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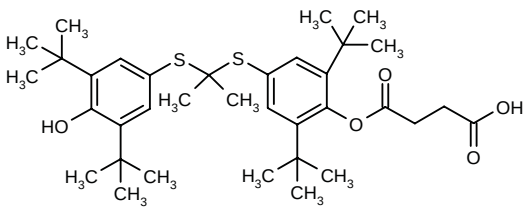
AGI-1067

Treatment of Atherosclerosis
VCAM-1 and MCP-1 Expression Inhibitor
Antioxidant

AGZ-1067

Succinic acid 2,6-di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenyl monoester



$C_{35}H_{52}O_5S_2$
Mol wt: 616.9228
CAS: 216167-82-7
EN: 260330

Abstract

AGI-1067 is a monosuccinate ester of probucol that exhibited marked lipid-lowering and antioxidant activity. AGI-1067 potently inhibited VCAM-1 and MCP-1 expression and smooth muscle cell proliferation and was effective in animal models of atherosclerosis and hyperlipidemia. The agent has shown efficacy in the prevention of atherosclerosis in patients with coronary artery disease and in preventing restenosis in patients undergoing percutaneous coronary interventions. AG-1067 is currently undergoing phase III trials with an indication for secondary prevention of atherosclerotic cardiovascular disease.

Synthesis

Esterification of probucol (I) with succinic anhydride (II) by means of NaH in THF produces a mixture of the monosuccinate and disuccinate esters and unreacted probucol, which are separated by column chromatography (1, 2). This procedure can also be performed in the presence of other bases, such as potassium *tert*-butoxide, KOH, DMAP and 1,4-diazabicyclo[2.2.2]octane (DABCO) (3). Scheme 1.

Introduction

Atherosclerosis is a chronic inflammatory disease of medium to large blood vessels that is the leading cause of death in the U.S. and developing countries. According to the American Heart Association, it accounts for 133,000 hospital discharges and 15,279 deaths per 100,000 population each year in the U.S. In addition, atherosclerosis is responsible for nearly 75% of all deaths from cardiovascular disease such as heart attacks and stroke (4).

Atherosclerosis involves both degenerative and regenerative processes that are thought to begin when vascular endothelium becomes damaged due to high levels of serum cholesterol and triglycerides, hypertension and/or environmental factors. The disease is characterized by focal accumulation of inflammatory cells (*e.g.*, excess monocyte-derived macrophages), elevated serum lipid levels (*e.g.*, cholesterol and triglyceride), smooth muscle cell proliferation and the presence of atheromatous plaques in arterial walls. These plaques may be calcified or soft and fatty and are formed by a buildup of lipids, cellular waste products and calcium on the inner artery walls, which eventually narrow the vessel and impede normal blood flow. Coronary arteries can become completely obstructed, which can lead to myocardial infarction. Fatty, soft plaques generally do not obstruct blood flow. However, they can break loose and form a blood clot and cause myocardial or cerebral infarction (4-6).

Atherosclerosis is a disease of oxidative stress and chronic inflammation. Abnormalities observed in oxygen-mediated signaling can induce formation of reactive intermediates by vascular endothelium, neutrophils and activated platelets, which can lead to accumulation and activation of macrophages in the arterial wall, endothelial dysfunction and progression to atherosclerosis. Oxidative stress and generation of reactive oxygen species (ROS) induce localized expression of vascular cell adhesion

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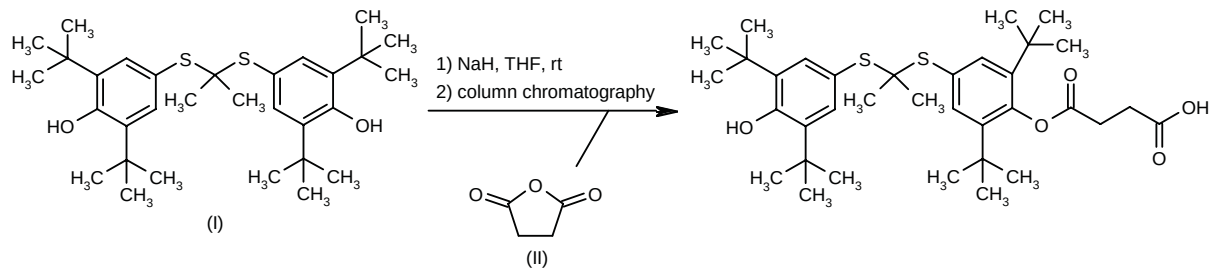
Scheme 1: Synthesis of AGI-1067



molecule-1 (VCAM-1) in the endothelium of early atherosclerotic lesions. VCAM-1 in turn recruits monocytes, which adhere to the developing lesions. Monocytes are converted to foamy macrophages, which synthesize various proinflammatory cytokines (*e.g.*, TNF-α, IL-1β), growth factors and chemoattractants (*e.g.*, monocyte chemoattractant protein-1 [MCP-1]) that aid in the formation of mature plaques. These pathological alterations are also implicated in the development of restenosis following percutaneous coronary interventions (PCI) in patients with coronary artery disease (CAD). Upregulated VCAM-1 expression, but not intracellular adhesion molecule-1 (ICAM-1), has been shown to be crucial in the development of atherosclerosis. Thus, based on the idea that atherosclerosis is a chronic inflammatory disease associated with abnormal oxidation-mediated vascular signals, a novel treatment approach has been proposed involving disruption of reduction/oxidation-sensitive signaling pathways. Selective suppression of expression of certain vascular inflammatory genes could interfere with the early stages of the pathophysiology of the disease without affecting immune integrity. This type of therapy would also have potential efficacy against other vascular inflammatory conditions and for preventing oxidative stress occurring after angioplasty (6-15).

In an effort to discover a novel class of antiatherosclerotic agents, researchers focused their attention on probucol, an antioxidant marketed as a lipid-lowering agent. The mechanism responsible for its lipid-lowering activity is unknown. Probucol was shown to have antioxidant activity and it reduced restenosis after coronary angioplasty. However, it was withdrawn from the U.S. market due to drawbacks such as induction of Q-Tc prolongation, significant and progressive HDL-lowering effects, its lack of effect on inducible endothelial cell VCAM-1 expression and its limited and variable oral bioavailability. Researchers therefore set out to improve probucol and discovered a novel class of agents referred to as composite vascular protectants, or v-protectants. These agents have a dual mechanism of action in that they protect the arterial lining (*i.e.*, antioxidant activity) and lower LDL cholesterol levels. One such agent is AGI-1067, the monosuccinate ester of probucol. AGI-1067 had a greater water solubility and cell permeability as compared to probucol and it exhibited marked lipid-lowering and antioxidant activity. Moreover, AGI-1067 potently inhibited VCAM-1 and MCP-1 (also upregulated in atherosclerotic lesions) expression, but not ICAM-1 expression, inhibited smooth muscle cell proliferation (which is involved in late-stage atherosclerosis) and was effective in animal models of atherosclerosis and hyperlipidemia. AG-1067 was selected for further development as a secondary prevention for atherosclerosis (*e.g.*, to improve survival, reduce recurrent events, reduce the need for interventional procedures, improve the quality of life) in patients with CAD (2, 16).

Pharmacological Actions

In contrast to probucol (IC_{50} > 50 μM), AGI-1067 potently inhibited TNF-α-inducible VCAM-1 expression (IC_{50} = 6 μM). AGI-1067 (up to 10 μM) had no effect on inducible ICAM-1 expression and little effect on E-selectin expression (IC_{50} = 25 μM) but potently inhibited inducible MCP-1 expression (IC_{50} = 10 μM). Both AGI-1067 and probucol (70 μM) inhibited 15-lipoxygenase-mediated oxidation of linoleic acid (about 85%) in a similar manner. However, only AGI-1067 (5 μM) significantly inhibited proliferation of human aortic smooth muscle cells by approximately 50% (2).

The efficacy and tolerability of AGI-1067 were demonstrated *in vivo*. Oral AGI-1067 was shown to be well tolerated in mice, hamsters, rabbits and monkeys, where the agent lowered LDL cholesterol levels and either increased or had no effect on HDL levels. Moreover, oral treatment of cholesterol-fed rabbits resulted in a 94% inhibition of the progression of atherosclerosis. The efficacy of AGI-1067 (150 mg/kg/day) was also examined using LDL receptor knockout and ApoE knockout mice and cholesterol-fed C57BL/6 mice as models. After 12 weeks of treatment, LDL levels were lowered by 57% in cholesterol-fed mice; no changes in plasma cholesterol levels were observed in treated LDL knockout mice, suggesting that the mechanism of action of AGI-1067 involves LDL receptor-dependent lipoprotein clearance.

After 2 weeks of treatment, cholesterol levels of ApoE knockout mice were reduced, although levels returned to high baseline values by 12 weeks. This reversal may have been due to downregulation of LDL receptors over time. However, AGI-1067 did inhibit progression of lesions in the arch, thoracic and abdominal regions of LDL receptor knockout mice (43%, 51% and 66%, respectively) and ApoE knockout mice (25%, 41% and 49%, respectively) as compared to untreated controls. Results suggest that AGI-1067 directly inhibits vascular inflammatory responses (2, 17).

Results from both preclinical animal studies and human clinical trials showing no significant metabolism of AGI-1067 to probucol have confirmed that AGI-1067 is not a prodrug of probucol. AGI-1067 was the major compound detected in plasma following single oral dosing with [^{14}C]-AGI-1067 in rats and dogs. Similarly, experiments in which single oral doses of [^{14}C]-AGI-1067 were administered to 6 healthy male volunteers found that the majority of radioactivity recovered in plasma (94.4-97.8%) was the unchanged compound (2).

Clinical Studies

Results from 7 phase I trials involving more than 150 healthy volunteers or patients up to 85 years of age have demonstrated the tolerability of AGI-1067. No dose- or use-limiting adverse events were reported (18).

The direct antiatherosclerotic effects of AGI-1067 (70, 140 or 280 mg p.o. once daily for 2 weeks before PCI and for 4 weeks after PCI) on coronary blood vessels and its efficacy in reducing restenosis as compared to placebo and probucol (500 mg b.i.d. p.o.) were demonstrated in the multicenter, randomized, double-blind, placebo-controlled Canadian Antioxidant Restenosis Trial (CART-1) involving 305 patients scheduled for PCIs with (85% of the patients) or without stenting. Both AGI-1067 (2.75 ± 1.76, 3.17 ± 2.26 and 3.36 ± 2.12 mm^2 for the respective doses) and probucol (3.69 ± 2.69 mm^2) dose-dependently and significantly prevented reductions in luminal area at the PCI site as compared to placebo (2.66 ± 1.58 mm^2). A significant enlargement of the reference segment lumen was observed with 140 mg AGI-1067 (+3.5 mm^3) at the 6-month follow-up, suggesting a direct antiatherosclerotic effect. In contrast, a significant mean narrowing was observed in placebo (–5.3 mm^3) and probucol (–0.2 mm^3) groups. The beneficial effects of AGI-1067 on CAD were observed as early as 2 weeks. Increases in Q-Tc intervals of more than 60 ms were noted in 17.4% of patients treated with probucol as compared to 4.8%, 4.8%, 2.4% and 2.5% of the patients treated with placebo and AGI-1067 at 70, 140 and 180 mg, respectively (19).

Based on results of this phase II trial, a multicenter phase III trial is planned to examine the efficacy of AGI-1067 as a secondary prevention of atherosclerotic cardiovascular disease in patients with CAD. The ARISE (Aggressive Reduction of Inflammation Stops Events) trial will examine the effects of AGI-1067 on death due to coronary disease, myocardial infarction, stroke, coronary revascularization and unstable angina in patients with CAD as compared to standard treatments. Approximately 4,000 patients will be included and followed for an average of 18 months or until a minimum of 1,160 patients have experienced primary events or outcomes. Patients will also be receiving cholesterol-lowering, hypertensive and/or anticlotting therapies (20).

Source

AtheroGenics, Inc. (US).

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