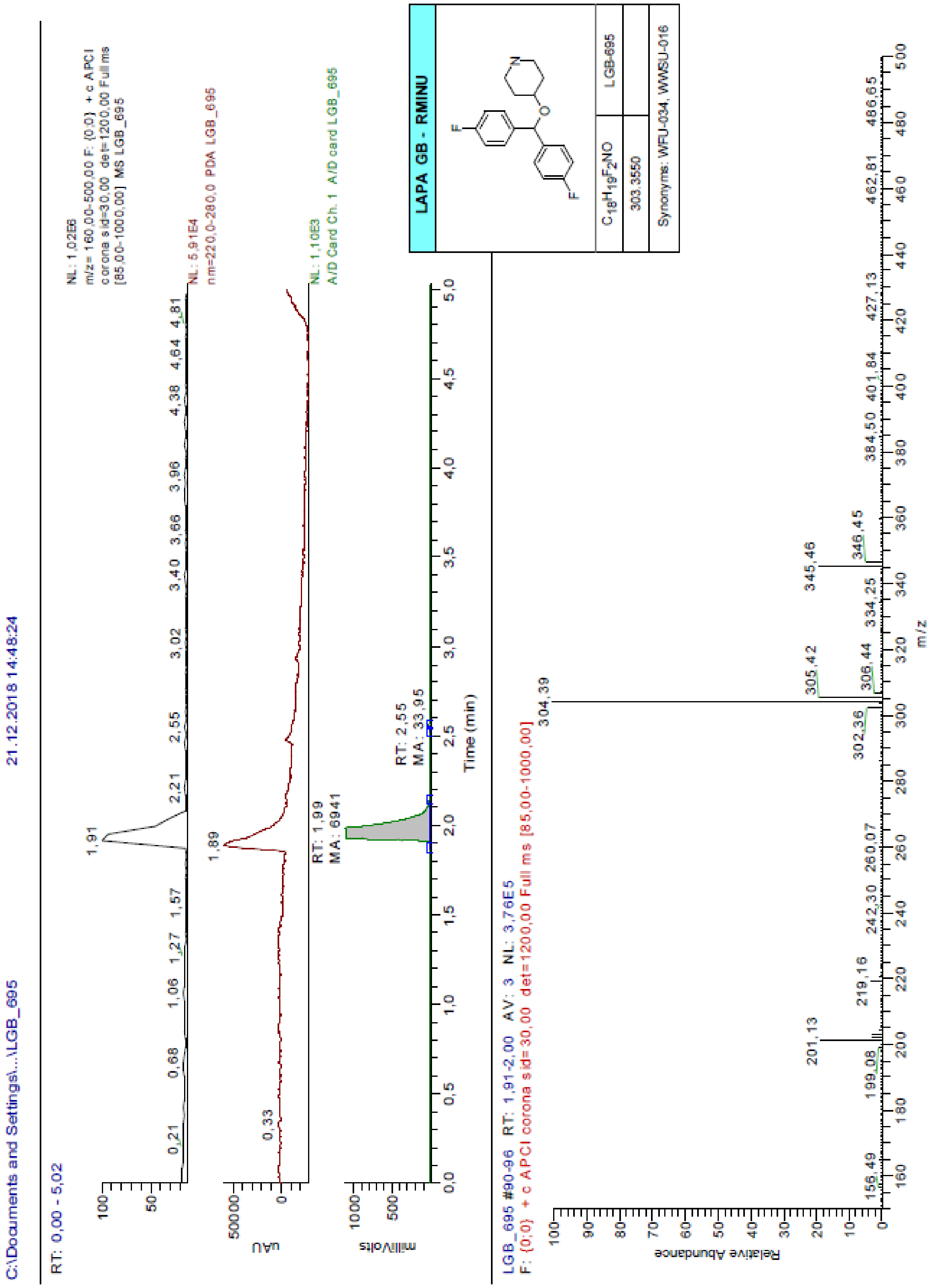


Figure S2 LC-MS spectral data for 4-[bis(4-fluorophenyl)methoxy]piperidine **14**.

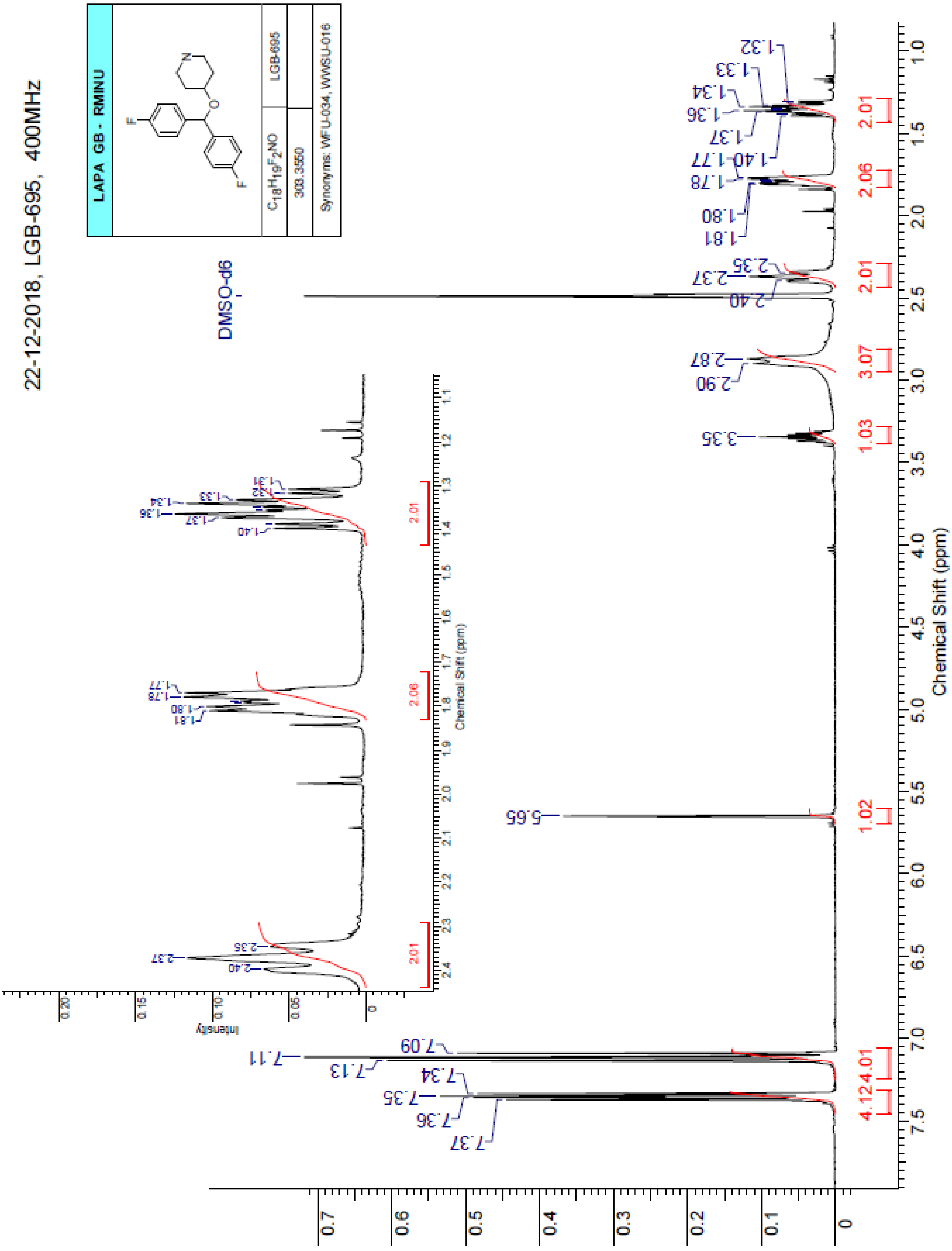


Figure S3 ^{1}H NMR data for 4-[bis(4-fluorophenyl)methoxy]piperidine **14**.

Biology

Potencies were calculated from displacement curves using seven concentrations of unlabeled compounds. All data are mean values ± SEM of at least three separate experiments, each of which was conducted in triplicate. Potencies of all unlabeled compounds in displacing [^{125}I]RTI-55 binding are expressed as IC50 values because the biphasic nature of [^{125}I]RTI-55 binding to striatal membranes makes determination of accurate Ki values difficult.

Animals. C57BL/6 mice (Jackson Laboratories, Bar Harbor, Maine) were housed in groups of three to four per cage with food and water ad libitum, and they were on a 12-h light–dark cycle. Experiments were performed in male and female mice (8–12 weeks old). Experimental protocols adhered to National Institutes of Health Animal Care guidelines and were approved by the Wake Forest University Institutional Animal Care and Use committee.

Locomotor activity testing. Locomotor activity was studied using plastic chambers (43.2 x 43.2 x 30.5 cm; MED Associates) equipped with photosensors spaced 2.5-cm apart along two perpendicular walls. One count of horizontal activity was registered each time the subject interrupted a single beam. Mice were habituated for 1 h and DPP (5 or 10 mg kg^{-1}), cocaine (6 mg kg^{-1}), or saline was injected. Horizontal activity counts were collected every 5 min within 1 h

Statistics. Statistical analyses were carried out by a one- and two way analysis of covariance (ANOVA) and Student’s t-test with GraphPad Prism (Graph Pad Software, San Diego, CA, USA). The data are presented as mean FS.E.M. The criterion of significance was set at Pb0.05.

For the details of biological experiments, see the following sources:

G. B. Lapa, T. A. Mathews, J. J. Harp, E. A. Budygin and S. R. Jones, *Eur. J. Pharmacol.,* 2005, **506**, 237.

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S. R. Letchworth, H. R. Smith, L. J. Porrino, B. A. Bennett, H. M. L. Davies, T. Sexton and S. R. Childers, *JPET*, 2000, **293**, 686.