(−)-UB006: a new fatty acid synthase inhibitor and cytotoxic agent without anorexic side effects

Kamil Makowski[a], *Joan Francesc Mir*[b], *Paula Mera*[b,c], *Xavier Ariza*[d,e], *Guillermina Asins*[b], *Fausto G. Hegardt*[b], *Laura Herrero*[b,e], *Jordi García*[d,e*], *Dolors Serra*[b,e*]

[a] Department of Chemistry, YachayTech University, Hacienda San Jose SN, San Miguel de Urcuqui 100119, Ecuador

[b] Department of Biochemistry and Physiology, Facultat de Farmàcia i Ciències de l'A limentació and Institut de Biomedicina de la Universitat de Barcelona (IBUB), Universitat de Barcelona, E-08028 Barcelona, Spain

[c] Columbia University Medical Center, 701 West 168th Street, New York, USA

[d] Department of Inorganic and Organic Chemistry, Section of Organic Chemistry, Facultat de Química, Universitat de Barcelona, E-08028 Barcelona, Spain; and Institut de Biomedicina de la Universitat de Barcelona (IBUB)

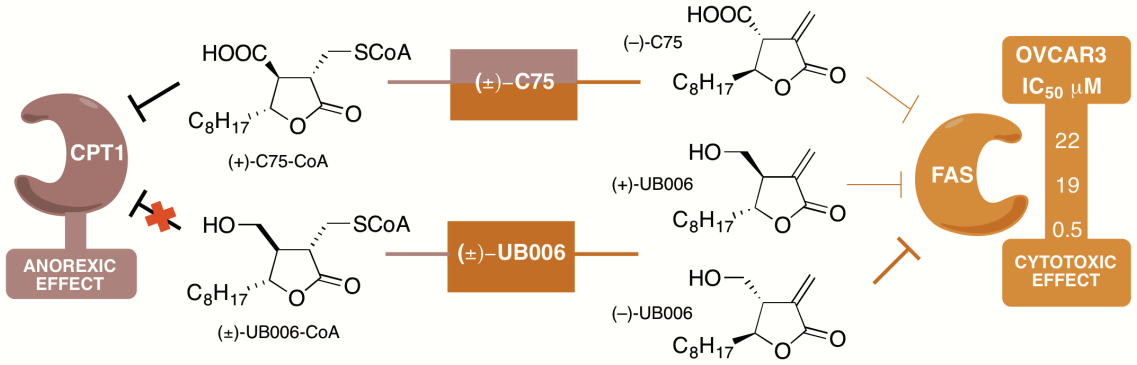
[e] Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, E-28029 Madrid, Spain

*Corresponding authors

E-mail addresses: **jordigarciagomez@ub.edu** and **dserra@ub.edu**

ABSTRACT

C75 is a synthetic anticancer drug that inhibits fatty acid synthase (FAS) and shows a potent anorexigenic side effect. In order to find new cytotoxic compounds that do not impact food intake, we synthesized a new family of C75 derivatives. The most promising anticancer compound among them was UB006 ((4*SR*,5*SR*)-4-(hydroxymethyl)-3-methylene-5-octyldihydrofuran-2(3*H*)-one). The effects of this compound on cytotoxicity, food intake and body weight were studied in UB006 racemic mixture and in both its enantiomers separately. The results showed that both enantiomers inhibit FAS activity and have potent cytotoxic effects in several tumour cell lines, such as the ovarian cell cancer line OVCAR-3. The (–)-UB006 enantiomer's cytotoxic effect on OVCAR-3 was 40-fold higher than that of racemic C75, and 2- and 38-fold higher than that of the racemic mixture and its opposite enantiomer, respectively. This cytotoxic effect on the OVCAR-3 cell line involves mechanisms that reduce mitochondrial respiratory capacity and ATP production, DDIT4/REDD1 upregulation, mTOR activity inhibition, and caspase-3 activation, resulting in apoptosis. In addition, central and peripheral administration of (+)-UB006 or (–)-UB006 into rats and mice did not affect food intake or body weight. Altogether, our data support the discovery of a new potential anticancer compound (–)-UB006 that has no anorexigenic side effects.



Keywords: FAS fatty acid synthase, C75 derivatives, CPT1 carnitine palmitoyltransferase 1, ovarian cell cancer

Abbreviations:

18S, 18S ribosomal RNA; BIP, binding immunoglobulin protein; BSA, bovine serum albumin; CASP3, caspase-3; CPT1, carnitine palmitoyltransferase 1; DDIT4/REDD1, DNA-damage-inducible transcript 4/ regulated in development and DNA damage responses 1 protein; DMSO, dimethyl sulfoxide; ETC, electron transport chain; FAO, fatty acid β-oxidation; FAS, fatty acid synthase; FCCP, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone; i.c.v., intracerebroventricular; i.p., intraperitoneal; IC_{50}, half maximal inhibitory concentration; LCFA, long chain fatty acid; mTOR, mammalian target of rapamycin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OCR, oxygen consumption rate; TIM-44, translocase of the mitochondrial inner membrane.

1. INTRODUCTION

Tumour cells present a typical phenotype of abnormally elevated fatty acid synthase (FAS) activity, which indicates that this enzyme is a good target for treating some malignancies [1,2]. FAS catalyses fatty acid synthesis from the substrates acetyl-CoA and malonyl-CoA [3]. There is increased interest in finding novel FAS inhibitors as antitumor drugs and many compounds have been evaluated for their cytotoxic capacity [4,5]. Among them, C75 is a chemically stable inhibitor of FAS that binds to the enzyme and inhibits its activity [6]. This synthetic compound has antitumor activity in cell lines and animal models, through mechanisms that reduce lipid synthesis and increase apoptosis [1,7].

Inside the cell, C75 is converted into its coenzyme A derivative, C75-CoA, a potent inhibitor of carnitine palmitoyltransferase 1 (CPT1) [8,9]. CPT1 catalyses the first step in the transport of long-chain fatty acyl-CoA (LFCA-CoA) from the cytoplasm to the mitochondria, which is the rate-limiting step in fatty acid β-oxidation (FAO) [10]. Due to its dual inhibitory effect on FA synthesis (FAS inhibition) and FA oxidation (CPT1 inhibition), C75 has antitumor activity. However, C75 also has undesirable side effects that limit its use for cancer therapy. C75 crosses the blood-brain barrier and affects the central nervous system. In the hypothalamus, the presence of the CoA derivative C75-CoA in the cell causes a profound reduction in food intake and drop in body weight in rodents, through CPT1 inhibition [9]. Therefore, appetite suppression and weight loss are undesirable side effects of the use of this drug in cancer therapy.

It is well-known that the stereochemistry of a drug could determine its biological activity [11,12]. The preparation of (–)-C75 and (+)-C75 enantiomers separately was not reported until recently [13]. We have previously shown that (–)-C75 inhibits FAS

activity *in vitro* and has cytotoxic effects on tumour cell lines, whereas (+)-C75 inhibits CPT1 *in vitro* and its administration to rodents produces anorexia [14].

The aim of this study was to investigate whether a modification in the C75 structure could lead to changes in its pharmacological activity, particularly in relation to its antitumor properties. We synthesized a series of new compounds that are structurally related to C75. Here we describe *de novo* synthesis of three alcohol C75 derivatives: (4*SR*,5*SR*)-4-(hydroxymethyl)-3-methylene-5-octyldihydrofuran-2(3*H*)-one, also called (±)-UB006, and its two homologues (±)-UB339 (one carbon extra) and (±)-UB340 (2 carbons extra). Based on our previous experience of the stereoselective synthesis of paraconic acids [15], we also synthesized both enantiomers of UB006 separately to study possible differences in their biological properties (Fig. 1).

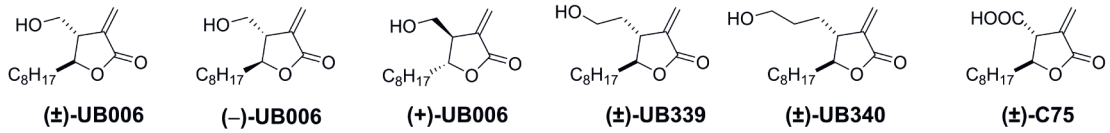


Figure 1. *Structure of (±)-UB006, (–)-UB006, (+)-UB006, (±)-UB339, (±)-UB340 and (±)-C75*

2. RESULTS AND DISCUSSION

2.1. Synthesis of new drugs

2.1.1. Synthesis of (±)-UB006

C75 was used as a starting material for the synthesis of (±)-UB006 (Fig. 2). In the first step, the exocyclic double bond was protected by a selenoether derivative. Then, the carboxylic group was reduced with borane, and finally the double bond was recovered under oxidizing conditions.

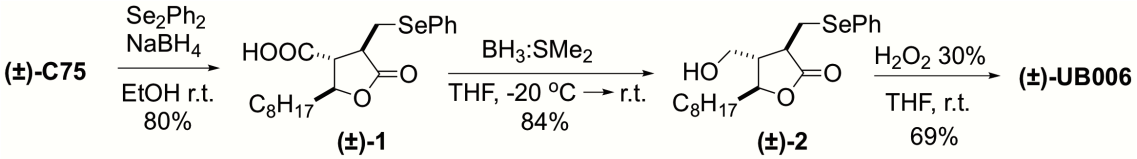


Figure 2. *Synthesis of (±)-UB006*

2.1.2. Synthesis of (–)-UB006 and (+)-UB006

We used two different routes to synthesize the enantiomers of UB006 (Fig. 3). In the first route, the enantiomers of C75 were used as a starting material [14], and the subsequent protocol was identical to those reported for the racemic mixture. The second route was more straightforward. In our previous studies, we obtained a useful chiral γ-butyrolactone [14]. We reductively cleaved this compound using $NaBH_4$ as a mild reagent. Next, we achieved α-methylenation of the resulting lactone by Greene's procedure [16], to yield (–)-UB006 (or (+)-UB006.

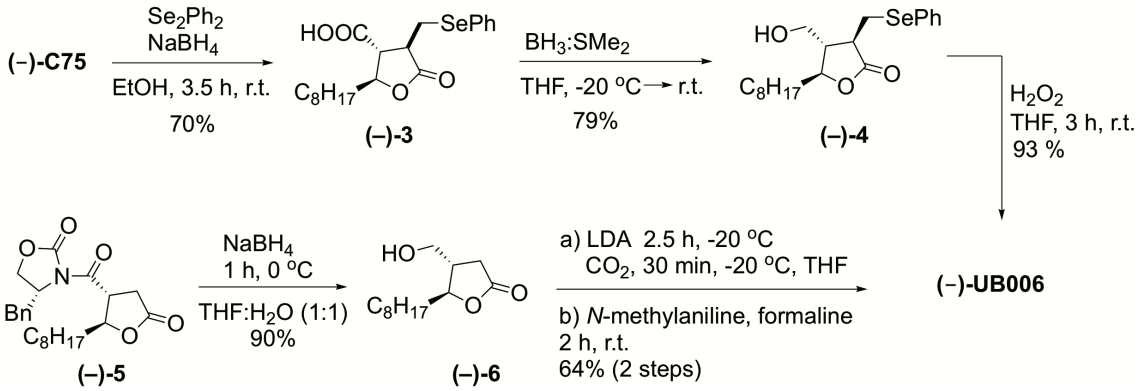


Figure 3. *Synthesis of (–)-UB006*

2.1.3. Synthesis of (±)-UB339

Lactone formation of (±)-UB339 was accomplished through Parker's protocol [17] from nonanoic anhydride and tricarballylic acid (Fig. 4). The carboxylic group was selectively reduced as explained before. We obtained the desired compound using borane, followed by α-methylenation of the resulting alcohol.

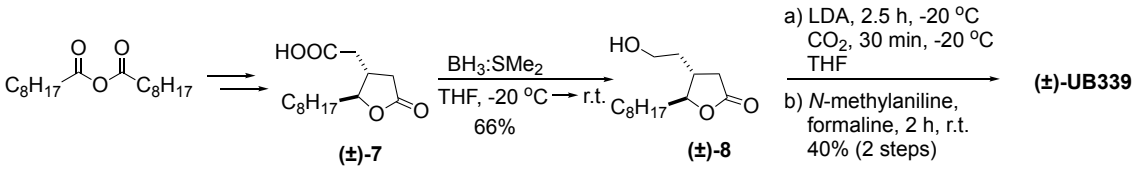


Figure 4. *Synthesis of (±)-UB339*

2.1.4. Synthesis of (±)-UB340

The synthesis of (±)-UB340 (Fig. 5) was started by known free radical addition of nonanal to dimethyl maleate [18]. Then, in three steps, we transformed the adduct to a functionalized *trans* butyrolactone bearing a methyl ester in β and a nonanyl group in γ. The methyl ester was hydrolysed in basic media without any appreciable

isomerization to the *cis* lactone. The carboxylic acid functionality was further changed to aldehyde by borane-mediated reduction, followed by Swern oxidation. Two-carbon homologation was carried out by a Wittig reaction using commercially available phosphorane to produce *E* olefin. In the next two steps, we hydrolysed *tert*-butyl ester and reduced the double bond with H_2/Pd. After that, we introduced a methylene group in the α position, and protected it with a selenoether derivate, which allowed us to reduce the carboxylic group with borane. In the final step, the double bond was recovered under oxidizing conditions.

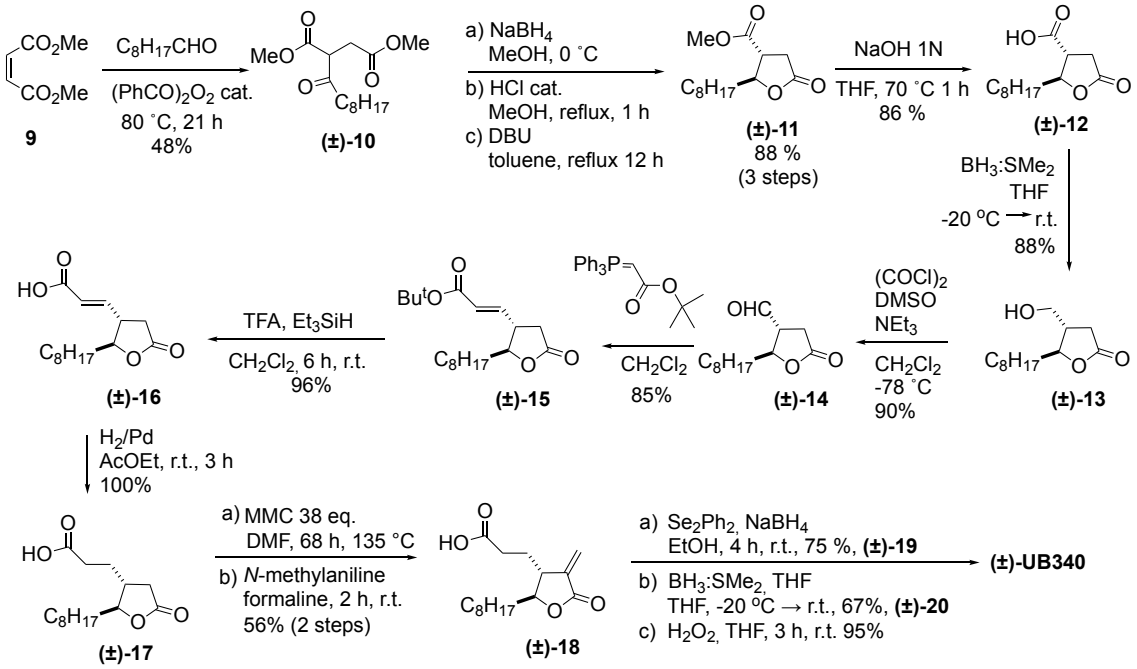


Figure 5. *Synthesis of (±)-UB340*

2.2. Inhibitory effect on in vitro FAS activity

We analysed the effect of the racemic mixtures of UB006, UB339 and UB340 and of the separated UB006 enantiomers on FAS activity. (±)-UB006 and its separated enantiomers inhibited FAS, but the (−)-UB006 form showed stronger inhibitory activity

than the (+) enantiomer or the racemic form (Fig. 6A). The (±)-UB339 and (±)-UB340 forms exhibited lower inhibitory activity on FAS than UB006 (Fig. 6B). Since our measure of IC_{50} of (–)-C75 was 460±44 μM [14], the data demonstrated that the (–)-UB006 form, IC_{50} 220±58, was a stronger FAS inhibitor than the other two UB006 chemicals or (–)-C75 (Fig. 6A and B). As expected, the results demonstrate that both enantiomers show different quantitative actions. While (–)-UB006 inhibits FAS *in vitro* with an IC_{50} value of 220 μM, the (+)-UB006 enantiomer has an IC_{50} value of 470 μM, which is close to that of racemic C75 with an IC_{50} value of 498 μM. In addition, UB006 homologues (±)-UB340 and (±)-UB339 showed less FAS inhibitory activity.

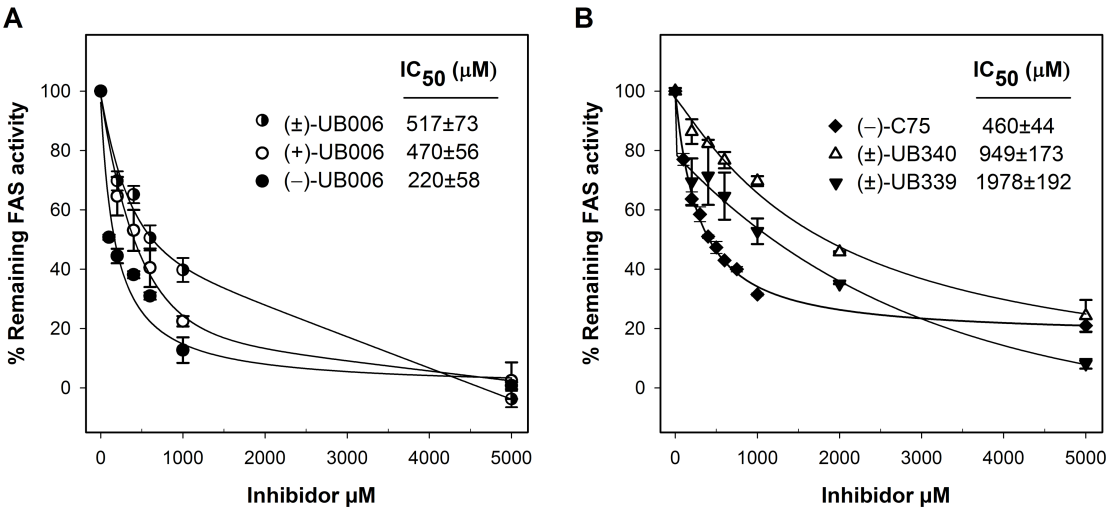


Figure 6*. Effects of (±)-UB006, (+)-UB006, (–)-UB006, (±)-UB339 and (±)-UB340 on in vitro FAS activity.* (**A**) Cytosolic hepatic extracts (315 μg) obtained from rat were preincubated for 30 min with increasing concentrations of (±)-UB006, (+)-UB006, (–)-UB006, (**B**) (±)-UB339 and (±)-UB340. FAS activity was measured and IC_{50} were calculated. Data are expressed as the mean ± SEM.

2.3. Cytotoxic effect on tumour cell lines

Normal tissues have low levels of fatty acid synthesis. However, some malignancies and their precursor lesions show high levels of FAS expression, which suggests that FAS is a target for anticancer drug development [1,7,19]. After the *in vitro* analysis of (+)-UB006, (−)-UB006, (±)-UB006, (±)-UB339 and (±)-UB340 on FAS activity, we studied the effect of these drugs on tumour cell viability. It has been reported that the inhibition of FAS in cancer cells diminishes their viability and has a cytotoxic effect. To this aim, cytotoxicity assays were performed with human cancer cell lines from different tissues: SKBr3, OVCAR3, HCT116, MCF-7, MiaPaca2 and MDA-MB-231, with different levels of FAS expression (Fig. 7) [1,20], and consequently different sensitivity to FAS inhibitors. Therefore, we performed an MTT cytotoxic assay with these seven cancer cell lines and fibroblasts as a control (Table 1).

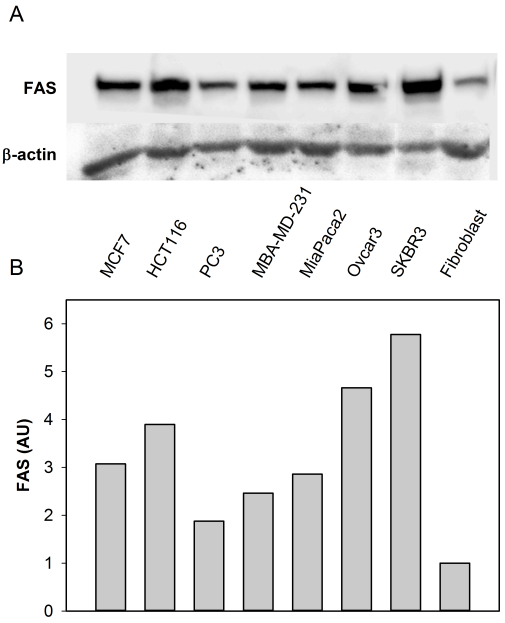


Figure 7. *Analysis of FAS protein levels in different cancer cell lines and fibroblasts.* (**A**) 30 ug of cytosolic extracts of the each cell line were used for the Western blot analysis. (**B**) Relative protein quantification.

The results of the MTT cytotoxic assay showed that all racemic alcohols, (±)-UB006, (±)-UB339 and (±)-UB340 were better cytotoxic agents than (–)-C75 in all cell lines used in this study. Interestingly, enantiomers of UB006 had different effects on cell viability (Table 1). We observed that (–)-UB006, the enantiomer showing stronger FAS inhibitory properties, had a cytotoxic effect on SKBr3, OVCAR3, HCT116, MCF-7, MiaPaca2 and MDA-MB-231 tumour cell lines with IC_{50} of 1.0, 0.5, 6.5, 15.0, 9.8, and 17.9 μM, respectively. All these IC_{50} values were lower than those determined for (–)-C75 (Table 1). (–)-UB006, with an IC_{50} of 1.0 (SKBR3) and 0.5 μM (OVCAR3), was almost 40 times more active than (–)-C75. However, (+)-UB006 had a higher IC_{50} in the cell lines tested than the (–) enantiomer. These results suggest that (–)-UB006 could be a good candidate for the treatment of some malignancies, because of its cytotoxic effect on tumour cells, particularly OVCAR3 cells whose IC_{50} value reached the minimum of 500 nM. A comparison of these IC_{50} values with those obtained by treating the cells with (–)-C75 shows the much stronger power of (–)-UB006 to act as an antitumor agent. This is especially relevant, as racemic C75 is considered a good candidate for improving cancer treatment [21].

Cell line	SKBR3	OVCAR3	HCT116	MCF-7	MiaPaca2	PC3	MDA-MB-231	Fibroblasts
Compound	**IC_{50} (μM)**							
C75	19.8±1.8	21.9±3.9	46.4±5.7	27.3±1.8	21.8±0.9	26.8±3.1	43.8±1.8	63.2±10.5
(–)-C75	38.2±1.4	17.9±2.7	74.8±2.3	46.4±2.3	25.3±1.6	34.3±1.6	57.7±0.4	>117
(±)-UB006	3.7±1.7	1.1±0.3	18.5±1.3	26.5±2.2	13.1±0.4	10.0±1.9	11.8±1.3	11.6±0.8
(–)-UB006	1.0±0.3	0.5±0.3	6.5±0.7	15.0±1.6	9.8±1.0	N.D	17.9±1.3	22.4±1.2
(+)-UB006	4.7±0.2	19.4±2.5	15.9±1.4	40.1 ±3.0	30.7±1.4	24.2±1.5	36.8±0.8	47.8±1.3
(±)-UB339	5.5±0.5	6.9±1.6	N.D	17.6±1.6	12.4±0.5	N.D	27.2±0.7	24.9±0.8
(±)-UB340	5.4±0.8	1.1±0.3	N.D	16.0±1.6	8.9±1.0	N.D	14.2±0.5	17.0±0.4

Table 1. *Effect of (–)-C75, (±)-UB006, (+)-UB006, (–)-UB006, (±)-UB339 and UB340 on MCF-7, SKBr3, Ovcar-3, MiaPaca2, PC3, HCT116 and MDA-MB-231 cell viability.* To calculate IC_{50}, cells were incubated for 72h with increasing concentrations of the compounds. Drug cytotoxicity was determined using a standard colorimetric MTT assay. Data are expressed as the mean ± SEM. N.D = not determined.

2.4. Effect on fatty acid synthesis

It has been reported that (±)-C75 inhibits fatty acid synthesis, measured as the incorporation of [$U^{14}C$]acetate into total lipids [22–24]. We demonstrated that the newly synthesized FAS inhibitors, (–)-UB006 and (+)-UB006, inhibited fatty acid synthesis pathway activity in the OVCAR3 cell more than (±)-C75. (–)-UB006 and (+)-UB006, at 2 μg/ mL concentration, produced an 85% and 68% reduction in fatty acid synthesis

activity respectively (Fig. 8), while the reduction produced by 8 µg/ mL of racemic C75 was 38%. Next, we confirmed that the observed effect of (–)-UB006 on fatty acid synthesis reduction is correlated with FAS inhibition. We achieved this by analysing FAS activity in OVCAR3 cells after 2-hour (–)-UB006 treatment or DMSO treatment. (–)-UB006 inhibits FAS activity by 24.1%. FAS activity in OVCAR3 cells was 23.6±1.9 pmol/min/mg prot after DMSO treatment, and 17.9±1.3 pmol/min/mg prot after (–)-UB006 treatment.

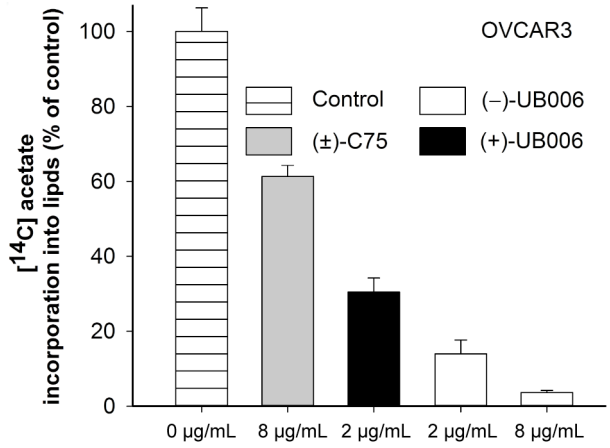


Figure 8. ***Effects of (+)-UB006, (–)-UB006 and (±)-C75 on fatty acid synthesis of OVCAR3 cells.*** OVCAR3 cells were incubated with different concentrations of (+)-UB006, (–)-UB006, (±)-C75 and DMSO as a control. After the addition of sodium [U-^{14}C]-acetate, lipids were extracted and the incorporation of ^{14}C was measured.

Our data agree with those of studies on other FAS inhibitors [25] of lipid biosynthesis, which deplete the palmitate and palmitate-derived lipids required in multiple cellular processes for tumour cell growth, proliferation and survival.

2.5. Effect of (−)-UB006 *on apoptosis*

It has been described that FAS inhibitors trigger cell death by apoptotic mechanisms [25,26]. In an attempt to elucidate the mechanism of action of (−)-UB006, we first measured the mRNA expression of apoptotic-marker caspase-3 and ER stress chaperone BiP or energy stress factor DDIT4/REDD1 [27] (Fig. 9). While BiP mRNA levels remained unchanged, we observed a 20% increase in caspase-3 mRNA levels and a remarkable 25-fold increase in DDIT4/REDD1 mRNA levels. DDIT4/REDD1 is an inducer of apoptosis [26]. In a recent study, this factor was involved in the induction of ovarian cancer cell death by activating apoptosis [26]. These authors demonstrated that orlistat, an inhibitor of FAS, mediates its apoptotic action by inducing DDIT4/REDD1, which in turn inhibits the mTOR pathway and cell proliferation, and activates 2-, 8-, 9- and 3-caspases, all resulting in cell death.

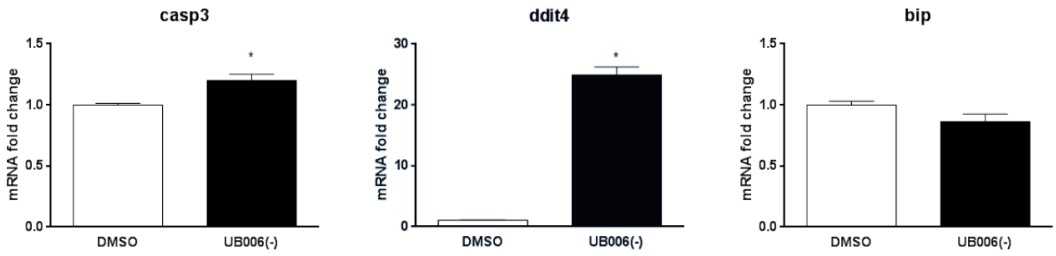


Figure 9. *Analysis of mRNA levels of caspase-3, DDIT4/REDD1 and ER stress Bip factor by qRT-PCR in OVCAR3 after (–)-UB006 treatment.*

The induction of apoptosis was confirmed by Western blot, through assessing caspase-3 cleavage. In line with the high upregulation of DDIT4/REDD1, we observed increased activation in both caspases (Fig. 10).

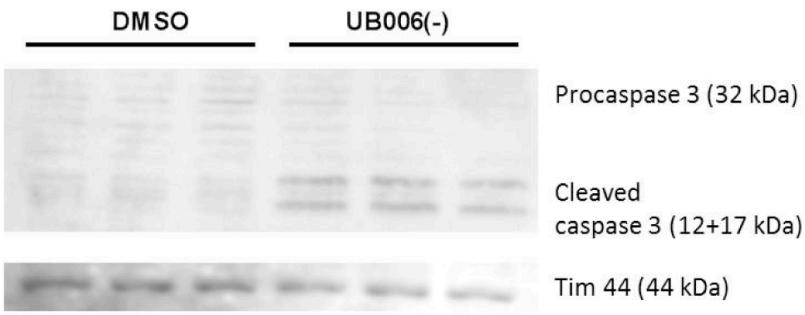


Figure 10. *Analysis of the activation of caspase-3 in OVCAR3 cell line after (–)-UB006 treatment by Western blot. Analysis of TIM-44 protein levels as a mitochondrial protein control.*

Furthermore, DDIT4/REDD1 is known to inhibit the mTOR pathway and cell proliferation [27]. To evaluate mTOR activity, we assessed the phosphorylation levels of mTOR substrate p70 S6 kinase. (–)-UB006 tends to reduce the S6K1 kinase phosphorylation level (Fig. 11), which might be due to the inhibition of mTOR activity, as a result of upregulated DDIT4/REDD1. Our results agree with the aforementioned findings [26]: treatment of OVCAR3 with (–)-UB006 increased DDIT4/REDD1 mRNA levels, which is correlated with decreased S6 kinase phosphorylation and mTOR inhibition. Concomitant with the increase in REDD1 expression, we also observed that caspase-3, which is required for robust death of ovarian cancer cells, is activated by (–)-UB006 treatment.

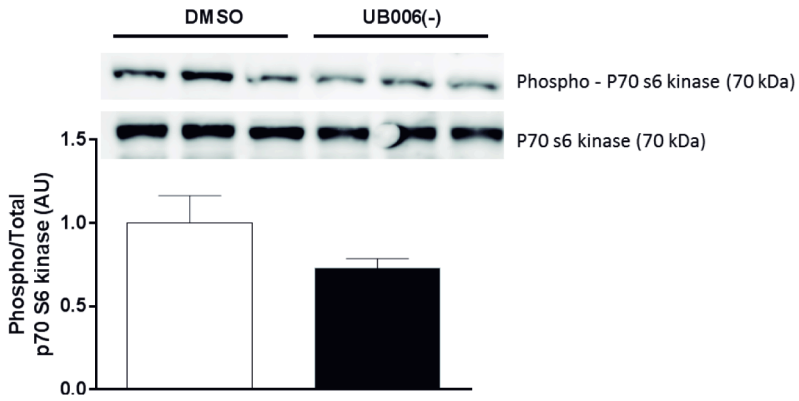


Figure 11. *Effect of (–)-UB006 on mTOR activity analysed by measuring mTOR substrate phosphorylation S6K(T389) levels by Western blot.*

2.7. Effect of (–)-UB006 in the metabolic extracellular flux analysis

Considering that DDIT4/REDD1 is a factor that is responsive to energy stress, we aimed to assess whether mitochondrial function was altered in OVCAR3 cells treated with (–)-UB006. We analysed metabolic extracellular flux changes produced by (–)-UB006 incubation. Mitochondrial respiration was reduced by 48.8% in OVCAR3 cells treated with (–)-UB006 (Fig. 12A and C). Interestingly, we observed a decrease in ATP production (Fig. 12D) and proton leak (Fig. 12E). When we analysed mitochondrial oxygen consumption ratios, (–)-UB006 did not change the percentages attributable to ATP-linked OCR, proton leak and spare capacity (Fig. 12B). However, when non-mitochondrial respiration was taken into account, there was a clear reduction in the percentage of mitochondrial oxygen consumption. These data suggest an overall reduction in mitochondrial function, regardless of the processes in which oxygen is consumed (Fig. 12B). Moreover, mitochondrion-dependent spare respiratory capacity is reduced in treated OVCAR3 cells (Fig. 12F and G). Nonetheless, non-mitochondrial

respiration remains unaltered (Fig. 12H), which suggests that only oxygen-consuming processes in mitochondria are affected. These energy disturbances might be the processes that trigger DDIT4/REDD1 upregulation and the consequent activation of apoptosis [28].

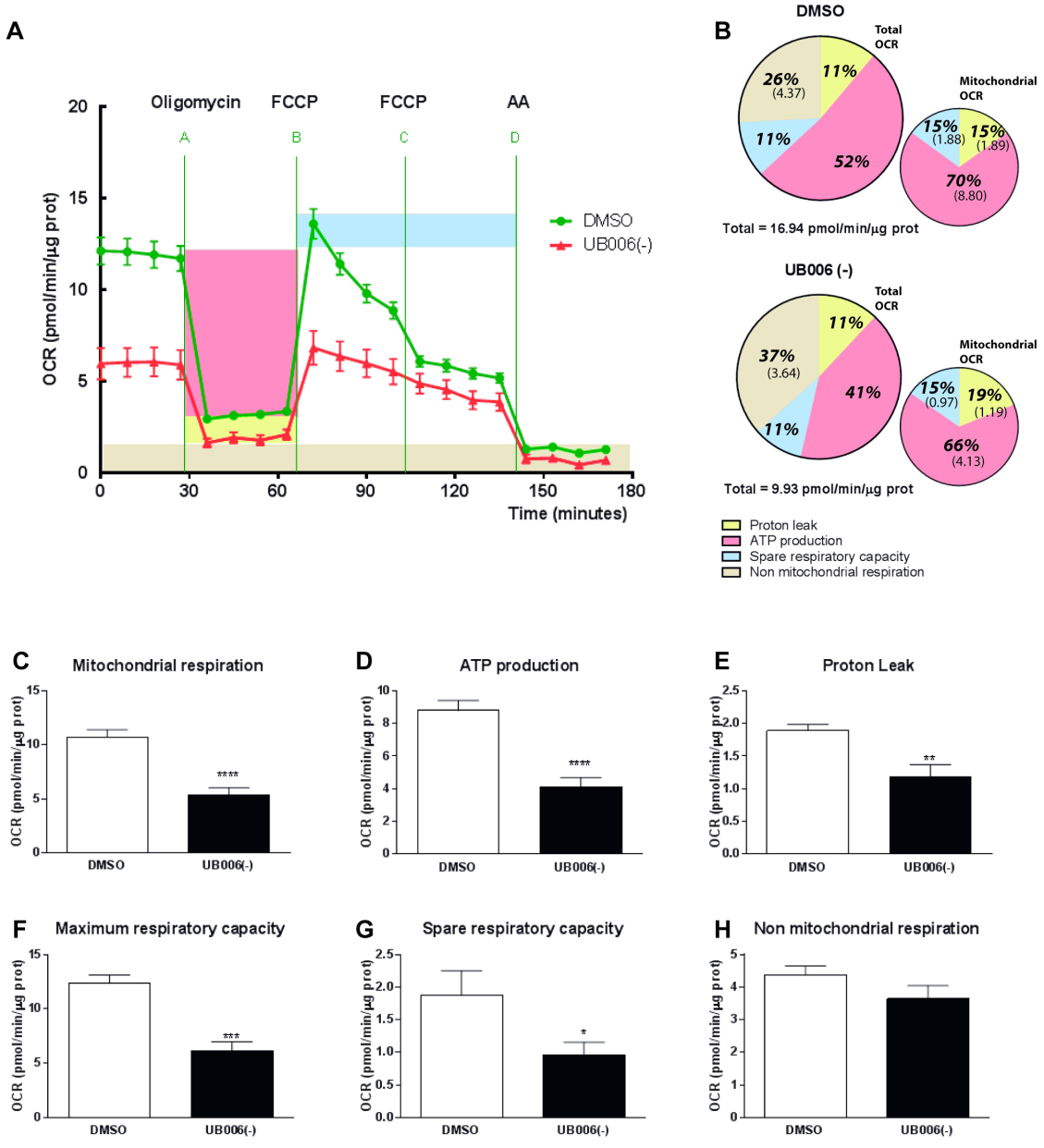


Figure 12. *(–)-UB006 effect on mitochondria bioenergetic profile in OVCAR3 cells.* (**A**) The injection of inhibitors over time is represented with lines, and their effect on the

average OCR trace is studied in the bioenergetic profile. (**B**) The ratios of the parameters are calculated for total OCR (bigger circles) and mitochondrial OCR (smaller circles). Taking into account the bioenergetic data, we calculated (**C**) basal mitochondrial oxygen consumption, (**D**) ATP-linked OCR and (**E**) proton leak after the addition of 2 μg/mL oligomycin. (**F**) Maximum respiratory rate and (**G**) spare capacity were calculated after the injection of 0.3 μM FCCP. Finally, 1 μM antimycin A was added to measure (**H**) non-mitochondrial respiration. The results are represented as mean ± SEM. $p<0.05$.

Although we do not know what triggers the energy depletion, we can speculate that it could be the result of a decreased amount of substrate (FA) and malonyl-CoA accumulation, both due to FAS inhibition. Alternatively, it has been described in HeLa cells that caspase-3 cleavage inhibits complexes I and II, which leads to a loss of mitochondrial function [29]. We cannot discard the possibility that the mitochondrial function loss observed in our model is triggered by caspase-3 activation.

2.8. Effect on CPT1 activity

One of the problems of using C75 as an anticancer drug is the undesirable side effect of reduction in body weight and appetite [30,31]. In fact, several laboratories are investigating the synthesis of FAS inhibitors that do not cause anorexia and a reduction in body weight [32]. It is known that the central nervous system (CNS) controls appetite and the maintenance of energy balance, and that lipid metabolism in hypothalamic neurons is crucial in mediating these actions [33,34]. C75 can modulate the activity of

enzymes involved in lipid metabolism, such as FAS [6] and CPT1 [8]. C75 is an inhibitor of FAS *in vitro*, and its inhibition in the hypothalamus could lead to an increase in malonyl-CoA levels, which, as a physiological inhibitor of CPT1, prevents the oxidation of newly synthesized FA. Both malonyl-CoA and LCFA-CoA have been proposed as molecular signals that control food intake and body weight [35,36]. Besides the effect on FAS activity, in a previous study we showed that C75-CoA is a potent inhibitor of CPT1, and that the hypothalamus can form the CoA adduct form of C75, that is, C75-CoA. In addition, we showed that hypothalamic C75-CoA actually inhibits CPT1 [9].

Since inhibition of CPT1 in the hypothalamus causes a decrease in food intake, we first analysed the effect of racemic (±)-UB006-CoA on CPT1 activity *in vitro* to determine whether it could act as a potential food intake regulator. (±)-UB006-CoA was prepared using (±)-UB006 and coenzyme A in slightly basic D_2O, as in previous descriptions [9,14]. Our results show that (±)-UB006 is a much weaker inhibitor of CPT1 activity *in vitro* than (±)-C75-CoA, with $IC_{50} > 10$ μM (Fig. 13).

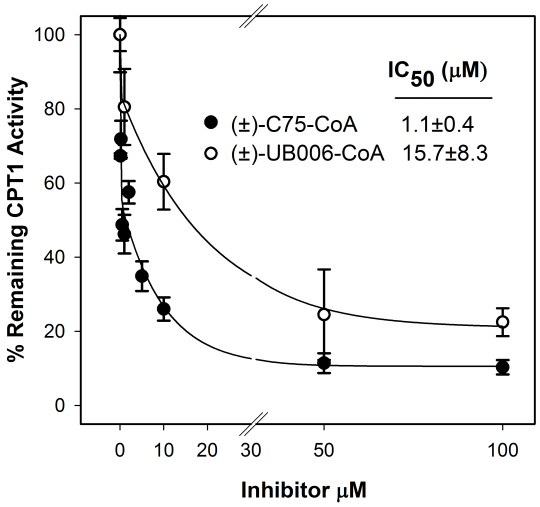


Figure 13. *Effect of (±)-C75-CoA and (±)-UB006-CoA on CPT1 activity*. Mitochondrial extracts (3-4 μg) from yeast expressing CPT1A-wt were preincubated for 5 min with increasing concentrations of (±)-C75-CoA and (±)-UB006-CoA, then CPT1 activity was measured and IC_{50} were calculated.

2.9. Effect of (−)-UB006 on food intake and body weight

Once we had synthesized both UB006 enantiomers separately, we analysed the effect of i.c.v. injection of these enantiomers on food intake and body weight. Male adult Sprague-Dawley rats received i.c.v. injections of (+)-UB006, (−)-UB006 or a vehicle (control animals). (±)-C75 was used as a control [9].

Consistent with previous results [8], (±)-C75 produced a decrease in body weight (Fig. 14A) and food intake (Fig. 14B). However, neither of the two compounds, (+)-UB006 or (−)-UB006, caused significant weight loss over three days.

We also investigated the peripheral action of both enantiomers of UB006. Male mice received i.p. injections (15 mg/kg/day) of (+)-UB006, or (−)-UB006, or a vehicle (control animals) or (±)-C75 (as a control). Similarly to the results of the i.c.v. injections, peripheral administration of the enantiomers did not affect body weight (Fig. 14C) or food intake (Fig. 14D) during the three-day treatment. Consistent with previous work, (±)-C75 produced a decrease in food intake and body weight, and after 2 days of treatment the animals exhibited resistance to C75-induced anorexia.

UB006, a novel derivative of C75 presents greatly enhanced potency as a cytotoxic agent, without affecting body weight. UB006-CoA produced by the reaction of (±)-UB006 with HSCoA does not inhibit CPT1 and does not produce weight lost in

animals. Both enantiomers of UB006 affect FAS activity and have cytotoxic activity. However, (–)-UB006 shows 40-fold higher activity than its (+)-enantiomer or C75. (–)-Consequently, UB006 should be examined in depth for cancer treatment.

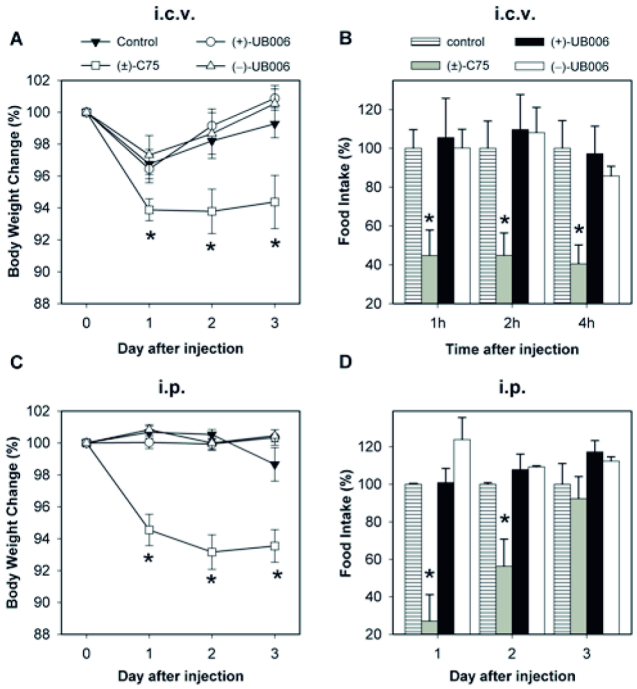


Figure 14. *Effect of (±)-C75, (+)-UB006 and (–)-UB006 on body weight and food intake after i.c.v. and i.p. injection.* (**A**) Body weight change measured in rats for 3 days every day after a unique i.c.v. injection of (±)-C75 (*n* = 8), (+)-UB006 (*n* = 8) and (–)-UB006 (*n* = 8) and control injections (n = 9; RPMI 1640 medium as a control). (**B**) Food intake measured in rats 1, 2, 4 and 22 h after i.c.v. injection as in (**A**). (**C**) The body weight change and (**D**) food intake measured in mice after daily i.p. injection (15 mg/kg) (±)-C75, (+)-UB006 and (–)-UB006 and control injections (DMSO dissolved in RPMI 1640 medium as a control). N=10; * $P < 0.05$.

UB006, a novel derivative of C75, presents greatly improved potency as a cytotoxic agent without affecting body weight. UB006-CoA produced by the reaction of (±)-UB006 with HSCoA does not inhibit CPT1 and does not produce weight loss in animals. Both enantiomers of UB006 affect FAS activity and have cytotoxic activity. However, (–)-UB006 shows 40-fold more activity than its (+)-enantiomer and C75. (–)-UB006 should be examined in depth for cancer treatment.

The present data demonstrate that racemic UB006-CoA cannot suppress CPT1 activity *in vitro*, and that neither of the two enantiomers of UB006 reduce food intake and body weight. Traditionally, the central effect of C75 on food intake has been attributed to FAS inhibition, but in our conditions, despite *in vitro* inhibition of FAS by UB006, this compound did not inhibit food intake or body weight. These results with the new compound UB006 confirm what we formerly observed: inhibition of hypothalamic FAS is not essential to reduce food intake [14]. In contrast, FAS inhibition produced a decrease in tumour development. Tumour cells died at increasing concentrations of either racemic UB006 or (–)-UB006.

3. CONCLUSIONS

We conclude that (–)-UB006 shows higher cytotoxic activity and specificity than C75. UB006 racemic and its enantiomers show inhibitory properties towards FAS. The incorporation of a hydroxyl group rather than maintaining the carboxylic group of C75 increases the inhibition of FAS and antitumor pharmacological action. (–)-UB006 acted as a strong FAS inhibitor and antitumor agent *in vitro,* but did not show anorexigenic effect when administered to animals. Moreover, (±)-UB006-CoA did not inhibit CPT1, which supports our previous results in which inhibition of CPT1, but not FAS, seems to be crucial in the central anorectic effect of C75. Altogether, these data provide further evidence that the use of the appropriate enantiomer significantly improves the effect of the racemic mixture (±)-UB006. The fact that (–)-UB006 has cytotoxic, but not anorexic, activity makes it a good candidate for the treatment of some types of cancer and encourages future synthesis of new, more specific drugs for the treatment of cancer.

4. MATERIAL AND METHODS

4.1. Materials

L-[Methyl-^{3}H]carnitine hydrochloride was purchased from Amersham Biosciences (GE Healthcare, Barcelona, Spain). [U-^{14}C]-acetate, [Malonyl-2-^{14}C]-malonyl-coenzyme A was from PerkinElmer Health Sciences (Boston, USA). Yeast culture media products were from DifcoTM Laboratories (Detroit, USA). Bradford solution for protein assays was from Bio-Rad Laboratories (Barcelona, Spain). RPMI 1640 was from Gibco-Invitrogen Corporation (Barcelona, Spain). Defatted bovine

serum albumin (BSA), palmitoyl-CoA, malonyl-CoA, (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and other chemicals were from Sigma-Aldrich (Madrid, Spain). (–)-C75 and (+)-C75 were prepared according to a known procedure [14].

4.2. Animals and treatments

Sprague-Dawley male rats (260–290 g) and C57/BL6J male mice (20–23 g) were purchased from Janvier. Animals were maintained under a 12 h dark/light cycle with free access to food and water. In accordance with current legislation, the Animal Ethics Committee at the University of Barcelona approved all experimental protocols.

After a 1-week acclimation period, chronic i.c.v. cannula were stereotaxically implanted into the lateral ventricle of rats under ketamine (Imalgene, 90 mg/kg) and xylazine (Rompun, 11 mg/kg) anesthesia. The coordinates were 1.0 mm posterior to bregma, 1.4 mm lateral of the sagittal sinus and 4 mm ventral to the dura mater [37]. Analgesics (buprenorphine, 0.3 mg/400 mL) and antibiotics (enrofloxacin, 10%) were added to the water for 7 days after surgery.

I.c.v. injections (10 μL, 33 mM final concentrations in RPMI 1640 medium) of (±) -C75, (+)-UB006, (−)-UB006 or vehicle (RMPI 1640) were performed with a microliter syringe (Hamilton) after 1 week of postsurgical recovery. For the feeding experiments, the rats received single injections of vehicle or compound dissolved in vehicle 30 min before the light was turned off. We measured intake of chow, corrected for spillage, at 1, 2, 4 and 22 h. Body weight was measured after 22 h.

I.p. injections of (±)-C75, (+)-UB006, (−)-UB006 (100 μL in RPMI 1640 medium, 15 mg/kg) or vehicle were performed in mice 3 h before the light was turned

off (17:00 h) every day, for 3 days. Body weight and food intake were measured after every injection.

4.3. Statistical analysis

Data are expressed as the mean ± SEM. The significance of differences was assessed using the unpaired Student's t-test.

4.4. Synthesis of compounds

4.4.1. Synthesis of (–)-UB006 via selenoether derivative

*(2S,3R,4R)-2-Octyl-5-oxo-4-((phenylselanyl)methyl)tetrahydrofuran-3-carboxylic acid, (–)-**3**:*

A solution of (−)-C75 (0.200 g, 0.78 mmol) in EtOH (3 mL), was added dropwise via cannula to a mixture of Ph_2Se_2 (0.135 g, 0.43 mmol) and $NaBH_4$ (0.035 g, 0.94 mmol) in EtOH (3 mL) under N_2 at r.t. The resulting yellow solution was stirred for 3.5 h and HCl 2 N was added until *p*H = 1. Then, the volatiles were evaporated and the aqueous residue was extracted with CH_2Cl_2 (3 x 8 mL). The combined organic extracts were washed with NH_4Cl 20% (2 x 5 mL) and NaCl sat. (2 x 5 mL), H_2O (5 mL), dried ($MgSO_4$), and the solvent was removed under vacuum. Purification of the residue by flash chromatography (hexane/AcOEt/AcOH 70:30:1) produced the desired product (0.224 g, 0.55 mmol, 70%). White solid; mp: 116-118ºC; R_f = 0.44 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D$ = –52.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.45-7.43 (m, 2H), 7.19-7.17 (m, 3H), 4.37 (td, *J* = 8.8, 4.0, 1H), 3.38-3.32 (m, 1H), 3.15-3.09 (m, 1H), 3.03 (dd, *J* = 9.2, 10.2, 1H), 1.80-1.72 (m, 2H), 1.70-1.61 (m, 2H), 1.48-1.16 (m, 12H), 0.81 (t, *J* = 6.6, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 176.2, 174.7, 133.0, 129.3, 129.0, 127.6, 79.7, 51.3, 45.3, 34.9, 31.8, 29.3, 29.2, 29.1, 26.9, 25.1, 22.6, 14.0; IR (KBr):

2951, 2922, 2852, 1763, 1697, 1162; HRMS (ESI+) calculated for $C_{20}H_{28}NaO_4Se$ $[M+Na]^+$ = 435.1045, found = 435.1039.

*(3R,4S,5S)-4-(Hydroxymethyl)-5-octyl-3-((phenylselanyl)methyl)dihydrofuran-2(3H)-one, (–)-**4**:*

BH_3:SMe_2 (0.066 mL, 0.66 mmol) was added under N_2 at –20ºC to a solution of *(2S,3R,4R)*-2-octyl-5-oxo-4-((phenylselanyl)methyl)tetrahydrofuran-3-carboxylic acid (0.210 g, 0.51 mmol) in THF anh. (3.5 mL). The resulting mixture was stirred at –20ºC for 1 h, 0 ºC for 3 h, and r.t. for 18 h. Then, CH_2Cl_2 (10 mL), buffer *p*H = 7 and NH_4Cl sat. at 0ºC were slowly added and stirred for 15 min at 0ºC and 10 min at r.t. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). Subsequently, the combined organic extracts were washed with NaCl (5 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue by *flash* chromatography (hexane/AcOEt 75:25) afforded the desired product (0.160 g, 0.403 mmol, 79%). Oil; R_f = 0.16 (hexane/AcOEt 80:20); $[\alpha]_D$ = –53.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60-7.49 (m, 2H), 7.26 (s, 3H), 4.23 (td, *J* = 8.4, 3.6, 1H), 3.80-3.66 (m, 2H), 3.47 (dd, *J* = 12.8, 3.9, 1H), 3.06 (dd, *J* = 12.8, 7.8, 1H), 2.94 (ddd, *J* = 10.0, 7.8, 3.9, 1H), 2.28-2.16 (m, 1H), 1.79-1.14 (m, 14H), 0.88 (t, *J* = 6.7, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.9, 132.8, 129.4, 127.5, 80.6, 61.2, 49.0, 43.3, 34.8, 31.8, 29.4, 29.3, 29.1, 27.2, 25.6, 22.6, 14.0; IR (film): 3468, 2924, 2853, 1755, 1467; HRMS (ESI+) calculated for $C_{20}H_{30}O_3NaSe$ $[M+Na]^+$ = 421.1252, found 421.1255.

(4S,5S)-4-(Hydroxymethyl)-3-methylene-5-octyldihydrofuran-2(3H)-one, (–)-UB006:

H_2O_2 30% (0.122 mL, 1.076 mmol) at r.t. was added to a solution of (3*R*,4*S*,5*S*)-4-(hydroxymethyl)-5-octyl-3-((phenylselanyl)methyl) dihydrofuran-2(3*H*)-one (0.107 g,

0.269 mmol) in THF anh. (1 mL). The resulting mixture was stirred for 3 h and then CH_2Cl_2 (5 mL) and H_2O (3 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL) and combined organic extracts were washed with Na_2SO_3 (3 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue by *flash* chromatography (hexane/AcOEt 60:40) yielded the desired product (0.060 g, 0.25 mmol, 93%). Oil; R_f = 0.28 (CH_2Cl_2/MeOH 97:3); $[\alpha]_D$ = – 13.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.33 (d, *J* = 2.4, 1H), 5.71 (d, *J* = 2.4, 1H), 4.43-4.36 (m, 1H), 3.76 (dd, *J* = 6.4, 2.1, 2H), 2.91-2.83 (m, 1H), 1.87-1.59 (m, 2H), 1.55-1.17 (m, 12H), 0.88 (t, *J* = 6.9, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 136.4, 123.6, 80.9, 64.1, 47.0, 36.2, 31.9, 29.5, 29.4, 29.3, 25.1, 22.8, 14.2; IR (film): 3453, 2855, 1764 1467, 1270; HRMS (ESI+) calculated for $C_{14}H_{25}O_3$ $[M+H]^+$ = 241.1798, found 241.1793.

4.4.2. Synthesis of (–)-UB006 via α-methylenation

*(4S,5S)-4-(Hydroxymethyl)-5-octyldihydrofuran-2(3H)-one, (–)-**6**:*

A cold solution of $NaBH_4$ (0.850 g, 22.37 mmol) in freshly prepared aqueous buffer *p*H = 8 was added to a solution of (*R*)-4-benzyl-3-((2*S*,3*S*)-2-octyl-5-oxotetrahydrofuran-3-carbonyl)oxazolidin-2-one ((–)-**5**, 1.5 g, 3.740 mmol) in THF (40 mL) at 0ºC. The mixture was stirred for 1 h, then HCl 2 N was added until *p*H = 4, and the THF was evaporated. The resulting residue was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were washed with NaCl (15 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue by *flash* chromatography (hexane/AcOEt 60:40) yielded the desired product (0.770 g, 3.377 mmol, 90%). Oil: R_f = 0.25 (hexane/AcOEt 1:1); $[\alpha]_D$ = – 32.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.39-4.32 (m, 1H), 3.70 (d, *J* = 5.6, 2H), 2.71-2.59 (m, 1H), 2.50-

2.30 (m, 2H), 1.82-1.16 (m, 14H), 0.88 (t, J = 6.7, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.1, 83.2, 62.9, 42.6, 35.3, 31.9, 31.6, 29.5, 29.4, 29.3, 25.5, 22.7, 14.2; IR (film): 3446, 2926, 2855, 1772, 1377, 1359, 1197; HRMS (ESI+) calculated for $C_{13}H_{25}O_3$ $[M+H]^+$ = 229.1798, found = 229.1797.

(4S,5S)-4-(Hydroxymethyl)-3-methylene-5-octyldihydrofuran-2(3H)-one, (–)-UB006:

A commercial solution of BuLi 2.5 M in THF (5.84, 14.6 mmol) was added to a stirred solution of diisopropylamine (2.05 mL, 14.6 mmol) in THF (4 mL) at –15ºC under N_2, and stirred for 15 min. The solution of (4*S*,5*S*)-4-(hydroxymethyl)-5-octyldihydrofuran-2(3*H*)-one (0.664 g, 2.91 mmol) in THF anh. (5 mL) was added slowly to a solution of LDA at –20ºC. After 3 h, the reaction mixture was treated with a slow stream of CO_2 gas for 30 min, and then quenched by addition of HCl 6 N until *p*H = 2. The resulting mixture was allowed to warm to room temperature, then CH_2Cl_2 (20 mL) and H_2O (5 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL) and combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The resulting residue was treated with 10 mL of stock solution, freshly prepared from 5 mL of acetic acid, 4.5 mL of formalin, 0.15 g of AcONa and 1.56 mL of *N*-methylaniline, and stirred at room temperature for 2 h. The reaction mixture was then treated with 10 mL of NaCl sat. and 1 mL of HCl conc. After 5 min, the resulting residue was thoroughly extracted with CH_2Cl_2. The organic extracts were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The purification of the residue by *flash* chromatography (hexane/AcOEt 80:20) yielded the desired product (–)-UB006 (0.450 g, 1.875 mmol, 64%).

4.4.3. Synthesis of (+)-UB006

For the synthesis of *(+)-UB006*, the same two synthetic procedures were used as for *(–)-UB006*.

The following intermediates were obtained for the procedure described in Section 4.4.1:

(2R,3S,4S)-2-Octyl-5-oxo-4-((phenylselanyl)methyl)tetrahydrofuran-3-carboxylic acid: $[\alpha]_D$ = +54.1 (*c* 1.0, $CHCl_3$).

(3S,4R,5R)-4-(Hydroxymethyl)-5-octyl-3-((phenylselanyl)methyl)dihydrofuran-2(3H)-one: $[\alpha]_D$ = +56.4 (*c* 1.0, $CHCl_3$).

The following intermediates were obtained for the procedure described in Section 4.4.2:

(4R,5R)-4-(Hydroxymethyl)-5-octyldihydrofuran-2(3H)-one: $[\alpha]_D$ = +31.0 (*c* 1.0, $CHCl_3$).

(4R,5R)-4-(Hydroxymethyl)-3-methylene-5-octyldihydrofuran-2(3H)-one, (+)-UB006: $[\alpha]_D$ = +14.2 (*c* 1.0, $CHCl_3$).

4.4.4. Synthesis of (±)-UB006

*(2SR,3RS,4RS)-2-Octyl-5-oxo-4-((phenylselanyl)methyl)tetrahydrofuran-3-carboxylic acid, (±)-**1**:*

A solution of (±)-C75 (0.200 g, 0.78 mmol) in EtOH (3 mL) was added dropwise via cannula to a mixture of Ph_2Se_2 (0.135 g, 0.43 mmol) and $NaBH_4$ (0.035 g, 0.94 mmol) in EtOH (3 mL) under N_2 at r.t. The resulting yellow solution was stirred for 3.5 h and HCl 2 N was added until *p*H = 1. Then, the volatiles were evaporated and the aqueous residue was extracted with CH_2Cl_2 (3 x 8 mL). The combined organic extracts were washed with NH_4Cl 20% (2 x 5 mL), NaCl sat. (2 x 5 mL) and H_2O (5 mL), dried ($MgSO_4$), and the solvent was removed under vacuum. Purification of the residue by *flash* chromatography (hexane/AcOEt/AcOH 70:30:1) yielded the desired product

(0.290 g, 0.62 mmol, 80%). The product showed identical analytical and spectroscopic data to *(2S,3R,4R)*-2-octyl-5-oxo-4-((phenylselanyl)methyl)tetrahydrofuran-3-carboxylic acid.

*(3SR,4RS,5RS)-4-(Hydroxymethyl)-5-octyl-3-((phenylselanyl)methyl)dihydrofuran-2(3H)-one, (±)-**2**:*

BH_3:SMe_2 (0.15 mL, 1.42 mmol) was added under N_2 at –20ºC to a solution of (2*SR*,3*RS*,4*RS*)-2-octyl-5-oxo-4-((phenylselanyl)methyl)tetrahydrofuran-3-carboxylic acid (0.45 g, 1.09 mmol) in THF anh. (3.5 mL). The resulting mixture was stirred at –20ºC for 1 h, 0ºC for 3 h, and r.t. for 18 h. Then, CH_2Cl_2 (10 mL), buffer *p*H = 7 and NH_4Cl sat. at 0ºC were slowly added and stirred for 15 min at 0ºC and 10 min at r.t. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). Afterwards, the combined organic extracts were washed with NaCl (5 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue by *flash* chromatography (hexane/AcOEt 75:25) yielded the desired product (0.357 g, 0.91 mmol, 84%). The product showed identical analytical and spectroscopic data to *(3R,4S,5S)*-4-(hydroxymethyl)-5-octyl-3-((phenylselanyl)methyl)dihydrofuran-2(3*H*)-one.

(4RS,5RS)-4-(Hydroxymethyl)-3-methylene-5-octyldihydrofuran-2(3H)-one, (±)-UB006:

H_2O_2 30% (0.061 mL, 0.52 mmol) at r.t. was added to a solution of (3*SR*,4*RS*,5*RS*)-4-(hydroxymethyl)-5-octyl-3-((phenylselanyl)methyl)dihydrofuran-2(3*H*)-one (0.053 g, 0.13 mmol) in THF anh. (0.5 mL). The resulting mixture was stirred for 3 h and then CH_2Cl_2 (5 mL) and H_2O (3 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL), and combined organic extracts were washed with Na_2SO_3 (3 mL),

dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue by *flash* chromatography (hexane/AcOEt 60:40) yielded the desired product (0.023 g, 0.096 mmol, 72%). The product showed identical analytical and spectroscopic data to (−)-UB006.

4.4.5. Synthesis of (±)-UB339

*(4RS,5SR)-4-(2-Hydroxyethyl)-5-octyldihydrofuran-2(3H)-one, (±)-**8**:*

BH_3:SMe_2 (0.055 mL, 0.55 mmol) was added slowly to a stirred solution of 2-((2*SR*,3*RS*)-2-octyl-5-oxotetrahydrofuran-3-yl)acetic acid ((±)-**7**, 0.100 g, 0.39 mmol) in THF anh. (2 mL) at –20ºC under N_2. The resulting mixture was stirred at –20ºC for 1 h, then 0ºC for 3 h, and r.t. for 18 h. Then, the second portion of BH_3:SMe_2 (0.055 mL, 0.55 mmol) at 0ºC was added. After 4 h, the mixture was diluted with CH_2Cl_2 (10 mL). Buffer *p*H = 7 and NH_4Cl sat. at 0ºC were slowly added, and the mixture was stirred for 15 min at 0ºC and 10 min at r.t. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). Afterwards, the combined organic extracts were washed with NaCl (5 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue by *flash* chromatography (hexane/AcOEt 1:1) yielded the desired product (0.062 g, 0.256 mmol, 66%). Oil; R_f = 0.4 (hexane:AcOEt, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.13 (td, *J* = 7.8, 3.9, 1H), 3.76-3.60 (m, 2H), 2.79-2.63 (m, 1H), 2.38-2.21 (m, 2H), 1.85-1.74 (m, 1H), 1.72-1.18 (m, 15H), 0.86 (t, *J* = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.9, 86.2, 60.6, 38.3, 35.6, 35.4, 34.6, 31.9, 29.5, 29.3, 25.8, 22.7, 14.2; IR (ATR): 3419, 2923, 2854, 1758, 1188, 1050; HRMS (ESI+) calculate for $C_{14}H_{27}O_3$ $[M+H]^+$ = 243.1955, found = 243.1963.

(4RS,5SR)-4-(2-Hydroxyethyl)-3-methylene-5-octyldihydrofuran-2(3H)-one, (±)-UB339:

A commercial solution of BuLi 2.5 M in THF (0.41 mL, 1.03 mmol) was added to a stirred solution of diisopropylamine (0.145 mL, 1.03 mmol) in THF (1 mL) at –15ºC under N_2 and stirred for 15 min. The solution of (4*RS*,5*SR*)-4-(2-hydroxyethyl)-5-octyldihydrofuran-2(3*H*)-one (0.050 g, 0.206 mmol) in THF anh. (1.5 mL) was added to a solution of LDA at –20ºC. After 3 h, the reaction mixture was treated with a slow stream of CO_2 gas for 30 min and then quenched by addition of HCl 6 N until *p*H = 2. The resulting mixture was allowed to warm to room temperature, then, CH_2Cl_2 (10 mL) and H_2O (5 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL) and combined organic extracts were washed with brine (10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The resulting residue was treated with 1.1 mL of stock solution that was freshly prepared from 1 mL of acetic acid, 0.75 mL of formalin, 0.03 g of AcONa, and 0.26 mL of *N*-methylaniline, and stirred at room temperature for 2 h. The reaction mixture was then treated with 10 mL of NaCl sat. and 1 mL of HCl con. After 5 min, the resulting residue was thoroughly extracted with CH_2Cl_2. The organic extracts were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue by *flash* chromatography (CH_2Cl_2:AcOEt, 97:3) yielded the desired product (0.021 g, 0.083 mmol, 40%). Oil; R_f = 0.39 (CH_2Cl_2:AcOEt, 95:5); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.28 (d, *J* = 2.5, 1H), 5.63 (d, *J* = 2.2, 1H), 4.25 (dt, *J* = 10.1, 5.2, 1H), 3.77 (t, *J* = 6.0, 2H), 2.95-2.80 (m, 1H), 1.84 (dd, *J* = 13.0, 6.4, 2H), 1.71-1.18 (m, 16H), 0.87 (t, *J* = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.5, 139.3, 122.6, 83.7, 59.6, 41.5, 36.7, 36.2, 32.0, 29.6, 29.4, 29.3, 25.3,

22.8, 14.2; IR (ATR): 3429, 2923, 2853, 1748, 1268, 1119, 1049; HRMS (ESI+) calculate for $C_{15}H_{30}NO_3$ $[M+NH_4]^+$ = 272.2220, found = 272.2221.

4.4.6. Synthesis of (±)-UB340

*Dimethyl 2-nonanoylsuccinate, (±)-**10**:*

Benzoyl peroxide (0.6 g, 1.9 mmol) was added under N_2 to a mixture of dimethyl maleate (**9**, 7.72 mL, 61.7 mmol) and *n*-nonanal (54 mL, 310 mmol). The resulting mixture was stirred for 18 h at 80ºC, then the second portion of benzoyl peroxide (0.1 g, 0.55 mmol) was added and stirred for an additional 3 h at 80ºC. Then, the mixture was treated with AcOEt (30 mL) and $NaHCO_3$ sat. (15 mL), and stirred for 15 min. The organic layer was washed with H_2O (2 x 15 mL) and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The *n*-nonanal was eliminated by distillation under reduced pressure and the residue was purified by *flash* chromatography (Hexane:AcOEt, 9:1) to yield the desired product (8.22 g, 28.7 mmol, 48%). Oil; R_f = 0.53 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.99 (dd, *J* = 6.3, 8.2, 1H), 3.74 (s, 3H), 3.68 (s, 3H), 2.98 (dd, *J* = 8.2, 17.6, 1H), 2.84 (dd, *J* = 6.3, 17.6, 1H), 2.75-2.57 (m, 2H), 1.64-1.27 (m, 12H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 204.1, 172.0, 169.2, 54.0, 52.9, 52.2, 43.0, 32.4, 32.0, 29.5, 29.3, 29.2, 23.6, 22.8, 14.3; IR (film): 2954, 2927, 2856, 1741, 1720, 1457, 1437, 1410, 1367, 1262, 1634, 1012, 668.

*(2SR,3RS)-Methyl 2-octyl-5-oxotetrahydrofuran-3-carboxylate, (±)-**11**:*

$NaBH_4$ (10 mg, 0.26 mmol) was added to a mixture of dimethyl 2-nonanoylsuccinate ((±)-**10**, 0.104 g, 0.36 mmol) in MeOH (5 mL) under N_2 at 0ºC. The resulting mixture was stirred for 1 h at r.t. Then, conc. HCl (1 drop, *p*H = 2-3) was added and heated to reflux for 1 h. After the volatiles had evaporated, the resulting residue was taken up in

CH_2Cl_2 (10 mL). The organic layer was washed with $NaHCO_3$ (5 mL), H_2O (2 x 15 mL) and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The resulting oil was diluted with toluene (5 mL) and DBU (54 μL, 0.36 mmol) was added. The mixture was stirred at 100ºC for 12 h. The resulting mixture was washed with HCl 0.02 N (5 mL) and brine. The organic extract was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by *flash* chromatography (hexane: AcOEt, 9:1) to afford the desired product (0.082 g, 0.32 mmol, 88%). Oil; R_f = 0.46 (hexane/AcOEt 7:3); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.57 (td, *J* = 7.3, 5.0, 1H), 3.77 (s, 3H), 2.92 (dd, *J* = 17.6, 8.7, 1H), 2.77 (dd, *J* = 17.6, 9.5, 1H), 1.84-1.64 (m, 2H), 1.59-1.18 (m, 12H), 0.93-0.84 (m, 3H); RMN de ^{13}C ($CDCl_3$, 101 MHz): δ 174.3, 171.5, 81.9, 52.6, 45.6, 35.2, 32.1, 31.7, 29.3, 29.1, 29.0, 25.1, 22.6, 14.2; IR (film): 2953, 2926, 2855, 1790, 1739, 1201; HRMS (ESI+) calculated for $C_{14}H_{28}O_4$ $[M+H]^+$ = 257.1747, found = 257.1746.

*(2SR,3RS)-2-Octyl-5-oxotetrahydrofuran-3-carboxylic acid, (±)-**12**:*

NaOH 1 N (8 mL) was added to a stirred solution of (2*SR*,3*RS*)-methyl 2-octyl-5-oxotetrahydrofuran-3-carboxylate ((±)-**11**, 0.820 g, 3.2 mmol) in THF (8 mL). The resulting mixture was stirred for 1 h at 70ºC. Then, CH_2Cl_2 (20 mL) was added and the aqueous layer was washed with CH_2Cl_2 (2 x 8 mL) and acidified by the addition of con. HCl, *p*H = 1, and heated at 70ºC for 1 h. Then, the mixture was extracted with CH_2Cl_2 (4 x 8 mL), and the combined organic extract was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure to give the desired product (0.670 g, 2.77 mmol, 86%). White solid; mp: 98-100ºC; R_f = 0.24 (hexane/AcOEt/AcOH 8:2:0.1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.65-4.60 (m, 1H), 3.14-3.07 (m, 1H), 2.95 (dd, *J* = 8.4, 17.9, 1H), 2.83 (dd, *J* = 9.6, 17.9, 1H), 1.86-1.70 (m, 2H), 1.56-1.28 (m, 12H), 0.88 (t, *J*

= 6.7, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 177.1, 175.7, 83.0, 46.4, 36.3, 32.9, 32.8, 30.3, 30.2, 30.1, 26.2, 23.6, 15.1; IR (film): 3000-3300, 2925, 2853, 1749, 1718, 1393, 1243, 1215, 1195, 759, 669; HRMS (ESI+) calculated for $C_{13}H_{22}NaO_4$ $[M+Na]^+$ = 265.1410, found = 265.1410.

*(4SR,5SR)-4-(Hydroxymethyl)-5-octyldihydrofuran-2(3H)-one, (±)-**13**:*

BH_3:SMe_2 (0.370 mL, 3.75 mmol) was added dropwise to a stirred solution of (2*SR*,3*RS*)-2-octyl-5-oxotetrahydrofuran-3-carboxylic acid ((±)-**12**, 0.650 g, 2.68 mmol) in THF anh. (12 mL) at –20ºC under N_2. The resulting mixture was stirred at –20ºC for 1 h, 0ºC for 3 h, and r.t. overnight. Then, H_2O (5 mL) at 0ºC was carefully added. After 10 min, Na_2CO_3 sat. (2 mL) and CH_2Cl_2 (15 mL) at 0ºC were slowly added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 8 mL). Afterwards, combined organic extracts were washed with NH_4Cl sat. (5 mL) and NaCl (5 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The purification of the residue by *flash* chromatography (hexane/AcOEt 1:1) yielded the desired product (0.540 g, 2.36 mmol, 88%). Oil; R_f = 0.25 (hexane/AcOEt 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.39-4.32 (m, 1H), 3.70 (d, *J* = 5.6, 2H), 2.71-2.59 (m, 1H), 2.50-2.30 (m, 2H), 1.82-1.16 (m, 14H), 0.88 (t, *J* = 6.7, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.1, 83.2, 62.9, 42.6, 35.3, 31.9, 31.6, 29.5, 29.5, 29.3, 25.5, 22.7, 14.2; IR (film): 3446, 2926, 2855, 1772, 1377, 1359, 1197; HRMS (ESI+) calculated for $C_{13}H_{25}O_3$ $[M+H]^+$ = 229.1798, found = 229.1797.

*(2SR,3RS)-2-Octyl-5-oxotetrahydrofuran-3-carbaldehyde, (±)-**14**:*

DMSO (0.478 mL, 6.72 mmol) was added dropwise at –78ºC to a stirred solution of $(COCl)_2$ (0.465 mL, 5.34 mmol) in CH_2Cl_2 anh. (5 mL). After 30 min, the solution of (4*SR*,5*SR*)-4-(hydroxymethyl)-5-octyldihydrofuran-2(3*H*)-one ((±)-**13**, 0.506 g, 0.221

mmol) in CH_2Cl_2 anh. (3.5 mL) at –78ºC was added via cannula and stirred for 30 min. Then, Et_3N anh. (1.5 mL, 10.90 mmol) was added dropwise and stirred at –78ºC for 20 min and for an additional 15 min at r.t. Subsequently, the inorganic salts were filtered with Celite and the volatiles were evaporated. The resulting residue was purified by *flash* chromatography (hexane/AcOEt 1:1) to give the corresponding aldehyde (0.445 g, 1.97 mmol, 90%). Oil; R_f = 0.52 (hexane/AcOEt 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.72 (d, *J* = 1.5, 1H), 4.68-4.60 (m, 1H), 3.10 (dddd, *J* = 9.6, 7.7, 6.4, 1.5, 1H), 2.89 (dd, *J* = 18.0, 7.7, 1H), 2.74 (dd, *J* = 18.0, 9.6, 1H), 1.88-1.61 (m, 2H), 1.55-1.04 (m, 12H), 0.86 (t, *J* = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 197.4, 174.4, 79.3, 52.6, 35.6, 31.9, 29.4, 29.3, 29.2, 29.1, 25.2, 22.7, 14.2; IR (film): 3420, 2958, 2925, 2852, 2727, 1772, 1730, 1362; HRMS (ESI+) calculated for $C_{13}H_{23}O_3$ $[M+H]^+$ = 227.1638, found = 227.1642.

*(E)-tert-Butyl 3-((2SR,3SR)-2-octyl-5-oxotetrahydrofuran-3-yl)acrylate, (±)-**15**:*

A solution of (2*SR*,3*RS*)-2-octyl-5-oxotetrahydrofuran-3-carbaldehyde ((±)-**14**, 0.400 g, 1.77 mmol) and *tert*-butyl 2-(triphenylphosphoranylidene)acetate (0.998 g, 2.65 mmol) in CH_2Cl_2 (10 mL) was stirred for 2.5 h at r.t. Then, the resulting solution was washed with H_2O (5 mL) and brine, dried ($MgSO_4$), and the volatiles were evaporated. *Flash* chromatography (hexane/AcOEt 9:1) of the residue furnished the desired product (0.485 g, 1.5 mmol 85%). Oil; R_f = 0.21 (hexane/AcOEt 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.70 (dd, *J* = 15.6, 8.4, 1H), 5.86 (d, *J* = 15.6, 1H), 4.21 (td, *J* = 8.3, 3.9, 1H), 2.98-2.84 (m, 1H), 2.73 (dd, *J* = 17.4, 8.3, 1H), 2.51 (dd, *J* = 17.4, 10.6, 1H), 1.78-1.59 (m, 2H), 1.49 (s, *J* = 0.6, 9H), 1.44-1.19 (m, 12H), 0.88 (t, *J* = 6.7, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.0, 165.0, 143.1, 126.1, 84.1, 81.2, 44.7, 35.3, 34.0, 31.9, 29.5, 29.4, 29.3,

28.2, 25.8, 22.8, 14.2; IR (film): 2928, 2856, 1785, 1715, 1655, 1153; HRMS (ESI+) calculated for $C_{19}H_{36}NO_4$ $[M+NH_4]^+$ = 342.2639, found = 342.2635.

*(E)-3-((2SR,3SR)-2-Octyl-5-oxotetrahydrofuran-3-yl)acrylic acid, (±)-**16**:*

Trifluoroacetic acid (0.185 mL, 2.40 mmol) and Et_3SiH (0.073 mL, 0.462) at room temperature were added to a solution of *(E)-tert*-butyl 3-((2*SR*,3*SR*)-2-octyl-5-oxotetrahydrofuran-3-yl)acrylate ((±)-**15**, 0.060 g, 0.185 mmol) in CH_2Cl_2 (1 mL). After 4 h, another portion of trifluoroacetic acid (0.042 mL, 0.55 mmol) and Et_3SiH (0.029 mL, 0.185 mmol) were added, and stirred for an additional 2 h. Then, the volatiles were evaporated and the almost pure product was used without further purification (0.047 g, 0.17 mmol, 96%). White solid; mp = 94.5-95 °C; R_f = 0.26 (hexane/AcOEt 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.48 (s, 1H), 6.94 (dd, *J* = 15.6, 8.5, 1H), 5.96 (d, *J* = 15.5, 1H), 4.25 (td, *J* = 8.0, 4.5, 1H), 3.07-2.84 (m, 1H), 2.78 (dd, *J* = 17.4, 8.4, 1H), 2.55 (dd, *J* = 17.5, 10.4, 1H), 1.75-1.60 (m, 2H), 1.58-1.16 (m, 12H), 0.87 (t, *J* = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.0, 170.7, 147.4, 123.5, 83.9, 44.9, 35.2, 34.1, 31.9, 29.8, 29.5, 29.4, 29.3, 25.7, 22.8, 14.2; IR (KBr): 3085, 3285-2598, 1770, 1695, 1625; HRMS (ESI-) calculated for $C_{15}H_{23}O_4$ $[M-H]^-$= 267.1602, found = 267.1592.

*3-((2SR,3RS)-2-Octyl-5-oxotetrahydrofuran-3-yl)propanoic acid, (±)-**17**:*

A solution of (*E*)-3-((2*SR*,3*SR*)-2-octyl-5-oxotetrahydrofuran-3-yl)acrylic acid ((±)-**16**, 0.245 g, 0.914 mmol) and Pt/C (5%, 0.071 g, 0.018 mmol) in AcOEt (3 mL, HPLC quality) was prepared under N_2 at room temperature. Then, the N_2 was replaced by H_2 and shaken vigorous for 2.5 h. The suspension was filtered on Celite and washed with AcOEt to remove traces of the catalyst. Subsequently, the volatiles were evaporated and the product was used without further purification (0.243 g, 0.914 mmol, 100%). White

solid; mp = 46.5-47.0 °C; R_f = 0.26 (hexane/AcOEt 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.11 (td, *J* = 7.8, 4.0, 1H), 2.78-2.64 (m, 1H), 2.48-2.34 (m, 2H), 2.27-2.14 (m, 2H), 1.98-1.87 (m, 1H), 1.79-1.57 (m, 3H), 1.57-1.12 (m, 12H), 0.88 (t, *J* = 6.9, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 178.0, 176.3, 85.9, 40.6, 35.1, 34.7, 32.1, 32.0, 29.8, 29.5, 29.5, 29.3, 27.9, 25.8, 22.8, 14.2; IR (KBr): 2955, 2920, 2855, 1760, 1695, 1219; HRMS (ESI+) calculated for $C_{15}H_{27}O_4$ $[M+H]^+$ =271.1904, found = 271.1899.

*3-((2SR,3RS)-4-Methylene-2-octyl-5-oxotetrahydrofuran-3-yl)propanoic acid, (±)-**18**:*

A mixture of 3-((2*SR*,3*RS*)-2-octyl-5-oxotetrahydrofuran-3-yl)propanoic acid ((±)-**17**, 0.230 g, 0.85 mmol) and a 2 M solution of dimethylmagnesium carbonate in DMF (15 mL, 30.6 mmol) was prepared and stirred at 135ºC under N_2 for 68 h. Subsequently, the solution was cooled to room temperature and 6 N HCl (20 mL) and CH_2Cl_2 (30 mL) were added carefully. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). Then, the combined organic extracts were washed with NaCl (5 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The resulting brown residue was treated with 5.3 mL of stock solution, freshly prepared from 2.5 mL of acetic acid, 2.25 mL of formalin, 0.075 g of AcONa and 0.78 mL of *N*-methylaniline, and stirred at room temperature for 2 h. The reaction mixture was then treated with 10 mL of NaCl sat. and 1 mL of HCl con. After 5 min, the resulting residue was thoroughly extracted with CH_2Cl_2. The organic extracts were washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The purification of the residue by *flash* chromatography (CH_2Cl_2:MeOH, 97:3) yielded the desired product (0.135 g, 0.476 mmol, 56%). Oil; R_f = 0.35 (CH_2Cl_2:MeOH 97:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.32 (d, *J* = 2.3, 1H), 5.65 (d, *J* = 2.3, 1H), 4.22-4.13 (m, 1H), 2.79-2.69 (m, 1H), 2.46 (t, *J* = 7.6, 2H), 1.99-1.87 (m, 2H), 1.69-1.55 (m, 2H), 1.53-1.16 (m, 12H), 0.88 (t, *J* =

6.7, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 178.1, 170.1, 138.5, 123.3, 83.2, 43.6, 36.2, 31.9, 30.6, 29.5, 29.4, 29.3, 28.6, 25.2, 22.8, 14.2; IR (film): 3225, 1762, 1712, 1410, 1270; HRMS (ESI+) calculated for $C_{16}H_{27}O_4$ $[M+H]^+$ = 283.1904, found = 283.1905.

*3-((2SR,3RS,4RS)-2-Octyl-5-oxo-4-((phenylselanyl)methyl)tetrahydrofuran-3-yl)propanoic acid, (±)-**19**:*

A solution of 3-((2*SR*,3*RS*)-4-methylene-2-octyl-5-oxotetrahydrofuran-3-yl)propanoic acid ((±)-**18**, 0.051 g, 0.18 mmol) in EtOH (1 mL, 1.3 mmol) was dropwise added via cannula to a mixture of Ph_2Se_2 (0.034 g, 0.11 mmol) and $NaBH_4$ (0.004 g, 0.11 mmol) in EtOH (2 mL) under N_2 at r.t. The resulting yellow solution was stirred for 4 h, and HCl 2 N was added until *p*H = 1. Then, the volatiles were evaporated and the aqueous residue was extracted with CH_2Cl_2 (3 x 8 mL). The combined organic extracts were washed with NH_4Cl 20% (2 x 5 mL), NaCl sat. (2 x 5 mL) and H_2O (5 mL), dried ($MgSO_4$), and the solvent was removed under vacuum. Purification of the residue by *flash* chromatography (CH_2Cl_2/MeOH, 97:3) yielded the desired product (0.060 g, 0.136 mmol, 75%). Oil; R_f = 0.45 (CH_2Cl_2:MeOH 97:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65-7.46 (m, 2H), 7.37-7.20 (m, 3H), 4.03 (td, *J* = 8.1, 3.5, 1H), 3.33 (dd, *J* = 13.0, 4.5, 1H), 3.18 (dd, *J* = 13.0, 6.2, 1H), 2.71 (ddd, *J* = 9.7, 6.2, 4.5, 1H), 2.30-2.21 (m, 2H), 2.20-2.08 (m, 1H), 1.86-1.77 (m, 2H), 1.74-1.12 (m, 14H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.9, 176.7, 133.3, 129.8, 129.5, 127.7, 83.8, 47.5, 45.0, 35.3, 32.0, 31.4, 29.8, 29.5, 29.5, 29.3, 27.8, 27.2, 25.8, 22.8, 14.2; IR (film): 3074, 3052, 1769, 170, 1189; HRMS (ESI+) calculated for $C_{22}H_{33}O_4Se$ $[M+H]^+$ = 441.1538, found = 441.1544.

*(3RS,4RS,5SR)-4-(3-Hydroxypropyl)-5-octyl-3-((phenylselanyl)methyl)dihydrofuran-2(3H)-one, (±)-**20**:*

BH_3:SMe_2 (0.016 mL, 0.16 mmol) was added slowly to a stirred solution of 3-((2*SR*,3*RS*,4*RS*)-2-octyl-5-oxo-4-((phenylselanyl)methyl)tetrahydrofuran-3-yl)propanoic acid ((±)-**19**, 0.050 g, 0.11 mmol) in THF anh. (2 mL) at –20ºC under N_2. The resulting mixture was stirred at –20ºC for 1 h, 0ºC for 3 h, and r.t for 18 h. Then, the second portion of BH_3:SMe_2 (0.016 mL, 0.16 mmol) was added at 0ºC. After 4 h, the mixture was diluted with CH_2Cl_2 (10 mL). Buffer *p*H = 7 and NH_4Cl sat. at 0ºC were slowly added and stirred for 15 min at 0ºC and 10 min at r.t. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). Subsequently, the combined organic extracts were washed with NaCl (5 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue by *flash* chromatography (hexane/AcOEt 1:1) yielded the desired product (0.032 g, 0.075 mmol, 67%). Oil; R_f = 0.52 (hexane:AcOEt, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58-7.49 (m, 2H), 7.32-7.22 (m, 3H), 4.04 (td, *J* = 8.3, 3.3, 1H), 3.56 (t, *J* = 6.2, 2H), 3.33 (dd, *J* = 12.9, 4.7, 1H), 3.20 (dd, *J* = 12.9, 5.9, 1H), 2.73 (ddd, *J* = 10.4, 5.8, 4.7, 1H), 2.16-2.06 (m, 1H), 1.75-1.19 (m, 18H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.9, 86.2, 60.6, 38.4, 35.6, 35.4, 34.6, 31.9, 29.5, 29.3, 25.8, 22.7, 14.2; IR (ATR): 3419, 2922, 2853, 1758, 1578, 1187, 1022; HRMS (ESI+) calculated for $C_{15}H_{30}O_3$ $[M+H]^+$ = 427.1745, found = 427.1747.

(4RS,5SR)-4-(3-Hydroxypropyl)-3-methylene-5-octyldihydrofuran-2(3H)-one UB340:

H_2O_2 30% (0.026 mL, 0.23 mmol) at r.t. was added to a solution of (3*RS*,4*RS*,5*SR*)-4-(3-hydroxypropyl)-5-octyl-3-((phenylselanyl)methyl)dihydrofuran-2(3*H*)-one (0.025 g, 0.059 mmol) in THF anh. (1 mL). The resulting mixture was stirred for 3.5 h and then CH_2Cl_2 (5 mL) and H_2O (3 mL) were added at 0ºC. The aqueous layer was extracted

with CH_2Cl_2 (2 x 5 mL), and the combined organic extracts were washed with Na_2SO_3 (3 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue by *flash* chromatography (hexane/AcOEt 70:30) yielded the desired product (0.015 g, 0.056 mmol, 95%). Oil; R_f = 0.45 (Hexane:AcOEt, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.27 (d, *J* = 2.5, 1H), 5.60 (d, *J* = 2.2, 1H), 4.18 (m, 1H), 3.68 (t, *J* = 5.7, 2H), 2.72-2.63 (m, 1H), 1.75-1.15 (m, 18H), 0.87 (t, *J* = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.5, 139.4, 122.5, 83.5, 62.6, 44.4, 36.3, 32.0, 30.5, 29.6, 29.5, 29.4, 29.3, 25.3, 22.8, 14.2; IR (ATR): 3422, 2925, 2855, 1761, 1268, 1119, 1058; HRMS (ESI+) calculated for $C_{16}H_{29}O_3$ $[M+H]^+$ = 269.2111, found = 269.2111.

4.3.7. Synthesis of (±)-UB006-CoA

A solution of hydrate of coenzyme A (13.4 mg, 0.017 mmol) and $Na_3PO_4 \cdot 12H_2O$ (3.5 mg) in D_2O (0.8 mL) was prepared. Then, the *p*H 8-8.5 was adjusted with KH_2PO_4. Subsequently, the solution was mixed with (±)-UB006 (4.0 mg, 0.017 mmol) and the *p*H was checked again. The progress of the reaction was monitored by ^{1}H NMR. ^{1}H NMR (300 MHz, D_2O): δ 8.56 (s, 1H), 8.27 (s, 1H), 6.17 (d, *J* = 6.8, 1H), 4.86-4.73 (m, HDO and 2H), 4.61-4.54 (m, 1H), 4.46-4.34 (m, 1H), 4.29-4.17 (m, 2H), 4.02 (s, 1H), 3.83 (dd, *J* = 10.0, 5.0, 1H), 3.74 (d, *J* = 5.0, 2H), 3.55 (dd, *J* = 9.8, 4.9, 1H), 3.47 (t, *J* = 6.5, 3H), 3.37 (t, *J* = 6.9, 3H), 2.95 (s, 2H), 2.77-2.65 (m, 2H), 2.47 (t, *J* = 6.6, 2H), 2.43-2.34 (m, 1H), 1.86-1.71 (m, 1H), 1.72-1.58 (m, 1H), 1.50-1.09 (m, 12H), 0.91-0.81 (m, 4H), 0.74 (s, 3H). HRMS (ESI-) calculated for $C_{35}H_{57}DN_7O_{19}P_3S$ $[M-2H]^{2-}$ = 503.1394, found = 503.1394.

4.5. Expression of CPT1A in Saccharomyces cerevisiae

Rat CPT1A was expressed in yeast cells, and mitochondrial cell extracts were obtained as previously described [38].

4.6. Determination of carnitine acyltransferase activity

Mitochondrial-enriched fractions were obtained by differential centrifugation [39], with minor modifications. All protein concentrations were determined using the Bio-Rad protein assay with bovine serum albumin as a standard.

A radiometric method was used for the assay of carnitine acyltransferase, as described previously [40]. The activity was assayed in mitochondrial-enriched fractions obtained from yeast (3–4 μg protein) and from rat hypothalamus (100 μg protein). Enzyme activity was assayed for 5 min at 30°C in a total volume of 200 μL. The substrates were 400 μM L-carnitine, and 50 μM palmitoyl-CoA. In the *in vitro* studies, the enzyme was preincubated with increasing concentration of drugs (0.1-100 μM) for 1 min. The values obtained were used to estimate the IC_{50} value. This value corresponds to the concentration that inhibits 50% of the enzymatic activity. In all cases, the molar ratio of acyl-CoA to albumin was kept at 5:1, to avoid the presence of free acyl-CoA and its deleterious detergent effects, and to prevent the formation of micelles.

4.7. Determination of spectrophotometric and radiometric FAS activity

For the *in vitro* experiments, FAS was purified from rat liver following the protocol described by Linn [41]. However, in our experimental conditions, DTT was not added. For the FAS activity assay, a spectrophotometric method was used [42]. Cytosolic hepatic extracts obtained from rat (315 μg) were preincubated at 30°C for 30 min with an increasing concentration of drugs: (±)-C75, (±)-UB006, (+)-UB006, (−)-UB006, (±)-UB339, (±)-UB340 dissolved in DMSO (100 – 5000 μM), using DMSO as

a blank. NADPH (121 μM) and acetyl-CoA (61 μM) in potassium phosphate buffer (*p*H = 7.2) were added to preincubated enzyme and equilibrated at 37°C for 3 min. The reaction was initiated by the addition of malonyl-CoA (55 μM). The total reaction volume was 0.33 mL. The oxidation of NADPH was followed at 340 nm and 37°C for 10 min. Drug concentrations ranging from 100 to 5000 μM were used to estimate the IC_{50} value.

The inhibitory effect of (−)-UB006 on FAS activity in OVCAR3 cell cultures was analysed by a radiometric method. FAS activity was assayed by measuring the incorporation of radiolabelled malonyl-CoA into palmitate [43]. 10-cm plates with 2.5 x 10^6 cells were treated with (−)-UB006 (29.7 μM) for 2 hours, using DMSO as a control. Tissue samples were homogenized into 20 mM Tris-HCl (pH 7.5), 1 mM DTT, 1 mM MgCl2 and 1 mM EDTA, and centrifuged at 14,000·*g* for 30 min at 4°C. Sample protein content was determined using the BCA protein assay (#23228, ThermoScientific). A total of 320 μg of protein extracts from each sample were preincubated for 10 min at 37ºC. Subsequently, reaction buffer components were added to a final concentration of 0.225 mM NADPH, 0.024 mM acetyl-CoA, 0.64 mM malonyl-CoA and 0.05 μCi/sample ^{14}C-malonyl-CoA (NEC6122005UC, Perkin Elmer) and incubated for 10 min at 37ºC. Reactions were stopped by adding 100 μL 0.5 N NaOH to the 500 μL reaction mix. Then samples were saponified by boiling them for 15 min at 100ºC and then neutralized by adding 100 μL 1 N HCl. After 3 *n*-pentane extractions, samples were dried with N_2 flow, resuspended in 0.5 mL pentane and analysed by scintillation counting.

4.8. Cell cultures and viability assays

MCF-7 (human breast adenocarcinoma), SKBr3 (human breast adenocarcinoma), OVCAR-3 (human ovary carcinoma), MIAPaCa-2 (human pancreas carcinoma), PC3 (human prostate adenocarcinoma), MDA-MB-231 (human breast cancer adenocarcinoma) and HCT116 (human colorectal carcinoma) cell lines were obtained from ATCC. Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 in complete medium composed of Ham's F12 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin and 100 μg/mL streptomycin. The cultures were passaged once or twice a week by gentle trypsinization, and cells were grown to confluence in 10 cm culture dishes.

To evaluate the cytotoxic effect of the drugs, an MTT cytotoxicity assay was performed. A total of 4–8 × 10^3 cells/well were plated in 96-well plates in 100 μL of culture medium. Once the cells had attached to the plate, the medium was removed and cells were incubated for 72 h in fresh medium with a different concentration (1.25, 2.5, 5, 10, 15, 20 and 30 μg/mL) of drugs. DMSO was used as a blank, at a final concentration ≤ 0.2%. Then, the cells were incubated for 3 h with 100 μL of fresh medium and 20 μL of MTT dissolved in PBS at 5 μg/mL concentration. Following treatment, the supernatants were carefully removed and the MTT-formazan crystals, formed by metabolically viable cells, were solubilized by adding 100 μL/well of DMSO. Absorbance was measured at 570 nm.

4.9. Determination of fatty acid synthesis pathway activity

OVCAR3 cells were plated at 5 x 10^4/well in 1 mL of the medium in 24-well plates and incubated overnight. Then, the medium was changed for a fresh medium without serum. Fatty acid synthesis was assayed with a 2-h pulse of [U-^{14}C]-acetate (1 μC) after 2 h of drug exposure. Then, Folch extraction of lipids and scintillation

counting was carried out [44]. All determinations were performed in triplicate. Data are represented as mean ± SEM.

4.10. Metabolic extracellular flux analysis

The Seahorse XF24 Analyzer (Seahorse Bioscience, Agilent) was used to measure oxygen consumption rate (OCR) in OVCAR3 cells. Cells were plated at 5 *x* 10^4/well 100 μL in 24-well plates and treated with (−)-UB006 (29.74 μM) (or equivalent DMSO as a control) for 24 h. For a typical bioenergetic profile, we used oligomycin to block ATP synthase; the uncoupler carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) to measure maximal respiratory capacity; and then antimycin-A to leave only non-mitochondrial activity to be measured (all from Sigma-Aldrich). Before the measurement, cells were incubated for 1 hour with XF Assay Medium (Seahorse Bioscience) plus 5 mM glucose. During the assay, we injected the following at the final concentrations in the injection port: 2 μg/mL oligomycin, 0.3 μM FCCP and 1 μM antimycin A. OCR was calculated by plotting the O_2 tension of media as a function of time (pmol/min), and data were normalized by the protein concentration measured in each individual well. The results were quantified as the average of 8–10 wells ± SEM per time point in at least three independent experiments.

4.11. Analysis of mRNA expression by quantitative real-time PCR

Total RNA was isolated from OVCAR-3 cells treated with (−)-UB006 (29.74 μM), using an Illustra MiniRNA Spin kit (GE Healthcare). cDNA was obtained using a Transcriptor First Strand cDNA Synthesis kit (Roche), following the manufacturer's instructions. Relative quantification of mRNA was performed with a LightCycler® 480 instrument (Roche) in 10 μL of reaction medium, using 8.25 ng of cDNA, forward and

reverse primers at 100 nM each, and a SYBR Green PCR Master Mix Reagent kit (Life Technologies). The primer pairs were as follows: casp3 (for: TGAGTGCTCGCAGCTCATA; rev: GGGCTCGCTAACTCCTCAC); ddit4 (for: GCTTAGGGGCCAACAAGG; rev: TCTGGATGTCACACCACTGTT); bip (for: AATGACCAGAATCGCCTGAC; rev: CGCTCCTTGAGGTTTTTGTC) and 18s (for: GCAATTATTCCCCATGAACG; rev: GGGACTTAATCAACGCAAGC). The mRNA levels were normalized to those of 18s and expressed as fold change.

4.12. Western blot analysis

OVCAR-3 cells were cultured at $1x10^5$ cells/well in 2 mL in 6-well plates and treated with (−)-UB006 (29.74 μM) (or equivalent DMSO as a control) for 24 h. Cells were harvested in lysis buffer and the protein concentration was determined using a BCA protein assay kit (Thermo). Samples were separated on 8% or 12% SDS-PAGE gels, depending on the molecular weight of the protein of interest, and then transferred onto PVDF membranes (Millipore). The following primary antibodies and dilutions were used: cleaved caspase-3 (1:1000; Cell Signalling, #9664), translocase of the mitochondrial inner membrane (TIM)-44 (1:5000; BD Biosciences, #612582), cleaved caspase-2 (1:1000; Cell Signalling, #2224), p70 S6 kinase (1:1000; Santa Cruz, sc-230), phospho-p70 S6 kinase (1:1000; Cell Signalling, #9205) and β-actin (1:10,000; Sigma A3854). Blots were incubated with the appropriate IgG-HRP-conjugated secondary antibody. Protein bands were visualized using the ECL immunoblotting detection system (GE Healthcare) and developed on an ImageQuant LAS4000 system (GE Healthcare). For the analysis of protein expression, bands from at least three independent experiments were quantified by densitometry, using Image J analysis

software.

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Conflict of interest

The authors declare no conflict of interest.

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