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A novel monoacylglycerol lipase inhibitor with analgesic and anti-inflammatory activity

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ABSTRACT

A variety of long chain 1,2-diamines and related compounds were synthesized and tested for their activity on fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL). (2*S*,9*Z*)-Octadec-9-ene-1,2-diamine selectively inhibits MGL (K_i 21.8 μM) without significantly affecting FAAH. This compound exhibited interesting in vivo analgesic and anti-inflammatory properties, suggesting that selective inhibitors of MGL may be valuable novel agents for the treatment of inflammatory pain.

The discovery of the CB1 and CB2 cannabinoid receptors as well as the endogenous ligand involved in their activation opened a new era in cannabinoid research.[1,2] The key endogenous ligands designated as endocannabinoids are anandamide (**1**) and 2-arachidonoylglycerol (2-AG, **2**) (Fig. 1). Of these, 2-AG has been shown to be the most abundant and more potent endocannabinoid.[3] 2-AG exhibits full agonist efficacy at both CB1 and CB2[4] and its physiological levels in rat brain exceed those of anandamide by several orders of magnitude.[3b] Although the endocannabinoid system has received much attention in recent years as a target for the discovery of novel therapeutic agents,[5] the role and function of 2-AG are less understood.

Anandamide and 2-AG are produced by neurons when the need arises, act near their site of biosynthesis, and are rapidly metabolized so that their biological activity is terminated. Both anandamide and 2-AG are hydrolyzed by fatty acid amide hydrolase (FAAH).[6] However, existing evidence indicates that the most likely candidate for 2-AG hydrolytic deactivation is monoacylglycerol lipase (MGL), a serine hydrolase that converts 1- and 2-monoacylglycerols into fatty acid and glycerol.[7] Dinh et al. cloned MGL from a rat brain cDNA,[8] and demonstrated that RNA interference suggested a primary role for MGL in the degradation of 2-AG.[9] More recently, Makriyannis and collaborators have characterized the binding domain of this enzyme using a proteomic approach.[10] Although a plethora of FAAH inhibitors have been developed,[1,2,11] only few MGL inhibiting ligands are available.

Various nonspecific serine hydrolase inhibitors such as methylarachidonoyl fluorophosphonate (**3**, MAFP) (Fig. 2), hexadecylsulfonyl fluoride (AM374) and phenylmethylsulfonyl fluoride (PMSF) inhibit purified MGL, or 2-AG-degrading enzymatic activity in rat cerebellar membranes.[6d,8,12] In addition, sulfhydryl-specific compounds, like *N*-arachidonylmaleimide (**4**, NAM) have been shown to inhibit MGL.[13] Arachidonoyl trifluoromethyl ketone (**5**, ATFMK)[12] as well as arachidonoyl glycerol derivatives such as compound **6** also interact with MGL.[14] It has been reported that MGL activity can be specifically and noncompetitively inhibited by two compounds, URB602 (**7**) and URB754 (**8**) (Fig. 2).[15] However,

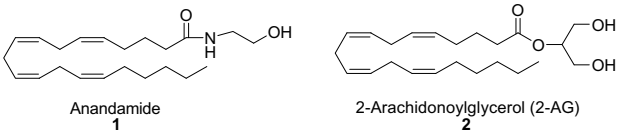

Figure 1. The most important endocannabinoids.

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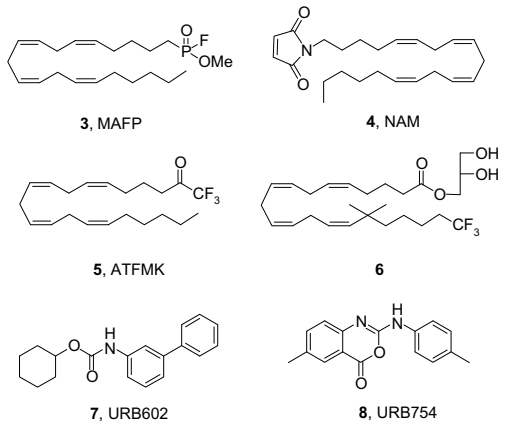


Figure 2. Inhibitors of monoacylglycerol lipase.

a recent report showed that both URB602 and URB754 had no effect on the hydrolysis or signaling capacity of 2-AG in rat brain.[16] Most recently, it has been reported that locally injected 2-AG decreased pain behavior in a dose-dependent manner in an inflammatory model of pain.[17] It is thought that the antinociceptive effect of 2-AG is mediated through the CB2 receptor.

The novel finding that the exogeneous application of 2-AG induces peripheral antinociception,[17] reveals the therapeutic potential of MGL inhibitors for the treatment of inflammatory pain.[18] Since both FAAH and MGL may hydrolyse 2-AG, it is of great importance to develop selective inhibitors of these enzymes in order to fully explore the physiological role for 2-AG. Here, we describe our efforts towards the synthesis of MGL selective inhibitors and the in vivo anti-inflammatory and analgesic properties of such a compound.

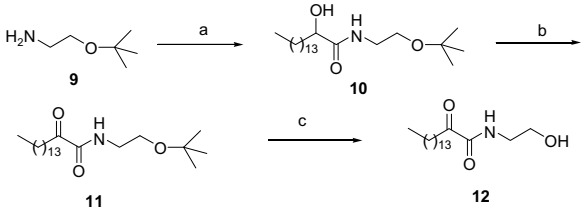
During the last years we have developed a variety of inhibitors of lipolytic enzymes (pancreatic and gastric lipases as well as phospholipases $cPLA_2$ and $iPLA_2$),[19–25] and we have shown that the cytosolic phospholipase A_2 inhibitor AX048 produces a potent anti-hyperalgesic action.[26] All of these lipolytic enzymes are serine hydrolases with a mechanism of action involving a serine residue at the catalytic site interacting with a 2-oxoamide functionality of the inhibitors. Since MGL is also a serine hydrolase, we have incorporated the 2-oxoamide functionality in our MGL inhibitor design. Towards this end, we synthesized a lipid analog carrying the 2-oxoamide of ethanolamine. 2-Oxoamide **12** was prepared from the *tert*-butyl ether of ethanolamine as described in Scheme 1. This compound exhibited weak inhibitory activity towards both FAAH and MGL, with no selectivity (Table 1).

At the same time, we synthesized a series of long chain 1,2-diamines and 1,2-amino alcohols containing a saturated chain or a chain corresponding to that of oleic acid chain. (*S*)- and (*R*)-2-aminohexadecanol[27] (**13**, **14**) and 1,2-diaminohexadecane[28] (**15**) were prepared as previously described. The synthesis of 1,2-diamine **21** is presented in Scheme 2. L-Glutamic acid was used as starting material and aminoaldehyde **16** was prepared according to literature procedure.[29] After a Horner–Wadsworth–Emmons reaction, hydrogenation with simultaneous reduction of the azide group and Boc protection, the key intermediate ester **18** was obtained. Compound **18** was then reduced to aldehyde **19**, which was allowed to react with the appropriate ylide to yield diamine

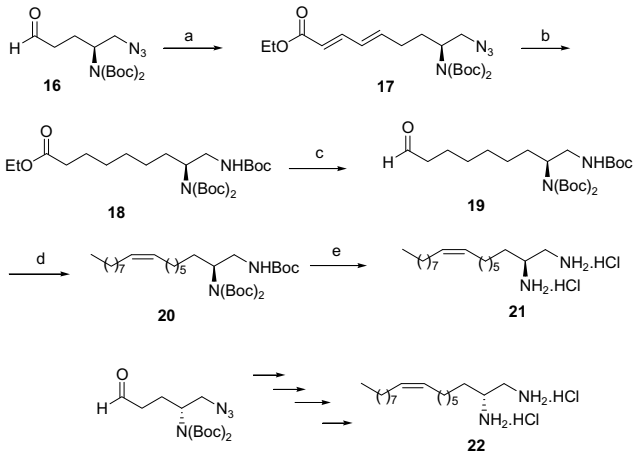
Table 1
Inhibitory results on FAAH and MGL[a]

Structure	FAAH inhibition (%)	MGL inhibition (%)	K_i for MGL (μM)
12	23.8	27.5	
13	33.6	31.5	
14	31.3	30.0	
15	31.2	30.3	58.7
21	4.9	49.9	21.8
22	25.9	42.2	39.0
25a	0.0	0.0	
25b	30.9	25.9	
29	24.0	0. 0	

[a] Percent inhibition of 2-AG hydrolysis using 100 μM concentrations.



Scheme 1. Synthesis of 2-oxoamide **12**. Reagents: (a) $CH_3(CH_2)_{13}CHOHCOOH$, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, HOBt, Et_3N, CH_2Cl_2; (b) AcNH-TEMPO, NaOCl, NaBr, EtOAc/$PhCH_3$/H_2O; (c) 50% TFA/CH_2Cl_2.



Scheme 2. Synthesis of 1,2-diamines **21** and **22**. Reagents: (a) $EtOOCCH{=}CHCH_2P(=O)(OEt)_2$, LiOH, THF; (b) H_2, 10% Pd/C, Boc_2O, MeOH; (c) DIBALH, Et_2O; (d) $C_9H_{19}P^+Ph_3Br^-$, KHMDS, toluene; (e) 4 N HCl/Et_2O.