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Synthesis and Pharmacological Activity of a Potent Inhibitor of the Biosynthesis of the Endocannabinoid 2-Arachidonoylglycerol

Tiziana Bisogno,[a] James J. Burston,[b] Ravi Rai,[c] Marco Allarà,[a] Bijali Saha,[c] Anu Mahadevan,[c] Raj K. Razdan,[c] Jenny L. Wiley,[b] and Vincenzo Di Marzo*[a]

Dedicated to the memory of Prof. Billy R. Martin

By binding to and activating the two cannabinoid receptor subtypes, CB_1 and CB_2, the endocannabinoid 2-arachidonoylglycerol (2-AG)[1,2] plays several physiological functions in both central and peripheral organs.[3] During either acute or chronic pathological conditions, the tissue levels of 2-AG, as well as of the other endocannabinoid anandamide, are altered, resulting in a change in cannabinoid receptor activation that acts to re-establish the homeostasis of other mediators (e.g., GABA and glutamate) and restore the cell to its original steady-state condition.[4] This initial protective function of the endocannabinoid system, and of 2-AG in particular, however, can be disrupted in individuals with certain chronic conditions causing a loss of specificity that may eventually contribute to the symptoms and/or progression of the disorder.[4] In these cases, inhibitors of endocannabinoid action, such as CB_1 and CB_2 receptor antagonists and inverse agonists, or biosynthesis might have therapeutic effects. Examples of such pathological states are obesity, metabolic syndrome, Alzheimer's and Parkinson's diseases, liver fibrosis and, under certain circumstances, allergic contact dermatitis.[4] Furthermore, localization of 2-AG biosynthetic enzymes on the postsynaptic dendritic spine suggests that, at least in the brain, 2-AG is the endocannabinoid that is most often involved in the modulation of the homeostasis of both excitatory and inhibitory neurotransmitters via "retrograde" activation of presynaptic CB_1 receptors[5] . Indeed, two enzymes involved in the biosynthesis of 2-AG from diacylglycerol precursors have been cloned and named *sn*-1-specific diacylglycerol lipase (DAGL) α and β.[6] At least one of these enzymes, DAGLα, has been found in the brain to be mostly localized in postsynaptic neurons.[5] Based on this background, the development of specific inhibitors of DAGLs is of great interest both because of the need for pharmacological tools to study the role of the endocannabinoid system in the brain, and from the point of view of drug development. In particular, given the specific role of up-regulated 2-AG expression in 1) hyperphagia of genetically obese rodents (hypothalamus),[7] and 2) intra-abdominal obesity and several parameters of the metabolic syndrome in obese individuals (peripheral organs),[8–11] DAGL inhibitors might provide a useful alternative to CB_1 receptor antagonists for the treatment of metabolic disorders.

We have previously described the development of two selective and rather potent fluorophosphonate inhibitors of DAGLα, O-3640 and O-3841 (Figure 1).[12] These compounds have been widely used in vitro as pharmacological tools to help distinguish the role of 2-AG from that of anandamide in retrograde modulation of neurotransmitter.[13–15] However, the applications of these two DAGL inhibitors, particularly of the more potent O-3841, have been limited so far by their relative lack of stability, and poor permeation of the plasma membrane as assessed through in vitro experiments with intact cells.[12] Therefore, we have undertaken the present study to improve the properties of the more potent compound O-3841 by substituting the methoxy for a *tert*-butoxy group, thus obtaining octadec-9-enoic acid 1-*tert*-butoxymethyl-2(fluoro-methyl-phosphinoyloxy)-ethyl-ester (O-5596) (Figure 1).

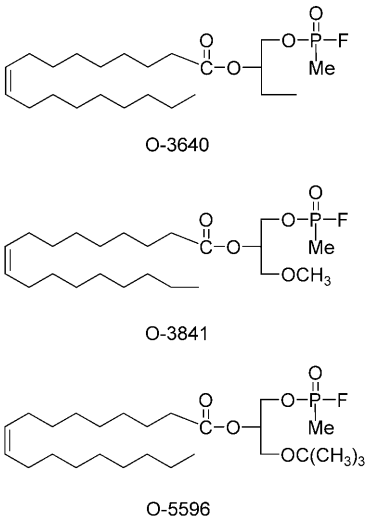


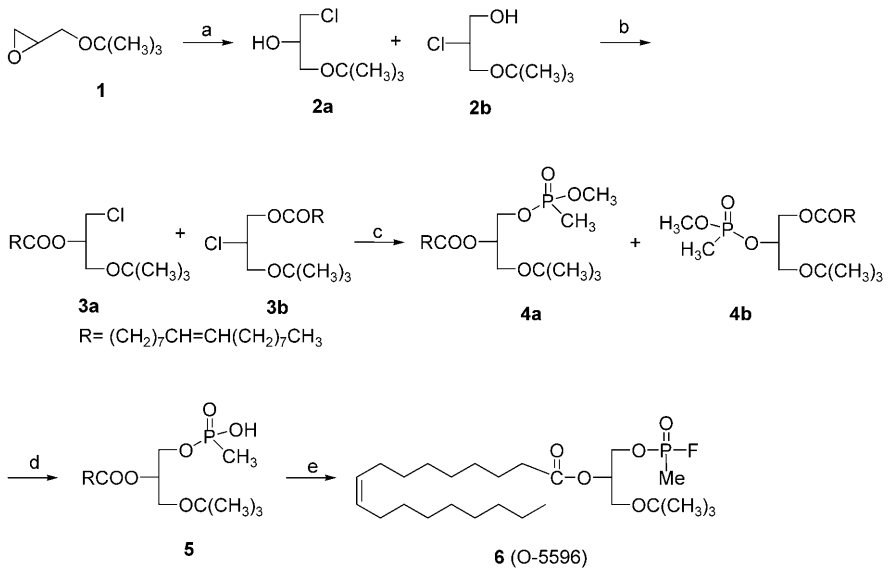
Figure 1. Chemical structures of O-5596 and other fluorophosphonate inhibitors of 2-AG biosynthesis.

We speculated that the oxygen on the O-3841 methoxy group (Figure 1) might be responsible for the compound instability by acting as a nucleophile and attacking the phosphorous atom and displacing the fluorine. Therefore, we hypothesized that a more sterically hindered *tert*-butoxy group (O-5596, Figure 1) would be more stable. O-5596 (**6**) was synthe-

[a] *T. Bisogno, M. Allarà, V. Di Marzo*
Endocannabinoid Research Group
Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche
Via Campi Flegrei 34, Comprensorio Olivetti, Pozzuoli (NA) (Italy)
Fax.: (+39) 081-8041770
E-mail: vdimarzo@icmib.na.cnr.it

[b] *J. J. Burston, J. L. Wiley*
Department of Pharmacology and Toxicology
Virginia Commonwealth University
410 North 12th St., Rm 746, P.O. Box 980613, Richmond, VA 23298 (USA)

[c] *R. Rai, B. Saha, A. Mahadevan, R. K. Razdan*
Organix Inc., 240 Salem St., Woburn, MA 01801 (USA)



Scheme 1. Synthesis of O-5596. *Reagents and conditions*: a) HCl in Et_2O, CH_2Cl_2, 0 °C; b) EDCI, oleic acid, DMAP, CH_2Cl_2; c) $CH_3PO(OCH_3)_2$,NaI, sealed tube, 160–170 °C, **4d**; d) TMSBr, CH_2Cl_2; e) Methyl DAST, CH_2Cl_2.

sized from commercially available *tert*-butyl glycidyl ether (**1**, Sigma–Aldrich) in five steps in an overall yield of 8.1% (Scheme 1). Treatment of compound **1** with HCl in Et_2O at 0 °C in CH_2Cl_2 afforded a 9:1 mixture of the desired isomer **2a** (90%) and isomer **2b** (10%). Treatment of **2** (mixture of isomers) with oleic acid in the presence of EDCI and DMAP gave **3** (97%, mixture of isomers). Further treatment with NaI and dimethyl methylphosphonate (DMMP) in a sealed tube at 160–170 °C for 4 days (or 3 h, 130 °C, 275 psi, under microwave conditions) gave compound **4** (54%, mixture of isomers). Compound **4a** was isolated by chromatography (25%).Treatment of **4a** with TMSBr gave the precursor **5** (79%), which on treatment with (dimethylamino)sulphur trifluoride (methyl DAST) gave compound **6** (O-5596, 47%).

The pharmacological profile of O-5596 was assessed in a variety of different assays (see Experimental Section for details). O-5596 exhibited an IC_{50} value of 100 nM in an assay measuring the hydrolysis of 1-[^{14}C]oleoyl-2-arachidonoylglycerol to [^{14}C]oleic acid in COS-7 cells stably expressing the human recombinant DAGLα (Table 1). O-5596 very weakly inhibited ($IC_{50} > 5$ μM) the hydrolysis of 2-[^{3}H]arachidonoylglycerol to [^{3}H]arachidonic acid by cytosolic fractions of COS-7 cells, which previous studies have shown to exhibit high monoacylglycerol lipase (MAGL) activity and high amounts of MAGL mRNA (Table 1). O-5596 also exhibited an IC_{50} value > 10 μM for the hydrolysis of [^{14}C]anandamide by rat brain membranes, which express high amounts of fatty acid amide hydrolase (FAAH)[16] (Table 1). Furthermore, in competition assays carried out using membranes from HEK-293 cells stably transfected with the human recombinant CB_1 or CB_2 receptors, O-5596 did not affect the specific binding of high affinity cannabinoid receptor ligands at concentrations lower than 10 μM (Table 1). When incubated with mouse neuroblastoma cells, O-5596 (1 μM) significantly inhibited the ionomycin-induced biosynthesis of 2-AG (Figure 2). Finally, when administered in vivo to mice, O-5596 (10 mg kg^{-1}, i.p.) caused a reduction in the consumption of palatable food (sweetened cereal) over a period of 21 h (Figure 3).

Table 1. Summary of the in vitro pharmacological activities of O-5596.[a]

Assay	IC_{50} [μM]	Inhibition [%]
2-AG formation[b]	0.10 ± 0.01	89 ± 4.8[e]
[^{3}H]2-AG hydrolysis[c]	> 5	28 ± 3.7[e]
[^{14}C]anandamide hydrolysis[d]	> 10	26 ± 1.8[f]
hCB_1 binding	> 10	–
hCB_2 binding	> 10	–

[a] Data are means ± SEM, $n = 4$. [b] 1-[^{14}C]oleoyl-2-arachidonoylglycerol in COS-7 cells expressing hDAGLα. [c] COS-7 cells cytosolic fractions. [d] rat brain. [e] max effect at 5 μ м. [f] max effect at 10 μM.

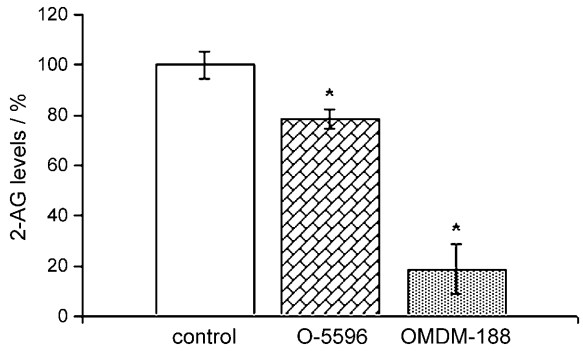


Figure 2. Effect of O-5596 on 2-AG levels in ionomycin-stimulated N18TG2 cells. The effect of both O-5596 (1 μM) and OMDM-188 (1 μM[17]) is reported as the maximum effect (%) observed with ionomycin (3 μM), and was significantly different from the control ($P < 0.01$, calculated by ANOVA followed by Bonferroni's correction).

Mice in both treatment conditions consumed similar amounts of regular feed but this amount was significantly greater than sweetened cereal consumption (Fruit Loops®). The amount of sweetened cereal consumed, however, differed between treatment groups, with O-5596-treated mice eating significantly less than vehicle-treated mice. Overt effects on mouse motor behavior were not observed at this dose, as assessed at the beginning and end of the 21 h feeding session.

The Stability of O-5596 and O-3841 was determined in DMSO at different temperatures and under physiological conditions (Tris-HCl buffer, pH 7, 37 °C). Table 2 details the degradation (%) observed under the different conditions. In aliquots kept in DMSO for one year at −20 °C, O-5596 was considerably

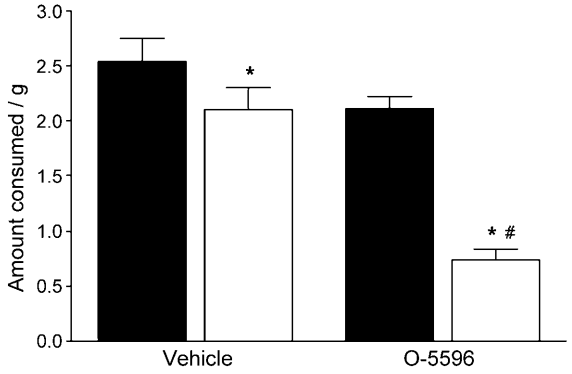


Figure 3. Effect of O-5596 (10 mg kg) on regular (■) and sweetened (□) feed consumption by mice. Data are means ± SEM, $n = 4$; *, as compared with regular feed after the same treatment; # as compared with sweetened cereal in vehicle-treated mice ($p < 0.05$, 2-factor split-plot ANOVA followed by Tukey's post hoc test).

Table 2. Stability of O-5596 versus O-3841

Compound	Degradation [%]					
	DMSO[a] 48 h		DMSO[a] 2 weeks		DMSO[a] 1 year	Tris-HCl buffer[b]
	25 °C	−20 °C	25 °C	−20 °C	−20 °C	37 °C
O-3841	35 ± 5	9 ± 2	79 ± 8	20 ± 4	85 ± 9	23 ± 5
O-5596	11 ± 3	< 5	35 ± 6	7 ± 2	18 ± 2	10 ± 3

[a] Drug concentration was 1 mg mL^{-1}. Values are means ± SD, $n = 2$. [b] Buffer conditions used: 50 mM, pH 7. Drug concentration was 200 μg mL^{-1}.

more stable than O-3841 (18 ± 2% vs. 85 ± 9% degradation). Generally speaking, O-5596 is significantly more stable than the almost equipotent analogue O-3841 at both room temperature and −20 °C and in both DMSO and physiological buffer.

Compared with previously identified irreversible DAGL inhibitors of the same class, O-3640 and O-3841,[12] O-5596 is selective for DAGLα over CB_1 and CB_2 receptors as well as FAAH and MAGL, and is more potent against human recombinant DAGLα. Furthermore, O-5596, as opposed to O-3640 and O-3841, is more cell membrane permeable. O-5596 inhibited the biosynthesis of 2-AG in intact cells to some extent, while O-3640 and O-3841 exerted no effect in the same assay at equal or higher concentrations.[12] However, O-5596 was less efficacious at inhibiting 2-AG biosynthesis in intact cells than OMDM-188, a DAGLα inhibitor with slightly higher potency and belonging to a different chemical class.[17]

Additionally, like other less selective and/or potent inhibitors of 2-AG biosynthesis, such as tetrahydrolipstatin and RHC80267,[6] O-5596 is suitable for in vivo use. The i.p. administration of O-5596 to mice reduced their intake of palatable food, as has been shown previously with the CB_1 receptor antagonists, rimonabant and AM251.[18,19] This finding is in agreement with the hypothesis that exposure to palatable food might stimulate endocannabinoid biosynthesis and CB_1 receptor activation in the nucleus accumbens shell, a brain area involved in motivation to consume food and reward.[20,21] Furthermore, hyperphagia in animal models of obesity has been linked to elevated levels of 2-AG in the hypothalamus,[7] and therefore, selective inhibitors of the biosynthesis of this endocannabinoid might be useful for the treatment of obesity.

In conclusion, the present data suggest that O-5596 is a useful pharmacological tool for the investigation of the physiopathological role of 2-AG in vitro and in vivo, and a promising template for the development of therapeutic alternatives to CB_1 antagonists in those pathological conditions that might benefit from a reduction in endocannabinoid levels (obesity, liver fibrosis, contact dermatitis, etc.).[4]

Experimental Section

Chemistry

1-*tert*-Butoxy-3-chloro-propan-2-ol (**2a**): HCl in Et_2O (2 M, 15 mL, 30 mmol) was added dropwise to a stirred solution of *tert*-butyl glycidyl ether (**1**, 2.6 g, 20 mmol) in CH_2Cl_2 (50 mL) at 0 °C and stirred until the reaction was complete. $NaHCO_{3(s)}$ was added to the reaction mixture, which was then extracted with CH_2Cl_2 (2× 40 mL). The organic layer was washed with H_2O and brine and dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column chromatography (5→15%, EtOAc/hexanes) afforded a mixture of **2a** and **2b** (9:1) as a colorless liquid (3 g, 90%): R_f = 0.5 (EtOAc/hexanes, 1:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.83–3.94 (m, 1H, CH), 3.54–3.66 (m, 2H, CH_2), 3.46 (d, J = 5.2 Hz, 2H, CH_2), 2.56 (td, J = 6, 0.84 Hz, 1H, OH), 1.19 ppm (s, 9H, $C(CH_3)_3$).

Octadec-9-enoic acid 2-*tert*-butoxy-1-chloromethyl ethyl ester (**3**): Compound **2** (3 g, 18 mmol), EDCI (5.17 g, 191.7 mmol), DMAP (catalytic amount) and oleic acid (6.1 g, 21.6 mmol) in CH_2Cl_2 (90 mL) was stirred at RT overnight. The reaction mixture was diluted with CH_2Cl_2, washed with H_2O, dilute HCl (1 N), brine and dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash column chromatography (5%, EtOAc/hexanes) afforded compound **3** (9:1 isomeric ration) as a colorless liquid (7.52 g, 97%): R_f = 0.7 (EtOAc/hexanes, 1:9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.31–5.35 (m, 2H, CH=CH), 5.02–5.09 (m,1H, CH), 3.64–3.79 (m, 2H, CH_2), 3.50 (d, J = 5.5 Hz, 2H, CH_2), 2.34 (td, J = 7.5, 0.9 Hz, $COCH_2$), 2.0 (m, 4H, CH_2), 1.57–1.63 (br t, 2H, CH_2), 1.26–1.34 (m, 20H, CH_2), 1.13 (s, 3H, $C(CH_3)_3$), 0.87 ppm (t, J = 6.6 Hz, 3H, CH_3); MS (CI) *m/z*: 395.4 $[M-Cl]^+$.

Octadec-9-enoic acid 1-*tert*-butoxymethyl-2(methoxy-methyl-phosphinoyloxy)ethyl ester (**4a**): Compound **3** (4 g, 9.27 mmol), NaI (2 g, 13.3 mmol) and DMMP (14 mL, 98.6 mmol) was heated in a sealed tube at 160–170 °C for 4 days. DMMP was removed under reduced pressure. The residue was purified by flash column chromatography (20→75%, EtOAc/hexanes) to give a mixture of **4a** and **4b** (2.62 g, 55%) followed by another column (20%, CH_2Cl_2/EtOAc), to afford pure isomer of **4a** (1.19 g, 25%): R_f = 0.4 (CH_2Cl_2/hexanes, 2:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.31–5.35 (m, 2H, CH=CH), 5.0–5.08 (m, 1H, CH), 4.15–4.24 (m, 2H, CH_2), 3.72 (dd, J = 11.2, 2.7 Hz, 3H, OCH_3), 3.46 (d, J = 5.2 Hz, 2H, CH_2), 2.32 (t, J = 7.4 Hz, 2H, $COCH_2$), 1.96–2.0 (m, 4H, CH_2), 1.58–1.64 (m, 2H, CH_2), 1.48 (dd, J = 17.6, 4.1 Hz, 3H, P-CH_3), 1.27 (br d, 20H, CH_2), 1.15 (s, 9H, $C(CH_3)_3$), 0.87 ppm (t, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) 173.17, 173.14, 130.02, 129.72, 73.41, 71.73, 71.64, 71.55, 64.35, 64.30, 64.27, 64.23, 59.69, 59.66, 52.13, 52.06, 52.04, 51.98, 34.30, 31.91, 29.77, 29.71, 29.52, 29.33, 29.20, 29.11, 29.07, 27.35, 27.22, 27.17, 24.94, 24.92, 22.68, 14.12, 11.39, 11.35, 9.47, 9.43 ppm; MS (CI) *m/z*: 505.3 $[M+H]^+$.

Octadec-9-enoic acid 1-*tert*-butoxymethyl-2(hydroxy-methyl-phosphinoyloxy)ethyl ester (**5**): TMSBr (1.4 mL, 0.99 mmol) was added in a single portion to a stirred solution of **4a** (0.5 g, 0.99 mmol) in anhydrous CH_2Cl_2 (10.8 mL). The reaction mixture was stirred for 30 min and the solvent was evaporated under reduced pressure at RT. The residue was dissolved in 95% MeOH (8 mL) and stirred for 1 h. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (10→30%, MeOH/$CHCl_3$) to afford **5** (0.384 g, 79%): $R_f = 0.05$ (MeOH/$CHCl_3$, 1:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.33 (br t, 2H, CH=CH), 5.05 (br s, 1H, CH), 4.06 (br s, 2H, CH_2), 3.46 (br s, 2H, CH_2), 2.34 (br t, 2H, CH_2), 1.99 (br d, 4H, CH_2), 1.61 (br s, 2H, CH_2), 1.27 (br d, 20H, CH_2), 1.15 (s, 9H, $C(CH_3)_3$), 0.87 ppm (t, $J = 6.3$ Hz, 3H, CH_3); MS (CI) *m/z*: 489.4 $[M-H]^-$.

Octadec-9-enoic acid 1-*tert*-butoxymethyl-2(fluoro-methyl-phosphinoyloxy)ethyl ester (**6**) O-5596: Methyl DAST (98.5 mg, 0.74 mmol) was added to a stirred solution of **5** (330 mg, 0.67 mmol) in CH_2Cl_2 (9 mL). The reaction mixture was stirred for 1 h and quenched with H_2O (10 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2×10 mL). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash chromatography with $CHCl_3$ to afford **6** (O-5596) (156 mg, 47%, purity >98%): $R_f = 0.6$ (MeOH/$CHCl_3$, 1:19); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.31–5.33 (m, 2H, CH=CH), 5.06–5.07 (m, 1H, CH), 4.35–4.38 (m, 2H, CH_2), 3.42–3.48 (m, 2H, CH_2), 2.0 (t, $J = 7.8$ Hz, 2H), 1.96–2.0 (m, 4H, CH_2), 1.65 (ddd, $J = 21$, 6, 1.65 Hz, 3H, PCH_3), 1.6 (br s, 2H, CH_2), 1.27 (br d, 20H, CH_2), 1.15 (s, 9H), 0.87 ppm (t, $J = 6.5$ Hz, 3H, CH_3); ^{19}F NMR (300 MHz, $CDCl_3$) δ −58.21 ppm (2d, $J = 1120$ Hz, 1F); MS (CI) *m/z*: 419.3 $[M-73]^+$; Anal. calcd for $C_{26}H_{50}O_5PF$: C, 63.39; H, 10.23. Found: C, 63.43; H, 10.34.

Stability experiments

Aliquots of O-3841 and O-5596 (1 mg mL^{-1}, 100 μL) were immediately analyzed or kept for 48 h or 2 weeks at RT (25 °C) or at −20 °C and then directly analyzed. Analysis was carried out by TLC on silica plates (Merck), using $CHCl_3$/CH_3OH (95:5, *v/v.*) as the developing solvent. With this solvent, the R_f values for O-3841 and O-5596 are 0.5 and 0.75, respectively. After each TLC analysis the corresponding bands of 5 μL of each solution were visualized by reaction with cerium sulfate and heating, and quantified with a densitometer using a digital camera working on grey levels (JCV FC 340FX, Leica) and a software Image Pro Plus® 6.0 for Windows (MediaCybernetics) working on logarithmic values scale of absorbance for densitometric evaluation. The amount of each compound at the beginning of the experiment was taken as 100%. This method allows the detection of changes in the amount of compound >5%. When the compounds (100 μg) were dissolved in Tris-HCl buffer (500 μL, 50 mM, pH 7), to be incubated at 37 °C for 0 min and 1 h, analyses were performed on aliquots of the lyophilized extracts obtained with $CHCl_3$/CH_3OH (2:1, *v/v*), loaded onto the TLC plates.

Biology

CB_1 and CB_2 binding assays: Membranes from HEK-293 cells transfected with either the human CB_1 or CB_2 receptor (Perkin–Elmer, Italia) were incubated with increasing concentration of compounds and [^{3}H]CP-55,940 ([^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)-cyclohexanol) was used as the high affinity ligand, as described by the manufacturer.

Fatty acid amide hydrolase assays: The effect of compounds on the enzymatic hydrolysis of [^{14}C]anandamide was studied as previously reported[22] by using membranes prepared from rat brain incubated with increasing concentrations of compounds in 50 mM Tris-HCl, pH 9, for 30 min at 37 °C. [^{14}C]Ethanolamine produced from [^{14}C]anandamide hydrolysis was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with two volumes of $CHCl_3$/CH_3OH (1:1, *v/v*).

DAGL assays: DAGL activity was detected as previously reported[6] by using membrane preparations (100 μg of protein) obtained from COS cells overexpressing human DAGLα, and 1-[^{14}C]oleoyl-2-arachidonoylglycerol (1.0 mCi mmol^{-1}, 25 μM, synthesized as reported[6]) or *sn*-1-stearoyl-2-[^{14}C]-arachidonoylglycerol (Amersham Biosciences, 56.0 mCi mmol^{-1}) as substrates and increasing concentrations of compounds in Tris-HCl buffer, pH 7 for 15 min. After the incubation, lipids were extracted with two volumes of $CHCl_3$/CH_3OH (2:1, *v/v*). The extracts, lyophilized under vacuum, were purified by using TLC on silica on polypropylene plates eluted in $CHCl_3$/CH_3OH/NH_4OH (85:15:0.1, *v/v*). Bands corresponding to either [^{14}C]oleic acid (when using 1-[^{14}C]oleoyl-2-arachidonoylglycerol as substrate) or [^{14}C]2-arachidonoylglycerol (when using *sn*-1-stearoyl-2-[^{14}C]-arachidonoylglycerol as substrate) were cut and their radioactivity measured with a β-counter.

MAGL assays: MAGL activity was measured as reported[6] using the cytosol derived from the 10,000 g fraction of homogenates from wild-type COS cells and synthetic 2-[^{3}H]arachidonoylglycerol (1.0 mCi mmol^{-1}, 25 mM) as substrate. Previous studies indicated that this fraction exhibits the highest MAGL activity and that COS cells express high amounts of MAGL mRNA (T. Bisogno and V. Di Marzo, unpublished observations). 100 μg of protein was used for each assay and increasing concentrations of compounds in Tris-HCl buffer, pH 7 for 20 min. After the incubation, lipids were extracted, purified and quantified as above. Bands corresponding to [^{14}C]arachidonic acid were cut and their radioactivity measured with a β-counter.

2-AG formation in intact cells: Confluent N18TG2 cells were stimulated for 20 min at 37 °C with ionomycin (3 μM) with O-5596 (1 μM, also pre-incubated with cells for 10 min) or OMDM-188 (1 μM, same conditions), or without any drug (control). After stimulation, cells plus medium were extracted with $CHCl_3$/CH_3OH 2:1 (*v/v*). Each extract was purified by open bed chromatography and 2-AG was quantified by LC-MS as previously reported.[23]

Effect of O-5596 on food intake in mice: Adult male ICR mice (27–35 g) (Harlan, Dublin, USA) were housed in a temperature-controlled environment (20–22 °C) with a 12-hour light–dark cycle (lights on at 7 a.m.). Each mouse ($n = 4$ per group) was injected intraperitoneally (at ~5 p.m.) with 10 mg kg^{-1} O-5596 or vehicle (1:1:18, ethanol/emulphor/saline) at a volume of 0.1 mL for every 10 g body weight. Subsequently, they were placed in a clear plastic cage with white tissue paper lining the bottom and allowed access to pre-measured amounts of their regular lab feed and sweetened cereal. The cereal was not a novel food item, as mice had previously received it in their home cages. After 21 h, all food was removed and weighed with a Mettler AT261 Delta Range scale (Toledo, USA) at 0.01 mg accuracy. The amount of each type of food consumed was calculated separately for each mouse. Data were analyzed with a split-plot ANOVA (within subject factor = type of food; between subject factor = drug) followed by Tukey post-hoc tests ($\alpha = 0.05$) on the significant interaction. The studies reported in this manuscript were carried out in accordance with guidelines pub-

lished in guide for the care and use of laboratory animals (National Research Council, 1996).

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*T. Bisogno, J. J. Burston, R. Rai, M. Allarà, B. Saha, A. Mahadevan, R. K. Razdan, J. L. Wiley, V. Di Marzo**

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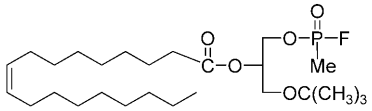
Synthesis and Pharmacological Activity of a Potent Inhibitor of the Biosynthesis of the Endocannabinoid 2-Arachidonoylglycerol



O-5596

Biosynthesis Inhibition: O-5596, a new inhibitor of the biosynthesis of the endocannabinoid, 2-arachidonoylglycerol, was synthesized and found to be potent ($IC_{50} = 100$ nM) and selective versus other proteins and enzymes of the endocannabinoid system in vitro and active in vivo at reducing food intake in mice.

