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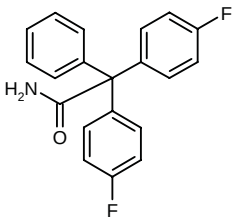
ICA-17043

Treatment of Sickle Cell Disease
Gardos Channel Inhibitor

2,2-Bis(4-fluorophenyl)-2-phenylacetamide



$C_{20}H_{15}F_2NO$
Mol wt: 323.3405
CAS: 289656-45-7
EN: 296717

Abstract

Sickle cell disease is a genetic hematologic condition characterized by the presence of abnormal hemoglobin within red blood cells (RBCs). The polymerization of these abnormal hemoglobin molecules leads to the formation of crescent-like or "sickle" shaped cells. These sickle shaped cells easily adhere to the vascular endothelium, obstructing normal blood flow and oxygen delivery to vital organs and tissues. Clinical manifestations include chronic anemia, susceptibility to infections, damage to multiple organ systems and a shortened lifespan. Currently, treatment options for sickle cell disease are limited. Hydroxyurea is used in some patients, but is associated with a low response rate and complicated by a range of side effects. Clotrimazole is effective in preventing sickling crises by stopping the sickling cascade from occurring, but is known to cause reversible liver damage. ICA-17043 is a novel Gardos channel inhibitor indicated for the treatment of sickle cell disease. ICA-17043 prevents RBC polymerization by blocking the cellular dehydration that normally precedes this event. ICA-17043 administration has been shown to successfully block the sickling pathway in human *in vitro* studies, as well as in transgenic animal models, and is now in phase II clinical trials for the treatment of sickle cell disease.

Synthesis

The Grignard condensation of 4,4'-difluorobenzophenone (I) with phenylmagnesium bromide (II) in refluxing *tert*-butyl methyl ether gives the corresponding carbinol (III), which is treated with acetyl chloride in dichloromethane to yield the methyl chloride (IV). Condensation of compound (IV) with copper cyanide by heating at 140 °C, followed by treatment with hot toluene affords 2,2-bis(4-fluorophenyl)-2-phenylacetonitrile (V), which is finally partially hydrolyzed with H_2SO_4 in refluxing glacial acetic acid (1). Scheme 1.

Introduction

Sickle cell disease is an autosomal recessive, inherited blood disorder affecting the structure and function of hemoglobin (Hb), an oxygen-carrying protein found within red blood cells (RBCs) comprising 4 globin polypeptide chains (2 α-like and 2 β-like chains). Patients with sickle cell disease inherit a point mutation in the gene encoding β-chain formation found on chromosome 11p15.4. The result of the point mutation is that hydrophilic amino acid glutamine is replaced by hydrophobic valine in position-6 of each β-chain. The mutation results in an abnormal structure of the Hb molecule (HbS).

The pathophysiology of sickle cell disease describes a complex sequence of biochemical events. Abnormal HbS molecules polymerize under conditions of low oxygen to form fibrous polymers. These changes in polymerization cause the normal disc-shaped RBC to take on the more crescent or "sickled" appearance characteristic of sickle cell disease. HbS molecules are more likely to polymerize when intracellular concentrations of HbS are increased, as is observed following RBC dehydration (2).

The effect of polymerization of the Hb molecule is that it works to stiffen the RBC membrane, making the HbS molecule more rigid than its Hb counterpart. HbS molecules, therefore, have a tendency to get stuck within the vasculature. Polymerized RBCs also have an increased

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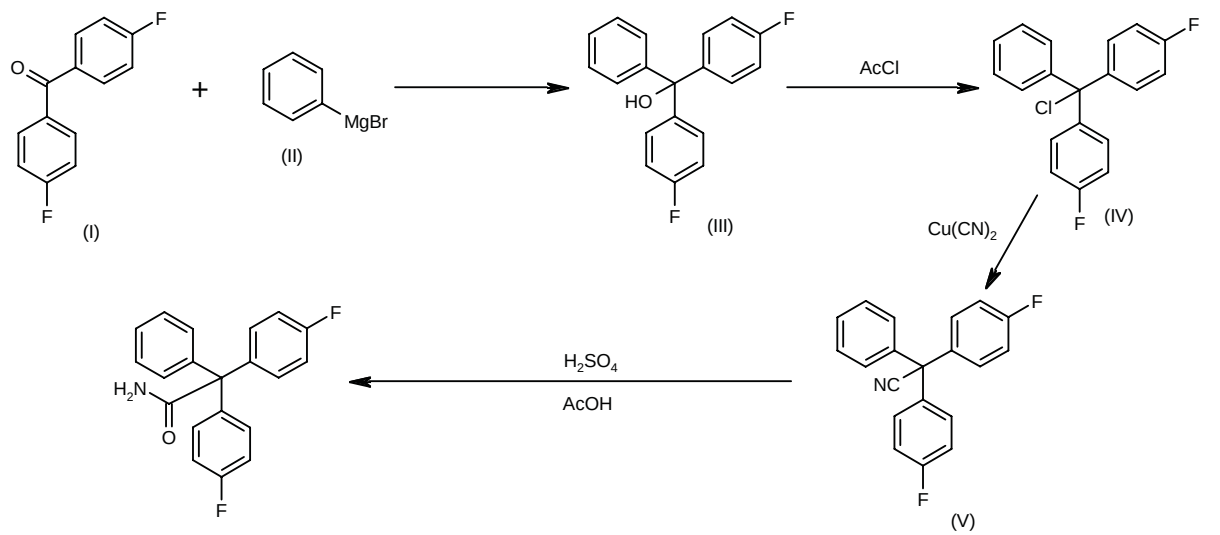
Scheme 1: Synthesis of ICA-17043



propensity to adhere to the endothelial cell layer within venules. They possess altered "sticky" membranes that increase blood viscosity. These abnormalities in HbS rigidity and viscosity result in the formation of vascular occlusions, bringing about end-organ damage through subsequent tissue ischemia and necrosis. Infarction commonly occurs in the spleen, bones, liver, kidneys, lungs and central nervous system, and is associated with significant chronic pain.

The abnormalities in Hb structure and function result in hemolytic anemia. RBCs containing HbS only survive for 10-20 days compared with the usual 120 days. The spleen recognizes abnormally shaped RBCs, and subsequently destroys them, provoking the anemia characteristic of sickle cell disease. Sickle cell disease can become life-threatening when an increased number of the sickled cells hemolyze, precipitating a hemolytic crisis. The two main manifestations of sickle cell disease are chronic anemia and pain from ischemic infarcts.

Sickle cell disease is the most common of the severe hemoglobinopathies, affecting 1 in 4,000 births per year in the U.K., 1 in 500 births in the U.S. and over 4 million people worldwide. A higher incidence is observed within the African population, with up to 25% of people being identified as carriers. The incidence of sickle cell disease is also increased in India, the Middle East and southern Europe (3, 4).

Currently, the most common treatments for sickle cell disease are symptomatic and supportive and include intravenous hydration, analgesics (NSAIDs and morphine), oxygen therapy, PCN prophylaxis and blood transfusions. While these methods can be used collectively to treat the symptoms of sickle cell disease, there is a significant need for an effective and safe prophylaxis therapy.

Hydroxyurea was the mainstay of prophylactic sickle cell disease therapy. It works by inhibiting formation of HbS through the production of fetal Hb (HbF). A rise in HbF inhibits the intracellular polymerization of HbS, and is inversely related to morbidity in patients with sickle cell disease. HbF inhibits the sickling process as it is an Hb molecule not involved in polymerization. It therefore acts by diluting the effective HbS concentration, thereby reducing hemolysis and ischemic pain. Hydroxyurea is only effective in 60% of patients, however, with 40% of patients being nonresponders. Also, hydroxyurea has been associated with toxic side effects including bone marrow suppression and progressive organ failure. The search for a more effective and less toxic solution to this problem was therefore sought (5-7).

Advances in the understanding of the sickling process led researchers to develop a more rational approach to the treatment of sickle cell disease. Sickling can be halted at a number of different places along its physiological pathway. Of particular interest has been the inhibition of the RBC dehydration that typically precipitates HbS polymerization. Membranous changes instituted by the abnormal HbS molecule predispose the RBCs to dehydration. RBC dehydration is a distinguishing characteristic between sickled and nonsickled RBCs, and is driven by the loss of intracellular potassium (8, 9).

Gardos first discovered the ion channel involved in RBC dehydration. The Gardos channel is a calcium-dependent, potassium channel found specifically in

RBCs. While Gardos channels are found in all RBCs, HbS molecules promote increased intracellular calcium concentrations, which act to amplify activation of the Gardos channel. This increased activation allows for a greater concentration of potassium to leave the cell, taking with it chloride and water, ultimately leading to RBC dehydration. Water loss from the RBC concentrates the remaining HbS, promoting sickling, which can eventually precipitate a sickle crisis. Researchers hypothesized that blockade of the Gardos channel could potentially decrease the flow of potassium ions leaving the cell, thereby limiting the subsequent sickling cascade (10).

Clotrimazole, an imidazole antifungal agent, was the first Gardos channel inhibitor to be designed. Clotrimazole was shown to reverse RBC dehydration in an *in vitro* model using human RBCs and in a transgenic mouse model of sickle cell disease. Its efficacy was subsequently tested in a human study involving 5 patients with sickle cell anemia, the results of which confirmed its ability to decrease RBC dehydration and sickling. However, clotrimazole administration was associated with increased levels of the enzymes AST and ALT, indicating a potential adverse effect on the liver. While liver enzyme levels returned to normal following discontinuation of the study drug, the long-term use of clotrimazole was questioned (10, 11).

Investigators considered that the liver toxicity was most likely due to the imidazole ring present within the clotrimazole molecular structure. These limitations led to the research and development of other Gardos channel blockers which could produce the same effects on RBC dehydration, but without the associated hepatic side effects. Structurally similar to clotrimazole, ICA-17043 works at the same ion channel but has a primary amide substituted for the imidazole ring and, as such, is not associated with adverse effects on the liver (12).

Pharmacological Actions

The rationale of ICA-17043 therapy is to halt the sickling process by inhibiting RBC dehydration. This in turn, should reduce the amount of HbS polymerization, thereby making the precipitation into a full-blown sickling crisis much less likely.

ICA-17043 inhibits ion transport through the Gardos channel with greater potency and selectivity than clotrimazole. It acts with high specificity at the Gardos channel, exhibiting receptor affinity values > 10 μM at 30 different receptor sites. The selectivity ratio for the Gardos channel was estimated to be over 900. *In vitro* analysis showed that ICA-17043 inhibited calcium-dependent transport of radiolabeled rubidium (Rb) across the Gardos channel, with an IC_{50} of 11 nM compared with clotrimazole (IC_{50} = 100 nM). ICA-17043 also inhibited RBC dehydration in a dose-dependent manner, measured as the drug's ability to inhibit the increase in intracellular HbS associated with RBC dehydration (12).

The hemologic effects of ICA-17043 were tested in a group of transgenic mice in a model of sickle cell disease. Transgenic knock-out sickle (SAD) mice are engineered to synthesize human HbS instead of their normal Hb and have been shown to mirror human pathology in their subsequent development of ischemia and hemolytic anemia, making them an ideal model for the research of human sickle cell disease (12-14).

Twice-daily oral administration of ICA-17043 10 mg/kg was delivered to SAD mice and was compared with mice receiving vehicle. Gardos channel activity, RBC density and potassium/sodium concentrations and other hematological parameters were recorded as primary outcome variables at 0, 11 and 21 days. Results showed that ICA-17043 was effective in reducing RBC dehydration and the subsequent sickling cascade. There was a significant dose-dependent reduction in Gardos channel activity following ICA-17043 administration, coinciding with a leftward shift in RBC density and an increase in intracellular potassium concentration. No changes in intracellular sodium were observed within this model. RBC dehydration was further accentuated in mice exposed to chronic hypoxia; however, ICA-17043 administration inhibited these hypoxic-induced changes (12).

Hematocrit was significantly increased (43.5 *vs.* 50.9), while MCHC was significantly decreased (34.0 *vs.* 30.0) after 21 days of ICA-17043 therapy. Blood morphology studies showed that the concentration of sickled RBCs was decreased in the venous circulation following ICA-17043 treatment. Vehicle mice did not exhibit any changes in any of the outcome variables studied (14).

Pharmacokinetics

The pharmacokinetics of ICA-17043 were tested in 36 healthy male volunteers. Pharmacokinetic parameters were measured following single-dose oral administration of ICA-17043 25-200 mg. C_{max} increased dose-dependently up to the 150 mg dose, with mean values of 21-170 ng/ml being reported. There was also a dose-dependent linear relationship between the study drug and systemic exposure, with AUC values increasing with ICA-17043 up to the 150 mg dose, then plateauing at 200 mg. T_{max} showed wide variability, ranging from 4-72 h. The half-life of ICA-17043 was estimated to be 10-13 days (15-17).

The pharmacodynamic profile of ICA-17043 was assessed in an ascending dose-finding study in 36 healthy male volunteers who were randomized to receive oral doses of ICA-17043 25-200 mg in this double-blind, placebo-controlled trial. Investigators intended to determine the minimal dose capable of inhibiting Gardos channel activity. The concentration of ICA-17043 needed to inhibit Gardos channel flux by 50% was 54.4 ng/ml, and 80-85% inhibition was exhibited following a single oral dose of ICA-17043 150 mg. Investigators reported an intersubject coefficient of variation of 83% (15).

Clinical Studies

The efficacy and tolerability of ICA-17043 has been assessed in 4 phase I studies involving a total of 115 healthy volunteers and patients with sickle cell disease. Clinical trials were initiated in 1999 and by 2000, ICA-17043 had been granted orphan drug status by the U.S. FDA.

Initial clinical results were described in a group of 42 healthy volunteers. Subjects received a single oral dose of ICA-17043 (25, 50, 100 or 150 mg). Gardos channel inhibition was achieved in < 20% of patients in the 25 mg group, compared with 80% in the 150 mg group. ICA-17043 was well tolerated up to the maximum 150 mg dose level tested in this study. There were no serious or dose-limiting adverse events observed in this group of volunteers (16).

A phase Ib trial was conducted to substantiate these initial promising results. A total of 28 patients with sickle cell disease (26 males and 2 females) aged 19-57 years were randomized to receive a single oral dose of ICA-17043 50 mg, 100 mg, 150 mg or placebo in this double-blind, placebo-controlled, dose-escalation study. Patients were followed up for 56 days after ICA-17043 administration. Results showed that single oral doses of ICA-17043 were effective in patients with sickle cell disease. Sustained and prolonged Gardos channel blockade was achieved at doses lower than that causing serious or dose-limiting toxicity. The most commonly reported adverse events were nausea and musculoskeletal pain. There were no differences in adverse events between placebo and active treatment recipients (17).

ICAgen has completed enrollment in its phase II trial of ICA-17043 for the treatment of sickle cell anemia. The 90-patient, double-blind, placebo-controlled, parallel-group study is being conducted at 21 U.S. sites. It has three arms, including high-dose, low-dose and placebo arms, and will assess the efficacy and safety of ICA-17043. Changes in certain hematological parameters and measures of pain will also be monitored and results will help establish a dose regimen for phase III studies. ICA-17043 recently received fast track designation from the U.S. FDA (18).

Conclusions

ICA-17043 is a novel Gardos channel inhibitor indicated for the treatment of sickle cell disease. ICA-17043 prevents RBC polymerization by blocking the cellular dehydration that normally precedes this event. ICA-17043 administration has successfully blocked the sickling pathway in human in vitro studies (in isolated RBCs), as well as in transgenic animal models of sickle cell disease.

Phase I and Ib trials have produced promising results in healthy volunteers and patients with sickle cell disease. ICA-17043 has been shown to be a potent and specific Gardos channel inhibitor in these studies. A phase II randomized, double-blind, placebo-controlled trial in patients with sickle cell disease is currently under way in order to confirm these preliminary findings.

Source

ICAgen, Inc. (US).

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