Cationic Peptidomimetic Amphiphiles Having a N-Aryl- or N-Naphthyl-1,2,3-Triazole Core Structure Targeting *Clostridioides* (*Clostridium*) *difficile*: Synthesis, Antibacterial Evaluation, and an In Vivo *C. difficile* Infection Model

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Abstract: *Clostridioides* (also known as *Clostridium*) *difficile* is a Gram-positive anaerobic, spore producing bacterial pathogen that causes severe gastrointestinal infection in humans. The current chemotherapeutic options are inadequate, expensive, and limited, and thus inexpensive drug treatments for *C. difficile* infection (CDI) with improved efficacy and specificity are urgently needed. To improve the solubility of our cationic amphiphilic 1,1′-binaphthylpeptidomimetics developed earlier that showed promise in an in vivo murine CDI model we have synthesized related compounds with an N-arytriazole or N-naphthyltriazole moiety instead of the 1,1′-biphenyl or 1,1′-binaphthyl moiety. This modification was made to increase the polarity and thus water solubility of the overall peptidomimetics, while maintaining the aromatic character. The dicationic N-naphthyltriazole derivative 40 was identified as a *C. difficile*-selective antibiotic with MIC values of 8 μg/mL against *C. difficile* strains ATCC 700507 and 132 (both ribotype 027). This compound displayed increased water solubility and reduced hemolytic activity (32 μg/mL) in an in vitro hemolysis assay and reduced cytotoxicity (CC50 32 μg/mL against HEK293 cells) relative to lead compound 2. Compound 40 exhibited mild efficacy (with 80% survival observed after 24 h compared to the DMSO control of 40%) in an in vivo murine model of *C. difficile* infection by reducing the severity and slowing the onset of disease.

Keywords: antibacterial; *Clostridioides* (*Clostridium*) *difficile*; peptidomimetic; triazole

1. Introduction

*Clostridioides* (also known as *Clostridium*) *difficile* is a Gram-positive, anaerobic spore-forming bacterium that causes mild to serious infections in the gastrointestinal tract (GIT) due to the production of potent exotoxins (TcdA, TcdB, and CDT) that cause severe gastrointestinal damage [1–3]. The resilient endospores contaminate healthcare environments and facilitate disease initiation, dissemination, and re-infection. In the GIT, spores require glycine and cholate derivatives for germination. In a healthy GIT, the microbiota metabolizes cholate derivatives preventing germination of *C. difficile* spores. CDI occurs when the...
normal GIT microbiota is disrupted or killed by conventional broad-spectrum antimicrobials [1]. Under these conditions the metabolism of cholate is significantly compromised, facilitating the germination of spores into \textit{C. difficile} vegetative cells [4,5].

CDI has a mortality rate of up to 8% [2] with the reoccurrence of infections occurring in up to 20% of cases treated with vancomycin or metronidazole [6]. A 2019 Antibiotic Resistance Threat Report from the US Centers for Disease Control and Prevention indicated that in the USA in 2017 an estimated 223,900 cases of CDI in hospitalized patients resulted in 12,800 deaths and $1 billion in attributed healthcare costs [7]. Thus, there is a significant and important incentive to develop novel therapeutics that show selectivity for \textit{C. difficile} over other gut bacteria to effectively combat CDI. While fecal microbiota transplantation can be effective for recurrent CDI, there can be adverse effects and the long-term impacts are unknown [1,2,8].

Fidaxomicin was specifically approved by the FDA in 2011 for treating CDI [9]; resulting in approximately 50% less CDI recurrence compared to vancomycin [10] most likely due to its greater selectivity for \textit{C. difficile}, less impact on commensal enteric microflora (i.e., \textit{Bacteroides} spp.), and its ability to reduce \textit{C. difficile} sporulation [11]. There are many potential chemotherapeutics undergoing clinical trials for the treatment of CDI [12]. Other small molecule chemotherapeutics currently under investigation for use against \textit{C. difficile}, include antimicrobial peptidomimetics [13–15], glycopeptides [16], bis-indoles [17], purine derivatives [18], tetramic acids [19], nitroheterocycles [20], macrocycles [21], and nylon-3 polymers [22]. Two vaccines are being investigated in clinical trials (Pfizer and Intercell [23]), whereas bezlotoxumab (a monoclonal antibody targeting \textit{C. difficile} TcdB) was given FDA approval in 2016 as adjunctive therapy for patients undergoing antimicrobial treatment who were at high risk of recurrent infection [24].

In our earlier work on the development of the cationic amphiphilic 1,1′-binaphthylpeptidomimetics, we established the pharmacophoric importance of a hydrophobic head group (e.g., a binaphthyl moiety) connected to a dicationic peptide in the development of broad-spectrum antibacterial agents. This led to the identification of compound 1 with potent antibacterial activity against drug resistant Gram-positive bacteria with potential for topical applications (Figure 1) [25]. More recent work in our laboratory has identified compounds 2–4 from a class of small molecule cationic amphiphilic 1,1′-biarylpeptidomimetics that exert antibacterial activity through cytoplasmic membrane disruption [13,14]. These compounds have IC$_{50}$ values of 4–8 µg/mL against \textit{C. difficile} (Figure 1). The efficacy of these compounds at treating CDI in an in vivo murine CDI model was assessed against vancomycin as a positive control with 10% DMSO as the negative control. Compound 2 appeared to protect the mice from disease at the 24 h point with a 50% survival rate (2/4 mice) vs. 0% survival in the 10% DMSO group; this was not statistically significant due to the small sample size. These results clearly showed that compound 2 exhibited a notable positive effect in the treatment of CDI. Unfortunately compound 3 showed poor solubility with precipitation during preparation in a 10% DMSO solution, and high in vitro hemolytic activity against HEK293 cells. While compound 4 showed promising in vitro properties, it performed poorly in the \textit{C. difficile} murine model with a survival rate of 60% after 24 h, but a 0% rate after 48 h [13], despite its low hemolytic activity. Despite some positive results, more water-soluble derivatives with lower hemolytic activity for further in vivo murine CDI model studies needed to be developed. To achieve this aim, we replaced the hydrophobic binaphthyl group found in 2 and 3 with an \textit{N}-aryltriazole or \textit{N}-naphthyltriazole moiety as shown in Figure 2. These modifications should retain the aromatic character of these molecules while inducing a better polarity profile and thereby increasing the water solubility of the overall peptidomimetics. It was not clear at the start what effect these modifications would have on the antibacterial activities of these newly proposed compounds or their specificity for \textit{C. difficile} over other pathogenic bacteria. Herein, we disclose the results of this investigation.
Figure 1. Previously published cationic amphiphilic hydrophobic anchored peptidomimetic antimicrobial agents. MIC values against *C. difficile* in µg/mL. SR = solubility ratio relative to that of compound 1—see Ref [13].

![Chemical structures](image)

**Figure 2.** Hydrophobic scaffold replacements of the binaphthyl moiety for the target peptidomimetics.

### 2. Results and Discussion

Preparation of the target *N*-aryltriazole or *N*-naphthyltriazole peptidomimetics required the synthesis of the carboxylic acid derivatives 5, 6, 17, 18, 27, and 28 based on scaffolds 1–4 (Figure 2); the syntheses of acid 17 is described in the experimental section with the other acid syntheses described in the Supporting Information.

The synthesis of the new peptidomimetic derivatives is described in Schemes 1–3. In a typical example, derivative 40 (Scheme 3) was generated starting from acid 17 coupling with the protected azidodipetide 29 under standard peptide coupling conditions (EDCI/HOBt) [26,27] to give amide 32 in 67% yield. This was followed by a standard copper-catalyzed azide-alkyne cycloaddition reactions [28] with ethenylcyclohexane to give the corresponding 1,4-disubstituted 1,2,3-triazole product which was deprotected using TFA/CH₂Cl₂/H₂O followed by treatment with ethereal HCl to yield the dicationic amphiphile 40 in 46% yield over two steps. The synthesis of the additional mono- and...
cationic peptidomimetic amphiphiles 10–16, 21–26, and 36–50 followed an analogous strategy and is summarized in Schemes 1–3 with experimental and characterization details provided in the Supporting Information.

**Scheme 1.** Synthesis of N-aryltriazole monocationic peptidomimetics 10–16. i. HOBr (1.1 eq), EDC.HCl (1.1 eq), Et3N (1.0 eq), CH2Cl2, rt, 16 h. ii. R3C≡CH, CuSO4.5H2O (0.2 eq), Naascorbate (0.4 eq), t-BuOH:H2O (4:1), rt, 16 h. iii. TFA/H2O/DCM, rt, 16 h; then HCl in Et2O.

| R1 | R2 | Yield % |
|----|----|---------|
| 5  | H  | 7-pent  |
| 6  | 7-pent | 58 |

**Scheme 2.** Synthesis of N-naphthyltriazole monocationic peptidomimetics 21–26. i. HOBr (1.1 eq), EDC.HCl (1.1 eq), Et3N (1.0 eq), CH2Cl2, rt, 16 h. ii. R3C≡CH, CuSO4.5H2O (0.2 eq), Naascorbate (0.4 eq), t-BuOH:H2O (4:1), rt, 16 h. iii. TFA/H2O/DCM, rt, 16 h; then HCl in Et2O.

| R1 | R2 | Yield % |
|----|----|---------|
| 17 | H  | 7-pent  |
| 18 | 7-pent | 58 |

Cy = cyclohexyl, Cp = cyclopentyl
**Scheme 3.** Synthesis of dicationic peptidomimetics 36–50-i. HOBt (1.1 eq), EDC.HCl (1.1 eq), Et₃N (1.0 eq), CH₂Cl₂, rt, 16 h. ii. R′C≡CH, CuSO₄·5H₂O (0.2 eq), Na.ascorbate (0.4 eq), t-BuOH:H₂O (4:1), rt, 16 h. iii. TFA/H₂O/DCM, rt, 16 h; then HCl in Et₂O.

The N-arytriazole and N-naphthyltriazole peptidomimetics were subjected to antimicrobial screening. In the first instance, minimum inhibitory concentrations (MICs) were determined against a panel of Gram-positive (including two strains of *C. difficile*) and Gram-negative pathogenic bacteria with vancomycin and the commercially available peptide colistin as positive controls, respectively; the MICs are displayed in Table 1. The compounds were then tested against a second panel of Gram-positive and Gram-negative pathogenic bacteria and two fungi strains at the Community for Open Antimicrobial Drug Discovery (CO-ADD)—these results are reported in the Supporting Information (Table S1) [29]. A cytotoxicity concentration (CC₅₀) assay was also performed by CO-ADD; the synthesized compounds were tested at concentrations ≤32 µg/mL on human embryonic kidney cells (HEK293 cells; ATCC CRL-1573) while hemolysis assays for lysis of human erythrocytes were also performed. Vancomycin, colistin, fluconazole, and tamoxifen were used as positive controls (see Table 1 for details). The CC₅₀ and HC₅₀ values are also shown in Table 1.
Preliminary antibiotic screening revealed that compared to the previously synthesized compounds 1–4, the new N-naphthyltriazole dicationic derivatives 40 and 42 showed the best activities against the two *C. difficile* RT 027 strains, ATCC 700,057 and 132 with a similar activity of 8 µg/mL compared to compounds 1, 3, and 4. However, they were generally less active against the other Gram-positive and Gram-negative bacteria (Table 1). The relative solubility ratios (relative to compound 1) [13] for 40 and 42 were 5 and 4 with CLogP values of 4.46 and 4.39, respectively, when compared to 1 with a ClogP of 7.47. Therefore, despite the better solubility profiles of these compounds, they failed to show better activity against *C. difficile*. However, the increased solubility (enhanced polarity) of derivatives 40–42 could be a factor in the reduced activities against the other bacteria, when compared to compounds 1–4 (see Table 2). None of the other derivatives synthesized in this study showed appreciable activity against *C. difficile* with MIC values ranging from 32 to 128 µg/mL (Table 1). Importantly, the remaining anti-bacterial results were generally poor, however for these specific derivatives, these reduced activities could indicate reduced capacity to interfere with normal GIT microbiota (Table 2). Compounds 40 and 42 showed a slight reduction in cytotoxicity against HEK293 cells compared to compounds 2 and 4. The hemolytic activity of these compounds was 32 µg/mL against human erythrocytes, 2-fold more than their IC50 values against *C. difficile*.

### Table 1. Preliminary antibacterial screening.

| Compound | *C. difficile* ATCC 700057 | *C. difficile* ATCC 29213 | S. aureus ATCC 29213 | S. aureus NCTC 10442 | *E. faecalis* ATCC 29212 | *S. pneumoniae* ATCC 49619 | *E. coli* ATCC 29222 | CC50 | HC50 |
|----------|---------------------------|---------------------------|---------------------|---------------------|-------------------------|-------------------------|------------------------|------|------|
| 1        | 10                        | 32                        | 64                  | 32                  | 32                      | 32                      | 16                     | 128  | >32  |
| 2        | 11                        | 32                        | 64                  | 32                  | 32                      | 32                      | 16                     | 128  | >32  |
| 3        | 12                        | 32                        | 64                  | 32                  | 32                      | 32                      | 16                     | 128  | >32  |
| 4        | 13                        | 32                        | 64                  | 32                  | 32                      | 32                      | 16                     | 128  | >32  |
| 5        | 14                        | 128                       | 128                 | 32                  | 32                      | 32                      | 32                     | >128 | >32  |
| 6        | 15                        | 64                        | 64                  | 16                  | 16                      | 16                      | 16                     | 128  | >32  |
| 7        | 16                        | 32                        | 32                  | 32                  | 32                      | 32                      | 32                     | >128 | >32  |
| 8        | 21                        | 128                       | >128                | 8                   | 8                       | 16                      | 16                     | 128  | >32  |
| 9        | 22                        | 32                        | 32                  | 4                   | 4                       | 8                       | 8                      | 32   | >32  |
| 10       | 23                        | 32                        | 32                  | 4                   | 4                       | 4                       | 4                      | 64   | >32  |
| 11       | 24                        | 32                        | 32                  | 8                   | 8                       | 8                       | 8                      | 32   | 21.9 |
| 12       | 25                        | 32                        | 64                  | 4                   | 4                       | 8                       | 8                      | 16   | >32  |
| 13       | 26                        | 64                        | >128                | 8                   | 8                       | 16                      | 4                      | 64   | 23.5 |
| 14       | 27                        | 64                        | 64                  | 16                  | 32                      | 16                      | 8                      | 64   | >32  |
| 15       | 28                        | 128                       | >128                | 128                 | >128                    | >128                    | >128                   | 128  | >32  |
| 16       | 29                        | 64                        | 32                  | 32                  | 64                      | 4                       | 64                     | >32  | >32  |
| 17       | 30                        | 8                         | 8                   | 16                  | 16                      | 32                      | 16                     | 128  | >32  |
| 18       | 31                        | 16                        | 16                  | 8                   | 8                       | 8                       | 8                      | 32   | 32   |
| 19       | 32                        | 8                         | 8                   | 8                   | 8                       | 8                       | 8                      | 32   | 32   |
| 20       | 33                        | 128                       | 128                 | 16                  | 16                      | 64                      | 4                       | 64   | >32  |
| 21       | 34                        | 32                        | 32                  | 8                   | 4                       | 16                      | 4                       | 64   | >32  |
| 22       | 35                        | 128                       | 128                 | 16                  | 16                      | 64                      | 4                       | 64   | >32  |
| 23       | 36                        | 64                        | 64                  | 16                  | 32                      | 16                      | 8                      | 64   | >32  |
| 24       | 37                        | 32                        | 32                  | 8                   | 4                       | 16                      | 4                       | 64   | >32  |
| 25       | 38                        | 128                       | >128                | 128                 | >128                    | >128                    | >128                   | 128  | >32  |
| 26       | 39                        | 64                        | 32                  | 32                  | 64                      | 16                     | 16                     | 128  | >32  |
| 27       | 40                        | 8                         | 8                   | 16                  | 16                      | 32                      | 16                     | 128  | >32  |
| 28       | 41                        | 16                        | 16                  | 8                   | 8                       | 8                       | 8                      | 32   | 32   |
| 29       | 42                        | 8                         | 8                   | 8                   | 8                       | 8                       | 8                      | 32   | 32   |
| 30       | 43                        | 128                       | 128                 | 16                  | 16                      | 64                      | 4                       | 64   | >32  |
| 31       | 44                        | 32                        | 32                  | 8                   | 4                       | 16                      | 4                       | 64   | >32  |
| 32       | 45                        | 128                       | 128                 | 16                  | 16                      | 64                      | 4                       | 64   | >32  |
| 33       | 46                        | 64                        | 32                  | 32                  | 64                      | 16                     | 16                     | 128  | >32  |
| 34       | 47                        | 128                       | 128                 | 32                  | 32                      | 64                      | >32                    | >32  | >32  |
| 35       | 48                        | >32                       | >32                 | >32                 | >32                     | >32                    | >32                    | >32  | >32  |
| 36       | 49                        | >32                       | >32                 | >32                 | >32                     | >32                    | >32                    | >32  | >32  |
| 37       | 50                        | >32                       | >32                 | >32                 | >32                     | >32                    | >32                    | >32  | >32  |

**Table 1.** Preliminary antibacterial screening.

- **Value**s are reported as MIC values in µg/mL.
- *C. difficile* PCR Ribotype (RT027).
- Methicillin resistant *S. aureus* (MRSA).
- Cytotoxicity; determined on HEK293 cells.
- Hemolysis; HC50 values determined by lysis of human erythrocytes and % hemolysis was determined by lysis of sheep erythrocytes. Vanc = vancomycin. Coloured cells refer to the same activities.
Table 2. Antimicrobial, cytotoxicity, and hemolytic activities of the three most active derivatives synthesized in this study.  

| Compound | C. difficile ATCC 700057 | C. difficile Ribotype 132 a | S. aureus ATCC 29213 | S. aureus ATCC 43300 c | S. aureus ATCC 39422 c,d | E. faecalis ATCC 29212 | S. pneumoniae ATCC 49619 | E. coli ATCC 25922 | CC50 e | HC50 f |
|----------|------------------------|---------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|--------|--------|
| 40       | 8                      | 8                         | 16                  | 8                   | 16                  | 32                  | 16                  | 64                  | 32     | 32     |
| 41       | 16                     | 16                        | 8                   | 4                   | 8                   | 8                   | 16                  | 128                 | 16     | 32     |
| 42       | 8                      | 8                         | 8                   | 8                   | 8                   | 8                   | 8                   | 32                  | 32     | 32     |
| 1 [30]   | 8                      | 8 h                       | 2                   | 2                   | 2                   | 2                   | 16                  | -                   | -      | -      |
| 2 [31]   | -                      | 32                        | 4                   | 2                   | 4                   | 4                   | 4                   | 8                   | 27.4   | 94%    |
| 3 [39]   | 8                      | 8 h                       | 2                   | 2                   | 4                   | 8                   | >128                 | -                   | -      | -      |
| 4 [31]   | -                      | 8                         | 8                   | 4                   | 4                   | 8                   | 8                   | 14.2                | 23%    | -      |
| vanc     | 0.5                    | 0.5                       | 1                   | 1                   | 1                   | 1                   | >16                  | -                   | -      | -      |

a Values are reported as MIC values in µg/mL. b C. difficile Ribotype (RT027). c Methicillin resistant S. aureus (MRSA). d Testing performed by the Community for Open Antimicrobial Drug Discovery (CO-ADD). e Cytotoxicity; determined on HEK293 cells. f Hemolysis; determined by lysis of sheep erythrocytes g HC50 values determined by lysis of human erythrocytes h C. difficile strain tested M7404 (RT027). i C. difficile strain tested R20291 (RT027). j % hemolysis at 50 µg/mL. vanc = vancomycin. Coloured cells refer to the same activities.

Analysis of the anti-bacterial activities against other bacterial species indicated that the monocationic naphthyltriazole derivatives 21–26 showed appreciable activity against Staphylococcus aureus (including an MRSA strain) with MIC values between 4 and 8 µg/mL (Table 1). Additionally, compound 21 had notable MIC values of 4 µg/mL against Enterococcus faecalis and Streptococcus pneumoniae. An overview of activity shown in Table 1 showed “pockets” of activities focused on the naphthyl-based derivatives (21–26 and 40–45, columns 1–4), with the monocationic examples (21–26) producing better outcomes against the Gram positive strains. The second screening results (Table S1, Supporting Information) were consistent with these results with analogous trends in activity against an additional S. aureus strain.

The secondary testing (Table S1, Supporting Information) also identified compounds 21, 25, and 40–46 as having activity against the fungal strain Cryptococcus neoformans var. grubii (ATCC208821) (MIC 4-8 µg/mL).

3. In Vivo Assay: Murine Model of CDI

Compound 40 was selected for further evaluation as an effective treatment for C. difficile using a murine model of CDI study because of its sustained antimicrobial potency against C. difficile and its better water solubility profile. The results from these studies are summarized in Figure 3.
The mice treated with compound 40 (red) showed delayed disease onset compared to mice treated with DMSO (blue; Figure 3), although they still succumbed to infection by day 2. Notably, at day 1 post-infection, mice treated with compound 40 showed 40% greater survival compared with mice treated with DMSO (Figure 3a), although there was no effect on mouse weight (Figure 3b), or spore numbers shed in the feces of these animals (Figure 3c), suggesting that compound 40 was not impacting C. difficile colonization. Furthermore, on day 1 post-infection, treatment with compound 40 resulted in a lower overall cage appearance score when compared to DMSO (Figure 3d), which suggested that this compound was delaying diarrheal onset although there was no significant difference in individual fecal score (Figure 3e) or physiological appearance score (Figure 3f) detected between the two groups of mice (Figure 3e). Thus, collectively these data suggest that compound 40 may reduce the severity of disease caused by C. difficile.

4. Materials and Methods

Synthetic methods and general characterization and analysis were as described previously [13].

Notes and other considerations. Known reagents that were not available commercially were prepared as reported using known methods and is detailed in the Supporting Information, [14,32–35].

4.1. General Synthesis Procedures

4.1.1. General Procedure I: Alkylation of Phenols (with Ethyl Bromoacetate)

A solution of the phenol (1 eq) in dry DMF (5 mL/mmol substrate) was stirred during the addition of K$_2$CO$_3$ (3 eq). Ethyl bromoacetate (1.3 eq) was added at room temperature and stirring was continued at rt for 12 h, before being diluted with EtOAc (2 × 50 mL). The resulting mixture was washed with water (2 × 50 mL), brine (2 × 50 mL), dried (MgSO$_4$), filtered, and concentrated under vacuum. The residue was subjected to silica gel flash column chromatography to afford the desired ester product.

4.1.2. General Procedure II: Ester Hydrolysis

A solution of the ester (1 eq) in ethanol (10 mL/mmol substrate) was stirred followed by the addition of 7% KOH solution (5 mL/mmol) at rt. The mixture was stirred at rt for
2 h, then acidified with 1 M HCl (25 mL). The resulting mixture was extracted with EtOAc (2 × 25 mL) and the combined extracts washed with brine (50 mL), dried (MgSO4), filtered, and concentrated under vacuum to afford the acid product.

4.1.3. General Procedure III: Amide Coupling

A mixture of the amine (1.0 eq), carboxylic acid (1.0 eq), EDC.HCl (1.2 eq), HOBt (1.1 eq), and TEA (1 eq) in dichloromethane/acetonitrile solution (10 mL/mmol amine) was stirred at rt for the specified time. The mixture was concentrated (if >5.0 mL dichloromethane/acetonitrile), and then the resulting residue dissolved in EtOAc (25 mL for reactions that contained ≤1.0 mmol amine or 25 mL/mmol amine for larger scale reactions) and washed with aqueous HCl (1.0 M–2 × 25 mL), saturated aqueous NaHCO3 (3 × 25 mL), and brine (1 × 25 mL). The organic solution was dried (MgSO4), filtered, concentrated and subjected to further purification via flash chromatography (if required) to furnish the targeted amide product.

4.1.4. General Procedure IV: Copper-Catalyzed Azide-Alkyne Cycloaddition

To a stirred solution of the azide (1.0 eq) and alkyne (2.0–3.0 eq in tert-butanol/water (4:1) at rt was added CuSO4·5H2O (0.2 eq), followed by sodium ascorbate (0.4 eq). The reaction was stirred at rt (unless noted otherwise) for the specified time. To the mixture was added aqueous saturated NH4Cl solution (1 mL), and water (20 mL) with the mixture then extracted with EtOAc (20 mL for reactions that contained ≤1.0 mmol azide or 20 mL/mmol azide for larger scale reactions). The organic layers were back-washed with water (2 × 25 mL), brine (2 × 25 mL), then dried (MgSO4), filtered, concentrated under vacuum and subjected to flash chromatography to afford the desired 1,4-disubstituted 1,2,3-triazole product.

4.1.5. General Procedure VII: Amine Deprotection (N-Boc and/or N-Pbf Removal)

To a solution of the N-protected amine (1.0 eq) in CH2Cl2 (30 mL/mmol substrate) (if the substrate contained an N-Pbf moiety, H2O (20.0 eq) was added to the solution) was added TFA (30.0 mL/mmol substrate) and then stirred at rt overnight (>16 h). The solvent was removed and the resulting residue dissolved in CH2Cl2 (30 mL/mmol substrate). Excess anhydrous HCl (2.0 M in Et2O, 15 mL/mmol substrate, 30.0 eq) was added and the solvent was then removed. The residue was then dissolved in a minimal volume of CH2Cl2 (or MeOH) and excess Et2O (25 mL for ≤0.1 mmol substrate) was added, resulting in a precipitate of the hydrochloride salt of the amine. The reaction mixture was filtered; the resulting filtrate collected, concentrated, triturated with Et2O (3 × 20 mL); and the solids then dissolved in MeOH. The solution was concentrated and dried in vacuo to yield the mono or di-hydrochloride salt as a thin, translucent film that usually required scratching with a spatula, producing a fine hygroscopic powder or amorphous gum.

4.2. Representative Synthesis of Compound 40

4.2.1. Ethyl 2-[[1-iodonaphthalen-2-yl]oxy]acetate

Following General Procedure I, 1-iodonaphthal (1.00 g, 3.70 mmol), K2CO3 (1.53 g, 11.11 mmol), and ethyl bromoacetate (0.80 g, 4.81 mmol) were stirred in DMF (8 mL) at rt for 16 h to give the titled ester (0.68 g, 52%) as a pale yellow waxy solid after flash chromatography over silica gel (EtOAc/n-hexane-10:90). TLC (EtOAc/n-hexane-20:80): Rf = 0.6; 1H NMR (400 MHz, CDCl3) δ 8.16 (d, J = 7.2 Hz, 1H, H8), 7.78 (d, J = 7.2 Hz, 1H, H5), 7.72 (d, J = 8.0 Hz, 1H, H4), 7.54 (t, J = 7.2 Hz, 1H, H7), 7.39 (t, J = 7.2 Hz, 1H, H6), 7.08 (d, J = 8.0 Hz, 1H, H3), 4.80 (s, 2H, H1′), 4.27 (q, J = 5.6 Hz, 2H, OCH2CH3),
1.29 (t, J = 5.6 Hz, 3H, OCH₂CH₃); ¹³C NMR (101 MHz, CDCl₃) δ 168.7 (C = O), 155.6 (C2), 135.8 (C8a), 131.7 (C4a), 130.6 (C4), 130.5 (C8), 128.4 (C7), 128.3 (C5), 121.1 (C6), 114.4 (C3), 89.4 (C1), 67.6 (C1′), 61.7 (OCH₂CH₃) 14.3 (OCH₂CH₃); IR (neat) νmax 2981, 1756, 1622, 1593, 1502, 1462, 1349, 1291, 1200, 1151, 1134, 1096, 1028, 801, 764, 747 cm⁻¹; MS (ESI +ve) m/z 379 ([M + Na]⁺, 100%); HRMS (ESI + ve TOF) calcd for C₁₄H₁₃O₃Na 378.9807, found 378.9801 ([M + Na]⁺).

4.2.2. Ethyl 2-((1-(4-isopentyl-1H-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetate

To a stirred solution of ethyl 2-(2-iodophenoxy)acetate (0.20 g, 0.54 mmol), 5-methyl-1-hexyne (0.16 g, 1.64 mmol), CuI (0.02 g, 0.11 mmol), NaN₃ (0.04 g, 0.60 mmol), and sodium ascorbate (0.04 g, 0.22 mmol) in DMSO (2.5 mL) in H₂O (0.5 mL) was added racemic trans-Ν,N′-dimethyl cyclohexane-1,2-diamine (0.016 g, 0.11 mmol) at rt under a nitrogen atmosphere. The reaction mixture was stirred and heated at 75 °C for 16 h. The reaction was cooled to rt and aqueous saturated NH₄Cl solution (3 mL) was added and the mixture was extracted with EtOAc (2 × 25 mL). The combined extracts were washed with water (25 mL), brine (25 mL) and dried (MgSO₄). The solution was filtered, concentrated under vacuum and the residue was subjected to silica gel flash column chromatography (EtOAc/n-hexane-10:90 → 100:0) to afford the titled compound (0.05 g, 25%) as a yellow waxy solid. TLC (EtOAc/n-hexane-33:67); Rf = 0.4; ¹H NMR (400 MHz, CDCl₃) δ 7.97 (d, J = 7.2 Hz, 1H, H8'), 7.84 (d, J = 6.4 Hz, 1H, H5'), 7.67 (s, 1H, H5), 7.49–7.41 (m, 2H, H6'/H7'), 7.27–7.25 (m, 2H, H3'/H4'), 4.67 (s, 2H, H1'''), 4.22 (q, J = 5.6 Hz, 2H, OCH₂CH₃), 2.89 (t, J = 5.6 Hz, 2H, H1'''), 1.73–1.67 (m, 3H, H2''/H3'''), 1.26 (t, J = 5.6 Hz, 3H, OCH₂OCH₃), 0.99 (d, J = 4.0 Hz, 6H, H4''/H5'''); ¹³C NMR (101 MHz, CDCl₃) δ 168.5 (C = O), 150.5 (C2'), 148.1 (C8a′), 131.6 (C4), 131.3 (C4a′), 129.5 (C4'), 128.5 (C5'), 127.9 (C7'), 125.3 (C8'), 124.7 (C6'), 122.1 (C5), 121.3 (C3'), 114.3 (C1'), 66.7 (C1'''), 61.6 (OCH₂CH₃), 38.6 (C2'), 27.9 (C1'), 23.8 (C3'''), 22.5 (C4''/C5'''); Observed by gHMBC), 14.2 (OCH₂CH₃); IR (neat) νmax 2954, 2928, 2868, 1748, 1632, 1600, 1513, 1483, 1454, 1430, 1366, 1288, 1206, 1150, 1117, 1087, 1042, 806, 749 cm⁻¹; MS (ESI +ve) m/z 390 ([M + Na]⁺, 100%); HRMS (ESI +ve TOF) calcd for C₂₁H₂₆N₃O₃ 368.1974, found 368.1985 ([M + H]⁺).

4.2.3. 2-((1-(4-Isopentyl-1H-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetic acid (17)

Following General Procedure II, ethyl 2-((1-(4-isopentyl-1H-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetate (0.07 g, 0.19 mmol) and 7% KOH solution (0.5 mL) were stirred in
ethanol (2 mL) at rt for 2 h to give after acidification the acid 17 (0.04 g, 62%) as a white solid. M.P. 152–154 °C. TLC (EtOAc/hexane-10:90): Rf = 0.2; 1H NMR (500 MHz, CDCl₃) δ 8.00 (d, J = 9.0 Hz, 1H, H8'), 7.88 (d, J = 7.5 Hz, 1H, H5'), 7.69 (s, 1H, H5), 7.54–7.46 (m, 2H, H6'/H7'), 7.47–7.29 (m, 2H, H3'/H4'), 4.78 (s, 2H, H1'''), 2.91–2.87 (m, 2H, H1'''), 1.71–1.68 (m, 3H, H2'/H3''), 0.98 (d, J = 6.0 Hz, 3H, H4'/H5''), COOH resonance was not observed; 13C NMR (126 MHz, CDCl₃) δ 170.6 (C = O), 150.4 (C2'), 148.4 (C8a'), 132.2 (C4), 130.7 (C4a'), 129.6 (C4'), 128.8 (C5'), 128.2 (C7'), 125.6 (C8'), 124.9 (C6'), 121.7 (C5), 120.9 (C3'), 114.4 (C1'), 66.8 (C1'''), 38.5 (C2'''), 28.0 (C3'''), 22.6 (C4''/C5'''); Observed by gHMBC; IR (neat) v max 3473, 2954, 2929, 2868, 1731, 1631, 1600, 1514, 1483, 1429, 1366, 1234, 1151, 1118, 1087, 1062, 923, 806, 748 cm⁻¹; MS (ESI +ve) m/z 362 ([M + Na]⁺, 40%), 340 ([M + H]⁺, 100%); HRMS (ESI + ve TOF) calculated for C₁₉H₂₂N₅O₃ 340.1661, found 340.1667 ([M + H]⁺).

4.2.4. (9H-Fluoren-9-yl)methyl tert-butyl [(R)-6-[[((R)-1-azido-5-(2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl]amino]-6-oxohexane-1,5-diyl]dicarbamate

To a reaction vessel charged with azide 7 [30] (1.38 g, 3.16 mmol), Fmoc-L-Lys(Boc)-OH (1.62 g, 3.50 mmol), EDCI (0.67 g, 3.50 mmol) and HOBt (0.53 g, 3.50 mmol) was added CH₂Cl₂ (10 mL) and the mixture was stirred at rt for 12 h. The mixture was concentrated and diluted with water (100 mL) and extracted with EtOAc (3 × 100 mL). The organic extracts were combined and washed with HCl (1 M–100 mL), aqueous NaHCO₃ (100 mL), brine (25 mL), dried (MgSO₄) and concentrated to give a pale-yellow residue. This residue was purified via flash chromatography over SiO₂ (MeOH/CH₂Cl₂ = 4:96) to afford the titled compound as an off-white foam (1.50 g, 54%). TLC (MeOH/CH₂Cl₂–10:90) Rf = 0.52; 1H-NMR (400 MHz, CDCl₃) δ 7.77–7.70 (m, 2H, H4'/H5''), 7.55 (d, J = 7.5 Hz, 2H, H1''/H8''), 7.55 (brs, 1H, βCONH), 3.71–3.32 (m, 2H, H1''/H8''), 7.29–7.21 (m, 2H, H3''/H6''), 7.17 (brs, 1H, αCONH), 6.31–6.24 (m, 2H, NH₂ (guanidine)), 5.99–6.09 (brs, 1H, N⁵-H), 4.82–4.72 (brs, 1H, LysN¹-H), 4.33 (d, J = 7.4 Hz, 2H, H10''), 4.25–4.07 (m, 2H, Lys5/H9''), 4.07–3.97 (m, 1H, H2), 3.41–3.23 (m, 2H, H1), 3.23–2.98 (m, 4H, H5/Lys1), 2.89 (s, 2H, H3'), 2.55 (s, 3H, C6'-CH₃), 2.48 (s, 3H, C4'-CH₃), 2.06 (s, 3H, C7'-CH₃), 1.67 (s, 6H, C2'-CH₃), 1.55–1.35 (m, 19H, H3/H4/Lys2/Lys3/Lys4/C(CH₃)₃); 13C NMR (101 MHz, CDCl₃) δ 172.7 (Cβ), 158.8 (C7a'), 156.7 (Cα), 156.4 (C = N), 156.2 (COOC(CH₃)₃), 143.85 (C1a' or C8a'), 143.83 (C8a' or C1a'), 143.82 (C4a' or C5a'), 143.6 (C5a' or C4a'), 138.3 (C3a'), 132.8 (C6'), 132.2 (C4'), 127.8 (C3'/C6''), 127.1 (C4''/C5''), 125.0 (C2''/C7''), 124.7 (C5'), 120.0 (C1''/C8''), 117.6 (C7'), 86.4 (C2'), 79.3 (C(CH₃)₂), 67.3 (C10''), 55.1 (Lys5), 54.8 (C1), 48.8 (C2), 47.0 (C9'), 43.2 (C3'), 40.9 (C5'), 39.9 (Lys1), 31.9 (Lys2), 29.5 (Lys4), 29.3 (C3), 28.6 (C2''-CH₃₂), 28.4 (C(CH₃)₃), 25.5 (C4), 22.5 (Lys3), 19.3 (C6'-CH₃), 17.9
(C₄'CH₃), 12.5 C₇'-CH₃); IR (neat) \( \tilde{\nu}_{\text{max}} \) 3322, 2101, 1634, 1548, 1450, 1248, 1165, 1092, 739, 567 cm⁻¹; MS (ESI +ve) \( m/z \) 888 ([M + H]⁺), 910 ([M + Na⁺]); HRMS (ESI +ve TOF) calcd for \( \text{C}_{45}\text{H}_{61}\text{N}_{6}\text{O}_{8}\text{Na} \) 910.4262, found 910.4218 ([M + Na⁺]).

4.2.5. Tert-butyl ((R)-5-amino-6-(((R)-1-azido-5-(2-(2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-6-oxohexane-1,5-diyl)dicarbamate (31)

![Chemical Structure of Compound 31](image)

To a solution of the above Fmoc-protected amine (1.50 g, 1.69 mmol) in acetonitrile (15 mL) was added piperidine (0.25 mL, 1.5 eq.) and the reaction was stirred vigorously at rt for 12 h. The reaction mixture was diluted with MeOH (50 mL) and extracted with hexane (50 mL) multiple times until TLC analysis showed no byproduct (dibenzofulvene piperidine adduct) present in the MeOH layer. The MeOH extract was concentrated under reduced pressure to give 31 as an off-white foam (0.80 g, 71%). TLC (MeOH/CH₂Cl₂–10:90) \( R_f = 0.2 \); \( ^1\text{H}-\text{NMR} \) (500 MHz, CDCl₃) \( \delta \) 7.61 (brs, 1H, N²-H), 6.42–6.20 (m, 3H, N⁵-H/NH₂ (guanidine)), 4.82–4.72 (m, 1H, Lys₁N-H), 3.46–3.29 (m, 3H, H1/H2), 3.29–3.14 (m, 2H, H3), 2.96 (s, 2H, C3'/CH₃), 2.58 (s, 3H, C6'-CH₃), 2.52 (s, 3H, C4'-CH₃), 2.10 (s, 3H, C7''-CH₃), 1.62–1.31 (m, 25H, H3/H4/Lys2/Lys3/Lys4/Lys5/C(CH₃)₃/C2'- (CH₃)₂), N³H₂ resonance was not observed; \( ^{13}\text{C}-\text{NMR} \) (126 MHz, CDCl₃) \( \delta \) 158.8 (C7'a'), 156.6 (C = O), 156.4 (C = N), 138.5 (C3'a'), 133.2 (C4'), 132.4 (C6'), 124.7 (C5'), 117.6 (C7'), 86.5 (C2'), 79.4 ((C(CH₃)₃), 55.1 (Lys5), 55.0 (C1), 46.9 (C2), 43.4 (C3'), 40.9 (C5), 40.4 (Lys1), 34.7 (Lys4), 30.1 (Lys2), 29.8 (C3), 28.8 (C2'-CH₃)₂, 28.6 (C(CH₃)₃), 25.8 (C4), 22.7 (Lys3), 19.4 (C6'-CH₃), 18.1 (C4'-CH₃), 12.6 (C7''-CH₃), COO(C(CH₃)₃) resonance was not observed; IR (neat) \( \tilde{\nu}_{\text{max}} \) 3327, 2101, 1685, 1620, 1551, 1454, 1366, 1278, 1250, 1168, 1094, 665, 569 cm⁻¹; MS (ESI +ve) \( m/z \) 666 ([M + H]⁺); HRMS (ESI +ve TOF) calcd for \( \text{C}_{30}\text{H}_{52}\text{N}_{6}\text{O}_{8} \) 666.3761, found 666.3741 ([M + H]⁺).
4.2.6. Tert-butyl ((R)-6-(((R)-1-azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-5-((1-(4-isopentyl-1H,1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamido)-6-oxohexyl)carbamate 37 (0.24 g, 0.35 mmol), EDCl.HCl (0.08 g, 0.39 mmol), HOBt (0.06 g, 0.39 mmol), and TEA (0.03 g, 0.35 mmol) were stirred in CH₂Cl₂ (5 mL) at rt for 12 h to give the acetamide 65 (0.22 g, 64%) as an off-white solid. M.P: 236–238 °C. TLC (MeOH/CH₂Cl₂-10:90): Rf = 0.5; 1H NMR (400 MHz, CDCl₃) δ 8.05 (d, J = 8.5 Hz, 1H, Ar8), 7.89 (d, J = 16.5 Hz, 2H, OCH₂B), 68.0 (OCH₂A), 138.4 (C7a′′′), 132.44 (C3a′′′), 132.46 (C6a′′′), 132.4 (C3′′), 129.4 (C4′′), 129.1 (Ar4a), 128.48 (C7′′′), 128.47 (Ar5), 125.7 (Ar7), 124.3 (Ar8), 121.1 (C5′′), 120.3 (Ar6), 117.5 (Ar3), 113.8 (Ar1), 86.5 (C2′′′), 79.2 (C(CH₃)₃), 68.0 (O(CH₂)₃), 54.8 (Lys5), 53.6 (C1′), 43.4 (C2′), 40.8 (C2′′), 40.2 (C5′), 38.6 (C3′), 38.5 (Lys1), 31.79 (Lys4), 31.74 (C3), 29.4 (Lys2), 28.7 (C2′′-(CH₃)₂), 28.6 ((CH₃)₃), 28.0 (C1′′′), 25.5 (C4′), 23.8 (C3′′′), 22.8 (C4′′/C5′′′), 22.6 (Lys3), 19.4 (C4′-CH₃), 18.1 (C6′-CH₃), 12.6 (C7′′′-CH₃); IR (neat) 𝜈̅max 3327, 2101, 1685, 1620, 1551, 1514, 1484, 1452, 1406, 1390, 1366, 1265, 1165, 1100, 1090, 1044, 994, 970, 852, 781, 733, 661, 641 cm⁻¹; MS (ESI +ve) m/z 987 ([M + H]⁺, 100%); HRMS (ESI +ve TOF) calcd for C₄₉H₇₁N₁₂O₅S 987.5239, found 987.5272 ([M + H]⁺).
Antibiotics 2021, 10, × FOR PEER REVIEW 13 of 16

4.2.7. (R)-6-Amino-N-((R)-1-(4-cyclohexyl-1H-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-((1-(4-isopentyl-1H-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamido)hexanamide dihydrochloride (40)

Following General Procedure IV, azide 32 (0.08 g, 0.08 mmol), cyclohexylacetylene (0.03 g, 0.24 mmol), CuSO₄·5H₂O (0.004 g, 0.01 mmol) and sodium ascorbate (0.006 g, 0.03 mmol) were stirred in R-BuOH (2.0 mL) and H₂O (0.5 mL) for 16 h to give the triazole product as an off-white gum after flash chromatography over SiO₂ gel (MeOH/CH₂Cl₂–H₂O:100 → 8:92). Following General Procedure VII, the intermediate (0.06 g, 0.05 mmol) was dissolved in CH₂Cl₂ (2 mL), treated with H₂O (0.02 g, 1.00 mmol) and CF₃COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt 40 (0.03 g, 46% over two steps) as an off-white solid that rapidly transitioned to a sticky gum. [a]₂⁵ + 59.1 (c 0.0052, MeOH); ¹H NMR (400 MHz, CD₃OD) δ 8.30 (s, 1H, H5), 8.29 (s, 1H, H4), 8.18 (d, J = 9.2 Hz, 1H, Ar8), 7.98 (d, J = 7.5 Hz, 1H, Ar5), 7.61 (ddd, J = 9.2, 9.2, 1.7 Hz, 1H, Ar7), 7.57–7.49 (m, 2H, Ar6/Ar4), 7.14 (d, J = 8.3 Hz, 1H, Ar3), 4.37–4.32 (m, 1H, Lys5), 4.12–4.09 (m, 1H, H2′′′), 3.88–3.83 (m, 2H, H5′′′), 2.95–2.91 (m, 2H, Lys1), 2.84–2.78 (m, 3H, H1‴′/H1‴), 2.00–1.96 (m, 2H, Lys4), 1.74–1.60 (m, 14H, 6H′′/6H′′′/Lys2/H3′/H4′/H2′′/H3′′′/H4′′/H5′/H6′′), 1.48–1.21 (m, 7H, 6H′/6H′′/H3′′′/H4′′′/H5′′′/H6′′′), 1.01 (d, J = 6.2 Hz, 6H, H4′″/H5′″/H6′″); ¹³C NMR (101 MHz, CD₃OD) δ 173.0 (C = O), 169.1 (αC = O), 157.1 (C = N), 150.8 (Ar2), 148.7 (C4), 147.6 (C6), 132.5 (Ar8a), 130.3 (Ar4), 129.1 (Ar4a), 128.6 (Ar5), 128.0 (Ar7), 126.9 (Ar8), 125.5 (C5), 125.1 (Cy), 120.2 (Ar6), 119.1 (Ar3), 113.9 (Ar1), 67.4 (OCH₃H₄), 55.7 (C1′), 53.5 (Lys5), 49.3 (C2′), 40.4 (C5′), 39.0 (Lys1), 37.9 (C2″″), 33.4 (C1‴′), 31.6 (C2″′′), 31.5 (C6″″), 30.9 (Lys4), 28.1 (Lys2), 27.5 (C1‴′′), 26.5 (C3′), 25.2 (C4″′), 25.0 (C3″′/C5″′), 24.8 (C3″″), 22.6 (C4″), 22.5 (C4″′/C5″′), 21.3 (Lys3); IR (neat) νmax 3348, 3265, 3202, 3066, 2932, 2860, 1662, 1544, 1518, 1431, 1384, 1366, 1349, 1279, 1220, 1168, 1117, 1081, 1049, 816, 749, 668, 585 cm⁻¹; MS (ESI + ve) m/z 743 ([M–2HCl + H]+, 60%), 372 ([M–2HCl + H]+, 100%); HRMS (ESI + ve TOF) calcd for C₃₉H₅₉N₁₂O₃ 743.4833, found 743.4866 ([M–2HCl + H]+).

4.3. Microbiological Assays

Primary screening (Gram-positive bacteria). Primary MIC assays were performed as described by the Clinical and Laboratory Standards Institute for aerobic [36] and anaerobic [37] bacteria. MIC values for vancomycin were within acceptable QC ranges [38].

Secondary screening (MRSA and Gram-negative bacteria) and cytotoxicity assays performed by the Community for Open Antimicrobial Drug Discovery (CO-ADD). Samples were provided to CO-ADD [29] for antimicrobial screening by whole cell growth inhibition assays.

Bacterial Inhibition–MIC Assay. These were performed as described previously [13,29].

Cytotoxicity Assay. These were performed as described previously [13,29].

Haemolysis assay (sheep erythrocytes). These were performed as described previously [13].
Hemolysis assay (human erythrocytes)–HC₅₀ determination. These were performed as described previously [13,29].

4.4. In Vivo Murine Model of CDI Treatment

Disease Treatment Model. These experiments were performed as previously described [39–42]. Mice were humanely killed at the onset of severe disease or at the end of the experiment (day 4), as previously described [43].

Statistical Analysis. Statistical analysis was performed using Prism 7 (GraphPad Software). The Kaplan–Meier survival curves were assessed using a log-rank (Mantel–Cox) test. Weight loss, spore shedding, fecal consistency, and physiological appearance data were analyzed by one-way ANOVA with a post hoc Tukey’s multiple comparison test. Differences in data values were considered significant at a p value of <0.05.

5. Conclusions

This study reported the next generation of hydrophobic anchored cationic peptidomimetics as antibacterial agents, with a focus on targeting CDI. A major aim was to improve the solubility profile of these compounds to allow for sufficient solubility for efficient administration of the drug while maintaining gut availability and antibacterial activity. The naphthyltriazole derivates containing either a monocationic or dicationic amino acid side chain were generally the most effective, with compounds 40 and 42, possessing terminal cyclohexyl and benzyl moieties, respectively, exhibiting MIC values of 8 µg/mL.

Naphthyltriazole 40 was selected for an in vivo murine model trials of CDI but exhibited only mild evidence of in vivo efficacy indicating that further investigation into the structural and biological parameters affecting the in vivo efficacy of these antibacterial peptidomimetics is required, as the observed in vitro efficacy did not translate directly into in vivo efficacy. We have already reported that a correlation exists between increased hemolytic activity and an increase in hydrophobic/cationic ratio [15]; unfortunately, compound 40 exhibited a slight increase in hemolytic activity relative to the majority of tested compounds in this class with an HC₅₀ value of 32 µg/mL. While the selectivity ratio could be more substantial, this is acceptable for the future development of these gastrointestinal focused compounds. We have previously reported a comparative solubility assay for this class of antimicrobial agents with increasing numerical values corresponding to better aqueous solubility relative to compound 1 (which possesses a value of 1) [13]. Compound 40 showed a better solubility ratio with an assay value of 5, relative to our lead compound 2 with a value 3—this is also reflected in the CLogP values of 4.46 and 5.76 for 41 vs. 2, respectively. These outcomes were confirmed with no issues during the mouse model trials with sufficient solubility in the dosage regimen. Variations on the triazole and O-naphthyl substituents could be made in future studies with the view of enhancing antibacterial activity against C. difficile.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/antibiotics10080913/s1, Figures S1–S85: Details of synthesis and characterization data for compounds; Table S1: Secondary antimicrobial screening a–(bacteria and fungi), Murine model studies experimental procedures.

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