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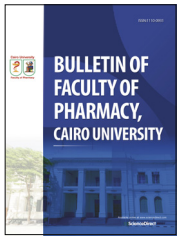

ORIGINAL ARTICLE

Adenine and benzimidazole-based mimics of REP-3123 as antibacterial agents against *Clostridium difficile* and *Bacillus anthracis*: Design, synthesis and biological evaluation

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Abstract As part of our ongoing research efforts to develop new antibacterial agents acting on novel molecular targets, a series of twenty-two adenine and benzimidazole-based mimics of the lead compound REP-3123 was designed to target methionyl-tRNA synthetase of *Clostridium difficile* and *Bacillus anthracis* based on a homology model. Structures of the target compounds were elucidated by means of ^{1}H and ^{13}C NMR spectral data and their purity was confirmed by HRMS or microanalyses. The target compounds were tested for their *in vitro* antibacterial activity against those two challenging organisms by the microdilution method in brain heart infusion broth. Unfortunately, six of the target compounds were not biologically tested due to inadequate solubility in DMSO under the assay conditions. Only the fluoro-substituted adenine-based sulfamide (**18**) showed activity against *C. difficile* with an MIC of 85.33 μg/mL. The adenine-based thiourea (**32**) and diamine (**36**) were the most promising antibacterial agents against *B. anthracis* with an MIC of 92.16 μg/mL. The rest of the tested compounds either showed inferior activity (MIC = 102.4 μg/mL) or were totally inactive. Although the compounds were not very active, the biological data are employed as a basis for our currently underway investigation for structure optimization.

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1. Introduction

The recognition of *Clostridium difficile* as an urgent threat to human health in 2013 CDC report and the potential use of *Bacillus anthracis* as a bioterrorist weapon, have accelerated medicinal chemistry efforts to search new antibacterial agents for those challenging organisms.[1–3] *C. difficile* is the most common cause of healthcare facility acquired diarrhoea. Improper use of broad-spectrum antibiotics results in disturbance in the healthy gut microbiome leading to colonization of *C. difficile* and emergence of gastrointestinal disorders.[2] *C. difficile* infection (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.[4] Recently CDI has exceeded MRSA as the most common healthcare facility-associated infection.[5] First-line treatment of *C. difficile* infections is usually metronidazole for mild to moderate infection or vancomycin for moderate to severe cases. Both drugs, however, do not effectively treat the infection or prevent relapsing infection.[6–8]

B. anthracis, the causative agent of Anthrax, is a Gram-positive rod sporulating bacteria that exists in the environment as spores. *B. anthracis* spores are stable for long periods and can remain viable in the soil for decades and grazing animals are their usual host.[9] Anthrax is a well-known zoonotic disease around the world that can infect humans as well as most animals, especially grazing animals. Normally, human infections may result from contact with infected animals or products, such as hides or wool from infected animals.[10] Anthrax can be highly lethal. Although the disease has been well studied since the nineteenth century, it has witnessed a renewed interest during the past decade. However, due to two recent outbreaks related to use as a bioterrorist agent in the USA through contaminated mail and heroin in UK and Europe, concerns over Anthrax have greatly increased.[11,12] The current vaccines are safe and effective but have limitations that justify the need for the development of new antimicrobial agents active against *B. anthracis* infections. The front-line antibiotics recommended for the treatment of Anthrax are ciprofloxacin, doxycycline and the β-lactams. However, *B. anthracis* strains are increasingly becoming resistant to the current generation of antibiotics.[3]

The steadily increasing incidence of bacterial resistance necessitates the continuous search for new antibacterial agents acting at novel molecular targets. Aminoacyl-tRNA synthetases represent a family of enzymes that are essential for protein biosynthesis. The development of the topical antibiotic mupirocin, which acts by inhibition of isoleucyl-tRNA synthetase, clinically validated this class of enzymes. In the past decade, there was a growing medicinal chemistry effort to target bacterial methionyl-tRNA synthetase that led to the discovery of a few lead compounds. So far, REP-3123 (Fig. 1), a novel diaryldiamine, is the only documented selective *C. difficile* methionyl-tRNA synthetase inhibitor that has good *in vitro* activity and selectivity against *C. difficile* with no activity against other anaerobes that compromise the intestinal flora.[13,14] In addition, REP3123 demonstrated superior *in vivo* efficacy compared to vancomycin in the hamster GI infection model.[15] On this basis, this particular lead compound was taken as a template for the design of target compounds previously reported by us.[16] In the present investigation, we continue our efforts to explore structural determinants of the antibacterial activity of this novel lead compound.

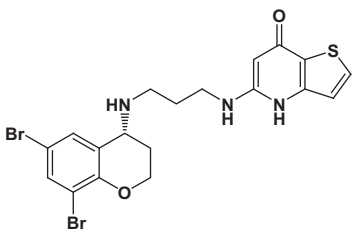


Figure 1 Chemical structure of the lead compound REP-3123.

To date, there is no reported crystal structure of *C. difficile* methionyl-tRNA synthetase. However, a homology model previously prepared was reported describing the active site to be formed of two pockets. Ile12, Asp51, Ala230 and Trp227 were the main residues of pocket 1 which is the methionine binding pocket, while Glu55, Ser133 and Tyr225 formed an adjacent pocket (pocket 2) with Lys56 bridging the two pockets.[17,18] Since methionyl tRNA synthetase enzyme sequences for *C. difficile* and *B. anthracis*[19] were found to display a high percentage identity (53.7%) and a high percentage similarity (79.1%),[20] our target compounds were tested against both organisms for *in vitro* antibacterial activity.

2. Materials and methods

2.1. Chemistry

Melting points were determined using a Gallenkamp melting point apparatus and were uncorrected. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Advance DP500 spectrometer operating at 500 MHz and 125 MHz, respectively. NMR solvents were chloroform-d ($CDCl_3$), methanol-d_4 (CD_3OD), and DMSO-d_6 ($(CD_3)_2SO$). Chemical shifts are given in parts per million (ppm) relative to the internal standard tetramethylsilane (Me4Si). Coupling constants (*J*-value) were calculated in hertz (Hz). Microanalysis data were obtained by MEDAC Ltd, Alpha 319, Chobham Business Centre, Chertsey Road, Chobham, Surrey, UK. For column chromatography, a glass column was slurry packed in the appropriate eluent with silica gel (Fluka Kieselgel 60), flash column chromatography was performed with the aid of a bellow. Analytical thin layer chromatography (TLC) was carried out on precoated silica plates (ALUGRAM® SIL G/UV254) with visualization via UV light (254 nm) and/or iodine stains.

2.1.1. *tert*-Butyl (2-bromoethyl)carbamate (**1**)

To a stirred and cooled (0 °C) solution of 2-bromoethylamine hydrobromide (10.0 g, 48.8 mmol) and triethylamine (13.6 mL, 97.6 mmol) in methanol (200 mL), was added di-*tert*-butyl dicarbonate (16.0 g, 73.2 mmol). The mixture was stirred at 60 °C for 1 h and then overnight at room temperature. The mixture was concentrated under vacuum and dissolved in dichloromethane (200 mL), then washed with 1 M aqueous HCl (2 × 100 mL) and brine (2 × 100 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to afford the title compound as colourless oil.[21]

Yield: 81%. ^{1}H NMR ($CDCl_3$) δ: 5.09 (bs, 1H, NH), 3.49 (m, 2H, CH_2), 3.41 (m, 2H, CH_2), 1.41 (s, 9H, *tert*-butyl).

^{13}C NMR ($CDCl_3$) δ: 155.60 (C-ester), 79.81 (C-*tert*-butyl), 42.37 (CH_2), 32.76 (CH_2), 28.34 ($3 \times CH_3$). The compound was used without further characterization for the synthesis of compounds (**2**,**3**).

*2.1.2. General procedure for the synthesis of tert-butyl [2-(adenyl or benzimidazolyl)ethyl]carbamate (**2**,**3**)*

To a suspension of adenine or benzimidazole (5.65 mmol), potassium carbonate (1.56 g, 11.30 mmol), and 18 crown 6 (0.15 g, 0.56 mmol) in anhydrous DMF (30 mL) was added (2-bromoethyl)carbamic acid *tert*-butyl ester (**1**; 2.50 g, 11.16 mmol). The suspension was then stirred at 60 °C overnight. The reaction mixture was allowed to cool to room temperature and inorganics removed by filtration, the filtrate was concentrated under vacuum. The residue was dissolved in ethyl acetate (100 mL) and washed with water (3×50 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum. The product was purified by recrystallization from methanol to give the title compounds as white crystals.

*2.1.2.1. tert-Butyl (2-(6-amino-9H-purin-9-yl)ethyl)carbamate (**2**)*. Yield: 92%. MP (°C): 203–208 (lit. 181 °C).[21] ^{1}H NMR (DMSO-d_6) δ: 8.14 (s, 1H, CH-Ar), 8.01 (s, 1H, CH-Ar), 7.15 (s, 2H, NH_2), 6.94 (t, J = 5.3, 1H, NH), 4.18 (t, J = 5.8, 2H, CH_2), 3.34 (CH_2, masked by H_2O), 1.32 (s, 9H, *tert*-butyl). ^{13}C NMR (DMSO-d_6) δ: 155.88 (C-ester), 155.52 (C-Ar), 152.22 (CH-Ar), 149.67 (C-Ar), 140.83 (CH-Ar), 118.74 (C-Ar), 77.77 (C-*tert*-butyl), 42.76 (CH_2), 39.00 (CH_2), 28.10 ($3 \times CH_3$, *tert*-butyl). The compound was used without further characterization for the synthesis of compound (**4**).

*2.1.2.2. tert-Butyl (2-(1H-benzimidazol-1-yl)ethyl)carbamate (**3**)*. Yield: 80%. MP (°C): 121–126. ^{1}H NMR (DMSO-d_6) δ: 8.12 (s, 1H, CH-imidazole), 7.64 (d, J = 7.9, 1H, CH-Ar), 7.57 (d, J = 7.9, 1H, CH-Ar), 7.25 (t, J = 8.1, 1H, CH-Ar), 7.19 (t, J = 8.0, 1H, CH-Ar), 6.98 (t, J = 5.0, 1H, NH), 4.29 (t, J = 5.9, 2H, CH_2), 3.31–3.34 (q, J = 5.9, 2H, CH_2), 1.32 (s, 9H, $3 \times CH_3$-*tert*-butyl). ^{13}C NMR (DMSO-d_6) δ: 155.59 (C-ester), 143.96 (C-imidazole), 143.36 (C-Ar), 134.10 (C-Ar), 122.11 (CH-Ar), 121.29 (CH-Ar), 119.32 (CH-Ar), 110.17 (CH-Ar), 77.82 (C-*tert*-butyl), 43.56 (CH_2), 39.69 (CH_2), 28.09 ($3 \times CH_3$-*tert*-butyl). HRMS (EI): Calcd for 261.1477 $[M]^+$, Found: 261.1476 $[M]^+$. The compound was used for the synthesis of compound (**5**).

2.1.3. General procedure for the synthesis of 9-(2-aminoethyl)-9H-purin-6-amine trifluoroacetate (4) and 2-(1H-benzimidazol-1-yl)ethanamine trifluoroacetate (4,5)

A solution of *tert*-butyl(2-(6-amino-9H-purin-9-yl)ethyl)carbamate (**2**; 2.0 mmol) or *tert*-butyl (2-(1H-benzimidazol-1-yl)ethyl)carbamate (**3**; 2.0 mmol) in trifluoroacetic acid/dichloromethane (20 mL, 75:25 v/v) was stirred overnight at room temperature. The solvent was then co-evaporated with ethanol several times. The residue was saturated with diethyl ether (75 mL), stirred for 2 h to afford the title compounds as white solids collected by filtration.

*2.1.3.1. 9-(2-aminoethyl)-9H-purin-6-amine trifluoroacetate (**4**)*. Yield: 94%. MP (°C): 158–163 (lit. 139 °C).[21] ^{1}H NMR (CD_3OD) δ: 8.40 (s, 1H, CH-Ar), 8.30 (s, 1H, CH-Ar), 4.65 (t, J = 5.9, 2H, CH_2), 3.55 (t, J = 5.9, 2H, CH_2). ^{13}C NMR (CD_3OD) δ: 152.97 (C-Ar), 150.93 (C-Ar), 147.02 (CH-Ar), 145.00 (CH-Ar), 120.19 (C-Ar), 43.12 (CH_2), 40.31 (CH_2). The compound was used without further characterization for the synthesis of the final target sulfamides (**13–19**).

*2.1.3.2. 2-(1H-benzimidazol-1-yl)ethanamine trifluoroacetate (**5**)*. Yield: quantitative. MP (°C): 214–219 (lit. 215–217 °C). ^{1}H NMR (DMSO-d_6) δ: 9.03 (s, 1H, CH-imidazole), 8.15 (s, 3H, NH_3^+, exchangeable), 7.90 (d, J = 8.0, 1H, CH-Ar), 7.84 (d, J = 7.5, 1H, CH-Ar), 7.47–7.54 (m, 2H, CH-Ar), 4.67 (t, J = 6.1, 2H, CH_2), 3.40 (t, J = 5.9, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 143.96 (CH-imidazole), 143.80 (C), 135.15 (C), 123.11 (CH-Ar), 122.74 (CH-Ar), 120.45 (CH-Ar), 111.13 (CH-Ar), 43.56 (CH_2), 39.69 (CH_2). The compound was used without further characterization for the synthesis of the final target sulfamides (**20–25**).

*2.1.4. General procedure for the synthesis of N-(un)substituted-benzyl-2-oxooxazolidine-3-sulfonamides (**6–12**)*

To a flask filled with dichloromethane (7.60 mL) under nitrogen, was added chlorosulphonyl isocyanate (0.5 mL, 5.74 mmol) at room temperature. The reaction mixture was cooled to about 0 °C and a solution of 2-bromoethanol (0.41 mL, 5.78 mmol) in dichloromethane (2.5 mL) was added dropwise while maintaining the temperature between 0 and 10 °C. The reaction mixture was stirred at the same temperature for 1 h. A mixture of the appropriate benzylamine (6.32 mmol) and triethylamine (2.0 mL, 14.40 mmol) in dichloromethane (5.5 mL) was then added dropwise while maintaining the temperature between 0 and 10 °C. The reaction mixture was then allowed to reach room temperature. 0.2 M aqueous HCl (100 mL) was then added and pH of the reaction was adjusted to 2 with concentrated HCl if necessary. The reaction mixture was decanted and the separated organic layer washed with 0.2 M aqueous HCl (100 mL) and then with water (100 mL). The organic layer was diluted with 100 mL water and as much as possible of dichloromethane was removed under vacuum at a temperature below 25 °C. The resulting suspension was stirred for 2.5 h at room temperature, filtered, rinsed twice with sufficient water and dried under vacuum at 60 °C overnight to afford the title compounds as white solids.

*2.1.4.1. N-benzyl-2-oxooxazolidine-3-sulfonamide (**6**)*. Yield: 50%. MP (°C): 144–147 (lit. 134–138 °C).[22] ^{1}H NMR (DMSO-d_6) δ: 8.96 (t, J = 6.0, 1H, NH), 7.29–7.38 (m, 5H, $5 \times$ CH-Ar), 4.24 (d, J = 6.1, 2H, CH_2-benzylic), 4.16 (dd, J = 7.6, 9.1, 2H, CH_2), 3.78 (dd, J = 6.7, 8.1, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 152.35 (C), 137.28 (C), 128.36 ($2 \times$ CH), 127.57 ($2 \times$ CH), 127.42 (CH), 62.24 (CH_2), 46.34 (CH_2), 45.07 (CH_2). The compound was used without further characterization for the synthesis of the target sulfamides (**13**,**20**).

2.1.4.2. N-(4-methoxybenzyl)-2-oxooxazolidine-3-sulfonamide (7). Yield: 60%. MP (°C): 108–112. ^{1}H NMR (DMSO-d_6) δ: 8.86 (t, J = 6.1, 1H, NH), 7.26 (d, J = 8.7, 2H, $2 \times$ CH-Ar), 6.92 (d, J = 8.7, 2H, $2 \times$ CH-Ar), 4.18 (dd, J = 7.6, 9.2, 2H, CH_2), 4.16 (d, J = 6.1, 2H, CH_2-benzylic), 3.79 (dd, J = 6.6, 8.1, 2H, CH_2), 3.75 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ: 158.67 (C), 152.35 (C), 129.10 (C), 128.98 ($2 \times$ CH), 113.75 ($2 \times$ CH), 62.23 (CH_2), 55.09 (CH_3), 45.86 (CH_2), 45.06

(CH_2). The compound was used without further characterization for the synthesis of the target sulfamides (**14**,**21**).

*2.1.4.3. N-(3-chlorobenzyl)-2-oxooxazolidine-3-sulfonamide (**8**)*. Yield: 74%. MP (°C): 102–108. ^{1}H NMR (DMSO-d_6) δ: 9.02 (t, J = 6.1, 1H, NH), 7.31–7.42 (m, 4H, 4 × CH-Ar), 4.24 (m, 4H, CH_2- benzylic and CH_2), 3.85 (dd, J = 6.7, 8.1, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 152.36 (C), 139.96 (C), 133.03 (C), 130.23 (CH), 127.37 (CH), 127.33 (CH), 126.20 (CH), 62.35 (CH_2), 45.60 (CH_2), 45.18 (CH_2). HRMS m/z (EI): Calcd for 289.0044 $[M-H]^-$, Found: 289.0040 $[M-H]^-$. The compound was used for the synthesis of the target sulfamides (**15**,**22**).

*2.1.4.4. N-(2,4-dichlorobenzyl)-2-oxooxazolidine-3-sulfonamide (**9**)*. Yield: 57%. MP (°C): 136–142. ^{1}H NMR (DMSO-d_6) δ: 9.02 (t, J = 5.9, 1H, NH), 7.63 (d, J = 2.0, 1H, CH-Ar), 7.52 (d, J = 8.4, 1H, CH-Ar), 7.48 (dd, J = 2.0, 8.4, 1H, CH-Ar), 4.34 (m, 4H, 2 × CH_2), 3.93 (dd, J = 7.6, 8.9, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 152.55 (C), 133.93 (C), 133.06 (C), 132.89 (C), 131.00 (CH), 128.65 (CH), 127.41 (CH), 62.47 (CH_2), 45.28 (CH_2), 43.46 (CH_2). HRMS m/z (EI): Calcd for 342.0077 $[M+NH_4]^+$, Found: 342.0075 $[M+NH_4]^+$. The compound was used for the synthesis of the target sulfamides (**16**,**23**).

*2.1.4.5. N-(3,4-dichlorobenzyl)-2-oxooxazolidine-3-sulfonamide (**10**)*. Yield: 76%. MP (°C): 123–128. ^{1}H NMR (DMSO-d_6) δ: 9.04 (s, 1H, NH), 7.63 (d, J = 8.3, 1H, CH-Ar), 7.61 (d, J = 1.7, 1H, CH-Ar), 7.34 (dd, J = 1.7, 8.3, 1H, CH-Ar), 4.27 (m, 4H, 2 × CH_2), 3.88 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 152.38 (C), 138.71 (C), 130.93 (C), 130.32 (CH), 129.95 (C), 129.52 (CH), 127.87 (CH), 62.41 (CH_2), 45.24 (CH_2), 44.99 (CH_2). The compound was used without further characterization for the synthesis of the target sulfamides (**17**,**24**).

*2.1.4.6. N-(4-fluorobenzyl)-2-oxooxazolidine-3-sulfonamide (**11**)*. Yield: 53%. MP (°C): 112–116. ^{1}H NMR (DMSO-d_6) δ: 8.95 (t, J = 6.1, 1H, NH), 7.39 (m, 2H, CH-Ar), 7.19 (m, 2H, CH-Ar), 4.23 (m, 4H, 2 × CH_2), 3.84 (ψt, J = 8.1, 7.7, 2H, CH_2). The compound was used without further characterization for the synthesis of the target sulfamides (**18**,**25**).

*2.1.4.7. N-(4-cyanobenzyl)-2-oxooxazolidine-3-sulfonamide (**12**)*. Yield: 89%. MP (°C): 160–164. ^{1}H NMR (DMSO-d_6) δ: 9.14 (t, J = 6.2, 1H, NH), 7.84 (d, J = 8.2, 2H, CH-Ar), 7.54 (d, J = 8.15, 2H, CH-Ar), 4.33 (d, J = 6.2, 2H, CH_2), 4.26 (ψt, J = 7.5, 8.2, 2H, CH_2), 3.85 (ψt, J = 7.5, 8.2, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 152.43 (C), 143.31 (C), 132.31 (2 × CH), 128.32 (2 × CH), 118.75 (C), 110.11 (C), 62.42 (CH_2), 45.73 (CH_2), 45.22 (CH_2). HRMS m/z (EI): Calcd for 280.0392 $[M-H]^-$, Found: 280.0379 $[M-H]^-$. The compound was used for the synthesis of the target sulfamide (**19**).

*2.1.5. General procedure for the synthesis of N-[2-(adenyl- or benzimidazolyl)ethyl]-N′-(un)substitutedbenzylsulfamides (**13–25**)*

To a suspension of 9-(2-aminoethyl)-9H-purin-6-amine trifluoroacetate **(4**; 1 eq.**)** or 2-(1H-benzimidazol-1-yl)ethanamine trifluoroacetate **(5**; 1 eq.) in anhydrous acetonitrile (10 mL) was added triethylamine (3.3 eq.) followed by the appropriate *N*-benzyl-2-oxooxazolidine-3-sulfonamide derivative **(6–12**; 1.1 eq.). The reaction was then stirred at 90 °C overnight. The reaction mixture was allowed to cool to room temperature and water (100 mL) was slowly added. Acetonitrile was removed under vacuum below 40 °C. The resulting suspension was stirred at room temperature for 2 h before it was collected by filtration to afford the title compounds white solids. The products were purified by recrystallization from methanol.

*2.1.5.1. N-[2-(6-amino-9H-purin-9-yl)ethyl]-N′-benzylsulfamide (**13**)*. Yield: 37%. MP (°C): 188–190. ^{1}H NMR (DMSO-d_6) δ: 8.16 (s, 1H, CH), 8.06 (s, 1H, CH), 7.43 (app. s, 1H, NH), 7.20–7.27 (m, 8H, 5 × CH-Ar, NH_2, NH), 4.26 (app.s, 2H, CH_2), 3.87 (app.s, 2H, CH_2), 3.29 (app.s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 155.94 (C), 152.31 (CH), 149.52 (C), 141.05 (CH), 138.15 (C), 128.14 (2 × CH), 127.60 (2 × CH), 126.97 (CH), 118.80 (C), 45.65 (CH_2), 42.76 (CH_2), 41.58 (CH_2). HRMS m/z (EI): Calcd for 348.1237 $[M+H]^+$, Found 348.1237 $[M+H]^+$.

*2.1.5.2. N-[2-(6-amino-9H-purin-9-yl)]-N′-(4-methoxybenzyl)sulfamide (**14**)*. Yield: 50%. MP (°C): 186–188. ^{1}H NMR (DMSO-d_6) δ: 8.16 (s, 1H, CH), 8.06 (s, 1H, CH), 7.34 (t, J = 5.5, 1H, NH), 7.22 (s, 2H, NH_2), 7.17 (d, J = 7.9, 3H, 2 × CH-Ar, NH), 6.86 (d, J = 8.3, 2H, 2 × CH-Ar), 4.25 (t, J = 5.5, 2H, CH_2), 3.80 (d, J = 5.6, 2H, CH_2), 3.73 (s, 3H, CH_3), 3.27 (d, J = 4.8, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 158.36 (C), 155.94 (C), 152.33 (CH), 149.51 (C), 141.08 (CH), 129.98 (C), 128.96 (2 × CH), 118.78 (C), 113.57 (2 × CH), 55.02 (CH_3), 45.15 (CH_2), 42.75 (CH_2), 41.57 (CH_2). HRMS m/z (EI): Calcd for 378.1343 $[M+H]^+$, Found: 378.1345 $[M+H]^+$.

*2.1.5.3. N-[2-(6-amino-9H-purin-9-yl)-ethyl]-N′-(3 chlorobenzyl)sulfamide (**15**)*. Yield: 15%. MP (°C): 182–184. ^{1}H NMR (DMSO-d_6) δ: 8.15 (s, 1H, CH), 8.07 (s, 1H, CH), 7.52 (t, J = 6.4, 1H, NH), 7.19–7.35 (m, 7H, NH, NH_2, 4 × CH-Ar), 4.25 (t, J = 6.0, 2H, CH_2), 3.89 (d, J = 6.4, 2H, CH_2), 3.29 (q, J = 6.0, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 155.94 (C), 152.33 (CH), 149.53 (C), 141.06 (CH), 140.94 (C), 132.86 (C), 130.03 (CH), 127.31 (CH), 126.91 (CH), 126.17 (CH), 118.79 (C), 44.95 (CH_2), 42.80 (CH_2), 41.55 (CH_2). HRMS m/z (EI): Calcd for 382.0847 $[M+H]^+$, Found: 382.0850 $[M+H]^+$.

*2.1.5.4. N-[2-(6-amino-9H-purin-9-yl)ethyl]-N′-(2,4-dichlorobenzyl)sulfamide (**16**)*. Yield: 45%. MP (°C): 164–168. ^{1}H NMR (DMSO-d_6) δ: 8.15 (s, 1H, CH), 8.07 (s, 1H, CH), 7.50 (m, 2H, NH + CH-Ar), 7.49 (d, J = 8.4, CH-Ar), 7.43(dd, J = 1.7, 8.4, 1H, CH-Ar), 7.34 (t, J = 5.75, 1H, NH), 7.19 (s, 2H, NH_2, ex), 4.2 (t, J = 5.9, 2H, CH_2), 3.9 (d, J = 5.9, 2H, CH_2), 3.31 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 155.94 (C), 152.31 (CH), 149.52 (C), 141.01 (CH), 134.77 (C), 132.86 (C), 132.37 (C), 130.72 (CH), 128.39 (CH), 127.21 (CH), 48.79 (C), 42.75 (CH_2), 42.65 (CH_2), 41.56 (CH_2). Anal. calcd for $C_{14}H_{15}N_7Cl_2O_2S$: C, 40.39; H, 3.63; N, 23.55. Found: C, 40.10; H: 3.80; N, 23.36.

*2.1.5.5. N-[2-(6-amino-9H-purin-9-yl)ethyl]-N′-(3,4-dichlorobenzyl)sulfamide (**17**)*. Yield: 40%. MP (°C): 192–197. ^{1}H NMR (DMSO-d_6) δ: 8.15 (s, 1H, CH), 8.07 (s, 1H, CH), 7.56

(m, 3H, 2 × CH-Ar + NH), 7.26 (m, 2H, CH-Ar + NH), 7.19 (s, 2H, NH_2), 4.25 (m, 2H, CH_2), 3.89 (s, 2H, CH_2), 3.29 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 155.91 (C), 152.33 (CH), 149.51 (C), 141.09 (CH), 139.66 (C), 130.76 (C), 130.32 (CH), 129.50 (C), 129.43 (CH), 127.83 (CH), 118.75 (C), 44.38 (CH_2), 42.81 (CH_2), 41.54 (CH_2). HRMS *m/z* (EI): Calcd for 416.0458 $[M+H]^+$, Found: 416.0453 $[M+H]^+$.

*2.1.5.6. N-[2-(6-amino-9H-purin-9-yl)ethyl]-N′-(4-fluorobenzyl)sulfamide (**18**).* Yield: 67%. MP (°C): 136–140. ^{1}H NMR (DMSO-d_6) δ: 8.16 (s, 1H, CH), 8.07 (s, 1H, CH), 7.44 (t, J = 6.25, 1H, NH), 7.29 (m, 2H, CH-Ar), 7.21 (m, 3H, NH + NH_2), 7.12 (t, J = 8.8, 2H, CH-Ar), 4.26 (t, J = 6.0, 2H, CH_2), 3.86 (d, J = 6.2, 2H, CH_2), 3.28 (q, J = 5.9, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 162.23 (C), 160.30 (C), 155.93 (C), 152.32 (CH), 149.51 (C), 141.09 (CH), 134.38 (C), 129.60 (CH), 129.53 (CH), 118.77 (C), 114.95 (CH), 114.78 (CH), 44.88 (CH_2), 42.76 (CH_2), 41.56 (CH_2). HRMS *m/z* (EI): Calcd for 365.1070 $[M]^+$, Found: 365.1073 $[M]^+$.

*2.1.5.7. N-[2-(6-amino-9H-purin-9-yl)-ethyl]-N′-(4-cyanobenzyl)sulfamide (**19**).* Yield: 59.5%. MP (°C): 202–206. ^{1}H NMR (DMSO-d_6) δ: 8.16 (s, 1H, CH), 8.07 (s, 1H, CH), 7.78 (d, J = 7.8, 2H, CH-Ar), 7.59 (bs, 1H, NH), 7.47 (d, J = 7.6, 2H, CH-Ar), 7.27 (bs, 1H, NH), 7.20 (bs, 2H, NH_2), 4.25 (app.s, 2H, CH_2), 3.97 (d, J = 5.1, 2H, CH_2), 3.29 (app. d, J = 5.0, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 155.94 (C), 152.33 (CH), 149.52 (C), 144.29 (C), 141.08 (CH), 132.12 (2 × CH), 128.30 (2 × CH), 118.82 (C), 118.78 (C), 109.74 (C), 45.14 (CH_2), 42.78 (CH_2), 41.55 (CH_2). HRMS *m/z* (EI): Calcd for 372.1117 $[M]^+$, Found: 372.1114 $[M]^+$.

*2.1.5.8. N-[2-(1H-benzimidazol-1-yl)ethyl]-N′-benzylsulfamide (**20**).* Yield: 82%. MP (°C): 181–186. ^{1}H NMR (DMSO-d_6) δ: 8.17 (s, 1H, CH-Imidazole), 7.67 (d, J = 7.9, 1H, CH-Ar), 7.62 (d, J = 8.0, 1H, CH-Ar), 7.46 (t, J = 6.1, 1H, NH), 7.2 (m, 8H, 7 × CH-Ar + NH), 4.3 (t, J = 6.0, 2H, CH_2), 3.8 (d, J = 6.1, 2H, CH_2), 3.2 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 144.24 (CH-Imidazole), 143.43 (C), 138.14 (C), 133.83 (C), 128.16 (2 × CH), 127.62 (CH), 127.00 (CH), 122.25 (CH), 121.44 (CH), 119.40 (2 × CH), 110.37 (CH), 45.59 (CH_2), 43.71 (CH_2), 41.89 (CH_2). HRMS *m/z* (EI): Calcd: 331.1223 $[M+H]^+$, Found: 331.1227 $[M+H]^+$.

*2.1.5.9. N-[2-(1H-benzimidazol-1-yl)ethyl]-N′-(4-methoxybenzyl)sulfamide (**21**).* Yield: 61%. MP (°C): 180–184. ^{1}H NMR (DMSO-d_6) δ: 8.18 (s, 1H, CH-Imidazole), 7.67 (d, J = 7.90, 1H, CH-Ar), 7.62 (d, J = 8.0, 1H, CH-Ar), 7.35 (t, J = 6.1, 1H, NH), 7.28 (ψt, J = 7.1, 7.8, 1H, CH-Ar), 7.23 (m,1H, CH-Ar), 7.17 (t, J = 5.9, 1H, NH), 7.13 (d, J = 8.5, 2H, 2 × CH-Ar), 6.85 (d, J = 8.6, 2H, 2 × CH-Ar), 4.35 (t, J = 6.1, 2H,CH_2), 3.73 (d, J = 5.95, 2H, CH_2), 3.72 (s, 3H, OCH_3), 3.2 (q, J = 5.65, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 158.36 (C), 144.22 (CH-Imidazole), 143.41 (C), 133.83 (C), 129.99 (C), 128.16 (2 × CH), 122.24 (CH), 121.43 (CH), 119.40 (CH), 113.57 (2 × CH), 110.35 (CH), 55.03 (CH_3) 45.11 (CH_2), 43.69 (CH_2), 41.88 (CH_2). Anal. calcd for $C_{17}H_{20}N_4O_3S$: C, 56.65; H, 5.59; N, 15.54. Found: C, 56.96; H, 5.58; N, 15.33.

*2.1.5.10. N-[2-(1H-benzimidazol-1-yl)ethyl]-N′-(3-chlorobenzyl)sulfamide (**22**).* Yield: 69%. MP (°C): 162–166. ^{1}H NMR (DMSO-d_6) δ: 8.18 (s, 1H, CH-Imidazole), 7.67 (d, J = 8.0, 1H, CH-Ar), 7.62 (d, J = 8.0, 1H, CH-Ar), 7.53 (t, J = 6.3, 1H, NH), 7.25 (m, 7H, 6 × CH-Ar + NH), 4.36 (t, J = 6.1, 2H, CH_2), 3.79 (d, J = 6.1, 2H, CH_2), 3.26 (q, J = 6.0, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 144.24 (CH-Imidazole), 143.42 (C), 140.88 (C), 133.83 (C), 132.85 (C), 130.03 (CH), 127.32 (CH), 126.92 (CH), 126.17 (CH), 122.26 (CH), 121.45 (CH), 119.39 (CH), 110.35 (CH), 44.89 (CH_2), 43.72 (CH_2), 41.89 (CH_2). Anal. calcd for $C_{16}H_{17}ClN_4O_2S$: C, 52.67; H, 4.70; N, 15.35. Found: C, 52.74; H, 4.79; N, 15.22.

*2.1.5.11. N-[2-(1H-benzimidazol-1-yl)ethyl]-N′-(2,4-dichlorobenzyl) sulfamide (**23**).* Yield: 64%. MP (°C): 158–162. ^{1}H NMR (DMSO-d_6) δ: 8.17 (s, 1H, CH-Imidazole), 7.65 (d, J = 7.9, 1H, CH-Ar), 7.61 (d, J = 8.0, 1H, CH-Ar), 7.55 (m, 2H, NH + CH-Ar), 7.41 (m, 2H, 2 × CH-Ar), 7.32 (t, J = 5.9, 1H, NH), 7.27 (t, J = 7.5, 1H, CH-Ar), 7.21 (t, J = 7.5, 1H, CH-Ar), 4.36 (t, J = 6.1, 2H, CH_2), 3.91 (d, J = 6.1, 2H, CH_2), 3.27 (q, J = 6.0, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 144.18 (CH-Imidazole), 143.38 (C), 134.71 (C), 133.80 (C), 132.91 (C), 132.40 (C), 130.79 (CH), 128.41 (CH), 127.20 (CH), 122.24 (CH), 121.42 (CH), 119.41 (CH), 110.29 (CH), 43.72 (CH_2), 42.80 (CH_2), 41.90 (CH_2). Anal. calcd for $C_{16}H_{16}Cl_2N_4O_2S$: C, 48.13; H, 4.04; N, 14.02. Found: C, 47.98; H, 4.06; N, 14.14.

*2.1.5.12. N-[2-(1H-Benzimidazol-1-yl)ethyl]-N′-(3,4-dichlorobenzyl)sulfamide (**24**).* Yield: 71%. MP (°C): 164–169. ^{1}H NMR (DMSO-d_6) δ: 8.17 (s, 1H, CH-imidazole), 7.67 (d, J = 8.0, 1H, CH-Ar), 7.62 (d, J = 8.0, 1H, CH-Ar), 7.51 (m, 1H, CH-Ar), 7.46 (s, 1H, CH-Ar), 7.2 (m, 5H, 3 × CH-Ar, 2 × NH, D_2O-exchangeable), 4.3 (t, J = 6.0, 2H, CH_2), 3.7 (d, J = 6.0, 2H, CH_2), 3.2 (q, J = 5.6, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 144.25 (CH-imidazole), 143.41 (C), 139.61 (C), 133.81 (C), 130.75 (C), 130.32 (CH), 129.50 (C), 129.42 (CH), 127.81 (CH), 122.26 (CH), 121.44 (CH), 119.39 (CH), 110.33 (CH), 44.51 (CH_2), 44.31 (CH_2), 43.72 (CH_2). HRMS *m/z* (EI): calcd for 399.0449 $[M+H]^+$, Found: 399.0446 $[M+H]^+$.

*2.1.5.13. N-[2-(1H-benzimidazol-1-yl)ethyl]-N′-(4-fluorobenzyl)sulfamide (**25**).* Yield: 80%. MP (°C): 176–180. ^{1}H NMR (DMSO-d_6) δ: 8.19 (s, 1H, CH-imidazole), 7.66 (d, J = 7.9, 1H, CH-Ar), 7.62 (d, J = 8.0, 1H, CH-Ar), 7.49 (t, J = 6.4, 1H, CH-Ar), 7.25 (m, 5H), 7.11 (m, 2H), 4.36 (t, J = 6.0, 2H, CH_2), 3.74 (d, J = 6.3, 2H, CH_2), 3.23 (q, J = 6.0, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 162.21 (C), 160.28 (C), 144.27 (CH), 143.36 (C), 134.34 (C), 133.79 (C), 129.60 (CH), 129.54 (CH), 122.27 (CH), 121.46 (CH), 119.38 (CH), 114.98 (CH), 114.81 (CH), 110.38 (CH), 44.77 (CH_2), 43.67 (CH_2), 41.86 (CH_2). HRMS (EI): Calcd for 349.1135 $[M+H]^+$, Found: 349.1136 $[M+H]^+$.

*2.1.6. General procedure for the synthesis of N-[2-(adenyl- or benzimidazolyl)ethyl]-N′-(un)substitutedbenzylureas or thioureas (**26–33**)*

To a solution of 9-(2-aminoethyl)-9H-purin-6-amine trifluoroacetate (**5**; 1.2 eq.) or 2-(1H-benzimidazol-1-yl)ethanamine trifluoroacetate (**6**; 1.2 eq.) in anhydrous acetonitrile (10 mL), was added triethylamine (2.4 eq.) followed by a solution of the appropriate benzylisocyanate (1 eq.) in anhydrous

acetonitrile (5 mL) while cooling at 0 °C (ice bath). After 30 min the reaction was stirred at 60 °C overnight. The reaction mixture was cooled to room temperature and stirred for 2 h. The suspension obtained was filtered and the precipitate was washed with water. The residue was dissolved in ethyl acetate (100 mL) and washed with H_2O (3 × 50 mL). The organic layer was dried over $MgSO_4$ and evaporated under vacuum. The residue was saturated with diethyl ether and stirred for 2 h. The suspension obtained was filtered and the precipitate was washed with water to provide the title compounds as white solids.

*2.1.6.1. N-[2-(6-amino-9H-purin-9-yl)ethyl]-N′-benzyl urea (**26**).* Yield: 71%. MP (°C): 212–216. ^{1}H NMR (DMSO-d_6) δ: 8.15 (s, 1H, CH-adenine), 8.02 (s, 1H, CH-adenine), 7.20 (m, 7H, CH-Ar + NH_2), 6.38 (t, J = 5.7, 1H, NH), 6.09 (t, J = 5.5, 1H, NH), 4.20 (m, 4H, 2 × CH_2), 3.48 (q, J = 5.5, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 157.89 (C), 155.92 (C), 152.30 (CH-adenine), 149.67 (C), 140.91 (CH-adenine), 140.76 (C), 128.14 (2 × CH-Ar), 126.88 (2 × CH-Ar), 126.46 (CH-Ar), 118.77 (C), 43.48 (CH_2), 42.82 (CH_2), CH_2 signal is obscured by DMSO-d_6 signal (39.01–39.85). HRMS m/z (EI): calcd for 312.1567 $[M+H]^+$, Found: 312.1565 $[M+H]^+$.

*2.1.6.2. N-[2-(6-amino-9H-purin-9-yl)ethyl]-N′-(4-methoxybenzyl) urea (**27**).* Yield: 91%. MP (°C): 206–210. ^{1}H NMR (DMSO-d_6) δ: 8.14 (s, 1H, CH-adenine), 8.02 (s, 1H, CH-adenine), 7.21 (bs, 2H, NH_2), 7.1 (d, J = 8.4, 2H, CH-Ar), 6.85 (d, J = 8.5, 2H, CH-Ar), 6.30 (t, J = 5.6, 1H, NH), 6.05 (t, J = 5.0, 1H, NH), 4.18 (t, J = 5.75, 2H, CH_2), 4.09 (d, J = 5.8, 2H, CH_2), 3.72 (s, 3H, OCH_3), 3.47 (q, J = 5.7, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 157.98 (C), 157.84 (C), 155.90 (C), 152.30 (CH), 149.62 (C), 140.93 (CH), 132.62 (C), 128.18 (2 × CH), 118.70 (C), 113.54 (2 × CH), 55.00 (CH_3), 43.48 (CH_2), 42.24 (CH_2), CH_2 signal is obscured by DMSO-d_6 signal (39.94–38.98). Anal. calcd for $C_{16}H_{19}N_7O_2$: C, 56.30; H: 5.61; N, 28.71. Found: C, 55.92; H, 5.62; N, 28.53.

*2.1.6.3. N-[2-(6-amino-9H-purin-9-yl)ethyl]-N′-(2,4-dichlorobenzyl)urea (**28**).* Yield: 80%. MP (°C): 228–232. ^{1}H NMR (DMSO-d_6) δ: 8.14 (s, 1H, CH-adenine), 8.03 (s, 1H, CH-adenine), 7.5 (d, J = 2.1, 1H, CH-Ar), 7.39 (dd, J = 2.0, 8.4, 1H, CH-Ar), 7.19 (d, J = 8.4, 1H, CH-Ar), 7.13 (bs, 2H, NH_2), 6.46 (t, J = 5.9, 1H, NH), 6.25 (t, J = 5.8, 1H, NH), 4.19 (m, 4H, 2 × CH_2), 3.47 (q, J = 5.6, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 157.74 (C), 155.88 (C), 152.30 (CH), 149.65 (C), 140.98 (CH), 136.99 (C), 132.51 (C), 131.79 (C), 129.71 (CH), 128.32 (CH), 127.17 (CH), 118.70 (C), 43.41 (CH_2), 40.34 (CH_2), CH_2 signal is obscured by DMSO-d6 signal (39.94–38.94). Anal. calcd for $C_{15}H_{15}Cl_2N_7O$: C, 47.38; H, 3.98; N, 25.79. Found: C, 47.11; H, 3.73; N, 25.79.

*2.1.6.4. N-[2-(1H-Benzimidazol-1-yl)ethyl]-N′-benzyl urea (**29**).* Yield: 75%. MP (°C): 158–162. ^{1}H NMR (DMSO-d_6) δ: 8.14 (s, 1H, CH-imidazole), 7.66 (d, J = 7.6, 1H, CH-Ar), 7.60 (d, J = 7.7, 1H, CH-Ar), 7.25 (m, 7H, CH-Ar), 6.47 (t, J = 5.8, 1H, NH), 6.16 (t, J = 5.4 Hz, 1H, NH), 4.30 (t, J = 6.0, 2H, CH_2), 4.20 (d, J = 5.9, 2H, CH_2), 3.45 (dd, J = 6.0, 11.9, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 158.00 (C), 144.05 (CH-imidazole), 143.40 (C), 140.77 (C), 134.02 (C), 128.16 (2 × CH), 126.94 (2 × CH), 126.49 (CH), 122.17 (CH), 121.34 (CH), 119.34 (CH), 110.34 (CH), 44.39 (CH_2), 42.82 (CH_2), CH_2 signal is obscured by DMSO-d_6 signal (39.01–39.85). HRMS m/z (EI): clacd for 295.1553 $[M+H]^+$, Found: 295.1557 $[M+H]^+$.

*2.1.6.5. N-[2-(1H-benzimidazol-1-yl)ethyl]-N′-(4-methoxybenzyl)urea (**30**).* Yield: 43%. MP (°C): 124–128. ^{1}H NMR (DMSO-d_6) δ: 8.13 (s, 1H, CH-imidazole), 7.66 (d, J = 7.4, 1H, CH-Ar), 7.60 (d, J = 7.6, 1H, CH-Ar), 7.22 (m, 2H, CH-Ar), 7.13 (d, J = 8.6, 2H, CH-Ar), 6.86 (m, 2H, CH-Ar), 6.34 (t, J = 5.8, 1H, NH), 6.05 (t, J = 5.7, 1H, NH), 4.29 (t, J = 6.0, 2H, CH_2), 4.12 (d, J = 5.9, 2H, CH_2), 3.73 (s, 3H, OCH_3), 3.43 (q, J = 6.0, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 158.02 (C), 157.92 (C), 144.06 (CH-imidazole), 143.38 (C), 133.99 (C), 132.63 (C), 128.16 (2 × CH), 122.19 (CH), 121.36 (CH), 119.34 (CH), 113.57 (2 × CH), 110.35 (CH), 55.00 (CH_3), 44.40 (CH_2), 42.39 (CH_2), CH_2 signal is obscured by DMSO-d_6 signal (39.95–38.95). Anal. calcd for $C_{18}H_{20}N_4O_2$: C, 66.65; H: 6.21; N, 17.26. Found: C, 66.62; H, 6.19; N, 16.91.

*2.1.6.6. N-[2-(1H-benzimidazol-1-yl)ethyl]-N′-(2,4-dichlorobenzyl)urea (**31**).* Yield: 64%. MP (°C): 148–152. ^{1}H NMR (DMSO-d_6) δ: 8.14 (s, 1H, CH-imidazole), 7.66 (d, J = 7.5, 1H, CH-Ar), 7.59 (d, J = 7.8, 1H, CH-Ar), 7.56 (d, J = 2.0, 1H, CH-Ar), 7.39 (dd, J = 2.0, 8.3, 1H, CH-Ar), 7.22 (m, 3H, CH-Ar), 6.50 (t, J = 5.9, 1H, NH), 6.25 (t, J = 5.6 Hz, 1H, NH), 4.30 (t, J = 6.0, 2H, CH_2), 4.22 (d, J = 5.9, 2H, CH_2), 3.45 (q, J = 5.9, 11.9, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 157.81 (C), 144.07 (CH-imidazole), 143.36 (C), 136.99 (C), 133.99 (C), 132.61 (C), 131.82 (C), 129.92 (CH), 128.43 (CH), 127.23 (CH), 122.19 (CH), 121.38 (CH), 119.34 (CH), 110.31 (CH), 44.35 (CH_2), 40.50 (CH_2), CH_2 signal is obscured by DMSO-d_6 signal (39.98–39.98). Anal. calcd for $C_{17}H_{16}Cl_2N_4O{\cdot}0.2\,H_2O$: C, 55.66; H, 4.50; N, 15.27. Found: C, 55.46; H, 4.33; N, 14.96.

*2.1.6.7. N-[2-(6-Amino-9H-purin-9-yl)ethyl]-N′-benzyl thiourea (**32**).* Yield: 75%. MP (°C): 176–182. ^{1}H NMR (DMSO-d_6) δ: 8.15 (s, 1H, CH), 8.01 (s, 1H, CH), 7.94 (bs, 1H, NH), 7.60 (bs, 1H, NH), 7.32 (t, J = 7.3, 2H, CH-Ar), 7.23 (m, 3H, CH-Ar), 7.19 (bs, 2H, NH_2), 4.64 (app.s, 2H, CH_2), 4.34 (app.s, 2H, CH_2), 3.90 (app.s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 155.93 (C), 152.33 (CH), 149.67 (C), 140.81 (CH), 139.14 (C), 128.20 (2 × CH), 127.11 (2 × CH), 126.75 (CH), 118.76 (C), 42.47 (CH_2), 2 × CH_2 signals are obscured by DMSO-d_6 signal (40.01–39.01). HRMS m/z (EI): calcd for 328.1339 $[M+H]^+$, Found: 328.1339 $[M+H]^+$.

*2.1.6.8. N-[2-(1H-benzimidazol-1-yl)ethyl]-N′-benzyl thiourea (**33**).* Yield: 93%. MP (°C): 158–162. ^{1}H NMR (DMSO-d_6) δ: 8.14 (s, 1H, CH-imidazole), 7.95 (bs, 1H, NH), 7.66 (ψt, J = 7.1, 5.8, 2H, CH-Ar), 7.57 (bs, 1H, NH), 7.33 (ψt, J = 7.9, 7.0, 2H-Ar), 7.26 (m, 5H, CH-Ar), 4.66 (app.s., 2H, CH_2), 4.47 (t, J = 6.0, 2H, CH_2), 3.87 (app.s., 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 143.85 (CH), 142.73 (C), 133.88 (C), 128.27 (2 × CH), 127.06 (2 × CH), 126.90 (CH), 122.57 (CH), 121.76 (CH), 119.20 (CH), 110.36 (CH), 43.25 (CH_2), 2 CH_2 signals are obscured by DMSO-d_6 signal (39.47–38.45). HRMS m/z (EI): calcd for 311.1325 $[M+H]^+$, Found: 311.1325 $[M+H]^+$.

2.1.7. 2-(6-amino-9H-purin-9-yl)ethanol (**34**)

Adenine (3) (5.60 g, 41.4 mmol), ethylene carbonate (4 g, 45.4 mmol), and a trace of sodium hydroxide were dissolved in anhydrous DMF (45 mL). The reaction mixture was refluxed for 3.5 h. then left stirring overnight at room temperature. The solvent was removed under vacuum and the residue recrystallized from methanol to afford [2-(6-amino-9H-purin-9-yl)ethanol] (13) as a white solid.

Yield: 70%. MP (°C): 240–242 °C (lit. 238.3–240.4).[23] ^{1}H NMR (DMSO-d_6): δ 8.15 (s, 1H, CH), 8.09 (s, 1H, CH), 7.20 (bs, 2H, NH_2), 5.02 (t, J = 5.4, 1H, OH), 4.20 (t, J = 5.5, 2H, CH_2), 3.75 (q, J = 5.4, 2H, CH_2). The product was used in the next step without further characterization.

2.1.8. 2-(6-amino-9H-purin-9-yl)ethyl methanesulfonate (**35**)

To a solution of 2-(6-amino-9H-purin-9-yl)ethanol **(34**; 4.94 g, 27.6 mmol) in dry pyridine (76 mL) cooled to 0 °C, methanesulfonyl chloride (2.35 mL, 30.3 mmol) was added. After 10 min the reaction was allowed to warm to room temperature. After 1 h, pyridine was evaporated and the residue dissolved in water (100 mL). The aqueous solution was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried with $MgSO_4$ and filtered. The solvent was removed under vacuum and remaining pyridine was removed by adding toluene to form an azeotropic mixture which was then removed under vacuum. 2-(6-amino-9H-purin-9-yl)ethyl methanesulfonate **(14)** was obtained as a white solid.

Yield: 51%. MP (°C): 186–190 (lit. 189–192).[24] ^{1}H NMR (DMSO-d_6): δ 8.17 (s, 1H, CH), 8.15 (s, 1H, CH), 7.26 (bs, 2H, NH_2), 4.61 (t, J = 5.2, 2H, CH_2), 4.51 (t, J = 5.1, 2H, CH_2), 3.12 (s, 3H, CH_3). The product was used in the next step without further characterization.

2.1.9. N^1-(2-(6-Amino-9H-purin-9-yl)ethyl)-N^2-benzylethane-1,2-diamine tri-trifluoroacetate (**36**)

To a solution of *N*-benzyl ethylene diamine (0.59 g, 3.92 mmol) in ethanol (40 mL) was added methanesulfonic acid 2-(6-amino-purin-9-yl)ethyl ester **(35**; 0.4 g, 1.57 mmol). The reaction mixture was refluxed for 3 days at 80 °C. The solvent was removed under vacuum to afford the crude product as a yellowish white solid which was dissolved in methanol and the solution was treated with triethylamine (0.6 g, 6.0 mmol) and di-*tert*-butyl dicarbonate (1.31 g, 6.0 mmol). The reaction mixture was refluxed for 1 h then stirred at room temperature overnight. The solvent was removed under vacuum and the residue dissolved in dichloromethane (50 mL) then washed with 1 N HCl (2 × 20 mL) and brine (2 × 20 mL). The organic layer was dried over $MgSO_4$ and evaporated under vacuum. The residue was saturated with diethyl ether and stirred for 2 h then the resulting suspension was filtered to afford the BOC protected intermediate as a white solid. A solution of the crude product in trifluoroacetic acid/dichloromethane (20 mL,75:25 v/v) was stirred overnight at room temperature. The solvent was then co-evaporated with ethanol several times. The residue was saturated with diethyl ether (75 mL), stirred for 2 h to give the title compound as a white solid collected by filtration.

Overall yield: 11%. MP (°C): 178–181. ^{1}H NMR (CD_3OD) δ: 8.25 (s, 1H, CH-Adenine), 8.22 (s, 1H, CH-Adenine), 7.51–7.46 (m, 5H, CH-Ar), 4.57 (t, J = 5.7, 2H, CH_2), 4.25 (s, 2H, CH_2), 3.48 (t, J = 5.7, 2H, CH_2), (2 × CH_2) peaks were obscured under CD_3OD peak. ^{13}C NMR (CD_3OD) δ: 155.77 (C), 151.26 (CH), 150.86 (C), 143.65 (CH), 132.43 (C), 130.97 (2 × CH), 130.82 (CH), 130.37 (2 × CH), 120.10 (C), 52.48 (CH_2), 45.40 (CH_2), 45.12 (CH_2), 43.17 (CH_2), CH_2 signal was obscured under CD_3OD signals (50.39–48.51). HRMS *m/z* (EI): Calcd for 652.1566 $[M-H]^-$, Found: 652.1566 $[M-H]^-$.

2.2. Microbiological assays

2.2.1. In vitro antibacterial activity against C. difficile strain 1813

Frozen stocks of *C. difficile* 1813 strain were thawed and streaked for purity on pre-prepared fastidious anaerobe agar plates and incubated at 37 °C for 48 h in an anaerobic cabinet (A95 Workstation, Don Whitley Ltd., UK). The gas mixture of the cabinet was composed of 10% CO_2, 10% H_2 and 80% N_2 as recommend by the CLSI (2003). Post incubation, plates were inspected for purity and visible CFUs were added to 3 mL of sterile brain heart infusion broth (BHI) using a 10 µL inoculation loop to give the broth turbidity equal to a McFarland standard of 1 (~108 cfu/mL). To ensure that suspensions of cultures with turbidity equal to a McFarland standard of 1 approximately contained 1 × 108 cfu/mL, viable counts were performed on strain 1813 using the Miles and Misra method.[25] McFarland turbidity standards were used to standardize cultures to all concentrations. A stock solution of each compound (256 µg/mL) was prepared. The method used plates for each concentration of bacteria (10^6, 10^5, 10^4, 10^3, 10^2 and 10^1). For each concentration of bacteria, doubling dilutions of compounds were used from the highest concentration 85.33 µg/mL down to 0.33 µg/mL. Finally, controls consisted of Vancomycin at 12 µg/mL – complete inhibition (of all strains)/Metronidazole at 8 µg/mL – complete inhibition (of all strains). In addition, a control of broth and 20% DMSO – no inhibition of growth, broth and water – no inhibition of growth, broth only - no inhibition of growth.

2.2.2. In vitro antibacterial activity against B. anthracis

B. anthracis was grown overnight at 37 °C within brain heart infusion (BHI) broth with shaking at 250 rpm. Again the concentration of bacteria was confirmed by the Miles and Misra

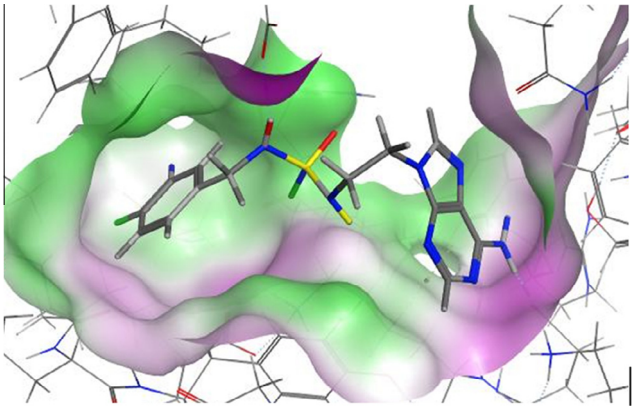


Figure 2 Adenine-based sulfamide (**18**) sitting in *C. difficile* methionyl-tRNA synthetase two active site pockets.

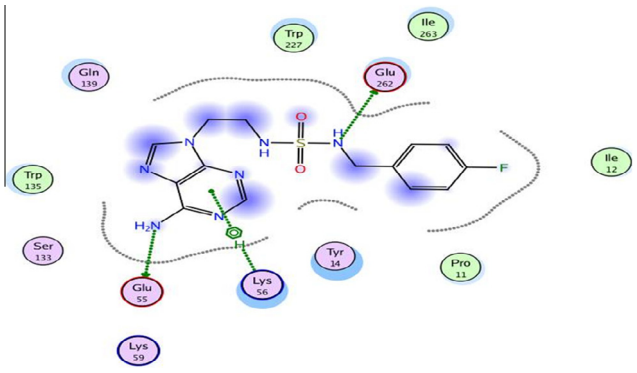


Figure 3 2D ligand interaction between sulfamide derivative **18** and the *C. difficile* methionyl-tRNA synthetase active site residues.

drop count and McFarland standard. For this test a single concentration of bacteria was used 1×10^4 cfu/mL. Different volumes of the stock solution of test compounds (256 μg/mL) were put in separate wells, diluted with an adequate amount of distilled water, mixed with 100 μL of BHI broth and 50 μL of *B. anthracis* (1×10^4 cfu/mL) to give 8 different final concentrations of the compounds: 102.4 μg/mL, 92.16 μg/mL, 81.92 μg/mL, 71.68 μg/mL, 61.44 μg/mL, 51.2 μg/mL, 40.96 μg/mL, and 30.72 μg/mL. Growth was then measured using a plate reader (Infinitie F2000 Pro) for 24 h with absorbance readings being taken at 595 nm every 5 min. Controls consisted of 12 μg/mL of ciprofloxacin, 20% DMSO diluted in the same way as the test solutions and BHI broth with bacteria only at 1×10^4 cfu/mL. Results were interpreted by comparison to the bacteria alone in BHI broth and inhibition could be observed by no increase in absorbance.

3. Results and discussion

3.1. Chemistry

The synthetic routes for the designed target compounds are illustrated in Schemes 1–3. The BOC-protected heteroaryl ethylamines (**2**,**3**) were prepared following reported procedures[21] through treatment of benzimidazole or adenine with *tert*-butyl (2-bromoethyl)carbamate (**1**) in the presence of potassium carbonate and 18-crown-6. BOC deprotection was effected by trifluoroacetic acid to afford the intermediate heteroaryl ethylamines (**4**,**5**) as the trifluoroacetate salts in almost quantitative yields. On the other hand, the sulfamoylating intermediates, *N*-sulfamoyloxazolidinones (**6–12**), were prepared following a reported procedure[22] by reacting chlorosulphonyl isocyanate

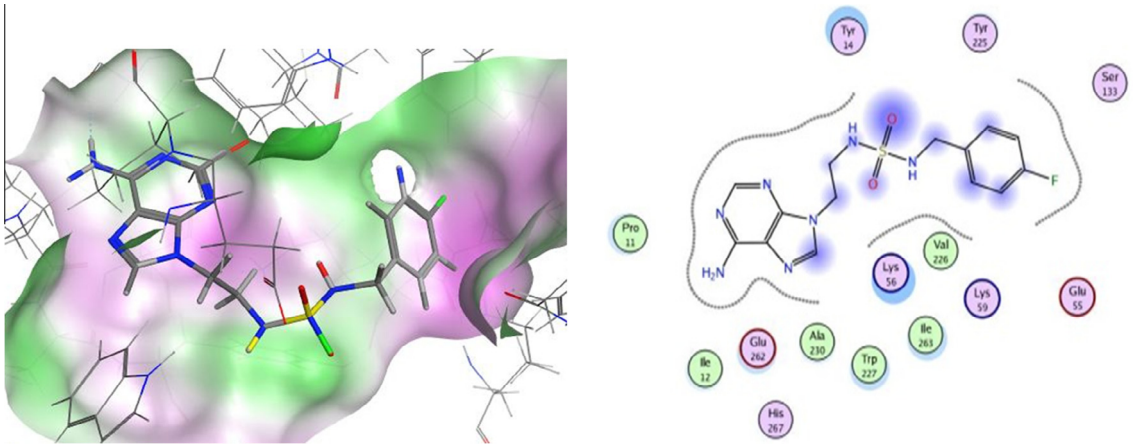
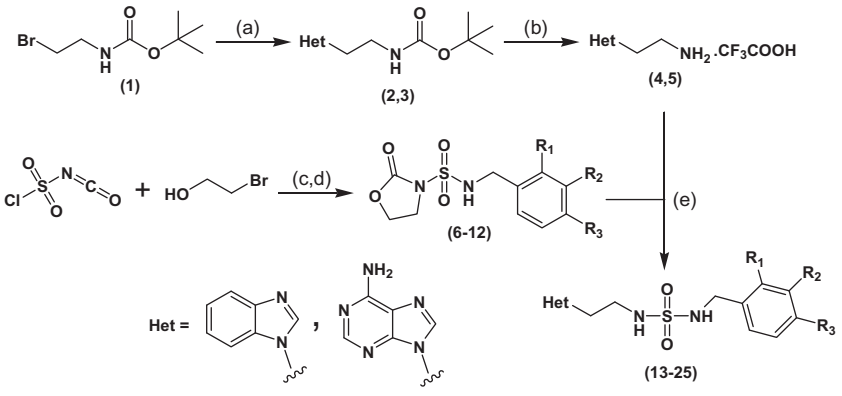
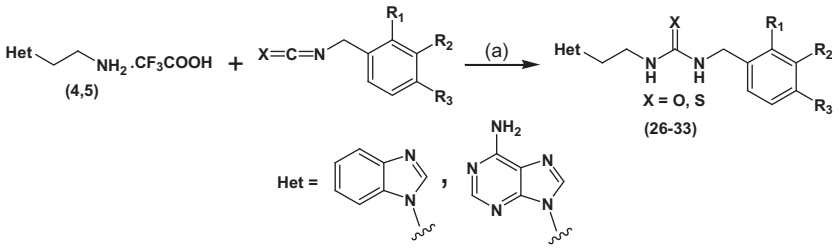


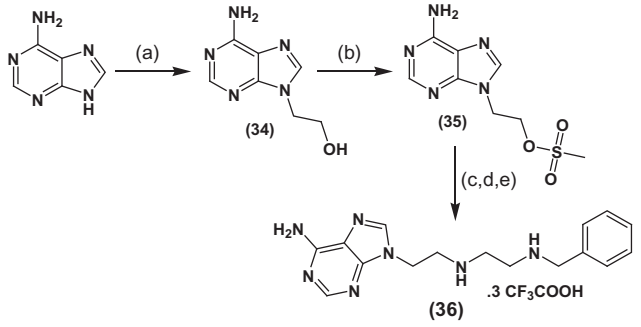
Figure 4 Compound (**18**) docked in the flipped position into *C. difficile* methionyl-tRNA synthetase two active site pockets.



Scheme 1 Reagents and conditions: (a) Benzimidazole or adenine, K_2CO_3, 18-crown-6, DMF, 60 °C, overnight. (b) TFA/DCM, room temperature, overnight. (c) DCM, 0–10 °C, 1 h. (d) Substituted benzylamines, TEA, DCM, 0 °C to room temperature. (e) TEA, acetonitrile, 90 °C, overnight.



Scheme 2 Reagents and conditions: (a) TEA, acetonitrile, 0–60 °C, overnight.



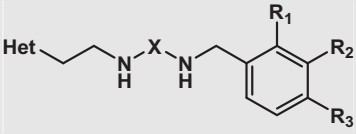
Scheme 3 Reagents and conditions: (a) Ethylene carbonate, NaOH, DMF, reflux, 3.5 h then room temperature, overnight. (b) Mesyl chloride, pyridine, 0 °C to room temperature, 1 h. (c) *N*-benzyl ethylenediamine, ethanol, 80 °C, 3 days. (d) Di-*tert*-butyl dicarbonate, TEA, methanol, reflux, 1 h then room temperature, overnight. (e) TFA, DCM, room temperature, overnight.

with 2-bromoethanol followed by treatment with substituted benzylamines in the presence of trimethylamine under nitrogen atmosphere as shown in Scheme 1. Sulfamoylation of the heteroaryl ethylamines (**4**,**5**) with the intermediates (**6–12**) under basic conditions of trimethylamine afforded the target sulfamides (**13–25**) in fairly good yields. In ^{1}H NMR spectra of the adenine-based sulfamides, two characteristic downfield singlets appeared at 8.15–8.16 and 8.06–8.07 ppm corresponding to adenine ring protons at C-8 and C-2, respectively. In case of the benzimidazole-based sulfamides, however, ring proton at C-2 apparently appeared more downfield (8.17–8.19 ppm) as compared to other ring protons. The sulfamide protons consistently appeared at 7.17–7.59 ppm within the aromatic region. In ^{13}C NMR spectra, the right number of carbon atoms appeared at their expected chemical shifts. In Scheme 2, the key intermediate heteroaryl ethylamines (**6–12**) were treated with substituted benzylisocyanates or benzylisothiocyanates under basic conditions to give the desired ureas and thioureas (**26–33**) in good yields. Finally, the target diamine compound (**36**) was obtained in three steps as illustrated in Scheme 3. Hydroxyethylation of adenine was achieved in 70% yield using ethylene carbonate in the presence of traces of sodium hydroxide.[23] The hydroxyethyl adenine (**34**) was mesylated under basic conditions of pyridine to afford the intermediate (**35**)[24] which was reacted with *N*-benzyl ethylene diamine to give the final diamine (**36**) in low overall yield due to chemical purification through BOC protection and deprotection. ^{1}H and ^{13}C NMR spectra of the target ureas, thioureas (**26–33**) as well as the target diamine (**36**) showed similar patterns to their sulfamide counterparts. Structures of all the synthesized compounds were characterized by means of ^{1}H NMR, ^{13}C NMR spectral data and purity of the final target compounds was confirmed by High Resolution Mass Spectroscopy or microanalysis. Docking interactions of the target compounds were performed using Molecular Operating Environment (MOE).[26] The target adenine-based sulfamide (**18**) was found to have reasonable docking result based on both visual inspection of the pose and the docking score. Compound (**18**) fitted well in the two pockets previously observed for REP-3123[17] with different hydrophobic and hydrogen bonding interactions (Fig. 2). Lys56, Glu262, Glu55, Lys59 and Ile12 were found to be the main interactions for this compound (Fig. 3). In addition, compound (**18**) docked in the flipped position with the same key interactions (Fig. 4).

3.2. Biological evaluation

Out of twenty-two compounds synthesized, only sixteen were available for biological testing due to insufficient solubility in DMSO under the assay conditions. The other six compounds, namely, **22–25**, **28** and **31**, precipitated on dilution to 20% DMSO with distilled water. Most of the inadequately soluble compounds were benzimidazole-based and chloro substituted. Compounds with adequate solubility in DMSO were tested against *C. difficile* strain 1813 and *B. anthracis*. The minimum inhibitory concentration (MIC) for the test compounds was determined by the microdilution method in brain heart infusion (BHI) broth. Reference drugs in the assays were metronidazole and vancomycin for *C. difficile* and ciprofloxacin for *B. anthracis*. Among the compounds tested, only the adenine-based sulfamide (**18**) showed activity against *C. difficile* with an MIC of 85.33 μg/mL. Against *B. anthracis*, however, all the tested adenine-based sulfamides, with the exception of the cyano substituted derivative (**19**), and the benzimidazole-based sulfamides (**20**, **21**) displayed activity with an MIC of 102.4 μg/mL. On the other hand, only the benzimidazole derivative (**29**) was active (MIC = 102.4 μg/mL) among the ureas in both series. The thioureas (**32**, **33**) exhibited more promising activity with the adenine-based derivative (**32**) slightly more potent (MIC = 92.16 μg/mL) than the benzimidazole counterpart (**33**; MIC = 102.4 μg/mL). Finally, the adenine-based diamine (**36**) showed equal activity to its thiourea analogue (**32**) with an MIC of 92.16 μg/mL. Biological results are summarized in Table 1. In summary, the adenine-based analogues were, in general, better antibacterial agents as compared to their benzimidazole-based counterparts. The adenine-based sulfamide (**18**) was the only target

Table 1 *In vitro* antibacterial activity (μg/ml) of the target compounds against *C. difficile* and *B. anthracis*.



Cpd	Het.	X	R_1	R_2	R_3	MIC (μg/ml)	
						C. difficile-1813	*B. anthracis*
13	Adenine	SO_2	H	H	H	a	102.4
14	Adenine	SO_2	H	H	OCH_3	a	102.4
15	Adenine	SO_2	H	Cl	H	a	102.4
16	Adenine	SO_2	Cl	H	Cl	a	102.4
17	Adenine	SO_2	Cl	Cl	H	a	102.4
18	Adenine	SO_2	H	H	F	85.33	102.4
19	Adenine	SO_2	H	Cl	CN	a	a
20	Benzimidazole	SO_2	H	H	H	a	102.4
21	Benzimidazole	SO_2	H	H	OCH_3	a	102.4
22	Benzimidazole	SO_2	H	Cl	H	b	b
23	Benzimidazole	SO_2	Cl	H	Cl	b	b
24	Benzimidazole	SO_2	Cl	Cl	H	b	b
25	Benzimidazole	SO_2	H	H	F	b	b
26	Adenine	C═O	H	H	H	a	a
27	Adenine	C═O	H	H	OCH_3	a	a
28	Adenine	C═O	Cl	H	Cl	b	b
29	Benzimidazole	C═O	H	H	H	a	102.4
30	Benzimidazole	C═O	H	H	OCH_3	a	a
31	Benzimidazole	C═O	Cl	H	Cl	b	b
32	Adenine	C═S	H	H	H	a	92.16
33	Benzimidazole	C═S	H	H	H	a	102.4
36	Adenine	Ethyl	H	H	H	a	92.16

[a]Inactive; [b]Not tested.

compound that displayed activity against both target organisms. The thioureas can act as a better scaffold than the ureas as evident from the biological results of the unsubstituted thiourea (**32**) as compared to its unsubstituted urea counterpart (**26**). In addition, the limited solubility of most of the benzimidazoles directs us to focus on the adenine-based scaffold in our currently underway investigation for structure optimization.

Conflict of interest

The authors report that they have no conflict of interest to declare.

Acknowledgement

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