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/NAP1/027[1]. 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 syndrome[2]. Recently CDI has exceeded MRSA as the most common healthcare facility-associated infection[3]. 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 metronidazole[4]. Unfortunately, recurrent disease occurs in 20 to 25% of patients receiving metronidazole and vancomycin[5]. 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* MetRS[6] 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 pocket[7]. 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 IC_{50} 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 assays[8].

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.diffcile* isolates[9]. REP3123 is reported to have good *in vitro* activity and selectivity against *C. difficile,* while sparing other gut flora[10].

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. $NaBH_4$ 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 ºC 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 NH_2 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

References

1. Lanis JM, Heinlen LD, James JA, et al. PLoS Pathog 2013; 9: e1003523.

2. Ananthakrishnan AN. Nat Rev Gastroenterol Hepatol 2011; 8: 17-26.

3. Miller BA, Chen LF, Sexton DJ, et al. Infect Control Hosp Epidemiol 2011; 32: 387-90.

4. Zar FA, Bakkanagari SR, Moorthi KM, et al. Clin Infect Dis 2007; 45: 302-7.

5. Bauer MP, Kuijper EJ, van Dissel JT. Clin Microbiol Infect 2009; 15: 1067-79.

6. Al-Moubarek E, Simons C. J Mol Mod 2011; 17: 1679-93.

7. Lee J, Kim SE, Lee JY, et al. Bioorg Med Chem Lett 2013; 13: 1087-92.

8. Green LS, Bullard JM, Ribble W, et al. Antimicrob Agents Chemother 2009; 53: 86-94.

9. Citron DM, Warren YA, Tyrrell KL, et al. J Antimicrob Chemother 2009; 63: 972-76.

10. Critchley IA, Green LS, Young CL, et al. J Antimicrob Chemother 2009; 63: 954-63.

11. Evans R, Green L, Sun X, et al. ICAAC 2007, Abstracts of the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Proceedings of the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy; Chicago, IL, USA. 17–20 September 2007; Washington, DC, USA: American Society for Microbiology; 2007, Poster No. F1-2114.

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Supplementary Material

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

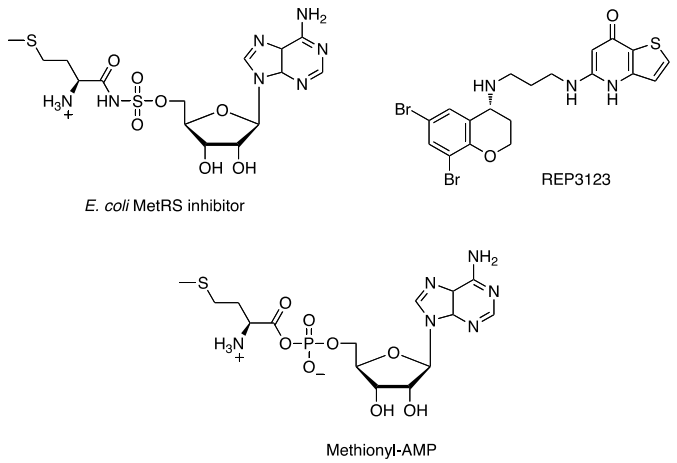


Figure 1. Adenosine analogue *E.coli* MetRS inhibitor and non-adenosine *C. difficile* MetRS inhibitor REP3123 and the natural substrate methionyl-AMP.

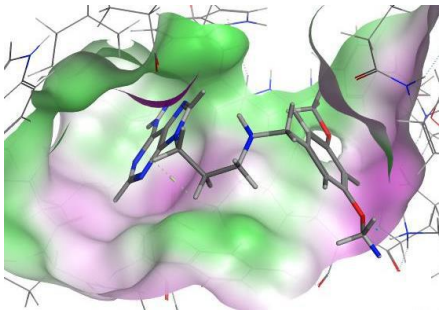


Figure 2. 3,5-Dimethoxy amine derivative (**10e**) sitting in *C. difficile* MetRS two active site pockets.

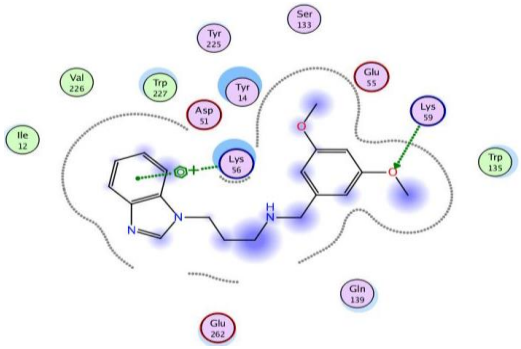
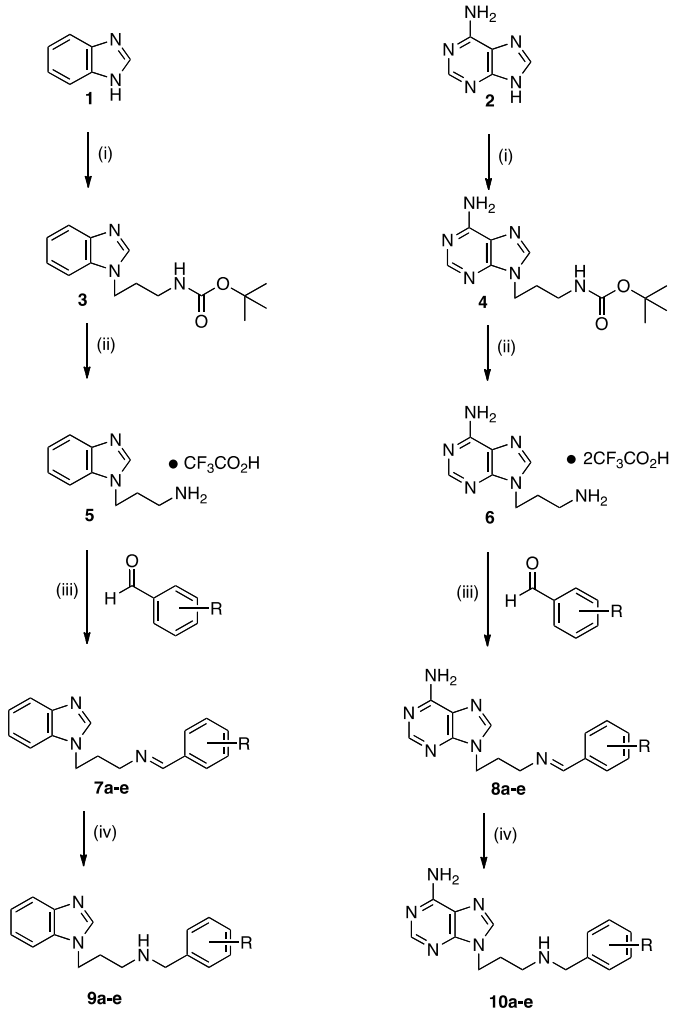
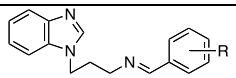
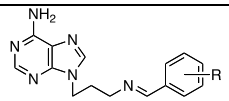
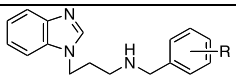
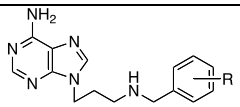


Figure 3. 2D Ligand interaction between amine derivative **9e** and the *C.difficile* MetRS active site residues.



Scheme 1. Reagents and conditions: (i) potassium carbonate, 18-crown-6, *tert*-butyl(3-bromopropyl)carbamate, DMF, 60 ºC overnight (ii) TFA/DCM (iii) TEA, methanol, different benzaldehydes, overnight (iv) $NaBH_4$, 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 (μg/mL) against *C. difficile* strain 1813

					
No.	R	MIC (μg/mL)	No.	R	MIC (μg/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 (μg/mL)	No	R	MIC (μg/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			