Pharmacology and Toxicology.

General Description.

PBR28 and DAA1106 are selective aryloxyanilide ligands for PBR (Figure 1). Although called the "peripheral" benzodiazepine receptor for historical reasons, PBR is located both in the central nervous system (CNS) and many peripheral organs, including endocrine tissues, kidney, heart, liver and blood cells. In the CNS, PBR exists mostly but not exclusively in glial cells. In most of these organs, PBR is located in the outer membrane of mitochondria. In some organs, PBR shows other subcellular localizations. For example, PBR has been localized in mitochondrial inner membrane of guinea pig lung (Mukherjee and Das 1989). In rat liver, PBR has been localized in two sites: a mitochondrial and an unidentified non-mitochondrial location (O'Beirne et al 1990). In heart, PBR is located in the plasma membrane, where it is reported to be coupled to calcium channels (Mestre et al 1985).

PBR has been proposed to be involved in cellular proliferation, calcium channel activity, immune responses, transport of porphyrin and anion and regulation of steroid biosynthesis (Zisterer and Williams 1997). Because of the low mass dose administered in this protocol (10 µg per PET scan), an acute toxicity experiment in mice by a single intravenous administration was obtained from Tetsuya Suhara, MD, PhD (National Radiological Sciences, Japan) and literature search was performed on other possible pharmacological/toxicological effects studied in cultured cells, mice, rats, rabbits, guinea pigs and humans.

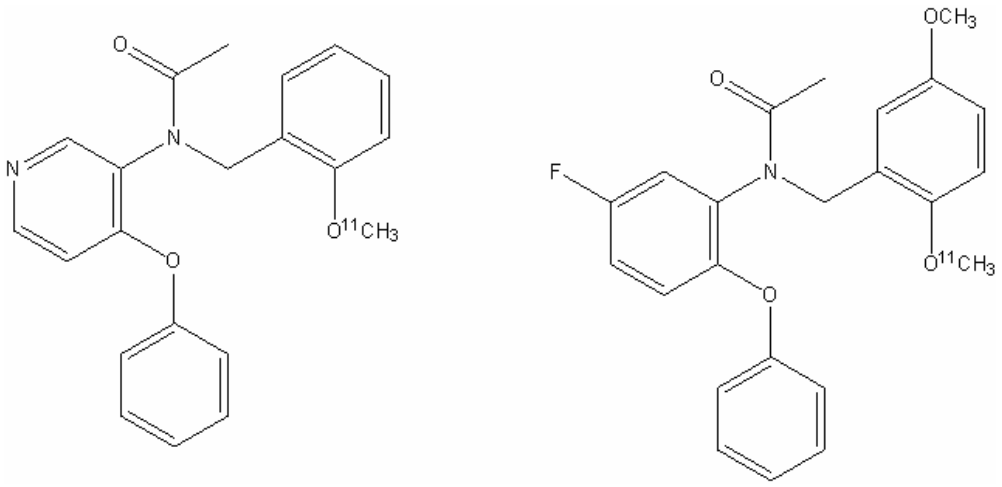
We have no formal toxicology data on PBR28. Instead, the following Section assesses the pharmacology and toxicology relative to two other PBR ligands:
a) DAA1106, which is a close chemical analog of PBR28 (Fig. 1).
b) PK11195, which is the prototypical PBR ligand and which has been used as a ^{11}C-labled probe in several human studies (see Section I) – and also studied in pharmacological doses in human subjects (see Section IV.F).

All three ligands (PK11195, DAA1106, and PBR28) are selective for PBR. Thus, the expected pharmacology of PBR28 is based upon the known effects of PBR agents. In addition, we have limited animal toxicology data on the closely related analog DAA1106.

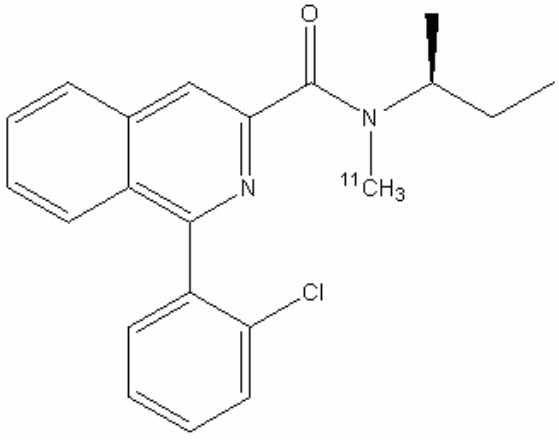
Comparison among these three ligands is made relative to their KI_i values. PBR28 was measured in our lab using *R*-(-)-[^{3}H]PK11195 as the radioligand and membranes prepared from rat brain, following a published procedure (Chaki et al 1999). The Ki values of the three compounds are fairly similar, with somewhat greater affinity for the two aryloxyanalides than PK11195:

PBR28: 0.2 nM (rat brain) (Briard et al 2005)
DAA1106: 0.04 (rat brain) & 0.2 nM (monkey brain) (Chaki et al 1999)
PK11195: 0.7 nM (rat brain) & 0.8 nM (monkey brain) (Chaki et al 1999)
[Unless otherwise noted, PK11195 refers to the racemic mixture.]

As described below (Section IV.F), PK11195 has been administered with no adverse effects to human subjects at doses of 10 mg IV and 100, 200, and 400 mg PO (with ~33% absolute bioavailability). By comparison, the currently proposed dose of up to 10 µg PBR28 is expected to be safe and lack pharmacological effects.



PBR28 DAA1106



K_i = 0.2 nM K_i = 0.04 – 0.2 nM

R-[^{11}C]-PK11195
K_i = 0.8 nM

Figure 1. Structures of three PBR-selective ligands.

In Vitro Receptor Binding.
Radioligand-binding assays were performed by using the resources of the NIMH-PDSP (Pharmacology Drug Screening Program). Detailed on-line protocols are available at the NIMH-PDSP web site (http://pdsp.cwru.edu). PBR28 was tested at 10 μM and found to have < 50% displacement of the target radioligand for the following receptors: 5HT1a, 5HT1b, 5HT1d, 5HT1e, 5HT2a, 5HT2b, 5HT2c, 5HT3, 5HT5a, 5JT6, 5HT7, alpa1a,

alpha1b, alpha2a, alpha2b, alpha2c, beta1, beta2, brya3, D1, D2, D3, D4, H1, H2, H3, H4, M2, M5DAT, NET and SERT.
As expected, PBR28 showed Ki > 10 μM for several "central" benzodiazepine receptor subtypes (i.e., the GABA-A receptor): alpha1beta1gamma2, alpha2beta2gamma2, alpga5beta2gamma2, and alpha6beta2gamma2.
Only one receptor showed >50% displacement at 10 μM: the kappa opiate receptor. A full displacement study showed Ki = 2.2 μM.

Acute 7-Day Intravenous Toxicity Study of DAA1106 in Mice

Dr. Tetsuya Suhara (Director of Neuroimaging at the National Institute of Radiological Sciences in Chiba, Japan) provided the following information on DAA1106.

Translation of the summary originally written in Japanese: Solutions of 20 mL/kg (10 mg/kg DAA) and the maximum injectable volume of 40 mL/kg (20 mg/kg DAA) were administered to mice through a tail vein at a rate of 1 mL/min. Three mice were used for each dose and DAA solution was administered once. To control mice, solutions of 20 and 40 mL/kg without DAA were administered. Mice were observed for 2 h after the administration, and once a day in the morning for seven days. Body weight was measured on the day of the administration and once a day in the morning for seven days after the administration.

Even the maximal dose of 20 mg/kg (corresponds to DAA1106 and PBR28 1.4 g/70 kg) DAA1106 caused only temporal decrease of locomotor activity and respiration, which were caused by the vehicle but not by DAA1106. No animal died. DAA1106 did not change body weight, and autopsy did not show significant changes. Therefore, it was concluded that the toxicity of DAA1106 was weak.

These results indicate that the mass dose of 740 MBq [^{11}C]DAA1106 with specific activity of more than 3.7 GBq/μmol to a 60 kg human subject is at least 1.5×10^4 smaller than the mass dose that did not show pharmacological effects. Therefore, no pharmacological effects are expected in human studies.