Bioorganic & Medicinal Chemistry 22 (2014) 1726–1735

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Reaction intermediate analogues as bisubstrate inhibitors of pantothenate synthetase

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ARTICLE INFO

Article history:
Received 18 October 2013
Revised 25 December 2013
Accepted 14 January 2014
Available online 23 January 2014

Keywords:
Pantothenate synthetase
Tuberculosis
Bisubstrate inhibitor
Adenylation
Coenzyme A

ABSTRACT

The biosynthesis of pantothenate, the core of coenzyme A (CoA), has been considered an attractive target for the development of antimicrobial agents since this pathway is essential in prokaryotes, but absent in mammals. Pantothenate synthetase, encoded by the gene *panC*, catalyzes the final condensation of pantoic acid with β-alanine to afford pantothenate via an intermediate pantoyl adenylate. We describe the synthesis and biochemical characterization of five PanC inhibitors that mimic the intermediate pantoyl adenylate. These inhibitors are competitive inhibitors with respect to pantoic acid and possess submicromolar to micromolar inhibition constants. The observed SAR is rationalized through molecular docking studies based on the reported co-crystal structure of **1a** with PanC. Finally, whole cell activity is assessed against wild-type *Mtb* as well as a PanC knockdown strain where PanC is depleted to less than 5% of wild-type levels.

1. Introduction

Tuberculosis (TB) caused by members of the *Mycobacterium tuberculosis* (*Mtb*) complex is an ancient scourge that remains the leading source of morbidity and mortality today with an estimated nine million new cases and 1.4 million deaths in 2011, primarily in the developing world.[1] The recommended therapy by the World Health Organization (WHO) for drug sensitive TB is known as directly observed treatment, short course (DOTS) and requires 6 months of treatment with isoniazid, rifampin, ethambutol, and pyrazinamide.[2] This extremely long duration of treatment is likely due to the ability of *Mtb* to switch its metabolism to a nonreplicating state,[3] the heterogeneous nature of the bacterial subpopulations residing in different lesions types,[3] and the lack of drug penetration into the site of infection.[4] In order to combat this global health threat, new drugs are needed to shorten the treatment duration and for drug resistant strains including multidrug-resistant (MDR) TB and extensively drug resistant (XDR) TB.[5,6]

Pantothenate, also known as vitamin B5 is a precursor to coenzyme A (CoA), an essential cofactor required in central and intermediary metabolism where it serves as an acyl group carrier and carbonyl activating group.[7,8] Bioinformatics analysis has identified the *de novo* biosynthetic pathway to pantothenate as an attractive target for the development of antimicrobial agents since this pathway is absent in mammals, but essential in prokaryotes.[9–11] Biosynthesis of pantothenate is accomplished by four enzymes encoded by the genes *panB*, *pancC*, *panD*, and *panE*. PanB and PanE are responsible for the synthesis of pantoic acid, while PanD, an aspartate α-decarboxylase, produces β-alanine. PanC (Rv3602c, pantothenate synthetase) then catalyzes the condensation of pantoic acid with β-alanine through a two-step adenylation–ligation reaction.[12] In the adenylation half-reaction, ATP and pantoic acid react to form a pantoyl-adenylate intermediate (Fig. 1A). Following the release of pyrophosphate, β-alanine binds and PanC catalyzes its ligation with the activated carbonyl of the pantoyl-adenylate to afford pantothenate. The detailed kinetic characterization of PanC from *Mtb* shows it uses a bi-uni-uni-bi ping pong kinetic mechanism with sequential ordered binding of ATP followed by pantoic acid and sequential ordered release of pantothenate followed by AMP (Fig. 1B).[12] The apparent K_M values for pantoic acid, ATP, and β-alanine are 0.13, 2.6, and 0.8 mM, respectively. PanC is a 33 kDa protein that exists as a homodimer in solution. The active site is located in the N-terminal domain (residues 1–186) while the C-terminal domain (residues 187–309) covers the active site.[13] Substrates must diffuse through a gate comprising residues 75–88, which are flexible and disordered, but becomes ordered upon formation of the pantoyl adenylate. The structures PanC from

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http://dx.doi.org/10.1016/j.bmc.2014.01.017

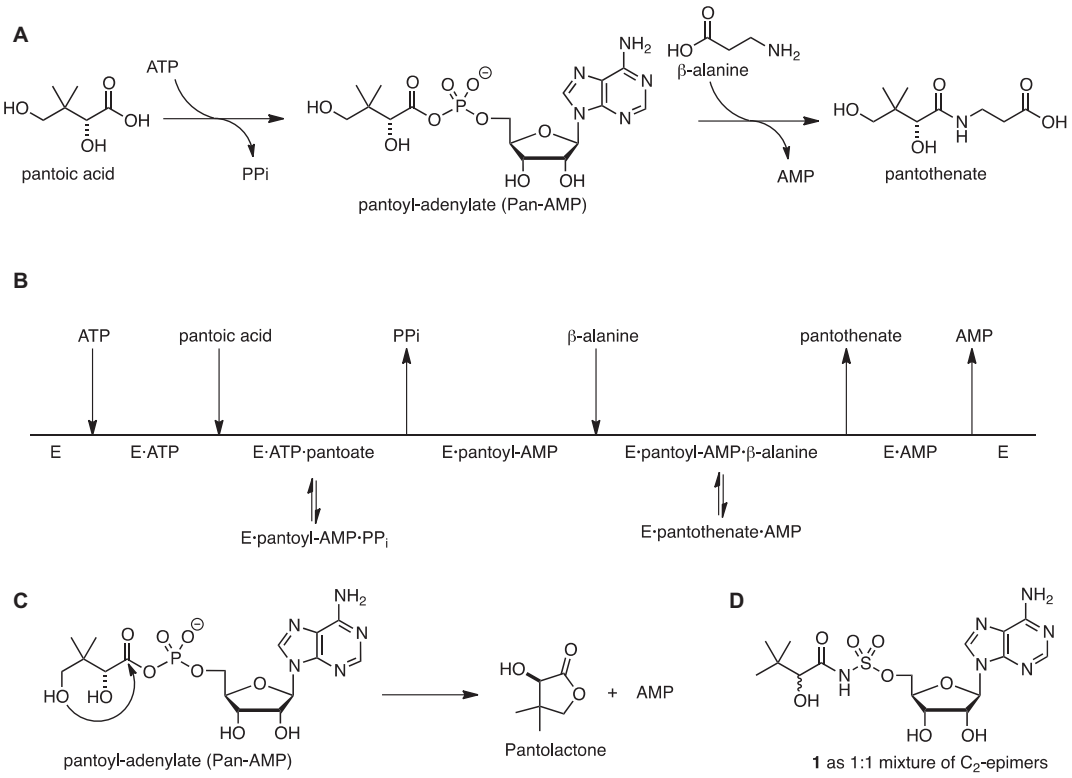
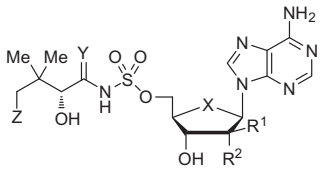


Figure 1. Pantothenate synthetase catalyzed reactions.

Mtb in complex with substrates, intermediates, and products have been solved providing a step-by-step view of the PanC reaction.[13,14]

Inhibitors of PanC have been identified by high-throughput screening,[15–17] fragment-based approaches,[18–20] dynamic combinatorial chemistry,[21] and through the rationale design of analogues of the pantoyl-adenylate intermediate.[22,23] The pantoyl-adenylate intermediate mimic **1**, which is epimeric at the C-2 position of the pantoyl fragment, reported by Ciulli and co-workers is the most potent inhibitor yet reported with a K_i of 0.22 μM with respect to ATP as the varied substrate at saturating concentrations of pantoic acid and β-alanine using a coupled biochemical assay that measures formation of AMP.[23] In this paper, we report the design, synthesis, and biochemical evaluation of five inhibitors with the C-2 stereocenter retaining the same configuration (i.e., *R*) as the reaction intermediate **1a** (Fig. 2). To prevent the intramolecular lactonization (Fig. 1C), the terminal C-4 hydroxyl of the pantoyl moiety was removed in **1a**–**4** while the pantoyl carbonyl group was deleted in **5**. All compounds were also evaluated against wild-type *Mtb* and a PanC depleted *Mtb* strain.



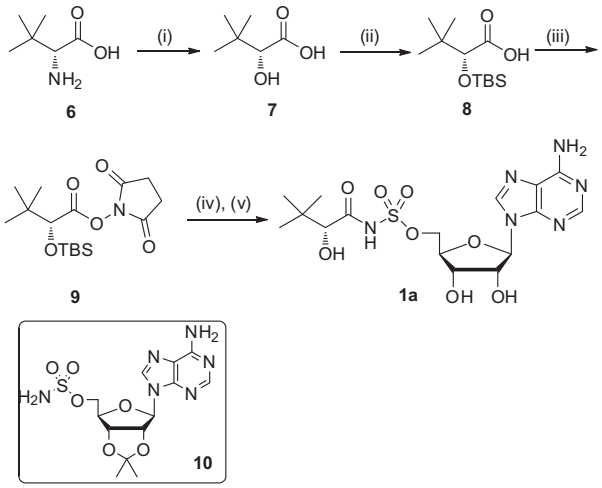
1a, R^1 = H, R^2 = OH, X = Y = O, Z = H
2, R^1 = OH, R^2 = H, X = Y = O, Z = H
3, R^1 = H, R^2 = F, X = Y = O, Z = H
4, R^1 = H, R^2 = OH, X = CH_2, Y = O, Z = H
5, R^1 = H, R^2 = OH, X = O, Y = H_2, Z = OH

Figure 2. Reaction intermediate analogues of pantoyl-adenylate.

2. Results and discussion

2.1. Chemistry

Synthesis of diastereomerically pure **1a** was achieved starting from commercially available (*R*)-2-amino-3,3-dimethylbutanoic acid **6** that was first converted to the corresponding α-hydroxy acid **7** with retention of configuration via an intermediate α-lactone (Scheme 1).[24] Protection of the resultant hydroxyl group as a TBS ether provided **8**, which was coupled with *N*-hydroxysuccinimide (NHS) using DCC to afford the common reaction intermediate **9**. The pantoyl-adenylate analogue **1a** was then prepared



Scheme 1. Reagents and conditions: (i) H_2SO_4, $NaNO_2$, H_2O, 0 °C → rt, 16 h, 81%; (ii) TBSCl, imidazole, DMF, 16 h, 81%; (iii) NHS, DCC, DME, 16 h, 60%; (iv) **10**, Cs_2CO_3, DMF, 24 h; (v) 4:1 TFA–H_2O, 8 °C, 48 h, 7% from **10**.