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[^{3}H]MFZ 2-12: A Novel Radioligand for the Dopamine Transporter

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Abstract—In an effort to develop a tritiated dopamine transporter radioligand with higher affinity than the widely used [^{3}H]WIN 35,428, we have synthesized [^{3}H]2β-carbomethoxy-3β-(3′,4′-dichlorophenyl)tropane ([^{3}H]MFZ 2-12). Unlabeled MFZ 2-12 and the *N*-demethylated intermediate (MFZ 2-13) inhibited dopamine uptake by the human dopamine transporter with IC_{50}'s of 1.1 and 1.4 nM, respectively. The N-nor-intermediate (MFZ 2-13) was treated with CT_3I resulting in [^{3}H]MFZ 2-12; S.A. = 80 Ci/mmol). [^{3}H]MFZ 2-12 reversibly bound with a K_D of 2.8 nM to human dopamine transporter expressed heterologously in EM4 cells. Published by Elsevier Science Ltd.

The inhibition of dopamine reuptake via the dopamine transporter has been characterized as the primary mechanism by which cocaine (**1**) produces its psychomotor stimulant actions.[1–4] In order to understand further the molecular mechanisms underlying the pharmacological actions of cocaine, as well as mechanisms that underlie its abuse, structure–function studies have been directed toward characterizing the dopamine transporter protein at a molecular level. Although the development of potent and selective radioligands has greatly aided in these studies, commercially available radiolabeled ligands are not ideal. Currently, the most commonly utilized radioligand for the dopamine transporter is [^{3}H]WIN 35,428.[5] Although this ligand has been useful for binding studies involving animal brain tissue, it has a relatively low affinity of ~40–50 nM.[6] This makes it less than ideal for binding studies with adherent heterologous cells expressing DAT, given the time required to manually wash the cells to remove free ligand. In contrast, [^{125}I]RTI 55, demonstrates significantly higher binding affinity and thus, in this way, overcomes the disadvantages of [^{3}H]WIN 35,428. Nevertheless, this radioiodinated ligand requires the use of ^{125}I, which also dramatically limits the useful lifetime of the ligand. Whereas other tritiated radioligands that may have advantages over [^{3}H]WIN 35,428 have been reported, they are not yet commercially available.[7–9]

In an effort to develop a novel tritiated radioligand with high affinity for the dopamine transporter, we reasoned that 2β-carbomethoxy-3β-(3′,4′-dichlorophenyl)tropane[10] would provide an excellent candidate for tritiation. This compound and its N-nor analogue had previously been reported to have high affinity at the dopamine transporter (K_i = 1.1 and 0.7 nM, respectively[10]) and could readily be prepared from cocaine.[11]

Compound MFZ 2-12 (**2**) was prepared according to Kozikowski et al.[11] from cocaine (**1**), in 34.5% yield. This yield is for the pure 2β-isomer,[12] which could be isolated from the 2α-isomer by column chromatography (Et_2O/TEA, 95:5). *N*-Demethylation using α-chloroethylchloroformate (ACE-Cl) in 1,2-dichloroethane, followed by methanolysis gave the N-nor compound MFZ 2-13 (**3**) in 76% yield.[13] Tritiation (American Radiolabeled Chemicals, St. Louis, MO) with CT_3I gave [^{3}H]MFZ 2-12 ([^{3}H] **2**) (99% pure by TLC: $CHCl_3$/MeOH, 85:15) with a S.A. = 80 Ci/mmol (Scheme 1).

MFZ 2-12 (**2**) and its N-nor-analogue MFZ 2-13 (**3**) inhibited [^{3}H]dopamine uptake in EM4 cells stably transfected with FlagHA-hDAT[14] with IC_{50}'s = 1.1 and

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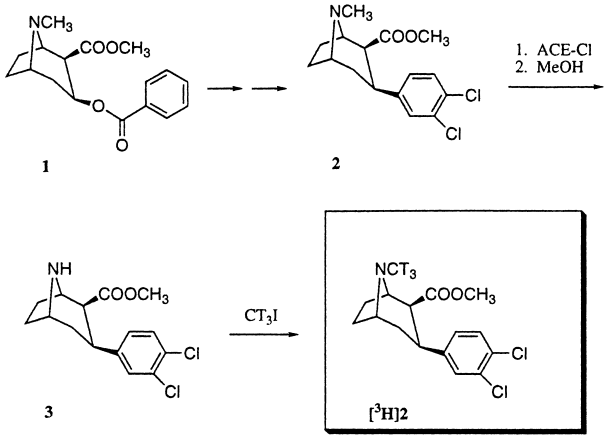
1.4 nM, respectively (Fig. 1). At room temperature, [^{3}H]MFZ 2-12 binding to EM4 cells stably expressing FlagHA-hDAT increased with time, reaching a plateau at 5 min (Fig. 2). Specific binding of [^{3}H]MFZ 2-12 to whole EM4 cells stably transfected with FlagHA synDAT was saturable with a $K_D = 2.8 \pm 0.98$ nM (Fig. 3). Cocaine competed with [^{3}H]MFZ 2-12 binding in EM4 cells expressing FlagHA-hDAT with a $K_i = 0.55 \pm 0.07$ μM (Fig. 4), which is comparable to its affinity to compete with [^{3}H]mazindol ($K_i = 0.54$ μM).[15]

In summary, [^{3}H]MFZ 2-12 is a novel, high affinity, tritiated ligand for the dopamine transporter. It has



Scheme 1.

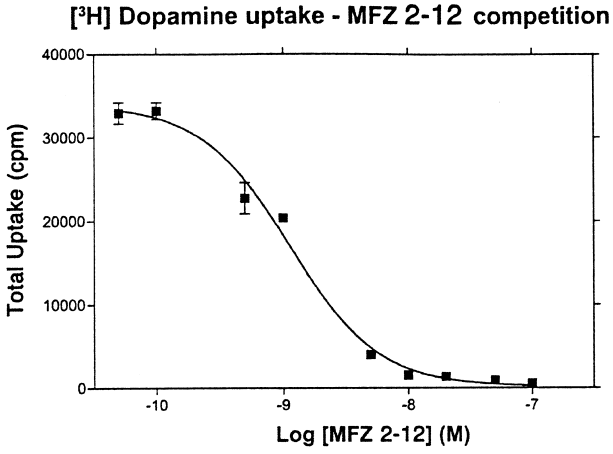


Figure 1. Inhibition of [^{3}H] dopamine uptake by cold MFZ 2-12. A 100% confluent 100 mm dish of EM4 cells stably transfected with FlagHA-hDAT were grown in 96 wells at 37 °C until they were confluent (48 h after plating). The FlagHA-hDAT cells were pre-incubated with 1 mM tropolone for 10 min at 37 °C. The plate was placed at 25 °C and washed twice with Hepes-Glucose buffer (130 mM NaCl,1.3 mM KCl, 10 mM Hepes, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.2 mM $CaCl_2$, and 10 mM glucose) containing 1 mM tropolone. In triplicate wells, the cells were pre-treated for 30 min at 25 °C with 10 different concentrations of cold MFZ 2-12 ranging from 50 pM to 100 nM. At the end of 30 min, 70 nM [^{3}H]dopamine (New England Nuclear, Boston, MA) was added to the wells to a final volume of 50 μL. The reaction mixture was incubated at 25 °C for 4 min and then was aspirated to terminate uptake. After 1 wash with ice cold buffer, cells were permeabilized with 50 μL 1% Triton X-100. Radioactivity was measured in a Trilux scintillation counter with Optiphase Supermix mixture (Wallac, Gaithersberg, MD). Specific uptake was defined as total uptake less nonspecific in the presence of 5 μM mazindol.

~20-fold higher affinity than [^{3}H]WIN 35,428, which has a K_D of approximately 40–50 nM.[14] Thus at a ligand concentration of 2.5 nM, ~50% occupancy is achieved with [^{3}H]MFZ 2-12 in contrast to ~6% by [^{3}H]WIN35,428. Since the nonspecific binding of these two ligands is a similar fraction of the total added counts and since they are of similar specific activity, at 2.5 nM the signal and the signal to noise ratio achieved with [^{3}H]MFZ 2-12 are approximately 10 times that

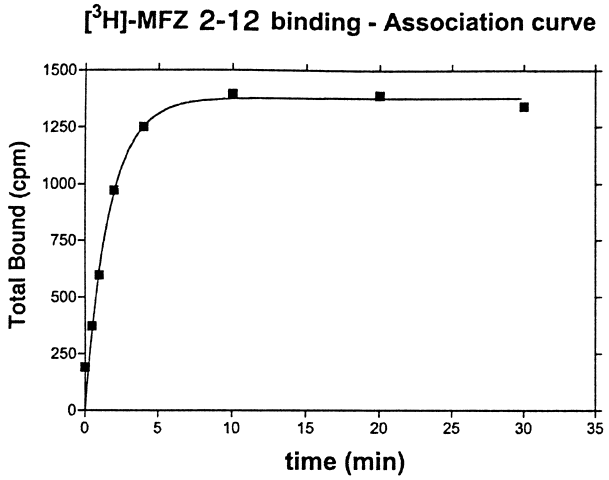


Figure 2. Association of [^{3}H]MFZ 2-12 to EM4 cells expressing FlagHA-hDAT. EM4 cells stably transfected with FlagHA synDAT were resuspended in 10 mL of Hepes-Glucose buffer. Triplicate polypropylene tubes contained 2.5 nM [^{3}H]MFZ 2-12 in a final volume of 75 μL. For association experiments, the mixture was incubated at 25 °C for the specified times and was then filtered, using Brandel cell harvester, through Whatman 934AH glass fiber filters (Brandel). The filter was presoaked in 0.25 polyethyleneimine (PEI) and was washed three times with 2 mL of cold 10 mM Tris–HCl, 120 mM NaCl (pH 7.4). Specific [^{3}H]MFZ 2-12 binding was defined as total binding less nonspecific binding in the presence of 5 μM mazindol.

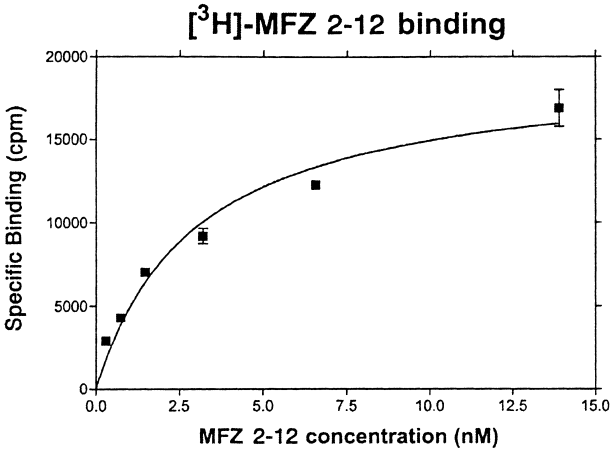


Figure 3. Saturation of specific [^{3}H]MFZ 2-12 binding to whole EM4 cells stably transfected with FlagHA synDAT. Duplicate polypropylene tubes contained six different concentrations of [^{3}H]MFZ 2-12 of between 0.5 and 15 nM with 125 μL of cell suspension in a final volume of 200 μL. The mixture was incubated at 25 °C for 40 min and then filtered, using a Brandel cell harvester, through PEI soaked Whatman 934AH glass fiber filters (Brandel). The filter was washed three times with 2 mL of cold 10 mM Tris–HCl, 120 mM NaCl (pH 7.4) at room temperature. Specific [^{3}H]MFZ 2-12 binding was defined as total binding less nonspecific binding in the presence of 5 μM mazindol.

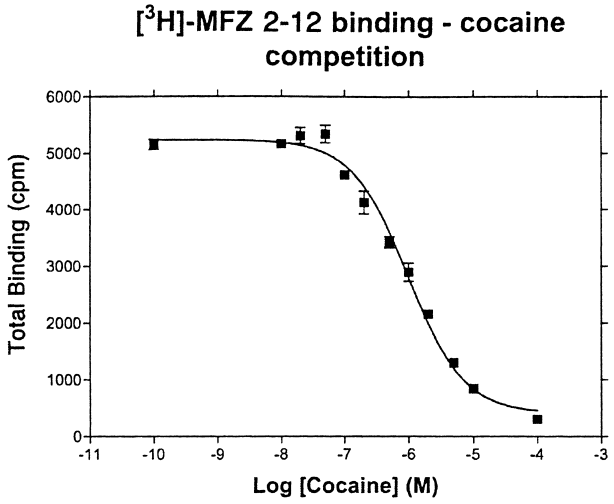


Figure 4. Competition of [^{3}H]MFZ 2-12 binding by cocaine. In duplicate tubes, EM4 cells expressing FlagHA-hDAT were treated with 2.5 nM [^{3}H]MFZ 2-12 in the presence of various concentrations of cocaine ranging from 10 nM to 10 μM. The reaction mixture was incubated for 40 min at 25 °C. Specific [^{3}H]MFZ 2-12 binding was defined as total binding less nonspecific binding in the presence of 5 μM mazindol.

observed with [^{3}H]WIN 35,428. For these reasons, [^{3}H]MFZ 2-12 is better suited for saturation analysis, filtration assays, and use with adherent cells expressing hDAT.

Acknowledgements

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12. Note 1: Compound **2** (MFZ 2-12): mp 83–84 °C. (lit.[10] mp 82.5–83.4 °C). ^{1}H NMR δ 1.55–1.72 (m, 2H), 2.02–2.28 (m, 2H), 2.22 (s, 3H, NCH_3), 2.50 (m, 1H), 2.83–3.00 (m, 2H), 3.38 (m, 1H), 3.53 (s, 3H, OCH_3), 3.58 (m, 1H), 7.10 (m, 1H), 7.30–7.43 (m, 2H) ppm; GC–MS *m*/*z* 327 (M^+); $[\alpha]_D^{23}$ −28.9° (*c* 1, MeOH), (lit. $[\alpha]_D^{21}$ −27.0°).[10]
13. Note 2: Compound **3** (MFZ 2-13): mp 101–102.5 °C, (lit.[10] mp 102–103 °C); ^{1}H NMR δ 1.58–1.80 (m, 3H), 1.95–2.40 (m, 4H), 2.74 (m, 1H), 3.18 (m, 1H), 3.44 (s, 3H, OCH_3), 3.72 (m, 2H), 7.08 (m, 1H), 7.26–7.36 (m, 2H) ppm; GC–MS *m*/*z* 313 (M^+); $[\alpha]_D^{24}$ −105.4° (*c* 1, MeOH), (lit $[\alpha]_D^{21}$ −108.1°).[10]
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