2-chloro-*N*-((*S*)-((*S*)-1-[^{11}C]methylpiperidine-2-yl)(thiophen-3-yl)methyl)-3-(trifluoromethyl)benzamide ([^{11}C]SA1) and derivatives

[^{11}C]SA1, [^{11}C]SA2, and [^{11}C]SA3

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Chemical name:	2-chloro-*N*-((*S*)-((*S*)-1-[^{11}C]methylpiperidine-2-yl)(thiophen-3-yl)methyl)-3-(trifluoromethyl)benzamide ([^{11}C]SA1) and derivatives
Abbreviated name:	[^{11}C]SA1, [^{11}C]SA2, and [^{11}C]SA3
Synonym:	
Agent Category:	Compounds
Target:	Glycine transporter 1 (GlyT-1)
Target Category:	Amino acid transporter
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	^{11}C
Activation:	No

Table continues on next page...

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Studies:	• *In vitro* • Rodents • Non-human primates	Structures of [^{11}C]SA1, [^{11}C]SA2 and [^{11}C]SA3 (1).

Background

[PubMed]

The amino acid (aa) glycine acts as a neurotransmitter in the mammalian central nervous system (CNS) and modulates the neuroexcitatory activity of the *N*-methyl-D-aspartate (NMDA) receptor (NMDAR) through strychnine-insensitive glycine binding sites located on the receptor (for details regarding structure and function of NMDAR, see Paoletti et al. (2)). Impaired functioning of the NMDAR is believed to be responsible for the cognitive dysfunction (e.g., loss of reasoning, hallucinations, etc.) observed in individuals suffering from neuropsychiatric ailments, such as schizophrenia and related disorders; improving the activity of this receptor has been shown to be therapeutic (3, 4). Because glycine is an obligatory co-agonist of the NMDAR in the presence of D-serine, the glycine binding site on the receptor is considered to be a suitable therapeutic target for the treatment of schizophrenia (4). Specific sodium chloride-dependent transporters are responsible for transporting glycine into the CNS. However, rapid sequestration into the nerve terminals and surrounding glial cells by two high-affinity transporters, designated as GlyT-1 and GlyT-2, block the activity of glycine on the NMDAR in the synapse (4). GlyT-1 has been shown to maintain low levels of glycine at the synapse, which indicates that the aa participates in controlling the process of neurotransmission through the NMDAR (4). It has been hypothesized that inhibition of GlyT-1 would increase glycine concentrations around the synapse (because by inhibiting the GlyT-1 transporter glycine will not be sequestered into the surrounding glial cells), which would enhance the activity of the NMDAR. The use of GlyT-1 inhibitors could be a good approach to treat schizophrenia and other related cognitive afflictions (5). In addition, researchers are interested in using noninvasive imaging techniques to study any changes in the brains of individuals suffering from neuropsychiatric disorders that may be associated with the GlyT-1. Therefore, several imaging agents, such as [^{18}F]2,4-dichloro-*N*-((1-(propylsulphony)-4-(6-fluoropyridine-2-yl)piperidine-4-yl)methyl)benzamide ([^{18}F]MK-6577 (6)) and

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[^{11}C]GSK931145 (7), that can be used with positron emission tomography (PET) were developed and successfully evaluated in non-human primates ([^{18}F]MK-6577 and [^{11}C]GSK931145) and humans ([^{11}C]GSK931145) for the *in vivo* visualization of GlyT-1. Toyohara et al. reported the development of three new ^{11}C-labeled non-sarcosine GlyT-1 inhibitors (2-chloro-*N*-((*S*)-((*S*)-1-[^{11}C]methylpiperidine-2-yl)(thiophen-3-yl)methyl)-3-(trifluoromethyl)benzamide ([^{11}C]SA1), *N*-((*S*)-((*S*)-1-[^{11}C]methylpiperidine-2-yl)(phenyl)methyl)thiophene-2-carboxamide ([^{11}C]SA2), and 2-chloro-*N*-((*S*)-((*S*)-1-[^{11}C]methylpiperidine-2-yl)(phenyl)methyl)-3-(trifluoromethyl)benzamide ([^{11}C]SA3)) and investigated the biodistribution of these tracers in mice (1). Among these radiolabeled compounds, [^{11}C]SA1 was evaluated with PET to visualize GlyT-1 in the brain of rhesus monkeys (*Macaca mulatta*).

Related Resource Links

Related chapters in MICAD

Human GlyT-1 variant 3 protein and nucleotide sequences (RefSeq database)

GlyT-1 in Online Mendelian Inheritance in Man (OMIM) Database

GlyT-1 in Kyoto Encyclopedia of Genes and Genomics Database

Clinical trials related to GlyT-1

NMDAR in PubMed

What is schizophrenia? (National Institute of Mental Health web site)

Treatment for schizophrenia (National Institute of Mental Health web site)

Synthesis

[PubMed]

The synthesis of SA1, SA2, and SA3 is detailed in international patent applications WO 03/089411 A1 (2003), WO 2005/037781 A2 (2005), and WO 2005/037792 A1 (2005) (1). The labeling of these compounds with ^{11}C has been described by Toyohara et al. (1). The radiochemical yields of [^{11}C]SA1, [^{11}C]SA2, and [^{11}C]SA3 were 50 ± 10%, 66 ± 22%, and 56 ± 3%, respectively. The radiochemical purity of each probe was >97%, as determined with high-performance liquid chromatography, and the specific activities of the tracers at 30 min after the end of bombardment (this was the total time taken for the synthesis, purification, and formulation of the labeled compounds) were 20–89 GBq/μmol (0.54–2.4 Ci/μmol).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The 50% inhibition concentrations of SA1, SA2, and SA3 that inhibited the uptake of [^{3}H]gly in mouse synaptosomes were determined to be 9.0 ± 2.0, 6,400 ± 1,930, and 39.7 ± 17.4 nM, respectively (1).

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{11}C]SA1, [^{11}C]SA2, and [^{11}C]SA3 was studied in normal mice (*n* = 4 animals/tracer per time point) after an intravenous injection of the labeled compounds (2.0 MBq/0.043–0.078 nmol (54 mCi/0.043–0.078 nmol) (1). The animals were euthanized at various time points ranging from 1 min to 60 min postinjection (p.i.), and the organs of interest were harvested to determine the amount of radioactivity accumulated in the various tissues (presented as percent of injected dose per gram tissue (% ID/g)). The amount of label detected in the different tissues is presented in Table 1.

Table 1: Amount of radioactivity from [^{11}C]SA1, [^{11}C]SA2, and [^{11}C]SA3 present in various tissues of mice.

Tissue	[^{11}C]SA1			[^{11}C]SA2			[^{11}C]SA3		
	Time (min) post injection								
	5	15	60	5	15	60	5	15	60
	% Injected dose/g tissue								
Blood*	0.7 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.0	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
Lung	29.0 ± 6.5	14.0 ± 1.4	3.6 ± 0.6	19.5 ± 1.7	8.0 ± 0.9	2.3 ± 0.4	24.4 ± 6.5	14.2 ± 2.1	3.8 ± 0.7
Liver	7.8 ± 1.0	12.5 ± 2.6	7.6 ± 1.4	4.6 ± 0.5	6.2 ± 0.8	6.4 ± 1.7	4.8 ± 0.5	5.1 ± 0.6	4.3 ± 0.4
Kidney	10.8 ± 1.0	6.2 ± 0.9	1.9 ± 0.4	12.8 ± 2.3	8.0 ± 0.7	3.4 ± 0.5	10.6 ± 0.7	7.5 ± 1.6	2.8 ± 0.6
Brain	2.1 ± 0.3	2.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.3 ± 0.1	1.6 ± 0.1	1.7 ± 0.2	0.9 ± 0.1

* **Data for other time points (1 min p.i. and 30 min p.i.) and tissues (heart, pancreas, spleen, small intestine, and muscle) is available in Toyohara et al.** (1)**.**

The patterns of uptake of radioactivity from the three probes in the different tissues were similar. In the brain, the uptake peaked at 15 min p.i. and gradually decreased thereafter. The uptake of label in the brain was higher with [^{11}C]SA1 than with either [^{11}C]SA2 (lowest uptake in brain) or [^{11}C]SA3. Time-activity curves for the medulla oblongata, cerebellum, and cortex for the three tracers showed there was a heterogeneous distribution of radioactivity in these regions of the brain, which was consistent with the level of GlyT-1 in these tissues: medulla oblongata > cerebellum > cortex.

In a blocking study, mice were injected with [^{11}C]SA1 (2.0 MBq/0.065 nmol (54 mCi/0.065 nmol)) and [^{11}C]SA3 (2.0 MBq/0.074 nmol (54 mCi/0.074 nmol)) in the presence

of 0.01, 0.1, and 1.0 mg/kg body weight unlabeled SA1 and SA3, respectively (n = 5 animals/concentration of unlabeled SA1 and SA3) (1). The animals were euthanized 15 min or 30 min later for [^{11}C]SA1 and [^{11}C]SA3, respectively, and the brains were harvested to determine the amount of radioactivity present in different regions of the organ (Table 2). Co-injection of [^{11}C]SA1 with unlabeled SA1 deceased uptake of the labeled probe significantly ($P < 0.05$) in the medulla oblongata and the cerebellum (GlyT-1 rich regions) compared to controls, whereas no such reduction in uptake was apparent with [^{11}C]SA3.

Table 2: Uptake of radioactivity in cerebellum and medulla oblongata from [^{11}C]SA1 and [^{11}C]SA3 in presence of different doses of unlabeled SA1 and SA3, respectively.

Probe	Brain tissue*	Control (no SA1 or SA3)	Unlabeled SA1 or SA3		
			0.01 mg/kg	0.1 mg/kg	1.0 mg/kg
		% Injected dose/g tissue			
[^{11}C]SA1	Cerebellum	1.98 ± 0.20	1.95 ± 0.33	1.76 ± 0.12	1.45 ± 0.30**
	Medulla oblongata	2.29 ± 0.30	2.38 ± 0.40	2.00 ± 0.23	1.72 ± 0.40**
[^{11}C]SA3	Cerebellum	1.72 ± 0.17	1.72 ± 0.26	1.62 ± 0.36	1.43 ± 0.21
	Medulla oblongata	2.18 ± 0.19	2.22 ± 0.36	2.27 ± 0.49	1.87 ± 0.34

* **For uptake in other tissues of the brain, see Toyohara et al.** (1). ** $P < 0.05$.

In another study, it was shown that pretreatment of the mice (n = 5 animals) with ALX-5407 (10 mg/kg body weight), a sarcosine-based irreversible inhibitor of GlyT-1, decreased the accumulation of radioactivity from [^{11}C]SA1 significantly ($P < 0.05$) in the cerebellum and the medulla oblongata of the animals compared to controls (1). No significant reduction in uptake of label in these brain tissues was noticed when the animals were pretreated with SSR504734, a non-sarcosine–based competitive inhibitor of GlyT-1. From these observations, the investigators concluded that endogenous concentrations of Gly in the mouse brain competed with SSR504734 and affected its binding to GlyT-1 in the tissues.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

Seven rhesus monkeys were used for PET imaging studies with [^{11}C]SA1 as described by Toyohara et al. (1). The animals were injected with 1,180–1,380 MBq/7.8–77.9 nmol (31.86–37.26 mCi/7.8–77.9 nmol) [^{11}C]SA1, and baseline PET images were acquired from conscious monkeys over the next 91 min. Another set of images were acquired for

the same duration after blocking with ALX-5407 (3 mg/kg body weight) or SSR504734 (3 mg/kg body weight) as described elsewhere (1). Magnetic resonance images were used to define the regions of interest (ROI) for the frontal cortex, thalamus, brain stem, and cerebellum on the PET images to calculate the standard uptake values (SUV) for each ROI. In addition, the total volume of distribution (V_T) of [^{11}C]SA1 for the brain tissues was evaluated as described elsewhere (1). From the baseline scans it was evident that the label peaked in the four regions of the brain between 10 min and 30 min p.i. and decreased slightly thereafter, indicating that the label exhibited reversible kinetics.

Table 3: Standardized uptake values (SUV) and total volume of distribution (V_T) of [^{11}C]SA1 at baseline and in rhesus monkeys brain tissue pretreated with ALX-5407 and SSR504734. Data obtained from Toyohara et al. (1).

Brain tissue	Standardized uptake value (SUV)			Total volume of distribution (V_T)		
	Baseline*	Pretreated (3 mg/kg)		Baseline	Pretreated (3 mg/kg)	
		ALX-5407	SSR504734		ALX-5407	SSR504734
Brainstem	1.5 ± 0.4	1.4 ± 0.2	1.3 ± 0.4	5.0 ± 2.3	4.3 ± 1.4	4.5 ± 2.1
Thalamus	1.7 ± 0.5	1.6 ± 0.1	1.6 ± 0.5	5.6 ± 2.6	5.0 ± 1.4	5.5 ± 2.6
Frontal cortex	1.3 ± 0.3	1.4 ± 0.1	1.3 ± 0.4	4.5 ± 2.0	4.5 ± 1.2	4.6 ± 2.1
Cerebellum	1.4 ± 0.3	1.3 ± 0.1	1.3 ± 0.3	4.5 ± 2.0	4.2 ± 1.0	4.4 ± 2.0

***For the baseline group, n = 7 animals; for the pretreated group, n = 4 monkeys/ GlyT-1 inhibitor.**

The baseline SUVs toward the end of the scans were thalamus ≈ brain stem > cerebellum ≈ frontal cortex (Table 3). The baseline parametric V_T images showed that the distribution of [^{11}C]SA1 in the brain was thalamus > brain stem > cerebellum ≈ frontal cortex (Table 3). All four regions of the brain from monkeys pretreated with ALX-5407 or SSR504734 showed little change in SUV or V_T of [^{11}C]SA1 compared to the baseline values (1). The investigators mentioned that the nearly complete lack of change in the SUV or the V_T values was probably because the dose of GlyT-1 inhibitors used in the monkey study (3 mg/kg body weight) was insufficient to block the transporter. For ethical reasons, higher doses of the inhibitors were not administered to the animals due to the possibility of inducing pharmacological side effects in the monkeys.

The investigators concluded that among the three ^{11}C-labeled GlyT-1 inhibitors evaluated in mice and non-human primates, only [^{11}C]SA1 showed a suitable imaging profile and could be used only in rodents (1). Blocking studies with this tracer in conscious monkeys showed that it was unsuitable to visualize GlyT-1 in the brain of non-human primates. Therefore, the chemical structure of [^{11}C]SA1 will have to be modified appropriately in order to use it as an *in vivo* imaging agent for the visualization of GlyT-1 in monkeys and humans (1).

Human Studies

[PubMed]

No publication is currently available.

Supplemental Information

[Disclaimers]

No information is currently available.

References

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