# *N*-(Morpholin-4-yl)-5-(4-[$^{123}$I]iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide

## [$^{123}$I]AM281

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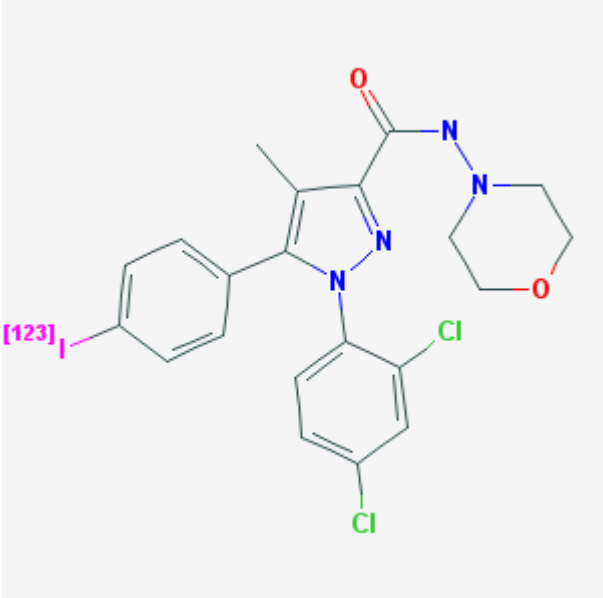
| **Chemical name:** | *N*-(Morpholin-4-yl)-5-(4-[$^{123}$I]iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide |
| **Abbreviated name:** | [$^{123}$I]AM281 |
| **Synonym:** | |
| **Agent Category:** | Compound |
| **Target:** | Cannabinoid CB1receptors |
| **Target Category:** | Receptor binding |
| **Method of detection:** | SPECT, gamma planar |
| **Source of signal:** | $^{123}$I |
| **Activation:** | No |
| **Studies:** | • *In vitro*<br>• Rodents<br>• Non-human primates<br>• Humans |

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## Background

[PubMed]

There are two subtypes of cannabinoid receptors in mammalian tissues: CB1 and CB2 (1, 2). CB1 receptors are expressed abundantly in neuronal terminals in the central nervous system (CNS) and peripheral tissues to inhibit neurotransmitter release. CB1 receptors are found predominately in the striatum, hippocampus, substantia nigra, globus pallidus, and cerebellum. CB2 receptors are present mainly on immune cells to modulate cytokine release. Both receptor subtypes are coupled through $G_{i/o}$ proteins to inhibit adenylate cyclase and to modulate potassium and calcium channels. CB1 receptors have been demonstrated to be involved in analgesia, regulation of food intake, and control of movement in normal subjects (3). Alteration of CB1 receptor function has been implicated in a number of human diseases such as depression, schizophrenia, and obesity (4-6).

Δ9-Tetrahydrocannabinol (THC) is a major active cannabinoid that is found in marijuana and activates CB1 receptors (7). THC has a very high lipophilicity (log *P*, 7), which causes imaging studies that use radiolabeled THC to be unsuccessful because of high nonspecific binding in the brain and slow brain entry. However, high lipophilicity is essential for binding to CB1 receptors, and moderate lipophilicity is required for crossing the blood–brain barrier (BBB). Existing radiolabeled ligands are mainly analogs of the antagonist rimonabant (SR141716A) and the agonist WIN 55,212-2; however, these ligands also exhibit high nonspecific binding, which limits their application in imaging (8). Therefore, there is a need to lower the lipophilicity of the radioligands with little effect on binding affinity and ability to cross the BBB. *N*-(Morpholin-4-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM281) is a potent and selective CB1 antagonist with nanomolar affinity and lower lipophilicity than its analog, SR141716A. *N*-(Morpholin-4-yl)-5-(4-[$^{123}$I]iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide ([$^{123}$I]AM281) is being developed as a single photon emission computed tomography (SPECT) agent for the non-invasive study of CB1 receptors in the brain (9, 10).

## Synthesis

[PubMed]

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Lan et al. (10) reported synthesis of [$^{123}$I]AM281 by radioiododestannylation reaction of the tributyltin precursor with [$^{123}$I]iodide in the presence of phosphoric acid and chloramine-T. Radiochemical yields were 50–60% after high-performance liquid chromatography purification. Specific activities were >185 GBq/μmol (>5 Ci/μmol) with a radiochemical purity of >95%.

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

Lan et al. (10) reported that AM281 had an inhibitory constant ($K_i$) value of 12 nM for CB1 receptors in rat forebrain membranes and a $K_i$ value of 4,200 nM for CB2 receptors in mouse spleen membranes, with a ratio of CB2/CB1 $K_i$ values of 340. THC had a $K_i$ value of 41 nM for CB1 receptors. The lipophilicity values were 3.7 for AM281 and 4.8 for SR141716A. Gatley et al. (9) reported that AM281 was 8-fold less potent than SR141716A in displacing [$^3$H]SR141716A from binding CB1 receptors in mouse cerebellum membranes. CB1 binding of [$^{123}$I]AM281 was inhibited by SR141716A, WIN 55,212-2, and THC with $K_i$ values of 1.8, 84, and 208, respectively.

## Animal Studies

### Rodents

[PubMed]

Gatley et al. (9) showed that there was good uptake of radioactivity in the mouse cerebellum after injection of [$^{123}$I]AM281, which reached a peak value of ~3.3% injected dose/g (ID/g) at 30 min and declining to ~1.9% ID/g at 60 min. The brain stem reached a peak value of ~2.6% ID/g at 30 min and declined more rapidly than the cerebellum to <1% ID/g at 60 min. Pretreatment with 2 mg/kg SR141716A before [$^{123}$I]AM281 injection reduced the cerebellum and brain stem uptakes to similar levels. *Ex vivo* autoradiography of rat brain showed the highest radioactivity in the substantia nigra, globus pallidus, and cerebellum, followed by cortex, striatum, thalamus, and brain stem at 120 min after [$^{123}$I]AM281 injection, consistent with the CB1 distribution in the brain.

### Other Non-Primate Mammals

[PubMed]

No publication is currently available.

### Non-Human Primates

[PubMed]

Biodistribution PET studies in anesthetized baboons injected with [$^{123}$I]AM281 were performed by Gatley et al. (9). These studies demonstrated the highest uptake in the

cerebellum, followed by the cortex and striatum. The peak level of radioactivity was reached in the cerebellum at ~20 min and declined with a half-life of 90 min. Pretreatment with SR141716A 10 min before [$^{123}$I]AM281 or 30 min after [$^{123}$I]AM281 accelerated the decline from the cerebellum between 20 and 60 min after injection.

## Human Studies

[PubMed]

Berding et al. (11) evaluated specific binding of [$^{123}$I]AM281 to CB1 receptors by SPECT imaging in six Tourette syndrome patients before and after THC treatment. The brain exhibited a low maximal uptake of 1.9% ID/g. Distribution volumes (DV) were obtained for the lentiform nuclei (3.5–3.8), occipital cortex (2.8–2.9), and white matter (3.0–3.1) using plasma input with the one-tissue compartment model and with Logan's graphical analysis. There were significantly lower DV values after therapy. However, using the occipital cortex or white matter as the reference region, no significant differences before or after therapy were detected in the lentiform nuclei by the specific to nonspecific partition coefficient $V_3$" (0.19–0.31). Only one patient with a marked clinical response showed a clear decline in $V_3$" value. After injection of [$^{123}$I]AM281, 70–75% of the total radioactivity in the blood was intact parent compound at 10 min, and 55–60% at 3 h. The main radioactive metabolite was more hydrophilic than [$^{123}$I]AM281. The organs that received the highest absorbed doses were found to be the upper large intestine (0.0461 mSv/MBq [170 mrem/mCi]), spleen (0.041 mSv/MBq [152 mrem/mCi]), and bone marrow (0.027 mSv/MBq [100 mrem/mCi]). The brain exhibited 0.0074 mSv/MBq (27 mrem/mCi). The effective dose was calculated as 0.011 mSv/MBq (40.7 mrem/mCi).

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## References

1. Howlett A.C., Barth F., Bonner T.I., Cabral G., Casellas P., Devane W.A., Felder C.C., Herkenham M., Mackie K., Martin B.R., Mechoulam R., Pertwee R.G.International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev. 2002;54(2):161–202. PubMed PMID: 12037135.
2. Pertwee R.G. Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol Ther. 1997;74(2):129–80. PubMed PMID: 9336020.
3. Pertwee R.G. Pharmacological actions of cannabinoids. Handb Exp Pharmacol, (168): p. 1-51. 2005.
4. Gambi F., De Berardis D., Sepede G., Quartesan R., Calcagni E., Salerno R.M., Conti C.M., Ferro F.M. Cannabinoid receptors and their relationships with neuropsychiatric disorders. Int J Immunopathol Pharmacol. 2005;18(1):15–9. PubMed PMID: 15698507.
5. De Vries T.J., Schoffelmeer A.N. Cannabinoid CB1 receptors control conditioned drug seeking. Trends Pharmacol Sci. 2005;26(8):420–6. PubMed PMID: 15992935.

6. Guzman M., Sanchez C. Effects of cannabinoids on energy metabolism. Life Sci. 1999;65(6-7):657–64. PubMed PMID: 10462066.
7. Martin B.R. Cellular effects of cannabinoids. Pharmacol Rev. 1986;38(1):45–74. PubMed PMID: 2872689.
8. Gifford A.N., Makriyannis A., Volkow N.D., Gatley S.J. In vivo imaging of the brain cannabinoid receptor. Chem Phys Lipids. 2002;121(1-2):65–72. PubMed PMID: 12505691.
9. Gatley S.J., Lan R., Volkow N.D., Pappas N., King P., Wong C.T., Gifford A.N., Pyatt B., Dewey S.L., Makriyannis A. Imaging the brain marijuana receptor: development of a radioligand that binds to cannabinoid CB1 receptors in vivo. J Neurochem. 1998;70(1):417–23. PubMed PMID: 9422389.
10. Lan R., Gatley J., Lu Q., Fan P., Fernando S.R., Volkow N.D., Pertwee R., Makriyannis A. Design and synthesis of the CB1 selective cannabinoid antagonist AM281: a potential human SPECT ligand. AAPS PharmSci. 1999;1(2):E4. PubMed PMID: 11741201.
11. Berding G., Muller-Vahl K., Schneider U., Gielow P., Fitschen J., Stuhrmann M., Harke H., Buchert R., Donnerstag F., Hofmann M., Knoop B.O., Brooks D.J., Emrich H.M., Knapp W.H. [(123)I]AM281 single-photon emission computed tomography imaging of central cannabinoid CB(1) receptors before and after Delta(9)-tetrahydrocannabinol therapy and whole-body scanning for assessment of radiation dose in tourette patients. Biol Psychiatry. 2004;55(9):904–15. PubMed PMID: 15110734.