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Sickle Cell Disease – Current Treatment and New Therapeutical Approaches

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Abstract

Sickle cell disease (SCD) is one of the most common genetic disorders worldwide. It is caused by a point mutation that changes glutamic acid (Glu6) to valine (Val6) in the β chain of hemoglobin. Vaso-occlusion is the most well-known problem associated with SCD. Despite recent advances in understanding the disease at the molecular level, few therapeutic strategies are available. Hydroxyurea is the only drug currently approved by the U.S. Food and Drug Administration for the disease, and it has serious adverse effects and lack of efficacy in some patients. However, new therapeutic approaches are under investigation in the hope of discovering new drugs to treat SCD. These include agents that: a) increase nitric oxide bioavailability; b) modify the rheological properties of the blood; c) bind covalently to hemoglobin; d) prevent hemoglobin dehydration; e) reduce iron overload; and f) induce the expression of gamma globin and fetal hemoglobin. In this chapter, we discuss the current treatment of SCD and the advances made in medicinal chemistry to find new drugs to treat this neglected hematological disease.

Keywords: Sickle cell disease, hemoglobinopathy, gamma globin, fetal hemoglobin, drug discovery

1. Introduction

1.1. General background

Sickle cell disease (SCD) is one of the most prevalent hemoglobinopathies worldwide. It has been hypothesized that this disease originated millions of years ago, in the sub-Saharan countries in mid-western Africa, eastern Asia, and some regions of India [1]. The distribution of the disease correlates with the malaria-endemic regions because it confers a protective effect against infection by the plasmodium [1-3].

Many biochemical and immunological mechanisms have been investigated to explain the protection conferred by hemoglobin S (HbS) against malaria. Infected sickle erythrocytes are known to be phagocytized faster than normal erythrocytes, thereby reducing parasitemia [4]; however, despite the many studies of the complex mechanisms involved, their relevance *in vivo* remains unclear [5].

According to the World Health Organization, about 5% of the world population carried a gene mutation for a hemoglobinopathy in 2011, particularly those mutations causing SCD and thalassemia. Today, SCD is not restricted to Africa and parts of India, but is found in the America and Europe, mainly as a result of migration and racial intermingling. In the United States, the disease afflicts approximately 1:500 Afro-American and 1:4000 Hispanic-American neonates [6].

The Brazilian National Program of Neonatal Screening estimates that around 2 million individuals carry the HbS trait in that country and 25,000–50,000 individuals are homozygous for HbS. About 3,500 children are thought to be born with SCD every year and 200,000 are heterozygous for the HbS gene [7-8].

1.2. Pathophysiology

Sickle cell disease is characterized by a point mutation in the sixth codon of the β -globin gene. The replacement of a thymine residue with an adenine (GTG to GAG) results in the substitution of glutamic acid for valine in the β -chain of hemoglobin, thus producing an anomalous hemoglobin (β^s -globin). After several cycles of deoxygenation and oxygenation, the HbS molecule polymerizes. This process is facilitated during the deoxygenation state of hemoglobin S (HbS) by hydrophobic interactions between the β subunits of the hemoglobin tetramer. The polymers thus formed can damage the erythrocyte structure, leading to sickle-shaped erythrocytes [9].

The polymerization of HbS represents the primary event in the molecular pathogenesis of the disease, and this process is dependent on several factors, including the concentrations of HbS and oxygen, the presence of high levels of normal hemoglobin, pH, temperature, and ionic strength [10]. HbS polymerization is responsible for: a) altering the structure and flexibility of the erythrocytes; b) promoting erythrocyte dehydration; and c) physical and oxidative stress [11, 12]. All of these events contribute to the hemolysis of the erythrocytes. The heme group present in the hemoglobin is then released into the circulation and can capture the nitric oxide

(NO) molecules present in the vascular endothelium, generating a “vasoconstriction effect” in the patient [13]. Low levels of NO contribute to the vasculopathy and hypercoagulability characteristic of the disease, and have been related to its clinical manifestations, including pulmonary hypertension, leg ulcers, priapism, and cerebrovascular disease [14, 15].

NO is an important mediator of cell functions, with various effects, including vasodilatation, the inhibition of platelet aggregation, and the reduced expression of adhesive molecules (Figure 1). This mediator also stimulates the expression of the gamma globin gene and consequently increases the production of fetal hemoglobin (HbF). This mechanism seems to involve soluble guanylyl cyclase (sGC), which increases the expression of γ -globin in erythroleukemic cells and primary erythroblasts [16, 17].

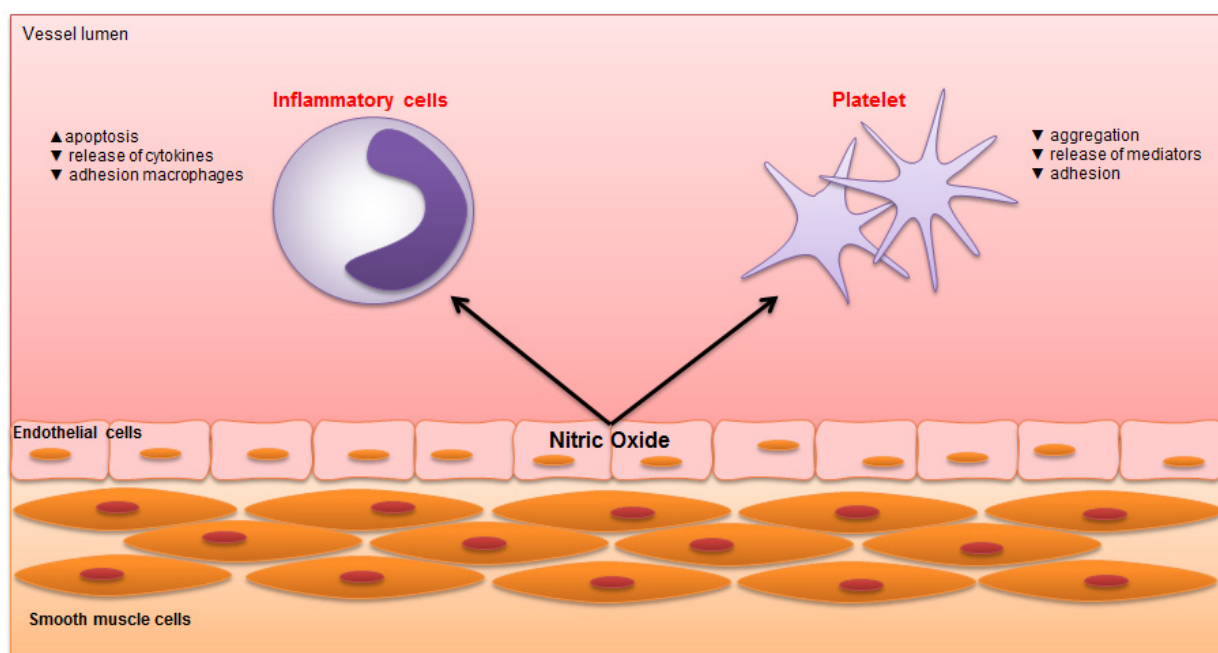


Figure 1. Effects of nitric oxide on the vascular endothelium, inflammatory cells, and platelets.

Another manifestation of SCD is vaso-occlusion (Figure 2), which is the major cause of morbidity in patients with SCD, causing tissue infarct, painful crises, acute thoracic syndrome, and nephropathy. The interactions of the sickle erythrocytes, leucocytes, neutrophils, and platelets with the vascular endothelium increase, leading to the formation of heterocellular aggregates, which are responsible for vaso-occlusion [18]. Mechanistically, the interaction between the erythrocytes and the endothelium involves $\alpha 4 \beta 1$ integrin, expressed on the erythrocyte surface, and fibronectin, vascular cell adhesion protein 1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1), and E-selectin, expressed on the endothelial cell surface [19]. Other ligands, including thrombospondin, von Willebrand factor, immunoglobulins, and fibrinogen, also seem to contribute to this adherence [20].

This vaso-occlusion is aggravated by ischemic cycles, which cause oxidative and inflammatory stress and increase the production of proinflammatory cytokines [21, 22].

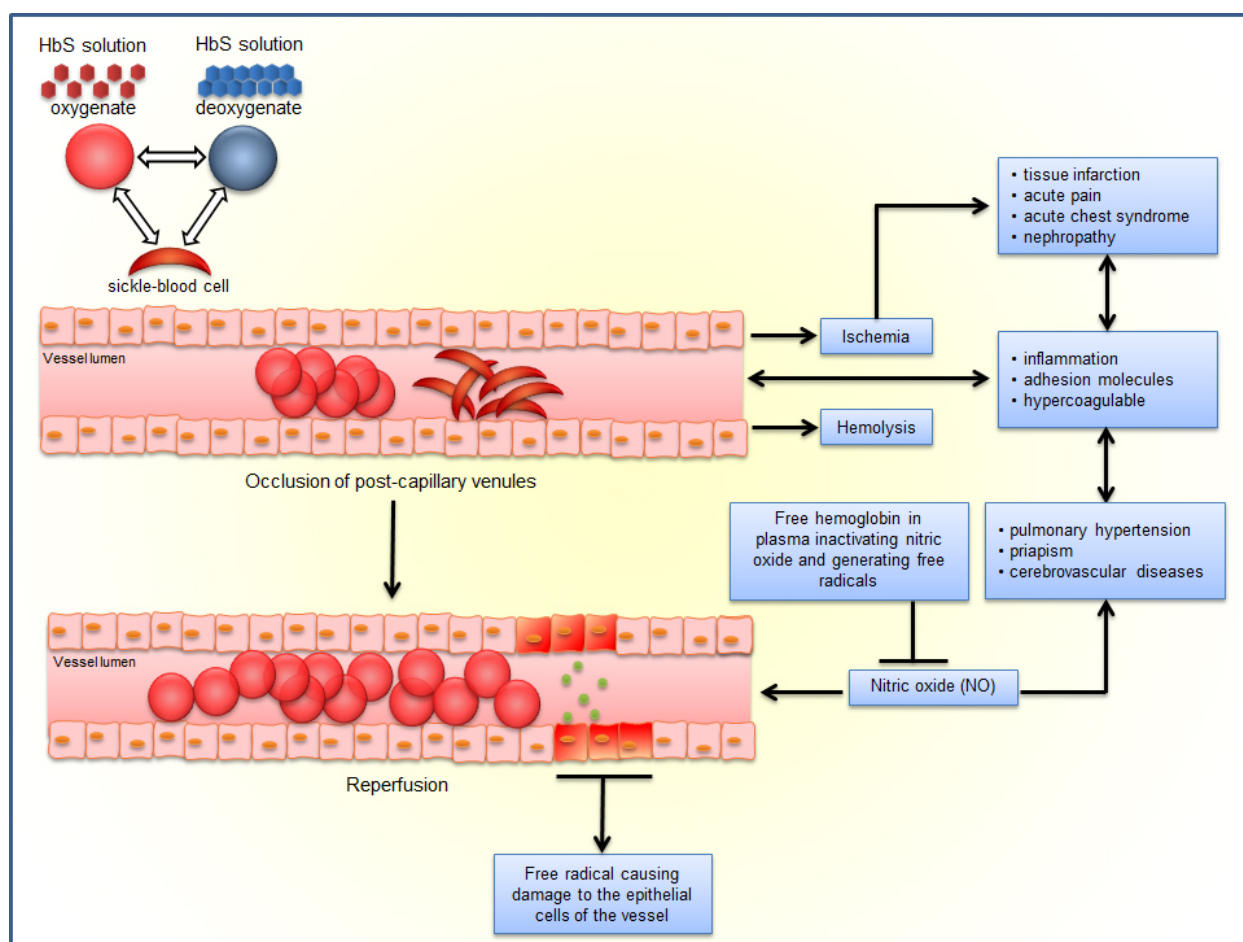


Figure 2. Pathophysiology of sickle cell disease.

Inflammation is another central feature of the vasculopathy of SCD (Figure 2). The adhesion and activation of leucocytes increase the production of proinflammatory cytokines, such as tumor necrosis factor α (TNF- α), interleukin 1 β (IL-1 β), and IL-8, which contribute to chronic inflammation and vaso-occlusive crises [23, 24]. SCD patients show increased levels of proinflammatory cytokines, including TNF- α [25], and high levels of TNF- α can increase the chemotactic properties of cells and amplify inflammation. This cytokine is also responsible for: a) increasing neutrophil adherence to the vascular endothelium; b) stimulating the production of free radicals; c) stimulating the synthesis of other inflammatory mediators, such as IL-1 β and prostaglandin E2 (PGE₂); and d) modulating coagulation and anticoagulation functions [26, 27]. Therefore, the increased plasma levels of TNF- α in SCD patients contribute to their vaso-occlusive crises and inflammatory episodes [25, 28-29].

1.3. Treatment

Despite current advances in medical technology, there is still no specific treatment for SCD. The drugs available can only reduce the symptoms and increase the patient's quality of life. The complexity of SCD is an obstacle to the scientific development of new selective and effective therapies. This is coupled with the lack of interest within the pharmaceutical industry

in searching for new drugs for this disease, another major impediment to the discovery of new treatments [30, 31]. Here, we discuss the main strategies and current advances in the search for new drugs with which to treat SCD.

Several strategies and therapies can be explored for the treatment of SCD. Among these, we include: a) the induction of HbF synthesis; b) the inhibition of erythrocyte dehydration; c) the inhibition of cellular adhesion; d) vasodilators; e) adenosine agonists; f) hemoglobin modifiers; g) Rho-kinase inhibition; and h) chelating agents (Figure 3). All of these strategies are discussed below.

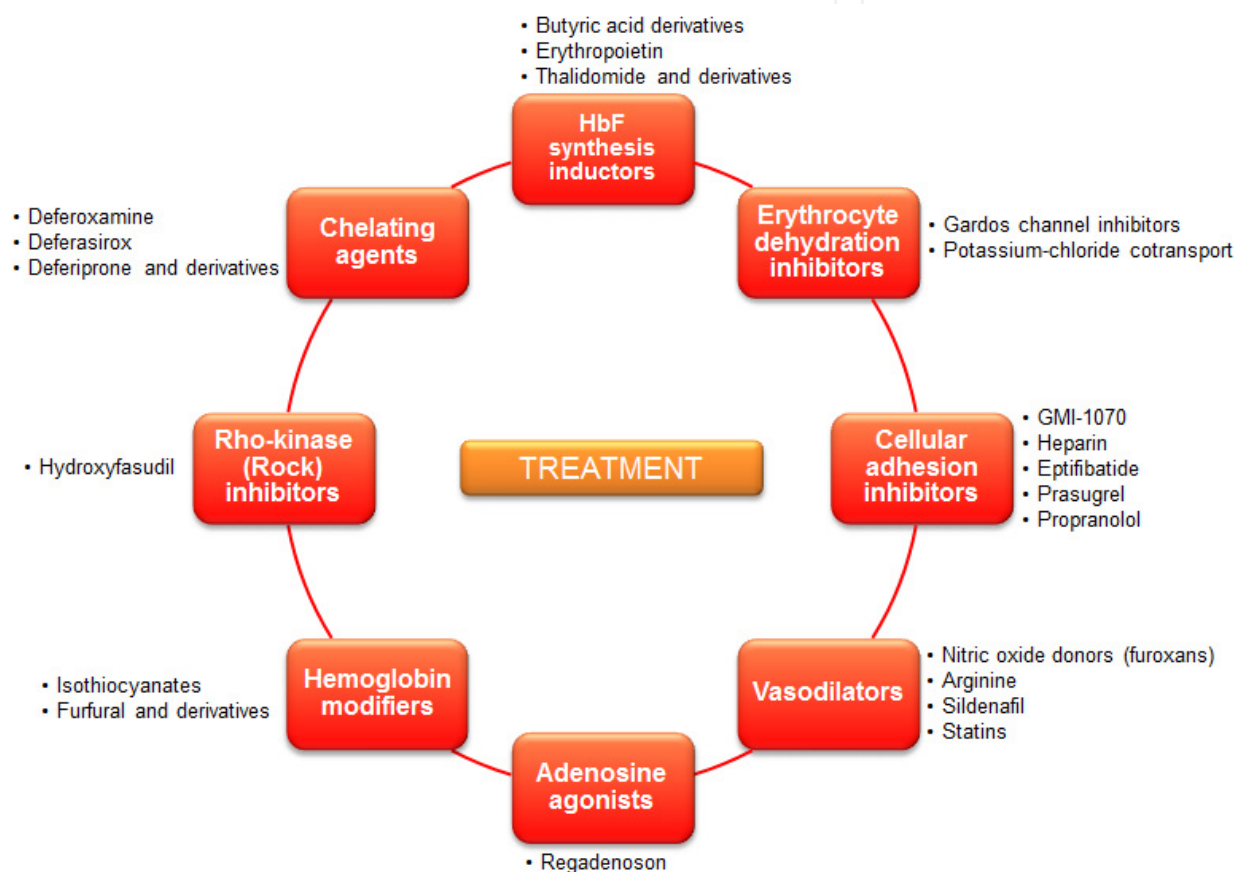


Figure 3. Therapeutic strategies for the treatment of SCD.

Induction of HbF synthesis

The induction of HbF synthesis is a promising strategy for the treatment of SCD [32, 33]. The elevated levels of HbS and low levels of HbF in patients with SCD are related to the clinical severity of the disease and the early mortality of the patients. This effect is related to high levels of HbS polymerization and its increased adherence to the vascular endothelium, which aggravate the vaso-occlusive process [34, 35]. Agents that increase HbF levels include hydroxyurea (HU), decitabine, azacitidine, NO donors, butyric acid and its derivatives, erythropoietin, and thalidomide and its derivatives.

- Hydroxyurea

Hydroxyurea (Figure 4) is a chemotherapeutic agent and selective inhibitor of ribonucleoside diphosphate reductase, an enzyme that converts ribonucleoside diphosphates to deoxyribonucleoside diphosphates. Therefore, HU inhibits the G1/S phase transition of the cell cycle [36]. Currently, it is the only drug approved by the U.S. Food and Drug Administration (FDA) for the treatment of SCD.

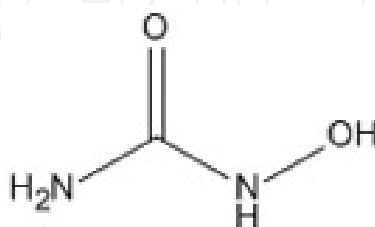


Figure 4. Chemical structure of hydroxyurea.

HU reduces the number of vaso-occlusive crises, episodes of acute thoracic syndrome, and urgent requirement for blood transfusion [37]. One nine-year clinical study demonstrated a reduction of 40% in the number of fatalities among patients treated with this drug [38].

The beneficial effects of HU in SCD are related to the increase of HbF levels. Some data suggest that the mechanism whereby HU increases the levels of HbF involves its biotransformation of NO, which activates the soluble guanylate cyclase (sGC) on erythroid cells [17, 39]. The activation of sGC increases the expression of γ -globin in erythroleukemic cells and primary human erythroblasts [16]. Other effects of HU include the reduction of leukocytes, reticulocytes, and platelets, and a reduction in the adhesiveness of erythrocytes and leukocytes to the vascular endothelium [40]. However, HU has several adverse effects, such as myelotoxicity, cutaneous hyperpigmentation, and ulcerative lesions on lower limbs [41].

Despite these adverse effects, the benefits of HU use are supported by evidence of its efficacy in reducing morbidity and mortality. However, importantly, about one third of patients do not respond to HU treatment [42]. In this context it is important to introduce new drugs that recapitulate the beneficial effects of HU without its potential toxicity.

- NO donors

Nitric oxide is a gas, synthesized from L-arginine by a family of enzymes called nitric oxide synthases [43], which have multiple regulatory functions in organisms, as transcription factor activators, in glycolysis and mitochondrial electron transport, hormone release, penile erection, and platelet and neutrophil adhesion, among others [44].

NO is the main endothelium-derived relaxant, with a central role in homeostasis and the inhibition of platelet aggregation [45]. It is also considered an epigenetic molecule because it bonds with the sulfhydryl groups of cysteine residues, generating S-nitrosyl groups, which can modify gene expression [46, 47]. The most important example of the modification of gene expression by NO involves the activity of histone deacetylase (HDAC2), in which cysteine

residues 262 and 274 are S-nitrosylated, causing the enzyme to dissociate from the chromatin, resulting in acetylation of the H3 and H4 histones [48].

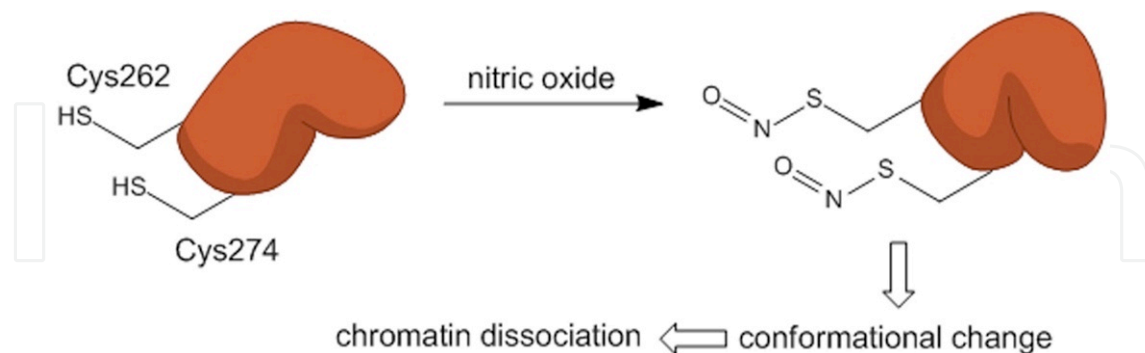


Figure 5. Representation of the NO effect on HDAC2 S-nitrosylation.

- Decitabine and azacitidine

Decitabine (5-aza-2'-deoxycytidine) (Figure 6) is a potent inducer of HbF synthesis, acting through the hypomethylation of the promoter of the γ -globin gene [49]. One clinical study conducted with a small group of patients showed that decitabine increases HbF production, even in patients unresponsive to HU [50]. In animal models, decitabine does not induce carcinogenesis and, curiously, has shown protective activity against cancer [51].

Azacitidine (5-azacytidine) (Figure 6) has also been shown to act as an inducer of HbF. However, serious adverse effects in humans and animals have restricted its use in the treatment of SCD, including carcinogenicity, neutropenia, thrombocytopenia, and leukopenia [52, 53].

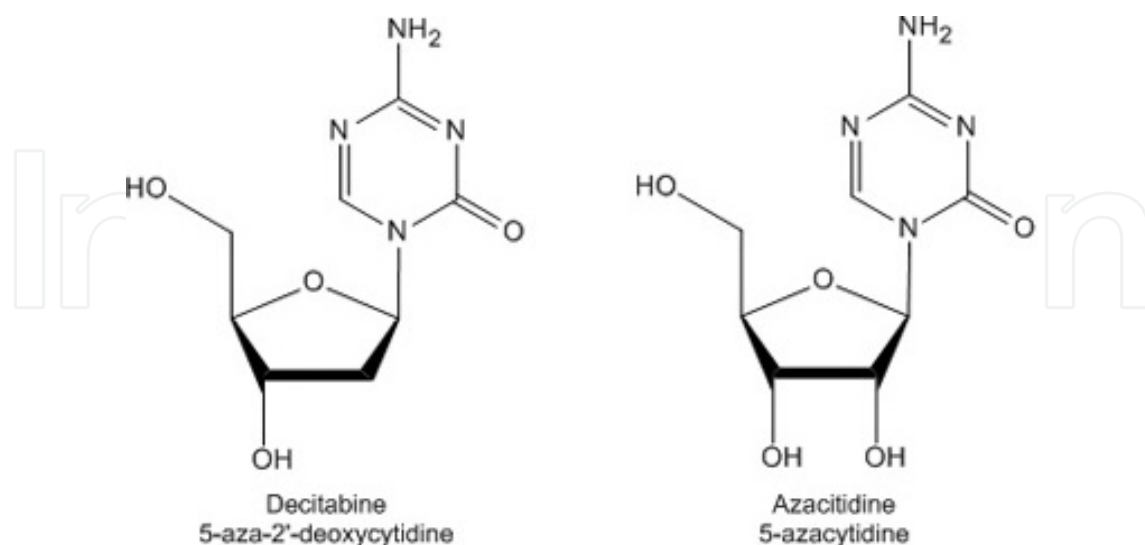


Figure 6. Chemical structure of decitabine and its analogue, azacitidine.

- Butyric acid and its derivatives

The butyrates (Figure 7) are short-chain fatty acids that inhibit the histone deacetylases, resulting in the induction of γ -globin gene expression and the synthesis of HbF [54]. The butyrates have been shown to produce a sustained increase in the HbF concentrations of SCD patients, but their short half-lives and low bioavailability have limited their use clinically. Therefore, new derivatives of butyric acid, with superior bioavailability and increased half-lives, are under investigation in animal models [33, 55].

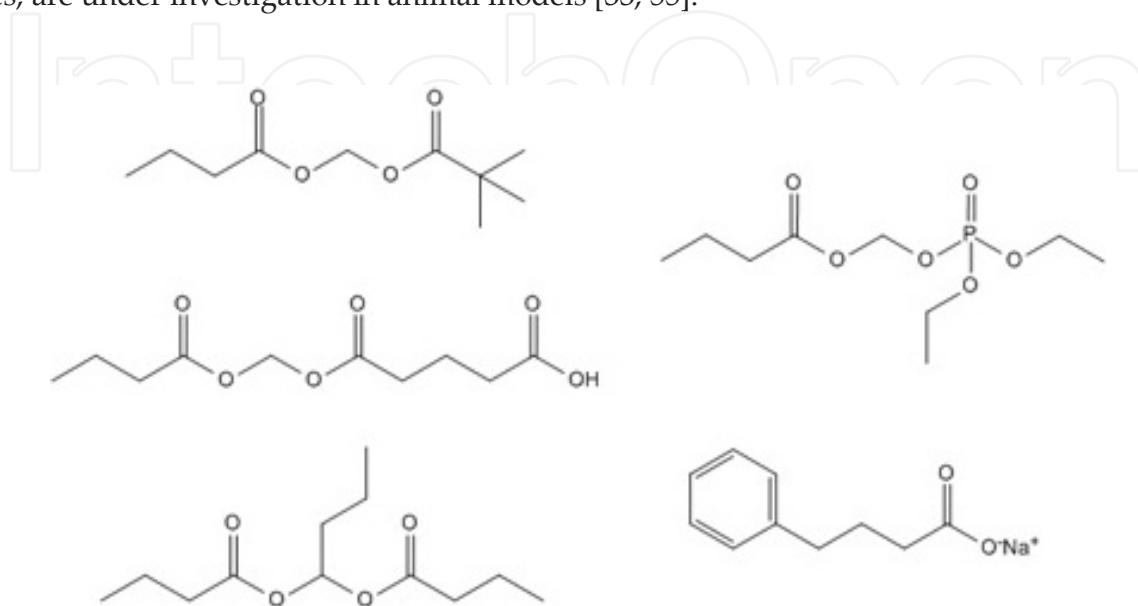


Figure 7. Chemical structures of some butyric acid derivatives.

- Erythropoietin

Recombinant human erythropoietin also increases HbF levels *in vivo* and *in vivo* with few adverse effects. This combination has shown good results, with a better tolerance profile than either agent alone, mainly in patients who are only weakly responsive to HU [56].

- Thalidomide and its derivatives

Thalidomide (Figure 8), originally used as a hypnotic/sedative and antiemetic agent, was withdrawn from the market in the 1960s because of its teratogenic effects [57]. However, it has proven useful in the treatment of other diseases, such as leprosy and multiple myeloma, based on its anti-inflammatory and immunomodulatory effects [58].

Thalidomide increases the production of reactive oxygen species (ROS) and induces γ -globin mRNA expression in a dose-dependent manner, *via* p38 MAPK signaling and histone H4 acetylation [59]. High levels of ROS act as signals that mediate the phosphorylation of tyrosine kinases, such as p38 MAPK, thereby regulating the expression of γ -globin [60].

Lenalidomide and pomalidomide (Figure 8) are thalidomide analogues with immunomodulatory effects related, in part, to the inhibition of TNF- α [61]. Moutouh-de Parseval et al. (2008) have shown that pomalidomide and lenalidomide induce HbF synthesis and modulate erythrocyte differentiation, and these effects were improved when the authors combined the

treatment with HU. The combination of HU with pomalidomide was more effective than its combination with lenalidomide [62]. *In vivo* studies of these two agents in transgenic animals have shown increased HbF expression, without any myelosuppressive effect, at levels similar to those in the HU-treated controls [63].

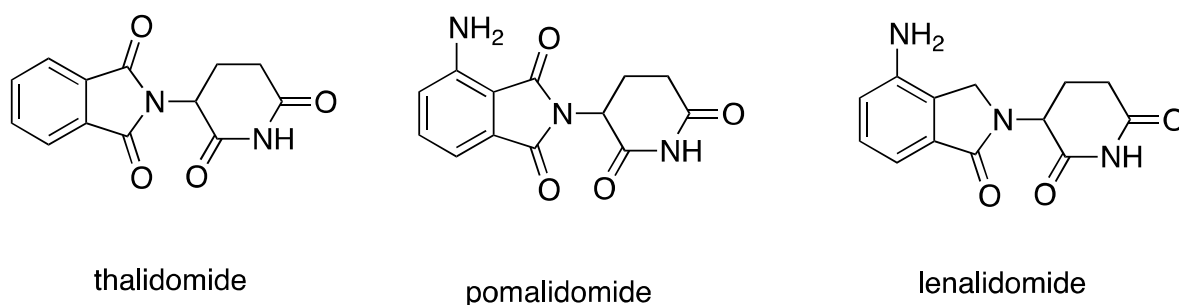


Figure 8. Chemical structures of thalidomide, pomalidomide and lenalidomide.

Inhibition of erythrocyte dehydration

HbS polymerization is dependent on the intracellular concentration of HbS, which is directly related to the hydration state of the cell. Therefore, strategies that prevent cellular dehydration should be explored for the treatment of SCD. The inhibition of potassium–chloride cotransport, in which potassium causes the movement of chloride ions and water, produces an osmotic imbalance and causes dehydration, with further polymerization of HbS [64]. The calcium-activated potassium channel known as the Gardos channel is also present on sickle erythrocytes and could be inhibited to promote an adequate osmotic balance [65]. Examples of compounds that inhibit this channel include magnesium, clotrimazole, and senicapoc.

- Magnesium

It has been reported that increased levels of intracellular Mg^{2+} inhibit the efflux of potassium from the erythrocyte, preventing its dehydration [66]. Preliminary studies in transgenic animals with SCD have shown that magnesium supplementation can substantially reduce the cotransport of KCl, and therefore reduce the mean corpuscular volume and the reticulocytes number [67]. Magnesium pidolate combined with HU was tested for six months in a clinical study (phase I) involving children. The results showed that magnesium pidolate reduces KCl cotransport activity. However, the authors found no changes in other hematological parameters [68].

- Clotrimazole

The antifungal drug clotrimazole (Figure 9) inhibits the Gardos channel in erythrocytes, but this drug is known for its adverse effects during long-term therapy [67]. Despite its toxicity, clotrimazole was used as a prototype molecule for molecular modification, which has generated compounds such as NS3623 and xlink652, which inhibit the Gardos channel. These compounds prevented hemolysis and sickle cell formation *in vivo*, in a transgenic mouse model [69, 70].

- ICA-17043 (Senicapoc)

Preclinical studies using the compound ICA-17043 (Figure 9) have shown that it reduces the activity of the Gardos channel, thus reducing the hemoglobin concentration and hemolysis. However, despite these beneficial effects, no reduction in the frequency of vaso-occlusion episodes was observed [71].

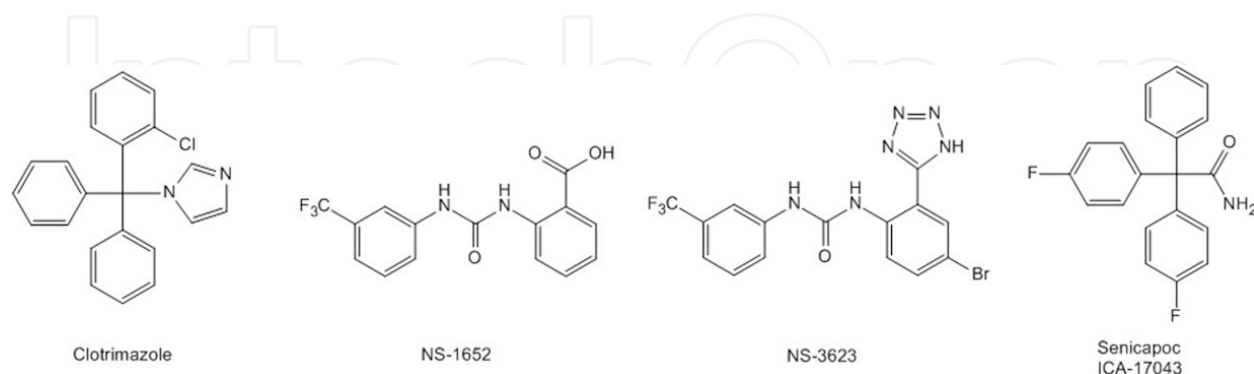


Figure 9. Chemical structures of erythrocyte dehydration inhibitors.

Inhibition of cellular adhesion

The adhesion of sickle cells to the vascular endothelium involves various mediators, including integrin $\alpha 4\beta 1$, CD36, and ICAM-4, which are responsible for the cellular interaction with the endothelium directly through E-selectin, P-selectin, integrins, and VCAM-1, or indirectly through molecules such as thrombospondin and von Willebrand factor [72, 73]. Several compounds have demonstrated a capacity to inhibit cellular adhesion, including rivipansel, heparin, eptifibatide, prasugrel, and propranolol.

Rivipansel (Figure 10), a synthetic glycomimetic molecule, is a pan-selectin inhibitor that acts on E-, P-, and L-selectin. It has been shown to restore blood flow during vaso-occlusion, increasing the survival rates in treated animals. An *in vivo* study indicated that this drug is a potent inhibitor of neutrophil adhesion through its interaction with E-selectin and ICAM-1 [74].

Heparin potentially interferes with the adhesion of sickle cells to the vascular endothelium through P-selectin. Clinical trials of low-molecular-weight heparin reported a reduction in the duration and severity of acute vaso-occlusive episodes [55, 73].

Another drug under investigation for the treatment of SCD is eptifibatide. Phase I clinical trials of eptifibatide, a synthetic cyclic peptide antagonist of glycoprotein IIb/IIIa (or integrin $\alpha_{IIb}\beta_3$), have reported reductions in platelet activation and inflammatory markers [75].

Vasodilators

Vasodilation is a desirable effect in the prevention of vaso-occlusive processes. NO is a vasodilator synthesized from L-arginine by endothelial cells and is responsible for maintaining vascular tonus [13]. It has been demonstrated that therapies that increase the bioavailability of NO may be beneficial to SCD patients, because 50% of patients showed endothelial dysfunction attributable to low endothelial levels of NO [76].

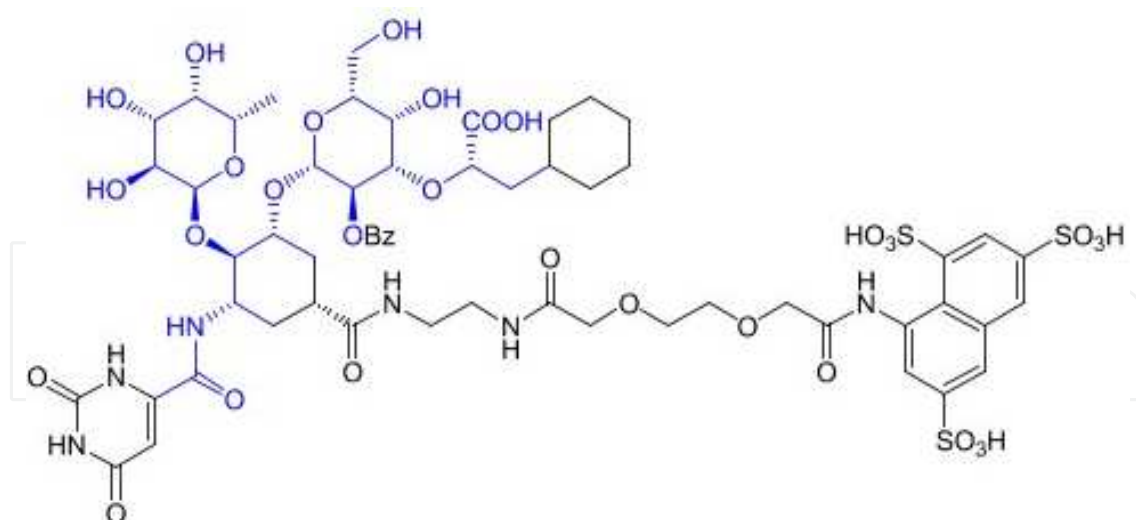


Figure 10. Chemical structure of rivipansel (GMI-1070).

NO is a soluble gas with a short half-life, used for pulmonary hypertension in newborn children. Its first use in SCD was reported by Atz and Wessel (1997), for the treatment of acute thoracic syndrome. NO inhalation reduces vascular pressure and resistance, and improves oxygenation in SCD patients [77].

NO donors containing the organic nitrate ester subunit and furoxan derivatives have been evaluated as potential compounds with which to treat SCD. Santos et al (2011 and 2012) synthesized new hybrid compounds containing the thalidomide subunit, an organic nitrate ester, and furoxan derivatives, as NO donors (Figure 11). All the molecules have shown NO-donor ability, with anti-inflammatory and analgesic effects. The compounds also induced gamma globin expression and HbF synthesis *in vivo* [78, 79].

Arginine supplementation can also increase NO levels, especially in patients suffering vaso-occlusive events [80]. Arginine also reduces the pulmonary arterial pressure in patients with pulmonary hypertension [81].

Sildenafil is a phosphodiesterase-5 inhibitor used to treat erectile dysfunction and pulmonary arterial hypertension [82]. Several studies have demonstrated that this drug reduces the activation of platelet-dependent glycoprotein IIb/IIIa in patients with SCD and pulmonary hypertension [83]. Sildenafil also increases the signalization of cGMP signaling which could be useful in the treatment of SCD patients with pulmonary hypertension [84, 85].

Statins efficiently prevent blood vessel damage *via* many mechanisms, including by increasing endothelial NO. These drugs also reduce vascular inflammation and restore endothelial relaxation in coronary diseases and stroke. Some studies have reported that lovastatin reduces the expression of platelet activation factor on the vascular endothelium [86].

Adenosine agonists

Adenosine is an endogenous purine nucleoside, whose signaling is responsible for promoting vasodilation, reducing inflammation, and protecting tissues during periods of hypoxia and

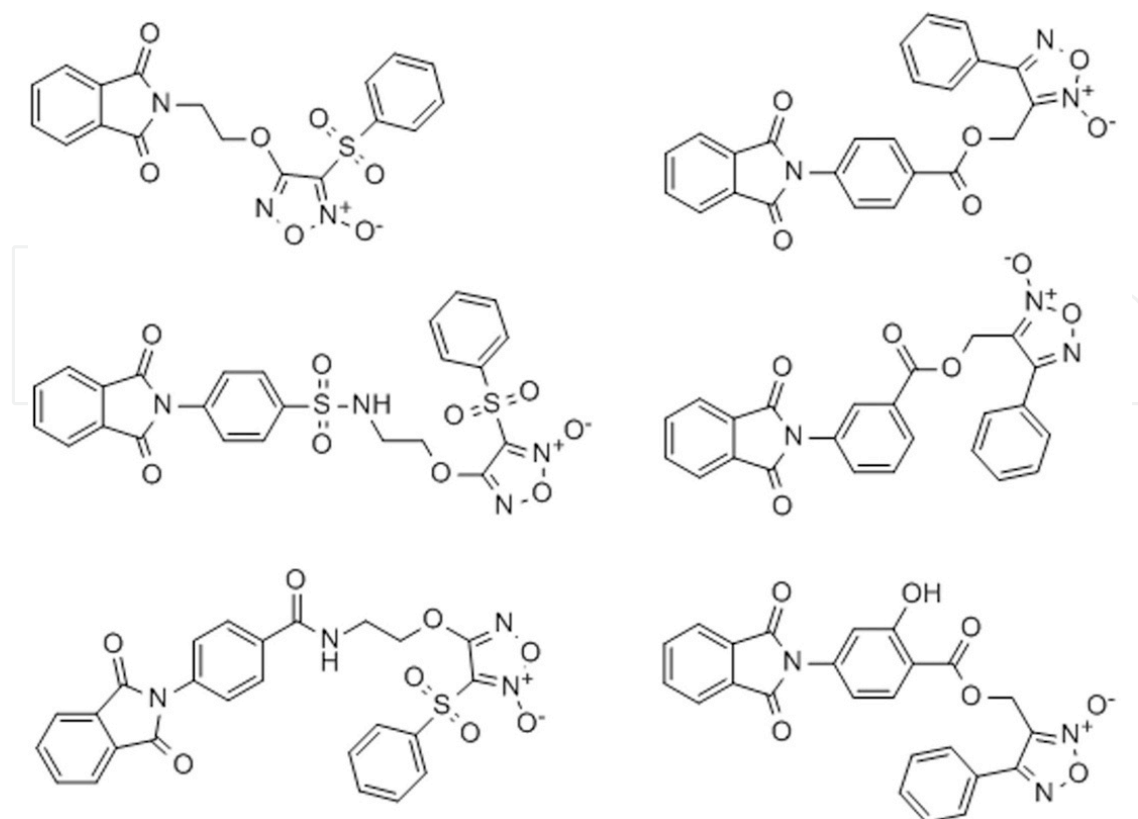


Figure 11. Furoxan derivatives with NO-donor ability.

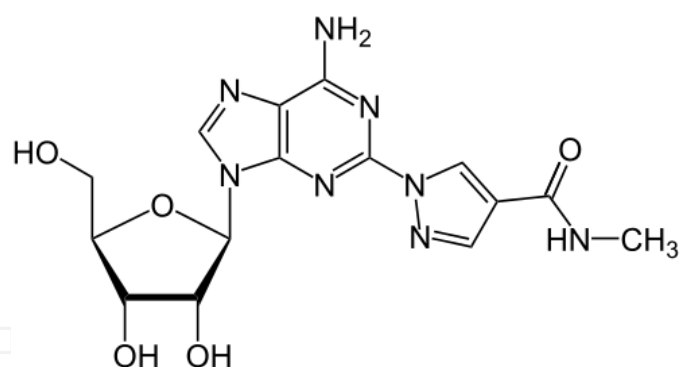


Figure 12. Chemical structure of regadenoson.

cellular stress [87]. In SCD patients, the tissue damage generated by ischemia–reperfusion may reduce the plasma levels of adenosine [88].

Some studies have suggested that receptors A_{2A} and A_{2B} are related to the pathogenesis of SCD. ATL146e is an A_{2A} adenosine agonist that reduces the activation of leukocytes, platelets, and invariant natural killer T cells (iNKT cells), inflammation, and pulmonary injury in transgenic animals with SCD [89]. Regadenoson (Figure 12) is a selective agonist of the A_{2a} adenosine receptor. During clinical trials (phase I), this compound reduced the activation of iNKT cells, with no toxic effects [90].

It has been reported that the activation of the adenosine A_{2B} receptor may increase the deleterious effects of SCD, promoting priapism and the sickling of erythrocytes [91]. Antagonists such as MS-1706 can reverse priapism [92]. Therefore, the adenosine signaling pathway is a promising target for the treatment of SCD. A double therapy with an A_{2A} agonist and an A_{2B} antagonist could have beneficial effects in patients, reducing inflammation, sickling, and priapism. However, more studies are required to understand the beneficial effects of these compounds in the treatment of SCD.

Hemoglobin modifiers

The hemoglobin modifiers are classified as either covalent or noncovalent. Although noncovalent modifiers have shown interesting activities, their use is still limited [93]. The modification of hemoglobin by covalent modifiers reduces erythrocyte sickling by two possible mechanisms: by increasing HbS solubility and/or by increasing its affinity for oxygen [94].

Isothiocyanates have been described as potential modifiers of HbS solubility, delaying its polymerization, specifically because they bind to the β subunit of HbS, which is responsible for the hydrophobic interactions that result in its polymerization [95].

Aldehyde compounds have the capacity to form adducts (Schiff bases) with the N-terminal amines on the amino acids in the HbS chain. Safo et al (2004) demonstrated that heterocyclic aldehydes, such as furfural, 5-methylfurfural, 5-ethylfurfural, and 5-hydroxymethylfurfural, increase the affinity of HbS for oxygen, thereby inhibiting sickling [96]. However, the low oral bioavailability of these drugs, the high doses required for a significant effect, and their dose-dependent toxicity limit their therapeutic use.

Rho-kinase (ROCK) inhibitors

The Rho-kinase protein (ROCK), identified as a Rho-GTPase effector, is involved in various cell processes, including contractility, chemotaxis, adhesion, and migration. This protein facilitates the infiltration of inflammatory cells, both *in vitro* and *in vivo* [97].

ROCK inhibition is beneficial in cardiovascular, neurological, and oncological diseases. The *in vivo* activities of these inhibitors include: a) the regulation of the arterial blood pressure; b) increased vascular resistance; c) the regression of atherosclerotic coronary lesions; d) the prevention of diabetes development; e) neurological repair; f) reduced formation of β -myeloid aggregates; and g) the inhibition of tumor growth, progression, and metastasis [98-105].

Rho-kinase inhibitors are also potential agents for the treatment of SCD. An *in vitro* study showed that Rho-kinase inhibitors, such as Y-27632 (Figure 13), reduced the activation of human endothelial cells and the adhesion of eosinophils in SCD patients. Fasudil (Figure 13) is a Rho-kinase inhibitor that is approved in Japan for the treatment and prevention of intracranial aneurysm and that has shown good results in preventing pulmonary complications in animals with SCD. The activities of fasudil include: a) the inhibition of eosinophil and chemokine recruitment, which promotes the progression of the pulmonary inflammatory response [106]; b) the reduction of proinflammatory cytokines levels, such as IL-6, IL1- β , and TNF- α , thus reducing inflammation [107]; and c) the reduction of the expression of adhesive molecules, such as ICAM-1, and therefore coagulation [108].

A comparison of HU and hydroxyfasudil demonstrated the superior activity of the ROCK inhibitor in reducing vaso-occlusion and inflammation. Therefore, this class of drug has been suggested as an alternative SCD treatment [109].

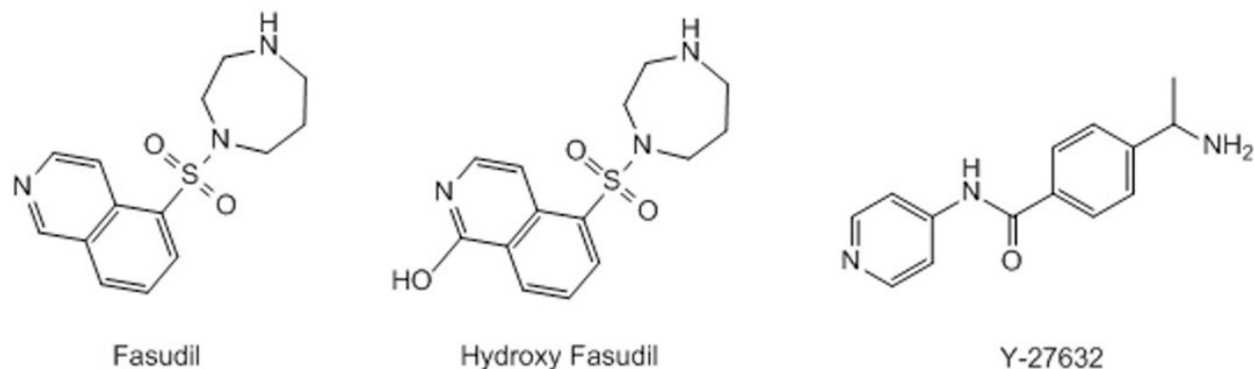


Figure 13. Chemical structures of Rho-kinase inhibitors.

Chelating agents

Iron-chelation therapies have been used to control iron overload in patients who have received several blood transfusions to reduce disease complications [110]. Iron overload can affect organs such as the liver, heart, and endocrine system, leading to tissue damage and even death [111].

Deferoxamine is a hexadentate chelating agent, introduced into the therapeutic context in 1963. It is still one of the drugs most frequently used to treat iron overload in hemoglobin disorders, such as SCD and thalassemia. Deferoxamine has a high molecular weight and a high affinity for Fe^{3+} , and the ratio between the drug and iron is 1:1. Because this drug has low oral/gastrointestinal absorption, it is administered *via* a subcutaneous or intravenous route [112].

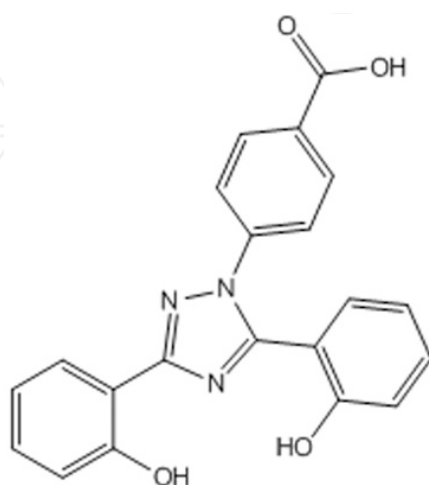


Figure 14. Chemical structure of deferasirox.

Deferasirox was approved by the FDA in November 2005 as an oral chelating agent. It is a trivalent molecule with great affinity for the iron atom. The chelating ratio is two drug molecules to each iron atom (Figure 14) [113]. The prolonged chelating effect produces a progressive reduction in free plasma iron. Its oral route of administration is the great advantage of this drug, but the treatment is expensive [114].

2. Conclusions

Sickle cell disease is one of the most prevalent hemoglobinopathies worldwide. Despite its importance, therapeutic resources are scarce and usually only control the main symptoms of the disease. The lack of interest by pharmaceutical companies in developing new drugs for SCD and the limited research undertaken in this area ensure that this disease still severely affects patients. The only drug currently available to treat SCD is HU, but its serious adverse effects limit its use. Moreover, around 1/3 of patients do not respond to HU treatment.

Therefore, research is urgently required to find new drugs for SCD. In this chapter, we have discussed the currently available treatments and their limitations, and have presented and discussed new approaches. Among these, the adenosine agonists/antagonists and ROCK inhibitors seem the most promising strategies, and therefore warrant further investigation.

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