Preparation of ^{18}F-Labeled Muscarinic Agonist with M2 Selectivity

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Introduction. Upon post mortem examination of brain tissue from Alzheimer's patients, Quirion et al. observed selective loss of muscarinic receptors of the M2 subtype from cortical regions, although the M1 subtype is preserved.[1] The receptor subtypes were quantitated using saturation analysis: [^{3}H]pirenzepine for M1 subtype and [^{3}H]acetylcholine and [^{3}H]AF-DX 116 for the M2 subtype.

We are interested in developing a positron emitting analog of an M2 selective ligand for use in positron emission tomography (PET) because, in theory, the more selective the ligand the simpler the interpretation of the imaging data. The use of PET may allow the determination of M2 receptor subtype quantities in the living human brain and therefore confirm the postmortem findings in patients with Alzheimer's disease. This could lead to early diagnosis and the noninvasive monitoring of drug therapy.

Sauerberg et al., in their search for M1 selective agonists, prepared a number of agonists containing a thiadiazolyltetrahydropyridine (TZTP) moiety.[2] Some of the analogs displayed the desired M1 selectivity; however, the shorter *S*-alkyl derivatives appeared to display high affinity and selectivity for the M2 subtype. The highest M2 selectivity was found with the propyl side chain (3-(3-(propylthio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine [P-TZTP]). Sauerberg et al. used a combination of the [^{3}H]pirenzepine binding and the [^{3}H]oxotremorine binding to determining M1 selectivity. They also used the relative response in two pharmacological tests to determine M2 selectivity. The inhibition of twitch height in rabbit vas deferens was used as an indication of M1 activity, and the inhibition of force of contraction in guinea pig atrai was an indication of M2 action. P-TZTP displayed an IC_{50} of 0.9 nM against [^{3}H]oxotremorine methiodide and 4.0 nM against [^{3}H]pirenzepine. P-TZTP was only a weak inhibitor of the twitch height in rabbit vas deferens (M1 activity), but showed a IC_{30} of 50 nM in the inhibition of force of contraction in guinea pig atria (M2 activity).

Based on these data, especially the ability to displace [^{3}H]pirenzepine, we have prepared three fluorinated derivatives of TZTP, 3-(3-((2-fluoroethyl)thio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine [FE-TZTP] (**11**), 3-(3-((3-fluoropropyl)thio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine [FP-TZTP] (**10**), and 3-(3-((5-fluoropentyl)thio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine [FT-TZTP] (**9**), and tested their *in vitro* subtype selectivity. In addition, we prepared [^{18}F]FP-TZTP, 3-(3-((3-[^{18}F]fluoropropyl)thio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine [[^{18}F]FP-TZTP] ([**^{18}F**]**10**). *In vivo* biodistribution studies of [^{18}F]FP-TZTP showed moderate uptake into brain tissues and inhibition of brain uptake when coinjected with the competitive inhibitor 3-(3-(propylthio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine [P-TZTP].

Chemistry. The *S*-(fluoroethyl) and *S*-(fluoropropyl) compounds, **11** and **10**, respectively, were prepared from 3-(3-chloro-1,2,5-thiadiazol-4-yl)pyridine (**1**) by modification of Sauerberg's method (Scheme 1).[2] Conversion of the chloro derivative, **1**, into the corresponding thiol derivative, **2**, was unsuccessful using the recommended sodium hydrosulfide monohydrate[2] but was effected by heating with lithium sulfide in DMF. The appropriate fluoroalkanol tosylate, **6** or **7**, was used as the alkylation reagent. Following the alkylation, the crude product was methylated on the pyridine nitrogen and reduced with $NaBH_4$ according to the literature method.[2]

The *S*-(fluoropentyl) derivative, **9**, was prepared using a slightly different route (Scheme 1). We prepared the known 3-(3-chloro-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine oxalate salt (**3**). This compound was then converted to **9** by first treating with Li_2S and then with 5-fluoropentyl methanesulfonate (**8**).

Subsequently we isolated the intermediate thiol product, SH-TZTP, **12** (Scheme 2). The Li_2S reaction was conducted in ethanol rather than DMF. Following heating, the reaction was cooled, centrifuged, and filtered. Oxalic acid was added to the supernate and the intermediate, **12**, collected as a precipitate. No attempt was made to further purify this thiol.

Radiochemistry. For the labeling reaction, we utilized a two step synthesis displayed in Scheme 2. First, we prepared 1-[^{18}F]fluoropropyl toluenesulfonate ([**^{18}F**]**6**) according to literature methods.[3] The reaction solution containing [**^{18}F**]**6** was added to a suspension of **12** in DMF containing 6 μL of 8 M KOH. The resulting solution was heated for 5 min. The product was passed through a 1 mL C-18 BondElut to remove the bulk of the unreacted **12**. Final purification was achieved by HPLC (Axxiom C-18, 9.4 × 250 mm, eluted at 7 mL/min with 50% CH_3CN and 50% buffer (5 mM Et_3N and 5 mM NaH_2PO_4). The product was recovered from the HPLC eluate by passing the eluate through a 3 mL C-18 BondElut, which trapped most of the product, and subsequently eluting the product with ethanol. The synthesis required about 85 min. The average decay corrected (end of bombardment) radiochemical yield after collection from the 3 mL BondElut was 34 ± 4% (n = 5). The specific activity averaged 2300 Ci/mmol (EOB) (n = 2).

***In Vitro* Affinity.** *In vitro* affinity constants (K_i) for M1, M2 and M3 subtypes were determined by NovaScreen[4] using the appropriate tissue subtype assay with six concentrations between 10^{-6} and 10^{-10} of the test compound (Table 1). No assay is available for the pharmacologic M4 subtype due to lack of a selective ligand for this receptor. [^{3}H]Pirenzepine was used with bovine striatal membranes for M1;[5] [^{3}H]AF-DX 384 was used with rat heart membranes for M2,[6] and [^{3}H]-*N*-

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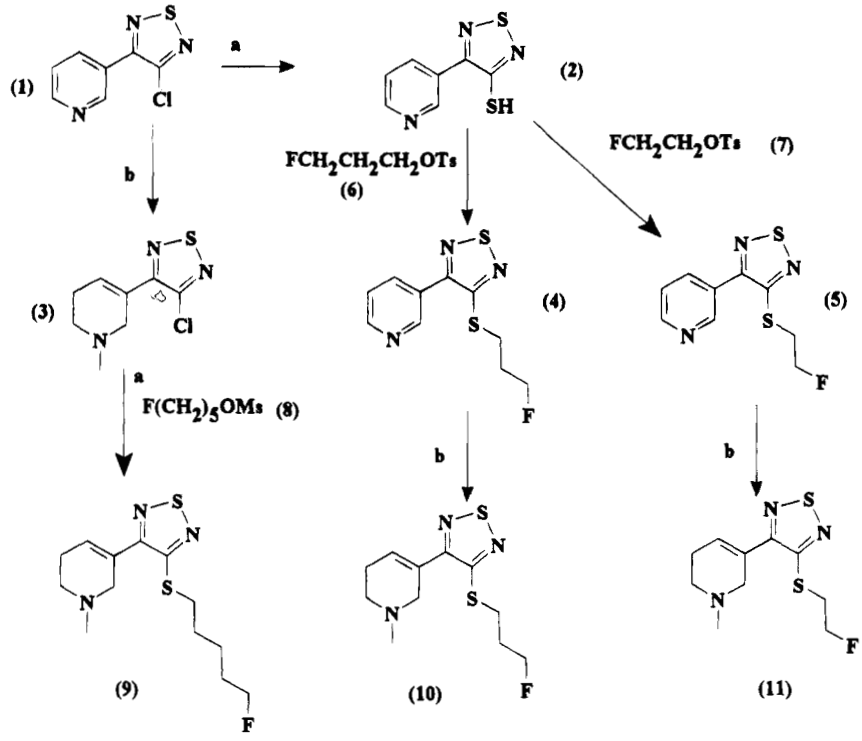
Scheme 1[a]



[a] (a) Li_2S, DMF; (b) CH_3I, $NaBH_4$.

Scheme 2

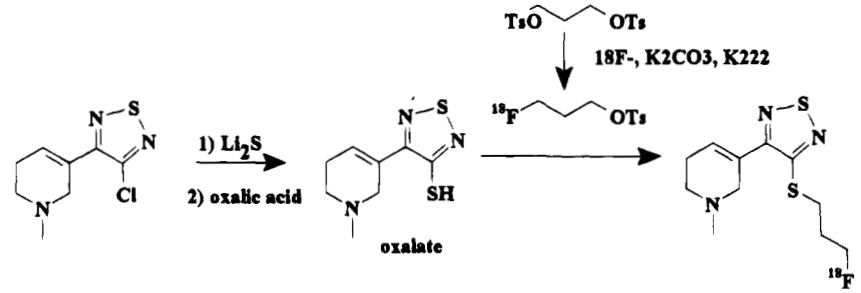


Table 1. *In Vitro* Affinity (K_i) for the Muscarinic Subtypes in nM As Determined by NovaScreen and Calculated by LIGAND[a]

	K_i (nM)			
compound	M1	M2	M3[b]	σ-1
P-TZTP	23 (40%)	1.5 (35%)	163	18.60 (0.9%)
FE-TZTP (**11**)	25 (13%)	1.8 (33%)	>10000	21.66 (19.2%)
FP-TZTP (**10**)	7.4 (16%)	2.2 (17%)	79.7	62.06 (5.5%)
FT-TZTP (**9**)	2.3 (69%)	6.3 (51%)	160	not assayed
T-TZTP	36 (35%)	4.0 (20%)	>10000	not assayed

[a] Values in parentheses are standard errors. [b] Values reported by NovaScreen. No recalculation was conducted.

methyl scopolamine was used with guinea pig ileum for M3.[7] We obtained *in vitro* affinity constants for P-TZTP and T-TZTP (3-(3-(pentylthio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine) for comparison. All compounds were screened for cross reactivity with other biogenic amine systems. The serotonin 1 system was the highest binding, but the K_i was only about 2 μM for all compounds except FT-TZTP which had a K_i of 0.3 μM. The affinity at σ-1 sites for compounds **10**, **11**, and P-TZTP were measured by displacement of [^{3}H]-(+)-pentazocine, a σ-1 selective probe, in guinea pig brain membrane[8] (Table 1). Sigma-2 affinity was assayed in rat liver tissue using the subtype nonselective ligand [^{3}H]-1,3-di-*o*-tolylguanidine in the presence of a blocking dose of σ-1 selective dextrallorphan.[9] The highest σ-2 affinity displayed by the three compounds was 400 nM for P-TZTP.

All samples were submitted for *in vitro* affinity to cells expressing cloned m1, m2, m3, and m4 subtypes.[10] All samples were reported to display K_i values of approximately 200 nM. These assays employ a labeled muscarinic antagonist ([^{3}H]-*N*-methylscopolamine). Agonists displace antagonists in these assays with low affinity.[11] The affinity state of the receptor and the coupling to the G protein may be important in obtaining meaningful inhibition data.

Biodistribution. The biodistribution of [^{18}F]**10** was determined in rats at 15, 60, and 120 min postinjection. The product was formulated in phosphate-buffered saline and contained less than 10% ethanol. [^{18}F]**10** (15 μCi) was injected into the tail vein of rats (Sprague–Dawley, 200–250 g), and at the appropriate time the animals were sacrificed in groups of five. The brain was dissected into 11 distinct regions. In addition, samples of blood, heart, bone, and lung were collected. The tissues were weighed, counted for activity, and % ID/g calculated. Co-injection studies of [^{18}F]**10** with 5, 50, and 500 nmol per rat of an appropriate inhibitor were evaluated at 60 min to assess selectivity of brain uptake.

Table 2. Brain Distribution (% ID/g) and Standard Deviation (std) of [^{18}F]FP-TZTP ([**^{18}F]10**) as a Function of Time (n = 5 or 6)[a]

tissue	15 min	std	60 min	std	120 min	std
blood	0.181	0.021	0.124	0.007	0.100	0.021
heart	0.324	0.031	0.121	0.005	0.096	0.016
bone	0.225	0.035	0.762	0.276	0.993	0.297
lung	0.871	0.114	0.261	0.019	0.156	0.025
LT-CTX	1.125	0.046	0.368	0.044	0.139	0.020
LT-HIPP	0.826	0.155	0.363	0.010	0.119	0.080
LT-STR	0.861	0.147	0.353	0.019	0.123	0.081
LT-THAL	0.667	0.063	0.293	0.013	0.153	0.017
PONS	0.824	0.089	0.385	0.080	0.238	0.078
MEDULL	0.788	0.741	0.438	0.029	0.226	0.034
CBLL	0.766	0.065	0.285	0.018	0.154	0.016

[a] Tissue abbreviations are defined as follows: LT, left; CTX, cortex; HIPP, hippocampus; STR, striatum; THAL, thalamus; MEDULL, medulla; CBLL, cerebellum.

Discussion. These compounds were evaluated using the pharmacological approach with subtype selective ligands and tissue known to contain high concentrations of that particular subtype. Levey[12] has compared the pharmacological approach, antibodies to the molecular subtype from cloning, and mRNA analysis. There is general agreement between the pharmacological approach and the cloned cell assay. The best agreement is in atria and cerebellum where the majority of the mACh receptors are of the m2/M2 subtype.

The *in vitro* affinites (K_i) for the muscarinic subtypes are shown in Table 1. P-TZTP displays a M2/M1 selectivity of 15, the highest of any tested. The introduction of the fluorine at the end of the chain in **10** did not affect the affinity constant but did reduce the selectivity of M2 over M1 to approximately 3.3. Compound **11** displays an *in vitro* selectivity of 13.9. Compound **9** displays a M2/M1 selectivity of 0.4.

The ability of some muscarinic ligands to interact at the σ sites has been observed.[13] The affinity of **10** at σ-1 sites should not interfere with muscarinic binding under the conditions of our *in vivo* experiments. The affinity for σ-1 sites is 27 times lower than for the muscarinic M2 subtype. In addition, the concentration of σ sites in the guinea pig and rat brain[14] are similar to the number of m2 sites.[15] Thus, one would expect no interference from σ sites in *in vivo* studies.

***In Vivo* Biodistribution.** We chose to label compound **10** over **11** based on Sauerberg's *in vivo and in vitro* data. Fortuitously, **10** displays lower σ-1 affinity that does **11**. The biodistribution in rats of **10** as a function of time is displayed in Table 2. The compound displays rapid uptake in the brain and also rapid clearance. The radioligand distributed relatively uniformly in the brain with uptakes ranging from 1.1% injected dose per gram (% ID/g) in the cortex to a low of 0.6% ID/g in the thalamus at 15 min postinjection. By 120 min the uptake is reduced to <0.2% in all regions except pons and medulla, which remain at slightly above 0.2%. The poor heart to blood ratio is most likely due to the rapidly clearing blood in the absence of a "blood-brain barrier" which prevents rapid efflux. Many of the high affinity β adrenoceptor ligands also show rapid clearance.[16] The displacement was carried out at 1 h after injection where the concentration in the heart had already decreased. The uptake in bone represents defluorination, which is often observed in rat studies with alkyl fluorides. The level is consistent with that found for fluorinated steroid studies in rats.[17] This high metabolic activity is not usually found in higher species.

Table 3. Brain Distribution of [^{18}F]FP-TZTP ([**^{18}F]10**) at 60 min as a function of Inhibitor Concentration (n = 5 or 6)[a]

tissue	nca	std	5 nmol	std	50 nmol	std	500 nmol	std
blood	0.126	0.012	0.121	0.011	0.112	0.002	0.123	0.008
heart	0.171	0.010	0.136	0.012	0.121	0.004	0.123	0.008
bone	0.501	0.055	0.520	0.034	0.519	0.036	0.441	0.032
lung	0.372	0.036	0.290	0.012	0.206	0.013	0.198	0.012
LT-CTX	0.484	0.154	0.315	0.052	0.115	0.006	0.080	0.005
LT-HIPP	0.429	0.078	0.408	0.072	0.160	0.022	0.106	0.008
LT-STR	0.448	0.021	0.342	0.062	0.146	0.012	0.092	0.009
LT-THAL	0.413	0.070	0.292	0.036	0.121	0.015	0.086	0.017
PONS	0.419	0.048	0.370	0.120	0.135	0.062	0.083	0.014
MEDULL	0.529	0.075	0.469[b]	0.083	0.158	0.016	0.074	0.006
CBLL	0.382	0.047	0.283	0.048	0.128	0.016	0.088	0.004

[a] The inhibitor used was P-TZTP. nca means no inhibitor was co-injected. For description of other abbreviations, see legend for Table 2. In the brain regions the statistical difference between each dose level was $p < 0.01$ unless noted. [b] $p < 0.06$.

Table 4. Brain Distribution of [^{18}F]FP-TZTP ([**^{18}F]10**) at 60 min as a function of Inhibitor Concentration (n = 5 or 6)[a]

tissue	nca	std	5 nmol	std	50 nmol	std	500 nmol	std
blood	0.13	0.00	0.13	0.01	0.12	0.01	0.11	0.01
LT-CTX	0.52	0.05	0.43	0.06	0.44	0.03	0.44	0.04
LT-HIPP	0.47	0.05	0.49	0.05	0.41	0.03	0.43	0.05
LT-STR	0.46	0.02	0.46	0.05	0.43	0.03	0.39	0.03
LT-THAL	0.45	0.08	0.44	0.04	0.42	0.03	0.47	0.04
PONS	0.55	0.10	0.54	0.05	0.52	0.03	0.53	0.06
MEDULL	0.63	0.05	0.63	0.07	0.61	0.03	0.58	0.04
CBLL	0.44	0.02	0.42	0.05	0.42	0.03	0.44	0.02

[a] The inhibitor used was (R,R) IQNB. nca means no inhibitor was co-injected. For description of other abbreviations, see legend for Table 2. All differences are not significant.

***In Vivo* Inhibition.** We chose to conduct co-injection studies at 60 min using P-TZTP, which exhibits high M2 selectivity *in vitro*. Co-injection with 5 nmol/rat of P-TZTP significantly reduced the uptake in all brain regions by 5–30% (Table 3). Injections of 50 and 500 nmol/rat resulted in proportionately larger reductions in the uptake of labeled compound in brain regions. The inhibition of uptake is nearly uniform across all brain regions.

The B_{max} for the various cloned subtypes has been determined in the literature and the m2 subtype, unlike m1, m3, and m4, has approximately the same concentration in all brain regions.[18] Since P-TZTP is M2 selective *in vitro*, the nearly uniform inhibition of the radioligands uptake suggests that [**^{18}F]10** is M2 selective *in vivo*. The dose dependent displacement of the radioactivity also argues for a receptor binding process. Because the affinity is low to other biogenic amine receptors, the binding in brain is dose dependent, and the distribution is uniform, we conclude that this saturable binding of compound **10** is to the M2 muscarinic cholinergic receptor.

Co-injection studies with the M1 selective antagonist (*R*,*R*)-IQNB showed no significant inhibition of uptake of [**^{18}F]10** in any brain tissue (Table 4). (*R*,*R*)-IQNB had affinities of 0.12 nM (SE 77%), and 3.6 nM (SE 28%) for M1 and M2, respectively, in the NovaScreen assay. This result is consistent with M2 selectivity for the radioligand. Co-injection with 500 nmol per rat of the M2 selective antagonist (*R*,*S*)-fluoromethyl QNB ((*R*)-azabicyclo[2.2.2]oct-3-yl-(*S*)-α-hydroxy-α-(4-(fluoromethyl)phenyl)benzeneacetate), did not show the expected

Table 5. Brain Distribution of [^{18}F]FP-TZTP ([**^{18}F]10**) at 60 min as a Function of Inhibitor Concentration (n = 5 or 6)[a]

tissue	nca	std	500 nmol	std
blood	0.18	0.01	0.17	0.02
CTX	0.70	0.12	0.44[b]	0.04
HIPP	0.65	0.06	0.43[c]	0.02
STR	0.64	0.10	0.39	0.02
THAL	0.56	0.11	0.48	0.03
PONS	0.63	0.05	0.56	0.05
MEDULL	0.71	0.12	0.61	0.01
CBLL	0.47	0.09	0.44	0.03

[a] The inhibitor used was (*R,S*)-fluoromethyl QNB. nca means no inhibitor was co-injected. For description of other abbreviations, see legend for Table 2. Differences between the two conditions were not statistically significant unless noted. [b] $p < 0.03$. [c] $p < 0.04$.

decrease in the uptake of **[^{18}F]10** (Table 5). This result may be explained by the difference in pharmacokinetics as the QNB analogs display a slower rate of uptake and clearance. (*R,S*)-Fluoromethyl QNB had affinities of 0.89 nM (SE 32%) and 0.13 nM (SE 35%) for M1 and M2, respectively, in the NovaScreen assay. There was a significant decrease in the cortex and hippocampus but not in other tissues. In addition, a pharmacological response to the antagonist was observed.

Alternatively, the binding model may be the primary factor. The binding of muscarinic agonists is more complicated than that of the antagonists.[19] From the *in vivo* studies, we cannot determine the exact binding state, but do know from experience[20] that the binding potential, i.e., the ratio of the receptor concentration to the K_d, must be high.[21] For muscarinic receptor concentrations in the rat brain, a K_d of less than 1 nM is required. The relatively low potency of (*R,S*)-fluoromethyl QNB to displace **[^{18}F]10** in the cortex and hippocampus may be due to these binding site differences.

Conclusion. We have synthesized a number of thiadiazole analogs of which **10** and **11** display M2 subtype selectivity *in vitro*. **[^{18}F]10** does not show inhibition *in vivo* by the M1 selective antagonist IQNB and shows only low inhibition by the M2 selective antagonist (*R,S*)-fluoromethyl QNB. We think the lack of inhibition by these antagonists is caused by the difference in agonist and antagonist binding sites and binding state of the receptor. Since the uptake of **[^{18}F]-10** and the inhibition of uptake by the M2 selective agonist P-TZTP is nearly uniform in all brain regions and the displacement is dose dependent, M2 selectivity is indicated for **[^{18}F]10**. This PET radiotracer displays high affinity with M2 selectivity *in vitro* and high blood brain barrier transport *in vivo*.

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Supplementary Material Available: Synthetic procedures and characterization of the unlabeled fluoroalkyl analogs, **9**, **10**, **11**, and of the preparation of **[^{18}F]10** are described (4 pages). Ordering information is given on any current masthead page.

References

(1) Quirion, R.; Aubert, I.; Lapchak, P. A.; Schaum, R. P.; Teolis, S.; Gauthier, S.; Araujo, D. M. Muscarinic receptor subtypes in human neurodegenerative disorders: focus on Alzheimer's disease. *Trends Pharmacol. Sci.* **1989** (Supplement), 80–84.
(2) Sauerberg, P.; Olesen P. H.; Nielsen, S.; Treppendahl, S.; Sheardown M. J.; Honoré, T.; Mitch, C. H.; Ward, J. S.; Pike, A. J.; Bymaster, F. P.; Sawyer, B. D.; Shannon, H. E. Novel functional M_1 selective muscarinic agonists. Synthesis and structure-activity relationships of 3-(1,2,5-thiadiazoyl)-1,2,5,6-tetrahydro-1-methylpyridines. *J. Med. Chem.* **1992**, *35*, 2274–2283.
(3) Block, D.; Coenen, H. H.; Stöcklin, G. The n.c.a. nucleophilic ^{18}F-fluorination of 1,N-disubstituted alkanes as fluoroalkylation agents. *J. Labelled Compd. Radiopharm.* **1987**, *24* (9), 1029–1042.
(4) NovaScreen, a division of Adheron Corp., 7170 Standard Dr., Hanover, MD 21706.
(5) Watson, M.; Roeske, W. R.; Yamamura, H. I. [^{3}H]pirenzepine and [^{3}H]quinuclidinyl benzilate binding to rat cerebral cortical and cardiac muscarinic cholinergic sites: II. Characterization and regulation of antagonist binding to putative muscarinic subtypes. *J. Pharmacol. Exp. Ther.* **1986**, *237*, 419–427.
(6) Eberlein, W. G.; Engel, W.; Mihm, G.; Rudolf, K.; Wetzel, B.; Entzeroth, M.; Mayer, N.; Doods, H. N. Structure-activity relationships and pharmacological profile of selective tricyclic antimuscarinics. *Trends Pharmacol. Sci.* **1989** Supplement, 50–54.
(7) Richards, M. H. Rat hippocampal muscarinic autoreceptors are similar to the M2 (cardiac) subtype: Comparison with hippocampal M1, atrial M2, and ileal M3. *Br. J. Pharmacol.* **1990**, *99*, 753–761.
(8) Bowen, W. D.; de Costa B. R.; Hellewell, S. B.; Walker, J. M.; Rice, K. C. [^{3}H](+)-Pentazocine: A potent and highly selective benzomorphan-based probe for σ-1 receptors. *Mol. Neuropharmacol.* **1993**, *3*, 117–126.
(9) Hellewell, S. B.; Bruce A.; Feinstein, G.; Orringer, J.; Williams, W., Bowen, W. D. Rat liver and kidney contain high densities of σ-1 and σ-2 receptors: Characterization by ligand binding and photoaffinity labeling. *Eur. J. Pharmacol. – Mol. Pharmacol.* **1994**, *Sect. 268*, 9–18.
(10) Zeeberg, B. R. Unpublished data.
(11) Freeman, S. B.; Harley, E. A.; Iverson, L. L. Biochemical measurement of muscarinic receptor efficacy and its role in receptor regulation. *Trends Pharmacol. Sci.* **1988**, Supplement, 54–60.
(12) Levey, A. I. Immunological localization of m1-m5 muscarinic acetylcholine receptors in perpheral tissues and brain. *Life Sci.* **1991**, *52*, 441–448.
(13) Hudkins, R. L.; DeHaven-Hudkins, D. L. M_1 muscarinic antagonists interact with σ recognition sites. *Life Sci.* **1991**, *49*, 1229–1235.
(14) Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R.; DeCosta B.; Rice, K. C. Sigma Receptors: Biology and Function. *Pharmacol. Rev.* **1990**, *42* (4), 355–402.
(15) Li, M.; Yasuda, R. P.; Wall, S. J.; Wellstein, A.; Wolfe, B. B. Distribution of m2 Muscarinic Receptors in Rat Brain using antisera selective for m2 Receptors. *Mol. Pharmacol.* **1991**, *40*, 28–35.
(16) Van Waarde, A.; Meeder, J. G.; Blanksma, P. K.; Bouwer, J.; Visser, G. M.; Elsinga, P. H.; Paans, A. M. J.; Vaalburg, W.; Lie, K. I. Suitability of CGP-12177 and CGP-26505 for quantitative imaging of β-adrenoceptors. *Nucl. Med. Biol.* **1992**, *19* (7), 711–718.
(17) French, A. N.; Napolitano, E.; VanBrocklin, H. F.; Hanson, R. N.; Welch, M. J.; Katzenellenbogen, J. A. Synthesis, radiolabeling and tissue distribution of 11β-fluoroalkyl- and 11β-fluoroalkoxy-substituted estrogens: target tissue uptake selectivity and defluorination of a homologous series of fluorine-18-labeled estrogens. *Nucl. Med. Biol.* **1993**, *20* (1), 31–47.
(18) Gitler, M. S.; Cohen, V. I.; De La Cruz, R.; Boulay, S. F.; Jin, B.; Zeeberg, B. R.; Reba, R. C. A novel muscarinic receptor ligand which penetrates the blood brain barrier and displays *in vivo* selectivity for the m2 subtype. *Life Sci.* **1993**, *53*, 1743–1751.
(19) Saunders, J. Ligand binding model for G-protein linked receptors: an update. In *Drug Design and Discovery*; Harwood Academic Pub. GmbH: New York, 1993; Vol. 9, pp 213–220.
(20) Eckelman, W. C.; Gibson, R. E. The design of site directed radiopharmceuticals for use in drug discovery. In *Nuclear Imaging in Drug Discovery, Development, and Approval*) Burns, H. D., Gibson, R. E., Dannals, R. F., Siegl, P. K. S., Eds.; Birkhauser Boston, Inc.: Boston, MA, 1992; pp 113–134.
(21) Mintun, M. A.; Raichle, M. E., Kilbourn, M. R.; Wooten, G. F.; Welch, M. J. A quantitative model for the *in vivo* assessment of drug binding sites with positron emission tomography. *Ann. Neurol.* **1984**, *15*, 217–227.

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