Received: 31 October 2018 | Revised: 4 December 2018 | Accepted: 5 December 2018

DOI: 10.1002/ddr.21508

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RESEARCH ARTICLE

Design, synthesis, and biological evaluation of 1,8-naphthyridine glucosamine conjugates as antimicrobial agents

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Funding information
The University of Jordan, Grant/Award Number: 2016

Abstract

In the quest for discovering potent antimicrobial agents with lower toxicity, we envisioned the design and synthesis of nalidixic acid-*D*-(+)-glucosamine conjugates. The novel compounds were synthesized and evaluated for their in vitro antimicrobial activity against Gram positive bacteria, Gram negative bacteria and fungi. Cytotoxicity using MTT assay over L6 skeletal myoblast cell line, ATCC CRL-1458 was carried out. In vitro antimicrobial assay revealed that 1-ethyl-7-methyl-4-oxo-N-(1,3,4,6-tetra-*O*-acetyl-2-deoxy-*D*-glucopyranose-2-yl)-[1,8]-naphthyridine-3-carboxamide **(5)** and 1-ethyl-7-methyl-4-oxo-N-(2-deoxy-*D*-glucopyranose-2-yl)-[1,8]-naphthyridine-3-carboxamide**(6)** possess growth inhibitory activity against resistant *Escherichia coli* NCTC, 11954 (MIC 0.1589 mM) and Methicillin resistant *Staphylococcus aureus* ATCC, 33591 (MIC 0.1589 mM). Compound **(5)** was more active against *Listeria monocytogenes* ATCC 19115 (MIC 0.1113 mM) in comparison with the reference nalidixic acid (MIC 1.0765 mM). Interestingly, compound **(6)** had potential antifungal activity against *Candida albicans* ATCC 10231 (MIC <0.0099 mM). Remarkably, the tested compounds had low cytotoxic effect. This study indicated that glucosamine moiety inclusion into the chemical structure of the marketed nalidixic acid enhances antimicrobial activity and safety.

KEYWORDS

antimicrobial, carboxamide, glucosamine, 1,8-napthyridine, nalidixic acid, resistant bacteria

1 | INTRODUCTION

Nalidixic acid is the first 4-quinolone antibacterial agent with α, β unsaturated carboxylic acid at the 2, 3 positions of the 1,8 naphthyridine nucleus (Assaleh, Chandu, Peraman, & Katakam, 2013; Nicolle, 2005). Following oral administration, nalidixic acid failed to achieve optimum plasma or tissue concentrations as it was rapidly excreted in the urine. Hence, nalidixic acid was commonly prescribed for the treatment of urinary tract infections (UTIs; Morrissey, Eustis, Haseman, Huff, & Bucher, 1991). However, patients with stones or obstructive uropathies or patients with catheter-related infections were usually diagnosed with complicated UTIs associated with nosocomial, antibiotic-resistant bacteria, and candidiasis (Hof, 2003; Nicolle, 2005). Nalidixic acid acts by inhibiting DNA synthesis during bacterial replication by interfering with DNA gyrase (topoisomerase 2) activity (Grover & Kini, 2006; Shen, Baranowski, Fostel, Montgomery, & Lartey, 1992). This enzyme is also found in *Aspergillus flavus* (*A. flavus*) fungi; aspergillosis-causative agent.

Previous structure activity relationship studies about 1,8-naphthyridine ring activity pointed out the potential inhibitory effect of substituents at Position 3 on bacterial DNA gyrase. Grover et al., developed novel agents by conjugating nalidixic acid with

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heterocyclic antifungal agents (Grover & Kini, 2006). Alternatively, bioisosteric replacement of C-3 carboxylic acid in ciprofloxacin with isothiazolo ring improved antibacterial activity by 4–10 folds (Chu & Fernandes, 1989). Lately, two naphthyl ester nalidixic acid derivatives have been reported with enhanced antibacterial activity against *Escherichia coli* (*E. coli*) upon irradiation (Vargas et al., 2008). Other structural variations bring about new tricyclic derivatives by modifying the β carbon of nalidixic acid α, β unsaturated carboxylic acid by Michaels addition (Assaleh et al., 2013) or by manipulating C-3 while retaining the biodynamic biheterocycle core (Grover & Kini, 2006).

The prevalance of bacterial resistance compelled medicinal chemists to design and synthesize new quinolones derivatives, either to enhance their activity and/or pharmacokinetics properties (Emmerson & Jones, 2003; Inoue et al., 1987). It is to be mentioned that these compounds mechanistic activity have been investigated, but their safety profile is still questionable. Thus, chemimanipulation of nalidixic acid is still essential to combat the emergence of microbial multidrug-resistance and to lower side effects.

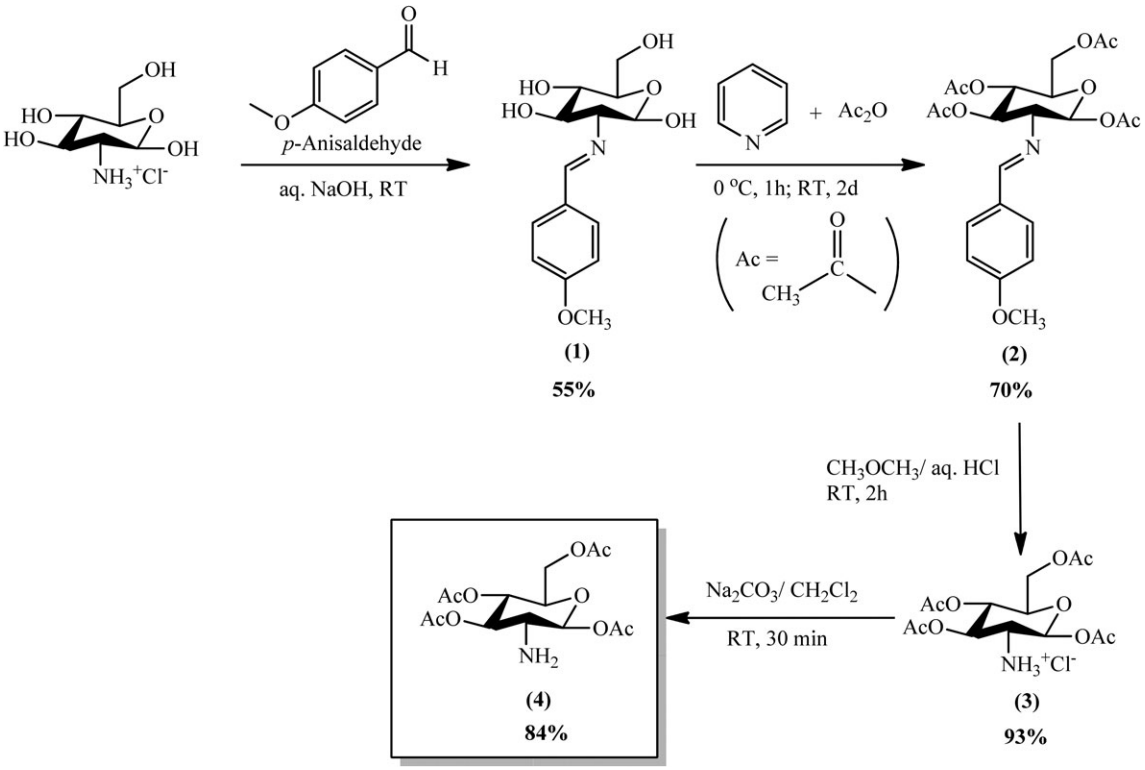
D-(+)-glucosamine amino sugar moiety is proposed to promote drug uptake by microbes (Laederach & Reilly, 2005) and thus, altering their antibacterial and/or antifungal activity. In addition, bacterial vital functions as cell division, energy conversion and motility were mediated by the glycosylated bacterial biogenic proteins (Laederach & Reilly, 2005; Ziegler & Hermann, 2008). Previous studies reported that chitosan polysaccharide; the N-acetyl glucosamine polymer had fungicidal activity against *A. flavus* (Roller & Covill, 1999). Other studies proposed lower cytotoxicity level with derivatives containing sugar carriers because of their selective uptake via the sugar-dependent phosphotransferase system in the bacteria (Laederach & Reilly, 2005). In this study, we designed and synthesized novel nalidixic acid-based-*D*-(+)-glucosamine conjugates as antimicrobial agents.

2 | MATERIALS AND METHODS

2.1 | Chemistry

2.1.1 | General

Nalidixic acid was obtained from Dar Al Dawa company (Jordanian Pharmaceutical Company). *D*-(+)-glucosamine hydrochloride, Reagent grade chemicals and solvents were purchased from Sigma–Aldrich (St. Louis, MO). TLC was performed on precoated TLC sheets ALUGRAM Xtra SIL G/UV_{254} and compounds were visualized under UV light. Melting points were measured manually by capillary method, using a Stuart melting point apparatus (SMP3, UK) and by Differential Scanning Calorimetry (DSC; Metler Toledo, Switzerland). FTIR spectra were recorded on Shimadzu FTIR spectrophotometer (Japan). ^{1}HNMR, ^{13}CNMR, ^{1}H-^{1}H COSY, DEPT 135, and HQMC spectra were recorded on Bruker Advance III spectrometers (500 MHz) (Switzerland) with deteriorated solvents. Chemical shifts were reported in parts permillion (δ) relative to tetra methyl silane as an internal standard. Significant ^{1}H NMR data were tabulated in the following order:number of proton(s) and multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet); *J* values are in Hertz (Hz). High resolution mass spectra (HRMS) were acquired (in positive and/or negative mode) using electrospray ion trap (ESI) technique by collision-induced dissociation on a Bruker Apex IV (7-Tesla) instrument (Germany). Samples were dissolved and



SCHEME 1 Synthesis of 1,3,4,6-tetra-*O*-acetyl-β-*D*-glucosamine (compound **4**)

infused using a syringe pump with a flow rate of 120 μL/min. External calibration was conducted using arginine cluster in mass range of 175–871 Da. Mass error: 0.00–0.50 mDa.

2.1.2 | General procedures for the synthesis of compounds (1–6) as reported in Schemes 1 and 2

2-Deoxy-2-[*p*-methoxybenzylidene(amino)]-*D*-glucopyranose (1)

General procedure was followed using 4-methoxybenzaldehyde and *D*-(+)-glucosamine hydrochloride to afford compound **(1)** (15.11 g, 54.7%) as a white solid: m.p. 164–168 °C (m.p.165–166 °C, Myszka, Bednarczyk, Najder, & Kaca, 2003): IRν_{max}: 1650 cm^{-1} (N═C), 3,200 cm^{-1} (O—H); ^{1}H-NMR (DMSO-d_6 500 MHz) δ_H 2.80 (1H, t, *J* = 8.4 Hz, CH), 3.13–3.18 (1H, m, CH), 3.22–3.25 (1H, m, CH), 3.41–3.43 (1H, m, CH), 3.45–3.52 (1H, m, CH), 3.73(1H, d, *J* = 5.4, 10.4 Hz, CH), 3.81 (3H, m, OCH_3), 4.53 (1H, t, *J* = 5.7 Hz, OH), 4.70 (1H, t, *J* = 7.2 Hz, OH), 4.80 (1H, d, *J* = 5.5 Hz, OH), 4.91 (1H, d, *J* = 5.2 Hz, OH), 6.50 (1H, d, *J* = 5.5 Hz, CH), 6.99 (2H, d, *J* = 8.5 Hz, Ar—H), 7.70 (2 H, d, *J* = 8.6 Hz, Ar—H), 8.12 (1H, s, N═CH).

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-[p-methoxybenzylidene (amino)] β-*D*-glucopyranose (2)

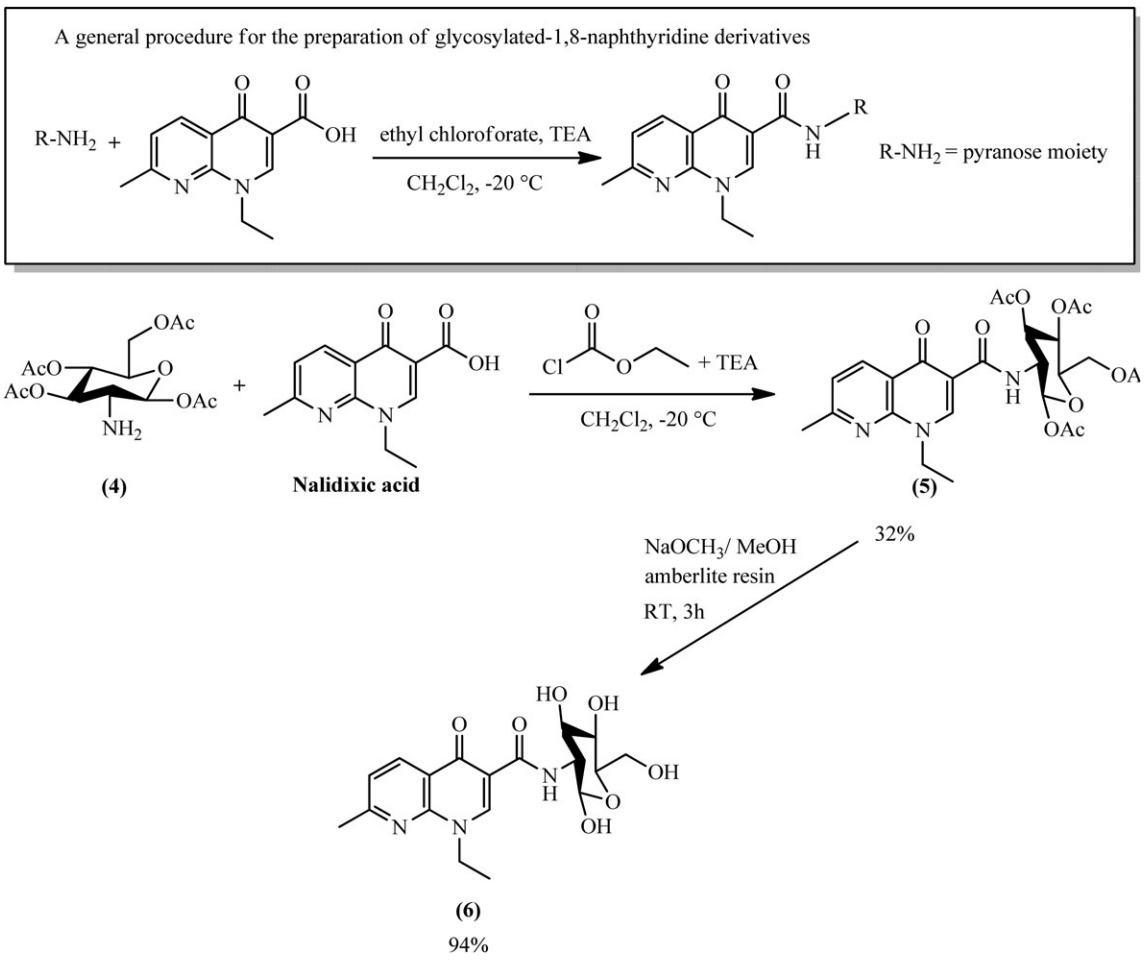
General procedure was followed using compound **1** acetic to afford compound **(2)** (16.58 g, 69.8%) as a white solid: m.p. 179–183.1 °C (m.p. 180–182 °C, Myszka et al., 2003), IRν_{max} 1750 cm^{-1} (C═O), 1,650 cm^{-1} (C═N); ^{1}H-NMR (DMSO-d_6. 500 MHz) δ_H 1.82 (3H, s, CH_3CO), 1.99 (6H, s, 2 × CH_3CO), 2.48 (3H, s, CH_3CO), 3.33 (1H, m, H-2), 3.77 (3H, s, CH_3O), 3.95–4.04 (1H, m, H-5), 4.20–4.40 (2H, m, H-6α, H-6β), 4.97 (1H, t, *J* = 9.7 Hz, H-4), 5.45 (1H, t, *J* = 9.7 Hz, H-3), 6.07 (1H, d, *J* = 8.2 Hz, H-1), 6.99 (2H, d, *J* = 8.6 Hz, Ar—H), 7.66 (2 H, d, *J* = 8.6 Hz, Ar—H), 8.29 (1H, s, N═CH).

1,3,4,6-Tetra-*O*-acetyl-β-*D*-glucosamine hydrochloride (3)

General procedure was followed using compound **2** and aq.5 M HCl to afford compound **(3)** (12.425 g, 92.5%) as a white solid: m.p. 229–230 °C (m.p. 235 °C, Myszka et al., 2003), IRν_{max}: 2800 cm^{-1} (NH_3Cl), 1,750 cm^{-1} (C═O); ^{1}H-NMR (DMSO-d_6. 500 MHz) δ_H 2.00 (3H, s, CH_3CO), 2.04 (6H, s, 2 × CH_3CO), 2.12 (3H, s, CH_3CO), 3.55 (2H, t, *J* = 9.5 Hz, H-2), 3.99–4.05 (2H, m, H-5, H-6), 4.20 (1H, dd, *J* = 4.1, 12.4 Hz, H-6), 4.95 (1H, t, *J* = 9.6 Hz, H-4), 5.37 (1H, t, *J* = 9.7 Hz, H-3), 5.94 (1H, d, *J* = 8.6 Hz, H-1), 8.87 (3H, s, NH_3Cl).

1,3,4,6-Tetra-*O*-acetyl-β-*D*-glucosamine (4)

General procedure was followed using compound **3** and aq. 1 M Na_2CO_3 to afford compound **(4)** (4.7 g, 84%) as white solid powder: m.p. 138–142 °C (m.p. 138 °C, Dang, Nguyen, Nguyen, & Im, 2014); ^{1}H-NMR (DMSO-d_6. 500 MHz) δ_H 1.65 (2H, s, NH_2), 2.00 (9H, s, 3 × CH_3CO), 2.12 (3H, s, CH_3CO), 2.76 (1H, dd, *J* = 8.8, 9.7 Hz, H-2),



SCHEME 2 Synthesis of glycosylated 1,8-napthyridine derivatives

3.96–3.98 (2H, m, H-5, H-6), 4.17 (1H, dd, *J* = 5.1, 13.0 Hz, H-6), 4.82 (1H, t, *J* = 9.6 Hz, H-4), 5.06 (1H, t, *J* = 9.6 Hz, H-3), 5.55 (1H, d, *J* = 8.5 Hz, H-1).

1-Ethyl-7-methyl-4-oxo-N-(1,3,4,6-tetra-*O*-acetyl-2-deoxy-*D*-glucopyranose-2-yl)-[1,8]-naphthyridine-3-carboxamide (5)

Ethyl chloroformate (0.530 mL, 5.7 mmol) was added drop-wise to a solution of TEA (0.600 mL, 4.3 mmol) and nalidixic acid (1.0 g, 4.3 mmol) in CH_2Cl_2 (100 mL) at −20 °C. The mixture was left stirring at −20 °C until reaction completion as revealed by TLC (EtOAc: n-hexane 10:1, R_f = 0.70). Following, compound **(4)** (1.5 g, 4.3 mmol) dissolved in cold CH_2Cl_2 (30 mL) was added drop-wise and the mixture was stirred at −20 °C for 3 h and then left stirring at RT for 24 h. Washing with H_2O (150 mL), aq. 1 M HCl (150 mL), aq. 5% $NaHCO_3$ (150 mL), H_2O (150 mL), drying over anhydrous Na_2SO_4, and evaporation yielded a gummy residue. Trituration with Et_2O (100 mL) and recrystallization with n-hexane and EtOAc afforded compound **(5)** (0.760 g, 32%) as a white solid m.p. 182.0–185.0 °C (DSC: 183.5 °C); IRν_{max} 3240.49 cm^{-1} (CONH), 1,746.08 (C═O), 1,660.77 cm^{-1} (C═O amide); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H 1.33 (3H, t, *J* = 6.7 Hz, —CH_3), 1.80 (3H, s, CH_3CO), 1.94 (6H, s, CH_3CO), 1.97 (3H, s, CH_3CO), 2.65 (3H, s, —CH_3Napth), 3.95–3.97 (1H, m, H-6), 4.10–4.17 (2H, m, H-5, H-6), 4.27 (1H, dd, *J* = 5.0, 12.4 Hz, H-2), 4.50 (2H, q, *J* = 6.7 Hz, —CH_2), 4.91 (1H, t, *J* = 8.8 Hz, H-4), 5.52 (1H, t, *J* = 9.2 Hz, H-3), 6.06 (1H, d, *J* = 8.0 Hz, H-1), 8.41 (1H, d, *J* = 7.4 Hz, H-6Napth), 8.46 (1H, d, *J* = 7.4 Hz, H-5Napth), 8.93 (1H, s, H-2Napth), 9.91 (1H, d, *J* = 8.7 Hz, CONH); ^{13}C-NMR (DMSO, 500 MHz) 15.44 ($CH_2\underline{CH_3}$), 20.77–21.01 (4 × $\underline{CH_3}CO$), 25.32 (CH_3), 46.54 ($\underline{CH_2}CH_3$), 52.37 (C-2), 62.07(C-6), 68.71 (C-4), 71.75 (C-3), 72.71 (C-5), 92.57 (C-1), 111.68 (C-6Napth), 120.03 (C-5Napth), 121.93 (C-8aNapth), 136.34 (C-3Napth), 148.59 (C-2Napth), 148.86 (C-4aNapth), 163.68 (C-7Napth), 164.74(CONH), 169.34–170.50 (5 × $CH_3\underline{CO}$), 176.12 (C-4Napth). (ESI +) *m/z* 584.18508 [M+Na] ($C_{26}H_{31}N_3NaO_{11}$ requires 584.18563).

1-Ethyl-7-methyl-4-oxo-N-(2-deoxy-*D*-glucopyranose-2-yl)-[1,8]-naphthyridine-3-carboxamide (6)

Compound **5** (0.350 g, 0.62 mmol) was dissolved in 30 mL MeOH. $NaOCH_3$ (0.200 g, 3.7 mmol) was added gradually and left stirring at room temperature for 3 h until the formation of precipitate. The suspension was neutralized with conc. HCl. Filtration, washing with cold MeOH and drying afforded compound **(6)** (0.228 g, 94%) as a yellow solid; DSC 245 °C; IRν_{max} 3,461.63 cm^{-1} (CONH), 2,829.43 cm^{-1} (OH), 1,582.47 cm^{-1} (C═O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H 1.50 (3H, t, *J* = 6.4 Hz, —CH_3), 2.62 (3H, s, —CH_3Napth), 3.13–3.60 (7H, m, H-3, H-4, H-5, H-6, H-6, 3 × OH, D_2O exchangeable), 3.83 (1H, q, *J* = 7.6 Hz, H-2), 4.42–4.56 (3H, m, —CH_2, OH, D_2O exchangeable), 5.10 (1H, m, H-1), 7.45 (1H, d, *J* = 8.1 Hz, H-6Napth), 8.51 (1H, d, *J* = 8.1 Hz, H-5Napth), 8.92 (1H, s, H-2Napth), 9.84 (1H, d, *J* = 8.45 Hz, CONH); ^{13}C-NMR (DMSO, 500 MHz) 15.49 ($CH_2\underline{CH_3}$), 25.32 (CH_3), 46.36 ($\underline{CH_2}CH_3$) 54.48 (C-2), 61.59 (C-6), 71.63 (C-4), 71.75 (C-3), 72.76 (C-5), 91.26 (C-1), 112.72 (C-6Napth), 120.16 (C-5Napth), 121.87 (C-8'a), 136.41 (C-3Napth), 148.44 (C-2Napth), 148.59 (C-4aNapth), 163.54 (C-7Napth), 164.63 (CONH), 176.20 (C-4′). (ESI+) *m/z* 416.14282 [M+Na] ($C_{18}H_{23}N_3NaO_7$ requires 416.14337).

2.2 | Biological activity

2.2.1 | Antimicrobial activity

Antimicrobial activities of the synthesized compounds were investigated in vitro against Gram negative bacteria including food-borne pathogens (*Escherichia coli* O157:H7, *Salmonella enterica* ATCC 13312 and *Listeria monocytogenes* ATCC 19115); Gram negative infectious bacteria (*Pseudomonas aeruginosa* ATCC 9627; *Escherichia coli* NCTC11954; *Escherichia coli* ATCC 8739 and resistant clinical *Escherichia coli* isolate (not susceptible to nalidixic acid, ciprofloxacin HCl, and Norfloxacin from Biolab® [Amman-Jordan]); Gram positive bacteria (Methicillin resistant *Staphylococcus aureus* ATCC 33591–MRSA and Methicillin sensitive *Staphylococcus aureus* ATCC 9253-MSSA); non spore forming fungi (*Candida albicans* ATCC 10231) and spore forming (*Aspergillus flavus* ATCC 9643; *Fusarium solani* ATCC 36031; *Stachybotrys chartarum* IBT 7711, which was provided by Professor Naresh Magan [Cramfield University, UK] and *Penicillium chrysogenum* ATCC 10106). All media were prepared according to manufacturer instructions under sterilized conditions. Bacterial broth cultures stocks were cultivated in the appropriate medium at 37 °C for 24 h prior to testing. 0.5 McFarland standard was used to visually approximate the concentration of cells in a suspension.

Fungi conidial suspension was prepared from 7-day cultures grown on malt extract agar at 30 °C. Fungi conidia was attained from the agar using cotton swap previously immerged in Tween 20 and transferred into 3 mL normal saline. The conidial suspension was vigorously vortexed for 20 s to prevent spore clumping and then left standing at RT for 15 min to settle down. Following, the supernatant was transferred into a sterile falcon tube and adjusted to 0.5 McFarland at 530 nm to yield 8.44–18.44 × 10^6 sporangiospore suspensions (*A. flavus*, 8.44 × 10^6; *F. solani*, 10.63 × 10^6; *S. chartarum*, 18.44 × 10^6; *P. chrysogenum*, 14.69 × 10^6). Mean spores count from three trials was determined using hemocytometer. The final working suspension was obtained after diluting the stock suspension by 1:50 with malt extract broth.

Minimal inhibitory concentrations (MICs, mM) of the targeted compounds against Gram positive, Gram negative bacteria and *candida* spp. were determined using micro-broth dilution method. MIC is defined as the lowest concentration of the tested compound showing no microbial viability. Compounds were first dissolved in DMSO at a stock solution of 2 mg mL^{-1}. Then 100 μL was added to first well of the 96-well plate having 100 μL of the adequate broth. From here, the solution was serially diluted resulting in two fold dilution of the test compound **5** (0.0070–0.8904 mM) and **6** (0.0099–1.2710 mM) in subsequent wells. Then, 10 μL of the microbial suspension (10^6 CFU mL^{-1}) and 90 μL of broth were added to each well and incubated at 37 °C for 24 h. Experiments were done in triplicates. Following, absorbance of light scattering at 625 nm was measured using Epoch spectrophotometer. Nalidixic acid (0.01682–2.1530 mM) was used as positive control and proper sterility and negative control (no compound) samples were prepared and evaluated.

TABLE 1 Antibacterial activities of the synthesized compounds versus nalidixic acid

Microorganism	Microorganism characteristic	Nalidixic acid MIC (mM ± *SD*)	Compound 5	Compound 6
E. coli NCTC 11954	Penicillinase without ESBL	0.5382 ± 0.0036	0.1113 ± 0.01131	0.1589 ± 0.0116
E. coli ATCC 8739		0.2691 ± 0.0191	0.4452 ± 0.0127	0.3177 ± 0.0311
E. coli O157:H7	Food-poisoning	<0.0168 ± 0.0064	0.0557 ± 0.0064	0.0397 ± 0.0170
Clinical *E. coli*	Resistant to nalidixic acid	ND	0.4452 ± 0.0074	0.6355 ± 0.0040
S. enterica ATCC 13312	Food-poisoning	0.1346 ± 0.0125	0.8904 ± 0.0382	0.1589 ± 0.0120
P. aeruginosa ATCC 13312		<0.0168 ± 0.0057	0.1113 ± 0.0107	0.0199 ± 0.0445
S. aureus ATCC 33591	MRSA	1.0765 ± 0.0086	0.1113 ± 0.0021	0.1589 ± 0.0169
S. aureus ATCC 9253	MSSA	0.0336 ± 0.0106	0.0557 ± 0.0625	0.1589 ± 0.0424
L. monocytogenes ATCC 19115	Food-poisoning	1.0765 ± 0.0074	0.1113 ± 0.0106	0.6355 ± 0.0087

Note. ND = not detected; *Escherichia coli* (*E. coli*) NCTC 11954; *Escherichia coli* (*E. coli*) ATCC 8739; *Escherichia coli* (*E. coli*) O157:H7; Clinical *Escherichia coli* (*E. coli*); *Salmonella enterica* (*S. enterica*) ATCC 13312; *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 13312; *Staphylococcus aureus* (*S. aureus*) ATCC 33591; *Staphylococcus aureus* (*S. aureus*) ATCC 9253; *Listeria monocytogenes* (*L. monocytogenes*) ATCC 19115.

MIC protocol for the spore-forming fungi was similar to bacterial protocol, but MICs were recorded after 48 h of incubation at 30 °C and the absorbance was measured at 405 nm. Compounds' stock solution was 4 mg mL^{-1}. The tested compound **5** (0.0070–1.7808 mM) and **6** (0.0099–2.5420 mM), and the positive control fluconazole (0.0128–3.2651 mM) were examined. DMSO was used as negative control along with proper sterility and growth control. Experiments were conducted in triplicates.

2.2.2 | Cell culture

L6 skeletal myoblast cell line (ATCC CRL—1,458) was procured from Bio-Focus Saintifik, Malaysia. Stock cells of L-6 were cultured in MEM (Minimum Essential Media) supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU mL^{-1}), streptomycin (100 mg mL^{-1}), and amphotericin B (5 mg mL^{-1}) in an humidified atmosphere of 5% CO_2 at 37 °C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 well plates.

2.2.3 | MTT

The tested compounds and their positive controls (nalidixic acid and ciprofloxacin HCl) were separately dissolved in distilled DMSO and the volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg mL^{-1} concentration and sterilized by filtration. Two folds serial dilutions for the tested compounds were prepared from this for carrying out cytotoxic studies.

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/mL using DMEM containing 10% FBS. To each well of the 96 well microtiter plate, 0.1 mL of the diluted cell suspension (approximately 20,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 μL of compounds **5** (0.1781–1.7808 mM/100–1,000 μg mL^{-1}), **6** (0.2542–2.5420 mM/100–1,000 μg mL^{-1}) and their positive controls were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37 °C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 μL of MTT in PBS was added to each well. The plates were gently shaking and incubated for 3 h at 37 °C in 5% CO_2 atmosphere. The supernatant was removed and 100 μL of DMSO was added and the plates were gently shaking to solubilize the formed formazan. The absorbance was measured using a micro plate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using formula below. The half maximal inhibitory concentration (IC_{50}) which is the concentration of test drug needed to inhibit cell growth by 50% value was generated from the dose–response curves for each cell line (Francis & Rita, 1986).

$$\%\text{Cell viability} = \frac{\text{Mean OD of the test sample}}{\text{Mean OD of the vehicle control (negative control)}}$$

Each test was repeated trice. IC_{50} was calculated from the dose effect curves and expressed as concentration (mM, μg mL^{-1}) of drug.

3 | RESULTS AND DISCUSSION

3.1 | Chemistry

Compound **4** was prepared by reacting *D*-(+)-glucosamine hydrochloride with 4-methoxybenzaldehyde to yield the glucosamine imine intermediate **1**. Following acetylation, amine deprotection, and neutralization, compound **4** was obtained (Dang et al., 2014; Myszka et al., 2003; Suaifan, Shehadeh, Darwish, Al-Ijel, & Abbate, 2015).

As illustrated in Scheme 2, the target carboxamide **5** was prepared by dissolving nalidixic acid in CH_2Cl_2 at −20 °C. Then, TEA was added to prepare the carboxylic acid-tertiary amine salt intermediate. Subsequent acid activation in situ, was achieved via drop-wise addition of ethyl chloroformate at −20 °C. Ester intermediate formation was monitored using thymol/sulfuric acid TLC visualizing reagent. Upon complete intermediate formation, compound **4** (1 Eq.) in CH_2Cl_2 was added drop-wise and the mixture was stirred for 4 h at −20 °C, and then left to warm gradually to room temperature. Amide bond formation must be carried at low temperature to improve ester intermediate stability (Kim, Lee, & Kim, 1985). Once carboxamide target **5** was confirmed by TLC, the reaction mixture was washed with water, aq.1 M HCl and aq. 5% $NaHCO_3$ to remove nonreacting TEA and to neutralize the N-amide. Trituration with

TABLE 2 Antifungal activities of the synthesized compounds versus fluconazole

Microorganism	Fluconazole MIC (mM ± *SD*)	Compound 5	Compound 6
C. albicans ATCC 10231	0.4081 ± 0.0375	0.2226 ± 0.0393	<0.0099 ± 0.0078
A. flavus ATCC 9643	0.0510 ± 0.0021	0.0557 ± 0.0014	0.6355 ± 0.03366
F. solani ATCC 36031	0.1020 ± 0.0099	ND	ND
S. chartarum IBT 7711	0.1020 ± 0.0007	0.4452 ± 0.01556	0.6355 ± 0.0120
P. chrysogenum ATCC 10106	0.2041 ± 0.0099	0.4452 ± 0.0047	ND

Note. ND = not detected; *Candida albicans* (*C. albicans*) ATCC 10231; *Aspergillus flavus* (*A. flavus*) ATCC 9643; *Fusarium solani* (*F. solani*) ATCC 36031; *Stachybotrys chartarum* (*S. chartarum*) IBT 7711; *Penicillium chrysogenum* (*P. chrysogenum*) ATCC 10106.

diethylether and recrystallization afforded targeted compound **5** in 32% yield as a white solid. The *O*-deacetyl glucosamine carboxamide **6** was obtained according to zemplén procedure (Fairweather, Liu, Karoli, & Ferro, 2012), via the reaction of compound **6** with sodium methoxide in methanol. TLC confirmed the end of the deprotection process using ninhydrin solution as a TLC visualizing reagent. The precipitated product was neutralized with aq. 1 M HCl, filtered, washed with cold MeOH to obtain compound **6** in high yield (94%) as a yellow solid. All synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, and HMQC and mass spectral data; IR spectra of the glycosylated carboxamide **5** showed strong absorption bands at 3240.49 cm^{-1} confirming amide bond formation. The characteristic signals in ^{1}H NMR displayed a doublet peak at δ_H = 9.91 ppm with a coupling constant of 8.7 Hz integrating one proton for the secondary amide. ^{13}C NMR indicated the presence of discrete peak at 164.74 ppm for the amide carbonyl. ^{1}H-^{1}H COSY and HMQC assisted the chemical shifts assignment of compound **6** glucosamine moiety protons and carbons. The mass spectra showed a molecular ion peak at *m*/*z* = 584.18508 (M+Na)$^{+}$ corresponding to the molecular formula $C_{26}H_{31}N_3NaO_{11}$.

Compound **6** IR displayed the appearance of a band at 2829.43 cm^{-1} corresponding to hydroxyl groups and a broad band at 3461.63 cm^{-1} corresponding to the amide bond formation. ^{1}H NMR displayed D_2O exchangeable peaks between 3.00 and 3.50 ppm corresponding to hydroxyl groups. ^{13}C NMR confirmed the disappearance of glucosamine acetyl carbonyl. Mass spectra

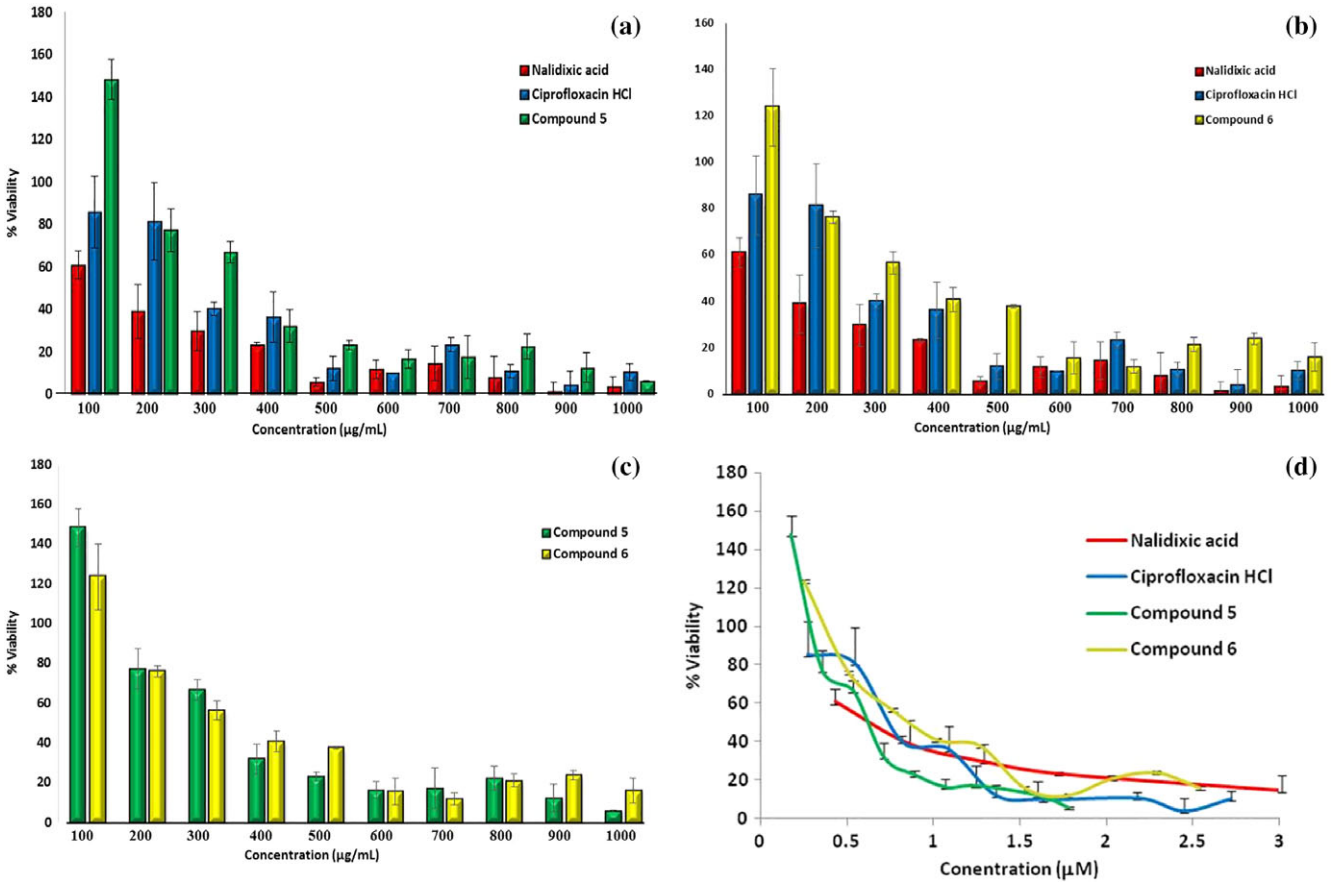


FIGURE 1 Toxicity profile of nalidixic acid, ciprofloxacin HCl, compound **5** and compound **6**; (a) percentage viability of nalidixic acid, ciprofloxacin HCl and compound **5**; (b) percentage viability of nalidixic acid, ciprofloxacin HCl and compound **6**; (c) comparison between percentage viability of compound **5** and compound **6**; (d) percentage viability of nalidixic acid, ciprofloxacin HCl, compound **5** and compound **6**. Error bars ± standard deviation

TABLE 3 IC_{50} (μM) for the controls (nalidixic acid, ciprofloxacin HCl) and tested compounds (**5**, **6**)

Compound	IC_{50} (μM)
Nalidixic acid	0.01934 ± 0.0385
Ciprofloxacin HCl	12.8330 ± 0.6177
Compound **5**	5.8856 ± 0.2916
Compound **6**	11.1340 ± 0.0839

Note. Error bars ± standard deviation.

showed a molecular ion peak m/z = 416.14282 $(M+Na)^+$ corresponding to a molecular formula $C_{18}H_{23}N_3NaO_7$.

3.2 | Antimicrobial evaluation

Target compounds antimicrobial activity was evaluated against Gram negative bacteria including *E. coli* O157:H7 (food poisoning); *Salmonella enterica* ATCC 13312 (food poisoning); *Listeria monocytogenes* ATCC 19115 (food poisoning); *Pseudomonas aeruginosa* ATCC 9627; *E. coli* NCTC 11954; *E. coli* ATCC 8739; resistant clinical *E. coli* strain (resistant to nalidixic acid, ciprofloxacin HCl, and norfloxacin antibiotics) and Gram positive bacteria including *S. aureus* ATCC 33591 and *S. aureus* ATCC 9253. Tables 1 and 2 show tested compounds antimicrobial activity. Nalidixic acid was used as a positive control.

As shown in Table 1, glycosylated 1,8-naphthyridine ring derivatives **5** and **6** exhibited potential antimicrobial activity against resistant *E. coli* NCTC 11954 with MIC values of 0.1113 ± 0.01131 mM and 0.1589 ± 0.0116 mM, respectively. Interestingly, tested compounds **5** and **6** showed growth inhibitory activity against nalidixic acid resistant clinical *E. coli* isolate. Moreover, compounds **5** and **6** had higher growth inhibitory activity against MRSA ATCC 33591 with MIC values of 0.1113 ± 0.0021 mM and 0.1589 ± 0.0169 mM, respectively, when compared to the parent nalidixic acid (MIC = 1.0765 ± 0.0086 mM). A plausible explanation would be the increased hydrophobicity of these analogues when compared to the standard which should improve drug uptake via lipid mediated pathway. Compound **6** had remarkably growth inhibitory activity against *P. aeruginosa* with an MIC of 0.0199 ± 0.0445 mM. Moreover, target compounds **5** and **6** possessed comparable and improved growth inhibitory activity against food poisoning pathogen (*L. monocytogenes* ATCC 19115), respectively. On the other hand, compound **6** exerted comparable growth inhibitory activity to the control against *Salmonella enterica* ATCC 13312 and slightly lower activity against *E. coli* O157:H7.

Targeted compounds antifungal activity was evaluated against *C. albicans* ATCC 10231 and other spore forming fungi including *A. flavus* ATCC 9643; *F. solani* ATCC 36031; *S. chartarum* IBT 7711; *P. chrysogenum* ATCC 10106 (Table 2). Compound **5** exerted antifungal activity against *A. flavus* in twice the concentration of fluconazole (MIC = 0.0557 ± 0.0014 mM). On the other hand, compound **6** showed potential antifungal activity against *C. albicans* ATCC 13312 (MIC <0.0099 ± 0.0078 mM).

3.3 | MTT

Target compounds were examined for their cytotoxicity at various concentrations. Interestingly, compounds **5** and **6** exerted potential viability. The viability was evaluated according to the conversion of MTT to formazan. The compounds were found to be less toxic than other quinolone marketed drug including nalidixic acid and ciprofloxacin hydrochloride. Percentage cell viability is twice as high as nalidixic acid even at higher concentrations as shown in Figure 1. It is to be mentioned that lower cytotoxicity level together with potential inhibitory activity, in particular against resistant clinical *E. coli* isolate supports the use of these derivatives as an alternative therapy to overcome nalidixic acid resistance. IC_{50} values which indicates the concentration upon which the compounds response is reduced by 50% was calculated from the dose effect curves. The IC_{50} of the synthesized compounds were nearly 100 folds higher than nalidixic acid as shown in Table 3. This supports the rationale that these compounds are of lower toxicity levels than nalidixic acid and ciprofloxacin hydrochloride.

4 | CONCLUSION

This study is not based on the discovery of new antibacterial scaffolds and so does not require tedious validation processes, which are difficult and time consuming tasks (Panda et al., 2015). In contrast, it is based on the chemipluation of the lead nalidixic acid to attain derivatives with modified antimicrobial spectrum. Nalidixic acid-based-*D*-(+)-glucosamine conjugates antimicrobial activity were evaluated in vitro using agar dilution method. Biological assays indicated the improved activity of the synthesized derivatives against various resistant bacterial strains while exhibiting lower cytotoxicity effect.

ACKNOWLEDGMENT

The authors would like to acknowledge the Deanship of the Scientific Research at The University of Jordan for financial support, Grants number (2016).

CONFLICT OF INTEREST

Disclosure relates to 1,8-naphthyridine glucosamine derivatives, and uses thereof for treating microbial infections have been registered for a patent at the Hashemite Kingdom of Jordan (Reg. No. P/JO/2018/000097).

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How to cite this article: Mohammed AAM, Suaifan GARY, Shehadeh MB, Okechukwu PN. Design, synthesis, and biological evaluation of 1,8-naphthyridine glucosamine conjugates as antimicrobial agents. *Drug Dev Res*. 2018;1–8. https://doi.org/10.1002/ddr.21508