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Synthesis, in vitro and in vivo evaluation of [*O*-methyl-^{11}C] 2-{4-[4-(3-methoxyphenyl)piperazin-1-yl]-butyl}-4-methyl-2*H*-[1,2,4]-triazine-3,5-dione: A novel agonist 5-HT$_{1A}$ receptor PET ligand

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Abstract—Synthesis and in vivo evaluation of 2-{4-[4-(3-methoxyphenyl)piperazin-1-yl]-butyl}-4-methyl-2*H*-[1,2,4]triazine-3,5-dione (**5** or MMT), a high affinity and selective serotonin 5-HT$_{1A}$R agonist PET tracer, are described. GTPγS assay shows that MMT is an agonist with an EC$_{50}$ comparable to 5-HT. Radiolabeling of **5** was achieved in 30% yield (EOS) from desmethyl-MMT (**4**) with >99% chemical and radiochemical purities and a specific activity >1000 Ci/mmol. PET studies in baboon show that [^{11}C]**5** penetrates the blood–brain barrier but, because of low specific binding and fast clearance of radioactivity it is not a suitable PET tracer for the in vivo quantification of 5-HT$_{1A}$R.

The serotonin$_{1A}$ receptor (5-HT$_{1A}$R) has been implicated in the pathophysiology of major psychiatric and neurological disorders and the action of psychotropic medications such as antidepressants.[1–6] Successful radioligands studied to date for 5-HT$_{1A}$R are antagonists ligands such as [carbonyl-^{11}C]WAY100635 (WAY) [carbonyl-^{11}C]desmethyl-WAY100635 (DWAY) or *p*-[^{18}F]MPPF.[7–9] The major limitation of using antagonist tracers in imaging the 5-HT$_{1A}$R is that these have comparable affinity to both the G-protein-coupled high affinity state and uncoupled low affinity state of 5-HT$_{1A}$R. In contrast, agonist PET radiotracers bind preferentially to the HA state of the receptor, thereby providing a more meaningful functional measure of 5-HT$_{1A}$R.[10–12] An agonist tracer may also be more sensitive to changes in endogenous serotonin concentrations and allow measurement of 5-HT$_{1A}$R occupancy by agonist drugs. We have recently reported [^{11}C]MPT as a 5-HT$_{1A}$R agonist PET ligand and this has been evaluated in baboons.[13] Parallel to these studies, structural variants of MPT were examined in order to find an agonist radioligand possessing more favorable kinetics to quantify 5-HT$_{1A}$R in vivo. Here, we report the synthesis and evaluation of 2-{4-[4-(3-methoxyphenyl)piperazin-1-yl]butyl}-4-methyl-2*H*-[1,2,4]triazine-3,5-dione (**5** or MMT), a phenyl analogue of MPT as an agonist PET ligand for 5-HT$_{1A}$R. We have synthesized **5** from 6-azaurazil in 5 steps (Scheme 1). Synthesis of the intermediate 4-methyl-2*H*-[1,2,4]triazine-3,5-dione (**2**) was achieved in 76% yield by treating acetylated 6-azaurazil with methyl iodide in the presence of sodium hydride followed by deprotection with *p*-TsOH. Substitution of 4-chlorobutyl group in compound **2** was achieved in 70% yield by the addition of 1-bromo-4-chlorobutane in the presence of sodium hydride. The synthesis of MMT (**5**) has been accomplished by the condensation of 2-(4-chlorobutyl)-4-methyl-2*H*-[1,2,4]-triazine-3,5-dione (**3**) with 1-(3-methoxyphenyl)piperazine in 86%

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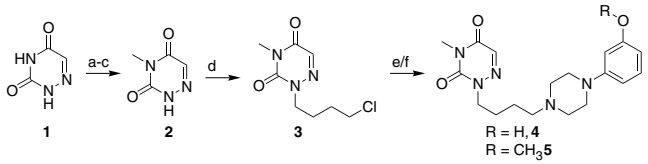


Scheme 1. Synthesis of **5** and desmethyl-MMT. Reagents and conditions: (a) $(CH_3CO)_2O$ (6 equiv), 130 °C, 1 h; (b) NaH, DMF, CH_3I, rt, 12 h; (c) *p*-TsOH, EtOH, 70 °C (3 steps, 85%); (d) NaH, DMF, $Br(CH_2)_4Cl$, 12 h, 75%; (e) 3-piperazin-1-ylphenol, TEA, BuOH, 70 °C, 75%; (f) 1-(3-methoxyphenyl)piperazine, TEA, BuOH, 70 °C, 86%.

yield. Under identical conditions, synthesis of desmethyl-MMT (**4**) was achieved by reacting **3** with 3-piperazin-1-yl-phenol in 75% yield.[14]

The affinity (K_i) and selectivity of **5** has been determined by radioligand binding assays through NIMH-psychoactive Drug Screening Program (PDSP). Results show that **5** has a K_i value of 1.1 nM for the 5-HT_{1A}R and has no appreciable affinity for a variety of biogenic amines, receptors, and transporters (Table 1).

We examined the agonist properties of **5** on 5-HT_{1A}R using [^{35}S]GTPγS binding in membranes of Chinese hamster ovary cells stably expressing the human 5-HT_{1A}R (CHO-h5-HT_{1A} cells).[15] Figure 1 shows the dose–response curves for 5-HT_{1A}R stimulated

Table 1. In vitro binding data of **5**

Target	K_i (nM)	Target	K_i (nM)
5-HT_{1A}	1.1 ± 0.09	5-HT_6	>10,000
5-HT_{2B}	33.7 ± 12.8	5-HT_{5a}	>10,000
Alpha $_{2C}$	109 ± 19	DOR	>10,000
Sigma1	180 ± 44	NET	>10,000
5-HT_{1B}	228 ± 77	AMPA	>10,000
Alpha $_{2A}$	250 ± 75	VMAT	>10,000
5-HT_7	328 ± 45	GABA	>10,000
Alpha$_{1B}$	378 ± 15	BZP	>10,000
Alpha $_{1A}$	413 ± 25	KO-R	>10,000
H_1	839 ± 90	MDR_1	>10,000
5-HT_{2A}	2100	EP	>10,000
H_2	2126	5-HT_{1E}	>10,000
D_2	2575	D_3	>10,000
D_5	>10,000	D_4	>10,000
A_1,A_2,A_3, A_4	>10,000	H_3, H_4	>10,000
HERG	>10,000	KA-R	>10,000
5-HT_3	>10,000	M	>10,000
mGluR	>10,000	NMDA	>10,000
NK	>10,000	NT	>10,000
Ca^+, Na^+ channels	>10,000	V	>10,000
DAT	>10,000	SERT	>10,000

A, adenosine; Alpha Beta; BZP, benzodiazepine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; V, vasopressin; CB, cannabinoid; D, dopamine; DAT, dopamine transporters; DOR: delta opioid receptors; EP, prostanoid receptors: GABA, Gamma-amino butyric acid; H, histamine; hERG, *Human Ether-a-go-go*; KOR, kappa opioid receptors; M, muscarinic; MDR, multidrug resist; MOR, mu opioid receptor; mGluR, metabotropic glutamate receptors; NMDA, *N*-methyl-D-aspartic acid; NK, neurokinin; SERT, serotonin transporter; VMAT, vesicular monoamine transporter; NET, norepinephrine transporter; NT, neurotrophin.

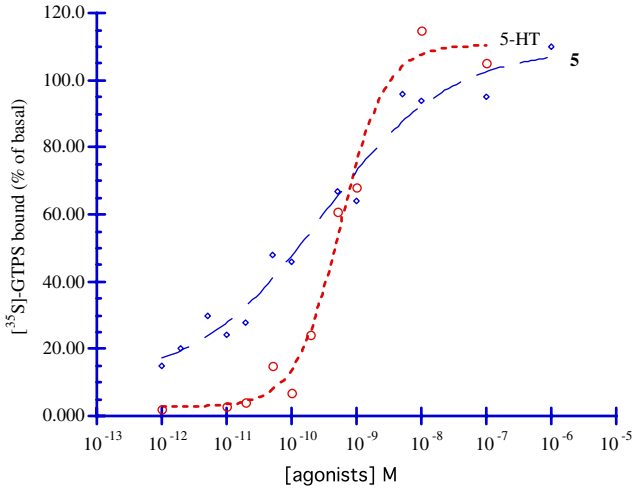
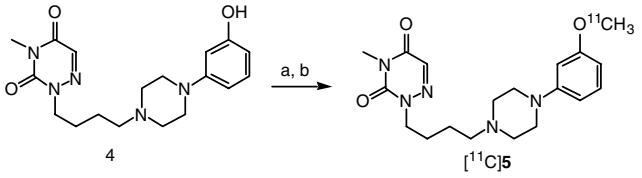


Figure 1. Effect of 5-HT_{1A}R agonist concentration on the stimulation of [^{35}S]GTPγS binding in CHO cells. Values are shown as expressed as a percentage above basal which is the binding of [^{35}S]GTPγS in the absence of agonists. Data points are means of duplicate determinations from representative experiments repeated on at least three independent occasions with similar results.

[^{35}S]GTPγS binding assay for **5** and 5-HT. The dose–response curves show an increased binding of [^{35}S]GTPγS with respect to the basal level. The maximum level of agonist stimulated binding of [^{35}S]GTPγS is comparable for **5** and 5-HT with an E_{max} 95% for **5** relative to 5-HT. The dose–response curve of MMT is also comparable to that of 5-HT with an EC_{50} of 0.3 and 0.7 nM, respectively. These results show that **5** is a 5-HT_{1A}R agonist with intrinsic activity similar to that of 5-HT. 5-HT, unlike **5**, showed a steeper slope at a lower concentration of the ligand.

Compound **5** was obtained by methylation of **4** using [^{11}C]MeOTf using standard radiolabeling procedures for phenols, that we previously optimized.[16] Optimum yields were obtained by treating **4** with [^{11}C]MeOTf in the presence of NaOH (Scheme 2).[17] The crude product was purified by reverse-phase HPLC followed by C-18 Sep-Pak® purification to obtain [^{11}C]**5** in 30% yield at the end of synthesis (EOS) (n = 4, SD = ±5). The formation of [^{11}C]**5** was confirmed by co-injecting the [^{11}C]-product with non-radioactive compound and comparing the HPLC retention times of the two compounds. Multiple mobile phases were used for determining the purities of the radioligand.[17] Specific activity obtained for [^{11}C]**5** was 1400 Ci/mmol (n = 3, SD = ±300) based on a standard mass curve.



Scheme 2. Radiosynthesis of [^{11}C]**5**. Reagents and conditions: (a) acetone, NaOH, [^{11}C]MeOTf, rt, 5′, heat 70 °C, 2′; (b) semi-PREP HPLC.