Neuropharmacology xxx (2014) 1–14

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Novel oxazolo-oxazole derivatives of FTY720 reduce endothelial cell permeability, immune cell chemotaxis and symptoms of experimental autoimmune encephalomyelitis in mice

Faik Imeri [a], Daniel Fallegger [a], Aleksandra Zivkovic [b], Stephanie Schwalm [c], Gaby Enzmann [d], Kira Blankenbach [c], Dagmar Meyer zu Heringdorf [c], Thomas Homann [e], Burkhard Kleuser [e], Josef Pfeilschifter [c], Britta Engelhardt [d], Holger Stark [b, f], Andrea Huwiler [a, c, *]

[a] Institute of Pharmacology, University of Bern, Friedbühlstrasse 49, CH-3011 Bern, Switzerland
[b] Institute of Pharmaceutical Chemistry, Goethe University Frankfurt am Main, Max-von-Laue-Str. 9, D-60438 Frankfurt am Main, Germany
[c] Pharmazentrum Frankfurt/ZAFES, University Hospital, Goethe University Frankfurt am Main, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany
[d] Theodor-Kocher Institute, University of Bern, Freiestrasse 1, CH-3012 Bern, Switzerland
[e] Institute of Nutritional Science, University of Potsdam, Arthur-Scheunert Allee 114–116, D-14558 Nuthetal, Germany
[f] Institute of Pharmaceutical and Medicinal Chemistry, Heinrich-Heine-University Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany

ARTICLE INFO

Article history:
Received 24 November 2013
Received in revised form
15 April 2014
Accepted 13 May 2014
Available online xxx

Keywords:
Fingolimod
St-968
St-1071
Sphingosine 1-phosphate
Endothelial cells
Permeability
Multiple sclerosis

ABSTRACT

The immunomodulatory FTY720 (fingolimod) is presently approved for the treatment of relapsing-remitting multiple sclerosis. It is a prodrug that acts by modulating sphingosine 1-phosphate (S1P) receptor signaling. In this study, we have developed and characterized two novel oxazolo-oxazole derivatives of FTY720, ST-968 and the oxy analog ST-1071, which according to molecular modeling studies require no preceding phosphorylation for activation and in vivo action. These novel compounds proved to be active in intact cells and triggered $S1P_1$ and $S1P_3$, but not $S1P_2$, receptor internalization as a result of receptor activation. In the human endothelial cell line EA.hy 926, they stimulated the activation of p42/p44-MAPKs which involved a $G_{o/i}$ protein and the $S1P_1$ and $S1P_3$ receptor subtypes.

Functionally, ST-968 and ST-1071 acted similar to FTY720 to abrogate S1P-triggered chemotaxis of mouse splenocytes, mouse T cells and human U937 cells. Additionally, endothelial cell permeability enhanced by TNFα and LPS was normalized in a dose-dependent manner by either S1P, FTY720, ST-968 or ST-1071. All compounds also reduced TNFα-induced ICAM-1 and VCAM-1 mRNA expression, but restored TNFα-mediated downregulation of PECAM-1 mRNA expression.

In an in vivo setting, the application of ST-968 or ST-1071 to mice resulted in a reduction of blood lymphocytes and significantly reduced the clinical symptoms of experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice comparable to FTY720 either by prophylactic or therapeutic treatment. In parallel to the reduced clinical symptoms, infiltration of immune cells in the brain was strongly reduced, and in isolated tissues of brain and spinal cord, the mRNA and protein expressions of ICAM-1 and VCAM-1, as well as of matrix metalloproteinase-9 were reduced by all compounds, whereas PECAM-1 and the tissue inhibitor of metalloproteinase TIMP-1 were upregulated.

In summary, the data suggest that these novel butterfly derivatives of FTY720 could have considerable implication for future therapies of multiple sclerosis and other autoimmune diseases.

Abbreviations used: AUC, area under the curve; BBB, blood brain barrier; BSA, bovine serum albumin; CHO, Chinese hamster ovary; DMEM, Dulbecco's modified Eagle medium; DMSO, dimethylsulfoxide; EAE, experimental autoimmune-induced encephalomyelitis; GPCR, G protein-coupled receptor; ICAM-1, intercellular adhesion molecule-1; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MMP-9, matrix metalloproteinase-9; MOG, myelin oligodendrocyte glycoprotein; PBS, phosphate-buffered saline; PECAM-1, platelet endothelial cell adhesion molecule-1; S1P, sphingosine 1-phosphate; SBE, Smad-binding element; TER, transendothelial resistance; TIE, TGFβ-inhibitory element; TIMP-1, tissue inhibitor of metalloproteinase-1; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.

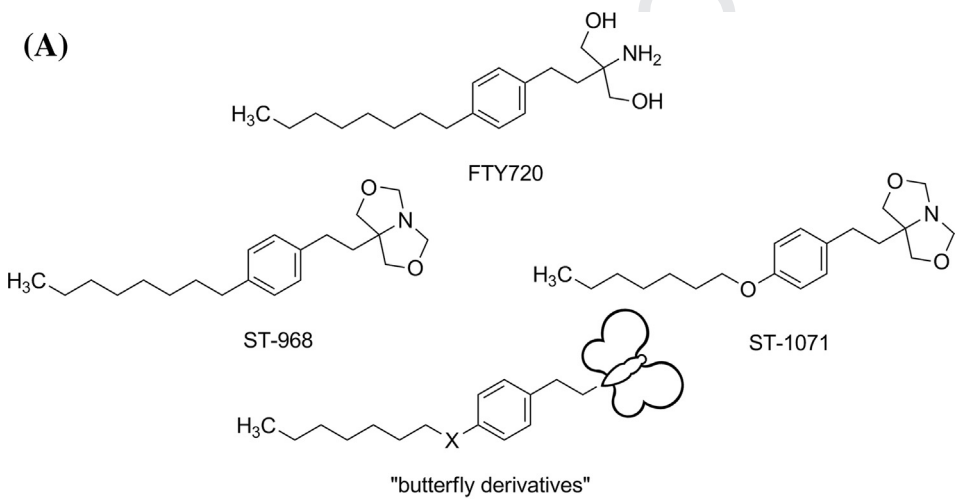
* Corresponding author. Institute of Pharmacology, University of Bern, Friedbühlstrasse 49, CH-3010 Bern, Switzerland. Tel.: +41 316323214; fax: +41 316324992.
E-mail address: Huwiler@pki.unibe.ch (A. Huwiler).

http://dx.doi.org/10.1016/j.neuropharm.2014.05.012
0028-3908/

1. Introduction

During the last decade it has become clear that certain sphingolipid subspecies exert important functions either as intracellular signaling molecules or as extracellular ligands to cell surface receptors. Especially sphingosine 1-phosphate (S1P) has attracted a lot of interest due to the existence of five different high affinity S1P receptors denoted $S1P_{1-5}$ (Sanchez and Hla, 2004; Brinkmann, 2007; Chun et al., 2010). These receptors belong to the superfamily of G protein-coupled receptors (GPCR) which are ubiquitously expressed and couple to diverse sets of signaling cascades (Brinkmann, 2007; Chun et al., 2010). This diversity of S1P receptors implicates a multitude of physiological and pathophysiological functions of extracellular S1P including the promotion of cell growth and survival, cell migration and the modulation of inflammatory reactions (Alvarez et al., 2007; Schuchardt et al., 2011; Maceyka et al., 2012). Consequently, the targeting of S1P signaling and the development of pharmacological S1P receptor agonists or antagonists has become a key interest for scientists involved in drug research (Huwiler and Zangemeister-Wittke, 2007; Huwiler and Pfeilschifter, 2008, 2009).

Recently, the immunomodulatory drug FTY720 (fingolimod, Gilenya^{R}) was launched for the treatment of multiple sclerosis (Brinkmann et al., 2010; Mehling et al., 2011). This compound is a prodrug that needs to be phosphorylated by sphingosine kinase-2 to the active form, which in turn acts as an unselective agonist at four of the five S1P receptor subtypes (Zemann et al., 2006; Chun and Brinkmann, 2011). Besides this agonistic effect, FTY720-



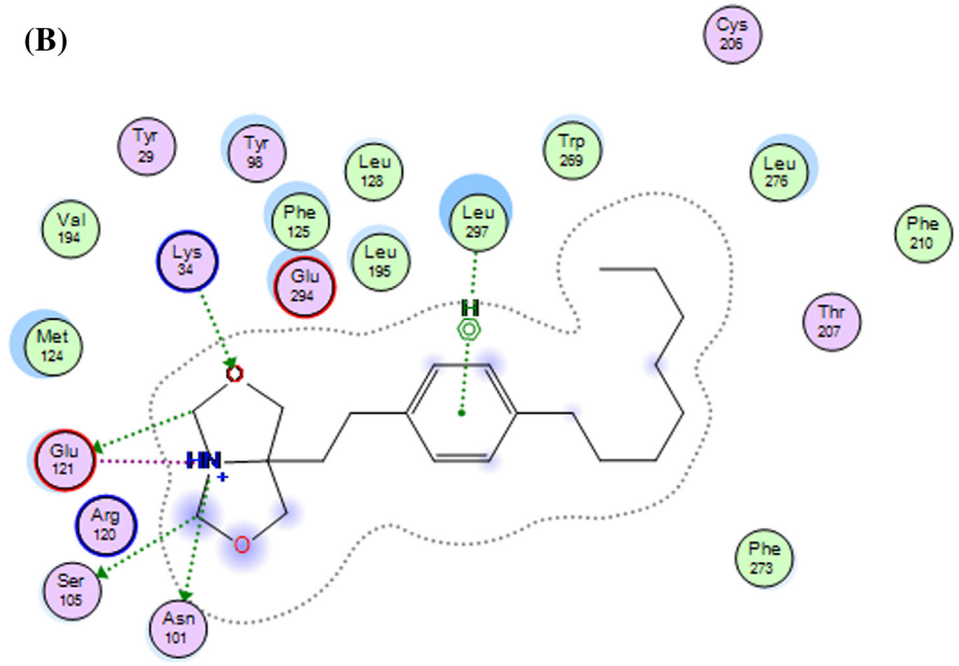


Fig. 1. Chemical structures of FTY720 (fingolimod), and the butterfly analogs ST-968 and ST-1071 and schematic docking model and interactions of ST-968 in the binding pocket of $S1P_1$.A:Chemical structures of ST-968 (7a-(4-octylphenethyl)tetrahydro-1H-oxazolo[3,4-*c*]oxazole), ST-1071 (7a-(4-(heptyloxy)phenethyl)tetrahydro-1H-oxazolo[3,4-*c*]oxazole), and FTY720; (B and C): Schematic representation of the interaction between ST-968 (B) and ST-1071 (C) and the surrounding amino acids in the binding pocket of $S1P_1$. The involved binding site residues are shown as follows: polar residues in pink, hydrophobic residues in green, acidic residues with a red contour ring, basic residues with a blue contour ring. Green arrows indicate hydrogen bonding to side chain atoms. Blue clouds on ligand atoms indicate the solvent exposed surface area of ligand atoms. Light blue halos around residues indicate the degree of interaction with ligand atom (larger and darker halos means more interaction). D: Docking of ST-968 into the ligand binding pocket of $S1P_1$ in a 3D model.

Please cite this article in press as: Imeri, F., et al., Novel oxazolo-oxazole derivatives of FTY720 reduce endothelial cell permeability, immune cell chemotaxis and symptoms of experimental autoimmune encephalomyelitis in mice, Neuropharmacology (2014), http://dx.doi.org/10.1016/j.neuropharm.2014.05.012