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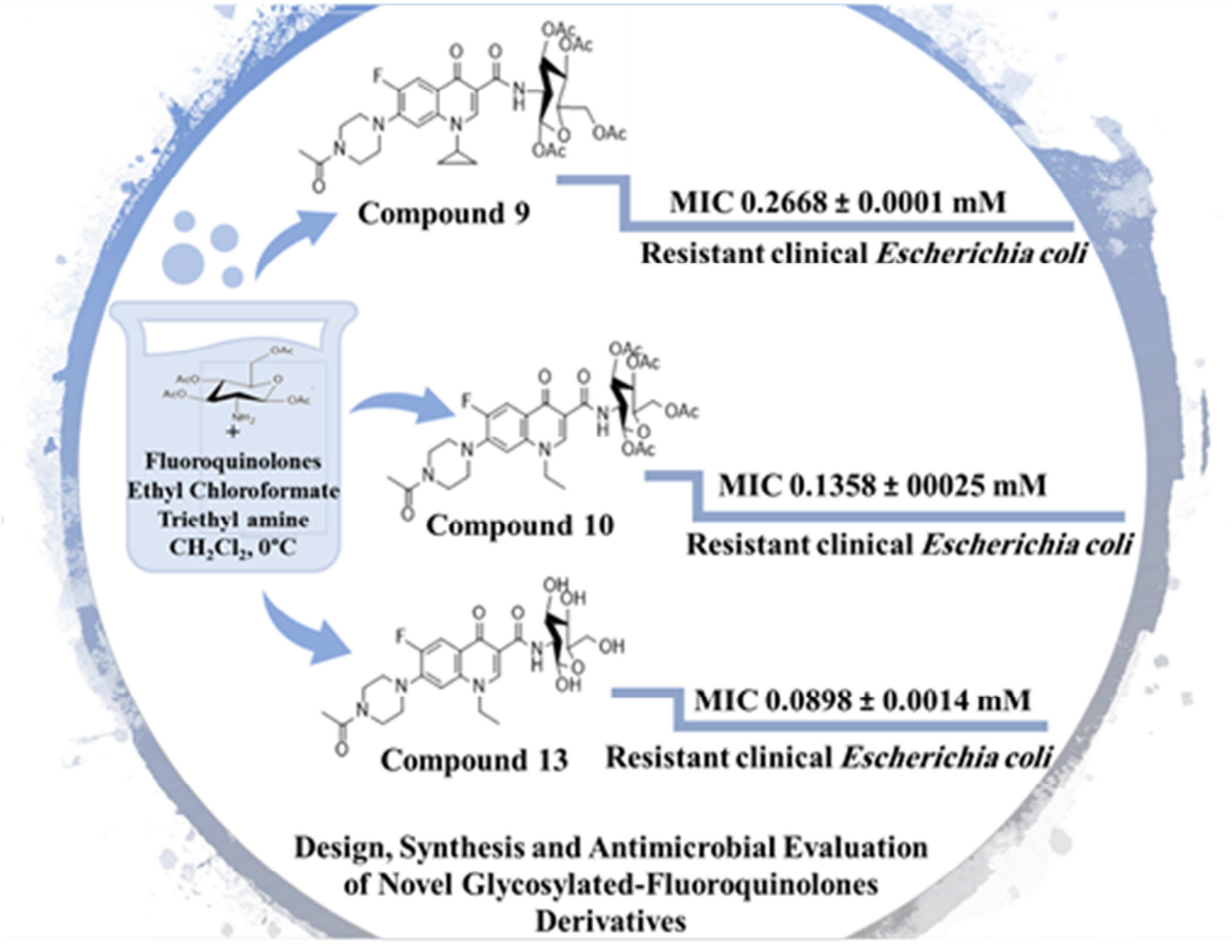
Compound 9
MIC 0.2668 ± 0.0001 mM
Resistant clinical Escherichia coli
Fluoroquinolones
Ethyl Chloroformate
Triethyl amine
CH2Cl2, 0°C
Compound 10
MIC 0.1358 ± 00025 mM
Resistant clinical Escherichia coli
Compound 13
MIC 0.0898 ± 0.0014 mM
Resistant clinical Escherichia coli
Design, Synthesis and Antimicrobial Evaluation
of Novel Glycosylated-Fluoroquinolones
Derivatives

Design, Synthesis and Antimicrobial Evaluation of Novel Glycosylated-fluoroquinolones Derivatives

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Abstract

Herein we report the design, synthesis and biological evaluation of structurally modified ciprofloxacin, norfloxacin and moxifloxacin standard drugs, featuring amide functional groups at C-3 of the fluoroquinolone scaffold. *In vitro* antimicrobial testing against various Gram-positive bacteria, Gram-negative bacteria and fungi revealed potential antibacterial and antifungal activity. Hybrid compounds **9** (MIC 0.2668 ± 0.0001 mM), **10** (MIC 0.1358 ± 00025 mM) and **13** (MIC 0.0898 ± 0.0014 mM) had potential antimicrobial activity against a fluoroquinolone-resistant *Escherichia coli* clinical isolate, compared to ciprofloxacin (MIC 0.5098 ± 0.0024 mM) and norfloxacin (MIC 0.2937 ± 0.0021 mM) standard drugs. Interestingly, compound **10** also exerted potential antifungal activity against *Candida albicans* (MIC 0.0056 ± 0.0014 mM) and *Penicillium chrysogenum* (MIC 0.0453 ± 0.0156 mM). Novel derivatives and standard fluoroquinolone drugs exhibited near-identical cytotoxicity levels against L6 muscle cell-line, when measured using the MTT assay.

Keywords

Glucosamine; ciprofloxacin; antibacterial; antifungal; MTT assay; structure-activity relationship.

1. Introduction

Since the discovery of nalidixic acid, the prototype of quinolone-based antibiotics (Fig. 1A) [1], extensive research has been conducted around the quinolone basic scaffold to develop novel derivatives of value as reserve antimicrobial agents with improved antibacterial activity [1, 2]. Of these, fluoroquinolones, such as norfloxacin and ciprofloxacin (Fig. 1B, 1C), were developed and considered to be clinically effective against a wide range of pathogenic microorganisms due to their broad spectrum of activity. Hence, fluoroquinolones are prescribed for the treatment of various infections including urinary tract, respiratory tract, gastrointestinal, skin and bone infections [3]. The widespread consumption of fluoroquinolones such as ciprofloxacin (the most dispensed antibacterial agent globally) has led to the emergence of resistant bacterial strains [4]. Therefore, medicinal chemists have often sought to design and synthesize novel fluoroquinolone derivatives.

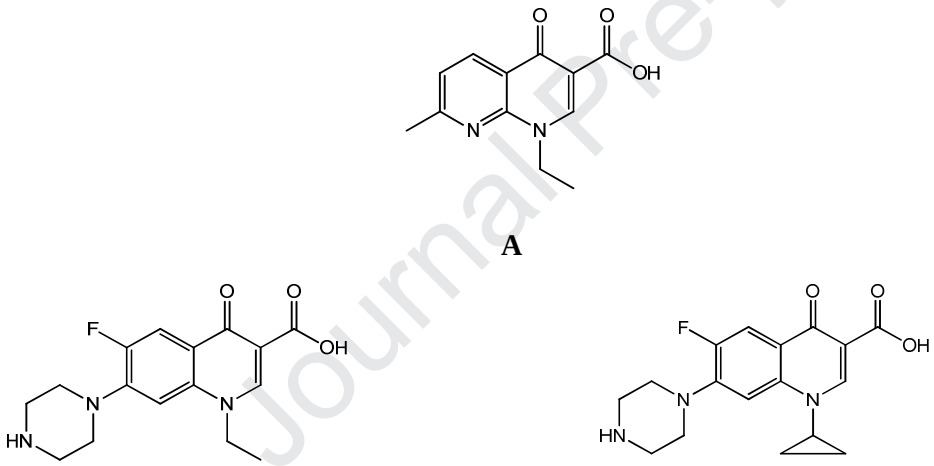


Fig. 1 Chemical structures of quinolones. (a) Nalidixic acid, (b) Norfloxacin and (c) Ciprofloxacin.

Recent structure-activity relationship (SAR) studies on the bacterial growth inhibitory activities of fluoroquinolones have been primarily related to molecular modification at C-6 and C-7 [2, 5]. Additionally, the researchers synthesized various derivatives by introducing new functional moieties to the secondary amine at the piperazinyl or hexahydropyrolo rings at C-7. However, this chemimanipulation does not always improve the antimicrobial activity [6]. Other studies revealed that bioisosteric replacement of C-3 carboxylic acid moiety enhances potency. Such modification involved amidation [1], esterification to form a prodrug [7] or conjugation with other compounds such as carbohydrates [8, 9]. Today, available

antifungal agents are limited and are known to cause toxicity to mammalian cells [10]. This encourages investigation of novel antifungal activity of fluoroquinolones, since these agents act by inhibiting bacterial DNA gyrase and topoisomerase IV [11, 12] which are also present in *Aspergillus flavus* (*A. flavus*), the fungus causing aspergillosis. Moreover, the emergence of resistant *Candida* strains against several antifungal agents (including fluconazole and ketoconazole) [13, 14] encourages investigating novel synthetic fluoroquinolone derivatives against various fungal strains.

Given the previous rationale, a new hybrid strategy was adopted for swift development of novel biologically active fluoroquinolone derivatives. Here, we describe modification of fluoroquinolone at C-3 by coupling with a glucosamine moiety (a carbohydrate analogue of N-Acetyl Glucosamine (NAG), one of the bacterial cell-wall constituents) to deliver hybrid derivatives with improved biological activities (Fig. 2). The present work describes the synthesis of a new series of derivatives of ciprofloxacin (**9** and **12**), norfloxacin (**10** and **13**), and moxifloxacin (**11** and **14**). The sugar moiety may act as a carrier in this proposed pro-drug approach, to enhance the selective uptake of fluoroquinolones into the microbial cells, hence enhancing selectivity and potency, and lowering cytotoxicity. Moreover, bacterial glycosylated biogenic proteins control bacterial cells' vital functions including cell division, energy conversion and motility [15, 16]. Accordingly, we synthesized novel glucosamine-fluoroquinolone conjugates as potential antibacterial/antifungal agents. Synthesized compounds were screened *in vitro* for their antimicrobial activity.

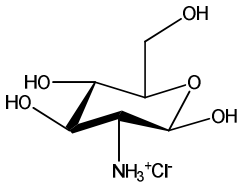


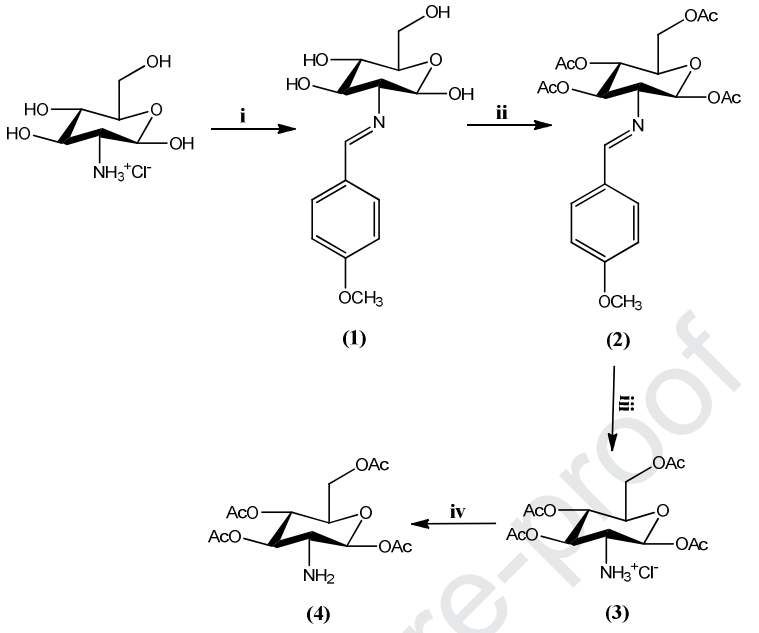
Fig. 2 Chemical Structure of *D*-(+)-Glucosamine hydrochloride.

2. Results and discussion

2.1 Chemistry (Synthesis)

Novel glycosylated fluoroquinolone derivatives were synthesized as depicted in Schemes 1 and 2. Compound **4** was prepared from the commercially available *D*-(+)-glucosamine hydrochloride as previously described [17, 18], with slight modifications. The primary amine of *D*-(+)-glucosamine was protected as an imine with *4-methoxybenzaldehyde* under basic conditions to afford compound **1** in a good yield. The alcohols of **1** were then esterified using

acetic anhydride to afford compound **2** as a white solid. The imine was selectively hydrolysed with dilute hydrochloric acid in warm acetone to expose the protonated primary amine in **3.** Neutralization with 1 M aq. Na_2CO_3 gave the free amine **4 (Scheme 1)**.



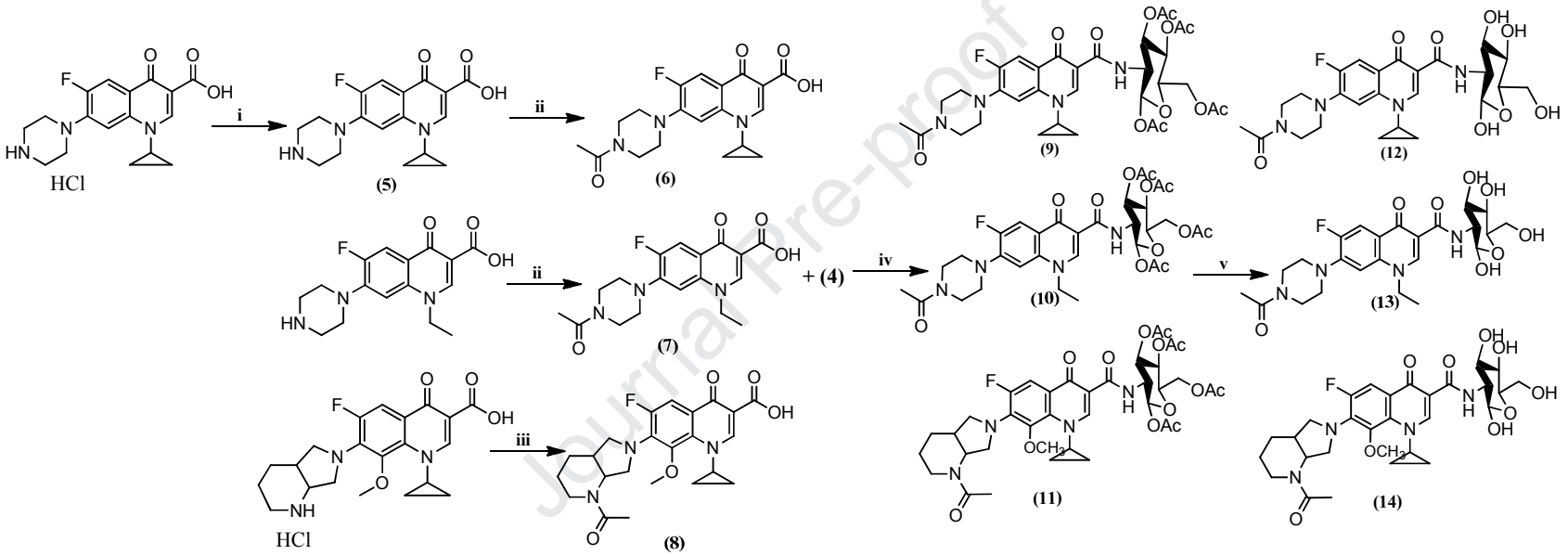
(i) aq. NaOH, p-anisaldehyde; (ii) Py, Ac_2O; (iii) acetone, 5 M HCl; (iv) CH_2Cl_2, 1 M Na_2CO_3

Scheme 1: Synthesis of 1,3,4,6-*O*-acetylated-*D*-glucosamine.

To prepare the quinolone coupling partners, ciprofloxacin HCl was converted to the free base form under basic conditions, followed by acetylation to afford compound **6** using acetic anhydride to avoid any potential dimerization during the subsequent coupling reaction (Scheme 2). Similarly, the norfloxacin piperazinyl amino group was protected to afford compound **7** in good yield. Moreover, the acetyl-derived moxifloxacin (compound **8)** was synthesized by dissolving moxifloxacin HCl in CH_2Cl_2 and then adding Ac_2O and triethylamine, where the latter acted to free the secondary amine of the side-chain [21].

Fluoroquinolone derivatives **6-8** were treated with ethyl chloroformate and Et_3N in CH_2Cl_2 at -20 °C to yield the mixed anhydride. Nucleophilic acyl substitution [23, 24] with compound **4** afforded the corresponding glycosylated targets **9-11**. The molar equivalents of the reactants and the durations of the reactions were adjusted according to monitoring by TLC. Work-up comprised washing the organic layer with water to deactivate unreacted ethyl chloroformate, followed by washing with aq. HCl and aq. $NaHCO_3$. Trituration with Et_2O afforded gummy products and compounds **9-11** were finally purified by column chromatography. The synthesized acyl-glycosylated fluoroquinolone derivatives **9-11** chemical structures were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR, COSY, HMQC, DEPT 135 and mass spectra.

Deprotection with six equivalents of NaOMe in MeOH, according to the the Zemplén procedure [25], led to the *O*-deacetylated compounds **12-14**.Once the TLC indicated the complete consumption of the starting material, the reaction was neutralized with amberlite resin and the aqueous solution of the products was freeze-dried to obtain **12-14** in excellent yields (90-96%). The structures of **12-14** were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR, COSY, HMQC, DEPT 135 and mass spectra.



(i) 5% $NaHCO_3$; (ii) Ac_2O,CH_3CO_2H; (iii)Ac_2O, TEA, CH_2Cl_2; (iv) $ClCOOC_2H_5$, TEA, CH_2Cl_2; (v) MeOH, NaOMe, amberlite resin

Scheme 2: Synthesis of novel glycosylated-based fluoroquinolones.

2.2 Antibacterial Activity

Target compounds **9-14** were evaluated *in vitro* for their antimicrobial activity using standard techniques against Gram-negative and Gram-positive bacteria including: (1) food poisoning bacteria *Eischerichia coli* (*E. coli*) O157:H7; *Listeria monocytogenes* (*L. monocytogenes*), ATCC 19115; *Salmonella enterica* (*S. enterica*), ATCC 13312); (2) Other Gram-negative species (*Pseudomonas aeruginosa* (*P. aeruginosa*), ATCC 9627; *E. coli*, NCTC 11954; *E. coli*, ATCC 8739 and clinical *E. coli* isolate (resistant to nalidixic acid, ciprofloxacin HCl and norfloxacin antibiotics); (3) Gram-positive (Methicillin-resistant *staphylococcus aureus* (MRSA), ATCC 33591 and Methicillin-sensitive *staphylococcus aureus* (MSSA), ATCC 9253). MICs of the synthesized compounds were compared with the positive controls; ciprofloxacin HCl, norfloxacin and moxifloxacin for antibacterial activity, as presented in Table **1**.

Corresponding to the broad antimicrobial spectrum of fluoroquinolones, tested compounds showed a wide-ranging antibacterial profile. Among the derivatives investigated, **9** displayed potent antibacterial activity against *S. enterica*, ATCC 13312 and *P. aeruginosa*, ATCC 9627 (MIC 0.0111 ± 0.0629 mM and 0.0111 ± 0.0177 mM, respectively) whereas **10** displayed potent antibacterial activity against *S. enterica*, ATCC 13312 (0.0113 ± 0.0269 mM). Interestingly, **9** was twice as potent as ciprofloxacin parent drug against a *E. coli* clinical isolate (MIC 0.2668 ± 0.0001 mM and 0.5098 ± 0.0024 mM, respectively). Similarly, **10** (MIC 0.1358 ± 00025 mM) was approximately twice as potent and **13** (MIC 0.0898 ± 0.0014 mM) was approximately three times more potent than the corresponding positive control norfloxacin (0.2937 ± 0.0021 mM) against *E. coli* clinical isolate. On the contrary, **11** and **14** were less active than their parent moxifloxacin drug against *E. coli* clinical isolate (MIC 0.4853 ± 0.0134 mM and 0.3103 ± 0.0007 mM, respectively). Notably, poor or no antibacterial activity was observed for **9** and **10** against the two Gram-positive *S. aureus* species **(**ATCC 33591 and ATCC 9253). In addition, **10** and **13** had lower antibacterial activity against *E. coli* O157:H7 (MIC 0.0453 ± 0.0049 and 0.0599 ± 0.0040 mM, respectively) compared to norfloxacin (MIC <0.0059 ± 0.0093 mM).

2.3 Antifungal activity

Most fungi are resistant to conventional antibacterial drugs. In this study, we aimed to investigate whether functional group modification at the C3 position induces antifungal properties in parent fluoroquinolones (ciprofloxacin, norfloxacin and moxifloxacin). Accordingly, the antifungal

activity of hybrid compounds was evaluated against *Candida albicans* (*C. albicans*), ATCC 10231 and other spore-forming fungi, including *A. flavus*, ATCC 9643; *Fusarium solani* (*F. solani*), ATCC 36031; *Stachybotrys chartarum* (*S. chartarum*), IBT 7711; *Penicillium chrysogenum* (*P. chrysogenum*),ATCC 10106.

As illustrated in Table 2, ciprofloxacin derivative **12** (MIC 0.2338 ± 0.0071 mM) and norfloxacin derivatives **10** (MIC 0.3620 ± 0.0099 mM) and **13** (MIC 0.4784 ± 0.0049 mM), and moxifloxacin derivatives **11** (MIC 0.1618 ± 0.0212 mM) and **14** (0.2069 ± 0.0106 mM) exhibited effective antifungal properties against *A.* flavus, compared to their parents ciprofloxacin (inactive) , norfloxacin (MIC 0.7829 ± 0.0926 mM) and moxifloxacin (MIC 0.2855 ± 0.0085 mM). Similarly, norfloxacin glucosamine hybrids **10** and **13** were found to be more active antifungal agents against *S. chartarum* and *P. chrysogenum*, compared to the parent norfloxacin. Likewise, ciprofloxacin hybrid **9** (MIC <0.0056 ± 0.0304 mM) had higher antifungal activity than its parent ciprofloxacin HCl (MIC <0.0106 ± 0.0191 mM) and the antifungal drug fluconazole (MIC 0.2041 ± 0.0099 mM) against the allergenic, toxic and pathogenic spore-forming *P. chrysogenum*.

Remarkably, compounds **10** (MIC 0.0056 ± 0.0014 mM), **11** (MIC 0.0809 ± 0.0098 mM), **13** (MIC 0.0599 ± 0.0007 mM) and **14** (MIC 0.2069 ± 0.0453 mM) had potential antifungal activity against *C. albicans*, in comparison to fluconazole (MIC 0.4081 ± 0.0375 mM). Notably, the acetylated norfloxacin derivative **10** and moxifloxacin derivative **11** were more potent than the deacetylated analogues **13** and **14.** Moreover, ciprofloxacin analogue **9** (MIC <0.0056 ± 0.0304 mM), norfloxacin analogues 1**0** (MIC 0.0453 ± 0.0156 mM) and **13** (MIC 0.1196 ± 0.0135 mM), and moxifloxacin analogues **11** (MIC 0.1618 ± 0.0214 mM) and **14** (MIC 0.0017 ± 0.0266 mM) had better antifungal activity against *P. chrysogenum* than the standard antifungal drug control fluconazole (MIC 0.2041± 0.0099 mM).

Notably, compound **14** (MIC 0.0017 ± 0.1181 mM) is 100 times more potent as an antifungal agent than the reference fluconazole control (MIC 0.1022 ± 0.0007 mM) against the life-threatening toxic *S. chartarum* fungi, commonly associated with damp building-related illnesses (DBRI) which trigger immunologic, neurologic and oncogenic disorders [26, 27]. Furthermore, **10** (MIC 0.0453 ± 0.0191 mM) is approximately twice as active and **13** (MIC 0.1196 ± 0.0099 mM) had a comparable activity against this toxic mould, compared to the control fluconazole, while ciprofloxacin analogues **9** and **12** were less active against *S. chartarum*, in comparison to

the control, as shown in Table 2. On the other hand, all tested compounds had lower antifungal activity against *A. flavus* and *F. solani* than did fluconazole.

Table 1: Minimum inhibitory concentrations (MIC (mM ±SD)) of target compounds and corresponding positive controls against different bacterial strains.

Microorganism		Compound								
		Ciprofloxacin HCl	**9**	**12**	**Norfloxacin**	**10**	**13**	**Moxifloxacin**	**11**	**14**
***E. coli* NCTC 11954**	G –ve*	<0.0052 ± 0.0332	ND	ND	0.0244 ± 0.0541	0.1810 ± 0.0070	0.2392 ± 0.0097	<0.0005 ± 0.0044	ND	0.2069 ±0.0644
***E. coli* ATCC 8739**	G -ve	<0.0052 ± 0.01474	ND	ND	0.0489 ± 0.0184	ND	ND	<0.0005 ± 0.0021	0.1618 ±0.0495	0.2069 ±0.0014
E. coli* *O157:H7	G –ve**	<0.0052 ± 0.0850	0.1779 ± 0.0082	0.2338 ± 0.0304	<0.0059 ± 0.0093	0.0453 ± 0.0049	0.0599 ± 0.0040	<0.0005 ± 0.0035	0.1618 ±0.0038	0.8275 ±0.0064
E. coli	G –ve***	0.5098 ± 0.0024	0.2668 ± 0.0001	0.7015 ± 0.0064	0.2937 ± 0.0021	0.1358 ± 00025	0.0898 ± 0.0014	0.0034 ± 0.0007	0.4853 ± 0.0134	0.3103 ±0.0007
***S. enterica* ATCC13312**	G –ve**	<0.0052 ± 0.0049	0.0111 ± 0.0629	ND	<0.0059 ± 0.0057	0.0113 ± 0.0269	0.0149 ± 0.0049	<0.0043 ± 0.0104	ND	ND
***P. aeruginosa* ATCC 9627**	G -ve	<0.0052 ± 0.0014	0.0111 ± 0.0177	ND	0.0980 ± 0.0070	ND	ND	0.0715 ± 0.0127	0.3235 ± 0.0071	0.0518 ±0.0028
***S. aureus* ATCC 33591**	G +ve****	<0.0052 ± 0.0042	ND	ND	0.0244 ± 0.0478	0.3620 ± 0.0113	0.4784 ± 0.0046	<0.0005 ± 0.0325	0.1618 ±0.0042	0.2069 ±0.0007
***S. aureus* ATCC 9253**	G +ve	<0.0052 ± 0.0078	ND	ND	0.0244 ± 0.0480	ND	ND	<0.0005 ± 0.0070	ND	ND
***L. monocytogenes* ATCC 19115**	G +ve**	0.0005 ± 0.0071	ND	ND	<0.0059 ±0.0339	ND	ND	<0.0005 ± 0.0095	<0.0050 ±0.0064	0.1034 ±0.0035

*Penicillinase without extended-spectrum β –lactamase.
** Food-poisoning bacterial strain.
*** Fluoroquinolones resistant *Escherichia coli* clinical isolate.
**** Methicillin-resistant *staphylococcus aureus*.
ND: Not Detected (No activity observed at the maximum tested concentration).

Table 2: Minimum inhibitory concentrations (MIC (mM ±SD)) of target compounds and corresponding positive controls against different fungi.

Microorganism	Compound									
	Fluconazole (mM)	**Ciprofloxacin HCl**	**9**	**12**	**Norfloxacin**	**10**	**13**	**Moxifloxacin**	**11**	**14**
C. albicans	0.4081 ± 0.0375	0.0003 ± 0.0297	ND	ND	<0.0059 ± 0.1181	0.0056 ± 0.0014	0.0599 ± 0.0007	0.0011 ± 0.0311	0.0809 ±0.0098	0.2069 ±0.0453
A. flavus	0.0510 ± 0.0021	ND	0.7116 ± 0.0078	0.2338 ± 0.0071	0.7829 ± 0.0926	0.3620 ± 0.0099	0.4784 ± 0.0049	0.2855 ± 0.0085	0.1618 ±0.0212	0.2069 ±0.0106
F. solani	0.1022 ± 0.0099	<0.0106 ± 0.0085	0.3558 ± 0.0127	0.4677 ± 0.0028	0.3914 ± 0.0136	0.1810 ± 0.0578	0.2392 ± 0.0092	0.2855 ± 0.0149	0.3235 ±0.0143	0.2069 ±0.0057
S. chartarum	0.1022 ± 0.0007	0.0850 ± 0.0283	0.3558 ± 0.0064	0.4677 ± 0.0028	0.1957 ± 0.0057	0.0453 ± 0.0191	0.1196 ± 0.0099	0.0023 ± 0.0481	0.3235 ±0.0068	0.0017 ±0.1181
P. chrysogenum	0.2041 ± 0.0099	<0.0106 ± 0.0191	<0.0056 ± 0.0304	0.2338 ± 0.0035	0.1957 ± 0219	0.0453 ± 0.0156	0.1196 ± 0.0135	0.0011 ± 0.0113	0.1618 ±0.0214	0.0017 ±0.0266

ND: Not Detected (No activity observed at the maximum tested concentration).

2.4. MTT assay

The cytotoxic activity of compounds **(9-14)** and the standard fluoroquinolones (ciprofloxacin HCl, norfloxacin and moxifloxacin) were evaluated using an MTT assay against L6 muscle cell line, according to Mossman protocol [22]. In brief, novel derivatives exhibited near-identical cytotoxic profiles against L6 muscle cells, as depicted in Table 3. Glycosylated ciprofloxacin derivatives **9** and **12** (3.6164 ± 0.0181 µM, 4.0270 ± 0.0100 µM, respectively) had higher IC_{50} compared to the lead ciprofloxacin HCl (3.3998±0.0083 µM). Similarly, **10** (5.3167 ± 0.0100 µM) and **13** (3.7249±0.0189 µM) had less cytotoxicity, when compared to norfloxacin (3.4311±0.0078 µM). Whereas, compounds **11** (5.5257 ± 0.6086 µM) and **14** (5.0765 ± 0.0064 µM) had decreased the proliferation of the cells, compared to moxifloxacin (5.7783 ± 0.0120 µM).

Table 3. *In vitro* cytotoxicity of tested compounds

Compound	**IC_{50} (µM)***
	L6- muscle cells
Ciprofloxacin HCl	3.3998 ± 0.0083
Compound 9	3.6164 ± 0.0181
Compound 12	4.0270 ± 0.0100
Norfloxacin	3.4311 ± 0.0078
Compound 10	5.3167 ± 0.0100
Compound 13	3.7249 ± 0.0189
Moxifloxacin	5.7783 ± 0.0120
Compound 11	5.5257 ± 0.6086
Compound 14	5.0765 ± 0.0064

*IC_{50} values from MTT assays after treatment. The values are mean ± SD of at least three independent experiments.

2.5. Structure-Activity Relationship

Modifications on fluoroquinolone 3-carboxylic acid group are rarely attempted due to the importance of C-3 carboxylate pharmacophore in the binding of the drugs to DNA topoisomerases. Dax cited the fusion of an isothiazolone ring, which serves as a carboxylic acid mimic, to afford derivatives with higher antibacterial activities [28]. Furthermore, the incorporation of aldehyde groups or labile carboxylate esters to quinolones can produce derivatives with potential antibacterial activity, following their conversion *in vivo* to the corresponding carboxylic acids [28]. Similarly, Bartzatt *et al.* synthesized ciprofloxacin ester analogues with modified hydrophobicity and polar surface area to improve tissue penetration, yet maintaining strong repression of penicillin-resistant *E. coli* [29, 30]. Accordingly, chemimanipulation of the fluoroquinolone C-3 position is challenging and requests a further investigation. The inspired use of carboxylic acid isosteres has redefined traditional quinolone structure-activity relationships. Following carbohydrate-based drug discovery, commercially available fluoroquinolones were modified by amidation with glucosamine moieties (acetylated and deacetylated) with different lipophilicity. The data presented here show that amidation of the fluoroquinolones with glucosamines altered the antimicrobial activity of the parent drugs. Moreover, amidation of ciprofloxacin and norfloxacin with glucosamines (acetylated and deacetylated) generated antibacterial activity against a quinolone-resistant *E. coli* clinical isolate. However, amidation of moxifloxacin with glucosamine lowered its growth inhibitory activity against this pathogen. Similarly, incorporation of glucosamine diminished the antibacterial activity of moxifloxacin against *S. aureus* ATCC 9253 and *S. enterica* ATCC13312. The molecular mechanism by which the tested compounds exert their antimicrobial activity remains to be determined and could be attributed to several factors such as steric effects, electronic effects, solubility effects, permeability/uptake, affinity to target enzyme, molecular masses, *etc*. Remarkably, norfloxacin conjugated with the *O*-acetylated glucosamine moiety (**10)** gave comparable or better antifungal activity, when compared to their deacetylated analogue (**13**) which may be attributed to its higher lipophilicity. These results are consistent with other studies in which an increase in the lipophilic character of fluoroquinolone analogues led to enhancements in antimycobacterial activity [31], due to the increased penetration and consequent concentration in the macrophages [32, 33].

3. Conclusion

A set of novel glycosylated ciprofloxacin, norfloxacin and moxifloxacin hybrids featuring amide functional groups at C-3 of the fluoroquinolone scaffold were successfully synthesized through various synthetic routes. Synthesized derivatives were *in vitro* evaluated for their antibacterial and antifungal activity. Hybrid compounds **9**, **10** and **13** were confirmed to be potential antimicrobial agents against the resistant clinical *E. coli*. The observed antifungal activity of hybrids **10** and **13** against *C. albicans, S. chartarum* and *P. chrysogenum* and compounds **10**, **11**, **12** and **13** against *A.* flavus are probably triggered by the functional group conversion at the C-3 position. Moreover, the cytotoxic activity of the synthesized compounds was evaluated against L6 muscle cell line *in vitro* using the MTT assay and showed near-identical cytotoxic profiles to the corresponding parent analogue. Accordingly, it could be proposed that the attachment of glucosamine *via* an amide linkage on the 3-position of fluoroquinolone core promoted the antibacterial and antifungal properties of the parent fluoroquinolones. Based on these results, glycosylated fluoroquinolone analogues could be identified as preferred hits, leading into further investigations, whereas detailed evaluation of the effect of these substituents on standard fluoroquinolones linked to microbial transporters remains to be determined.

4. Experimental

4.1 *Materials and Equipment*

Ciprofloxacin hydrochloride, norfloxacin and moxifloxacin hydrochloride were obtained from Dar Al Dawa, Jordanian Pharmaceutical Manufacturing Company and Al Taqaddom Pharmaceutical Industries, respectively. *D*-(+)-Glucosamine hydrochloride, solvents and other reagent grade chemicals were purchased from Sigma-Aldrich and used without any further purification. TLC was performed on pre-coated TLC sheets ALUGRAM Xtra SIL G/UV_{254} and compounds were visualized under UV light. The melting points were recorded with the Stuart melting point apparatus (SMP3, UK) and Differential Scanning Calorimetry (DSC) (Mettler Toledo, Switzerland). FTIR spectra were recorded using Shimadzu FTIR spectrophotometer (Japan). ^{1}H NMR,^{13}C NMR, COSY, DEPT 135 and HQMC spectra were acquired using Bruker Advance III spectrometers (500 MHz) (Switzerland) with deuterated solvents. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS) as an internal standard. Significant ^{1}H NMR data were tabulated in the following order: the 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 recorded (in positive or negative mode) using the electrospray ion trap (ESI) technique by collision-induced dissociation on a Bruker Apex IV (7 T) instrument (Germany). Samples were dissolved and infused using a syringe pump with a flow rate of 120 µL min^{-1}. External calibration was conducted using the arginine cluster in the mass range of 175-871 Da. Mass error: 0.00-0.50 mDa.

4.2. Chemical synthesis

*4.2.1. 2-Deoxy-2-(4-methoxybenzylideneamino)-D-glucopyranose **(1)***

4-Methoxybenzaldehyde (12 mL, 93 mmol) was added dropwise to *D*-(+)-glucosamine hydrochloride (20 g, 93 mmol) in aq. NaOH (1.0 M, 96 mL). Stirring was continued at room temperature until complete crystallization indicated the end of the reaction. The crystals were filtered, washed with cold water (100 mL), washed with EtOH:Et_2O (1:1, 100 mL) and dried in a desiccator under vacuum overnight to obtain **1** (15.2 g, 55%) as a white powder: mp 164-168 °C (Lit. [17]: mp165-166 °C); IR $\nu_{max:}$ 1650 (N=C), 3200 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, dd, *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).

*4.2.2. 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-(4-methoxybenzylidene(amino)-ß-D-glucopyranose **(2)***

Compound **1** (15.1 g, 51 mmol) was added portionwise to a cold solution of pyridine (83 mL) and Ac_2O (45 mL). Stirring at 0 °C was continued for 1 h, followed by 2-d stirring at room temperature. The reaction was monitored using TLC (n-hexane: EtOAc). Ice and cold water (300 mL) were added to induce precipitation. The precipitate was filtered, washed with cold water (185 mL) and dried in a desiccator under vacuum overnight, yielding **2** (16.6 g, 70%) as a white powder; mp 179-183.1 °C (Lit. [17]: mp 180-182 °C), IR ν_{max} 1750 (C=O), 1650 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), 3.45 (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).

4.2.3. 1,3,4,6-Tetra-O-acetyl-ß-D-glucosamine hydrochloride ***(3)***

Compound **2** (16.5 g, 35 mmol) was added to a mixture of aq. HCl (5.0 M, 8.3 mL) and warm acetone (150 mL), leading to the formation of a white precipitate after a few min. The mixture was cooled. Stirring was resumed for 2 h after adding Et_2O (150 mL). Filtration and washing with Et_2O (50 mL) afforded **3** (12.4 g, 92 %) as a white precipitate; mp 292-230 °C (Lit. [17]: mp 235 °C), IR ν_{max}: 2800 (N^+H_3), 1750 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, N^+H_3).

4.2.4. 1,3,4,6-Tetra-O-acetyl-ß-D-glucosamine ***(4)***

Compound **3** (6.0 g, 16 mmol) was dissolved in CH_2Cl_2 (85 mL). Aq. Na_2CO_3 (1.0 M, 85 mL) was added and the solution was stirred for 30 min. Extraction with CH_2Cl_2, washing with water, drying over anhydrous Na_2SO_4 and evaporation afforded **4** (4.7 g, 84%) as a white powder; mp 138-142 °C (Lit. [18]: mp.138 °C); ^{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), 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).

4.2.5. 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid ***(5)***

Ciprofloxacin HCl (8.00 g, 21.7 mmol) was dissolved in water (267 mL), followed by the addition of excess aq. $NaHCO_3$ (5%, 300 mL). The precipitate was filtered, washed with cold water and dried in a desiccator under reduced pressure to produce ciprofloxacin **5** (6.8 g, 95%) as a white solid: mp 253-255 °C (Lit. [19]; m.p. 255-257 °C); ^{1}H NMR (DMSO-d_6, 500 MHz) δ_H 1.14 (2H, m, cyclopropane 2,3-H_2), 1.28 (2H, q, *J* = 6.2 Hz, cyclopropane 2,3-H_2), 2.87 (4H, m, 2 × NC$\underline{H}_2$), 3.20 (4H, m, 2 × NC$\underline{H}_2$), 3.78 (1H, m, cyclopropane 1-H), 7.49 (1H, d, *J* = 7.4 Hz, H-8), 7.85 (1H, d, *J* = 13.4 Hz, H-5), 8.62 (1H, s, H-2).

*4.2.6. 7-(4-Acetylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6)***

Ac_2O (0.5 mL) was added to ciprofloxacin **5** (1.5 g, 4.5 mmol) in CH_3CO_2H (12.0 mL). The reaction was warmed to no more than 35°C for 1 h and left for a few min to crystallize at room temperature. The crystals were filtered, washed with Et_2O and dried overnight in a desiccator to afford **6** (0.94 g, 56%) as a white solid; mp 253-256 °C (Lit. [20] mp 255 °C); IR ν_{max} 1716 cm^{-1} (C=O); ^1H NMR (DMSO-d_6, 500 MHz) δ_H 1.15 (2H, m, cyclopropane 2,3-H_2), 1.27 (2H, q, *J* = 6.2 Hz, cyclopropane 2,3-H_2), 2.02 (3H, s, CH_3CO), 3.32 (4H, m, 2 × NC$\underline{H_2}$), 3.63 (4H, m, 2 × NC$\underline{H_2}$), 3.77 (1H, m, cyclopropane 1-H), 7.53 (1H, d, *J* = 7.4 Hz, H-8), 7.89 (1H, d, *J* = 13.2 Hz, H-5), 8.61 (1H, s, H-2), 15.02 (1H, s, CO$\underline{OH}$).

*4.2.7. 7-(4-(Acetyl)-piperazin)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(7)***

Ac_2O (1.8 mL, 19.5 mmol) was added dropwise to a solution of norfloxacin (4.0 g, 13 mmol) in glacial acetic acid (150 mL). The reaction was warmed to no more than 35°C for 1 h and left for a few minutes to crystallize at room temperature. The crystals were filtered, washed with Et_2O and dried overnight in a desiccator to afford **7** (3.1 g, 69%) as a white powder; mp 297-302 °C (Lit. [20, 21] 300 °C); IR ν_{max} 1722 cm^{-1} (C=O); ^1H NMR (DMSO-d_6, 500 MHz) δ_H 1.37 (3H, t, *J* = 6.9 Hz, $CH_2\underline{CH_3}$), 2.02 (3H,s, C$\underline{H_3}$CO), 3.35 (4H, m, 2 × NC$\underline{H_2}$), 3.61 (4H, m, 2 × NC$\underline{H_2}$), 4.54 (2H, q, *J* = 6.9 Hz, C$\underline{H_2}CH_3$), 7.16 (1H, s, H-8), 7.90 (1H, d, *J* = 12.0 Hz, H-5), 8.92 (1H, s, NC$\underline{H}$=C), 15.27 (1H, s, CO$\underline{OH}$).

*4.2.8. 7-(1-Acetylhexahydro-1H-pyrrolo[3,4-*b*]pyridin-6(2H)-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic* acid ***(8)***

Moxifloxacin hydrochloride (4.85 g, 11 mmol) was dissolved in CH_2Cl_2 (30 mL). Ac_2O (1.6 mL, 16.5 mmol) was added along with Et_3N (3.1 mL, 22 mmol). The reaction was slightly warmed while stirring was continued until TLC (chloroform ($CHCl_3$): methanol (MeOH)) indicated the end of the reaction. The solvent was evaporated under reduced pressure, MeOH was added and the reaction mixture was left standing overnight. Evaporation and trituration with Et_2O afforded **8** (quant., [21]) as a white solid; IR_{vmax} 1746.86 cm^{-1} (C=O); ^1H NMR (DMSO-d_6, 500 MHz) δ_H 0.77, 1.08 and 1.25 (4H, m, cyclopropyl 2,3-H_4), 1.39 (2H, m, CH_2), 1.83 (4H, m, CH_2), 2.11

(3H, s, CH_3CO), 2.22 (1H, m, CH), 3.22-4.06 (9H, m, CH_2, CH, OCH_3) 7.73 (1H, d, *J* =13.8 Hz, H-5), 8.72 (1H, s, NCH=C), 14.96 (1H, bs, COOH).

4.2.9. 7-(4-(Acetylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-N-(1,3,4,6-tetra-O-acetyl-2-deoxy-D-glucopyranose-2-yl)-1,4-dihydroquinoline-3-carboxamide ***(9)***

Ethyl chloroformate (0.5 mL, 5.4 mmol) was added dropwise to Et_3N (0.8 mL, 5.4 mmol) and compound **6** (2 g, 5.4 mmol) in cold CH_2Cl_2 (150 mL, -20 °C). The stirred solution was monitored by TLC ($CHCl_3$: MeOH) until formation of the mixed anhydride was complete. Compound **4** (1.88 g, 5.4 mmol) in cold CH_2Cl_2 (30 mL, -20 °C) was then added dropwise and the mixture was stirred at -20 °C for 5 h and at room temperature for 2 d. The solution was washed with water (180 mL), aq. HCl (1.0 M, 180 mL), aq. $NaHCO_3$ (1.0 M, 180 mL) and water (180 mL). The organic layer was dried over anhydrous Na_2SO_4. The gummy evaporation residue was triturated with Et_2O and purified using silica gel column chromatography ($CHCl_3$: MeOH) to afford **9** (0.50 g, 15%) as a white solid; m.p 151-153 °C; IR ν_{max}; 1741 (C=O amide), 1654 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 1.14 (2H, m, cyclopropyl 2,3-H_2), 1.39 (2H, m, cyclopropyl 2,3-H_2), 1.92 (3H,s, CH_3CO), 1.99 (3H, s, CH_3CO), 2.01 (3H, s, CH_3CO), 2.06 (3H,s, CH_3CO), 2.13 (3H, s, CH_3CO), 3.20 (2H, s, NCH_2), 3.26 (1H, m, cyclopropyl 1-H), 3.41 (2H, s, NCH_2), 3.66 (2H, s, NCH_2), 3.82 (2H, s, NCH_2), 3.89 (1H, m Gluc H-5), 4.11 (2H, m, Gluc H-2, Gluc H-6), 4.30 (1H, m, Gluc H-6), 5.10 (1H, t, *J* = 8.9 Hz, Gluc H-4), 5.62 (1H, t, *J* = 8.9 Hz, Gluc H-3), 6.11 (1H, d, *J* = 6.2 Hz, Gluc H-1),7.28 (1H, bs, H-8), 7.99 (1H, d, *J* = 11.5 Hz, H-5), 8.75 (1H, s, N<u>CH</u>=C), 10.2 (1H, d, *J* = 8.2 Hz, CONH); ^{13}C NMR ($CDCl_3$, 125 MHz) 8.20 (cyclopropane 2,3-C_2), 20.65-21.30 (5 × <u>C</u>H_3CO), 34.84 (cyclopropane C-1), 41.12 (N<u>C</u>H_2), 46.18 (N<u>C</u>H_2), 49.54 (N<u>C</u>H_2), 50.49 (N<u>C</u>H_2), 53.74 (Gluc 2-C), 61.83 (Gluc 6-C), 68.41 (Gluc 4-C), 72.05 (Gluc 3-C), 72.64 (Gluc 5-C), 91.84 (Gluc 1-C), 105.02 (C-8), 110.61 (C_q), 112.97 (C-5), 121.00 (C_q), 138.41 (C_q), 146.00 (C_q), 147.11 (N<u>C</u>H=C), 152.00-154.00 (2 × C_q), 165.49, 169.07, 169.61, 170.09, 170.70, 175.30 (5 × CH_3<u>C</u>O, <u>C</u>ONH); MS (ESI +) ***m/z*** 666.23835 [M + Na – $MeCO_2H$]$^+$ ($^{12}C_{30}{}^{13}C_1H_{35}FN_4NaO_{10}$ requires 666.22685), 665.23717 [M + Na – $MeCO_2H$]$^+$ ($^{12}C_{31}H_{35}FN_4NaO_{10}$ requires 665.22350).

4.2.10. 7-(4-Acetylpiperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-N-(1,3,4,6-tetra-O-acetyl-2-deoxy-D-glucopyranose-2-yl)-1,4-dihydroquinoline-3-carboxamide ***(10)***

Ethyl chloroformate (0.2 mL, 2.1 mmol) was added drop-wise to Et_3N (0.3 mL, 2.1 mmol) and **7** (0.500 g, 1.39 mmol) in cold CH_2Cl_2 (250 mL, -20 °C). The stirred solution was monitored by TLC until formation of the mixed anhydride was complete. Then **4** (0.7250 g, 2.025 mmol) in cold CH_2Cl_2 (30 mL, -20 °C) was added dropwise. The mixture was stirred at -20 °C for 5 h and at room temperature for 2 d. The mixture was washed with water (300 mL), aq. HCl (1.0 M, 300 mL), aq. $NaHCO_3$ (1.0 M, 300 mL) and water (300 mL). The organic layer was dried over anhydrous Na_2SO_4. The gummy evaporation residue was triturated with Et_2O and purified using silica gel column chromatography ($CHCl_3$: MeOH) to afford **10** (0.65 g, 47%) as a white powder; mp 135-139 °C; IR ν_{max}; 3226 (NH), 1650 cm^{-1} (C=O); ^1H NMR ($CDCl_3$, 500 MHz) δ_H 1.50 (3H, t, *J* = 7.2 Hz, $CH_2\underline{CH_3}$), 1.91 (3H, s, $\underline{CH_3}CO$), 1.97 (3H, m, $\underline{CH_3}CO$), 2.01 (3H, s, $\underline{CH_3}CO$), 2.05 (3H, s, $\underline{CH_3}CO$), 2.11 (3H, s, $\underline{CH_3}CO$), 3.17 (2H, m, $N\underline{CH_2}$), 3.23 (2H, m, $N\underline{CH_2}$), 3.65 (2H, m, $N\underline{CH_2}$), 3.80 (2H, m, $N\underline{CH_2}$), 3.90 (1H, m, Gluc H-5), 4.10 (2H, m, Gluc H-2, Gluc H-6), 4.22 (2H, q, *J* = 7.2 Hz, $\underline{CH_2}CH_3$), 4.31 (1H, dd, *J* = 4.5, 12.3 Hz, Gluc H-6), 5.10 (1H, t, *J* = 9.6 Hz, Gluc H-4), 5.58 (1H, t, *J* = 9.6 Hz, Gluc H-3), 6.10 (1H, d, *J* = 8.6 Hz, Gluc H-1), 6.75 (1H, d, *J* = 6.7 Hz, H-8), 8.02 (1H, d, *J* = 13.0 Hz, H-5), 8.60 (1H, s, $N\underline{CH}$=C), 10.25 (1H, d, *J* = 8.4 Hz, CONH); ^{13}C NMR ($CDCl_3$, 500 MHz) δ_C 14.41 ($NCH_2\underline{C}H_3$), 20.63-21.28 (5 × $\underline{CH_3}CO$), 41.09 (2 × $N\underline{C}H_2$), 46.13 (2 × $N\underline{C}H_2$), 49.25 ($N\underline{CH_2}CH_3$), 53.55 (Gluc C-2), 61.83 (Gluc C-6), 68.40 (Gluc C-4), 72.10 (Gluc C-3), 72.62 (Gluc C-5), 92.15 (Gluc C-1), 104.06 (C-8), 110.80 (C_q), 113.23 (C-5), 123.02 (C_q), 136.44 (C_q), 144.79 (C_q), 146.92 ($N\underline{C}H$=C), 152.00-154.00 (2 × C_q), 165.58, 169.06, 196.60, 170.56, 170.68, 175.15 (5 × $CH_3\underline{CO}$, CONH); MS (ESI +) *m/z* 716.2748 $[M + Na]^+$ ($^{12}C_{29}{}^{13}C_3H_{39}FN_4NaO_{12}$ requires 716.2547), 715.2686 $[M + Na]^+$ ($^{12}C_{30}{}^{13}C_2H_{39}FN_4NaO_{12}$ requires 715.2513), 714.2661 $[M + Na]^+$ ($^{12}C_{31}{}^{13}C_1H_{39}FN_4NaO_{12}$ requires 714.2480), 713.2637 $[M + Na]^+$ ($^{12}C_{32}H_{39}FN_4NaO_{12}$ requires 713.2446), 672.2551 $[M + Na - H_2CCO]^+$ ($^{12}C_{29}{}^{13}C_1H_{37}FN_4NaO_{11}$ requires 672.2374), 671.2500 $[M + Na - H_2CCO]^+$ ($^{12}C_{30}H_{37}FN_4NaO_{11}$ requires 671.2341).

*4.2.11. 7-(1-Acetylhexahydro-1H-pyrrolo[3,4-*b*]-pyridin-6(2*H*)-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-N-(1,3,4,6-tetra-O-acetyl-2-deoxy-D-glucopyranose-2-yl)1,4-dihydroquinoline-3-carboxamide (**11**)*

Ethyl chloroformate (0.266 mL, 2.8 mmol) was added to Et_3N (0.291 mL, 2.1 mmol) and **8** (1.0 g, 2.3 mmol) in cold CH_2Cl_2 (40 mL, -20 °C). The stirred solution was monitored by TLC until

formation of the mixed anhydride was complete. Then **4** (0.945 g, 3.45 mmol) in cold CH_2Cl_2 (30.0 mL, -20 °C) was added dropwise. The mixture was stirred at -20 °C for 5 h and then left to stir at room temperature for 2 d. The solution was washed with water (75 mL), aq. HCl (1.0 M, 75 mL), aq. $NaHCO_3$ (1.0 M , 75 mL) and water (75 mL). The organic layer was dried over anhydrous Na_2SO_4, The evaporation residue was triturated with Et_2O to afford solid material and purified using silica gel column chromatography ($CHCl_3$: MeOH) to afford **11** (196 mg, 11%) as a white powder; mp 150-154 °C; IR ν_{max}: 2829 (NH), 1724 (C=O), 1583 cm^{-1} (C=O, amide); ^1H NMR (DMSO-d_6, 500 MHz) δ_H 0.81, 1.03, 1.13 1.25 (4H, m, cyclopropane 2,3-H_4), 1.60 (2H, m, CH_2), 1.87 (2H, m, CH_2), 1.98 (3H, s, CH_3CO), 2.05 (3H, s, CH_3CO) 2.08 (3H, s, CH_3CO), 2.12 (3H, s, CH_3CO), 2.18 (3H, s, CH_3CO), 2.21 (1H, m, C<u>H</u>), 3.24-4.17 (13H, m, 3 × CH_2, -OC<u>H</u>$_3$, C<u>H</u>, Glu-H-2, Glu-H-5, Glu-H-6), 4.36 (1H, dd, *J* = 4.6, 9.4 Hz, Gluc H-6), 5.16 (1H, t, *J* = 9.4 Hz, Gluc H-4), 5.29 (1H, m, CH), 5.63 (1H, t, *J* = 9.6 Hz, Gluc H-3), 6.17 (1H, d, *J* = 8.4 Hz, Gluc H-1), 7.81 (1H, d, *J* = 13.6 Hz, H-5), 8.77 (1H, s, N<u>CH</u>=C), 10.29 (1H, d, *J* = 7.8 Hz, CONH); ^{13}C NMR (DMSO-d_6, 500 MHz) δ_C 3.75 (CH_2), 5.64 (CH_2), 15.92 (<u>CH_3</u>CO), 16.02 (<u>CH_3</u>CO), 16.22 (<u>CH_3</u>CO), 16.85 (<u>CH_3</u>CO), 17.47 (<u>CH_3</u>CO), 19.96 (CH_2), 20.45 (CH_2), 30.28 (CH), 35.00 (CH), 37.36 (CH_2), 43.25 (CH_2), 45.40 (CH), 51.27 (CH_2), 50.65 (Glu-2), 56.27 (-O<u>CH_3</u>), 57.10 (Gluc C-6), 67.56 (Gluc C-3), 63.67 (Gluc C-4), 67.85 (Gluc C-5), 87.34 (Gluc C-1), 103.75 (C-5), 105.17 (C_q), 117.39 (C_q), 129.03 (C_q), 136.52 (C_q), 144.48 (N<u>C</u>H=C), 161.80, 164.34, 164.89, 165.33, 165.82, 165.96 (5 CH_3<u>CO</u>, CONH) ; MS (ESI +) *m/z* 773.3047 $[M+H]^+$ ($C_{37}H_{46}FN_4O_{13}$ requires 773.3045), 795.2846 $[M+Na]^+$ ($C_{37}H_{45}FN_4NaO_{13}$ requires 795.2865), 796.28873 [M + Na + H] ($^{12}C_{36}{}^{13}C_1H_{46}FN_4NaO_{13}$ requires 796.2898.

3.2.12. 7-(4-Acetylpiperazin-1-yl)-1-cyclopropyl-N-(2-deoxy-D-glucopyranose-2-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide ***(12)***

NaOMe (0.1 g, 2.1 mmol) was added portionwise to **9** (0.30 g, 0.35 mmol) in MeOH (30 mL). The mixture was stirred for 2 h. The solution was neutralized using Amberlite resin, filtered, evaporated and redissolved in water. The solution was freeze-dried to obtain **12** (0.20 g, 90%) as a white powder; mp 154-165 °C; IR ν_{max} 3289 (CONH), 1621 (<u>CO</u>NH) cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ_H 1.06 (2H, m, cyclopropane 2,3-H_2), 1.26 (2H, m, cyclopropane 2,3-H_2), 2.02 (3H, s, CH_3CO), 3.12 (1H, m, Gluc H-4), 3.15-3.19 (4 H, m, 2 × NCH_2),), 3.26 (1H, m, cyclopropane 1-H), 3.29 (1H, m, Gluc H-6), 3.47-3.53 (2H, m, Gluc H-3, Gluc H-5), 3.62 (4 H,

m, 2 × NCH_2), 3.73-6.54 (7H, m, Gluc H-6, Gluc H-2, Gluc H-1, 4 × OH), 7.46 (1H, bs, H-8), 7.82 (1H, d, *J* = 13.4 Hz, H-5), 8.59 (1H, bs, NCH=C), 9.92 (1H, d, *J* = 8.5 Hz, CONH); ^{13}C-NMR (DMSO-d_6, 500 MHz) δ_C 7.76 (cyclopropane 2,3-C_2), 21.37 (CH_3CO), 39.99 (cyclopropane C-1), 50.23 (3H, s, CH_3CO), 49.98 (4 × NCH_2), 54.49 (Gluc C-2), 61.59 (Gluc C-6), 71.65 (Gluc C-4), 71.78 (Gluc C-3), 72.72 (Gluc C-5), 91.27 (Gluc C-1), 106.87 (C-8), 110.76 (C_q), 117.85 (C-5), 121.53 (C_q), 138.80 (C_q), 144.37 (C_q), 147.24 (NCH=C), 147.61 (C_q), 152.13 (C_q-quat), 164.44, 168.86, 174.64 (2 × CH_3CO, CONH); MS (ESI +) *m/z* 557.2018 $[M + Na]^+$ ($C_{25}H_{31}N_4NaO_8$ requires 557.2024).

4.2.13. 7-(4-Acetylpiperazin-1-yl)-N-(2-deoxy-D-glucopyranose-2-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamide ***(13)***

NaOMe (0.200 g, 2.58 mmol) was added portionwise to **10** (0.40 g, 0.46 mmol) in MeOH (50 mL). After 2 h, TLCindicated the formation of product. The solution was then neutralized using Amberlite resin, filtered, evaporated and redissolved in water. The solution was freez dried to obtain **12** (0.20 g, 96%) as a pale yellow powder; mp 170-172 °C; IR ν_{max} 3315 (CONH), 1540 (CONH) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H 1.35 (3H, t, *J* = 7.1 Hz, CH_2CH_3), 2.01 (3H, s, CH_3CO), 3.19-3.70 (8H, m, 2 × NCH_2, OH, Gluc H-3, Gluc H-4, Gluc H-5), 3.60 (6H, m, 2 × NCH_2, 2 × Gluc H-6), 3.81 (1 H, m, Gluc H-2), 4.44 (2H, q, *J* = 7.1 Hz, CH_2CH_3), 4.50 (1H, m, OH), 4.99 (1H, m, Gluc H-1), 7.08 (1H, bs, H-8), 7.85 (1H, d, *J* = 13.5 Hz, H-5), 8.72 (1H, s, NCH=C), 9.97 (1H, d, *J* = 8.6 Hz, CONH); ^{13}C-NMR (DMSO-d_6. 500 MHz) δ_C 14.85 (CH_2CH_3), 21.62 (CH_3CO), 40.81(NCH_2), 45.49 (CH_2CH_3), 48.54, 49.69, 50.06 (3 × NCH_2), 54.31 (Gluc), 61.00 (Gluc C-6), 71.57 (Gluc), 71.62 (Gluc), 74.84 (Gluc), 91.08 (Gluc), 106.12 (C-8), 111.67 (C-5), 147.53 (NCH=C), 165.85 (CH_3CO); (ESI +) *m/z* 545.2181 $[M + Na]^+$ ($C_{24}H_{31}FN_4NaO_7$ requires 545.2024), 527.2050 $[M + Na - H_2O]^+$ ($C_{24}H_{29}FN_4NaO_7$ requires 527.1919).

*4.2.14. 7-(1-Acetylhexahydro-1*H*-pyrrolo[3,4-*b*]-pyridin-6(2*H*)-yl)-1-cyclopropyl-N-(2-deoxy-D-glucopyranose-2-yl)-6-fluoro-8-methoxy-4-oxo- 1,4-dihydroquinoline-3-carboxamide* ***(14)***

NaOMe (0.21 g, 3.9 mmol) was added portionwise to **11** (0.50 g, 0.65 mmol) MeOH (30 mL). After 2 h, TLC indicated the formation of product. The solution was neutralized using Amberlite resin. The evaporation residue was recrystallized from MeOH/Et_2O to afford **14** (0.40 g, 90%) as

a white powder; mp 156-169 °C; IR ν_{max} 3274 (OH) 2934 (NH), 1643 (CONH), 1536 cm^{-1} (C=O, amide); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 0.74, 0.90, 0.99, 1.15 (4H, m, cyclopropane 2,3-H_4), 1.41, 1.73 (4H, m, 2 × CH_2), 2.01 (3H, s, CH_3CO), 2.13 - 2.23 (1H, m, CH), 3.11- 4.01 (17H, m, 3 x× CH_2, CH, Gluc H-2, Gluc H-4, Gluc H-5, 2 × Gluc H-6, -O$\underline{CH_3}$, 2 × OH), 4.32-4.55 (2H, m, Gluc H-3, OH), 4.99 (2H, m, Gluc H-1, CH), 6.55 (1H, bs, OH), 7.55 (1H, dd, *J* = 7.5, 11.0 Hz, H-5), 8.57 (1H, s, N$\underline{CH}$=C), 10.00 (1H, d, *J* = 8.6 Hz, CONH); ^{13}C-NMR ($CDCl_3$, 500 MHz) δ_C 8.59 (CH_2), 10.23 (CH_2), 21.63 ($\underline{CH_3}CO$), 23.82, 24.43, 24.92, 25.07 (2 × CH_2), 35.23 (CH), 39.82 (CH), 41.66 (CH_2), 48.07 (CH_2), 48.47 (CH_2), 50.10 (CH), 54.20 (Gluc), 56.07 (Gluc C-6), 61.42 (O$\underline{CH_3}$), 71.40 (Gluc), 71.54 (Gluc), 72.45 (Gluc), 91.01 (Gluc C-1), 106.82 (C-5), 110.24 (C_q), 120.50 (C_q), 136.00 (C_q), 142.00 (C_q), 154.20 (C_q), 149.49 (N$\underline{C}$H=C), 164.39, 170.33, 174.28 ($CH_3\underline{CO}$, CONH)); MS (ESI +) *m/z* 627.2576 $[M + Na]^+$ ($C_{29}H_{37}FN_4NaO_9$ requires 627.2442).

4.3. *In vitro* antimicrobial testing

Target compounds **9**-**14** were evaluated *in vitro* for their antimicrobial activities against Gram-negative bacteria *E. coli*, O157:H7; *S. enterica*, ATCC 13312; *L. monocytogenes*, ATCC 19115; *P. aeruginosa*, ATCC 9627; *E. coli*, NCTC 11954; *E. coli*, ATCC 8739 and clinical *E. coli* isolate (Resistant to nalidixic acid, ciprofloxacin HCl and norfloxacin and acquired from Biolab® (Amman-Jordan)); Gram-positive bacteria (MRSA, ATCC 33591 and MSSA, ATCC 9253); non-spore-forming fungi *C. albicans*, ATCC 10231) and spore-forming fungi *A. flavus*, ATCC 9643; *F. solani*, ATCC 36031; *S. chartarum* provided by Professor Naresh Magan (Cranfield University, UK)) and *P. chrysogenum*, ATCC 10106. All media were prepared under sterile conditions following manufacturers' instructions. Bacterial broth cultures stocks were cultivated in the appropriate medium at 37 °C for 24 h before testing. 0.5 McFarland standard were used to approximate visually the concentration of cells in a suspension.

Fungal conidia were attained from fungi stock previously cultivated on malt extract agar at 30 °C for 7 d. Fungal conidia were transferred into normal saline (3.0 mL) with a cotton swap previously impregnated in Tween 20. Then, conidial suspensions were vortexed vigorously for 20 sec, to prevent spore clumping and were left standing at RT for 15 min. The upper layer then was transferred into a sterile falcon tube and adjusted to 0.5 McFarland at 530 nm to yield 4.38-10.00 × 10^6 suspensions of sporangiospores (*A. flavus*, 5.00 × 10^6; *F. solani*, 4.38 × 10^6; *S.*

chartarum, 7.50×10^6; *P. chrysogenum*, 10.00×10^6). Mean spore counts from three trials were determined using a hemocytometer. 1:50 Dilutions of the stock suspensions with malt extract broth gave the final working suspension.

The minimal inhibitory concentrations (MICs) against study pathogens were determined for each of the target compounds using the micro-broth dilution method. MIC was defined as the lowest concentration of the tested compound showing no microbial viability. A stock solution of the target compounds in DMSO was prepared, then 100 µL was transferred into each well of 96-well plate having 100 µL of the appropriate broth, followed by two-fold serial dilution in subsequent wells. 10 µL of the microbial suspension (10^6 strength) was added to each well, incubated at 37°C for 24 h and the volume was adjusted to 200 µL with appropriate broth in each well. All experiments were conducted in triplicate. The absorbance of light at 625 nm was measured using an Epoch spectrophotometer. Ciprofloxacin HCl, norfloxacin and moxifloxacin HCl were used as positive controls and negative control (DMSO) samples, along with proper sterility and growth controls, were prepared and evaluated.

The MIC protocol for the spore-forming fungi was similar to the bacterial protocol; however, MICs were recorded after 48 h of incubation at 30°C and absorbance was measured at 405 nm. Stock solutions of compounds had a 4.0 mg mL^{-1} concentration. Fluconazole was used as a positive control; DMSO was used as negative control along with proper sterility and growth controls. Experiments were conducted in triplicate.

4.4. Cell viability testing with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide] assay

L6 rat muscle myoblast cells (ATCC CRL-1458) were cultured as a monolayer under 95% air, 5% CO_2 at 37°C in glucose-DMEM supplemented with 10% FBS, 1 mM L-glutamine, 100 U mL^{-1} penicillin and 0.1 mg mL^{-1} streptomycin. Cells were sub-cultured at a ratio of 1:20. The **cytotoxicity** study was carried out according to the Mossman protocol [22] using L6 rat muscle cells with a final density of 2×10^4 cells mL^{-1}. Cells were suspended (20 µL $well^{-1}$) and seeded into a 96-well plate at a density of 2×10^4 cells/well and incubated at 37°C, 5% CO_2, 95% air, and 100% relative humidity to allow attachment. After 24 h, cells were treated with different concentrations of glycosylated fluoroquinolones **9-14,** standard fluoroquinolones (ciprofloxacin

HCl, norfloxacin and moxifloxacin) and DMSO (0.1%) as a negative control. Aliquots of novel derivatives and standards (100 µL) at different concentrations were added to the appropriate wells and the plates were incubated for 24 h. The medium was then replaced in the dark with 100 μL of fresh medium/well containing 0.5 mg mL^{-1} MTT (filtered with 0.22 µm nylon) and incubated for another 4 h. The supernatant was removed and 100 μL of 100% DMSO was added to each well. Positive-control wells contained cells in complete media without analogues or drugs. The absorbance was read at 570 nm using the FLUO star Omega microplate reader. The cytotoxic effect of the analogues was expressed as cell viability using the following formula:

$$\%\text{Cell viability} = \frac{\text{Mean OD of the test sample}}{\text{Mean OD of the vehicle control (negative control)}} \text{ X } 100\%$$

The tests were conducted in triplicate and IC_{50} values were calculated using dose-effect curves and expressed as a concentration (µM) of the drug.

Declaration of competing interest

Disclosures related to glycosylated 3-substituted fluoroquinolone derivatives, preparation methods thereof, and their use in the treatment of antimicrobial infections have been registered for patents (Reg. No. P/JO/2019/000097) and (Reg. No. PCT/JO2019/050011).

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Design, Synthesis and Antimicrobial Evaluation of Novel Glycosylated-fluoroquinolones Derivatives

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Highlights

1- Glucosamine-fluoroquinolone conjugation was made possible through a simple synthetic pathway
2- Several novel hybrids showed potential growth inhibitory activity against the clinical fluoroquinolones resistant *Escherichia coli* isolate.
3- Compound **10** exerted potential antifungal activity against *Candida albicans* and *P. chrysogenum*.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Disclosure relates to glycosylated 3-substituted fluoroquinolone derivatives, preparation methods thereof, and their use in the treatment of antimicrobial infections have been registered for a patent (Reg. No. P/JO/2019/240) and (Reg. No. PCT/JO2019/050011).