Brown, Alistair and Aljohani, Ahmed and Gill, Jason and Steel, Patrick and Sellars, Jonathan (2019) 'Identification of novel benzoza-[2,1,3]-diazole substituted amino acid hydrazides as potential anti-tubercular agents.', Molecules., 24 (4). p. 811.

Further information on publisher’s website:
https://doi.org/10.3390/molecules24040811

Publisher’s copyright statement:
© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Use policy

The full-text may be used and/or reproduced, and given to third parties in any format or medium, without prior permission or charge, for personal research or study, educational, or not-for-profit purposes provided that:
- a full bibliographic reference is made to the original source
- a link is made to the metadata record in DRO
- the full-text is not changed in any way

The full-text must not be sold in any format or medium without the formal permission of the copyright holders.

Please consult the full DRO policy for further details.
Identification of Novel Benzoxa-[2,1,3]-diazole Substituted Amino Acid Hydrazides as Potential Anti-Tubercular Agents

Alistair K. Brown 1,2,*, Ahmed K. B. Aljohani 1, Jason H. Gill 2,3, Patrick G. Steel 4 and Jonathan D. Sellars 1,2,5,*

1 Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, Catherine Cookson Building, Framlington Place, Newcastle upon Tyne NE2 4HH, UK; alistair.brown2@newcastle.ac.uk (A.K.B.); a.aljohani2@newcastle.ac.uk (A.K.B.A.)
2 School of Pharmacy, Faculty of Medical Sciences, King George VI Building, Newcastle upon Tyne NE1 7RU, UK
3 Northern Institute for Cancer Research, Paul O’Gorman Building, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne NE2 4HH, UK; jason.gill@newcastle.ac.uk
4 Department of Chemistry, Durham University, Lower Mountjoy, Stockton Road, Durham DH1 3LE, UK; p.g.steel@durham.ac.uk
5 Chemistry, School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne NE1 7RU, UK
* Correspondence: jon.sellars@newcastle.ac.uk; Tel.: +44-(191)-2082357

Abstract: Discovery and development of new therapeutic options for the treatment of Mycobacterium tuberculosis (Mtb) infection are desperately needed to tackle the continuing global burden of this disease and the efficacy and cost limitations associated with current medicines. Herein, we report the synthesis of a series of novel benzoxa-[2,1,3]-diazole substituted amino acid hydrazides in a two-step synthesis and evaluate their inhibitory activity against Mtb and selected bacterial strains of clinical importance utilising an end point-determined REMA assay. Alongside this, their potential for undesired cytotoxicity against mammalian cells was assessed employing standard MTT assay methodologies. It has been demonstrated using modification at three sites (the hydrazine, amino acid, and the benzodiazole) it is possible to change both the antibacterial activity and cytotoxicity of these molecules whilst not affecting their microbial selectivity, making them attractive architectures for further exploitation as novel antibacterial agents.

Keywords: benzoxa-[2,1,3]-diazole; antibacterial; Mycobacterium tuberculosis; amino acid; hydrazide

1. Introduction

Tuberculosis (TB) remains the predominant bacterial infectious disease globally, with approximately 10.4 million new cases and 1.7 million deaths recorded in 2016 [1]. Additionally, estimates suggest that over 20% of the population is latently infected with Mycobacterium tuberculosis (Mtb), the causative agent of TB [2]. Current treatment regimens of TB require patients to administer drug combinations for an extended period of time, at least six months, which is costly, prone to differential response rates, and exhibits significant problems with patient compliance and adherence [3]. The current treatment regimen involves combinations of rifampicin (RIF), isoniazid (INH), ethambutol (EMB), and pyrazinamide. However, resistance to these drug therapies has exacerbated the management issues of TB. In 2016 alone, almost 500,000 new cases of multidrug-resistant TB (resistant to both RIF and INH) and over 100,000 cases of RIF-resistant TB were identified [1]. Consequently, the identification of...
novel therapeutic options is an absolute necessity for the management of TB in the future [4]. In this context, in recent years several new drugs and/or regimens have been reported for TB, including bedaquiline [5], delamanid [6], clofazimine [7], bedazuline-protonamid-linezolid [8]. Worryingly, resistance to several of these approaches has already been reported, primarily because of similar drug resistance mechanisms and target pathways [9, 10]. Consequently, the development of new drug therapies for TB requires the discovery of new drug targets and novel structures which circumvent resistance mechanisms, whilst also enabling shorter treatment regimens.

Benzoxa-[2,1,3]-diazole, benzothia-[2,1,3]-diazole and benzotriazoles are a class of heterocycle which exhibit widespread therapeutic opportunities [11]. In addition, no studies have evaluated these compounds or their derivatives against Mtb. Inspired by virtual screening studies which identified simple substituted nitro benzoxa-[2,1,3]-diazoles as potential inhibitors of Mtb ATP phosphoribosyl transferase (HisG) and APS reductase (APSR) [12,13], we were attracted to this scaffold as a new starting point for the development of anti-TB drugs.

In this report we describe a series of novel benzoxa-[2,1,3]-diazole substituted amino acid hydrazides as selective drugs for the treatment of TB, highlighting the importance of the benzo-[2,1,3]-diazole, amino acid (AA) and the substituted aryl hydrazine (R₁), towards Mtb selectivity, potency, efficacy, and avoidance of toxicity against mammalian cells (Figure 1).

**Figure 1.** Inhibitors of *M. tuberculosis* based on the benzoxa-[2,1,3]-diazole framework highlighting the key modifications.

2. Results and Discussion

In the context of a study to identify novel antibacterial agents designed to overcome antimicrobial resistance, a small library of diverse bioactive compounds had previously been synthesised within our team. Using the Resazurin Microtiter Assay (REMA) [14–16], these compounds were screened for antibacterial activity at a fixed concentration (128 µg/mL) against a range of drug-susceptible bacteria including Gram-positive, Gram-negative and *mycolata* bacteria (Supporting Information, Table S1) which revealed that many possessed little utility, even at these high concentrations. However, benzo-[2,1,3]-diazole architectures 1–12 were shown to possess antibacterial activity, including activity against *mycolata* bacteria and *Mtb* (Figure 2).
To gain an improved understanding of the antibacterial potency and scope of these compounds, a dose-range REMA assay was performed (128–0.125 µg/mL, converted to µM if active) (Table 1).

**Table 1.** Selective antibacterial activity of benzodiazole compounds present in the compound library, expressed as mean inhibitory concentration (MIC) (µM). (-) = No activity in the REMA assay.

| Compound | S. aureus | E. coli | S. equi subsp. equi | S. agalactiae | S. pyogenes | R. equi | M. tuberculosis mc²7000 |
|----------|-----------|---------|---------------------|---------------|-------------|---------|-----------------------|
| 1        | -         | 11.9    | -                   | -             | 11.9        | 128.9   |                       |
| 2        | 0.7       | 29.8    | 5.6                 | 0.9           | -           | -       | 17.16                 |
| 3        | 3.4       | 3.4     | 1.7                 | 20.3          | -           | 5.1     | 0.59                  |
| 4        | 7.1       | -       | 0.9                 | 2.4           | -           | 9.5     | 80.7                  |
| 5        | -         | -       | -                   | -             | -           | -       | 109.7                 |
| 6        | -         | -       | -                   | -             | -           | -       | 23.0                  |
| 7        | -         | -       | -                   | -             | -           | -       | -                     |
| 8        | -         | -       | -                   | -             | -           | -       | -                     |
| 9        | -         | -       | -                   | -             | 3.6         | -       | 31.1                  |
| 10       | -         | -       | -                   | -             | -           | -       | 36.7                  |
| 11       | -         | -       | 27.6                | -             | -           | 10.4    | 148.4                 |
| 12       | -         | -       | -                   | -             | -           | 11.6    | 44.0                  |
| Isoniazid| -         | -       | -                   | -             | -           | -       | 0.91                  |
| Rifampin | 0.12      | 7.78    | 0.03                | 0.97          | 0.49        | 7.78    | 0.03                  |
| Ethambutol| -         | -       | -                   | -             | -           | -       | 9.79                  |
The results of the endpoint REMA assay revealed a mix of activity against the organisms tested, with simple substituted benzodiazole compounds 1–4 providing broad spectrum activity. Whilst the nitrobenzoa-[2,1,3]-diazole 3 showed the highest levels of activity against \textit{Mtb}, the lack of selectivity was a cause for concern. Replacing the nitro group with a sulphonamido amino acid moiety greatly improved specificity for \textit{mycolata} bacteria, with substituted benzoa-[2,1,3]-diazole 6 showing much higher activity than 5, suggesting poor cell wall penetration of 5 is due to the carboxylic acid moiety. Notwithstanding this, replacement of the benzoa-[2,1,3]-diazole with benzothia-[2,1,3]-diazoles 7 and 8 led to a complete loss of activity suggesting that the benzoa-[2,1,3]-diazole plays a crucial role in these compounds antibacterial activity.

Further analysis of the results revealed that conversion of the ester to an aryl hydrazide 9–12 provided compounds more consistent activity across a range of structures. Consequently, substituted benzoa-[2,1,3]-diazoles were chosen as the partner to amino acid hydrazides for further investigation, via a SAR study to further understand the importance of the amino acid (AA) and the hydrazine (R₁) on anti-mycobacterial activity.

### 2.1. Chemical Synthesis of Benzoxa-[2,1,3]-diazole Amino Acid Hydrazides

To undertake this investigation, a two-step synthesis was engaged starting from N-Boc amino acids (Scheme 1). DCC coupling with a monosubstituted hydrazine produced the intermediate protected amino acid hydrazide which, following deprotection and condensation with benzoa-[2,1,3]-diazole sulphonyl chloride afforded the desired products in moderate to good overall yields following flash chromatography purification (Table 2). Interestingly, several of the compounds exhibited restricted rotation as demonstrated by VT-\(^{1}\)H-NMR spectroscopy (Supporting Information, Figure S2).

Scheme 1. Synthesis of amino acid hydrazides and the desired benzoa-[2,1,3]-diazole amino acid hydrazides.

### 2.2. Structure–Activity Relationships

Following the synthetic approach, Boc amino acid hydrazides 13a–22a and benzoa-[2,1,3]-diazole amino acid hydrazide 9, 10, 14b–22b were screened in the same way against the same range of drug-susceptible bacteria as described above. Importantly, in line with the preliminary screening data these compounds showed high selectivity for \textit{mycolata} bacteria (Supporting Information, Table S3). Focusing on the \textit{Mtb} response, initially exploring the role of the amino acid, fixing the hydrazide and increasing the bulk of the amino acid substituent 13a–17a resulted in diminished antibacterial activity of this component (Table 2). Subsequently, fixing the amino acid to glycine, we then evaluated the role of the hydrazine component (18a–22a). Introduction of an unsubstituted aromatic hydrazine 18a alongside halogenated hydrazines 19a–22a did not provide any significant enhancement in activity although a marked increase in cytotoxicity was observed. For both series, enhanced antibacterial activity was restored on coupling to the benzoa-[2,1,3]-diazole 9, 10, 14b–22b albeit at the cost of increased cytotoxicity, as noted for this subunit [17].
Table 2. Results of synthesis of amino acid hydrazides and benzoxa-[2,1,3]-diazole amino acid hydrazides and the antibacterial activity and cytotoxicity.

| Amino Acid (AA) | Hydrazine (R₁) | Compound | Yield | Rotamer Ratio | M. tuberculosis mc²7000 (μM) | Mammalian Cell Toxicity |
|-----------------|---------------|----------|-------|---------------|-----------------------------|------------------------|
| Gly             | 4-CF₃         | 13a      | 47%   | -             | 12.01                       | 0%                     |
|                 |               | 9        | 7%    | 7:1           | 31.13                       | >90%                   |
| Phg             | 4-CF₃         | 14a      | 95%   | -             | 112.74                      | 0%                     |
|                 |               | 14b      | 63%   | -             | 60.85                       | >90%                   |
| Pro             | 4-CF₃         | 15a      | 85%   | -             | 214.26                      | 0%                     |
|                 |               | 15b      | 85%   | -             | 16.33                       | >90%                   |
| Phe             | 4-CF₃         | 16a      | 83%   | -             | 151.14                      | <10%                   |
|                 |               | 16b      | 85%   | -             | 59.27                       | >90%                   |
| Ala             | 4-CF₃         | 17a      | 93%   | -             | 46.06                       | 0%                     |
|                 |               | 17b      | 60%   | -             | 17.25                       | >90%                   |
| Gly             | H             | 18a      | 76%   | 5:1           | 90.46                       | 57%                    |
|                 |               | 19a      | 78%   | 4:1           | 112.95                      | 59%                    |
|                 |               | 19b      | 57%   | 4:1           | 20.00                       | 60%                    |
| Gly             | 4-CF₃         | 20a      | 96%   | 4:1           | 106.75                      | 43%                    |
|                 |               | 20b      | 43%   | 6:1           | 19.22                       | 80%                    |
| Gly             | 3-CF₃         | 21a      | 80%   | 4:1           | 213.50                      | 55%                    |
|                 |               | 21b      | 22%   | 5:1           | 19.22                       | 45%                    |
| Gly             | 2-CF₃         | 22a      | 95%   | 3:1           | 106.75                      | <20%                   |
|                 |               | 22b      | 29%   | 4:1           | 19.22                       | 70%                    |

3. Discussion

Worryingly, as drug-resistant bacterial infections are on the rise and with the recent removal of antibiotic drug discovery programmes, there will be a significant demand for new chemical entities to address this condition. This study has identified that benzoxa-[2,1,3]-diazole substituted amino acid hydrazides have considerable potential as selective and potent agents against Mtb.

Throughout this study, the benzoxa-[2,1,3]-diazole core appears to be essential for activity. Whilst, as observed in some examples, the use of this unit is commonly associated with cytotoxicity this can be effectively modulated through the addition of the amino acid hydrazine. For example, we observed that conjugation with the amino acid hydrazides 19a or 21a provides a reduction in cytotoxicity (19b, 21b, Table 2).

Excitingly, this modulation led to the use of a simple unsubstituted hydrazide 18a which, although in isolation showed significant cytotoxicity, when conjugated with the benzoxadiazole 10 provides a compound with good level of activity against Mtb and no observable cytotoxicity.
4. Materials and Methods

4.1. Chemistry

4.1.1. Synthesis of Hydrazides—General Procedure

A solution of N-Boc amino acid (0.25 g, 1.43 mmol, 1 equiv.), HOBt (2 equiv.) and DCC (1.2 equiv.) was dissolved in THF (7.5 mL), cooled to 0 °C and stirred for 15 min. The solution was treated with N-Aryl/Alkyl hydrazine * (1.2 equiv.) before warming to room temperature and stirring for a further 1.5 h. The mixture was then poured into sat. aq. NH₄Cl (20 mL) before separating and extracting the aqueous layer with EtOAc (40 mL). The organic layer was further washed with sat. aq. NaHCO₃ (20 mL) and then brine (20 mL). The combined organic layers were dried over MgSO₄, filtered, concentrated and dried in vacuo. Flash chromatography (DCM/EtOH/NH₃ [200:8:1], [400:8:1], [200:8:1]) afforded the desired N-Boc amino acid hydrazides.

* When the hydrazine hydrochlorides were used, Et₃N (1.2 equiv.) is added to neutralise the salt

tert-Butyl 2-oxo-2-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)ethylcarbamate, 13a

Following the general procedure outlined, N-Boc-L-Glycine (0.50 g, 2.9 mmol) and 4-trifluorophenyl hydrazine (0.61 g, 3.5 MMOL) was transformed following flash chromatography into the title compound which was isolated as a white solid (0.45 g, 47%); m.p. 185–187 °C; v_{max} (ATR) 3278 (NH), 3234 (NH), 3108, 3058, 2996, 1649 (C=O), 1615, 1518 (C=O), 1330, 1245, 1156, 1107, 1052, 828, 559 cm⁻¹; δH (700 MHz, DMSO-d₆) 9.78 (1H, s, Ar-NH), 8.32 (1H, s, Ar-NH), 7.40 (2H, d, J 9, Ar-H), 7.07 (1H, t, J 6, BocNH), 6.78 (2H, d, J 9, Ar-H), 3.60 (2H, d, J 6, NHCH₂), 1.37 (9H, s, (CH₃)₂CO), 59.30; m/z (ES⁺) 356 (MNa⁺), 689 (2M + Na⁺); HRMS (ES⁺) Found MH⁺, 334.13722 (C₁₇H₁₉F₃N₅O₃ requires 334.13730).

tert-Butyl (S)-(2-oxo-2-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)ethyl)carbamate, 14a

Following the general procedure outlined, N-Boc-L-phenylglycine (0.250 g, 0.99 mmol) and 4-trifluorophenyl hydrazine (0.30 g, 1.75 mmol) was transformed following flash chromatography into the title compound as a brown solid (0.366 g, 85%); Rf 0.44 (DCM/ EtOH/NH₃ [200:8:1]); m.p. 138–143 °C; v_{max} (ATR) 3328 (N-H), 2982, 2930, 2851, 1678 (C=O), 1655 (C=O), 1524, 1322, 1158, 1113, 1067, 834, 696, 558, 488 cm⁻¹; δH (400 MHz, CDCl₃) 8.00 (1H, bs, NH), 7.46–7.37 (7H, m, Ar-H), 6.68 (2H, d, J 8, Ar-H), 6.24 (1H, bs, NH), 5.69 (1H, d, J 7, NHCH₂), 5.35 (1H, bs, NH), 1.42 (9H, s, C(CH₃)₃); δC (176 MHz, CDCl₃) 170.2 (CONH), 156.6 ((CH₃)₂CO), 153.1 (Ar-C), 126.7 (Ar-C), 126.4 (Ar-C), 118.82 (Ar-C), 118.63 (Ar-C), 112.1 (Ar-C), 78.8 (NHCH₂), 42.7 ((CH₃)₂CO), 28.8 ((CH₃)₂CO); δF (658 MHz, DMSO-d₆) -59.30; m/z (ES⁺) 356 (MNa⁺), 689 (2M + Na⁺); HRMS (ES⁺) Found MH⁺, 340.13722 (C₁₉H₁₉F₃N₅O₃Na requires 340.13730).

tert-Butyl (S)-2-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)-1-carbonyl-l-tryptophol-1-carboxylate, 15a

Following the general procedure outlined, N-Boc-L-proline (0.250 g, 1.16 mmol) and 4-trifluorophenyl hydrazine (0.30 g, 1.75 mmol) was transformed following flash chromatography into the title compound as a golden-brown solid (0.366 g, 85%); Rf 0.38 (DCM/EtOH/NH₃ [200:8:1]); m.p. 116–120 °C; v_{max} (ATR) 3265 (N-H), 2979, 2932, 2851, 1672 (C=O), 1618, 1399, 1324, 1107, 1065, 833, 591 cm⁻¹; δH (400 MHz, CDCl₃) 8.91 (1H, bs, NH), 7.45 (2H, d, J 8, Ar-H), 6.87 (2H, d, J 8, Ar-H), 6.34 (1H, bs, NH), 4.45–4.34 (1H, m, NCH₂CO), 3.52–3.35 (2H, m, N(CH₂)₃), 2.06–1.87 (4H, m, N(CH₂)₃), 1.53 (9H, s, C(CH₃)₃); δC (176 MHz, CDCl₃) 172.2 (NCOO), 156.1 (NCOO), 150.8 (Ar-C), 126.4 (Ar-C), 125.2 (Ar-C), 123.7 (CF₃), 112.7 (Ar-C), 81.0 (C(CH₃)₃), 58.4 (NCH₂CO), 47.2 (N(CH₂)₂), 28.4 (C(CH₃)₃), 25.5, (NCH₂(CH₂)₂), 24.8 (NCH₂(CH₂)₂); m/z (ES⁺) 374 (M⁺H⁺), 396 (MNa⁺), 769 (2M + Na⁺); HRMS (ES⁺) Found MNa⁺, 396.1497 (C₁₇H₂₂F₃N₅O₃Na requires 396.1505).
**Molecules 2019, 24, 811**

**7 of 14**

** tert-Butyl (S)-(1-oxo-3-phenyl-1-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)propan-2-yl)carbamate, 16a**

Following the general procedure outlined, N-Boc-L-phenylalanine (0.250 g, 0.94 mmol) and 4-trifluorophenyl hydrazine (0.30 g, 1.75 mmol) was transformed following flash chromatography into the title compound as a golden-brown solid (0.330 g, 83%); Rf 0.39 (DCM/EtOH/NaH₂PO₄ [200:8:1]; mp. 157–159 °C; υmax (ATR) 3324 (N-H), 2924, 2851, 1686 (C=O), 1660 (C=O), 1520, 1328, 1156, 1103, 1067, 835, 716, 515 cm⁻¹; δH (400 MHz, CDCl₃) 7.93 (1H, bs, NH), 7.39 (2H, d, J 8, Ar-H), 7.36–7.32 (3H, m, Ar-H), 7.26–7.22 (2H, m, Ar-H), 6.61 (2H, d, J 8, Ar-H), 6.18 (1H, bs, NH), 5.09 (1H, bd, J 7, NH), 4.46 (1H, q, J 8, CH₂CH₂CO), 3.12 (2H, d, J 8, CH₂CH₂CO), 1.47 (9H, s, C(CH₃)₃); δC (176 MHz, CDCl₃) 171.6 (NHCHCO), 150.3 (NHCOO), 135.96 (Ar-C), 129.3 (Ar-C), 128.9 (Ar-C), 127.2 (Ar-C), 126.4 (Ar-C), 125.2 (Ar-C), 123.2 (CH₃), 112.7 (Ar-C), 80.9 (C(CH₃)₃), 54.5 (NHCHCO), 33.5 (CH₂CH₂CO), 28.3 (C(CH₃)₃); δF (376 MHz, CDCl₃) -61.56 (3F, s, CF₃); m/z (ES⁺) 424 (MH⁺), 446 (MNa⁺), 869 (2M + Na⁺); HRMS (ES⁺) Found MH⁺, 424.1847 (C₂₁H₂₅F₃N₃O₃ requires 424.1843).

** tert-Butyl (S)-(1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)propan-2-yl)carbamate, 17a**

Following the general procedure outlined, N-Boc-L-alanine (0.250 g, 1.32 mmol) and 4-trifluorophenyl hydrazine (0.30 g, 1.75 mmol) was transformed following flash chromatography into the title compound as a brown solid (0.35 g, 93%); Rf 0.37 (DCM/EtOH/NaH₂PO₄ [200:8:1]; mp. 126–131 °C; υmax (ATR) 3321 (N-H), 2929, 2851, 1683 (C=O), 1617, 1524, 1322, 1157, 1066, 830, 641 cm⁻¹; δH (400 MHz, CDCl₃) 8.50 (1H, bs, NH), 7.46 (2H, d, J 8, Ar-H), 6.87 (2H, d, J 8, Ar-H), 6.34 (1H, bs, NH), 5.07 (1H, bd, J 6, NH), 4.40 (1H, m, CH₂CH), 1.50 (9H, s, C(CH₃)₃), 1.43 (3H, d, J 7, CH₃CH); δC (176 MHz, CDCl₃) 171.7 (NHCHCO), 158.7 (NHCOO), 129.3 (Ar-C), 128.9 (Ar-C), 127.2 (Ar-C), 126.4 (CF₃), 112.8 (Ar-C), 80.9 (C(CH₃)₃), 54.6 (NHCHCO), 33.7 (CH₂CH₂CO), 28.3 (C(CH₃)₃); δF (376 MHz, CDCl₃) -61.55 (3F, s, CF₃); m/z (ES⁺) 348 (MH⁺), 370 (MNa⁺), 717 (2M + Na⁺); HRMS (ES⁺) Found MNa⁺, 370.1532 (C₁₅H₁₇F₃N₂O₃Na requires 370.1349).

** tert-Butyl 2-oxo-2-(2-phenylhydrazinyl)ethylcarbamate, 18a**

Following the general procedure outlined, N-Boc-L-Glycine (0.50 g, 2.9 mmol) and phenyl hydrazine (0.30 mL, 3.1 mmol) was transformed following flash chromatography into the title compound as a white solid (0.30 g, 39%); Rf 0.1 (n-hexane/EtOAc 7:3) as a mixture of rotamers in the ratio [5:1] by NMR @ 25 °C; mp. 120–122 °C; υmax (ATR) 3348 (NH), 3274 (NH), 2922, 2852, 1746, 1652 (C=O), 1640 (C=O), 1519, 1488, 1418, 1375, 1260, 1192, 1106, 1036, 874, 760 cm⁻¹; δH (700 MHz, DMSO-d₆) 9.59 (1H, s, NHNHPh), 9.08 (0.2H, s, NHNHPh), 7.82 (0.85H, s, NHNHPh), 7.65 (1H, s, NHNHPh), 7.17 (0.45H, t, J 8, Ar-H), 7.08 (2H, t, J 8, Ar-H), 7.02 (1H, t, J 5, BocNH), 6.74 (0.23H, t, J 8, Ar-H), 6.71 – 6.63 (3H, m, Ar-H), 6.61 (0.23H, m, Ar-H), 3.68 (0.34H, d, J 6, BocNHCH₂), 3.58 (2H, d, J 5, BocNHCH₂), 1.37 (9H, s, (CH₃)₃COC(O)NH), 1.34 (1.33H, s, (CH₃)₃COC(O)N); δC (176 MHz, DMSO-d₆) 174.2 (CONH), 169.9 (CONH), 156.5 ((CH₃)₃COC(O)NH), 149.9 (Ar-C), 149.1 (Ar-C), 129.3 (Ar-C), 119.0 (Ar-C), 112.8 (Ar-C), 78.7 (NHCH₂), 78.5 (NHCH₂), 42.7 ((CH₃)₃CO), 31.9 ((CH₃)₃CO), 28.8 ((CH₃)₃CO), 22.8 ((CH₃)₃CO); m/z (ES⁺) 266 (MH⁺), 531 (2M + H⁺); HRMS (ES⁺) Found MH⁺, 266.24985 (C₁₃H₂₀O₂N₃ requires 266.14992).

** tert-Butyl (2-(2-(4-fluorophenyl)hydrazinyl)-2-oxoethyl)carbamate, 19a**

Following the general procedure outlined, N-Boc-L-Glycine (0.25 g, 1.45 mmol) and 4-fluorophenyl hydrazine (0.20 g, 1.57 mmol) was transformed following flash chromatography (DCM/EtOH/NaH₂PO₄ [600:8:1], [400:8:1], [200:8:1]) into a brown light solid (0.32 g, 78%) as a mixture of rotamers [4:1] by NMR @ 25 °C; Rf 0.32 (DCM/EtOH/NaH₂PO₄ [200:8:1]); mp. 127–129 °C; υmax (ATR) 3364 (NH), 3269 (NH), 3132, 2984, 1652 (C=O), 1505, 1393, 1370, 1269, 1225, 1224, 1162, 1029, 1007 cm⁻¹; NMR data given for major rotamer δH (700 MHz, CDCl₃) 8.35 (1H, bs, (CH₃)₃COC=ONH), 6.89 (2H, t, J 17, Ar-H), 6.75 (2H, dd, J 9, 4, Ar-H), 5.30 δH, bs, NH, C=O), 4.07 (1H, bs, NH), 3.85 (2H, d, J 6, CH₂NH), 1.45 (9H, s, (CH₃)₃COC=ONH); δC (176 MHz, CDCl₃) 170.4 (C=O), 157.4 (C=O), 158.6 (Ar-C) 143.7
Following the general procedure outlined, N-Boc-L-Glycine (0.25 g, 1.45 mmol) and 4-chlorophenyl hydrazine (0.22 g, 1.57 mmol) was transformed following flash chromatography into the title compound as a light brown gum (0.41 g, 95%) as a mixture of rotamers [3:1] by NMR @ 25 °C; Rf 0.50 (DCM/EtOH/NH3 [200:8:1]); νmax (ATR) 3262 (NH), 3074, 2960, 2928, 2851, 2118, 1668 (C=O), 1590, 1536, 1498, 1456, 1365, 1281, 1254, 1171, 1053, 1035 cm⁻¹; NMR data given for major rotamer δH (700 MHz, CDC13) 7.09 (1H, bs, NH), 7.12 (1H, t, J 8, Ar-H), 6.85 (1H, ddd, J 8, 2, 1, Ar-H), 6.81 (1H, dd, J 2, Ar-H), 6.70 (1H, ddd, J 8, 2, 1, Ar-H), 5.17 (1H, bs, NH), 3.88 (2H, d, J 6, CH2NH), 1.47 (9H, s, (CH3)3COC=ONH); δC (176 MHz, CDC13) 169.0 (C=O), 145.2 (Ar-Cl), 142.6 (Ar-CN), 141.8 (Ar-Cr), 134.0 (CH2), 128.3 (CH2), 127.6 (Ar-Cl), 126.2 (Ar-CN), 115.8 (Ar-H), 113.9 (Ar-Cr), 111.8 (Ar-CN), 111.4 (Ar-Cr), 104.9 (CH2), 80.4 ((CH3)3COC=ONH); 30.0 (C35Cl)[MH]+, 302 (C35Cl)[MNa]+, 324 (C35Cl)[MNa]+, 623 (C35Cl)[2M+Na]+, 625 (C35Cl)[2M + Na]+, 627 (C35Cl)[2M + Na]+; HRMS (ES+) Found [C35Cl][MH]+, 300.1119 (C13H19N3O335Cl requires 300.1115).

tert-Butyl (2-(2-(2-chlorophenyl)hydrazineyl)-2-oxoethyl)carbamate, 22a

Following the general procedure outlined, N-Boc-L-Glycine (0.25 g, 1.45 mmol) and 2-chlorophenyl hydrazine hydrochloride (0.28 g, 1.57 mmol) was transformed following flash chromatography into the title compound as a pale brown solid (0.34 g, 80%) as a mixture of rotamers [4:1] by NMR @ 25 °C; Rf 0.38 (DCM/EtOH/NH3 [200:8:1]); νmax (ATR) 3343 (NH), 3269 (NH), 3077, 2980, 2932, 1659 (C=O), 1597, 1512, 1490, 1368, 1242, 1230, 1156, 1046, 1030 cm⁻¹; NMR data given for major rotamer δH (700 MHz, CDC13) 7.09 (1H, bs, NH), 7.12 (1H, t, J 8, Ar-H), 6.85 (1H, ddd, J 8, 2, 1, Ar-H), 6.81 (1H, dd, J 2, Ar-H), 6.70 (1H, ddd, J 8, 2, 1, Ar-H), 5.17 (1H, bs, NH), 3.88 (2H, d, J 6, CH2NH), 1.47 (9H, s, (CH3)3COC=ONH); δC (176 MHz, CDC13) 170.0 (C=O), 157.5 (C-O), 148.9 (Ar-C), 135.1 (Ar-CN), 130.2 (Ar-C), 121.3 (Ar-C), 113.4 (Ar-C), 111.8 (Ar-C), 81.2 ((CH3)3COC=ONH), 28.3 ((CH3)3COC=ONH); 30.0 (C35Cl)[MH]+, 302 (C35Cl)[MNa]+, 324 (C35Cl)[MNa]+, 623 (C35Cl)[2M+Na]+, 625 (C35Cl)[2M + Na]+, 627 (C35Cl)[2M + Na]+; HRMS (ES+) Found [C35Cl][MH]+, 300.1119 (C13H19N3O335Cl requires 300.1115).

4.1.2. Synthesis of Benzoxa-[2,1,3]-Diazole Peptidomimetics—General Procedure

N-Boc amino acid hydrazides (1.20 equiv.) were dissolved in 4 M HCl solution in dioxane (3 mL) and stirred for 30 min at room temperature. The solvent was then removed in vacuo, the resulting solid was suspended in THF (3 mL) and Et3N (3 equiv.) was added. The solution was cooled to 0 °C, before treating with 7-chlorobenzoa-[2,1,3]-diazole-4-sulphonyl chloride (50 mg, 0.20 mmols,
1 equiv.) before warming to room temperature and stirring for 2 h. The mixture was then poured into sat. aq. NH₄Cl (20 mL). The aqueous layer was separated and extracted with EtOAc (20 mL). The combined organic layers were dried over MgSO₄, filtered, concentrated and dried in vacuo. Flash chromatography (DCM/ EtOH/ NH₃ [600:8:1], [400:8:1], [200:8:1]) afforded the desired sulphonamides.

7-Chloro-N-(2-oxo-2-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)ethyl)benzoc[1,2,5]oxadiazole-4-sulfonamide, 9

Following the general procedure outlined, tert-Butyl 2-oxo-2-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)ethyl carbamate, 13a (79 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a pale brown solid (6 mg, 7%); Rₜ 0.6 (DCM/MeOH 9:1) as a mixture of rotamers in the ratio [7:1] by NMR @ 25 °C; m.p. 211–213 °C; v_max (ATR) 332, 3158, 1685 (C=O), 1614, 1524, 1473, 1418, 1326, 1155, 1107, 1062, 952, 834, 632 cm⁻¹; δ_H (500 MHz, DMSO-d₆) 9.88 (1H, s, Ar-NH,NH), 9.31 (0.13H, s, Ar-NH,NH), 8.73 (1H, t, J 6, SO₂NH), 8.46 (0.14H, s, Ar-NH,NH), 8.41 (0.14H, t, J 6, SO₂NH), 8.26 (1H, s, Ar-NH,NH), 8.02 (1H, d, J 6, Ar-H), 7.95 (0.16H, d, J 7, Ar-H), 7.91 (0.17H, d, J 7, Ar-H), 7.85 (1H, d, J 8, Ar-H), 7.57 (0.34H, d, J 8, Ar-H), 7.43 (2H, d, J 8, Ar-H), 6.79 (0.32H, d, J 8, Ar-H), 6.68 (2H, d, J 8, Ar-H), 3.94 (0.25H, d, J 6, NHCH₂), 3.88 (2H, d, J 6, NHCH₂), δ_C (126 MHz, DMSO-d₆) 168.4 (C=O), 152.6 (Ar-C), 149.4 (Ar-C), 146.2 (Ar-C), 134.5 (Ar-C), 131.5 (Ar-C), 129.1 (Ar-C), 126.7 (Ar-C), 125.6 (Ar-C), 121.1 (Ar-C), 44.3 (NHCH₂); δ_p (376 MHz, DMSO) -59.71, -59.89; m/z (ES⁺) 448 ([M+Cl]⁺), 450 ([M+Cl]⁻), 897 ([M+3Cl]⁻), 899 ([M+3Cl]+), 900 ([M+3Cl]²M⁻); HRMS (ES⁺) Found [M+3Cl]⁻, 448.00931 (C₁₅H₁₀O₁₅F₃N₃O₂S requires 448.00996).

(S)-7-Chloro-N-(2-oxo-2-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)ethyl)benzoc[1,2,5]oxadiazole-4-sulfonamide, 14b

Following the general procedure outlined, tert-butyl (S)-(2-oxo-1-phenyl-2-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)ethyl)carbamate, 14a (97 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a pale yellow solid (66 mg, 63%); Rₜ 0.46 (DCM/ EtOH/ NH₃ [200:8:1]); m.p. 183–187 °C; v_max (ATR) 3275 (N-H), 2930, 1693 (C=O), 1618, 1521, 1325, 1161, 1105, 1067, 840, 800, 636, 619, 590, 570, 530, 491 cm⁻¹; δ_H (700 MHz, DMSO-d₆) 10.20 (1H, s, NH, N), 9.35 (1H, bs, NH), 8.36 (1H, s, NH), 7.97 (1H, d, J 7, Ar-H), 7.82 (1H, d, J 7, Ar-H), 7.32–7.27 (4H, m, Ar-H), 7.18–7.15 (3H, m, Ar-H), 6.44 (2H, d, J 8, Ar-H), 5.30 (1H, s, NHCHCO); δ_C (176 MHz, DMSO-d₆) 169.8 (NHCHCO), 152.3 (Ar-C), 148.9 (Ar-CNO), 145.3 (Ar-CNO), 137.1 (Ar-C), 134.9 (Ar-C), 131.1 (Ar-C), 128.9 (Ar-C), 128.5 (Ar-C), 128.3 (Ar-C), 127.9 (Ar-C), 127.7 (Ar-C), 126.4 (Ar-C), 125.6 (Ar-C), 124.5 (CF₂), 111.6 (Ar-C), 59.0 (NHCHCO); m/z (ES⁺) 526 ([M+5Cl]⁺), 528 ([M+3Cl]⁺), 548 ([M+5Cl]+), 550 ([M+7Cl]⁻MNa⁺), 1073 ([M+35Cl]+ 2M + Na⁺), 1075 ([M+37Cl]⁺ 2M + Na⁺), 1077 ([M+37Cl]⁺ 2M+Na⁺); HRMS (ES⁺) Found [M+3Cl]⁺MNa⁺, 526.05665 (C₂₁H₁₄F₃N₃O₂SCl requires 526.0558).

(S)-1-(7-Chlorobenzo[c][1,2,5]oxadiazol-4-yl)sulfonfonyl)-N’-(4-(trifluoromethyl)phenyl)pyrrolidine-2-carboxyhydrazide, 15b

Following the general procedure outlined, tert-butyl (S)-2-(2-(4-(trifluoromethyl)phenyl)hydrazine-1-carbonyl)pyrrolidine-1-carboxylate, 15a (89 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a yellow solid (82 mg, 85%); Rₜ 0.46 (DCM/ EtOH/ NH₃ [200:8:1]); m.p. 166–170 °C; v_max (ATR) 3324 (N-H), 2929, 2851, 1682 (C=O), 1621, 1574, 1325, 1146, 1102, 1066, 946, 836, 616 cm⁻¹; δ_H (700 MHz, DMSO-d₆) 10.09 (1H, s, N), 8.39 (1H, bs, NH), 8.08 (1H, d, J 7, Ar-H), 7.86 (1H, d, J 7, Ar-H), 7.44 (2H, d, J 9, Ar-H), 6.82 (2H, d, J 9, Ar-H), 4.43 (1H, dd, J 9, 4, NCHCO), 3.61–3.57 (1H, ddd, J 10, 7, 5, NCH₂(CH₂)₂), 3.47–3.41 (1H, dt, J 10, 7, NCH₂(CH₂)₂), 1.26–0.96 (4H, m, NCH₂(CH₂)₂); δ_C (176 MHz, DMSO-d₆) 171.4 (NHCHCO), 152.8 (Ar-C), 149.5 (Ar-CNO), 146.3 (Ar-CNO), 136.8 (Ar-C), 131.2 (Ar-C), 127.7 (Ar-C), 126.5 (Ar-C), 126.3 (Ar-C), 125.5 (Ar-C), 124.7 (CF₃), 111.9 (Ar-C), 60.7 (NCHCO), 49.3 (NCH₂(CH₂)₂), 33.9 (NCH₂(CH₂)₂), 24.9 (NCH₂(CH₂)₂); m/z (ES⁺) 490 ([M+3Cl]⁺), 492 ([M+37Cl]⁺); HRMS (ES⁺) Found [M+3Cl]⁺, 490.0555 (C₁₈H₁₆F₃N₃O₄S³Cl requires 490.0558).
(S)-7-Chloro-N-(1-oxo-3-phenyl-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide, 16b

Following the general procedure outlined tert-butyl (S)-(1-oxo-3-phenyl-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)carbamate, 16a (100 mg, 0.24 mmol) was transformed following flash chromatography into the title compound which was isolated as a brown solid (91 mg, 85%); R_\text{f} 0.41 (DCM/EtOH/NH_3 [200:8:1]); m.p. 80–84 °C; v_{\text{max}} (ATR) 3321 (N-H), 2932, 2854, 1677 (C=O), 1617, 1323, 1156, 1095, 1065, 834, 700, 632, 577, 488 cm⁻¹; δ_1 H (700 MHz, DMSO-d_6) 10.13 (1H, s, NH), 9.06 (1H, d, J = 9, NH), 8.43 (1H, bs, NH), 7.76 (1H, d, J = 7, Ar-H), 7.71 (1H, d, J = 7, Ar-H), 7.51 (2H, d, J = 9, Ar-H), 7.00–6.96 (2H, m, Ar-H), 6.84–6.79 (3H, m, Ar-H), 6.70 (2H, d, J = 9, Ar-H), 4.16–4.12 (1H, m, CH_2CHCO), 2.87 (1H, dd, J = 14, 4, CH_2CHCO), 2.68 (1H, dd, J = 14, 11, CH_2CHCO); δ_c (176 MHz, DMSO-d_6) 170.9 (NHCHCO), 152.6 (Ar-C), 148.9 (Ar-CNO), 144.7 (Ar-CNO), 136.9 (Ar-C), 133.9 (Ar-C), 130.7 (Ar-C), 129.5 (Ar-C), 128.2 (Ar-C), 127.6 (Ar-C), 126.5 (Ar-C), 126.2 (Ar-C), 125.5 (Ar-C), 124.6 (CF_3), 111.8 (Ar-C), 57.4 (NHCHCO), 38.0 (CH_2CHCO); m/z (ES⁺) 540 ([35Cl]MH⁺), 542 ([37Cl]MH⁺), 562 ([35Cl]MNa⁺), 564 ([37Cl]MNa⁺), 1101 ([35,35Cl]2M + Na⁺), 1103 ([37,37Cl]2M + Na⁺), 1105 ([37,37Cl]2M + Na⁺). HRMS (ES⁺) Found [35Cl]MH⁺, 540.0642 (C_22H_18ClF_3N_3O_5S requires 540.0715).

(S)-7-Chloro-N-(1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide, 17b

Following the general procedure outlined, tert-butyl (S)-(1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)carbamate, 17a (82 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a pale yellow solid (55 mg, 60%); R_\text{f} 0.34 (DCM/EtOH/NH_3 [200:8:1]); m.p. 127–131 °C; v_{\text{max}} (ATR) 3388 (N-H), 3315 (N-H), 3274, 2929, 2851, 1667 (C=O), 1619, 1323, 1159, 1110, 1067, 949, 836, 633, 597, 579, 511 cm⁻¹; δ_1 H (700 MHz, DMSO-d_6) 9.90 (1H, bs, NH), 8.80 (1H, d, J = 8, NH), 8.24 (1H, bs, NH), 8.00 (1H, d, J = 7, Ar-H), 7.82 (1H, d, J = 7, Ar-H), 7.39 (2H, d, J = 9, Ar-H), 6.61 (2H, d, J = 9, Ar-H), 4.20–4.15 (1H, m, CH_2CHCO), 1.26 (3H, d, J = 7, CH_2CHCO); δ_c (176 MHz, DMSO-d_6) 171.4 (NHCHCO), 152.5 (Ar-C), 149.2 (Ar-CNO), 145.6 (Ar-CNO), 134.4 (Ar-C), 131.2 (Ar-C), 128.9 (Ar-C), 128.3 (Ar-C), 126.5 (Ar-C), 125.4 (Ar-C), 124.6 (CF_3), 111.7 (Ar-C), 51.2 (NHCHCO), 19.8 (CHCH_3); m/z (ES⁺) 464 ([35Cl]MH⁺), 466 ([37Cl]MH⁺); HRMS (ES⁺) Found [35Cl]MH⁺, 464.0400 (C_16H_14F_3N_3O_5S [35Cl] requires 464.0402).

7-Chloro-N-(2-oxo-2-(2-phenylhydrazinyl)ethyl)benzo[c][1,2,5]oxadiazole-4-sulfonamide, 10

Following the general procedure outlined, tert-butyl 2-oxo-2-(2-phenylhydrazinyl)ethylcarbamate, 18a (64 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a yellow solid (41 mg, 55%); R_\text{f} 0.4 (DCM/MeOH 9:1) as a mixture of rotomers in the ratio [4:1] by NMR @ 25 °C; m.p. 209–211 °C; v_{\text{max}} (ATR) 3262 (NH), 1738, 1647 (C=O), 1606, 1530, 1494, 1410, 1346, 1190, 1157, 947, 834, 747 cm⁻¹; δ_1 H (500 MHz, DMSO-d_6) 9.70 (1H, s, Ar-NHNH), 9.13 (0.24H, s, Ar-NHNH), 8.68 (1H, t, J = 6, SO_2NH), 8.35 (0.24H, t, J = 6, SO_2NH), 8.00 (1H, d, J = 7, Ar-H), 7.94 (0.29H, d, J = 7, Ar-H), 7.90 (0.27H, d, J = 7, Ar-H), 7.84 (1H, d, J = 7, Ar-H), 7.60 (1H, s, Ar-NHNH), 7.22 (0.55H, t, J = 7, Ar-H), 7.10 (2H, t, J = 7, Ar-H), 6.80 (0.25H, t, J = 7, Ar-H), 6.71–6.66 (1.6H, m, Ar-H), 6.58 (2H, d, J = 7, Ar-H), 3.96 (2H, d, J = 6, NHCH_2), 3.85 (2H, d, J = 6, NHCH_2); δ_c (126 MHz, DMSO-d_6) 168.2 (C=O), 149.47 (Ar-C), 149.43 (Ar-C), 146.1 (Ar-C), 134.4 (Ar-C), 131.5 (Ar-C), 129.8 (Ar-C), 129.3 (Ar-C), 125.5 (Ar-C), 119.2 (Ar-C), 112.7 (Ar-C), 44.4 (NHCH_2); m/z (ES⁺) 382 ([35Cl]MH⁺), 384 ([37Cl]MH⁺); HRMS (ES⁺) Found [35Cl]MH⁺, 382.03702 (C_14H_13Cl_3N_3O_5S requires 382.03713).

7-Chloro-N-(2-(2-(4-fluorophenyl)hydrazinyl)-2-oxoethyl)benzo[c][1,2,5]oxadiazole-4-sulfonamide, 19b

Following the general procedure outlined, tert-butyl 2-(2-(4-fluorophenyl)hydrazinyl)-2-oxoethyl carbamate, 19a (68 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a brown solid (46 mg, 57%) as a mixture of rotomers [4:1] by NMR @ 25 °C; R_\text{f} 0.48 (DCM/EtOH/NH_3 [200:8:1]); m.p. 180–185 °C; v_{\text{max}} (ATR) 3364, 3245 (NH), 2918, 2850, 1673.
Following the general procedure outlined, tert-butyl (2-(2-(4-chlorophenyl)hydrazineyl)-2-oxoethyl) carbamate, 20a (72 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a pale yellow solid (29 mg, 22%) as a mixture of rotamers [5:1] by NMR @ 25 °C.

Following the general procedure outlined, tert-butyl (2-(2-(3-chlorophenyl)hydrazineyl)-2-oxoethyl) carbamate, 21a (72 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a pale yellow solid (29 mg, 22%) as a mixture of rotamers [5:1] by NMR @ 25 °C.
6.58 (1H, dd, J 8, 1, Ar-H), 3.85 (2H, d, J 6, CH₂NH); δC (176 MHz, DMSO-d₆) 168.1 (C=O), 149.2 (Ar-C), 145.8 (Ar-C), 144.5 (Ar-C), 134.2 (Ar-C), 131.2 (Ar-C), 129.5 (Ar-C), 128.9 (Ar-C), 125.3 (Ar-C), 120.6 (Ar-C), 117.5 (Ar-C), 113.2 (Ar-C), 44.1 (CH₂NH); m/z (ES⁺) 416 ([35,35Cl]MH⁺), 418 ([35,37Cl]MH⁺), 420 ([37,37Cl]MH⁺), 471 ([35,35Cl]MNa + MeOH), 473 ([35,37Cl]MNa + MeOH), 475 ([37,37Cl]MNa + MeOH); HRMS (ES⁺) Found [35,35Cl]MH⁺, 415.9972 (C₁₄H₁₂N₂O₄S₂Cl₂ requires 415.9987).

4.2. Biological Assessment

Bacterial strains and growth media used in this study (Supporting Information, Table S4).

4.2.1. Bacterial Growth Inhibition Assays

The minimum inhibitory concentration of the compounds against all strains using stand REMA assay protocols [14]. Briefly, 100 µL of relevant growth media was added to all wells of a sterile 96-well plate (Corning Incorporated, Corning, NY, USA). The wells in rows A to H in columns 1 received 94.88 µL of growth medium (7H9 media was supplemented with 0.2% casamino acids, 24 µg/mL pantothenate and 10% OADC, Beckton Dickinson, Sparks, MD, USA). Compounds were added to rows A1-H1 (quadruplet per compound) followed by 1:2 serial dilutions across the plate to column 11 were 100 µL of excess medium was discarded from the wells in column 11. The bacterial cultures at 0.5 McFarland standard diluted 1:25 was added to the wells in rows A to H in columns 1 to 11 (100 µL), where the wells in column 12 served as drug-free controls (positive and negative). The plates were sealed with parafilmTM and incubated at 37 °C, unless 30 °C was stated as the optimum for the organism. Freshly prepared filter sterilised resazurin (0.2% w/v, Sigma Aldrich, Dorset, UK) was filter sterilised and 10 µL added to all wells and re-incubated at 37 °C or 30 °C for 24 h or until the positive and negative controls showed a clear result.

4.2.2. Mammalian Cytotoxicity Determination Using the MTT Assay

In vitro chemosensitivity of Human NCI-H460 lung carcinoma cells to the agents were determined using the MTT assay, described elsewhere [18]. Cells were exposed to the amino acid hydrazides or benzoxa-[2,1,3]-diazole amino acid hydrazides (10 µM), or solvent (dimethyl sulphoxide; DMSO) in quadruplicate. Solvent concentrations did not exceed 0.1% and were not cytotoxic. Chemosensitivity and cell survival was assessed following 96 h compound exposure, with cytotoxicity relative to vehicle control subsequently determined.

5. Conclusions

As shown by the present study, this interplay between cytotoxicity and antibacterial activity can be readily manipulated through the substitution patterns on each component, aromatic hydrazides, the size of the amino acid side chain and the benzoxa-[2,1,3]-diazole (Figure 3). The ease of manipulation makes this an attractive template and a full examination of all these parameters is the subject of ongoing efforts which will be reported in due course.

**Figure 3.** SAR profile of benzoxa-[2,1,3]-diazole substituted amino acid hydrazides.
### Supplementary Materials:
The following are available online, Table S1: Initial screen of 99 compounds against a range of Gram-positive, Gram-negative and mycolata bacterial in a 96-well plate REMA assay at 128 µg/mL. Blue cells indicate no bacterial growth. Empty cells indicate bacterial growth, Figure S1: Several of the amino acid hydrazides presented as a mixture of rotamers by NMR, Table S2: Antibacterial activity against Gram-positive, -negative and mycolata bacteria and Mammalian Cell Toxicity of amino acid hydrazides and benzoza-[2,1,3]-diazole amino acid hydrazides, expressed as MIC (µM) or percentage relative to control (100%). - = No activity from the REMA assay at 128 µg/mL, Table S3: Bacterial strains and growth media used in the REMA assays, 1H NMR, 13C NMR for all compounds.

### Author Contributions:
Conceptualization and design of research, A.K.B., J.H.G., J.D.S.; methodology, A.K.B., J.H.G., P.G.S., J.D.S.; experimental investigation, A.K.B., A.K.B.A., J.H.G., and J.D.S.; interpreted results of experiments, A.K.B., J.H.G., and J.D.S.; writing—original draft, J.D.S.; preparation; writing—review and editing, A.K.B., J.H.G., and P.G.S.; project administration, J.D.S.; approval of final version of the manuscript, A.K.B., A.K.B.A., J.H.G., P.G.S., and J.D.S.

### Funding:
This research received no external funding.

### Acknowledgments:
We would also like to thank A. M. Kenwright (University of Durham) and Corinne Wills for assistance with NMR spectroscopy and J. A. Mosely (University of Durham) for mass spectra.

### Conflicts of Interest:
The authors declare no conflict of interest.

### References
1. O’Neill, J. Review on Antimicrobial Resistance, Tackling Drug-Resistant Infections Globally: Final Report and Recommendations; Wellcome Trust and UK Government: London, UK, 2016.
2. Houben, R.M.; Dodd, P.J. The Global Burden of Latent Tuberculosis Infection: A Re-estimation Using Mathematical Modelling. PLoS Med. 2016, 13, e1002152. [CrossRef] [PubMed]
3. World Health Organization. Guidelines on the Management of Latent Tuberculosis Infection; World Health Organization: Geneva, Switzerland, 2015.
4. Byrne, A.; Fox, G.J.; Marais, B.J. Better than a pound of cure: Preventing the development of multidrug-resistant tuberculosis. Future Microbiol. 2018, 13, 577–588. [CrossRef] [PubMed]
5. Patel, R.V.; Riyaz, S.; Park, S. Bedaquiline: A new hope to treat multi-drug resistant tuberculosis. Curr. Top. Med. Chem. 2014, 14, 1866–1874. [CrossRef] [PubMed]
6. Baptista, R.; Fazakerley, D.M.; Beckmann, M.; Baillie, L.; Mur, L.A. Untargeted metabolomics reveals a new mode of action of pretomanid (PA-824). Sci. Rep. 2018, 8, 5084. [CrossRef] [PubMed]
7. Lechartier, B.; Cole, S.T. Mode of Action of Clofazimine and Combination Therapy with Benzothiazinones against Mycobacterium tuberculosis. Antimicrob. Agents Chemother. 2015, 59, 4457–4463. [CrossRef] [PubMed]
8. Boeree, M.J.; Heinrich, N.; Aarnoutse, R.; Diacon, A.H.; Dawson, R.; Rehal, S.; Kibiki, G.S.; Churchyard, G.; Sanne, I.; Nttinginya, N.E.; et al. High-dose rifampicin, moxifloxacin, and SQ109 for treating tuberculosis: A multi-arm, multi-stage randomised controlled trial. Lancet Infect. Dis. 2017, 17, 39–49. [CrossRef]
9. Hu, Y-Q.; Zhang, S.; Zhao, E.; Gao, C.; Feng, L-S.; Lv, Z-S.; Xu, Z.; Wu, X. Isoniazid derivatives and their anti-tubercular activity. Eur. J. Med. Chem. 2017, 133, 255–267. [CrossRef] [PubMed]
10. Rivera, B.; Castellsagué, E.; Bah, I.; van Kempen, L.C.; Foulkes, W.D. Biallelic NTHL1 Mutations in a Woman with Multiple Primary Tumors. N. Engl. J. Med. 2015, 373, 1985–1986. [CrossRef] [PubMed]
11. Piccionello, A.; Guarcello, A. Bioactive Compounds Containing Benzoxadiazole, Benzothiadiazole, Benzotriiazole. Curr. Bioact. Compd. 2010, 6, 266–283. [CrossRef]
12. Cho, Y.; Ioerger, T.R.; Sacchettini, J.C. Discovery of Novel Nitrobenzothiazole Inhibitors for Mycobacterium tuberculosis ATP Phosphoribosyl Transferase (HisG) through Virtual Screening. J. Med. Chem. 2008, 51, 5984–5992. [CrossRef] [PubMed]
13. Cosconati, S.; Hong, J.A.; Novellino, E.; Carroll, K.S.; Goodsell, D.S.; Olson, A.J. Structure-Based Virtual Screening and Biological Evaluation of Mycobacterium tuberculosis Adenosine 5’-Phosphosulfate Reductase Inhibitors. J. Med. Chem. 2008, 51, 6627–6630. [CrossRef] [PubMed]
14. Palomino, J.-C.; Martin, A.; Camacho, M.; Guerra, H.; Swings, J.; Portela, F. Resazurin microtiter assay plate: Simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis. Antimicrob. Agents Chemother. 2002, 46, 2720–2722. [CrossRef] [PubMed]
15. Scalacci, N.; Brown, A.K.; Pavan, F.R.; Ribeiro, C.M.; Manetti, F.; Bhakta, S.; Maitra, A.; Smith, D.L.; Petricci, E.; Castagnolo, D. Synthesis and SAR evaluation of novel thioridazine derivatives active against drug-resistant tuberculosis. *Eur. J. Med. Chem.* 2017, 127, 147–158. [CrossRef] [PubMed]

16. Bhakta, S.; Scalacci, N.; Maitra, A.; Brown, A.K.; Dasugari, S.; Evangelopoulos, D.; McHugh, T.D.; Mortazavi, P.N.; Twist, A.; Petricci, E.; et al. Design and Synthesis of 1-((1,5-Bis(4-chlorophenyl)-2-methyl-1H-pyrrol-3-yl)methyl)-4-methylpiperazine (BM212) and N-Adamantan-2-yl-N′-((E)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (SQ109) Pyrrole Hybrid Derivatives: Discovery of Potent Antitubercular Agents Effective against Multidrug-Resistant Mycobacteria. *J. Med. Chem.* 2016, 59, 2780–2793. [PubMed]

17. Ricci, G.; Maria, F.; Antonini, G.; Turella, P.; Bullo, A.; Stella, L.; Filomeni, G.; Federici, G.; Caccuri, A. 7-Nitro-2,1,3-benzoxadiazole Derivatives, a New Class of Suicide Inhibitors for Glutathione S-Transferases. Mechanism of Action of Potential Anticancer Drugs. *J. Biol. Chem.* 2005, 280, 26397–26405. [CrossRef] [PubMed]

18. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 1983, 65, 55–63. [CrossRef]

**Sample Availability:** Samples of all compounds are available from the authors.