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Research paper

Discovery of phenylsulfonylfuroxan derivatives as gamma globin inducers by histone acetylation

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ABSTRACT

N-oxide derivatives **5(a–b)**, **8(a–b)**, and **11(a–c)** were designed, synthesized and evaluated *in vitro* and *in vivo* as potential drugs that are able to ameliorate sickle cell disease (SCD) symptoms. All of the compounds demonstrated the capacity to releasing nitric oxide at different levels ranging from 0.8 to 30.1%, *in vivo* analgesic activity and ability to reduce TNF-α levels in the supernatants of monocyte cultures. The most active compound (**8b**) protected 50.1% against acetic acid-induced abdominal constrictions, while dipyrone, which was used as a control only protected 35%. Compounds **8a** and **8b** inhibited ADP-induced platelet aggregation by 84% and 76.1%, respectively. Both compounds increased γ-globin in K562 cells at 100 μM. The mechanisms involved in the γ-globin increase are related to the acetylation of histones H3 and H4 that is induced by these compounds. *In vitro*, the most promising compound (**8b**) was not cytotoxic, mutagenic and genotoxic.

1. Introduction

Sickle Cell Disease (SCD) is one of the most prevalent genetic hemoglobinopathies worldwide. The disease is caused by a single nucleotide polymorphism (GTC to GAC) at the sixth codon of the β-globin gene, which causes the substitution of glutamic acid (Glu6) to valine (Val6) [1]. Under low oxygen stress, the interactions of hydrophobic β-chains promote the polymerization of sickle hemoglobin (HbS) inside red blood cells (RBCs) and cause changes in the erythrocyte cytoskeleton that result in rigid and irregularly shaped cells that are prone to hemolysis [2]. Erythrocyte damage leads to the release of cell-free hemoglobin and arginase. The heme group sequesters nitric oxide (NO) from the vascular endothelium and causes the 'vasoconstriction crisis' that is commonly found in sickle cell patients [3]. Furthermore, NO deficiency contributes to vasculopathy and hypercoagulability [4,5].

Abnormal adhesion of red blood cells to the vascular endothelium and their increased interaction with leucocytes and platelets leads to the formation of heterocellular aggregates, which are responsible for the vaso-occlusion phenomenon that leads to the main clinical symptoms of SCD, including ischemia, acute chest syndrome, priapism, pain crisis and strokes [6,7].

Hydroxyurea (HU) and glutamine are the only therapeutic alternatives approved by the Food and Drug Administration (FDA) to treat sickle cell disease [8,9]. HU acts as a selective inhibitor of

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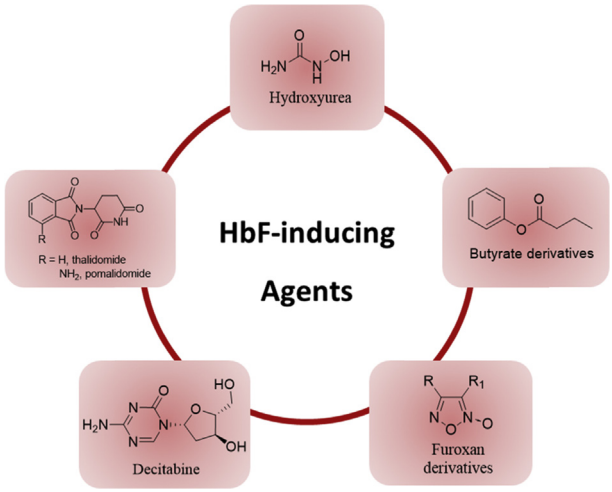

Fig. 1. Structures of current state-of-the-art of HbF-inducing agents in SCD treatment.

ribonucleotide diphosphate reductase synthesis, an enzyme required to convert diphosphate ribonucleotides to diphosphate deoxyribonucleotides. This drug prevents the cells leaving the G1/S phase of the cell cycle, being used mainly in neoplasias of the hematopoietic system [8]. For SCD patients, HU demonstrates multiple beneficial effects, including an increase of fetal hemoglobin levels and reduction of cellular adhesion to the vessel endothelium [9]; however, serious adverse effects, such as myelosuppression, limit its long-term use. In addition, approximately one-third of adult patients do not respond to HU therapy, justifying the search for new drugs [6,10].

The current 'state-of-art' for discovery new drugs to treat SCD include several approaches such as: a) inhibition of erythrocyte dehydratation (eg. senicapoc); b) cellular adhesion inhibitors (e.g., rivipansel); c) adenosine agonists (e.g., regadenoson); d) Rho-kinase (Rock) inhibitors (e.g., hydroxy-fasudil); e) chelating agents (e.g., deferasirox) and f) fetal hemoglobin (HbF) inducers (Fig. 1). Among these strategies, that aiming to increase HbF is the most promising [11] and several agents that act through this mechanism have been described. Reactivation of HbF in adults ameliorates the clinical symptoms of sickle cell disease [12]. Currently, experimental HbF inducers include a) nucleoside analogues with inhibitory effects on DNA methyltransferases (e.g., decitabine), b) histone deacetylase inhibitors (e.g., butyric acid), c) thalidomide and pomalidomide and; d) NO-donors (e.g., furoxan) among others [12,13].

Several molecular mechanisms are involved in γ-globin gene silencing in adult erythroid cells [14]. These mechanisms include epigenetic DNA modifications, such as histone modifications and DNA methylation [15]. Studies have shown that thalidomide induces γ-globin expression through the p38 MAPK phosphorylation pathway as well as histone H4 acetylation [16,17]. Its analogue pomalidomide induces HbF synthesis and modulates erythrocyte differentiation by decreasing the levels of the transcriptional factors BCL11-A and SOX-6 during erythropoiesis [18,19].

Other HbF inducer compounds include NO-donors. *N*-oxide compounds (e.g., furoxan) are widely known for their ability to release NO [20–23]. Experimental data revealed that NO-released from furoxan occurs due to the nucleophilic attack of thiol groups present in L-cysteine residues into furoxan nucleus leading to the formation of intermediate that undergo ring oppening to a nitroso derivative. Further, NO is formed by oxidation of eliminated nitrosyl anions [24,25]. NO contributes to increase fetal hemoglobin levels through activation of the soluble guanylyl cyclase (sGC) pathway [26]. Therefore, the synergistic combination of thalidomide within *N*-oxide subunits is a useful strategy to increase HbF [10].

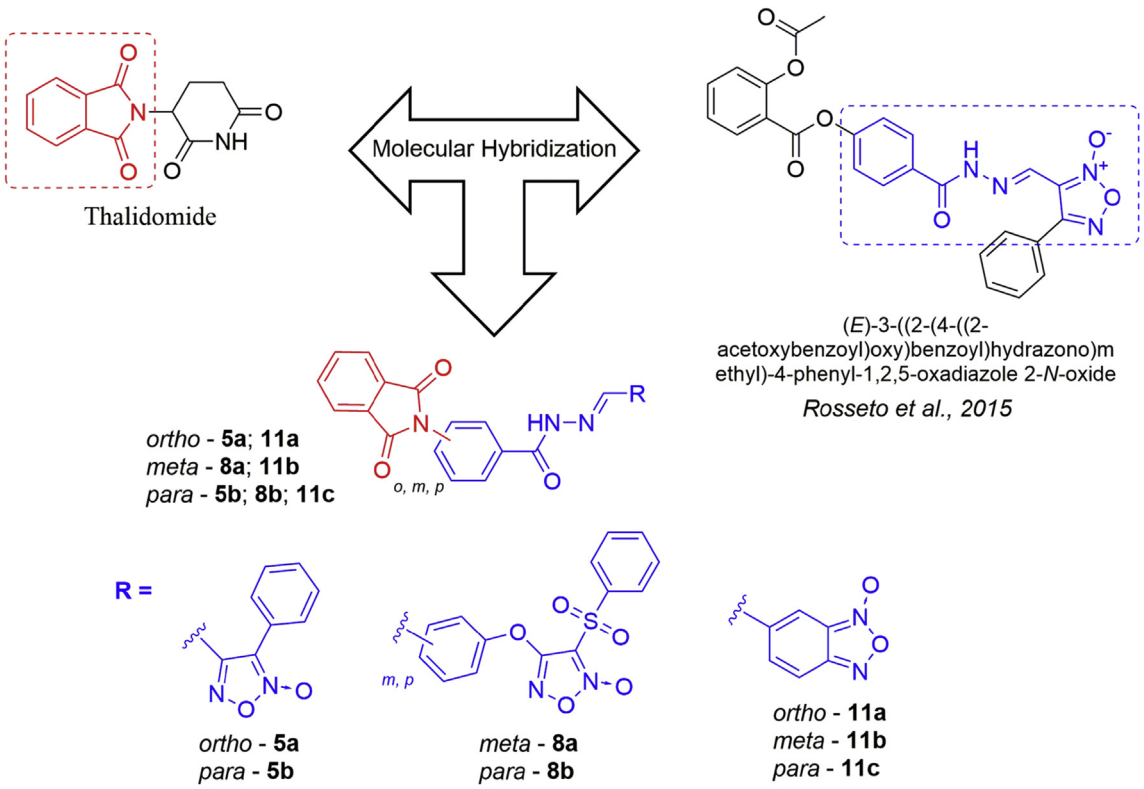

Fig. 2. Design of the hybrid furoxan derivatives.