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

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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

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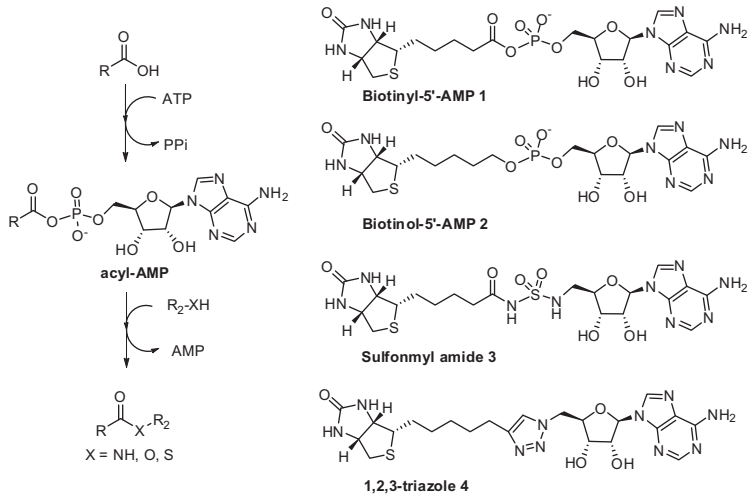


Figure 1. General reaction mechanism of adenylate forming enzymes (left); acyl AMP analogues of biotin protein ligase (right).

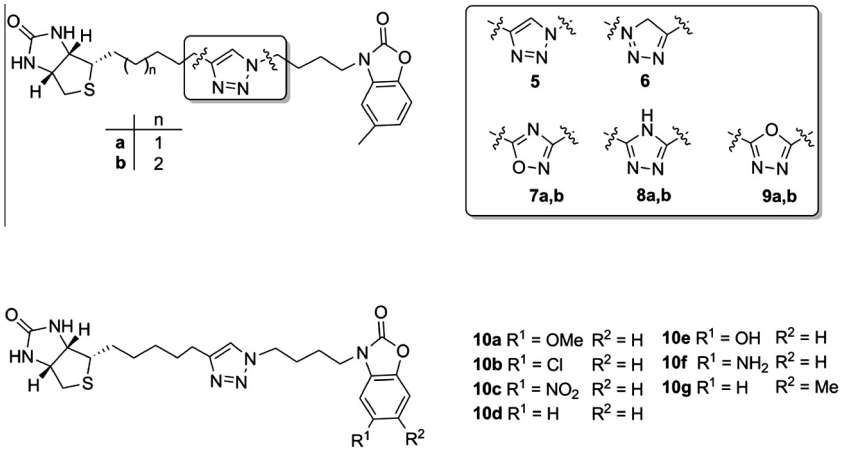


Figure 2. Heterocyclic analogues derived from biotinyl-5′-AMP **1**.

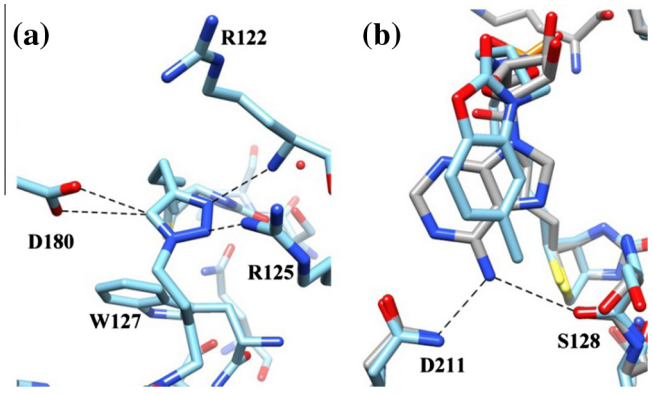
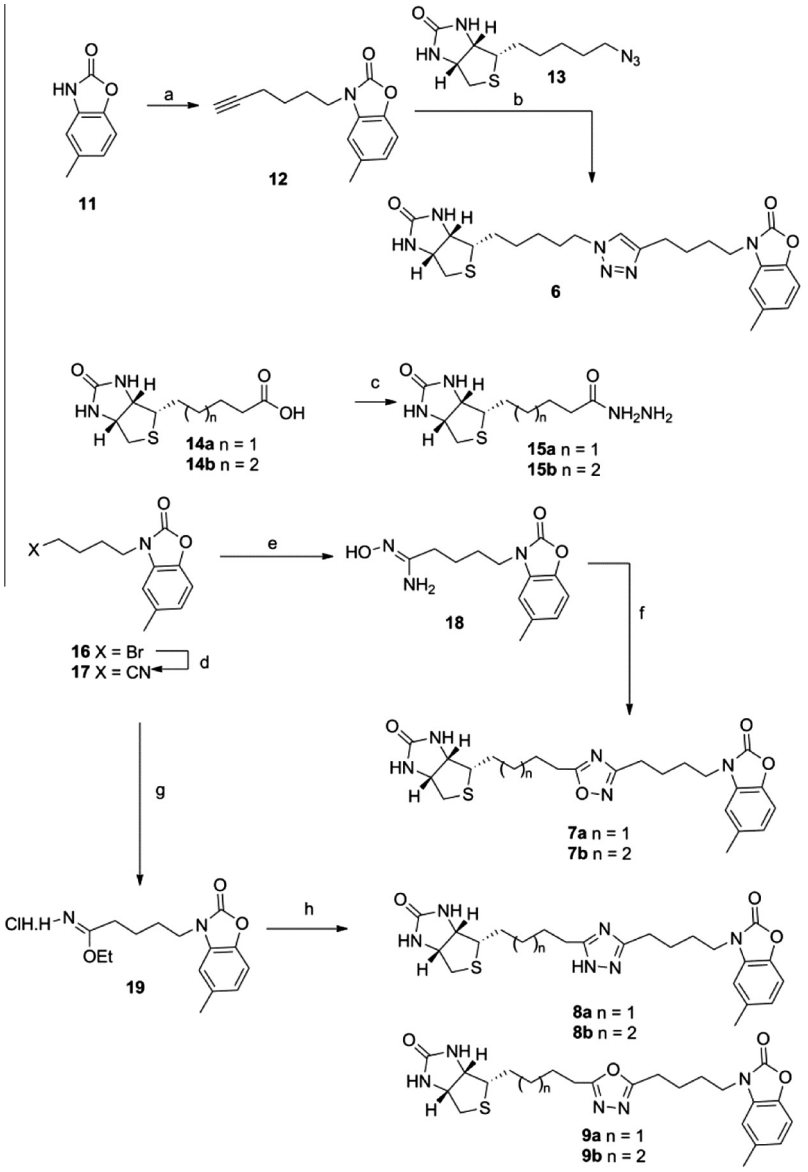


Figure 3. (a) X-ray crystal structure of **5** bound to *Sa*BPL. Interactions between the 1,2,3-triazole ring and residues are highlighted with black dashes. The edge to face pi interaction between 1,2,3-triazole ring and W127 is not shown. (b) An overlay of X-ray crystal structure of biotinyl-5′-AMP **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.

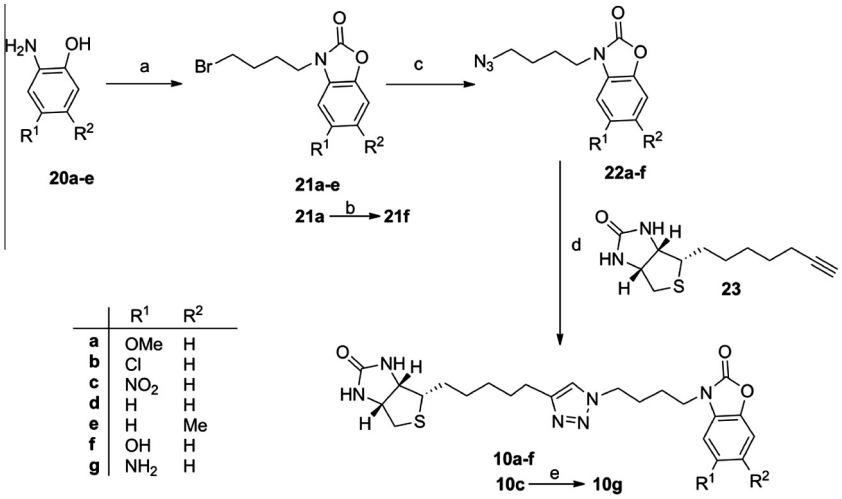
The synthesis of the 1,2,3-triazoles **10a**–**g** is summarized in Scheme 2. The key benzoxazolone azides **22a**–**e** were obtained on reacting the respective 2-amino phenol (**20a**–**e**) with



Scheme 1. Reagents and conditions: (a) 5-hexynyl tosylate, K_2CO_3, DMF; (b) Cu nano powder, MeCN/H_2O; (c) (i) $SOCl_2$, MeOH, (ii) $NH_2NH_2{\cdot}H_2O$, Δ; (d) KCN, DMF; (e) NH_2OH, EtOH, Δ; (f) (i) **14**, EDCI·HCl, DIPEA, DMF, (ii) PhMe, Δ; (g) AcCl, EtOH; (h) **15**, K_2CO_3, 1:1 DMF/PhMe, 150 °C, MW, 2 h (gave **9** (18%) and **8** (32%)) or AcOH, EtOH, Δ, 4 h (gave **9** (21%)).

1,1-carbonyldiimidazole, followed by alkylation with dibromobutane and subsequent conversion of the bromide to azide. Benzoxazolone **22f** was prepared by demethylation of **21a** with NaI in aqueous HBr, followed by treatment with sodium azide. Copper catalyzed cycloaddition of biotin alkyne **23** and benzoxazolone azides **22a**–**f** in the presence of copper nano powder gave the corresponding 1,2,3-triazole **10a**–**f**. Reduction of 1,2,3-triazole **10c** with aqueous titanium trichloride gave **10g**.

The complete activity profiles of heterocycles **6**–**9** and the new derivatives of **5** (i.e., **10a**–**g**) were determined against *Sa*BPL and *Hs*BPL using an in vitro biotinylation assay[5,28] that measures the enzymatic incorporation of radiolabelled biotin onto an acceptor protein, see Tables 1 and 2. A 1,2,4-oxadiazole heterocycle as in **7b** provided an effective acyl phosphate bioisostere for the generation of inhibitors of *Sa*BPL (**7b** had a K_i = 1.2 ± 0.4 μM). While this compound is somewhat less active than the parent 1,2,3-triazole **5** (K_i of 0.09 ± 0.01 μM)[5] it does provide an important new lead for further optimisation. Interestingly, 1,2,3-triazole **6**, 1,3,4-triazole **8b** and 1,3,4-oxadiazole **9b** were all inactive against *Sa*BPL. These results are somewhat surprising given the close similarities of the constituent heterocycles (i.e., **5** vs **6** and **9b** vs **7b**). We suggest that the relative ability of each heterocycle to hydrogen bond with *Sa*BPL residues Arg125, Asp180 and Lys187 (see Fig. 3a) may account for the observed difference. These three residues undergo significant conformational changes on inhibitor binding and are responsible for creating the tight association between ligand and enzyme.[3,5,29] Any disruption to these key interactions, by a particular heterocycle, would be expected to significantly impact on binding and hence potency, as in **5** and **6**. In support, we know that certain biotin-based analogues are more effective than others at inducing the conformational change.[3,29] Specifically, biotin acetylene **23**, the synthetic precursor for **5**, is a more potent inhibitor of *Sa*BPL than is biotin azide **13**, the precursor for **6** (K_i of 0.3 μM and >10 μM respectively).[3] Based on this observation alone it is clear that the position of the nitrogen relative to the biotin heterocycle is critical for activity. Whilst 1,2,3-triazole **5** and the 1,2,4-oxadiazole **7b** were active against *Sa*BPL, both were inactive against human BPL (*Hs*BPL). This is an important observation for



Scheme 2. Reagents and conditions: (a) CDI, DCM; $Br(CH_2)_4Br$, K_2CO_3, DMF; (b) NaI, HBr (aq); (c) NaN_3, DMF; (d) Cu nano powder, $MeCN/H_2O$; (e) 15% $TiCl_3$ (aq).

Table 1
Inhibition assay results of **4**–**12** against *Sa*BPL and *Hs*BPL[a,b,c]

	Heterocycle	*Sa*BPL K_i (μM)	*Hs*BPL K_i (μM)
5	1,2,3-Triazole	0.09 ± 0.01	NI
6	1,2,3-Triazole[d]	NI	NI
7a	1,2,4-Oxadiazole	NI	NI
7b	1,2,4-Oxadiazole	1.2 ± 0.4	NI
8a	1,2,4-Triazole	NI	NI
8b	1,2,4-Triazole	NI	NI
9a	1,3,4-Oxadiazole	NI	NI
9b	1,3,4-Oxadiazole	NI	NI

[a] See Figure 2 for structures and see Supplementary material for procedure.
[b] All compounds possessed no inhibition against *Hs*BPL at ⩾80 μM.
[c] NI: No inhibition at concentrations ⩾80 μM.
[d] This heterocycle possesses a different configuration than **5**, see Figure 2.

Table 2
Inhibition assay results of **5** and **10a**–**g** against *Sa*BPL and *Hs*BPL[a,b,c]

	R_1	R_2	*Sa*BPL K_i (μM)	*Hs*BPL K_i (μM)
5	Me	H	0.09 ± 0.01	NI
10a	OMe	H	1.87 ± 0.11	NI
10b	Cl	H	0.60 ± 0.05	NI
10c	NO_2	H	NI	NI
10d	H	H	NI	NI
10e	H	Me	NI	NI
10f	OH	H	NI	NI
10g	NH_2	H	NI	NI

[a] See Figure 2 for structures and see Supplementary material for procedure.
[b] All compounds possessed no inhibition against *Hs*BPL at ⩾80 μM.
[c] NI: No inhibition at concentrations ⩾80 μM.

ongoing development of new antibiotics based on the inhibition of BPL. Finally, the length of the tether between the biotin bicycle and the bioisostere is critical for activity. The corresponding truncated 1,2,4-oxadiazole **7a** (cf. **7b**) was devoid of inhibitory activity against *Sa*BPL.

The results for the second series of compounds revealed that for the best base heterocycle (1,2,3-triazole), both methyl (**5**, K_i = 0.09 ± 0.01 μM) and chloro substituents (**10b**, K_i = 0.60 ± 0.05 μM) were well tolerated at R_1, as was methoxy to a lesser extent (**10a**, K_i = 1.87 ± 0.11 μM) (Table 2). Interestingly, neither a hydroxyl nor amino group were tolerated at R_1 (see **10f** and **10g**), despite molecular modeling suggesting these groups could potentially hydrogen bond with Asn212 and Ser128 residues within the ATP binding pocket of *Sa*BPL, see Figure 3b.[30] The position of the methyl substituent on the 1,2,3-triazole heterocycle is critical, where methyl at the alternative R_2 position gives rise to an inactive compound (see **10e**). Interestingly the unsubstituted derivative (**10d**) is also inactive.

Importantly the two new active 1,2,3-triazoles **10a** and **10b** both displayed bacteriostatic activity against *S. aureus* ATCC 49755 in a antibacterial microdilution broth assay (see Fig. 4). At 8 μg/mL inhibitor concentration, *S. aureus* growth was reduced to 55% for **10a** and 60% for **10b** relative to 58% for **5** and the streptomycin control (MIC = 8 μg/mL). This compares to a value of 24% for biotin acetylene **23**. MIC values could not be obtained for these compounds because of limited solubility at concentrations greater than 64 μg/ml.

In summary, heterocyclic-based bioisosteres of the acyl phosphate of biotinyl-5′-AMP **1** are reported. 1,2,3-Triazole (see **5**, **10a** and **10b**) and 1,2,4-oxadiazole (see **7b**) heterocycles provide potent and selective inhibitors of *Sa*BPL These heterocycles are, in general, easier to prepare than classic phosphodiester and sulfomylamides bioisosteres and provide improved selectivity for *Sa*BPL over *Hs*BPL. A second series of analogues containing the optimum 1,2,3-triazole isostere, were also prepared, to investigate binding of the terminal benzoxazolone to the ATP binding pocket of *Sa*BPL. A hydrophobic substituent at R_1 on the benzoxazolone

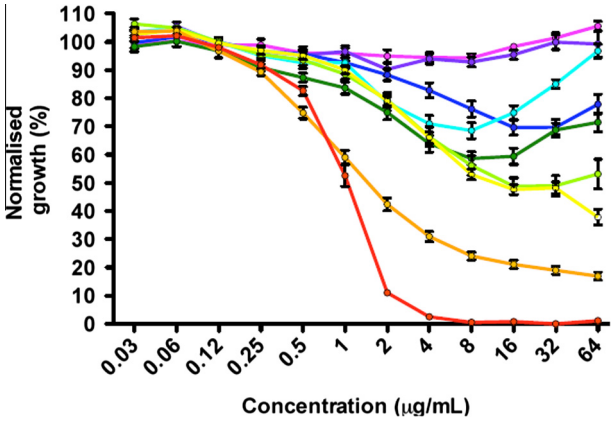


Figure 4. Inhibition of *S. aureus* growth in vitro. Compounds **5** (yellow), **23** (orange), **10a** (lime), **10b** (green), **10c** (fuchsia), **10d** (purple), **10f** (azure), **10g** (blue) and positive control streptomycin (red). See Supplementary data for conditions.

is favored, with chloro being particularly active (K_i = 0.6 μM). Work on optimization the 1,2,3-triazoles with a more expansive SAR study and developing these isosteres to other clinically relevant adenylate forming enzyme targets is currently underway.

Acknowledgments

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Supplementary data

Supplementary data (details on chemical synthesis, enzyme inhibition assay and antimicrobial broth assay) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.08.030.

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