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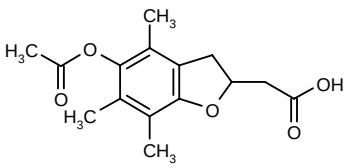
Raxofelast

Rec INN

Treatment of Diabetic Complications
Antioxidant

IRFI-016

(±)-5-Acetoxy-2,3-dihydro-4,6,7-trimethyl-2-benzofuran-acetic acid



$C_{15}H_{18}O_5$ Mol wt: 278.3022

CAS: 128232-14-4

EN: 168329

Synthesis

Raxofelast has been synthesised by a short route involving Friedel-Crafts alkylation of trimethylhydroquinone (I) by methyl 4-bromocrotonate (II) (1). The crude reaction mixture containing methyl 4-(2,5-dihydroxy-3,4,6-trimethylphenyl)-2(*E*)-butenoate (III) was directly submitted to a hydrolytic cyclization in alkaline environment in the presence of a strong reducing agent to afford (±)-2,3-dihydro-5-hydroxy-4,6,7-trimethyl-2-benzofuranacetic acid (IV) (IRFI-005). Acetylation of phenolic hydroxyl by routine methods, followed by recrystallization by isopropyl acetate, gave the final product. Scheme 1.

Description

Colorless crystalline powder, m.p. 184-5 °C.

Introduction

Recent years have seen increasing interest in outlining the role of free radical oxidative damage in human diseases. Free radicals and reactive oxygen metabolites, including superoxide anion, hydroxyl radical and hydrogen peroxide, have the potential to cause cellular injury and tissue damage by oxidizing biological molecules such as membrane lipids, proteins and nucleic acids. Moreover, impairment of endothelial-dependent vascular relaxation, which is found in several pathological conditions, was shown to be, at least in part, mediated by free radicals, most probably by interfering with the nitric oxide pathway (2).

The overoxidation processes – often associated with a deficit of natural antioxidative mechanisms – are central pathophysiological events in many disease states, including ischemia/reperfusion injury, chronic inflammatory diseases, atherosclerosis, diabetes mellitus and their sequelae. For these reasons, exogenous antioxidant supply could be a promising approach to limit oxidative stress, thus preventing and hindering the disease progression (3).

Pharmacological Actions

Raxofelast is an acetoxy prodrug of a vitamin E-like hydrophilic phenolic antioxidant. Ingold and coworkers showed that 2,3-dihydro-5-benzofuranols exhibit higher antioxidant activity than the corresponding 6-chromanols, due to stereoelectronic effects stabilizing the aryloxyl radical ArO· (4).

The antioxidant/radical scavenging potential of raxofelast has been investigated in a wide array of *in vitro* and *in vivo* studies, with the first ones being performed with the 5-hydroxy active metabolite, IRFI-005.

In vitro *studies*

Superoxide anion (O_2^-) in aqueous medium was destroyed in the presence of increasing concentrations of IRFI-005, as indicated by the progressive disappearance of electron spin resonance O_2^- signal. Similar experiments revealed that IRFI-005 has a good scavenger activity toward different organic radical species, such as bipyridinium radical from paraquat and anthracycline radical from daunomycin (5).

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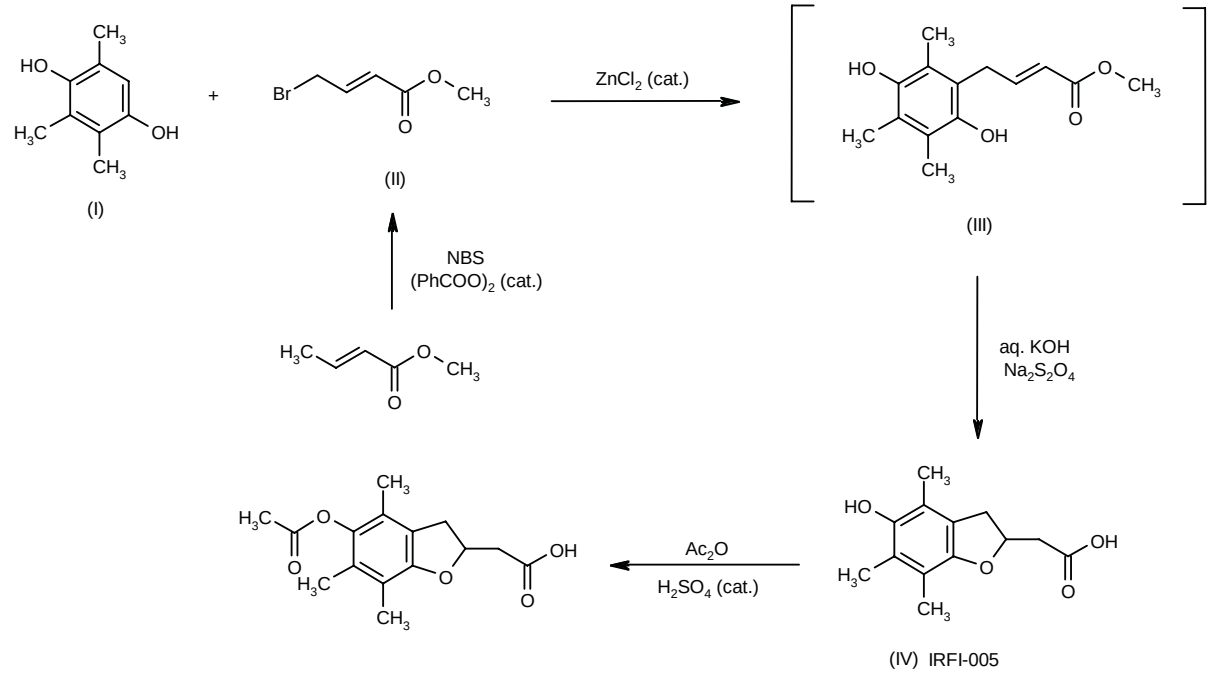
Scheme 1: Synthesis of Raxofelast



Antioxidant activity of IRFI-005 in biological systems in vitro has been especially evaluated in terms of inhibition of lipid peroxidation. In rat liver mitochondria IRFI-005 dose-dependently increased the lag time and decreased the extent of lipid peroxidation induced by Fe^{2+}/ascorbate, with an IC_{50} of 12 μM. In rat liver microsomes it inhibited the lipid peroxidation induced by both NADPH/ Fe^{2+}/ADP and cumene hydroperoxide, with IC_{50}s of 25 and 30 μM, respectively. In the latter models, trolox C exhibited lower antioxidant potencies, its IC_{50}s being in both cases 40 μM (6). In another study, the extent of hyperoxia-induced lipid peroxidation in human bronchial epithelial cells and pulmonary macrophages was significantly reduced by IRFI-005. The inhibition of generation of thiobarbiturate-reactive substances at 1 μM was 65% and 62% in epithelial cells and in macrophages, respectively. In these cultured cell systems, IRFI-005 was also shown to exert a cytoprotective action, as indicated by a concentration-dependent inhibition of the hyperoxia-induced release of the lysosomal enzyme β-glucuronidase (7).

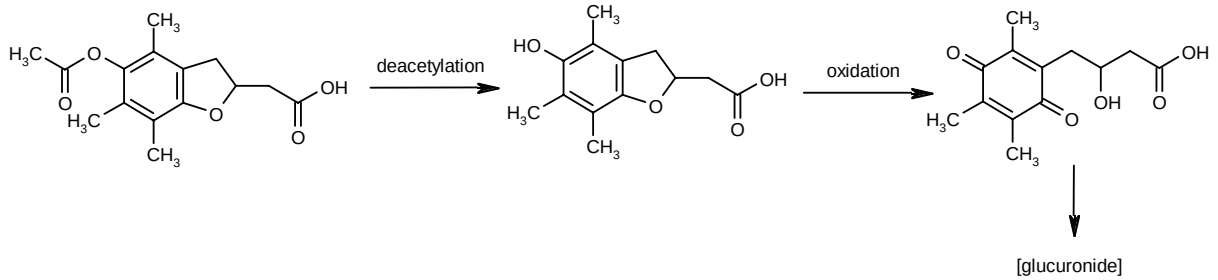
The chain-breaking antioxidant activity of IRFI-005 was demonstrated in a system of peroxyl radical-mediated methyl linoleate oxidation, monitored electrochemically through the oxygen consumption: the bimolecular rate constant $k_{(IRFI\text{-}005 + LOO\bullet)}$ is 1.8 x 10^6 $M^{-1}s^{-1}$ at 37 °C, higher than the rate constant for the reaction of α-tocopherol with LOO·, $k_{(\alpha\text{-tocopherol} + LOO\bullet)}$, which was found to be 1.1 x 10^6 $M^{-1}s^{-1}$ in identical conditions (8).

IRFI-005 (5-20 μM) inhibited LDL oxidation driven by Cu^{2+}/H_2O_2, as evaluated by the thiobarbituric acid reaction and diene evolution at 235 nm. The compound induced a concentration-dependent increase in the lag phase, which is a characteristic behavior of antioxidants. Oxidation of LDL by Cu^{2+}/H_2O_2 is accompanied by an increased electrophoretic mobility due to a modification of the protein component. A reduced electrophoretic mobility was seen in the presence of 20 μM IRFI-005. Modification of Apo B100 during LDL oxidation is also associated with a strong increase in protein fluorescence at 430 nm, which can be followed kinetically. IRFI-005 (20 μM) limited the formation of this protein-bound fluorophore in copper-driven LDL oxidation. Moreover, IRFI-005 substantially preserved LDL-associated antioxidants α-tocopherol and carotenoids, and when coincubated with physiologic levels of ascorbate provoked a synergistic inhibition of LDL oxidation. A synergistic inhibition of lipid peroxidation was also demonstrated by coincubating IRFI-005 and α-tocopherol incorporated in linoleic acid micelles (8).

In vivo *studies*

The protective action of raxofelast against ischemia-reperfusion injury was evaluated by several well-established protocols. IRFI-016 was assessed in a rat model of long-lasting myocardial ischemia (6 h of left coronary artery occlusion) (9) and after 1 h of left coronary artery

Scheme 2: Biotransformation Pathway of Raxofelast



occlusion followed by 30 min of reperfusion in the rat (10). In these models the compound was shown to limit the extension and severity of myocardial injury and to improve the survival rate of the animals. Raxofelast was further investigated in a challenging rat model of ischemia/reperfusion injury with the antioxidant being given after the onset of occlusion. The damage was induced by 1 h of left coronary artery occlusion followed by 6 h of reperfusion. Administration of raxofelast (25, 50 and 100 mg/kg i.p. 5 min after occlusion) dose-dependently limited myocardial necrosis (p <0.005 *vs*. the sham group following the highest dose), reduced lipid peroxidation (cardiac malondialdehyde and plasma conjugated dienes), restored the endogenous antioxidants vitamin E, SOD and GSH, improved hemodynamic parameters MEP, HR, LV dP/dt_{max} and reduced myocardial neutrophil infiltration as evaluated by myeloperoxidase activity (11).

Peroxynitrite, a cytotoxic oxidant species that results from reaction between NO and superoxide, has recently been proposed as an agent that mediates, at least in part, the oxidative injury associated with simultaneous production of NO and oxyradicals. Peroxynitrite formation has been demonstrated in several pathological states associated with an uncontrolled oxidative stress and particularly in circulatory shock and in various inflammatory disorders.

The therapeutic efficacy of raxofelast was investigated in rats subjected to carrageenan-induced pleurisy. Treatment with raxofelast (5, 10 and 20 mg/kg i.p. 5 min before carrageenan) dose-dependently prevented carrageenan-induced pleural exudation and polymorphonuclear migration in this rat model. Lung myeloperoxidase activity and malondialdehyde levels, as well as histological organ injury, were significantly (p <0.01) reduced by raxofelast. Immunohistochemical analysis for nitrotyrosine, an index of the nitrosylation of proteins and/or oxygen-derived free radicals, revealed a positive staining in lungs from carrageenan-treated rats. No positive nitrotyrosine staining was found in the lungs of the carrageenan-treated rats which had been administered raxofelast 20 mg/kg.

Furthermore, raxofelast treatment significantly reduced peroxynitrite formation as measured by oxidation of the fluorescent dihydrorhodamine 123, prevented the appearance of DNA single-strand breaks, significantly inhibited the decrease in mitochondrial respiration and partially restored the depletion of intracellular levels of NAD^+ in *ex vivo* macrophages harvested from the pleural cavity of rats subjected to carrageenan-induced pleurisy (12).

Pharmacokinetics and Metabolism

Raxofelast is rapidly and almost completely absorbed. It is quantitatively deacylated to the active metabolite IRFI-005. This compound is partially oxidized to afford the benzoquinone-like metabolite IRFI-045, which is detected at low levels in plasma (Scheme 2). The absence of a detectable conjugation for IRFI-005 is a noteworthy feature of the compound since the metabolic inactivation due to the easy formation of O-glucuronide and O-sulphate on the phenolic hydroxyl group was considered to be a major disadvantage of this class of compounds (13).

In rats and dogs, plasma levels of the unchanged drug were very low, whereas high levels of the active metabolite IRFI-005 were found in the systemic circulation, with a corresponding rapid distribution to tissues in rats. The plasma half-life ($t_{½}$) of the parent compound varied from 0.59 h in rats to < 0.1 h in dogs and humans, while the half-life of the active metabolite was 1.44, 4.62 and 1.65 h in rats, dogs and humans, respectively. There were no marked differences between the plasma concentrations of enantiomers of IRFI-005 after oral administration of the racemic mixture (IRFI-016) in rats, indicating a substantial bioequivalence of the enantiomers (14).

A tissue distribution study in rats using [^{14}C]-IRFI-016 showed high levels of radioactivity, particularly in the kidneys and along the routes of urinary clearance, in line with the prevailing renal excretion. Concentrations of radioactivity were also high in stomach and seminal vesicles and lowest in the brain, indicating that IRFI-005 has little, if any, tendency to cross the blood-brain barrier. The

active metabolite did not appear to significantly penetrate erythrocytes, as suggested by the equivalence of the blood and plasma $t_{½}$s and the ratio of the concentrations of IRFI-016 equivalents in plasma to whole blood.

No unchanged raxofelast was found in the urine. IRFI-005 was excreted mainly by the renal route, probably through a saturable process, with a substantial proportion being eliminated in the first 4 h. Urinary recovery accounted for the majority of the administered dose, and in rats < 15% of the radiolabeled material was recovered from the feces. The main pharmacokinetic parameters were similar after acute and repeated administration, indicating that neither accumulation nor tolerance occurs. In the excretion study with radiolabeled IRFI-016, elimination from the body was substantially complete within 48 h in male and female rats (15).

Clinical Studies

A pharmacokinetic study with 3 single oral doses (400, 800 and 1200 mg) of raxofelast in 12 healthy volunteers found that both C_{max} and AUC for IRFI-005 were proportional to the administered dose; respective C_{max} values were 12.7 ± 5.2, 26.6 ± 6.7 and 40.7 ± 14.8 μg/kg and respective AUCs were 51.7 ± 17.4, 106.2 ± 47.4 and 172.5 ± 62.1 h.μg/kg. The t_{max} for IRFI-005 ranged from 1-2 h at all doses (unpublished data).

In a double-blind, randomized, placebo-controlled phase I clinical study, multiple oral doses (400 or 800 mg b.i.d. for 10 d) of IRFI-016 were given to 7 healthy male volunteers. The plasma concentration-time data for IRFI-005 and IRFI-045 for the 400 mg dose regimen indicated that there was no significant change in the mean $t_{½}$, mean C_{max} or mean AUC at the end of 10 days. At the 800 mg b.i.d. regimen, a moderate but significant increase in the IRFI-005 $t_{½}$ and AUC was observed from day 1 to day 10. Analysis of urine samples also confirmed a predominance of renal excretion, recovery being mainly IRFI-005 (80.3-95.7% of the total). After both single and repeated administration a high proportion of the administered dose was excreted within 6 h (unpublished data).

As regards safety and tolerance, there were no clinically significant changes in vital signs, ECG, hematology, clinical chemistry or urinalysis after 400 or 800 mg b.i.d. IRFI-016. Adverse events were mild, with headache and abdominal symptoms being the most common. Most of them occurred in the group taking the lower dose. In another study in healthy males, single oral doses of up to 1600 mg IRFI-016 were well tolerated, without any adverse events or clinically significant changes in vital signs or laboratory parameters. No systemic or gastrointestinal adverse reactions attributable to the drug were observed (unpublished data).

A pharmacodynamic study was conducted in patients with noninsulin-dependent diabetes mellitus (NIDDM) and age-matched healthy volunteers to assess the effects of multiple oral dosing (7 days) of raxofelast on endothelial function and lipid peroxidation. The increase in forearm blood flow response to acetylcholine infusion in patients with NIDDM was significantly lower than in control subjects, indicating the presence of endothelial dysfunction. On the other hand, diabetic patients treated with raxofelast showed a marked improvement (p <0.05) of endothelial-dependent vasodilation to acetylcholine, an effect that may be linked to the counteraction of nitric oxide inactivation by oxygen free radicals. Treatment with raxofelast in NIDDM patients also reduced by 37% the plasma concentrations of epi-$PGF_{2\alpha}$, an isoprostane generated by oxidation of arachidonic acid and a marker of lipid peroxidation (unpublished results).

Toxicology

Preclinical toxicity studies were carried out acutely (single dose in mice and rats), subchronically and chronically (rats, rabbits and dogs). The overall profile of raxofelast was favorable. In 6-month toxicology studies, no adverse effects were noted except for mild decreases in erythrocytes. At the highest dose tested in both rats (300 mg/kg p.o.) and dogs (120 mg/kg p.o.), histological signs typical of an increased turnover of erythrocytes were found in the spleen and in the liver. Raxofelast was shown to have no effects on teratogenecity or reproduction (16).

Manufacturer

Biomedica Foscama Industria Chimico-Farmaceutica SpA (IT).

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