Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 9, pp. 1213-1218, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00193-5

FURTHER EVIDENCE FOR A DOPAMINE REUPTAKE PHARMACOPHORE. THE EFFECT OF N-METHYLATION ON *THREO*-METHYLPHENIDATE AND ITS ANALOGS

Mark Froimowitz,[a]* Howard M. Deutsch,[b]* Quing Shi,[b] Kuo-Ming Wu,[a] Robert Glaser,[c] Itay Adin,[c] Clifford George,[d] and Margaret M. Schweri[e]*

[a]*Pharm-Eco Laboratories, 128 Spring Street, Lexington, MA 02173-7800*

[b]*School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400*

[c]*Department of Chemistry, Ben-Gurion University of the Negev, Beer Sheva 84105 Israel*

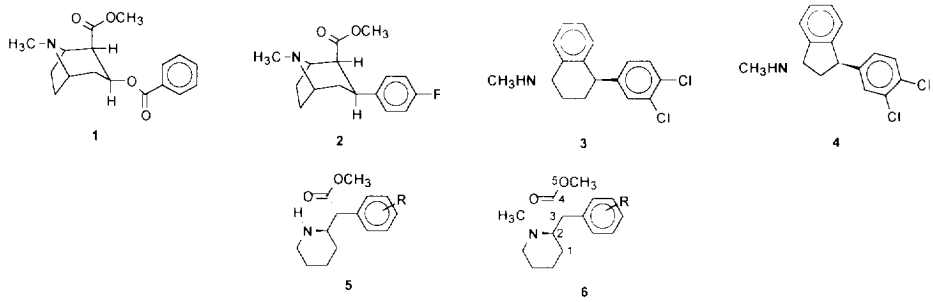
[d]*Laboratory for the Structure of Matter, Naval Research Laboratory, Washington D.C. 20375-5341*

[e]*Division of Basic Medical Sciences, School of Medicine, Mercer University, Macon, GA 31207-0001*

Abstract. N-methyl derivatives of methylphenidate and four analogs were synthesized and assayed for affinity at the dopamine transporter. The binding affinities of the N-methyl compounds were consistently lower by a factor ranging from 4 to 30 as compared with the corresponding secondary amine. This is consistent with the predictions of a pharmacophore model of compounds that bind to the dopamine transporter.

There appears to be a divergence in the structure–activity relationships of dopamine reuptake blockers with respect to substitution on the nitrogen. In cocaine **1** and CFT **2**, the optimal compound is a tertiary amine and the secondary amine has reduced activity.[1,2] More recently, additional 3-phenyltropanes have been synthesized, including ones in which the carbomethoxyl has been replaced by a ketone, and there have been mixed results regarding the effect of N-methylation.[3–5] However, in other series, such as 1-amino-4-phenyltetralins **3** and 3-phenyl-1-indanamines **4**, secondary amines are optimal and the addition of an N-methyl group consistently reduces activity.[6,7] Based on a conformational analysis of these compounds and the development of a pharmacophore model, an explanation was proposed for the decrease in the potency of the tertiary amines of **3** and **4**.[8] That is, an added N-methyl group to **3** and **4** was found to preferentially occupy the space preferred by the ammonium hydrogen in cocaine and 3-phenyltropanes. While the directionality of the ammonium hydrogen is likely to modulate the activity of dopamine reuptake blockers, it should be noted that a basic amine or even a nitrogen is not always required for potent activity.[9–11]

Recently, a conformational analysis was performed on *threo*-methylphenidate **5a** and the compound was incorporated into the pharmacophore model.[12] Superimposing the preferred conformers of the active enantiomers of **5a**[12] and **2**, a near perfect fit was found for the sequence of atoms from the nitrogen through the ester group. It was also noted that an added N-methyl group should preferentially be placed in the position



a R = H, b R = 3-Cl, c R = 3-Me, d R = 4-Me, e R = 4-OH

required for the ammonium hydrogen as defined by the pharmacophore model. This predicts that N-methyl derivatives of **5a** and its analogs should have reduced activity relative to the corresponding secondary amine. Here we report that this prediction is indeed correct.

threo-Methylphenidate and four of its phenyl substituted analogs were synthesized as previously described.[13] These were N-methylated by a reductive amination procedure which consisted of treatment of a methanol solution of **5a–5e** with HCHO, HOAc, H_2, and Pd/C. The products were purified by chromatography and converted to HCl salts. These analogs were screened for inhibition of [^{3}H]-WIN 35,428 binding and the results for five pairs of compounds with and without an N-methyl group are shown in Table I. As can be seen, there is a consistent decrease in affinity, ranging from 4- to 30-fold, for the corresponding compound with an N-methyl group, consistent with the prediction of the pharmacophore model.

Table I. Inhibition of [^{3}H]-WIN 35,428 Binding of Compounds With and Without an N-methyl Group

	unsubstituted (**5**)		N-methyl substituted (**6**)		
Compound	IC_{50} ± SEM (n) (nM)[a]	Hill coefficient ± SEM (n)[a]	IC_{50} ± SEM (n) (nM)	Hill coefficient ± SEM (n)	ratio (sub/unsub)
methylphenidate (**a**)	83 ± 8 (4)	0.90 ± 0.09 (4)	500 ± 25 (3)	1.00 ± 0.01 (3)	6.0
3-chloro (**b**)	5.1 ± 1.6 (3)	0.95 ± 0.12 (3)	161 ± 18 (4)	0.96 ± 0.04 (3)	32.
3-methyl (**c**)	21.4 ± 1.1 (2)	1.01 ± 0.12 (2)	108 ± 16 (3)	1.00 ± 0.04 (3)	5.0
4-methyl (**d**)	33 ± 1.2 (2)	1.05 ± 0.02 (2)	139 ± 13 (3)	1.03 ± 0.04 (3)	4.2
4-hydroxy (**e**)	98 ± 10 (2)	1.07 ± 0.12 (2)	1220 ± 140 (2)	1.06 ± 0.01 (2)	12.

[a]Ref 13

The preferred conformer of *threo*-methylphenidate **5a** by MM2 calculations and X-ray crystallography is one in which there is a hydrogen bond between the carbonyl oxygen and the equatorial ammonium hydrogen.[12] The addition of an equatorial N-methyl group disrupts this so that the carbomethoxy group is rotated away from its previous position (Figure 1). In addition, superimposing this conformer (using the four atoms between the nitrogen and carbonyl carbon) of N-methylmethylphenidate **6a** with CFT **2**, it is clear that an equatorial N-methyl group of the former occupies the position of the ammonium hydrogen in the latter (Figure 2).

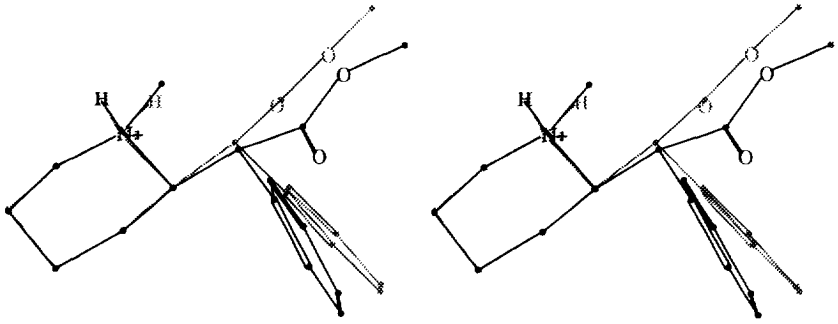


Figure 1. Stereoscopic image of superposition of preferred conformer of *threo*-methylphenidate **5a** (light line) with *threo*-N-methylmethylphenidate **6a** (dark line) with an equatorial N-methyl group. Most hydrogens have been omitted for clarity.

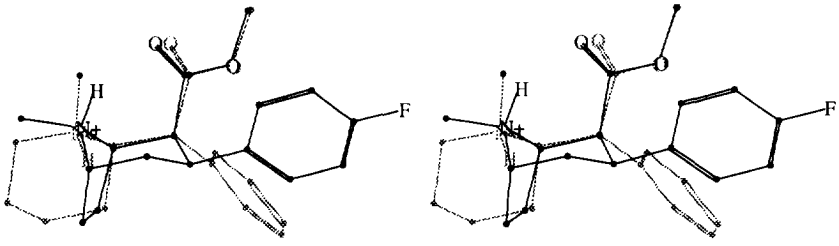


Figure 2. Stereoscopic image of superposition of N-methylmethylphenidate **6a** (light line) with an equatorial N-methyl group and preferred conformer of CFT **2** (dark line). Most hydrogens have been omitted for clarity.

Normally, groups on a piperidine ring will preferentially occupy equatorial positions. However, the conformational equilibria of the N-methyl compounds were found to be highly dependent on the molecular environment. Energy calculations of the conformations/diastereomers of **6a** with the MM3-92[14,15] indicate that species in which the N-methyl/CH(Ph)COOMe groups are axial have about the same energy as those with the groups equatorial for both low or high values of the dielectric constant (Table II). Calculations with a high dielectric constant would be qualitatively relevant in a polar solvent such as water which damps out intramolecular electrostatic interactions whereas those with a low dielectric would be more relevant in a more nonpolar solvent. In both D_2O and CD_2Cl_2, two sets of NMR signals were found for HCl salts of **6a–6e** indicating distinct species at the slow exchange limit to interconversion via a prototropic shift/nitrogen inversion mechanism.[16,17] ^1H and ^{13}C NMR spectroscopy were then used to study the protonated species in solution. In CD_2Cl_2, the major species (87%) had both groups equatorial while the minor species had an axial N-methyl group. In D_2O, the minor species (45%) had both groups equatorial while the the major species had an axial N-methyl group. Crystal structures were also obtained for HCl salts of **6a** and **6d** by X-ray crystallography. In both of these, the N-methyl group was axial. However, the CH(4'-MePh)COOMe group

was also axial in **6d** whereas the former was a disordered crystal in which the CH(Ph)COOMe group was in both the axial and equatorial positions. Finally, noncrystalline forms of **6a** and **6d** were studied by solid state NMR and the N-methyl groups were equatorial and the CH(Ar)COOMe groups were axial. Thus, all four possible equatorial/equatorial, equatorial/axial, axial/equatorial, and axial/axial species were experimentally observed depending on the molecular environment. These unusual results will be presented in more detail elsewhere.[18] Nevertheless, since the major species (87%) by NMR in CD_2Cl_2, is equatorial/equatorial and this species is also present in high (45%) concentration in D_2O, this suggests that an equatorial N-methyl group impairs binding to the dopamine transporter. The likely explanation for this is the positioning of the N-methyl group in the region required for the ammonium hydrogen as previously proposed.

Table II. Computed energies of species[a] of 6a with low and high values of the dielectric constant

minimized species D = 1.5	steric energy (kcal/mole)	minimized species D = 80	steric energy (kcal/mole)
CH(Ph)COOMe equatorial/N-methyl equatorial			
[69,176]	28.5	[73,174]	22.0
[164,160]	29.0	[162,156]	22.1
[-67,154]	27.2	[-64,154]	25.2
CH(Ph)COOMe equatorial/N-methyl axial			
[71,172]	32.9	[74,170]	26.0
[-177,165]	27.2	[179,157]	22.0
[-48,164]	30.0	[-48,172]	27.1
CH(Ph)COOMe axial/N-methyl equatorial			
[73,169]	33.9	[74,169]	27.1
[167,157]	29.9	[166,151]	23.6
[-67,142]	35.5	[-65,139]	32.4
CH(Ph)COOMe axial/N-methyl axial			
[70,170]	35.5	[73,169]	28.2
[180,167]	28.4	[176,159]	22.9
[-55,146]	34.6	[-55,151]	31.8

[a]Energy minimized values for torsion angles [C1-C2-C3-C4, C2-C3-C4-O5] (see **6** in Scheme) in degrees. These are for the active *R,R*-enantiomer and are the same as the same torsion angles used in ref 12. Note that the two torsion angles in ref 12 were accidentally interchanged so that the global minimum is [-177,172] rather than [172,-177] as previously reported.

Chemistry. Reagents and solvents were mostly reagent grade and used without further purification. Column chromatography was carried out with Fisher Scientific silica gel (Grade 62) or neutral alumina (60-325 mesh). Melting points were obtained using a Laboratory Devices Mel-Temp II without corrections. NMR spectra (300 and 500 MHz) were measured on Varian Gemini 300 and Bruker DMX-500 spectrometers. Mass spectra were measured on a VG 70-SE, 2 sector, forward geometry instrument. IR spectra were recorded on a Nicolet 520 FT spectrophotometer. Elemental analyses were obtained from Atlantic Microlabs, Atlanta, GA.

(±)-*threo*-N-Methylmethylphenidate (6a). A solution of 230 mg (0.99 mmol) of (±)-*threo*-**5a** in 20 mL of MeOH was treated with 0.095 mL (1.28 mmol) 37% HCHO solution and allowed to stand at room temperature for 20 min. MeOH (5 mL), HOAc (0.03 mL) and 5% Pd/C (45 mg) were added and the resultant mixture was treated with H_2 gas at 35 psi for 1.5 h at room temperature. (Mild catalytic hydrogenation was used to N-methylate rather than standard Eschweiler-Clarke conditions with HCHO/HCOOH which produced a complex mixture of products.) The mixture was filtered and the filtrate and washings were evaporated in vacuo. The residue was mixed with 5 mL of water made basic with 15% NaOH solution and extracted with EtOAc (3 x 100 mL). The extracts were combined, washed with water, dried with $MgSO_4$, and evaporated in vacuo. The residue was chromatographed on 5 g of silica gel (2% MeOH/$CHCl_3$) to give 190 mg (80%) of pure title compound (TLC, one spot R_f = 0.38 in 2% MeOH/$CHCl_3$) as a solid, mp 31-32°. ^{1}H NMR ($CDCl_3$) δ 1.6–1.1 (m, 6H), 2.40 (s, 3H, CH_3), 2.98–2.90 (m, 1H), 2.59–2.42 (m, 1H), 3.1–3.08 (m, 1H), 3.62 (s, 3H, OCH_3), 3.88–3.73 (d, 1H), 7.4–7.2 (m, 5H, aromatic); MS-CI *m/z* 248.160, calcd for $C_{15}H_{22}NO_2$ (MH^+) 248.165. The HCl salt that was prepared from this material was not solid. However, a solid was obtained from HPLC purified material. In another experiment, a mixture of the *threo* and *erythro* isomers was dissolved in EtOAc (100 mg/mL) and separated on an HPLC column (Phenomenex Primesphere, 5m Silica 110, 21.2 mm x 250 mm; mobile phase 0.05% Et_2NH/EtOAc; flow rate 9 mL/min; UV detector at 268 nm). With a loading of ~100 mg, the less polar *erythro* isomer appeared at 12.1 min and the *threo* isomer at 14.5 min. The free base of the *threo* isomer was then dissolved (250 mg/10mL) in ether. To this was slowly added 1 M HCl/ether with swirling until no further precipitation occured. Solvent evaporation and vacuum drying gave an off-white solid. Anal. Calcd for $C_{15}H_{22}NO_2Cl$: C, 61.53; H, 7.92; N, 4.78; Cl, 12.11. Found: C, 61.90; H, 7.73; N, 4.69; Cl, 12.22.

(±)-Methyl *threo*-(4-methylphenyl)-(2-N-methylpiperidyl)acetate (6d). Similarly, 0.10 g (0.35 mmoles) of pure (±)-*threo*-**5d** was converted to the title compound yielding 0.080 g (88%) of a colorless oil which was pure by NMR; ^{1}H NMR ($CDCl_3$) δ 7.2–7.1 (m, 4H), 3.83 (d, *J*=9.9 Hz, 1H), 3.68 (s, 3H), 3.22–3.1 (m, 1H), 3.05–2.9 (m,1H), 2.6–2.5 (m, 1H), 2.4 (s, 3H), 1.8–1.0 (m, 6H). The material was dissolved in 2 mL of MeOH, a small excess of 12 M HCl added, and the solution evaporated to dryness. Treatment with Et_2O/acetone gave 0.080 g (87%) of a colorless crystalline solid, mp 195–196°. Anal. Calcd for $C_{16}H_{24}ClNO_2$ $0.25H_2O$: C, 63.56; H, 8.17; N, 4.63; Cl, 11.73. Found: C, 63.52; H, 8.12; N, 4.62, Cl, 11.80.

[^{3}H]-WIN 35,428 binding. Compounds were screened for affinity at the rat striatal dopamine transporter as previously described.[13]

Acknowledgment This work was supported by National Institute on Drug Abuse contract N01DA-4-8313 to MF, grant DA 06305 to HMD and MMS, and contract DA 09045 to CG. CG would also like to acknowledge the support of the Naval Research Laboratory. We would like to acknowledge the superb technical assistance of Adam Eason in the conduct of the [^{3}H]-WIN 35,428 binding assays.

References and Notes

1. Reith, M. E. A; Meisler, B. E.; Sershen, H.; Lajtha, A. *Biochem. Pharmacol.* **1986**, *35*, 1123.
2. Madras, B. K.; Kamien, J. B.; Fahey, M. A.; Canfield, D. R.; Milius, R. A.; Saha, J. K.; Neumeyer, J. L.; Spealman, R. D. *Pharmacol. Biochem. Behav.* **1990**, *35*, 949.
3. Boja, J. W.; Kuhar, M. J.; Kopajtic, T.; Yang, E.; Abraham, P.; Lewin, A. H.; Carroll, F. I. *J. Med. Chem.* **1994**, *37*, 1220.
4. Davies, H. M. L.; Kuhn, L. A.; Thornley, C.; Matasi, J. J.; Sexton, T.; Childers, S. R. *J. Med. Chem.* **1996**, *39*, 2554.
5. Blough, B. E.; Abraham, P.; Lewin, A. H.; Kuhar, M. J.; Boja, J. W.; Carroll, F. I. *J. Med. Chem.* **1996**, *39*, 4027.
6. Welch, W. M.; Kraska, A. R.; Sarges, R.; Koe, B. K. *J. Med. Chem.* **1984**, *27*, 1508.
7. Bøgesø, K. P.; Christensen, A. V.; Hyttel, J.; Liljefors, T. *J. Med. Chem.* **1985**, *28*, 1817.
8. Froimowitz, M. *J. Computat. Chem.* **1993**, *14*, 934-943.
9. Stoelwinder, J.; Roberti, M.; Kozikowski, A. P.; Johnson, K. M.; Bergmann, J. S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 303.
10. Kozikowski, A. P.; Saiah, M. K. E.; Bergmann, J. S.; Johnson, K. M. *J. Med. Chem.* **1994**, *37*, 3440.
11. Madras, B. K.; Pristupa, Z. B.; Niznik, H. B.; Liang, A. Y.; Blundell, P.; Gonzalez, Meltzer, P. C. *Synapse* **1996**, 24, 340.
12. Froimowitz, M.; Patrick, K. S.; Cody, V. *Pharm. Res.* **1995**, *12*, 1430.
13. Deutsch, H. M.; Shi, Q.; Gruszecka-Kowalik, E.; Schweri, M. M. *J. Med. Chem.* **1996**, *39*, 1201.
14. Allinger, N. L.; Yuh, Y. H.; Lii, J.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8551.
15. Technical Utilization Corporation, Inc., 235 Glen Village Court, Powell, OH 43065.
16. Glaser, R.; Peng, Q.-J.; Perlin, A. S. *J. Org. Chem.* **1988**, *53*, 2172.
17. Glaser, R.; Blumenfeld, J.; Geresh, S. *Magn. Reson. Chem.* **1993**, *31*, 845.
18. Glaser, R.; Shiftan, D.; Adin, I.; Deutsch, H. M.; Shi, Q.; George, C.; Wu, K.-M.; Froimowitz, M. (manuscript in preparation).

(*Received in USA* 28 *February* 1997; *accepted* 7 *April* 1997)