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Research paper

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

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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.

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

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.

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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).

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.

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.

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.

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.

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,

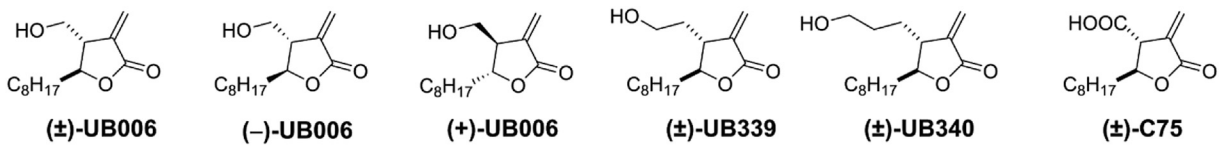

Fig. 1. Structure of (±)-UB006, (−)-UB006, (+)-UB006, (±)-UB339, (±)-UB340 and (±)-C75.

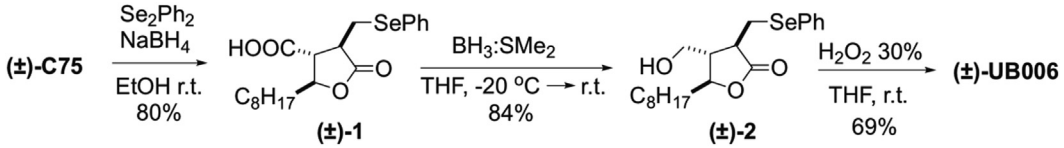

Fig. 2. Synthesis of (±)-UB006.