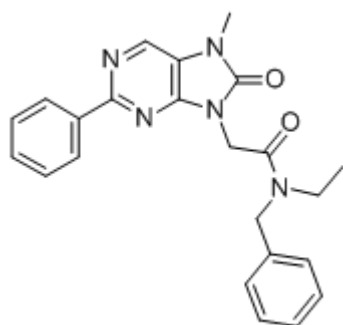


XBD173 (AC-5216, Emapunil)

XBD173 (AC-5216, Emapunil) acts as a selective agonist at the peripheral benzodiazepine receptor, also known as the mitochondrial 18 kDa translocator protein or TSPO. This protein has multiple functions, among which is regulation of steroidogenesis, particularly the production of neuroactive steroids such as allopregnanolone in the brain. In both animal and human trials, XBD173 produced rapid anxiolytic and anti-panic effects probably via newly synthesized neurosteroids, without producing sedation or withdrawal symptoms, and may represent a promising target for the development of fast-acting anxiolytics with a more favourable side-effect profile than benzodiazepines. TSPO expression may constitute a biomarker of brain inflammation and reactive gliosis that could be monitored by using TSPO ligands as neuroimaging agents.

N-benzyl-N-ethyl-2-(7,8-dihydro-7-methyl-8-oxo-2-phenyl-9H-purin-9-yl)acetamide



Biodistribution, Binding Affinity and Selectivity

<p>AC-5216 showed high affinity for mitochondrial benzodiazepine receptors in the crude mitochondrial fraction prepared from rat whole brain (K_i 0.297 nM), rat glioma cells (IC_{50} 3.04 nM) and human glioma cells (IC_{50} 2.73 nM), but only negligible affinity for central benzodiazepine receptors. AC-5216 bound to both the human and the rat glial mitochondrial benzodiazepine receptors with similar affinity, indicating that its binding properties do not differ between species.</p> <p>To further investigate the selectivity of AC-5216 for the mitochondrial benzodiazepine receptors, the binding affinity of this compound for other receptors, transporters or ion channels was examined in a total of 90 assays. AC-5216 at a concentration of 1 μM showed no noticeable affinity for any of the receptors, transporters or ion channels tested.</p>	<p>Kita A, Kohayakawa H, Kinoshita T, Ochi Y, Nakamichi K, Kurumiya S, Furukawa K, Oka M. Antianxiety and antidepressant-like effects of AC-5216, a novel mitochondrial benzodiazepine receptor ligand. Br J Pharmacol. 2004 Aug;142(7):1059-72.</p>
<p>After injection of ^{11}C-AC-5216 into mice, a high accumulation of radioactivity was found in the lungs, heart, adrenal glands, and other peripheral benzodiazepine receptor-rich organs. In the mouse brain, high radioactivity was observed in the olfactory bulb and cerebellum.</p> <p>A PET study of the monkey brain determined that ^{11}C-AC-5216 had a relatively high uptake in the occipital cortex, a rich peripheral benzodiazepine receptor-dense area in the primate brain.</p> <p>Pretreatment with nonradioactive AC-5216 and PK11195 reduced the radioactivity of ^{11}C-AC-5216 in the occipital cortex significantly, suggesting its high specific binding with peripheral benzodiazepine receptor in the brain.</p>	<p>Zhang MR, Kumata K, Maeda J, Yanamoto K, Hatori A, Okada M, Higuchi M, Obayashi S, Suhara T, Suzuki K. ^{11}C-AC-5216: a novel PET ligand for peripheral benzodiazepine receptors in the primate brain. J Nucl Med. 2007 Nov;48(11):1853-61.</p>

<p><i>In vitro</i> autoradiography of normal rat brain showed that ¹¹C-AC-5216 accumulated highly in the olfactory bulb, choroid plexus and cerebellum. The distribution pattern agreed with the localization of peripheral benzodiazepine receptor in the rodent brain.</p> <p>Infusion of kainic acid (1, 2.5 and 5 nmol) into the rat striatum resulted in neuroinflammation. <i>In vitro</i> and <i>ex vivo</i> autoradiography revealed that the radioactivity level of ¹¹C-AC-5216 was increased significantly in the kainic acid-lesioned striatum compared to the non-lesioned striatum.</p> <p>Increasing the amount of kainic acid infused into the striatum augmented radioactivity in the striatum as well as the cerebral cortex and hippocampus of the lesioned side.</p>	<p>Yanamoto K, Zhang MR, Kumata K, Hatori A, Okada M, Suzuki K. <i>In vitro</i> and <i>ex vivo</i> autoradiography studies on peripheral-type benzodiazepine receptor binding using [¹¹C]AC-5216 in normal and kainic acid-lesioned rats. <i>Neurosci Lett</i>. 2007 Nov 27;428(2-3):59-63.</p>
<p>The distribution of radioactivity in the living human brain was widespread and fairly uniform in the gray matter of the cerebral cortices and cerebellum, striatum, and thalamus as studied with ¹¹C-AC-5216 PET measurement, which was in good agreement with previous PET studies using peripheral benzodiazepine receptor ligand in the human brain.</p> <p>After an iv injection of ¹¹C-AC-5216, radioactivity peaked at about 2–3 min, followed by slow washout. The average percentage of unchanged ¹¹C-AC-5216 in plasma was 99.8% ± 0.2% at 3 min, 89.1% ± 4.5% at 30 min, and 69.6% ± 12.0% at 90 min.</p>	<p>Miyoshi M, Ito H, Arakawa R, Takahashi H, Takano H, Higuchi M, Okumura M, Otsuka T, Kodaka F, Sekine M, Sasaki T, Fujie S, Seki C, Maeda J, Nakao R, Zhang MR, Fukumura T, Matsumoto M, Suhara T. Quantitative analysis of peripheral benzodiazepine receptor in the human brain using PET with (11)C-AC-5216. <i>J Nucl Med</i>. 2009 Jul;50(7):1095-101.</p>
<p>The binding affinity of XBD173 for the Translocator Protein (TSPO) in human brain <i>in vitro</i> was investigated using tissue samples that have been previously characterized as high-, mixed- or low-affinity binders. The mean K_i value of XBD173 for high-affinity binders was 2.4 ± 0.67 nM, for mixed affinity binders was 10.5 ± 0.99 nM, and for low-affinity binders was 30.3 ± 8.7 nM. In summary, XBD173 binds to the TSPO protein with significantly different affinity, and this has important implications for the further clinical development of this compound.</p>	<p>Owen DR, Lewis AJ, Reynolds R, Rupprecht R, Eser D, Wilkins MR, Bennacef I, Nutt DJ, Parker CA. Variation in binding affinity of the novel anxiolytic XBD173 for the 18 kDa translocator protein in human brain. <i>Synapse</i>. 2011 Mar;65(3):257-9.</p>

Neuroinflammation

Studies concerning the potential of TSPO ligands in the CNS have focused primarily on its neuroprotective and anti-inflammatory actions in experimental models of excitotoxic and traumatic brain injury. TSPO expression may constitute a biomarker of brain inflammation and reactive gliosis that could be monitored. Whereas TSPO ligands as neuroimaging agents have become an important diagnostic tool for ischaemic stroke, there is only indirect evidence that TSPO ligands may also be useful for reducing cerebral infarction. TSPO is also an attractive drug target for controlling neuroinflammation.

There are ongoing trials with TSPO ligands for the treatment of chemotherapy-induced peripheral neuropathy and as an adjunct treatment in amyotrophic lateral sclerosis. Moreover, clinical studies with various TSPO ligands have been performed in patients suffering from diabetic neuropathy.

Reference:

Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D, Schumacher M (2010) Translocator protein (18 kDa) as a therapeutic target for neurological and psychiatric disorders. *Nature Reviews Drug Discovery* 9: 971-988

Anxiety, Panic Disorder and Depression

The selective TSPO ligand XBD173 enhanced gamma-aminobutyric acid-mediated neurotransmission and counteracted induced panic attacks in rodents in the absence of sedation and tolerance development. XBD173 also exerted antipanic activity in humans and, in contrast to benzodiazepines, did not cause sedation or withdrawal symptoms. Thus, translocator protein (18 kD) ligands are promising candidates for fast-acting anxiolytic drugs with less severe side effects than benzodiazepines.

<p>AC-5216 as well as diazepam produced anti-anxiety effects in one conditioned model of anxiety (Vogel-type conflict test) in rats and two unconditioned models of anxiety (light/dark box and social interaction tests) in mice. These effects of AC-5216 were antagonized by PK11195, a translocator protein (18 kD) antagonist.</p> <p>In the forced swimming test in rats, AC-5216 reduced the immobility time (comparable to that of desipramine), and this effect was blocked by PK11195.</p> <p>Unlike undesirable effects associated with conventional benzodiazepines, AC-5216 had no myorelaxant effects, did not affect the memory (passive avoidance response) or prolong hexobarbitone-induced sleep in mice, even at doses as high as 1000 mg/kg, p.o., although it did slightly prolong the ethanol-induced sleep time at 1000 mg/kg.</p> <p>AC-5216 (1–100 mg/kg, p.o.) slightly changed the EEG power density distribution in rat. However, these changes were not dose-related, frequency-specific or time-dependent. Whereas, diazepam, at an oral dose of 5 mg/kg, decreased the power of the low frequencies (below 10 Hz) and increased the power of the high frequencies (above 10 Hz).</p>	<p>Kita A, Kohayakawa H, Kinoshita T, Ochi Y, Nakamichi K, Kurumiya S, Furukawa K, Oka M. Antianxiety and antidepressant-like effects of AC-5216, a novel mitochondrial benzodiazepine receptor ligand. <i>Br J Pharmacol.</i> 2004 Aug;142(7):1059-72.</p>
<p>AC-5216 when repeatedly administered for two weeks does not induce tolerance to its anxiolytic-like effects or emotional and somatic withdrawal symptoms. In contrast, diazepam treatment withdrawal not only induced anxiogenic-like effects on the second day of the withdrawal period, but also decreased body weight gain and brought about body weight loss in mice.</p>	<p>Kita A, Kinoshita T, Kohayakawa H, Furukawa K, Akaike A. Lack of tolerance to anxiolysis and withdrawal symptoms in mice repeatedly treated with AC-5216, a selective TSPO ligand. <i>Prog Neuropsychopharmacol Biol Psychiatry.</i> 2009 Aug 31;33(6):1040-5.</p>
<p>XBD173 exerted acute anxiolytic effects, which were prevented by the TSPO antagonist PK11195, in the social exploration test and the elevated plus maze test in rats. Also, the subchronic administration of XBD173 (twice daily for 5 days) in the rat social exploration test could retain its anxiolytic activity.</p> <p>Both the benzodiazepine alprazolam and XBD173 effectively counteracted lactate- or cholecystokinin tetrapeptide (CCK4)-induced panic in rodent paradigms. No sedation was observed after treatment with XBD173, whereas alprazolam caused a marked reduction in locomotor activity. These preclinical studies suggest that XBD173 exerts rapid anxiolytic and antipanic effects with a more favourable side-effect profile than that of benzodiazepines.</p>	<p>Rupprecht R, Rammes G, Eser D, Baghai TC, Schüle C, Nothdurfter C, Troxler T, Gentsch C, Kalkman HO, Chaperon F, Uzunov V, McAllister KH, Bertaina-Anglade V, La Rochelle CD, Tuerck D, Floesser A, Kiese B, Schumacher M, Landgraf R, Holsboer F, Kucher K. Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. <i>Science.</i> 2009 Jul 24;325(5939):490-3.</p>

Pharmacology of XBD173

TSPO is a five transmembrane domain protein (18 kDa) that is expressed predominantly in steroid-synthesizing tissues. At the subcellular level, TSPO is localized at contact sites between the outer and inner mitochondrial membranes and mediates the rate-limiting step of neurosteroidogenesis by translocating cholesterol across the mitochondrial membrane.

TSPO drug ligands are able to stimulate the primary neurosteroid formations that enhance GABA_A receptor activity, pregnenolone and allopregnenalone, both in in vitro steroidogenic cells and in vivo animal models.

At the cellular level, the selective TSPO ligand XBD173 potentiated the amplitude and duration of GABA-mediated inhibitory postsynaptic currents in mouse medial prefrontal cortical neurons, which was prevented by finasteride.

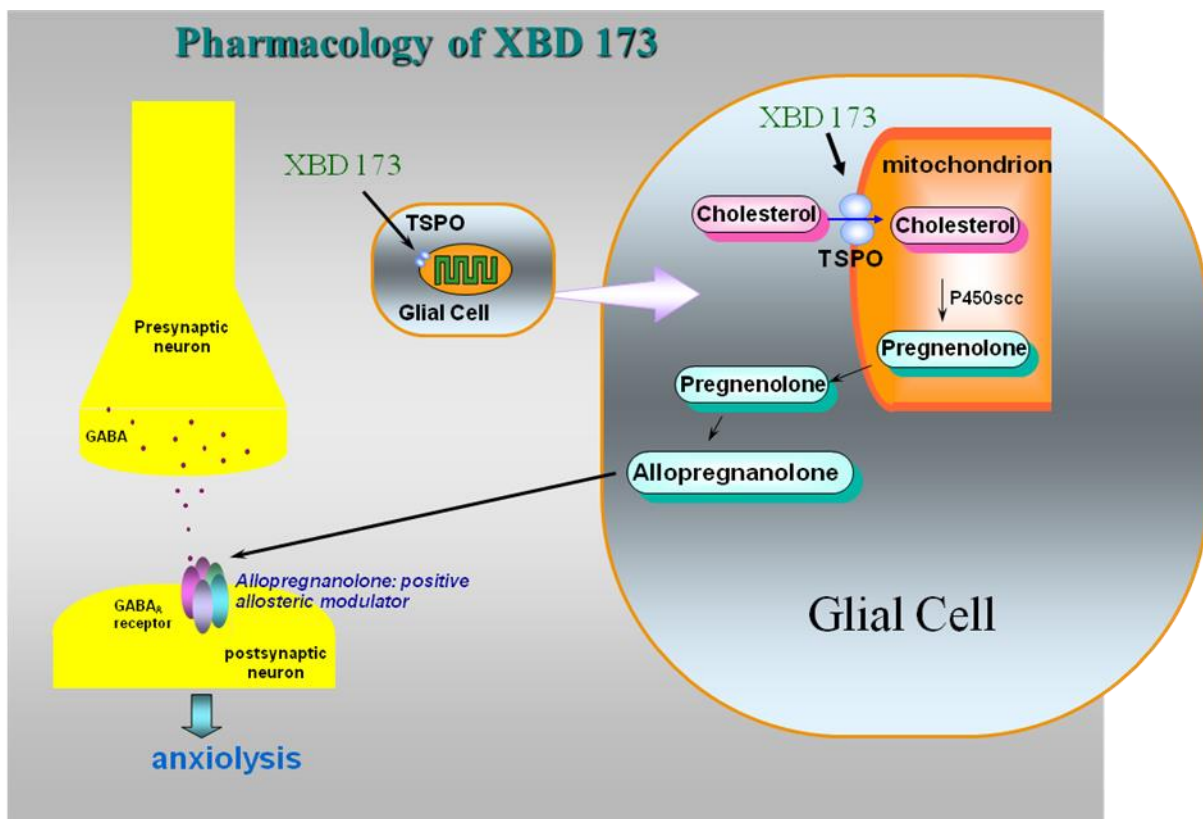
In contrast to diazepam, XBD173 did not act directly on postsynaptic GABA_A receptors expressed in human WSS1 cells expressing rat $\alpha_1\gamma_2$ and human β_3 GABA_A receptor subunits. These data provide further evidence that neurosteroidogenesis is involved in the differential effects of TSPO ligands on GABAergic neurotransmission.

Neurosteroids modulate GABA_A receptors via an allosteric site different from that targeted by benzodiazepines. These distinct sites of action at the GABA_A receptor might explain the lack of tolerance development and withdrawal symptoms after XBD173-induced neurosteroidogenesis.

References:

Rupprecht R, Rammes G, Eser D, Baghai TC, Schüle C, Nothdurfter C, Troxler T, Gentsch C, Kalkman HO, Chaperon F, Uzunov V, McAllister KH, Bertaina-Anglade V, La Rochelle CD, Tuerck D, Floesser A, Kiese B, Schumacher M, Landgraf R, Holsboer F, Kucher K. Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. *Science*. 2009;325(5939):490-3.

Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D, Schumacher M (2010) Translocator protein (18 kDa) as a therapeutic target for neurological and psychiatric disorders. *Nature Reviews Drug Discovery* 9: 971-988



Owen DR, Rupprecht R, Nutt DJ (2012) Stratified Medicine in psychiatry: a worrying example or new opportunity in the treatment of anxiety? *Journal of Psychopharmacology* (in press)

Clinical Trials

Panic Disorder

To investigate the anxiolytic potential, The TSPO agonist, XBD173, has been investigated in healthy male volunteers using the cholecystokinin tetrapeptide (CCK4) challenge – a human model of panic-like anxiety. CCK4 fulfills the criteria for an ideal panicogenic agent and the panic induction by CCK4 is sensitive to clinically effective anxiolytic agents, including benzodiazepines.

Out of 85 subjects, 71 healthy volunteers that showed a clear panic response to the neuropeptide CCK4 were randomized into one of five treatment arms (n=14 in each group) which consisted of 7 days treatment with either placebo, the benzodiazepine alprazolam (2 mg/day), or one of three doses of XBD173 (10, 30, or 90 mg/day). A second CCK4 challenge was performed following the dosing period. At the highest dose (90 mg/day), XBD173 attenuated CCK4-induced anxiety compared to placebo (p<0.036) to a degree similar in magnitude to the benzodiazepine.

The number of side effects reported with XBD173 was comparable to the incidence in the placebo group. In contrast, a much higher incidence was reported by the alprazolam treated group, in particular dizziness and somnolence. While 57% of the subjects treated with alprazolam complained of withdrawal symptoms such as sleep disturbances or restlessness, these were almost absent in the XBD173 treated groups.

Generalized Anxiety Disorder

A Phase II study on the efficacy, safety and tolerability of XBD173 has been conducted by Novartis in patients with generalized anxiety disorder (ClinicalTrials.gov identifier: NCT00108836).

Primary Outcome:

Mean reduction from baseline to week 6 in the combined score of items 1 and 2 of the Hamilton Anxiety Rating Scale (HAM-A). The difference on day 4 in effect between placebo and the individual doses of XBD173 on reduction in anxiety and depression. Pharmacokinetic assessments at baseline. Pharmacogenetic assessments at baseline. Pharmacogenomic and proteomic assessments at baseline. Metabonomic assessments at visits 4, 7 and 10.

Start Date: May 2005; Enrolment: 400 (18 Years to 65 Years); Status: completed on May 2006;

In this unpublished study, XBD173 showed no reduction compared with placebo in a variety of anxiety measures.

The lack of efficacy in this Phase II trial may lie in the choice of an inappropriate disease model – the CCK4 challenge resembles panic disorder much more closely than it does generalized anxiety disorder. But perhaps more important was a failure to appreciate differences between patients regarding the way in which the drug interacts with its target.

As with the majority of TSPO radioligands, XBD173 binding is also affected by the A147T substitution – the polymorphism reduces XBD173 binding affinity to TSPO by a factor of roughly 15 fold. This is highly relevant to the failed XBD173 generalized anxiety disorder study, as the preceding XBD173 CCK4 study showed efficacy only at the highest dose of 90mg, with no effect seen at doses of 30mg and below. The 15 fold reduction in binding affinity means that the same dose will produce huge differences in TSPO occupancy between subjects who differ with respect to the A147T substitution.

The clinical trial of XBD173 in generalized anxiety disorder was performed in a chiefly Caucasian population. Because 50% of Caucasians express at least one copy of the mutant allele, this means that half of the subjects in the XBD173 arm of the study were in fact receiving a sub-therapeutic dose of the drug.

In conclusion, repeating the study with dosing appropriate to genotype may yield more promising results. An alternative approach would be to enrol only those subjects likely to respond (ie AA subjects).

Source Data:

Rupprecht R, Rammes G, Eser D, Baghai TC, Schüle C, Nothdurfter C, Troxler T, Gentsch C, Kalkman HO, Chaperon F, Uzunov V, McAllister KH, Bertaina-Anglade V, La Rochelle CD, Tuerck D, Floesser A, Kiese B, Schumacher M, Landgraf R, Holsboer F, Kucher K. Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. *Science*. 2009;325(5939):490-3.

Owen DR, Lewis AJ, Reynolds R, Rupprecht R, Eser D, Wilkins MR, Bennacef I, Nutt DJ, Parker CA. (2011) Variation in binding affinity of the novel anxiolytic XBD173 for the 18 kDa translocator protein in human brain. *Synapse* 65(3):257-9.

Owen DR, Rupprecht R, Nutt DJ (2012) Stratified Medicine in psychiatry: a worrying example or new opportunity in the treatment of anxiety? *Journal of Psychopharmacology* (in press)