European Journal of Medicinal Chemistry 148 (2018) 210–220

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Cyclic peptide inhibitors of lysine-specific demethylase 1 with improved potency identified by alanine scanning mutagenesis

Isuru R. Kumarasinghe, Patrick M. Woster*

Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, 70 President St., Charleston, SC 29425, United States

ARTICLE INFO

Article history:
Received 7 December 2017
Received in revised form
29 January 2018
Accepted 31 January 2018
Available online 7 February 2018

Keywords:
Alanine scanning
Chromatin remodeling
Lysine-specific demethylase 1
Cyclic peptide
Epigenetic modulator
Histone demethylation

ABSTRACT

Lysine-specific demethylase 1 (LSD1) is a chromatin-remodeling enzyme that plays an important role in cancer. Over-expression of LSD1 decreases methylation at histone 3 lysine 4, and aberrantly silences tumor suppressor genes. Inhibitors of LSD1 have been designed as chemical probes and potential anti-tumor agents. We recently reported the cyclic peptide **9**, which potently and reversibly inhibits LSD1 (IC_{50} 2.1 μM; K_i 385 nM). Systematic alanine mutagenesis of **9** revealed residues that are critical for LSD1 inhibition, and these mutated peptides were evaluated as LSD1 inhibitors. Alanine substitution at positions 2, 3, 4, 6 and 11−17 preserved inhibition, while substitution of alanine at positions 8 and 9 resulted in complete loss of activity. Cyclic mutant peptides **11** and **16** produced the greatest LSD1 inhibition, and **11**, **16**, **27** and **28** increased global H3K4me2 in K562 cells. In addition, **16**, **27** and **28** promoted significant increases in H3K4me2 levels at the promoter sites of the genes IGFBP2 and FEZ1. Data from these LSD1 inhibitors will aid in the design of peptidomimetics with improved stability and pharmacokinetics.

© 2018 Elsevier Masson SAS. All rights reserved.

1. Introduction

Lysine and arginine residues on nucleosomal histone protein tails undergo reversible mono-, di- and trimethylation that serves to regulate gene expression. Unlike histone acetylation, which activates gene transcription, histone methylation can either activate or silence gene expression, depending on the specific chromatin mark involved. The primary function of the flavin-dependent amine oxidase lysine-specific demethylase 1, (LSD1, also known KDM1A) is to remove methyl groups from the activating chromatin marks monomethyl histone 3 lysine 4 (H3K4me2) and dimethyl histone 3 lysine 4 (H3K4me2). LSD1 is also known to demethylate histone 3 lysine 9 (H3K9) when co-localized with the androgen receptor in prostate tumors [3], and demethylates non-histone protein substrates such as p53 and deoxynucleic acid methyltransferase 1 (Dnmt1) [5]. Over-expression of LSD1 has been observed in a variety of tumor cell lines, and promotes the aberrant silencing of tumor suppressor genes. Thus LSD1 is regarded as an attractive target for therapeutic intervention. Effective LSD1 inhibitors have been described (Fig. 1), including tranylcypromine-based irreversible inhibitors such as GSK2879552 (**1**) [6] and ORY-1001 (**2**) [7–9], oligoamines such as verlindamycin **3** [10–13] and related isosteric ureas and thioureas [13,14], reversible benzohydrazide inhibitors such as SP-2509 (**4**) [9], reversible 1,2,4-triazoles such as **5** [15], dithiocarbamate-urea hybrid LSD1 inactivators related to **6** [16] and peptide based LSD1 inhibitors such as **7** [17–20]. Compounds **1**, **2** and **4** are currently the subjects of human clinical trials.

Forneris et al. described a 21-mer peptide analogous to the histone 3 lysine 4 substrate region of LSD1, wherein Lys4 was replaced by a methionine (compound **8**, Fig. 2) [4]. This linear peptide was a potent inhibitor of recombinant LSD1 with a K_i value of 0.04 μM, and inhibited LSD1 bound to CoREST with a K_i value of 0.05 μM [4]. The X-ray conformation of **8** bound to LSD1/CoREST (PDB ID: 2V1D) reveals that the side chains of some amino acid residues in **8** (Arg2 and Gln5; Arg2 and Ser10; Arg2 and Gly12; Arg2 and Lys14; Gln5 and Ser10) are in close proximity to each other in three-dimensional space when it is bound to the catalytic pocket. In order to mimic the bound conformation of **8**, we replaced these amino acids with Lys and Glu residues and made a series of cyclic peptides containing a lactam bridge [1]. The most active LSD1 inhibitor in this series, compound **9** (Fig. 2A), exhibited an IC_{50} value of 2.1 μM and a Ki of 385 nM against purified recombinant LSD1/CoREST. The global least energy conformation of **9** obtained using

* Corresponding author.
E-mail address: woster@musc.edu (P.M. Woster).

https://doi.org/10.1016/j.ejmech.2018.01.098
0223-5234/© 2018 Elsevier Masson SAS. All rights reserved.

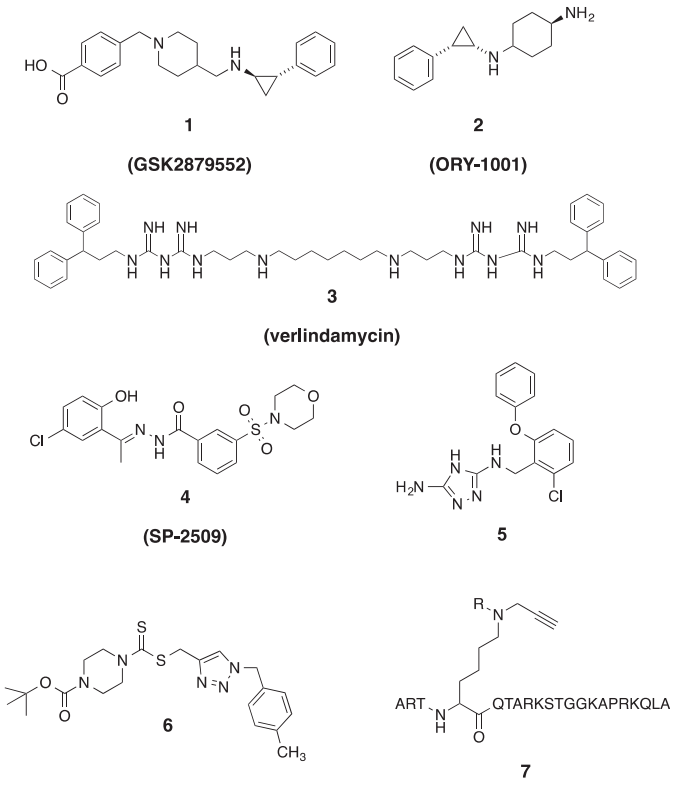

Fig. 1. Chemotypes of known reversible and irreversible LSD1 inhibitors.

the MacroModel Monte Carlo Multiple Minimum (MCMM) search algorithm [21,22] features a right-handed alpha helical section and a beta sheet section, and assumes very similar backbone and local side chain conformations to **8** (Fig. 2B). This similarity in the least energy conformations of **8** and **9** could explain their similar ability to inhibit recombinant LSD1.

Alanine scanning mutagenesis is a powerful tool used to identify key amino acid residues in a peptide that are important for biological activity. We thus completed systematic alanine mutagenesis involving residues 2–4, 6, 8–9, 11–14 and 16 of the cyclic peptide LSD1 inhibitor **9** to identify those residues in the ligand important for LSD1 inhibition.

2. Materials and methods

2.1. Synthesis

All reagents and dry solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI), Sigma Chemical Co. (St. Louis, MO), VWR (Radnor, PA) or Fisher Scientific (Chicago, IL) and were used without further purification except as noted below. Dry methanol, ethyl acetate, tetrahydrofuran, dimethyl formamide and hexane were prepared using a Glass Contour Solvent Purification System (Pure Process Technology, LLC, Nashua, NH). Routine chromatographic purification on reversed phase silica gel and preparative scale chromatographic procedures were carried out using a Teledyne Isco CombiFlash Rf200 chromatography system (Teledyne-Isco, Lincoln, NE) fitted with silica gel 60 cartridges (230–440 mesh). Thin layer chromatography was conducted on Merck precoated silica gel 60 F-254.

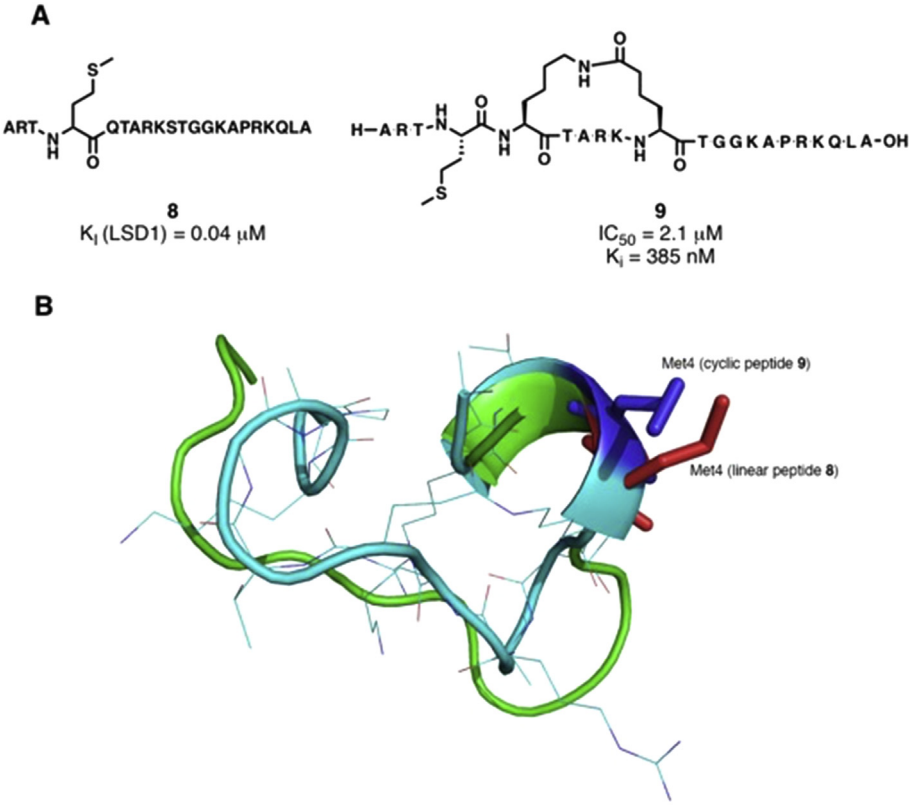

Fig. 2. A. Structures of the linear peptide LSD1 inhibitor **8** and the cyclic peptide LSD1 inhibitor **9**. B. Overlay of the least-energy conformations of **8** and **9**.

Download English Version:

https://daneshyari.com/en/article/7796736

Download Persian Version:

https://daneshyari.com/article/7796736

Daneshyari.com