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Pharmacological Profile of the Selective FAAH Inhibitor KDS-4103 (URB597)

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ABSTRACT

In the present article, we review the pharmacological properties of KDS-4103 (URB597), a highly potent and selective inhibitor of the enzyme fatty-acid amide hydrolase (FAAH), which catalyzes the intracellular hydrolysis of the endocannabinoid anandamide. *In vitro*, KDS-4103 inhibits FAAH activity with median inhibitory concentrations (IC_{50}) of 5 nM in rat brain membranes and 3 nM in human liver microsomes. *In vivo*, KDS-4103 inhibits rat brain FAAH activity after intraperitoneal (i.p.) administration with a median inhibitory dose (ID_{50}) of 0.15 mg/kg. The compound does not significantly interact with other cannabinoid-related targets, including cannabinoid receptors and anandamide transport, or with a broad panel of receptors, ion channels, transporters and enzymes. By i.p. administration to rats and mice KDS-4103 elicits significant, anxiolytic-like, antidepressant-like and analgesic effects, which are prevented by treatment with CB1 receptor antagonists. By contrast, at doses that significantly inhibit FAAH activity and substantially raise brain anandamide levels, KDS-4103 does not evoke classical cannabinoid-like effects (e.g., catalepsy, hypothermia, hyperphagia), does not cause place preference, and does not produce generalization to the discriminative effects of the active ingredient of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC). These findings suggest that KDS-4103 acts by enhancing the tonic actions of anandamide on a subset of CB_1 receptors, which may

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normally be engaged in controlling emotions and pain. KDS-4103 is orally available in rats and cynomolgus monkeys. Sub-chronic repeated dose studies (1500 mg/kg, *per os*) in these two species have not demonstrated systemic toxicity. Likewise, no toxicity was noted in bacterial cytotoxicity tests *in vitro* and in the Ames test. Furthermore, no deficits were observed in rats on the rotarod test after acute i.p. treatment with KDS-4103 at doses up to 5 mg/kg or in a functional observation battery after oral doses up to 1500 mg/kg. The results suggest that KDS-4103 will offer a novel approach with a favorable therapeutic window for the treatment of anxiety, depression and pain.

INTRODUCTION

The Endocannabinoid System

The brain endocannabinoid signaling system is composed of three main elements. The first is represented by the G protein-coupled receptors that bind endogenous and exogenous cannabinoid ligands. Two such receptors have been molecularly cloned — the CB_1, which is found almost everywhere in the body, but is most abundant in the central nervous system (CNS) (15); and the CB_2, which is primarily expressed in immune cells, but is also present at low levels in the brain (38,51). The second element is represented by the endocannabinoids, naturally occurring lipid molecules that bind to and activate cannabinoid receptors (11,35,46), are generated on demand by neurons and other cells (12,17,45), and are rapidly eliminated (3,45). The third element is represented by the proteins involved in the formation and elimination of the various endocannabinoid ligands identified thus far (42).

Anandamide (arachidonoylethanolamide) was the first endocannabinoid substance to be discovered (11,42). Current evidence indicates that this lipid-derived mediator is released upon demand by stimulated neurons (12,17); activates cannabinoid receptors with high potency (11), and is rapidly eliminated through a two-step process consisting of carrier-mediated internalization followed by intracellular hydrolysis (4,12, 22).

Fatty-Acid Amide Hydrolase (FAAH)

Intracellular anandamide hydrolysis is primarily catalyzed by a membrane-bound serine hydrolase enzyme, whose existence was first suggested by biochemical experiments (10,21,44,49) and then demonstrated by purification, molecular cloning and heterologous expression (7). This enzyme, called FAAH in recognition of the large number of endogenous fatty-acid amides that are accepted as substrates, displays significant homology with the "amidase signature family" of enzymes. *In vitro*, FAAH can act as a hydrolytic enzyme not only for fatty-acid ethanolamides such as anandamide, but also for primary amides such as oleamide and even for esters such as 2-arachidonoylglycerol (2-AG), a major endocannabinoid ligand in the brain (35,45,46). Site-directed mutagenesis experiments have shown that this broad substrate preference may be due to a previously undescribed catalytic mechanism involving the amino acid residues lysine 142, serine 241, and serine 217 (34), whose energetic aspects have been investigated by computational methods (29).

FAAH is abundantly expressed throughout the CNS, with particularly high levels in neocortex, hippocampus, and basal ganglia (15). *In situ* hybridization studies in the rat have shown that FAAH mRNA levels are highest in the neocortex and hippocampus; intermediate in the cerebellum, thalamus, olfactory bulb and striatum; and lowest in hypothalamus, brain stem and pituitary gland. Immunohistochemical experiments confirmed these findings, showing that large principal neurons in the cerebral cortex, hippocampus, cerebellum and olfactory bulb have the highest levels of FAAH immunoreactivity. Moderate immunostaining has been also observed in amygdala, basal ganglia, ventral and posterior thalamus, deep cerebellar nuclei, superior colliculus, red nucleus, and motor neurons of the spinal cord (15).

Many FAAH-positive neurons in the brain are found in proximity of nerve terminals that contain CB_1 cannabinoid receptors, supporting a role of FAAH in anandamide deactivation. There are, however, several regions of the brain where no such correlation can be demonstrated. This discrepancy is likely to reflect the participation of FAAH in the catabolism of non-cannabinoid fatty-acid ethanolamides, such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) (6,33,43), which are endogenous ligands for the nuclear receptor $PPAR_\alpha$ (peroxisome proliferator-activated receptor type-α) (16,19,30). Consistent with this idea, pharmacological inhibition of FAAH activity or genetic disruption of the *faah* gene results in marked increases in the levels of these fatty acid ethanolamides along with anandamide (8,27).

FAAH inhibitors

A number of FAAH inhibitors have been described, including fatty-acid trifluoromethylketones, fluorophosphonates, α-keto esters and α-keto amides, bromoenol lactones, and non-steroidal antiinflammatory drugs. These compounds are exemplified by the bromoenol lactone BTNP (US patent 5,925,672 issued 7/20/1999), which inhibits FAAH activity with a half-maximal inhibitory concentration (IC_{50}) of 800 nM (3) and the fatty acid sulfonyl fluoride palmitylsulfonylfluoride (AM374), which inhibits FAAH activity with an IC_{50} of 10 nM (US patent 5,688,825 issued 11/18/1997). These compounds lack, however, the target selectivity and/or biological availability needed in a therapeutic drug.

An emerging second generation of FAAH inhibitors comprises several structurally diverse groups of compounds (Fig. 1). These include substituted α-ketoheterocycles, developed at the Scripps Research Institute (US patent 6,462,054 issued 10/8/2002) (5); carbamate oxime (US patent 6,949,574 issued 9/27/05) and bisarylimidazolyl derivatives (US patent 6,562,846 issued 5/13/2003; patent application WO 03/065989, 8/14/2003), developed at Bristol-Myers Squibb; dioxane-2-alkyl carbamates, developed at Sanofi-Synthélabo (patent application WO 04/020430, 11/3/2004), aryl alkyl carbamates (patent applications WO 04/067498 published 8/12/2004 and WO 05/033066 published 4/14/2005) and piperidinyl- and piperazinyl-alkyl carbamates (patent application WO 04/099176 published 11/18/2004), developed at Sanofi-Synthélabo (Sanofi Aventis); tetrazole-based inhibitors, developed at Eli-Lilly (36); and alkylcarbamic acid aryl esters, developed at the Universities of California-Irvine, Urbino and Parma (patent application WO 04/033422, published 4/22/2004) (1,27,37,47,48). The index member of the latter

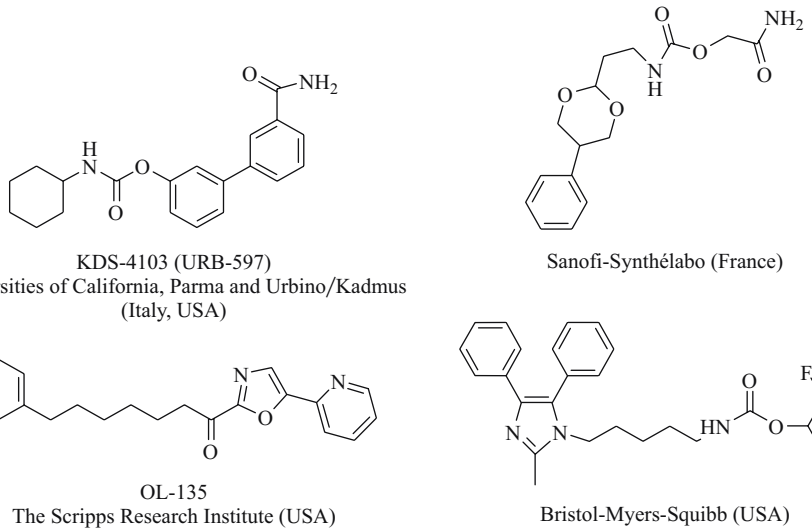

Fig. 1. Chemical structures of various FAAH inhibitors.

group of compounds, the compound URB597 (KDS-4103) (Fig. 1) has been characterized both *in vitro* and *in vivo* and its properties are the subject of the present review.

CHEMISTRY

KDS-4103 (cyclohexylcarbamic acid 3′-carbamoylbiphenyl-3-yl ester) is a crystalline white solid with a molecular weight of 338.4. The compound has two hydrogen bond donors and five hydrogen bond acceptors. Its solubility in phosphate-buffered saline (PBS, pH 7.4) is 523 ng/mL and it has a log *D* of 3.93 (*n*-octanol/PBS, pH 7.4). KDS-4103 has a high degree of membrane permeability, as determined in CaCo II TC7 cells, with an apparent permeability coefficient (A-B Permeability) of 45.3×10^{-6} cm/sec. It is stable under various conditions (heat, acid, peroxide, light, and simulated gastric fluid) (Kadmus Pharmaceuticals, unpublished results). The overall yield of a 3-kg bulk synthesis of the product is >80% with a purity of 98.5%.

PHARMACOLOGY

FAAH Inhibition *in Vitro*

When FAAH activity was assayed in membrane fractions using [^{3}H]anandamide (anandamide[ethanolamine-^{3}H]) as a substrate, KDS-4103 was found to inhibit rat brain activity with an IC_{50} of ~5 nM (27) and human liver activity with an IC_{50} of ≈2.5 nM (Kadmus Pharmaceuticals, unpublished results). KDS-4103 was also found to prevent the

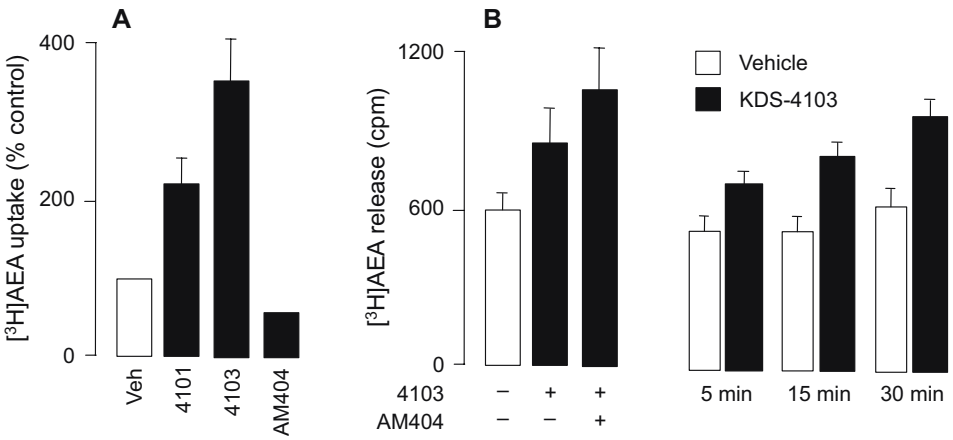

Fig. 2. Effects of the FAAH inhibitors, KDS-4103, 10 nM, and KDS-4101, 3 μM, as well as anandamide transport inhibitor AM404 on (**A**) anandamide uptake into and (**B**) anandamide release from rat brain cortical neurons in primary cultures. Modified from ref. 27.

FAAH-catalyzed hydrolysis of [^{3}H]anandamide by primary cultures of rat cortical neurons with an IC_{50} value of ~0.50 nM (27).

Notably, KDS-4103 selectively blocks the breakdown of [^{3}H]anandamide without reducing its carrier-mediated uptake, causing non-metabolized [^{3}H]anandamide to accumulate in, and eventually exit from, the neurons. Thus, after a 4-min incubation with [^{3}H]anandamide, the intracellular content of non-metabolized [^{3}H]anandamide is markedly higher in inhibitor-treated than in control neurons (Fig. 2A). Similar results were obtained with KDS-4101, a carbamate-based FAAH inhibitor that is weaker than KDS-4103 at inhibiting FAAH activity (Fig. 2A). Importantly, the anandamide transport blocker *N*-(4-hydroxyphenyl) eicosa-5,8,11,15-tetraenamide (AM404) has an opposite effect, significantly reducing [^{3}H]anandamide internalization (Fig. 2A). When neurons treated with KDS-4103 are exposed for 4 min to [^{3}H]anandamide and then incubated for 15 min in an [^{3}H]anandamide-free solution, ~43% of the accumulated [^{3}H]anandamide is released back into the medium (Fig. 2B). This process is linear with time (Fig. 2B) and is not inhibited by AM404 (Fig. 2A), suggesting that it is due to passive diffusion rather than reverse transport. No such time-dependent release is observed in control untreated neurons, the medium of which only contains residual levels of [^{3}H]anandamide carried over from the preincubation period (27). Together, these studies demonstrate that KDS-4103 is potent at blocking anandamide hydrolysis in rat brain neurons without having any inhibitory effect on anandamide transport. As a result of its ability to block FAAH activity, KDS-4103 causes extracellular levels of anandamide to increase.

FAAH Inhibition *in Vivo*

By intraperitoneal (i.p.) injections KDS-4103 produced a profound dose-dependent inhibition of brain FAAH activity in rats, which is half-maximal at ~0.15 mg/kg (27). After injection of KDS-4103 (0.3 mg/kg, i.p.), FAAH inhibition is rapid in onset (<15 min), persistent (>12 h) (Fig. 3A), and accompanied by significant elevations in the brain

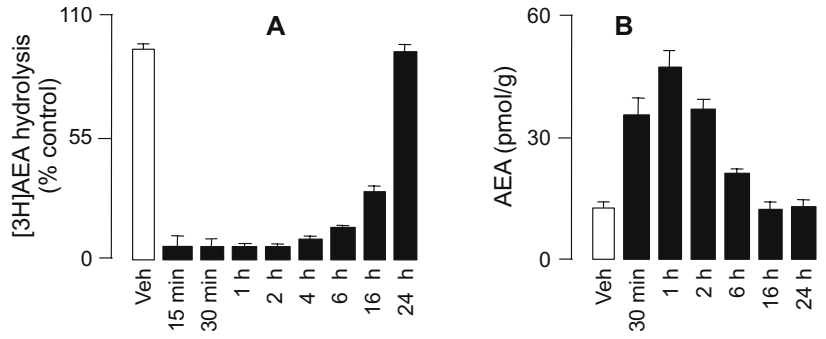

Fig. 3. Effects of parenteral administration of KDS-4103 (0.3 mg/kg, i.p.) on (**A**) rat brain FAAH activity, assessed *ex vivo* by enzyme assay; and (**B**) brain anandamide levels, assessed *ex vivo* by liquid chromatography/mass spectrometry. Modified from ref. 14.

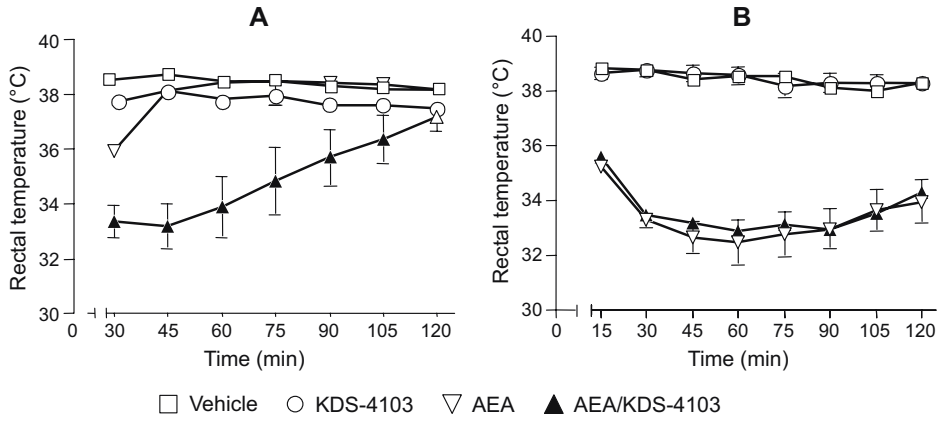

Fig. 4. Effects of parenteral administration of anandamide (AEA, 5 mg/kg, i.p.), KDS-4103 (0.3 mg/kg, i.p.) or anandamide plus KDS-4103 on body temperature in wild-type mice (+/+, left panel) or FAAH-null mice (–/–, right panel). Modified from ref. 14.

content of anandamide (Fig. 3B) and other fatty-acid ethanolamides that are substrates for FAAH (14). Similar changes in FAAH activity and fatty-acid ethanolamide levels are also observed in peripheral tissues (14). Significantly elevated anandamide levels are also observed in the brains of $FAAH^{-/-}$ mice, and KDS-4103 (0.3 mg/kg, i.p.) does not further alter these levels (14). In agreement with its inability to affect monoglyceride lipase (MGL) activity (Table 1), KDS-4103 does not change the brain content of the endocannabinoid 2-arachidonoylglycerol (2-AG), a primary substrate for this enzyme (13).

As previously observed in mutant $FAAH^{-/-}$ mice, FAAH inhibition is associated with increased sensitivity to the administration of exogenous anandamide. Thus, KDS-4103 (0.3 mg/kg, i.p.) markedly enhances the hypothermic response produced by a sub-threshold dose of anandamide (5 mg/kg, i.p.), although it has no effect on body temperature when injected alone (Fig. 4A) (14). The effect of anandamide plus KDS-4103 is mediated by CB_1 receptors, since it is prevented by the CB_1 antagonist rimonabant (27). Notably,

TABLE 1. In vitro *pharmacology of KDS-4103.*
Effects of KDS-4103 in various in vitro *receptor binding and enzyme assays*

Assay	Result
Receptors	
Adenosine: A_1 (h), A_{2A} (h), A_3 (h)	NSI
Adrenergic: α_1 (non-selective), α_2 (non-selective), β_1 (h), β_2 (h)	NSI
Angiotensin-II: AT_1 (h), AT_2 (h)	NSI
Benzodiazepine: BZD (central), BZD (peripheral)	NSI
Bombesin: BB (non-selective)	NSI
Bradykinin: B_2 (h)	NSI
Calcitonin gene-related peptide: CGRP (h)	NSI
Cannabinoid: CB_1 (h), CB_2 (h)	NSI
Cholecystokinin: CCK_A (h) (CCK_1), CCK_B (h) (CCK_2)	NSI
Dopamine: D_1 (h), D_{2S} (h), D_3 (h), D_4 (h), D_5 (h)	NSI
Endothelin: ET_A (h), ET_B (h)	NSI
GABA (non-selective)	NSI
Galanin: GAL_1 (h), GAL_2 (h)	NSI
Growth factors, cytokines, chemokines: $CXCR_2$ (h) (IL-8B), TNF-α (h), CCR_1 (h)	NSI
Histamine: H_1 (h), H_2 (h)	NSI
Melanocortin: MC_4 (h)	NSI
Melatonin: MT_1	NSI
Muscarinic: M_1 (h), M_2 (h), M_{23} (h), M_4 (h), M_5 (h)	NSI
Neurokinin: NK_1 (h), NK_2 (h), NK_3 (h)	NSI
Neuropeptide Y: Y_1 (h), Y_2 (h)	NSI
Neurotensin: NT_1 (h) (NTS_1)	NSI
Opiate: δ (h) (DOP), κ (KOP), μ (h) (MOP) (agonist site)	NSI
Orphanin: ORL_1 (h) (NOP)	NSI
PACAP: (PAC_1) (h)	NSI
Phencyclidine: PCP	NSI
Prostanoid: TXA_2/PGH_2 (h) (TP)	NSI
Purinergic: P_{2X}, P_{2Y}	NSI
Serotonin: 5-HT_{1A} (h),5-HT_{1B}, 5-HT_{2A} (h), 5-HT_{2C} (h), 5-HT_3 (h), 5-HT_{5A} (h) (5-ht5A), 5-HT_6 (h), 5-HT_7 (h)	NSI
σ (non-selective)	NSI
Somatostatin: Sst (non-selective)	NSI
Vasopressin: V_{1a} (h)	NSI
Vasoactive intestinal Peptide: VIP_1 (h) ($VPAC_1$)	NSI
Ion Channels	
Ca^{2+} channel (L, verapamil site) (phenylalkylamines)	NSI
K_V^+ channel	NSI
SK_{Ca}^+ channel	NSI
Na^+ channel (site 2)	NSI
Cl^- channel	NSI

TABLE 1 (continued)

Assay	Result
Transporters	
DA transporter (h)	NSI
NE transporter (h)	NSI
5-HT transporter (h)	NSI
Anandamide transport (human astrocytoma cells)*	NSI
Enzymes	
COX-1 (h), COX-2 (h)	NSI
Acetylcholinesterase (human and electric eel*)	NSI
Butyryl cholinesterase (horse plasma)*	NSI
Monoglyceride lipase (rat brain)*	NSI

Unless marked with an asterisk (*), assays were performed at CEREP (Paris, France). FOR BINDING ASSAYS: Specific ligand binding (radiolabeled reference ligand) to the receptors was determined as the difference between total binding and nonspecific binding in the presence of an excess of unlabelled ligand. Percent (%) inhibition of control specific binding of each reference compound was determined in the presence of KDS-4103 (at 10 μM unless marked with an asterisk. *). For enzymatic assays: COX-1, COX-2 and human acetylcholinesterase assays, enzymatic activity was determined under standard conditions and results were expressed as percent inhibition of control values obtained in the presence of KDS-4103 (10 μM). For binding and enzymatic assays, results showing an inhibition higher than 50% were considered significant. In each experiment, a reference compound was tested concurrently with KDS-4103 at several concentrations (for IC_{50} determinations) in order to assess assay suitability. Methods and results for anandamide transport, electric eel acetylcholinesterase, butyryl cholinesterase, and MGL (all marked with an asterisk, *) were previously reported in Kathuria et al., 2003. h, human; NSI, no significant inhibition.

KDS-4103 does not further increase the sensitivity to anandamide in $FAAH^{-/-}$ mice (Fig. 4B) (14), supporting the notion that FAAH inhibition plays an exclusive role in mediating the actions of KDS-4103.

Target Selectivity *in Vitro*

In vitro pharmacology screening showed that KDS-4103 does not affect the activities of various serine hydrolases including human and electric-eel acetylcholinesterase, horse plasma butyryl cholinesterase, and rat brain MGL (Table 1). As noted above, the lack of MGL inhibition is particularly important because of the involvement of this enzyme in the biological inactivation of the endocannabinoid 2-AG (13,23,31). *In vitro* screening for interactions with other members of the endocannabinoid system demonstrated that KDS-4103 has no effect on anandamide transport or CB_1 and CB_2 receptor binding (see above) (27). Furthermore, at a test concentration of 10 μM, KDS-4103 was found not to significantly interact with a broad panel of receptors, ion channels, neurotransmitter transporters and enzymes (Table 1), cytochrome P450 isoforms (Table 2), or HERG channel activity (not shown). In a proteomic-based selectivity screen based on the displacement of fluorophosphonate-rhodamine (FPR) from mouse brain protein extracts, KDS-4103 was found to prevent FPR binding to triacylglycerol hydrolase (TGH) with an IC_{50} of 192 nM

TABLE 2. Effects of KDS-4103 on cytochrome P450 enzymes activities

Assay	Result
CYP1A2 inhibition (CEC substrate)	No significant inhibition
CYP2C9 inhibition (MFC substrate)	No significant inhibition
CYP2C19 inhibition (CEC substrate)	No significant inhibition
CYP2D6 inhibition (MFC substrate)	No significant inhibition
CYP3A4 inhibition (BFC substrate)	No significant inhibition

Activities of human cytochrome P450 enzymes (CYP) were assayed under standard conditions by CEREP (Paris, France) in the absence or presence of KDS-4103 (10 μM). Conversion of each substrate to each corresponding product (see below) was determined by fluorimetry. In each experiment, a reference compound was tested concurrently with KDS-4103 at several concentrations (for IC_{50} determinations) in order to assess assay suitability. Substrates and detected products were: for CYP1A2, CEC substrate (3-cyano-7-ethoxycoumarin) and CHC product (3-cyano-7-hydroxycoumarin); for CYP2C9, MFC substrate (7-methoxy-4-trifluoromethylcoumarin) and HFC product (7-hydroxy-4-trifluoromethylcoumarin); for CYP2C19, CEC substrate and CHC product; for CYP2D6, MFC substrate and HFC product; and for CYP3A4, BFC substrate [7-benzyloxy-4-trifluoromethylcoumarin] and HFC product.

(28). However, direct *in vitro* measurements of enzyme activity showed that KDS-4103 has no effect on either TGH (rat liver) or triacylglycerol lipase (rat white adipose tissue) activity at concentrations as high as 10 μM. Moreover, *in vivo* administration of KDS-4103 (3 mg/kg, i.p.) failed to alter triacylglycerol levels in rat liver and white adipose tissue, although it significantly inhibited FAAH activity in the same tissues (Clapper and Piomelli, unpublished results). Collectively, these results indicate that KDS-4103 is remarkably selective for FAAH.

Anxiolytic-Like Activity

Three lines of evidence suggest that the endocannabinoid system may be involved in the regulation of emotional reactivity. Firstly, CB_1 receptors are expressed at high levels in brain structures, such as the basolateral nucleus of the amygdala, which are implicated in the control of mood and anxiety (15). Secondly, administration of cannabinoid drugs produces marked emotional responses in both experimental animals and humans (25). Finally, pharmacological or genetic disruption of CB_1 receptor activity elicits anxiety-like behaviors in rodents, suggesting the existence of an intrinsic anxiolytic tone mediated by the endocannabinoid system (20,39,41,50).

To determine whether KDS-4103 affects anxiety-like behaviors, two pharmacologically validated animal models of anxiety were used: the elevated zero maze test and the isolation-induced ultrasonic emission test (which is also utilized to model antidepressant-like drug activity). The elevated zero maze consists of an elevated annular platform with two open and two closed quadrants and is based on the conflict between an animal's instinct to explore its environment and its fear of open spaces, where it may be attacked by predators. Clinically used anxiolytic drugs, such as the benzodiazepines, increase the proportion of time spent in, and the number of entries made into, the open compartments. Similarly, KDS-4103 (0.05–0.1 mg/kg, i.p.) evoked anxiolytic-like responses at doses that correspond to those required to inhibit FAAH activity *in vivo* (Fig. 5). Consistent with an

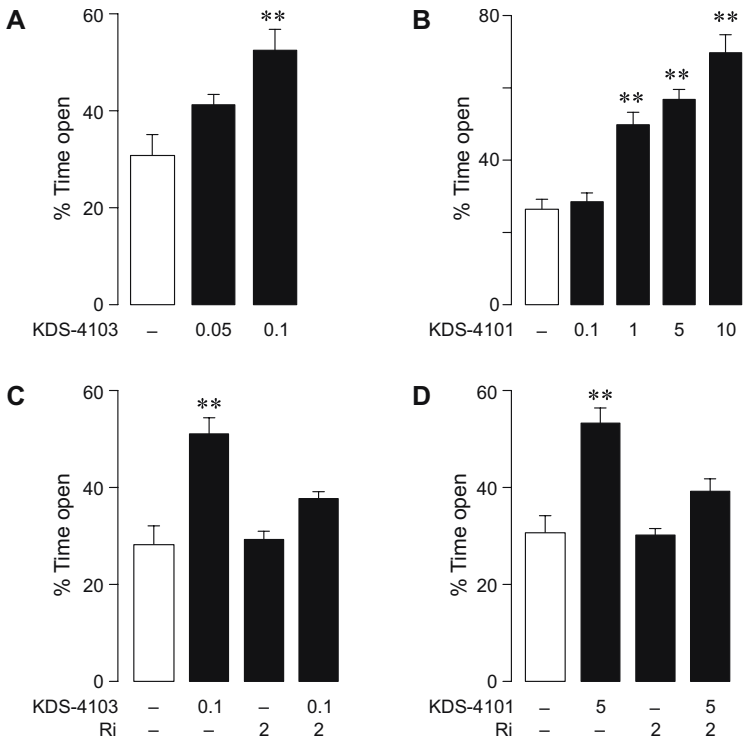

Fig. 5. (**A**, **B**) Effects of various doses (mg/kg, i.p.) of KDS-4103 on anxiety-like behavior (percent time in open quadrant of an elevated zero maze) in rats. (**C**, **D**) The CB_1 receptor antagonist rimonabant (Ri, 2 mg/kg, i.p.) prevents the anxiolytic-like actions of KDS-4103. The carbamate-based FAAH inhibitor, KDS-4101, which is weaker than KDS-4103 at inhibiting FAAH activity *in vitro*, also elicited anxiety-like behavior. Modified from ref. 27.

involvement of anandamide, the anxiolytic-like effects of the FAAH inhibitor were attenuated by rimonabant (2 mg/kg, i.p.) (Fig. 5B). These anxiolytic-like effects were confirmed by testing KDS-4103 in the ultrasonic vocalization emission model, which measures the number of stress-induced vocalizations emitted by rat pups removed from their nest. As seen with anxiolytic drugs, KDS-4103 (0.1 mg/kg, i.p.) strongly reduced ultrasonic calls in rat pups and this effect was antagonized by rimonabant (27).

Antidepressant-Like Activity

The positive responses elicited by KDS-4103 in the isolation-induced ultrasonic vocalization test suggested the possibility that FAAH blockade might also result in antidepressant-like effects. To test this idea, the actions of KDS-4103 were examined in two validated animal models of antidepressant drug activity — the tail suspension test in C57Bl6 mice and the Porsolt's forced swimming test in Wistar rats (18). In the tail suspension test, acute administration of KDS-4103 (0.03–0.3 mg/kg, i.p.) produced a dose-dependent increase in struggling time, which is characteristic of clinically used antidepressant agents such as desipramine and paroxetine (Fig. 6A). This effect was blocked by rimonabant

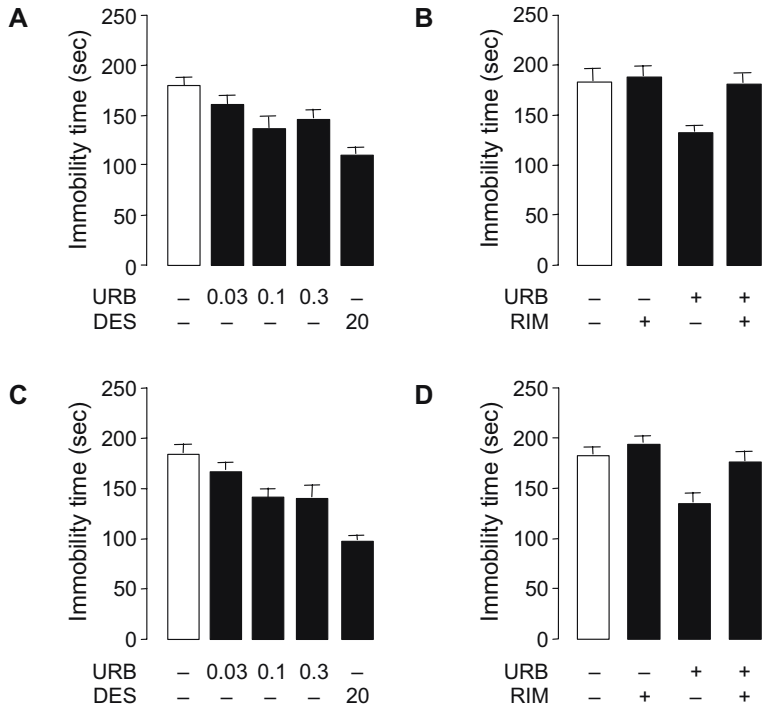

Fig. 6. Antidepressant-like effects of KDS-4103 in the mouse tail-suspension test (TST). (**A**, **C**) Effects of KDS-4103 (mg/kg, i.p.) and desipramine (DES,20 mg/kg, i.p.) in the TST after (**A**) single or (**C**) repeated administration (once daily for 4 days). (**B**, **D**) Single injection of rimonabant (RIM) (1 mg/kg, i.p.) prevents the effects of (**B**) single or (**D**) repeated URB597 administration. Modified from ref. 18.

(Fig. 6B). Importantly, a 4-day treatment with KDS-4103 (0.03–0.3 mg/kg, i.p., once daily) caused similar effects, indicating lack of tolerance to the effects of the drug (Fig. 6C, D). In the Porsolt test, acute administration of KDS-4103 (0.1 mg/kg, i.p.) elicited a significant decrease in floating time and a parallel increase in swimming time. An even more marked effect was obtained following a 4-day treatment with KDS-4103 (0.1 mg/kg, i.p., once daily) (18). It is worth noting that the effects of KDS-4103 in the Porsolt test resemble those produced by serotonergic antidepressants such as paroxetine, which increase swimming time, rather than noradrenergic antidepressants such as desipramine, which increase struggling time (40).

Accordingly, we found that KDS-4103 markedly stimulates firing activity in serotonergic neurons of the dorsal raphe nucleus (DRN) of anesthetized rats (18). After acute administration, KDS-4103 (0.03–0.3 mg/kg, i.v.) produced a dose-dependent increase in mean firing activity of DRN neurons, an effect that was prevented by rimonabant (1 mg/kg). Similar results were noted after subchronic administration (0.1 mg/kg, i.p., once daily for 4 or 21 days). The subchronic effects of KDS-4103 were accompanied by augmented serotonin outflow in the hippocampus and the basolateral amygdala, as assessed by *in vivo* microdialysis in awake rats. Unlike most antidepressants, however, KDS-4103, by acute or subchronic administration, did not reduce firing activity in noradrenergic neurons of the locus ceruleus, but rather increased such an activity (18). Collec-

tively, the behavioral and electrophysiological experiments outlined above indicate that KDS-4103 exerts profound antidepressant-like effects in rats and mice.

Analgesic Activity

KDS-4103 was shown to exert moderate antinociceptive actions in the mouse hot-plate test, which measures the response of the animal to a noxious thermal stimulus. In this model, the compound modestly but significantly lengthened response latencies at a dose of 0.5 mg/kg, i.p. (27). These effects were prevented by the CB_1 antagonist rimonabant (0.2 mg/kg, i.v.) (27). More marked analgesic effects of acute doses of KDS-4103 (0.1–0.3 mg/kg, i.p.) were recently observed in the complete Freund's adjuvant model of arthritis pain in the rat (26). These effects were prevented by the CB_1 antagonist AM251, indicating that blockade of FAAH activity may result in CB_1-mediated antiallodynic effects in an inflammatory pain model (26). Finally, administration of KDS-4103 enhanced a form of foot shock-induced analgesia in rats, which is mediated by release of anandamide and 2-AG in the brainstem periaqueductal gray (23).

Absence of Cannabinoid-Like Activity and Abuse Liability

Though KDS-4103 increases brain anandamide levels, this compound does not reproduce the spectrum of pharmacological responses produced by exogenous anandamide or other cannabinoid agonists. Systemic doses of KDS-4103 (0.3 mg/kg, i.p.) that maximally block FAAH activity were found to produce no catalepsy (rigid immobility), hypothermia or hyperphagia, three typical signs of CB_1 receptor activation (27). Even more importantly, KDS-4103 was shown to have no effect on two rat models of abuse liability, the conditioned place preference (CPP) test and the drug discrimination (DD) test (18). In the CPP test, acute administration of KDS-4103 (0.03–0.3 mg/kg, i.p.) produced no preference compared to vehicle or to the direct cannabinoid agonist Win-55212-2. Likewise, in the DD test KDS-4103 (0.1–3 mg/kg, i.p.) did not substitute for the plant-derived cannabinoid Δ^9-THC or the synthetic cannabinoid agonist Win-55212-2 (Fig. 7).

Additional Pharmacological Properties

The availability of KDS-4103 has prompted a broad range of efforts to investigate the pharmacological properties of FAAH inhibitors. For example, experiments in spontaneously hypertensive rats have shown that by parenteral administration KDS-4103 (0.1–1 mg/kg, i.p.) reduces blood pressure, cardiac contractility, and vascular resistance to levels similar to those found in normotensive animals. These effects were prevented by administration of CB_1 antagonists. Similar responses were observed in two mechanistically distinct models of hypertension — Dahl salt-sensitive rats and chronic angiotensin II infusion in rats — but not in normotensive control rats. These results suggest that KDS-4103 may normalize blood pressure in hypertensive rats by amplifying the cardiodepressor and vasodilator effects of endogenous anandamide and, as such, may offer a novel therapeutic strategy for the treatment of hypertension (2). In another study, KDS-4103 (0.1–3 mg/kg, i.p.) was found to reduce dose-dependently carrageenan-induced edema formation in mice, an effect that was blocked by the CB_2 antagonist SR144528, but not by the CB_1 antagonist AM251 (24). A possible proinflammatory role

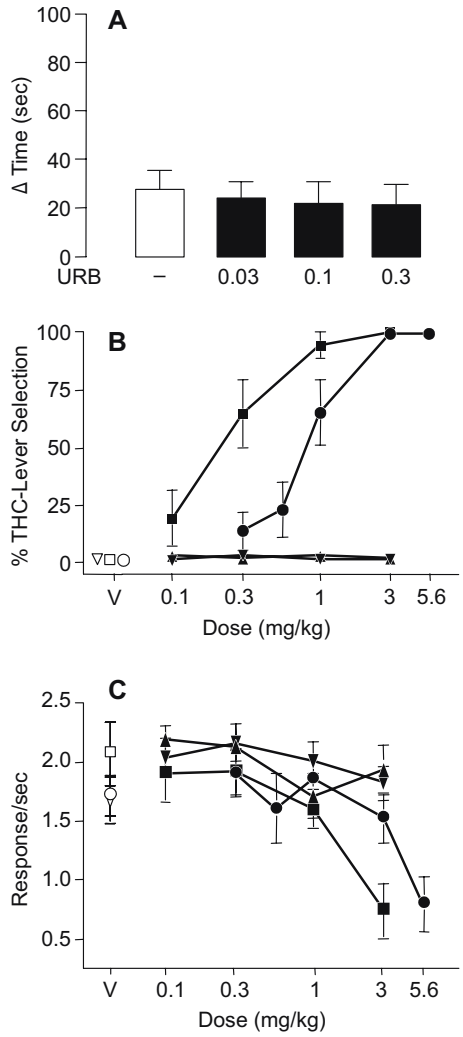

Fig. 7. Motivational profile of KDS-4103. (**A**) Effects of KDS-4103 (0.03–0.3 mg/kg, i.p., 2 h treatment) in the rat conditioned place preference test. Δ Time: difference in time spent in the non-preferred compartment between post- and pre-conditioning sessions. (**B**, **C**) Effects of WIN55,212-2 (■), Δ^9-THC (●), KDS-4103 2 h before test session (▼), and KDS-4103 40 min before test session (▲) in rats trained to discriminate 3 mg/kg Δ^9-THC from vehicle. (**B**) Percent of responses on the Δ^9-THC associated lever and (**C**) rate of lever pressing over the entire 30 min session. Open symbols represent respective vehicles. Modified from ref. 18.

of FAAH is further suggested by the reduced response to inflammatory stimuli observed in FAAH-null mice (9).

Cellular Mechanism of Action

In vitro experiments show that KDS-4103 causes non-metabolized anandamide to accumulate and, eventually, leak out of brain neurons (Fig. 2) (27). *In vivo*, this action is expected to result in the accumulation of anandamide outside cells and, consequently, in the increased local activation of CB_1 receptors. If anandamide was primarily generated in brain areas engaged in the processing of emotional information, this might help explain the restricted spectrum of actions, and more specifically the lack of cannabinoid-like side effects of KDS-4103. This possibility is confirmed by studies, which show that ananda-

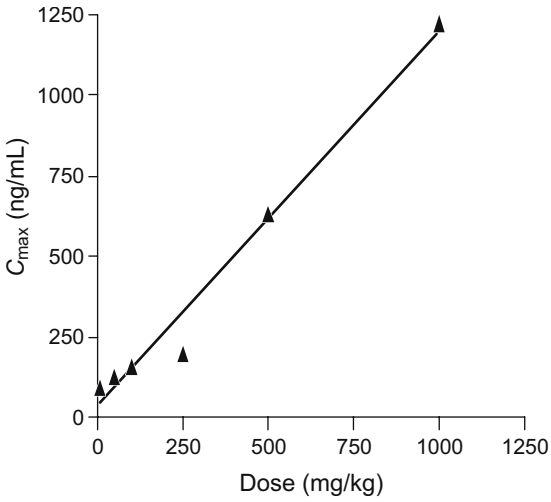

Fig. 8. Exposure to KDS-4103 following oral administration in rats. Animals received KDS-4103 as a suspension (0.5% carboxymethyl cellulose, 0.5% simethicone, and 0.4% polysorbate-80 in water). Exposure is plotted as C_{max} vs. dose. (Kadmus Pharmaceuticals, unpublished results)

mide levels in the amygdala and periacqueductal gray matter rise when animals are exposed to stressful stimuli (23,32).

PHARMACOKINETICS

The pharmacokinetic properties of KDS-4103 were assessed in rats following oral administration as a suspension. KDS-4103 was absorbed at a moderate rate with peak plasma concentrations (C_{max}) achieved at 1.2 h after treatment. The oral elimination half-life of KDS-4103 was approximately 2 h. Linear exposure (Area Under the Curve, AUC, and C_{max}) of KDS-4103 was observed at doses of 10 to 1000 mg/kg (Fig. 8). In the brain, peak concentrations and AUC values for KDS-4103 were similar to those observed in plasma. Maximum brain levels of KDS-4103 were reached approximately 1 hour after administration. Accordingly, FAAH inhibition in the brain was rapid (≤1 h), sustained at >90% through 12 h and >60% through 24 h after an oral dose of 10 mg/kg (Fig. 9).

PRECLINICAL SAFETY EVALUATION

Formal preclinical safety studies to support an Investigational New Drug Application (IND) for KDS-4103 have been initiated. To date, single-dose and 7- and 28-day repeated dose studies have been completed in rats; single-dose and 7-day repeated dose studies have been completed in cynomolgus monkeys and a 28-day repeated dose study is ongoing. Monkeys were chosen as the non-rodent species due to limited oral bioavailability in dogs. No signs of systemic toxicity have been noted at single oral doses up to 2000 mg/kg in rats or 1500 mg/kg in monkeys. In rats, this dose is at least 40-fold greater than that required to inhibit brain FAAH activity to less than 10% of baseline levels. Considering that plasma exposure at 1000 mg/kg p.o. is 20-fold greater than the effective

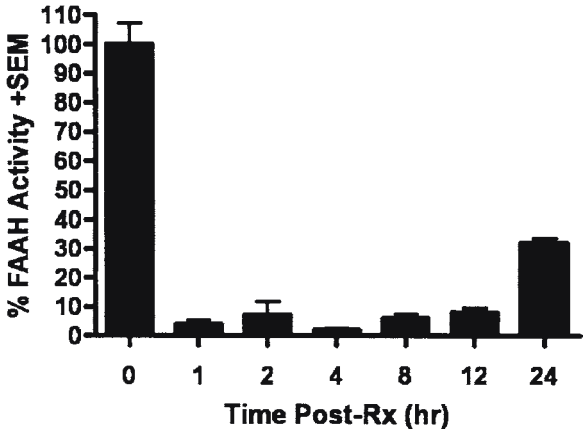

Fig. 9. Effects of orally administrated KDS-4103 (10 mg/kg, p.o.) on rat brain FAAH activity, assessed *ex vivo* by enzyme assay. (Kadmus Pharmaceuticals, unpublished results)

dose, these results suggest a therapeutic index of at least 20. No treatment-related clinical observations were noted during repeated daily dosing at 1500 mg/kg in rats (28 days) and monkeys (through 21 days to date). Terminal evaluations (following 7 days in monkeys and 28 days in rats) including blood chemistry, hematology, and gross necropsy showed no signs of toxicity. CNS safety pharmacology assessment involving a complete functional observation battery in rats revealed no overt effects following oral doses up to 1500 mg/kg. No deficits were observed on the rotarod test in rats after treatment with KDS-4103 at doses up to 5 mg/kg, i.p. (33-fold over the ID_{50} for FAAH activity in the brain). Furthermore, *in vitro* bacterial cytotoxicity and Ames testing yielded negative results. Cardiovascular and respiratory safety pharmacology studies evaluating oral doses of 50, 275, and 1500 mg/kg will be completed in the first half of 2006.

CONCLUSIONS

KDS-4103 and its analogs represent a novel class of agents that prevent anandamide deactivation by targeting the intracellular enzymatic activity of FAAH. KDS-4103 inhibits FAAH activity with an IC_{50} value of approximately 5 nM in rat brain membranes *in vitro*, 0.5 nM in intact rat neurons *in vitro*, 3 nM in human liver microsomes *in vitro*, and an ID_{50} value of 0.15 mg/kg following i.p. administration in the rat.

KDS-4103 has a remarkable selectivity for FAAH with no activity on other cannabinoid-related targets, including cannabinoid receptors, anandamide transport and monoglycerol lipase (the enzyme involved in the deactivation of the endocannabinoid ester 2-AG). Such target discrimination is matched by a lack of overt cannabimimetic effects *in vivo*. Thus, at doses that almost abolish FAAH activity and substantially raise brain anandamide levels, KDS-4103 does not evoke catalepsy, reduce body temperature or stimulate feeding, three key signs of cannabinoid receptor activation. Moreover, the compound does not display abuse liability in two animal models. KDS-4103 does elicit anxiolytic-like, antidepressant-like and analgesic responses, which parallel its ability to inactivate FAAH and are prevented by CB_1 receptor blockade. These findings indicate that KDS-4103 acts

by enhancing the tonic actions of anandamide on a subset of CB_1 receptors, which may normally be engaged in controlling emotions and pain. In addition, KDS-4103 reduces carageenan-induced inflammation in mice and normalizes blood pressure in rat models of hypertension. To date, the ease of large-scale manufacturing, oral bioavailability, and safety profile indicate that KDS-4103 is a promising new therapeutic agent for a variety of significant medical conditions, including anxiety, depression and pain.

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Conflict of interest statement. Timothy R. Compton, Olivier Dasse, Edward P. Monaghan, Jeff A. Parrott and David Putman are employees of, and Daniele Piomelli is a consultant for Kadmus Pharmaceuticals Inc., the developer of KDS-4103.

Addendum. Chemical names.

AM251, *N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide;

SR144528, N-[1S]-endo-1,3,3-[trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazol 3-carboxamide;

Win-55212-2, (*R*)-(+)-[2.3-Dihydro-5-methyl-3-(4-morpholino-metyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate.

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