European Journal of Medicinal Chemistry 123 (2016) 905–915

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Studies on the inhibition of sphingosine-1-phosphate lyase by stabilized reaction intermediates and stereodefined azido phosphates

Pol Sanllehí [a, c], José-Luís Abad [a], Jordi Bujons [b], Josefina Casas [a, **], Antonio Delgado [a, c, *]

[a] Spanish National Research Council (CSIC), Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Research Unit on Bioactive Molecules (RUBAM), Department of Biomedicinal Chemistry, Jordi Girona 18-26, 08034, Barcelona, Spain
[b] Spanish National Research Council (CSIC), Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Department of Biological Chemistry and Molecular Modelling, Jordi Girona 18-26, 08034, Barcelona, Spain
[c] University of Barcelona (UB), Faculty of Pharmacy, Department of Pharmacology, Toxicology and Medicinal Chemistry, Unit of Pharmaceutical Chemistry (Associated Unit to CSIC), Avda. Joan XXIII s/n, 08028, Barcelona, Spain

ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form
4 August 2016
Accepted 5 August 2016
Available online 6 August 2016

Keywords:
Sphingolipids
Enzyme inhibitor
Sphingosine lyase
Synthesis
Stereocontrolled

ABSTRACT

Two kinds of inhibitors of the PLP-dependent enzyme sphingosine-1-phosphate lyase have been designed and tested on the bacterial (StS1PL) and the human (hS1PL) enzymes. Amino phosphates **1**, **12**, and **32**, mimicking the intermediate aldimines of the catalytic process, were weak inhibitors on both enzyme sources. On the other hand, a series of stereodefined azido phosphates, resulting from the replacement of the amino group of the natural substrates with an azido group, afforded competitive inhibitors in the low micromolar range on both enzyme sources. This similar behavior represents an experimental evidence of the reported structural similarities for both enzymes at their active site level. Interestingly, the *anti*-isomers of the non-natural enantiomeric series where the most potent inhibitors on hS1PL.

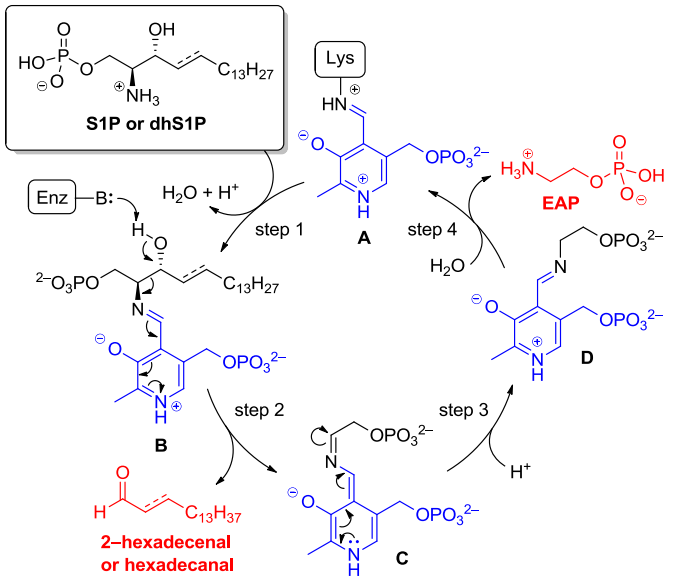
1. Introduction

Phosphorylated sphingolipids are an important group of sphingoid metabolites that present a terminal phosphate group. Among them, sphingosine-1-phosphate (S1P) is a well-recognized signaling molecule that regulates cell differentiation, survival, inflammation, angiogenesis, calcium homeostasis and immunity, among other functions [1]. Recent findings have associated high S1P levels with neuroprotective effects that counteract many noxious processes that follow CNS injury [2,3] (apoptotic cell death, lipid hydrolysis, oxidative stress and tissue damage), while supporting growth and trophic factor activities. In general, the roles of phosphorylated sphingolipids are opposed to those of ceramides (Cer), which are potent inducers of cell cycle arrest and apoptosis [4]. Thus, the control of the S1P/Cer balance might be exploitable for the discovery of potential neuro-restorative therapies with applications in traumatic brain injury, spinal cord injury, and stroke. These tissue protective properties make S1P a suitable cellular survival signaling agent, in contraposition to Cer, which is a well-recognized mediator of cellular stress responses. In addition to the intracellular functions, paracrine functions of S1P through its binding to specific lipid G-protein coupled $S1P_{1-5}$ receptors are also known. In this way, S1P becomes essential for vascular development and endothelial integrity, control of cardiac rhythm, and immunity responses [5–10].

Sphingosine-1-phosphate lyase (S1PL) is a pyridoxal 5′-phosphate (PLP) dependent enzyme, localized in the endoplasmic reticulum (ER), that catalyzes the irreversible degradation of S1P into ethanolamine phosphate (EAP) and *trans*-2-hexadecenal (Scheme 1) [11]. According to the proposed mechanism of cleavage [11], the incoming S1P replaces the catalytic Lys in internal aldimine **A** (step 1, Scheme 1) to form the external aldimine **B**. Retro-aldol cleavage takes place at the C3–OH group of S1P (step 2) to release the corresponding aldehyde. Reprotonation of the transient quinonoid intermediate **C** (step 3) leads to external aldimine **D**, from which EAP is released after hydrolysis (step 4) and the internal aldimine **A** is formed again for a second enzyme turnover. An

* Corresponding author. Spanish National Research Council (CSIC), Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Research Unit on Bioactive Molecules (RUBAM), Department of Biomedicinal Chemistry, Jordi Girona 18-26, 08034, Barcelona, Spain.
** Corresponding author.
E-mail address: delgado@rubam.net (A. Delgado).

http://dx.doi.org/10.1016/j.ejmech.2016.08.008
0223-5234/



Scheme 1. Catalytic cycle of sphingosine-1-phosphate lyase (S1PL). 1) S1P or dhS1P replaces Lys from **A** to form the external aldimine **B**; 2) Retro-aldol cleavage with generation of 2-hexadecenal or hexadecanal and the quinonoid intermediate **C**; 3) Formation of the aldimine system **D** by protonation of **C**; 4) Hydrolysis of **D** with release of EAP and final restoration of **A**.

identical mechanism is accepted for the alternative S1PL substrate 4,5-dihydro-S1P (dhS1P), giving rise to EAP and hexadecanal as reaction products. The degradation of S1P by S1PL has been considered as the "exit gate" of sphingolipid metabolism and the natural connection with phospholipid metabolism through the conversion of EAP into phosphatidylethanolamine, another signaling molecule in lipid metabolism [12]. In combination with S1P phosphatase and sphingosine kinases (SK) [13], the levels of intracellular S1P can be efficiently regulated.

So far, the repertoire of available S1PL inhibitors structurally related to the enzyme substrate is scarce. In this context, 1-deoxysphinganine 1-phosphonate [14] and the 2-vinyl dihydrosphingosine 1-phosphate (2-vinyl dhS1P) (Fig. 1) are the only ones reported in the literature [15]. The structurally related **FTY720**, a well-known S1P receptor agonist after the enantioselective phosphorylation to the (*S*) isomer, has also been reported as a modest S1PL inhibitor *in vitro* [16,17]. On the other hand, the PLP antagonist 4-deoxypyridoxine (DOP) has been described as a functional S1PL inhibitor *in vivo* [18–20], as well as THI and related analogs [20,21], whose mechanism of action is controversial [22–24]. Finally, some heterocyclic compounds resulting from massive HTS programs have been discovered as potent and selective S1PL inhibitors [18–20,25], some of them with IC_{50} values in the nM range [26,27].

As a result of our interest in the development of sphingolipid modulators by targeting S1P metabolism [28], we undertook the design of new S1PL inhibitors based on S1PL mechanistic considerations. Thus, compounds **12** and **32** (Fig. 2) were designed as non-reactive analogs of the intermediate aldimine **B** (Scheme 1), whereas compound **1** (Fig. 2) can be regarded as a non-hydrolyzable analog of the putative intermediate **D** (Scheme 1). Interestingly, dephosphorylated **32** had been reported in the literature as a potential S1PL inhibitor. However, its lack of activity in cells was attributed to an inadequate phosphorylation *in vivo* [20].

As PLP analogs, compounds **1**, **12**, and **32** were expected to displace the cofactor in the enzyme active site. In a second approach, a series of configurationally defined azide analogs of both enzyme substrates (S1P and dhS1P) were designed as non-reactive, potential S1PL competitive inhibitors (compounds **89**, **98**, **103**, and **114**, Fig. 2). Since the C2-amino group in S1P is replaced by an azido group, formation of the external aldimine **B** is expected to be precluded at the enzyme active site. Apart from the inherent lack of reactivity towards imine formation and its remarkable stability *in cellulo* [29], the azido group has also been used as a "pseudohalide" in drug design [30]. In our case, the replacement of the amino group present in S1P with an azido group is expected to cause a dramatic effect in the electronic properties and on the interactions with the enzyme active site, without disturbing the overall shape of the molecule. Moreover, to gain insight into the enzyme stereoselectivity towards this type of analogs, all the configurations around the C2 and C3 carbon atoms have been considered (ie. compounds ***ent*-89**, ***ent*-98**, ***ent*-103** and ***ent*-114**).

2. Results and discussion

2.1. Synthesis

Compounds **1**, **12** and **32** were obtained as outlined in Scheme 2 by reductive amination of PLP with either *O*-phosphorylethanolamine, dhS1P or S1P, respectively. Following an adaptation of reported protocols [31,32], the corresponding dipotassium salts were generated *in situ* with KO*t*-Bu in order to ensure their total solubility. After the consumption of the starting amine was evidenced by ^{1}H NMR, reduction of the corresponding imines with $NaBH_4$ afforded the desired diphosphates in acceptable overall yields, together with pyridoxine-5′-phosphate, which was also included in our S1PL inhibition assays due to its high structural similarity with PLP and DOP. On the other hand, crude diphosphates **12** and **32** presented a very low solubility in different organic solvents and in aqueous mixtures. Nevertheless, preparation of the corresponding triethylammonium salts afforded highly water-soluble species, which could be properly characterized in terms of chemical identity and biological activity.

The synthesis of the differently configured azidophosphates was carried out from the corresponding stereodefined sphingosines, whose synthesis from D- or L-Garner's aldehyde is depicted in Scheme 3. For the 2*S* series, the *anti*-adducts resulted from the diastereoselective addition of vinylmagnesium bromide to the starting aldehyde, following a well-established reported protocol in which the *anti*-configuration of the amino diol moiety was secured by chemical correlation with a stereodefined reference compound [33]. Cross-metathesis of **23** with 1-pentadecene, in the presence of Grubbs catalyst (second generation) [34,35], followed by isopropylidene removal afforded the key sphingoid intermediate **25**, whose configuration was in agreement with the optical rotation data reported for this compound in the literature [36]. On the other

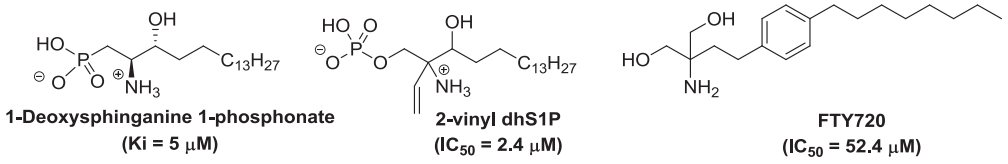


Fig. 1. Reported S1PL inhibitors structurally related with the enzyme substrate.