Biological Evaluation of a Mitochondrial Phosphoenolpyruvate Carboxykinase Inhibitor †

Sergio Rodríguez-Arévalo 1*, Sònia Abás 1, Marc Aragó 2, Juan Moreno-Felici 2, Petra Hyroššová 2, Jose Carlos Perales 2, Carles Galdeano 3, Tiziana Ginex 4, Francisco Javier Luque 4 and Carmen Escolano 1

1 Laboratory of Medicinal Chemistry (Associated unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, Institute of Biomedicine (IBUB), University of Barcelona, Barcelona 08028, Spain; soniaabas88@gmail.com (S.A.); cescolano@ub.edu (C.E.)
2 Department of Physiological Sciences, Faculty of Medicine, University of Barcelona, Barcelona 08907, Spain; marc.arago@gmail.com (M.A.); juanmorenofelici@gmail.com (J.M.-F.); petra.hyrossova@gmail.com (P.H.); jcperales@outlook.com (J.C.P.)
3 Department of Pharmacy, Pharmaceutical Technology and Physical Chemistry, Faculty of Pharmacy and Food Sciences, Institute of Biomedicine of the University of Barcelona (IBUB), University of Barcelona, Barcelona 08028, Spain; carlesgaldeano@gmail.com
4 Departament de Nutrició, Ciències de l’Alimentació i Gastronomia, Faculty of Pharmacy, Institute of Biomedicine (IBUB), University of Barcelona, Barcelona 08921, Spain; fjluque@ub.edu (F.J.L.); tiziana.ginex@gmail.com (T.G.)
* Correspondence: sergio6_6@hotmail.com

Published: 10 September 2019

Keywords: PEPCK; mitochondria; CETSA; GSIS

Phosphoenolpyruvate carboxykinase (PEPCK) is a key enzyme in gluconeogenesis, catalyzing the decarboxylation of oxaloacetate to phosphoenolpyruvate. In eukaryotes, there are two isozymes present either in the cytosol (PEPCK-C, PCK1) or in the mitochondria (PEPCK-M, PCK2). PCK2 is highly expressed in pancreatic β-cells, where it contributes to the regulation of the TCA cycle flux by coupling it to mitochondria GDP recycling. This flux has been shown to regulate glucose stimulated insulin secretion (GSIS) [1].

In order to obtain high purity and efficacy compounds, we synthetized, through lineal synthesis, a group of C-8 modified 3-alkyl-1,8-dibenzylxanthines, starting from 6-aminouracil. These compounds were described as potent PEPCK-C inhibitors by Roche [2]. Structural and docking analysis of both PEPCK isoforms showed that both enzymes are structurally very close and might be inhibited by the same family of inhibitors.

The lead inhibitor (INH-2) was compared with 3-mecaptopicolinic acid, a classic PCK1 inhibitor, in a kinetic assay, and the results confirmed the cross-inhibitory capacity between PCK1/PCK2 and their increased potency as inhibitors. Moreover, we studied PCK2 target engagement of our candidates through cellular thermal shift assays (CETSA).

INH-2 successfully inhibits GSIS in INS-1 cell line through PEPCK-M inhibition, validating the cross-inhibition between PEPCK-C and PEPCK-M. Furthermore, this compound inhibited insulin secretion In vivo, on an impaired glucose tolerance test (IGTT) in mice.
Reference


© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).