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Citation for final published version:

Eissa, Ahmed G., Blaxland, James A., Williams, Rhodri O., Metwally, Kamel A., El-Adl, Sobhy M., Lashine, El-Sayed M., Baillie, Les ORCID: <https://orcid.org/0000-0002-8186-223X> and Simons, Claire ORCID: <https://orcid.org/0000-0002-9487-1100> 2016. Targeting methionyl tRNA synthetase: design, synthesis and antibacterial activity against *Clostridium difficile* of novel 3-biaryl-N-benzylpropan-1-amine derivatives. *Journal of Enzyme Inhibition and Medicinal Chemistry* 31 (6) , pp. 1694-1697. 10.3109/14756366.2016.1140754 file

Publishers page: <http://dx.doi.org/10.3109/14756366.2016.1140754>
<<http://dx.doi.org/10.3109/14756366.2016.1140754>>

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Targeting methionyl tRNA synthetase: design, synthesis and antibacterial activity against *Clostridium difficile* of novel 3-biaryl-*N*-benzylpropan-1-amine derivatives

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Abstract

The synthesis of a series of benzimidazole-*N*-benzylpropan-1-amines and adenine-*N*-benzylpropan-1-amines is described. Subsequent evaluation against two strains of the anaerobic bacterium *Clostridium difficile* was performed with three amine derivatives displaying MIC values of 16 µg/mL. Molecular docking studies of the described amines determined that the amines interact within two active site pockets of *Clostridium difficile* methionyl tRNA synthetase with methoxy substituents in the benzyl ring and an adenine biaryl moiety resulting in optimal binding interactions.

Keywords

Methionyl-tRNA synthetase, *Clostridium difficile*, 3-biaryl-*N*-benzylpropan-1-amines, molecular modeling, MetRS-ligand interactions

Introduction

The *Clostridium difficile* infection (CDI) rate has shown a dramatic increase over the past decade and this increase is associated with the emergence of hypervirulent strain B1/NAP1/027¹. CDI can also now be found more commonly outside healthcare facilities and in previously low-risk groups, such as children, pregnant women, and individuals with irritable bowel syndrome². Recently CDI has exceeded MRSA as the most common healthcare facility-associated infection³. First-line treatment for CDI is usually metronidazole or vancomycin, for patients with severe infection, oral vancomycin is the preferred therapy as the clinical cure rate for vancomycin in patients with severe CDI was significantly better than that for metronidazole⁴. Unfortunately, recurrent disease occurs in 20 to 25% of patients receiving metronidazole and vancomycin⁵. For this reason there is a need to exploit other *C. difficile* targets in the development of new therapies. A promising target is aminoacyl-tRNA synthetase (aaRS).

Research in our lab in Cardiff has focused on methionyl tRNA (MetRS) as an antibacterial target, using our published homology model of *Clostridium difficile* MetRS⁶ to aid the design process. Most MetRS inhibitors studied are against *Staphylococcus aureus* and *Escherichia coli*. These inhibitors are divided into either adenosine or non-adenosine analogues. Adenosine analogues (Figure 1) are thought to mimic the high-energy intermediate aminoacyl-AMP, thus blocking the aaRS pocket⁷. Non-adenosine analogues may have vast structural differences compared with the natural substrate aminoacyl-AMP, but they still have good enzyme inhibitory activity. The challenge for MetRS inhibitors however is not biochemical activity but whole-cell activity. The most potent examples at the biochemical level, with IC₅₀ values in the low nanomolar range, including oxazolone dipeptides and methionyl adenylate

isosteres, have shown good selectivity versus human MetRS but lack antibacterial activity in whole-cell assays⁸.

Figure 1 here

To date, REP3123 (Figure 1), a novel diaryldiamine, is the only documented selective *C.difficile* MetRS inhibitor with whole cell activity in a range of clinical *C.difficile* isolates⁹. REP3123 is reported to have good *in vitro* activity and selectivity against *C. difficile*, while sparing other gut flora¹⁰.

We report the efficient synthesis (Scheme 1) and whole cell activity against *C. difficile* (Table 1) of a series of novel inhibitors incorporating pharmacophore elements of both REP3123 and adenosine analogues, namely: benzyl and biaryl moiety (adenine or benzimidazole) and flexible amine linker chain.

Results and Discussion

Chemistry

Treatment of benzimidazole (**1**) or adenine (**2**) with potassium carbonate followed by the addition of *tert*-butyl(3-bromopropyl)carbamate resulted in N-alkylation products **3** and **4** in yields of 76 and 68% respectively. Subsequent deprotection of BOC under acidic conditions gave the trifluoroacetic acid salts **5** and **6** in quantitative and 93% yield respectively. Imine formation was achieved on base catalyzed reaction of **5** and **6** with a range of benzaldehydes, giving the imine derivatives **7** and **8** in variable yields. NaBH₄ reduction of the imines gave the required benzimidazole and adenine *N*-benzylpropan-1-amines **9** and **10** in good yields.

Scheme 1 here

Microbiology

A stock solution of each amine was prepared in 5% DMSO at a concentration of 256 µg/mL. These were then diluted with nutrient broth to final concentrations of 128 µg/mL, 64 µg/mL, 32 µg/mL, 16 µg/mL, 8 µg/mL, 4 µg/mL, 2 µg/mL, 1 µg/mL or 0.5 µg/mL and inoculated with a bacterial suspension of either *C. difficile* strain 1813 or strain R20291. Growth was assessed after incubation at 37 °C for 48 h in an anaerobic cabinet. Metronidazole as a control showed an MIC of <0.5 µg/mL for both *C. difficile* strain 1813 and R20291.

Table 1 here

Three amines **9e**, **10a** and **10e** displayed MIC values of 16 µg/mL against *C. difficile* strain 1813 (Table 1) and one of the imines **8d** showed activity with an MIC value of 16 µg/mL. None of the imines or amines showed inhibitory activity against strain R20291.

Molecular modeling

Docking interactions of the imines and amines were explored using molecular modeling software. REP3123 had previously been shown to fit in two pockets within the *C. difficile* MetRS active site. Pocket 1 (the methionine binding pocket) has Ile12, Asp51, Ala230 and Trp227 as the main residues, while Glu55, Ser133 and Tyr225 form an adjacent pocket (pocket 2) with Lys56 bridging the two pockets^{6,11}.

Figure 2 here

All the amines and imines had reasonable docking results based on both visual inspection of the poses and the docking score and occupied the two pockets previously observed for REP3123 (Figure 2). The adenine derivatives were found to give better interactions in terms of hydrogen bonding via the NH₂ and the 4-methoxy

and 3,5-dimethoxy derivatives were able to better fill the two pockets with additional hydrogen bonding interactions between a methoxy group and Lys59 (Figure 3). Ile12 was found to be the main key interaction in the methionine pocket, while Glu55, Lys59, Ala230 were found to be the main interactions in the adjacent pocket with Lys56 and Glu262 bridging these two pockets.

Figure 3 here

Conclusions

This study provides some insight into the functionality required for improved binding interactions within the two pockets of the MetRS active site, although there is still scope for further extension within the pockets. The whole cell activity observed for the imine **8d** and amines **9e**, **10a** and **10e** is promising for further development, however the different inhibitory activity observed with the two *C. difficile* strains highlights the challenges of developing a MetRS inhibitor that can inhibit a range of *C. difficile* clinical isolates.

Declaration of interest

No interests to declare

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Acknowledgments

We acknowledge the EPSRC Mass Spectrometry Centre, Swansea, U.K. for mass spectroscopy data.

Supplementary Material

Synthetic methods and analytical data for all described compounds and microbiological assay method are provided.

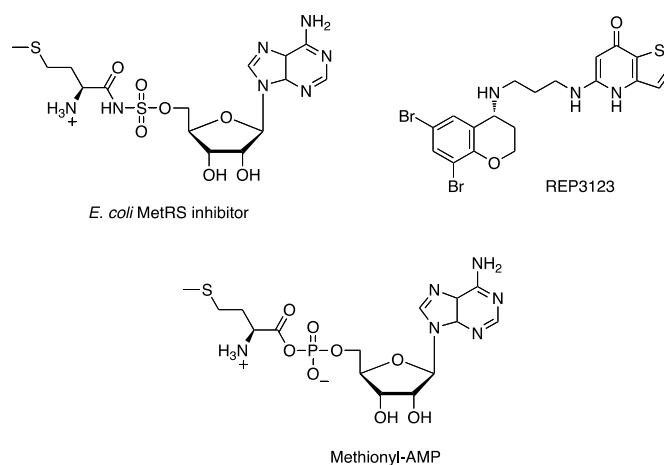


Figure 1. Adenosine analogue *E.coli* MetRS inhibitor and non-adenosine *C. difficile* MetRS inhibitor REP3123 and the natural substrate methionyl-AMP.

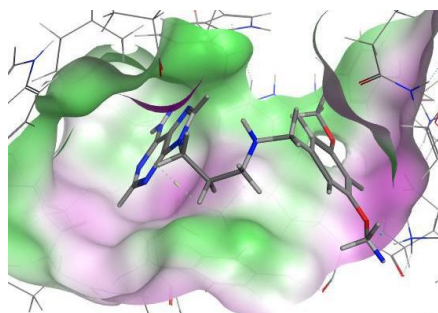


Figure 2. 3,5-Dimethoxy amine derivative (**10e**) sitting in *C. difficile* MetRS two active site pockets.

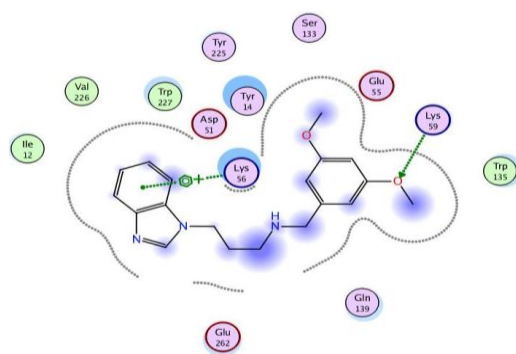
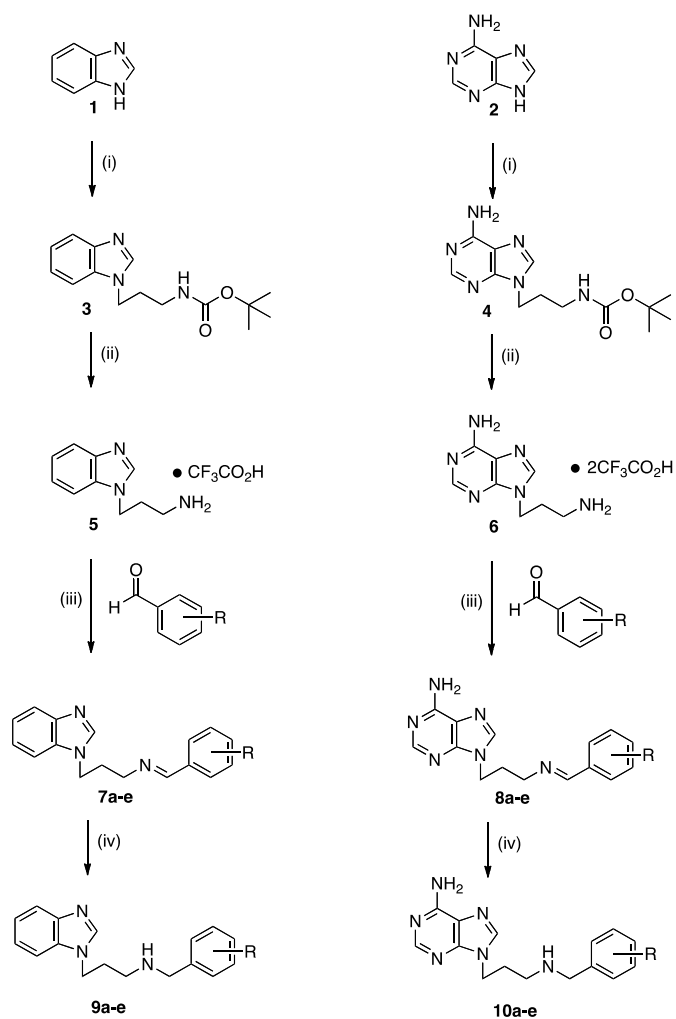
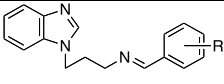
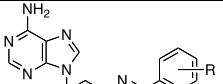
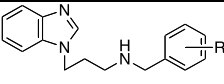
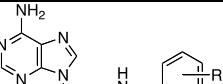


Figure 3. 2D Ligand interaction between amine derivative **9e** and the *C. difficile* MetRS active site residues.



Scheme 1. Reagents and conditions: (i) potassium carbonate, 18-crown-6, *tert*-butyl(3-bromopropyl)carbamate, DMF, 60 °C overnight (ii) TFA/DCM (iii) TEA, methanol, different benzaldehydes, overnight (iv) NaBH₄, methanol, overnight. [**a**, R = H; **b**, R = 4-Cl ; **c**, R = 4-Br; **d**, R = 4-OMe ; **e**, R = 3,5-diOMe]

Table 1. MIC ($\mu\text{g/mL}$) against *C. difficile* strain 1813

					
No.	R	MIC ($\mu\text{g/mL}$)	No.	R	MIC ($\mu\text{g/mL}$)
7a	H	> 128	8a	H	> 128
7b	4-Cl	> 128	8b	4-Cl	> 128
7c	4-Br	>128	8c	4-Br	128
7d	4-OMe	> 128	8d	4-OMe	16
7e	3,5-di-OMe	128	8e	3,5-di-OMe	> 128
					
No.	R	MIC ($\mu\text{g/mL}$)	No.	R	MIC ($\mu\text{g/mL}$)
9a	H	128	10a	H	16
9b	4-Cl	128	10b	4-Cl	128
9c	4-Br	128	10c	4-Br	128
9d	4-OMe	128	10d	4-OMe	128
9e	3,5-di-OMe	16	10e	3,5-di-OMe	16
Metronidazole		<0.5			