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Erythroid differentiation ability of butyric acid analogues: Identification of basal chemical structures of new inducers of foetal haemoglobin

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ABSTRACT

Several investigations have demonstrated a mild clinical status in patients with β-globin disorders and congenital high persistence of foetal haemoglobin. This can be mimicked by a pharmacological increase of foetal γ-globin genes expression and foetal haemoglobin production. Our goal was to apply a multistep assay including few screening methods (benzidine staining, RT-PCR and HPLC analyses) and erythroid cellular model systems (the K562 cell line and erythroid precursors collected from peripheral blood) to select erythroid differentiation agents with foetal haemoglobin inducing potential.

With this methodology, we have identified a butyric acid derivative, namely the 4174 cyclopropane-carboxylic acid compound, able to induce erythroid differentiation without antiproliferative effect in K562 cells and increase of γ-globin gene expression in erythroid precursor cells. The results are relevant for pharmacological treatments of haemoglobinopathies, including β-thalassaemia and sickle cell anaemia.

1. Introduction

Several investigations have demonstrated how the clinical status of patients with β-globin disorders can be improved by pharmacologically increased expression of foetal γ-globin genes (Jouini et al., 2012, Musielak, 2011, Huisman, 1979, Stamatoyannopoulos and Nienhuis, 1992 and Gallo et al., 1979). Moreover, foetal haemoglobin levels greater than 9% could reduce early mortality (Platt et al., 1994).

A large number of compounds stimulating foetal haemoglobin production, such as chemotherapeutic agents, 5-azacytidine and hydroxyurea (Bunn, 1997, Ferster et al., 1996, Ballas et al., 2006 and Italia et al., 2013), was considered; however, citotoxicity, potential carcinogenicity and the moderate effects obtained have limited their clinical use (Domenica Cappellini et al., 2000).

The ability to induce foetal haemoglobin was investigated using several molecules, including hematopoietic growth factors and cytokines. For instance, the effects of erythropoietin and interleukin-3 (IL-3) (Breymann et al., 1999 and Reinhardt et al., 2001), as well as the effects of interferon-γ (INF-γ) (Miller et al., 1990) were reported for treatment of the anaemia. In addition, interleukin-4, interleukin-8 and interleukin-18 were found involved with γ-globin gene expression (Kato et al., 2004).

The effects of butyric acid have been investigated since long time in the K562 cell line demonstrating its ability to induce the expression of embryonic globin genes (Cioè et al., 1981). The activity of sodium butyrate and α-amino-n-butyric acid (ABA) to enhance γ-globin synthesis *in vitro* in erythroid progenitors of patients with sickle cell anaemia and β-thalassaemia, suggested these molecules as relevant for therapy (Perrine et al., 1989). However, there are evidences that the activity of butyric acid and some related compounds can be associated with neurologic toxicity. Therefore, in adult primates, several studies were performed to define the doses able to

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induce foetal haemoglobin production at lower concentrations and toxic conditions. In addition, the efficacy and toxicity of butyric acid in the presence of other inducers were evaluated (Blau et al., 1993).

In the pharmaceutical field of investigation, butyrates represent a prodrug both to develop less cytotoxic compounds (Faller et al., 1995) and increase aqueous solubility for potential clinical applications (Nudelman et al., 2001). For instance, a novel family of butyric acid (acyloxyalkyl analogues) has been synthetised to increase potency ameliorating transport to the cells (Raphaeli et al., 2000). Among the most studied foetal haemoglobin inducers, histone deacetylase inhibitors (*i.e.* trichostatin A, arginine butyrate, sodium butyrate, sodium phenylbutyrate) were found to be active *in vitro* using established cell lines as well as primary erythroid cultures (Glauber et al., 1991, Fibach et al., 1993, Berkovitch-Luria et al., 2012, Muralidhar et al., 2011 and Marianna et al., 2001). The interest in applied biomedicine of butyrates is demonstrated by the several patent applications (few examples of this translational activity are US4822821A, US5645852A, EP0627220A1 and the more recent US8618068).

Several studies report *in vivo* experiments using both mouse (Pace et al., 1996, Partington et al., 1984 and Perrine et al., 1988), both primates and humans (Constantoulakis et al., 1989a, Lavelle et al., 1993, Constantoulakis et al., 1988, Fucharoen et al., 2013a and Kutlar et al., 2013) models demonstrating the expected clinical effects of this class of foetal haemoglobin inducers. The activity of sodium butyrate and sodium phenylbutyrate and analogues exhibiting similar mechanism of action have been evaluated in clinical trials (Dover et al., 1994, Sher et al., 1995, Collins et al., 1995, Perrine et al., 2011 and Patthamalai et al., 2014) demonstrating induction of foetal haemoglobin in some patients with thalassaemia. A further example in the phase II study made by Domenica Cappellini et al., 2000 made on patients with thalassaemia intermedia treated with oral isobutyramide to evaluate the ability of this butyric acid analogue to stimulate foetal haemoglobin production.

In conclusion, it is well established that the γ-globin gene modulation by butyrate leads to clinically beneficial increasing the endogenous content of foetal haemoglobin. These effects are based on the inhibition of histone deacetylases, causing an accumulation of acetylated histone species in a variety of vertebrate cell lines and hyperacetylation of H3 and H4 (Candido et al., 1978), accompanied with deep modification of the chromatin structure and transcriptional events (Gabbianelli et al., 2000, Gul et al., 2009, Turner and O'Neill, 1995 and Turner, 1991).

Since the effects of butyrates have been investigates on K562 cells and on primary erythroid progenitor cells (Cioè et al., 1981, McCaffrey et al., 1997), the *in vitro* cultures of erythroid precursors represent, in the first step, a model to study haemoglobin production under physiological conditions (Travers et al., 2002; Fibach et al., 2006; Gambari and Fibach, 2007).

The object of our investigation are some analogues presenting chemical structures derivate from a compound described in the U.S. Patent number 5,700,640 (23rd December 1997) (Voss and Caron, 1997) and described in a lot of chemical modification in Fig. 1A.

2. Materials and methods

2.1. Compounds employed in the study

The chemical structure of the compound 5049 (Ethyl 4,4,4-trifluoro-butyrate) described in the U.S. Patent number 5,700,640 (23rd December 1997) (Voss and Caron, 1997) and the modified analogues were synthetised by Chiesi Farmaceutici SpA (Parma, Italy) and reported in Fig. 1B. These small molecules are similar to butyric

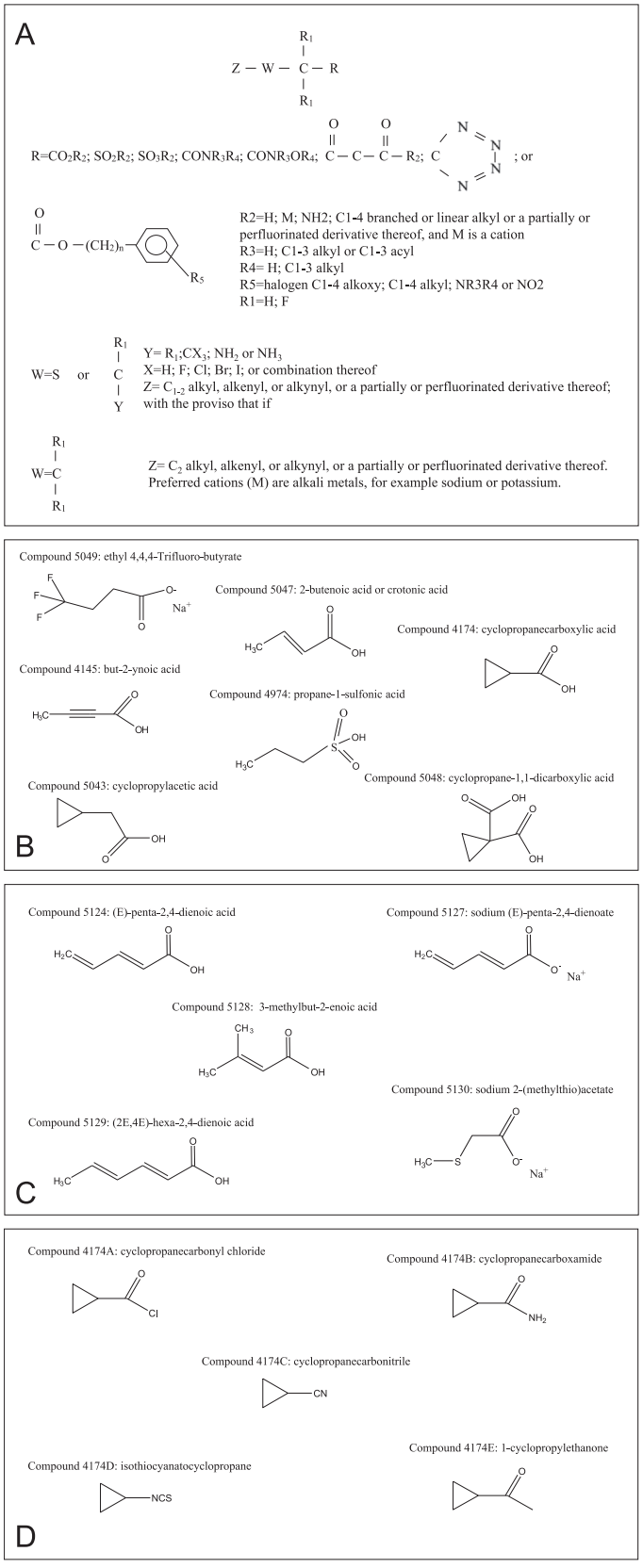

Fig. 1. (A) General formula of the compounds described in U.S. Patent number 5,700,640 (23rd December, 1997 by Voss and Caron). (B–D) Chemical structure of the compound 5049 (ethyl 4,4,4-trifluoro-butyrate) and the modified analogues 5047, 4174, 4145, 4974, 5043, 5048 (B), of the compounds 5124, 5127, 5128, 5129 and 5130 (C) and of the 4174A, 4174B, 4174C, 4174D and 4174E derivatives (D).