Bioorganic & Medicinal Chemistry Letters 24 (2014) 4689–4693

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Heterocyclic acyl-phosphate bioisostere-based inhibitors of *Staphylococcus aureus* biotin protein ligase

William Tieu [a,*,†], Angie M. Jarrad [b,†,§], Ashleigh S. Paparella [b], Kelly A. Keeling [a], Tatiana P. Soares da Costa [b,‡], John C. Wallace [b], Grant W. Booker [b], Steven W. Polyak [b], Andrew D. Abell [a,*]

[a] School of Chemistry and Physics, University of Adelaide, Adelaide, South Australia 5005, Australia
[b] School of Molecular and Biomedical Science, University of Adelaide, Adelaide, South Australia 5005, Australia

ARTICLE INFO

Article history:
Received 29 May 2014
Revised 7 August 2014
Accepted 11 August 2014
Available online 19 August 2014

Keywords:
Antibiotics
Bioisosteres
Inhibitors
Ligases
Drug design

ABSTRACT

Inhibitors of *Staphylococcus aureus* biotin protein ligase (*Sa*BPL) are generated by replacing the acyl phosphate group of biotinyl-5′-AMP with either a 1,2,3-triazole (see **5**/**10a**/**10b**) or a 1,2,4-oxadiazole (see **7**) bioisostere. Importantly, the inhibitors are inactive against the human BPL. The nature of the 5-substituent in the component benzoxazolone of the optimum 1,2,3-triazole series is critical to activity, where this group binds in the ATP binding pocket of the enzyme.

Biotin protein ligase (BPL) is an adenylate forming enzyme that catalyses the reaction of biotin and ATP to form an acyl AMP intermediate known as biotinyl-5′-AMP (**1**). This intermediate is then employed in the biotinylation and subsequent activation of acetyl CoA carboxylase; a key metabolic enzyme that is central to membrane biogenesis and, hence, the viability of all organisms.[1,2] Thus, the inhibition of BPL has been identified as a viable drug target for pathogens resistant to existing chemotherapies.[3–8] Recent efforts in this area have focused on developing mimics of biotinyl-5′-AMP **1**, where the reactive acyl phosphate group is replaced with a stable bioisosteres,[9–24] but to date, only a handful of acyl phosphate bioisosteres have been reported that mimic biotinyl-5'-AMP.[5–8] For example, biotinol-5′-AMP **2** with its phosphodiester bioisostere, is a potent inhibitor of BPL from *Staphylococcus aureus* (*Sa*BPL), *Escherichia coli* and *Homo sapiens* (*Hs*BPL).[5,24] Importantly, this compound also inhibits the growth of *Staphylococcus aureus* with a minimal inhibitory concentration (MIC) of 8 μg/μL.[5] Sulfomylamide isosteres, as found in **3**, have also been reported to be active against *Mycobacterium tuberculosis* BPL, with no data reported on other BPLs.[6,7]

We also recently reported a 1,2,3-triazole as an effective bioisostere of the hydrolytically unstable acyl phosphate of **1**, for example, see **4**, Figure 1.[5] A 1,2,3-triazole heterocycle as in **4** offers significant advantages over other reported acyl phosphate bioisosteres in that it allows for both facile synthesis by Huisgen cycloaddition and also combinatorial in situ approaches to inhibitor discovery and optimization.[5,25] This work identified 1,2,3-triazole **5** as the most potent (K_i = 0.09 ± 0.01 μM) and selective (>1100 fold in K_i *Sa*BPL vs *Hs*BPL) inhibitor of *Sa*BPL reported to date.[5] The triazole **5** inhibits the growth of *S. aureus*, while being devoid of cytotoxicity against cultured human liver cells.[5] X-ray crystal structures of *Sa*BPL in complex with **5**, in combination with mutagenesis studies, identified a key role for active site amino acids Arg122, Arg125 and Asp180 in selective binding to *Sa*BPL (see Fig. 3). X-ray crystallography also confirmed that the benzoxazolone group of 1,2,3-triazole **5** binds into the ATP pocket of *Sa*BPL thereby functioning as a replacement of the adenine group present in **1**–**4**.

This paper reports the synthesis of analogues of 1,2,3-triazole **5** and their inhibition against *Sa*BPL and *Hs*BPL. The inverted 1,2,3-triazole **6**, 1,2,4-oxadiazole **7**, 1,2,4-triazole **8** and 1,3,4-oxadiazole **9** heterocycles were compared as possible bioisosteres of the reactive acyl phosphate group of **1** (see Fig. 2). All compounds

* Corresponding authors. Tel.: +61 88 3135360 (W.T.); tel.: +61 88 313 5652; fax: +61 88 303 4358 (A.D.A.).
E-mail addresses: william.tieu@adelaide.edu.au (W. Tieu), andrew.abell@adelaide.edu.au (A.D. Abell).
† These authors contributed equally.
‡ Current address: School of Biomedical Sciences, Charles Sturt University, Booroma St, Wagga Wagga, New South Wales 2678, Australia.
§ Current address: Institute for Molecular Bioscience, The University of Queensland, Brisbane 4072, Australia.

http://dx.doi.org/10.1016/j.bmcl.2014.08.030
0960-894X/

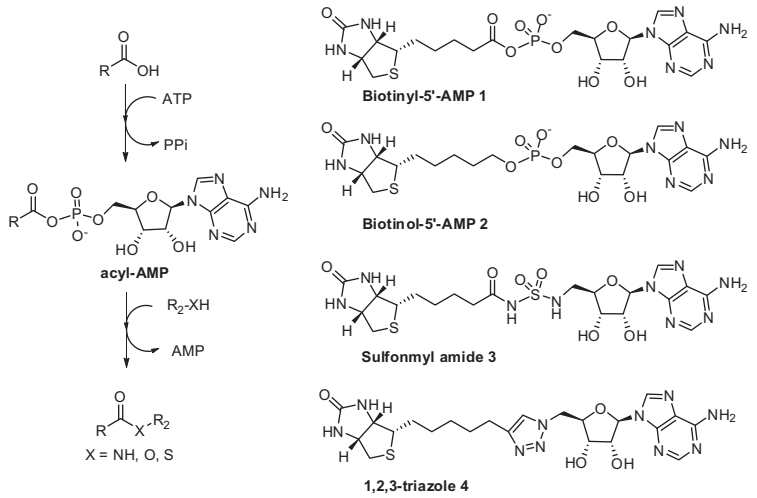

Figure 1. General reaction mechanism of adenylate forming enzymes (left); acyl AMP analogues of biotin protein ligase (right).

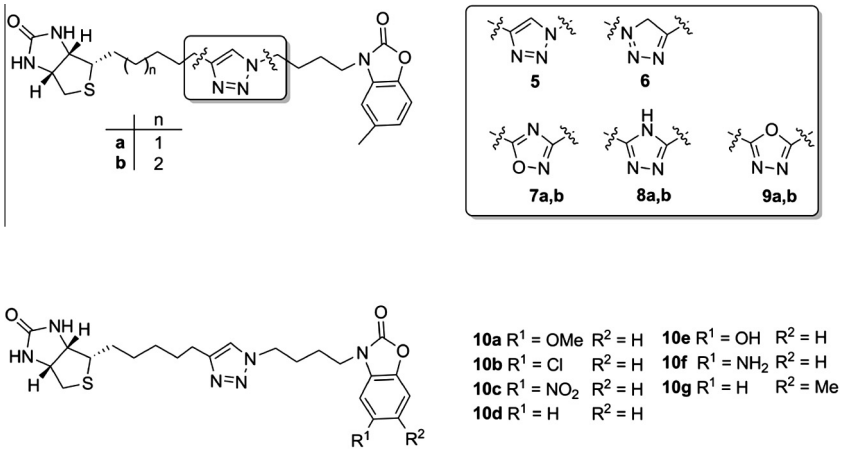

Figure 2. Heterocyclic analogues derived from biotinyl-5′-AMP **1**.

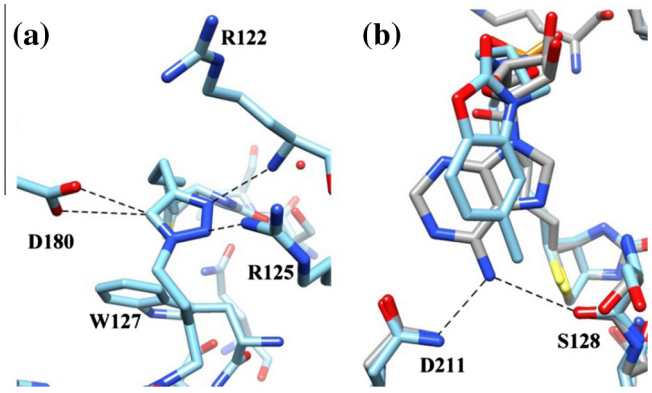

Figure 3. (a) X-ray crystal structure of **5** bound to *Sa*BPL. Interactions between the 1,2,3-triazole ring and residues are highlighted with black dashes. The edge to face pi interaction between 1,2,3-triazole ring and W127 is not shown. (b) An overlay of X-ray crystal structure of biotinyl-5′-AMP **1** and 1,2,3-triazole **5**. The hydrogen bonding interactions between amine of **1** and D211 (3.06 Å) and S128 (3.41 Å) are highlighted with black dashes. Methyl substituent of **5** is 4.00 Å and 3.02 Å away from D211 and S128, respectively.

share the benzoxazolone group and optimum tether linkers either side of the bioisostere as found in **5**, where a simple methylene-based tether can replace the ribose group of **1** and **4** without compromising inhibitory activity. A range of substituents on the benzoxazolone group of **5** were also investigated in order to begin to explore interactions with the ATP binding pocket of *Sa*BPL, see compounds **10** Figure 2.

The 1,2,3-triazole **6** was prepared by alkylation of benzoxazolone **11**,[5] followed by copper-catalyzed cycloaddition with biotin azide **13**[3] in the presence of copper nano powder. Heterocycles **7**–**9** were each prepared in two steps from a common starting nitrile **17** as shown in Scheme 1. In particular, oxime **18**, prepared on reaction of **17** with hydroxylamine, was treated with biotin **14b**[26] and EDCI with subsequent dehydration under reflux to give 1,2,4-oxadiazole **7b**. Conversely, conversion of nitrile **17** to imidic ester **19**, followed by microwave reaction with hydrazide **15b** in the presence of K_2CO_3, gave 1,2,4-triazole **8b** in 32% yield. 1,3,4-Oxadiazole **9b** was also isolated from this reaction in 17% yield. Interestingly, reaction of **14** with **19** under acidic conditions (acetic acid) gave solely the 1,3,4-oxadiazole **9b** and not the 1,2,4-triazole **8b** based on analysis by analytical HPLC and mass spectrometry. Truncated analogues **7a**, **8a** and **9a** were prepared in the same fashion as shown in Scheme 1. The key building blocks **15a**,[27] **15b** and **17** used in these syntheses were prepared as shown in Scheme 1.

The synthesis of the 1,2,3-triazoles **10a**–**g** is summarized in Scheme 2. The key benzoxazolone azides **22a**–**e** were obtained on reacting the respective 2-amino phenol (**20a**–**e**) with