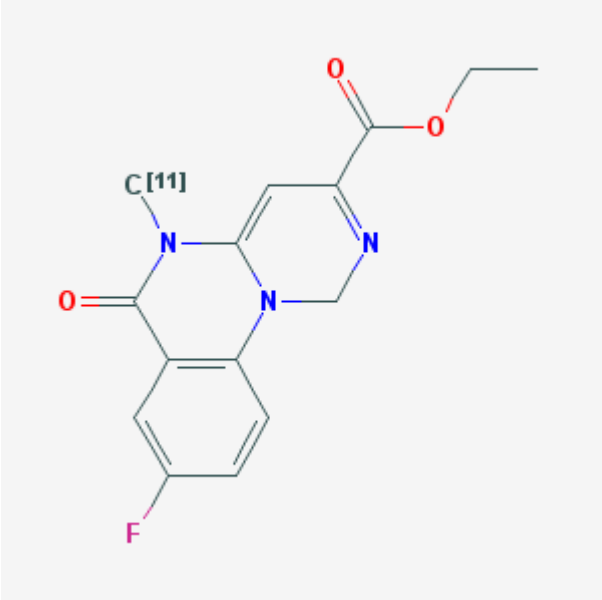
Ethyl 8-fluoro-5-[^{11}C]methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate

[^{11}C]FMZ

Arvind Chopra, PhD[1]

Created: April 28, 2008; Updated: June 10, 2008.

Chemical name:	Ethyl 8-fluoro-5-[^{11}C]methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate
Abbreviated name:	[^{11}C]FMZ
Synonym:	[^{11}C]Flumazenil; Romazicon®; Ro 15-5528
Agent Category:	Compound
Target:	Gamma-aminobutyric acid type A receptors ($GABA_A$ receptors)
Target Category:	Ligand-receptor binding
Method of detection:	Positron emission tomography (PET)
Source of Signal/ Contrast:	^{11}C
Activation:	No



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Studies:	• *In vitro* • Rodents • Non-human primates • Humans	Click on the above structure for additional information in PubChem.

Background

[PubMed]

The development of epilepsy has been associated with the impairment of gamma-aminobutyric acid (GABA) neurotransmission in the central nervous system because a reduced GABA release has been observed in the mesial temporal lobe during epilepsy episodes in animals and a GABA-mediated inhibition loss was reported in human epileptic hippocampal sclerosis (1, 2). The GABA receptors (GABAR) comprise several different pharmacological subtypes depending on the type of subunits constituting the receptor complex, and GABA mediates its effects primarily through the $GABA_A$ receptors (3). Also, individuals with the Angleman syndrome (AS) have a neurodevelopmental disorder that results in severe mental retardation, delayed motor development, and epilepsy (4). It has been shown that surviving *gabrag3*-knockout mice are epileptic and have a phenotype that is similar to AS patients, indicating that the *GABRβ3* gene in humans could have a role in the development of AS (5). Interestingly, genes coding for the various GABAR subunits (β3, α5, and γ3) are located within the same 15q11-q13 region of the human chromosome that is believed to have a function in the development of AS (5, 6), suggesting that the *GABARβ3* gene may have a role in the development of AS in humans. In addition, the $GABA_A$knockout mice were shown to have reduced levels of the $GABA_A$ receptor.

A benzodiazepine (BDZ) site antagonist labeled with radioactive carbon ([^{11}C]), ethyl 8-fluoro-5-[^{11}C]methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate ([^{11}C]flumazenil ([^{11}C]FMZ)), which has a high affinity for the $GABA_A$ receptors, has been used widely with positron emission tomography (PET) for the investigation of the

1 National Center for Biotechnology Information, NLM, NIH, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

NLM Citation: Chopra A. Ethyl 8-fluoro-5-[^{11}C]methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate. 2008 Apr 28 [Updated 2008 Jun 10]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

these receptors (7). Flumazenil is available commercially in the United States and has been approved by the United States Food and Drug Administration for use in various clinical trials.

Synthesis

[PubMed]

The synthesis of [^{11}C]FMZ has been described by Nagren and Halldin (8). Briefly, 8-fluoro-5,6-dihydro-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylic acid (also known as desmethylflumazenil or desmethyl-FMZ or Ro 15-5528) was mixed with [^{11}C]methyl triflate in a sealed reaction vessel at 0°C containing sodium hydroxide and acetone. The vessel was heated to 60°C for 1 min, and a mixture of acetonitrile:0.01 M phosphoric acid was injected into the vessel. The mixture was separated on a Waters reverse-phase high-performance liquid chromatography μ-Bondapak-C_{18} column, and the purified product was collected in a propylene glycol/ethanol mixture. The mobile phase was evaporated, and the residue was dissolved in propylene glycol/ethanol contained in physiological phosphate buffer (pH 7.4) and sterile filtered. The radiochemical yield (decay corrected) of this reaction was reported to be 65–75%. The radiochemical purity and specific activity of the product were not provided (8).

A robotic method for the synthesis of [^{11}C]FMZ has also been published (9). The synthesis was performed on an Anatech RB 86 robotic system in a vial containing Ro 15-5528, potassium fluoride, and aluminum oxide in acetonitrile (9). [^{11}C]Methyl iodide was trapped for 90 s in the vial, and the reaction mixture was kept at room temperature for another 120 s. A solid-phase extraction technique was used to separate [^{11}C]FMZ from its precursor (Ro 15-5528). This was followed by removal of the solvent in a stream of nitrogen at 130°C. The purified [^{11}C]FMZ was dissolved in an ethanol:saline mixture, and the solution was sterile filtered. The synthesis time was reported to be 30 min from the time of [^{11}C]methyl iodide trapping. The radiochemical purity of [^{11}C]FMZ was reported to be >99% with a specific activity of 11.1–59.2 GBq/μmol (0.3–1.6 Ci/μmol). The stability of the radiochemical was not reported (9).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The *in vitro* characteristics of [^{11}C]Ro 15-1788 were studied in membranes isolated from the rat forebrain (10). The various kinetic constants were determined with two different techniques involving a phosphorimager and γ-counter measurements. The dissociation constants (K_D) were reported to be 2.0 ± 0.9 and 2.1 ± 0.5 nM, respectively, by the two methods, and the number of binding sites (B_{max}) was determined to be 1.3 ± 0.5 and 1.7 ± 0.3 pmol/mg protein by the respective methods. In competition experiments the 50% inhibitory concentration (IC_{50}) of unlabelled Ro 15-1788 was reported to be 4.03 ± 0.75 nM.

Animal Studies

Rodents

[PubMed]

Using [^{11}C]FMZ, investigators have shown that the binding potential (B_{max}/K_D) of the $GABA_A$ receptors is reduced significantly over a 12-h period in the trauma-site cortex of rats subjected to a moderate lateral fluid percussion injury (11). This demonstrated that the GABA receptors were involved in cellular dysfunction as a result of the fluid percussion injury in these animals.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

Matsunaga et al. investigated the effect of yohimbine administration on BDZ receptor binding in the central nervous system of non-human primates (rhesus monkeys) (12). Using [^{11}C]FMZ, the investigators reported that yohimbine, an α2-adrenoceptor antagonist, increased the binding potential for the BDZ receptors in the monkey brain, indicating that a state of anxiety potentiated the effects of anxiolytics.

Human Studies

[PubMed]

Using [^{11}C]FMZ with PET in 11 patients with acute hemispheric ischemic stroke, Heiss et al. showed that this radiopharmaceutical is a suitable agent to distinguish between irreversibly damaged and viable neuronal tissue during early onset of the condition (13). In another study in 12 patients, the probability of predicting the cortical infarction in early ischemic stroke using PET *versus* diffusion-weighted magnetic resonance imaging (based on movement of water molecules, which is the method of choice to detect ischemic lesions) for the central BDZ receptors with [^{11}C]FMZ was determined (14). With results obtained from this study, the investigators concluded that, although the two methods were comparable, the probability of false-positive predictions were lower when PET with [^{11}C]FMZ was used.

Koepp et al. used [^{11}C]FMZ to demonstrate a reduction in the number of GABA/central BDZ receptors in the hippocampus of patients with mesial temporal lobe epilepsy caused by unilateral hippocampal sclerosis (15). These observations were later confirmed by

comparing *in vivo* [^{11}C]FMZ and *ex vivo* [^{3}H]FMZ binding in patients with hippocampal sclerosis with anterior temporal lobe resections (7).

[^{11}C]FMZ has also been used to estimate the synaptic density of BZD in five individuals who became blind at an early age (16). Compared with sighted control subjects, a significantly lower ($P < 0.01$) BDZ density was reported in the cerebellum of the blind subjects. However, the BZD density was comparable in the visual areas and other cortical regions of the blind patients and the controls, indicating a modification of the cerebellar neural circuitry in the blind individuals.

The binding of [^{11}C]FMZ was studied in four AS patients (17). Among these patients, three had a maternal deletion of 15q11-q13 leading to a loss of the β_3 subunit of the GABAR, and the fourth patient had a mutation in the ubiquitin protein ligase (*UBE3A*) but had a normal β_3 subunit gene. Compared to the fourth patient, a lower binding of [^{11}C]FMZ was observed in the frontal, parietal, hippocampal, and cerebellar regions of the brain of the first three patients, indicating that the 15q11-q13 deletion leads to a reduced number of GABAR in the AS patients. However, using [^{11}C]FMZ, Asahina et al. reported an increase in total expression of $GABA_A$ receptors in the cerebral cortex and the cerebellum of five AS patients with a deletion and one patient with a *UBE3A* mutation (18).

Supplemental Information

[Disclaimers]

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