RESEARCHS / INVESTIGACIÓN

Synthesis of new Carnitine Palmitoyltransferase I inhibitors derivatives of C75.

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Abstract: Carnitine Palmitoyltransferase (CPT1) is an enzyme that catalyzes the transport of fatty acids from the cytosol into the mitochondria. CPT1 inhibition in the hypothalamus increases fatty acid levels, which produces an increased expression of anorexigenic neuropeptides, a sign of satiety. C75 acts as an antiobesity predrug. *In vivo* C75, is converted into C75-CoA adduct, which is a potent inhibitor of CPT1 and produces a loss of appetite and body weight. In this work, we present three new derivatives of C75, where the carboxylic group is replaced by a carnitine unit, malonic group, and a hydroxyl group with changes from *trans* to *cis* relative stereochemistry. Our results suggest that introducing a bigger group than carboxylic in β position or *cis* relative configuration of the lactone leads to a decrease of CPT1 inhibitory activity.

Key words: C75, CPT1, Carnitine Palmitoyltransferase 1 inhibitor, α-Methylene-γ-butyrolactones.

Introduction

Obesity is a global problem that is increasing at epidemic rates. According to the World Health Organization, in 2016, more than 1.9 billion adults were overweight, and more than 650 million are classified as truly obese[1]. Obesity is a risk factor for health as it is related to serious diseases such as type 2 diabetes mellitus, cardiovascular and Alzheimer's disease, and even some types of cancer[2]. Carnitine palmitoyltransferase 1 (CPT1), is an enzyme belonging to the family of carnitine acyltransferases whose function is to transport the long chain fatty acids coupled with coenzyme A (LCFA-CoA), from the cytosol to the mitochondria where LCFA are β-oxidized to satisfy the need for the energy required in the body[3]. Energy homeostasis is specifically regulated by the hypothalamic neurons, which can detect increased LCFA levels and regulate food intake by modulating appetite[4]. The inhibition of hypothalamic CPT1 increases cytosolic LCFA-CoA levels of hypothalamic neurons, which triggers the mRNA expression of anorexigenic neuropeptides and decreases the mRNA expression of orexigenic neuropeptides. This is a signal of satiety and nutrient abundance which produces a loss of appetite[5]. The activity of CPT1 can be modulated by its physiological inhibitor malonyl-CoA, which controls the balance between synthesis and oxidation of LCFAs[6].

C75 is an α-methylene-γ-butyrolactone, which acts as an appetite suppressor when it is coupled with coenzyme A (C75-CoA) by inhibiting CPT1 enzyme. Studies *in vivo* showed that injection of free C75 is transformed into C75-CoA and causes suppression of appetite and loss of body weight in animals[5]. The majority of the studies were carried out with a racemic mixture of C75, however (+)-C75-CoA enantiomer is responsible for the strong inhibition of CPT1 and a decrease in food intake in animals[7]. Herein, we show the synthesis of some racemic C75 derivatives and the evaluation of their CPT1 inhibitory activity with the aim of better understanding the structural requirements for stronger CPT1 inhibition.

Materials and methods

The organic synthesis was carried out by standard procedures. A thin layer, column chromatography, melting point, NMR, IR, and high-resolution mass spectrometry were used to characterize and identify the obtained products (*data not shown*).

The anorexigenic activity of the C75 derivatives was tested *in vivo* in Sprague-Dawley rats, with intracerebroventricular (i.c.v.) administrations[5]. To perform *in vitro* CPT1 inhibitory studies, all derivatives of C75 were previously converted into coenzyme A adducts. The determination of the CPT1 inhibitory activity was carried out using a radiometric method[7].

Results

We started the design of new derivatives of C75 with changes in the β position of lactone. Since malonyl-CoA is a physiological inhibitor of CPT1 and carnitine is a substrate of that enzyme, we synthesized two derivatives which substitute carboxylic group by malonic and carnitine moiety. On the other hand, the importance of relative lactone stereochemistry is unknown, and *cis*-C75 is unstable due to the migration of exocyclic double bond into endocyclic. Thus, we synthesized an alcoholic derivative of *cis*-C75, which can be compared with the already reported trans compound (UB006)[8].

The synthesis of the C75 derivatives is initiated by the reduction using BH_3: SMe_2 of the advanced acid mixture reported previously in the synthesis of C75[9] with a protected double bond as selenoether.

The mixture of alcoholic diastereomers could be separated with fewer difficulties than acids and were the straining point in the synthesis of designed derivatives. The final alcoholic product (±)-*cis*-UB006 where obtained straightforwardly by recovering the exocyclic double bond in oxidative conditions.

The malonic derivative (±)-UB001 was synthesized in

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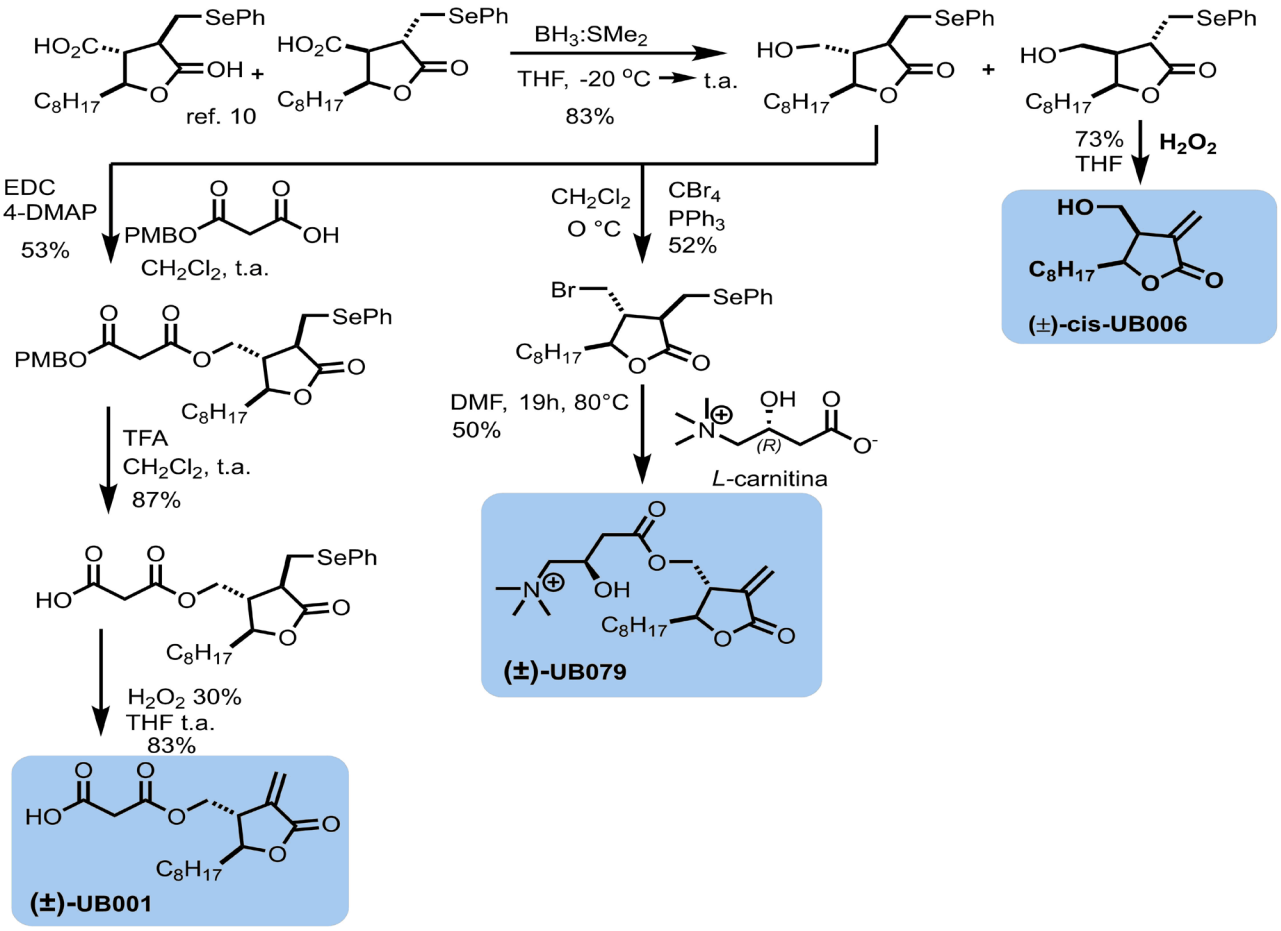

Figure 1. Synthesis of three new derivatives of C75.

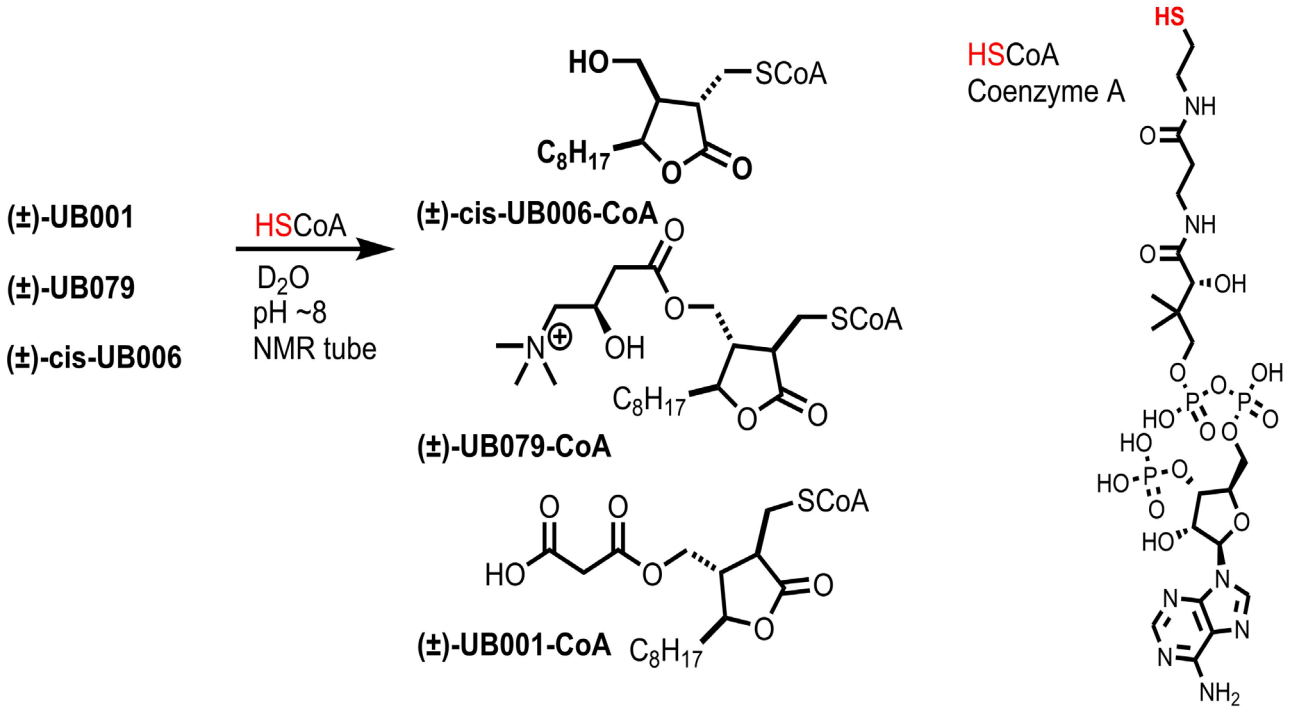

Figure 2. Preparation of the adducts of derivatives with coenzyme A.

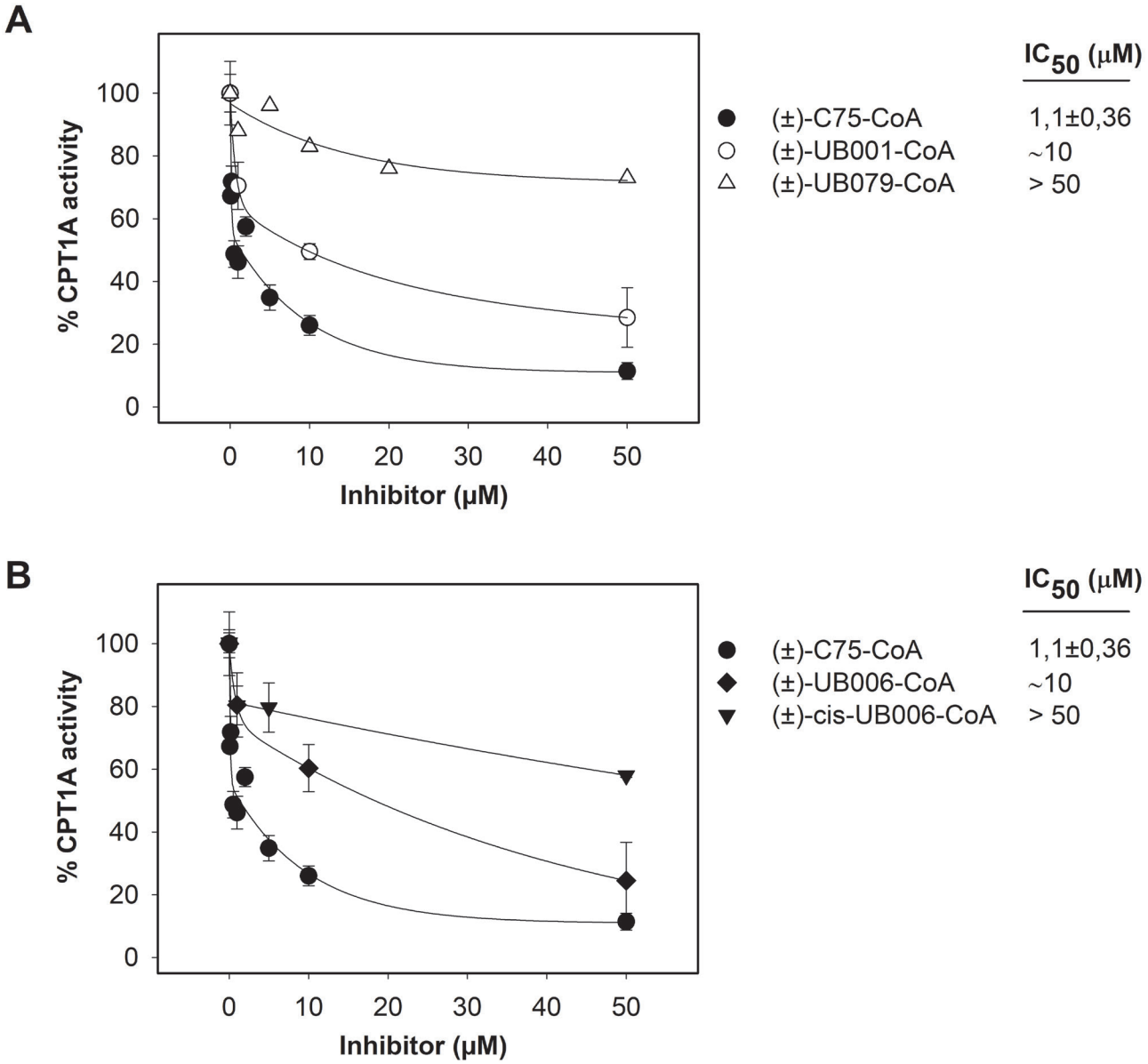

Figure 3. Effect of C75 derivatives on CPT1 activity was assayed in the presence of increasing concentrations of UB001-CoA, UB079-CoA (A), UB006-CoA, *cis*-UB006-CoA (B). C75-CoA was used as a positive control. The results are represented as the mean ± S.E.M.

three steps, condensation of starting *trans* alcohol with a monoprotected malonic acid using carbodiimide and 4-DMAP as a catalyst. Next, the benzyl group was removed with TFA. Finally, under oxidizing conditions, the double bond of the lactone was recovered obtaining the derivative UB001.

The last derivatives with carnitine moiety were obtained in two steps. The first hydroxyl group was replaced by bromine using Appel reaction conditions, and then *L*-carnitine was coupled. The reaction required relatively high temperature and in the same step double bond was formed indicating that selenoether is unstable during the heating. The product called UB079 is a mixture of diastereomers due using enantiopure carnitine, and this mixture was used for preliminary CPT1 activity studies.

To test the inhibitory power *in vitro* of the synthesized derivatives, these must be previously converted into their coenzyme A adducts. The synthesis of the coenzyme A adduct is carried out by mixing the derivative and the HSCoA in D_2O at a pH ~ 8 in an NMR tube and reaction was finished before 4h in all cases.

Once coenzyme A adduct of the derivatives was prepared, the inhibitory activity of these compounds was tested *in vitro*. The malonic derivative, UB001-CoA showed a decrease in the inhibitory activity concerning C75. Whereas, the carnitine derivative, UB079-CoA, was inactive in the inhibition of CPT1 at concentrations IC_{50} > 50 mM. On the other hand, it is observed that the alcoholic analogous, compound (±)-cis-UB006-CoA, present much worst inhibitory capacity than C75-CoA and *trans* UB006-CoA.

Of the three derivatives presented, the compound UB001-CoA was selected for *in vivo* study. For the analysis, the free C75 (positive control) and UB001 were administered by i.c.v. Injection as well as a vehicle without any drug as a control. Then, body weight and food intake were measured. The UB001, which present about 10-fold decrease of inhibitory *in vitro* activity, compared to C75, present complete loss of appetite suppressing activity (Figure 4B) when administered at the same concentration as C75 i.c.v. and did not cause a loss of body weight (Figure 4A).

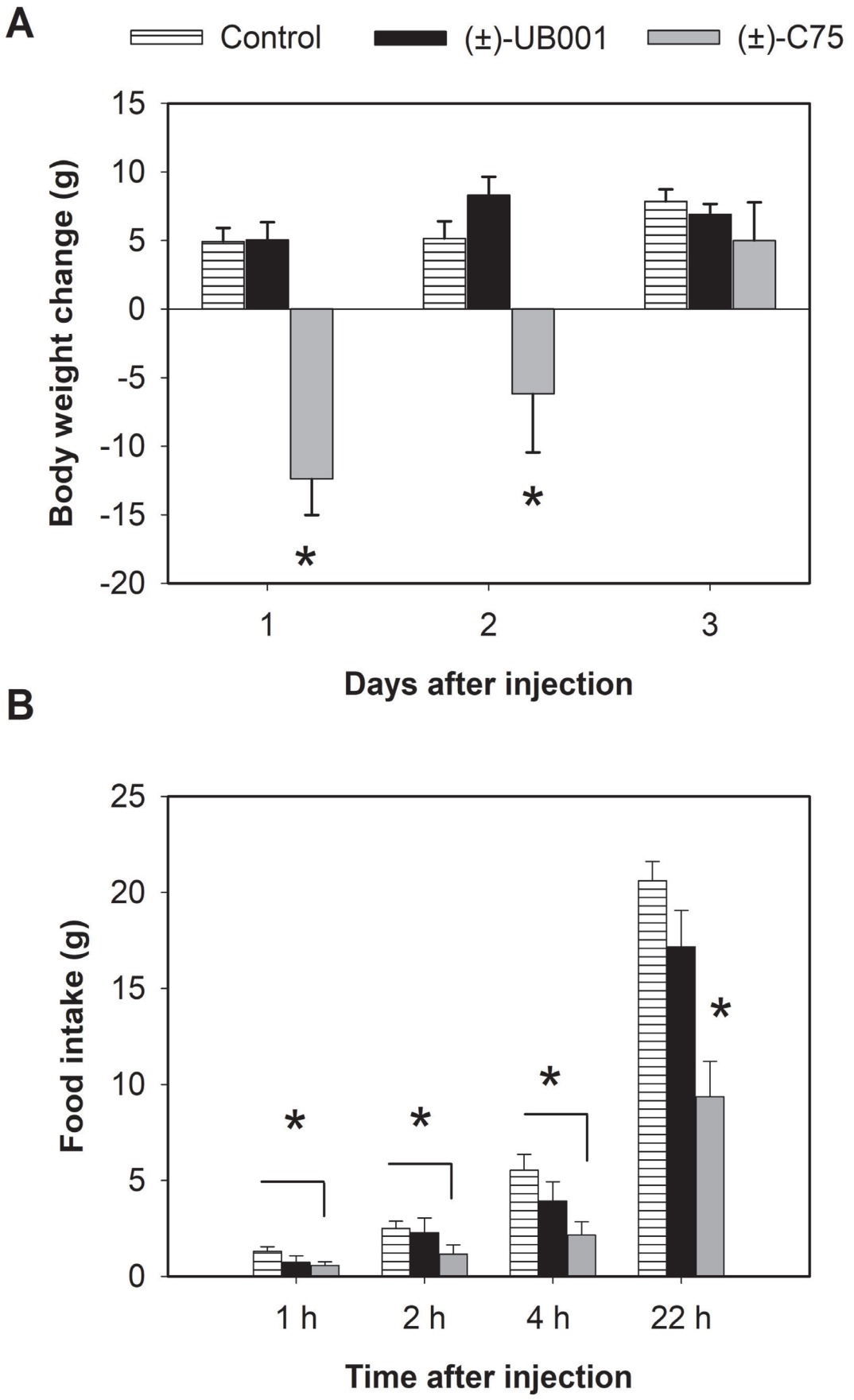

Figure 4. Effect of UB001 on body weight and food intake. (A). Accumulated food intake was measured after 1, 2, and 22 h after the injection (B). The results are expressed as the average ± S.E.M.

Conclusions

WWe conclude that the groups tested in the β position of the derivatives, the carnitine unit in UB079 and the malonic group in UB001 cause the loss of CPT1 inhibitory activity and seems that active center of that enzyme does not support bulkier group than carboxylic on the β position of the lactone. On the other hand, the change of *trans* to *cis* relative stereochemistry produces a loss of CPT1 inhibitory activity.

These new findings suggest that the length of the spacer between coenzyme A and the carboxylic group should be less than seven bonds, and it will be considered in future studies of CPT1 inhibitors derivatives of C75.

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