Synthesis, antibacterial evaluation and in silico study of DOTA-fluoroquinolone derivatives

Weitian Li1 · Ge Hong1 · Lina Mao1 · Zengping Xu1 · Jiawen Wang1 · Wenzhi Wang1 · Tianjun Liu1

Received: 10 November 2021 / Accepted: 21 February 2022 / Published online: 12 March 2022
© The Author(s), under exclusive licence to Springer Science + Business Media, LLC, part of Springer Nature 2022

Abstract
A series of water-soluble fluoroquinolones based upon DOTA (1,4,7,10-tetraazacyclododecane -1,4,7,10-tetraacetic acid) modification were synthesized and characterized by NMR and HRMS spectra. All the newly prepared quinolones compounds exhibited potent antimicrobial activities against MRSA (methicillin-resistant Staphylococcus aureus), P. aeruginosa and E. coli. Molecular docking study indicated they could form stable complex with DNA gyrase and topoisomerase IV-DNA respectively, ADMET prediction showed they were low toxicity to the mice as whole. Among them, water-soluble quinolone 4c exhibited promising antibacterial potency, its MIC, MBC value for MRSA and P. aeruginosa was (1.56, 6.25) µg/mL, (3.1, 12.5) µg/mL respectively. Atomic Force Microscope (AFM) imaging revealed 4c could effectively destroy MRSA bacterial membrane and wall, causing its contents to leak out. Cytotoxicity assay showed 4c had low toxicity to L-02, A549 and MCF-7, over 80% cell viability even at 100 µmol/L. These results showed that water-soluble compound 4c was a promising antibacterial candidate.

Keywords Fluoroquinolone · Water-soluble · Antimicrobial activities · ADMET · Cytotoxicity

Introduction
Fluoroquinolone, as a major class of synthetic antibiotics since the mid-1980s, had many advantages like good chemical stability, broad antibacterial spectrum as well as excellent antibacterial activity, high biological half lifetime, widely distributed ability in tissues and body fluids, excellent penetration rate and therapeutic ratio [1–9]. Up to now, fourth-generation quinolone antibiotics had been applied in clinics. Some third- and fourth-generation quinolone drugs were listed as follows (Fig. 1).

However fluoroquinolone drugs had limited water-solubility [10, 11], although salt-formation like Ciprofloxacin hydrochloride, Pazufl oxacin mesilate and Moxifloxacin hydrochloride, etc., could partially resolve this problem, the solubility-increase was still limited. Conjugating a hydrophilic group on the piperazine ring of the quinolone would be an efficient chemical method [12, 13], the typical example was introducing amino acids [14, 15], N-linked glycosylation, or PEGylation [16, 17]. DOTA [18–20] ,ag o o dc h e l a t o ru s e di n MRI (magnetic resonance imaging) contrast agent, with merits of both good water solubility and good biocompatibility, could greatly improve the water solubility of poorly soluble drugs [21]. Teichoic acid [22], one of the main components of the bacterial cell wall, could enrich the Mg2+ around the cell via the interaction with a large amount of negative charge on the surface, resulting in an increase in the activity of some synthetases on the cell membrane [23, 24]. DOTA modification could enhance or tune the targeting of the DOTA-drug to the surface of the bacteria by metal chelation. In addition, teichoic acid, glycopeptide at the bacterial wall interaction with DOTA via hydrogen bonding would also raise the concentration of DOTA modified drug at the bacteria wall. The ensemble effect of DOTA modification could promote the drug potency towards bacterial strains, even lead to morphological changes in the bacterial cell wall. Atomic force microscope (AFM) was often used to confirm the structural changes in bacterial surface.
DNA gyrase and topoisomerase IV were two type II topoisomerases presented in bacteria [3]. They both played important roles in many cellular processes including bacterial DNA replication, transcription, recombination, etc. [25]. For most gram-negative bacteria, DNA gyrase was thought to be the primary quinolone target, whereas topoisomerase IV appeared as the primary quinolone target for gram-positive bacteria [26]. Since the crystal structure of them had been elucidated, they were often used as molecular targets of quinolone derivatives for virtual screening and protein-ligand interaction simulation in silico to identify the potent antibiotics.

In this paper, we would introduce DOTA moiety into the fluoroquinolones at C-7 position and evaluate the effect on their antibacterial activity against sensitive and resistant bacteria strains. In silico ADMET prediction and molecular docking study were then employed to explore the drug-likeness character and the protein-ligand interaction of the newly prepared DOTA-modified compounds. Finally, the titled compound 4c with the most potent antibacterial activity among the synthesized DOTA-fluoroquinolones were subjected to bacterial morphology and cytotoxicity studies to see the effect of structural modification on compound activity and toxicity. 2a-g were obtained quantitatively by TFA (Trifluoroacetic acid) deprotection of the tert-butyl group of 3a-g. The purity of the compounds were over 95.00% by HPLC, and among them, 4c was 98.56% (Fig. 3).

**MIC and MBC determinations in vitro**

MIC and MBC values of 2a-4g along with the control drugs of 1a-g against MRSA, *P. aeruginosa* and *E. coli*, were listed in Table 1.

The general trend of the antibiotic activity in Table 1 changed in the order of 1a-g > 2a-g > 3a-g > 4a-g. The corresponding fluoroquinolone mother drug showed the potent antibiotic ability, while modification in this paper would lower this ability, the larger the modification skeleton size, the lower the antibiotic ability. In 2a-g and 3a-g, the modification moieties were hydrophobic, the size effect dominated their antibiotic potency. And the antimicrobial activity of compound 3a-g were less than that of 2a-g. The possible reason was the large molecular skeleton of compound 3a-g weakened them to pass across the cell membrane and bind to its target site. In 4a-g, DOTA modification enhanced their water solubility at the cost of their antibiotic activity. Although the molecular skeleton of the compound 3a-g is larger than their corresponding

**Results and discussion**

**Chemistry**

DOTA had four carboxylic groups, direct reaction with fluoroquinolone gave complex products, which was difficult to purify. Therefore, the target compound was synthesized according to the method outlined in Scheme 1. The first cyclen reacted with t-butyl bromoacetate to form tris-N-(t-butylamidomethyl) cyclen, 2, in a yield of 89% in DMA (Dimethoxyamphetamine) solution containing CH3COONa. N-alkylation of commercially available fluoroquinolones 1a-g (in Fig. 1) gave intermediates 2a-g.

The reaction of 2 with fluoroquinolone derivative 2a-g in acetonitrile in the presence of K2CO3 under heating condition formed the target fluoroquinolones 3a-g. The final product 4a-g were obtained quantitatively by TFA (Trifluoroacetic acid) deprotection of the tert-butyl group of 3a-g. The purity of the compounds were over 95.00% by HPLC, and among them, 4c was 98.56% (Fig. 3).

**Fig. 1** The structure of fluoroquinolones modified in this paper
deprotection product 4a-g, their antibacterial activity was higher. These differences possibly came from the balance of the hydrophilicity and lipophilicity, which influenced the cellular uptake and the following function site binding, finally determined the antibacterial potency. However, the extraordinary was antibacterial potency of 2a-b better than 1a-b for MRSA and P. aeruginosa: the MIC value of 2a against P. aeruginosa reduced 5 times compared with 1a, while the MBC value reduced 2 times; the MIC value of 2b against P. aeruginosa reduced 2 times compared with 1b, while the MBC value reduced 2 times. The possible explanation was the N-alkylation of amines in the fluoroquinolone mother drug changed the alkalinity and water solubility of the reference antibiotics. Due to intrinsic differences between various amine groups, the antibiotic activity of some synthesized derivatives in 2a-g was found to be better than the reference antibiotics. While for E. coli, the potency changed in the order of 1b > 2b, 1a > 2a. This indicated that the antibacterial potency of the compound was also dependent on the strain type. In the whole system studied here, the compound whose antibiotic potency changed less after DOTA modification were 3c and 4c, 3e and 4e. 3c and 4c showed the same MIC and MBC value for MRSA and P. aeruginosa. For E. coli, 3c and 4c almost lost their potency, at the level of 100 μg/mL. While 3e and 4e possessed the strong antibacterial potency for the three strains studied here, the MIC and MBC of 3e and 4e for MRSA or P. aeruginosa was (3.13, 6.25) μg/mL, and for E. coli was (6.25, 12.5) μg/mL respectively.

In 1a-e, with piperazine ring at the C-7 position in common, so the antibiotic trend for their derivative like 2a-e, 3a-e, 4a-e changed roughly in the same way. While in 1f...
and 1g, with fused bicyclic or cyclopropyl ring at C-7 respectively, their antibiotic potency changed differently, modification with DOTA made them almost lose completely their antibiotic activity. For the three strains reported here, MRSA, P. aeruginosa and E. coli, the MIC value of 3f, 4f increased 1282 times compared with 1f, while the MBC value increased 2564 times; the MIC and MBC value of 3g, 4g increased more than 1282 times and 2564 times compared with 1g respectively. The possible interpretation is due to the function of fluoroquinolone antibiotics on topoisomerase IV and DNA gyrase [3, 27, 28], inner of the cell, the large molecular skeleton would weaken the molecule ensemble pass across the cell membrane.

The DOTA-quinolone (4a-g) could complex with metal ions such as Fe$^{2+}$, Mg$^{2+}$, Mn$^{2+}$ in the form of 1:1 ratio, which was confirmed by ESI-MS. However, the metal ions’ effect on the antibacterial of DOTA-modified quinolone compounds revealed that these metal ions docking just improved a little in antibacterial activity, such as the MIC, MBC data of 4a for MRSA was (12.5, 50) $\mu$g/mL, for P. aeruginosa (25, 100) $\mu$g/mL, while the data of 4a-Fe was (5, 20) $\mu$g/mL, (10, 20) $\mu$g/mL, respectively. These results supported our original suggestion: these complexes could enrich the DOTA-quinolone compounds at the surface of bacterial walls. But their potency just improved a limited bit, was still weaker than the mother drug 1a-g, so the detailed study for the other DOTA compounds was stopped.

### Prediction of ADMET property

The prediction of ADMET (absorption, distribution, metabolism, excretion and toxicity) properties of all the target compounds 1a-4g were presented in Table 2. The solubility of the compounds correlated tightly with its ADMET. LogP values could reflect the lipophilicity of compounds [32], the smaller the logP value, the more hydrophilic the molecule. Similarly, the opposite was the change of lipophilicity. Table 2 showed that the logP of compounds 2a-g and 3a-g were larger than zero, suggesting their lipophilic properties, while less zero data for 4a-g and these data were even less 1a-g suggesting its good hydrophilicity. 3a-4g did not meet Lipinski’s rule of five, their molecular weight was over 500, the number of HBA and total rotation bonds were more than 10. The cytochrome P450 2D6 (CYP2D6) was involved in the metabolism of a wide range of substrates in the liver and its inhibition by a drug constitutes a majority case of drug-drug interaction [33, 34]. Based upon Pre-ADMET ver 2.0 computation, the target compounds 1a-4g interaction with CYP2D6 was conducted. Results showed that except for 1e, 1g, compounds 1a-4g were found to be non-inhibitors of cytochrome P450 2D6. No carcinogenicity to mice was predicted for compounds of 1a-1g, 2g, 3a-3g and 4a-4g compounds. Except for 3d and 4d, medium risk in toxicity to heart was predicted for 3a-4g, while 4g showed more risk in this case. In general, DOTA modification of fluoroquinolone in this paper actually increased the water solubility of the ensemble system 4a-g, no carcinogenicity, no toxicity to heart and no toxicity to P450 were predicted, however the change in molecular weight, the binding site as well as the ADMET, caused an ensemble effect in antibacterial activity listed in Table 1.

### Molecular docking studies

Type IIA topoisomerases played an important role in the regulation of DNA replication, recombination and transcription for all bacteria [25]. It was well known that two

| Comp. | MRSA MIC | MBC | P. aeruginosa MIC | MBC | E. coli MIC | MBC |
|-------|-----------|-----|-------------------|-----|-------------|-----|
| 1a    | 1.56      | 3.13| 0.78              | 1.56| 0.39        | 0.39|
| 2a    | 0.078     | 0.39| 0.156             | 0.78| 0.78        | 1.56|
| 3a    | 6.25      | 25  | 12.5              | 12.5| 50          | 100 |
| 4a    | 12.5      | 50  | 25                | 100 | 50          | 100 |
| 1b    | 0.78      | 3.13| 0.78              | 1.56| 0.39        | 0.39|
| 2b    | 0.39      | 0.39| 0.39              | 0.78| 1.56        | 3.12 |
| 3b    | 6.25      | 25  | 6.25              | 12.5| 200         | 200 |
| 4b    | 200       | 200 | 200               | >200| >200        | >200|
| 1c    | 0.078     | 0.156| 0.078            | 0.156| 0.156     | 0.156|
| 2c    | 0.39      | 0.78| 0.39              | 0.78| 3.12        | 6.25 |
| 3c    | 1.56      | 6.25| 3.13              | 12.5| 100         | 100 |
| 4c    | 1.56      | 6.25| 3.13              | 12.5| 100         | 100 |
| 1d    | 0.78      | 1.56| 0.78              | 1.56| 1.56        | 1.56|
| 2d    | 0.312     | 0.625| 0.312            | 0.625| 2.5        | 5   |
| 3d    | 12.5      | 25  | 12.5              | 25  | 12.5        | 25  |
| 4d    | 25        | 50  | 25                | 25  | 25          | 50  |
| 1e    | 0.078     | 0.156| 0.078            | 0.156| 0.078     | 0.156|
| 2e    | 2.5       | 5   | 0.078             | 0.156| 5          | 10  |
| 3e    | 3.13      | 6.25| 3.13              | 6.25| 3.13        | 6.25|
| 4e    | 6.25      | 12.5| 6.25              | 12.5| 6.25        | 12.5|
| 1f    | 0.039     | 0.039| 0.039            | 0.039| 0.039     | 0.039|
| 2f    | 0.156     | 0.312| 0.078            | 0.156| 10         | 20  |
| 3f    | 50        | 100 | 50                | 100 | 50          | 100 |
| 4f    | 50        | 100 | 50                | 100 | 50          | 100 |
| 1g    | 0.156     | 0.313| 0.313            | 0.78| 0.156       | 0.313|
| 2g    | 12.5      | 50  | 25                | 50  | 12.5        | 50  |
| 3g    | >200      | >200| >200              | >200| >200        | >200|
| 4g    | >200      | >200| >200              | >200| >200        | >200|

Table 1 In vitro antimicrobial data as MIC ($\mu$g/mL) and MBC ($\mu$g/mL) for compounds 1a-g, 2a-g, 3a-g and 4a-g.

*No inhibition

 Springer
bacterial type IIA topoisomerases, DNA gyrase and topoisomerase IV, were therapeutic targets for quinolone antibiotics [3], which were very potent against both Gram-negative and Gram-positive bacteria [26]. Study on interaction between type IIA topoisomerases and their inhibitors were crucial for novel quinolones design. To rationalize the observed antibacterial activity of the compounds 1a-4g, a flexible ligand docking study was conducted. The crystal structure data of DNA gyrase [PDB ID: 2XCT] [26] and topoisomerase IV-DNA [PDB ID: 4KPF] [35] were obtained from a protein database, and compound 4c interaction with 2XCT and 4KPF was outlined as an example. Table 3 listed the molecular docking results.

The data of Table 3 docking score and glide energy [36] revealed that most synthesized quinolones in this paper exhibited high binding affinity towards the two proteins, 2XCT and 4KPF, lower binding energy than the co-crystallized neutral compounds.

### Table 2 The ADMET properties of compound 1a-4g

| Comp. | Drug Likeness | ADME | Toxicity |
|-------|---------------|------|----------|
|       | MW<sup>a</sup> | clogP<sup>c</sup> | HBD<sup>b</sup> | HBA<sup>b</sup> | RBN<sup>b</sup> | ASA<sup>b</sup> | ASA<sub>P</sub><sup>b</sup> | Rule of Five<sup>b</sup> | CYP2D6<sup>c</sup> | PPB<sup>c</sup> | Carcino_M<sup>c</sup> | hERG<sup>c</sup> |
| 1a    | 331.13        | −0.73 | 2 | 6 | 3 | 301.52 | 216.83 | 0 | 0 | 31.05 | 0 | 0 |
| 1b    | 334.16        | −0.78 | 2 | 6 | 3 | 298.57 | 193.46 | 0 | 0 | 24.29 | 0 | 0 |
| 1c    | 375.16        | −0.27 | 2 | 7 | 4 | 314.16 | 216.06 | 0 | 0 | 32.76 | 0 | 0 |
| 1d    | 320.13        | −1.60 | 2 | 7 | 3 | 301.23 | 209.59 | 0 | 0 | 23.33 | 0 | 0 |
| 1e    | 389.18        | −0.36 | 2 | 7 | 5 | 326.11 | 227.66 | 0 | 1 | 39.22 | 0 | 0 |
| 1f    | 401.18        | −0.08 | 2 | 7 | 4 | 337.22 | 221.10 | 0 | 0 | 42.99 | 0 | 0 |
| 1g    | 318.10        | −1.19 | 2 | 6 | 2 | 284.15 | 220.20 | 0 | 1 | 19.23 | 0 | 0 |
| 2a    | 407.10        | 1.75  | 1 | 7 | 5 | 346.07 | 293.87 | 0 | 0 | 78.39 | 1 | 0 |
| 2b    | 395.10        | 1.70  | 1 | 7 | 5 | 343.12 | 270.21 | 0 | 0 | 71.64 | 1 | 0 |
| 2c    | 451.13        | 2.21  | 1 | 8 | 6 | 350.15 | 291.09 | 0 | 0 | 75.47 | 1 | 0 |
| 2d    | 396.10        | 0.88  | 1 | 8 | 5 | 345.78 | 289.08 | 0 | 0 | 66.46 | 1 | 0 |
| 2e    | 465.15        | 2.48  | 1 | 8 | 7 | 358.63 | 304.80 | 0 | 0 | 80.17 | 1 | 0 |
| 2f    | 477.15        | 2.30  | 1 | 8 | 6 | 362.62 | 289.57 | 0 | 0 | 81.65 | 1 | 0 |
| 2g    | 394.07        | 1.55  | 2 | 7 | 5 | 304.57 | 274.88 | 0 | 0 | 68.93 | 0 | 0 |
| 3a    | 885.50        | 2.06  | 1 | 17 | 18 | 611.86 | 280.21 | 3 | 0 | 25.67 | 0 | 0 |
| 3b    | 873.50        | 2.01  | 1 | 17 | 18 | 612.15 | 245.65 | 3 | 0 | 24.57 | 0 | 0 |
| 4a    | 717.31        | −5.51 | 4 | 17 | 12 | 512.70 | 521.57 | 3 | 0 | 11.36 | 0 | 0 |
| 4b    | 705.31        | −5.50 | 4 | 17 | 12 | 510.50 | 476.65 | 3 | 0 | 11.14 | 0 | 0 |
| 4c    | 761.34        | −4.99 | 4 | 18 | 13 | 515.44 | 515.30 | 3 | 0 | 11.42 | 0 | 0 |
| 4d    | 706.30        | −6.32 | 4 | 18 | 12 | 513.16 | 515.53 | 3 | 0 | 11.01 | 0 | 0 |
| 4e    | 775.36        | −4.85 | 4 | 18 | 14 | 515.77 | 489.39 | 3 | 0 | 11.90 | 0 | 0 |
| 4f    | 787.36        | −4.90 | 4 | 18 | 13 | 520.94 | 478.92 | 3 | 0 | 11.63 | 0 | 0 |

<sup>a</sup>MW (molecular weight) and clogP<sup>c</sup> values were calculated by Chem Draw Ultra 14.0

<sup>b</sup>HBD (number of hydrogen bond donors), HBA (number of hydrogen bond acceptors), RBN (number of rotatable bonds), ASA (water accessible surface area) and ASA<sub>P</sub> (total polar surface area), Lipinski’s rule of five are: MW < 500, clogP < 5, HBD ≤ 5, HBA ≤ 10, RBN ≤ 10. Compounds that satisfy these rules are considered druglike. Here the prediction was conducted using MOE 2008.10 [29, 30]. 0, 3 indicated zero or three violation items of Lipinski’s rule of five.

<sup>c</sup>CYP2D6 (CYP2D6 inhibition) Cytochrome P450 2D6 inhibition in vitro. 0 = non inhibitor, 1 = inhibitor. PPB (plasma protein binding) plasma protein binding (%) in vitro., Carcino_M (Carcinogenicity Mouse) Carcinogenicity bioassay in mouse. 0 = negative, 1 = positive and hERG (hERG inhibition) human ether-a-go-go-related gene channel inhibition in vitro. 0 = low risk, 1 = medium risk, 2 = high risk. were calculated via Pre-ADMET ver 2.0 (Yonsei University, Seoul, Korea, https://preadmet.bmdrc.kr) [31].
ligands revealed a more stable complex than that of the co-crystallized ligand. And these energy trends did not match well with the antibacterial results in Table 1. The ensemble binding energy was dependent upon the total interaction area, the larger total interaction area caused lower energy and resulted in strong affinity. However, the energy contribution for each group was unequal, the rationalized design should outline each moiety contribution, i.e., each group was necessary other than auxiliary. Based upon this consideration, the ligand efficiency (LE) [37] for each group in the quinolones 1a−4g was evaluated again, and LE ran in the order of 1a−1g < 2a−2g < 3a−3g = 4a−4g, which was roughly consistent with antibacterial results in Table 1.

As an example of the good docking poses, compound 4c in the active site of DNA gyrase and topoisomerase IV were shown (in Fig. 4). Although compounds 1a−g, 2a−g had more potent activity than 4c, the mother compounds like 1a−g were the clinical drugs, the reaction intermediate like 2a−g was reported before [38], in this paper we tried to investigate the DOTA modification effect on the fluorquinolone activity, and 4c was the most potent one among DOTA-fluorquinolone series, so a rational explanation based upon the 3D and 2D docking model was given to 4c. Compound 4c was well accommodated inside the active site of DNA gyrase (A, B) in the predicted model. The DOTA ring bearing carboxyl moiety formed four hydrogen bonds with amino acid residues ARG-1033 (1.91 Å, 2.07 Å), LYS-1043 (1.83 Å) and SER-1085 (2.11 Å). Moreover, the quinolone parent ring bearing carboxyligic group was closely linked to the adjacent residues ALA-439 (2.04 Å) and GLY-584 (1.88 Å) via another two hydrogen bonds. In the predicted model of topoisomerase IV (C, D), compound 4c was also well accommodated inside the active site, the quinolone parent intercalated into the hydrophobic pocket of the receptor with a hydrogen bond between the oxygen atom of carboxyl moiety and SER-79 (1.72 Å). The DOTA ring bearing carboxyligic group was closely linked to the adjacent residues LYS-415 (1.67 Å) via another hydrogen bond. Overall, the 3D and 2D docking models provided a rational explanation for why compound 4c had a strong inhibitory efficacy against bacteria.

**Damaged cell wall monitored by atomic force microscope (AFM)**

AFM images of strains could reveal some detailed information. The representative results are shown (in Fig. 5). Under normal condition without any treatment, MRSA (in Fig. 5) (a) showed a normal ball-shaped structure, the shape and size were similar to that reported in previous studies [39, 40]. However, MRSA ruptured into fragments upon being treated with compounds 1c and 4c (in Fig. 5) (b–f). Treated by 10 μmol/L gatifloxacin (1c), MRSA showed obvious cracks on the surface (in Fig. 5) (b), although its ball shape was similar to that of untreated in general, some of them was broken into irregular fragments. These morphologic changes correlated tightly with their death and division ability. While treated by 10 μmol/L gatifloxacin-DOTA (4c), the spherical shape of MRSA strain was completely broken into fragments and clustered together, with apparent leakage of the cell contents (in Fig. 5) (d–f). The possible interpretation was that the damage of 4c to the strains broke the balance between the inner and outer osmotic pressures of the cell, resulting in the loss of inner contents and consequently cell death. Generally, the AFM images of the size, shape and leakage with the cell contents
of the bacteria agreed well with the report in the previous study [41, 42]. The morphological imaging result demonstrated the potent antibacterial activity of 4c.

**Cytotoxicity assay in vitro with L-02 cell, A549 cell, and MCF-7 cell**

Compound 4c showed potent antibacterial activity, so its cytotoxicity towards different cell lines was studied (shown in Fig. 6). For clarity the mother compound 1c as control was studied. Both 4c and 1c did not show significant toxicity to normal hepatocytes L-02 cell (A), A549 cell (B) and MCF-7 cell (C), except a limited difference in more than 0.25 μmol/L. In L-02 cell and MCF-7 cell 4c had low toxicity than the reference antibiotic 1c [43], more than 80% cell viability was obtained even at 100 μmol/L, which was consistent with the toxicity of ADMET prediction results in Table 2. The antibacterial MIC and MBC value of 4c were (1.56, 6.25) μg/mL for MRSA and (3.13, 12.5) μg/mL for P. aeruginosa respectively, at 2–16 μmol/L level, much less than 100 μmol/L, so 4c was safe and promising as an antibacterial agent.

**Conclusion**

In summary, a series of novel water-soluble quinolone antimicrobial based upon DOTA modification were synthesized and characterized, with their bacteriostatic and bactericidal activities evaluated. Based upon Pre-ADMET ver 2.0 computation, most of the compounds obtained were predicted no toxicity to mice, slightly toxicity to the heart. Molecular docking studies indicated its strong binding affinity towards DNA gyrase and topoisomerase IV, correlated with its antibacterial mechanism. Among them, a novel water-soluble quinolone compound 4c with good antibacterial activity was discovered, the value of MIC and MBC was (1.56, 6.25) μg/mL against MRSA, (3.13, 12.5) μg/mL against P. aeruginosa. It had low cellular toxicity, over 80% cellular availability even at over 100 μM. AFM revealed that 4c could destroy bacterial walls and membranes. Overall, these results suggested that DOTA modification actually could improve the water-soluble of quinolone structure, although at cost of limited antibacterial activity. Compound 4c exhibited promising characteristics worth of studying further as a novel antibacterial agent.
Experimental section

Chemistry

High resolution mass spectra (HRMS) were recorded on Agilent 6520 Q-TOF LC/MS or Varian 7.0 T FTMS (MALDI). $^1$H NMR spectra and $^{13}$C NMR were acquired on a Mercury Vx-300 (300 MHz) or Bruker AVANCE III (400 MHz) and referenced to tetramethylsilane (TMS). The residual solvent line was used as an internal standard and the chemical shift was reported in ppm ($\delta$). Compounds were purified by silica gel column (200–300 mesh). The reaction process was detected by thin-layer chromatography (TLC) on silica gel GF254 plates and...
the spots were observed with iodine or UV lamps ($\lambda = 254\text{ nm}$, 365 nm). The purity of the compounds was tested over 95.00% by Waters e2695 HPLC, with Kromasil 100-5-C18 column 4.6 × 250 mm, eluted by A/B = 32:68 (A, acetonitrile: methanol = 70:30; B, water: methanol = 95:5) containing 0.1% TFA at a flow rate of 1 mL/min.

**Synthesis of tri-tert-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (2)**

10.0 g (1 eq, 58.05 mmol) cyclen (1,4,7,10-tetraazacyclododecane) and 14.3 g (3 eq, 174.14 mmol sodium acetate were dissolved in 134 mL DMA, stirred in an ice water bath at 0 °C for 30 minutes, then 26 mL (3 eq, 174.14 mmol) tert-butyl bromoacetate in 50 mL DMA was added dropwise in 30 minutes. The slurry was stirred at room temperature for 5 days. 10.0 g (1 eq, 58.05 mmol) potassium bicarbonate in 660 mL of deionized water was added. After reaction completion detected by TLC, 30 mL of methanol was added. After the reaction was cooled to 0 °C, the mixture was washed with deionized water and dried overnight in the vacuum chamber, 0.27 g of the target compound 2a was obtained, white solid, yield 73%.

Compound 2b-g was synthesized in the same way as compound 2a in yields of 70–88%.

**7-(4-(2-Chloroacetyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2b)**

Compound 2b, 0.29 g was obtained, white solid, yield 83%, 1H NMR (300 MHz, CDCl3) δ 14.92 (s, 1H, -COOH), 8.70 (s, 1H, -C2H), 8.13 (d, J = 11.1 Hz, 1H, -C3H), 6.85 (d, J = 7.5 Hz, 1H, -C4H), 4.36 - 4.33 (m, 2H, -N-CH2), 4.14 (s, 2H, -CH2Cl), 3.91 - 3.88 (m, 2H, -piperazine-H), 3.82 - 3.78 (m, 2H, 2'-piperazine-H), 3.73 - 3.37 (m, 2H, 3'-piperazine-H), 3.34 - 3.30 (m, 2H, 3', -piperazine-H), 1.28 - 1.27(m, 3H, - NCH2CH2). HRMS (ESI) m/z 415.1173 [M + H]+, calcd. for [C19H20ClF3N3O4]+, 415.1162.

**7-(4-(2-Chloroacetyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-4-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2c)**

Compound 2c, 0.30 g was obtained, white solid, yield 71%, 1H NMR (300 MHz, CDCl3), δ 14.67 (s, 1H, -COOH), 8.81 (s, 1H, -C2H), 7.88 (d, J = 11.9 Hz, 1H, -C4H), 4.13 (s, 2H, -CH2Cl), 4.06 - 3.99 (m, 1H, -N-CH2), 3.75 (s, 3H, -OCH3), 3.74 - 3.71 (m, 2H, 2', -piperazine-H), 3.53 - 3.48 (m, 2H, 2'-piperazine-H), 3.39 - 3.35 (m, 2H, 2', -piperazine-H), 3.31 - 3.27 (m, 1H, 3', -piperazine-H), 1.46 - 1.40 (m, 2H, -NCH2CH2), 1.26 - 1.20 (m, 2H, -NCH2CH2), 1.08 - 0.96 (m, 3H, 3', -piperazine-CH2). HRMS (ESI) m/z 452.1380 [M + H]+, calcd. for [C21H24ClFN3O5]+, 452.1389.

**7-(4-(2-Chloroacetyl)-3-methylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2d)**

Compound 2d, 0.32 g was obtained, white solid, yield 81%, 1H NMR (300 MHz, CDCl3), δ 14.83 (s, 1H, -COOH), 8.69 (s, 1H, 1H), 8.15 (d, J = 12.9 Hz, 1H), 4.44 - 4.36 (m, 2H), 4.11 (s, 2H), 3.93 - 3.85 (m, 4H), 3.83 - 3.73 (m, 4H), 1.53 - 1.48 (t, J = 7.1 Hz, 3H). HRMS (ESI) m/z 397.1072 [M + H]+, calcd. for [C17H19ClFN3O4]+, 397.1079.

**7-(3-(2-Chloro-N-methylacetamido)piperidin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2e)**

Compound 2e, 0.41 g was obtained, white solid, yield 88%, 1H NMR (300 MHz, CDCl3), δ 14.76 (s, 1H), 8.79 (dd, J = 3 Hz, 1H), 7.86 - 7.80 (m, 1H), 4.69 - 4.62 (m, 1H), 4.07 - 4.02 (m, 1H), 3.85 (s, 2H), 3.50 - 3.47 (br, 3H), 3.63n - 3.26 (m, 1H), 3.23 - 3.20 (m, 1H), 3.05 (s, 3H), 2.07 - 1.74 (m, 6H), 1.27 - 1.21 (m, 2H), 1.06 - 0.97 (m, 2H). HRMS (ESI)
Compound 3a, 0.29 g was obtained, light yellow oil, yield 81%. ¹H NMR (300 MHz, CDCl₃), δ 8.56 (s, 1H, -C₈H), 7.77 (d, J = 13.1 Hz, 1H), 7.40 (br, 1H), 4.97 (s, 1H), 3.72 (br, 4H), 3.39 (br, 8H), 3.29 (d, J = 7.5 Hz, 4H), 3.06 - 2.75 (m, 16H), 1.40 (s, 27H), 1.30 (s, 1H), 1.0 (s, 1H), 0.99 (s, 2H). HRMS (ESI) m/z 886.5084 [M + H]⁺, calcd. for [C₄₅H₇₀F₅N₇O₁₀]⁺, 886.5090.

1-Ethyl-6-fluoro-4-oxo-7-(4-(2-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetetyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (3b)

Compound 3b, 0.29 g was obtained, light yellow oil, yield 88%. ¹H NMR (300 MHz, CDCl₃), δ 8.48 (s, 1H, -C₂H), 7.76 (d, J = 12.8 Hz, 1H, -C₅H), 6.97 (s, 1H, -C₅H), 4.44 - 4.35 (m, 2H, -NCH₂), 3.65 (br, 8H), 3.36 - 3.22 (m, 8H), 2.91 - 2.72 (m, 16H), 1.35 (s, 30H, -CH₃). HRMS (ESI) m/z 896.4912 [M + Na]⁺, calcd. for [C₄₄H₇₀F₅N₇NaO₁₁]⁺, 896.4909.

1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(2-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetetyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3c)

Compound 3c, 0.27 g was obtained, light yellow oil, yield 88%. ¹H NMR (300 MHz, CDCl₃), δ 8.60 (s, 1H, -C₂H), 7.69 (d, J = 12.3 Hz, 1H, -C₅H), 4.37 - 4.24 (m, 1H, -NCH₂), 4.17 - 4.07 (m, 1H, -piperazine-H), 3.85 (s, 3H, -OCH₃), 3.68 - 3.56 (m, 6H, -C₅H), 3.2 - 3.0 (m, 8H, -NCH₂CH₂N-), 2.98 - 2.70 (m, 8H, -NCH₂CH₂N-), 1.40 (s, 27H, -CH₃), 1.28 - 1.26 (m, 2H, -NCH₂), 1.06 - 0.84 (m, 2H, -CH₂), 0.82 (s, 3H, -CH₃). HRMS (ESI) m/z 952.5165 [M + Na]⁺, calcd. for [C₄₇H₇₂F₅N₇NaO₁₁]⁺, 952.5157.

1-Ethyl-6-fluoro-4-oxo-7-(4-(2-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetetyl)piperazin-1-yl)-4,1-dihydro-1,8-naphthyridine-3-carboxylic acid (3d)

Compound 3d, 0.30 g was obtained, light yellow oil, yield 90%. ¹H NMR (300 MHz, CDCl₃), δ 15.01 (s, 1H, -C₂H), 8.68 (s, 1H, -C₂H), 8.08 (d, J = 13.1 Hz, 1H), 4.49 - 4.42 (m, 2H, -piperazine-H), 4.03 - 3.77 (m, 8H, -C₂H), 3.71 (s, 2H), 3.59 (br, 4H), 3.38 (s, 2H), 3.12 - 3.10 (m, 4H), 2.96 - 2.88 (m, 8H), 2.54 (br, 4H), 1.46 (s, 18H), 1.25 (s, 9H), 0.87 (t, J = 6.7 Hz, 3H). HRMS (ESI) m/z 875.5054 [M + H]⁺, calcd. for [C₄₇H₇₂F₅N₇NaO₁₁]⁺, 875.5044.

1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-(N-methyl-2-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetetyl)piperizin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3e)

Compound 3e, 0.26 g was obtained, light yellow oil, yield 86%. ¹H NMR (300 MHz, CDCl₃), δ 14.76 (s, 1H, -C₂H), 8.72 (t, J = 2.8 Hz, 1H), 7.77 - 7.69 (m, 1H), 5.25 (br, 1H), 4.65 - 4.54 (br, 1H), 4.06 - 3.99 (m, 1H), 3.76 - 3.75 (m, 1H), 3.72 (s, 2H), 3.69 - 3.66 (m, 1H), 3.58 (br, 1H), 3.46 - 3.42 (br, 2H), 3.33 (s, 3H), 3.23 (s, 2H), 3.10 (br, 3H), 3.03 (s, 2H), 2.97 (s, 3H), 2.94 (s, 3H), 2.91 - 2.72 (m, 16H), 1.40 (s, 27H), 1.30 (s, 1H), 1.20 (s, 1H), 0.99 (s, 2H).
2.92 - 2.91 (br, 2H), 2.88 (s, 2H), 2.19 (br, 8H), 1.84 (br, 8H), 1.42 - 1.40 (m, 27H), 1.24 - 1.23 (m, 2H), 1.19 (br, 2H). HRMS (ESI) m/z 944.5515 [M + H]^+ calcd. for [C₄₈H₇₅FN₇O₁₁]^+. 944.5509.

1-Cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(2-(4-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetyl)octahydro-6H-pyrrrolo[3,4-b]pyridin-6-yl)-1,4-dihydroquinoline-3-carboxylic acid (3f)

Compound 3f, 0.30 g was obtained, light yellow oil, yield 90%. ¹H NMR (300 MHz, CDCl₃), δ 8.74 (d, J = 4.2 Hz, 1H), 7.77 - 7.72 (m, 1H), 5.18 - 5.10 (m, 1H), 4.29 - 4.22 (m, 1H), 4.00 (br, 3H), 3.69 (s, 2H), 3.58 (s, 2H), 3.55 (s, 2H), 3.35 (s, 2H), 3.28 (br, 2H), 3.12 - 3.09 (m, 2H), 3.09 - 3.00 (m, 2H), 2.44 - 2.00 (m, 16H), 2.71 - 1.70 (m, 1H), 1.85 - 1.81 (m, 4H), 1.45 - 1.43 (m, 27H), 1.13 - 1.06 (m, 2H), 0.83 - 0.77 (m, 2H). HRMS (ESI) m/z 956.5515 [M + H]^+ calcd. for [C₄₈H₇₅FN₇O₁₁]^+. 956.5509.

9-Fluoro-3-methyl-7-oxo-10-(1-(2-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetamido)cyclopropyl)-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid (3g)

Compound 3g, 0.27 g was obtained, light yellow oil, yield 82%. ¹H NMR (300 MHz, CDCl₃), δ 14.86 (s, 1H), 9.67 (s, 1H), 8.64 (d, J = 3.6 Hz, 1H), 7.60 (d, J = 10.1 Hz, 1H), 4.89 - 4.83 (m, 1H), 4.56 - 4.52 (m, 1H), 3.48 - 3.45 (m, 1H), 3.38 (s, 2H), 3.36 (s, 2H), 3.31 (s, 2H), 3.28 (s, 2H), 2.87 (br, 8H), 2.77 - 2.69 (m, 8H), 1.45 - 1.43 (m, 27H), 1.32 (br, 1H), 1.24 (br, 3H), 1.08 (br, 2H), 0.89 - 0.85 (m, 1H). HRMS (ESI) m/z 873.4769[M + H]^+ calcd. for [C₄₈H₆₆FN₅O₁₁]^+. 873.4774.

Synthesis of compound 4a-g

0.2 g (1 eq, 0.23 mmol) 3a was dissolved in 10 mL of TFA and stirred for about 40~48 h to remove the tert-butyl groups on the carboxylic acids in DO3A. Then the excess TFA was removed under vacuum. The 0.2 g final deprotected product 4a was precipitated with cold ether, filtered and dried under vacuum, in the yield of 80%.

Compound 4b-g was synthesized in the same way as compound 4a, in the yields of 70~90%.

2,2',2''-(10-(2-(4-(3-Carboxy-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinolin-7-yl)piperazin-1-yl)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1,4,7-triyl)triacetic acid (4a)

Compound 4a, 0.13 g was obtained, powdery light yellow solid, yield 80%. ¹H NMR (300 MHz, CD₃OD), δ 8.73 (s, 1H), 7.86 (d, J = 13.1 Hz, 1H), 7.54 (br, 1H), 4.16 (s, 1H), 3.78 - 3.65 (br, 16H), 3.46 - 3.31 (br, 16H), 1.38 (br, 2H), 1.20 (br, 2H). HRMS (ESI) m/z 756.2663 [M + K]^+ calcd. for [C₃₂H₄₃FKN₃O₁₀]^+. 756.2771.

Compound 4b, 0.12 g was obtained, powdery light yellow solid, yield 75%. ¹H NMR (300 MHz, CD₃OD), δ 8.79 (s, 1H), 7.92 (brs, 1H), 7.14 (br, 1H), 4.16 (br, 2H), 3.76 (br, 16H), 3.31 (br, 16H), 1.52 (br, 3H). HRMS (ESI) m/z 744.2667 [M + K]^+ calcd. for [C₃₂H₄₃FKN₃O₁₀]^+. 744.2771.

Compound 4c, 0.13 g was obtained, powdery light yellow solid, yield 79%. ¹H NMR (400 MHz, DMSO) δ 14.87 (s, 1H), 8.69 (s, 1H), 7.72 (d, J = 11.9 Hz, 1H), 4.62 - 4.60 (m, 1H), 4.16 (brs, 3H), 4.07 (br, 2H), 3.73 (br, 4H), 3.54 (br, 1H), 3.45 (br, 8H), 3.37 - 3.34 (m, 4H), 3.17 (s, 10H), 3.03 (s, 2H), 1.30 - 1.28 (m, 2H), 1.13 - 1.12 (m, 2H), 1.02 (brs, 3H). ¹³C NMR (101 MHz, DMSO) δ 176.38, 176.36, 172.87, 165.70, 158.57, 158.25, 150.70, 146.23, 139.70, 139.59, 134.23, 121.37, 118.38, 115.41, 106.77, 64.96, 63.72, 54.75, 54.71, 54.51, 54.05, 53.09, 52.94, 51.65, 50.95, 50.83, 50.49, 50.30, 50.15, 45.49, 37.18, 15.90, 15.19, 9.10, 9.03. HRMS (ESI) m/z 800.2921 [M + K]^+ calcd. for [C₃₂H₄₃FKN₃O₁₀]^+. 800.3033.

2,2',2''-(10-(2-(4-(6,8-dihydro-1,8-naphthyridin-2-yl)piperazin-1-yl)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1,4,7-triyl)triacetic acid (4d)

Compound 4d, 0.13 g was obtained, powdery light yellow solid, yield 81%. ¹H NMR (300 MHz, CD₃OD), δ 8.82 (s, 1H), 8.03 (d, J = 11.1 Hz, 1H), 4.49 (br, 2H), 4.12 (br, 4H), 3.92 (br, 4H), 3.73 (br, 8H), 3.48 - 3.41 (m, 8H), 3.25 - 3.14 (m, 8H), 1.46 (br, 3H). HRMS (ESI) m/z 745.2623 [M + K]^+ calcd. for [C₃₂H₄₃FKN₃O₁₀]^+. 745.2723.

2,2',2''-(10-(2-(1-(3-Carboxy-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinolin-7-yl)piperidin-3-yl)(methyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1,4,7-triyl)triacetic acid (4e)

Compound 4e, 0.12 g was obtained, powdery light yellow solid, yield 73%. ¹H NMR (300 MHz, CD₃OD),
δ 8.76 (d, J = 2.0 Hz, 1H), 7.71 - 7.63 (m, 1H), 4.50 (br, 1H), 4.14 (s, 3H), 4.02 - 3.99 (m, 1H), 3.81 (s, 2H), 3.75 (s, 2H), 3.71 (s, 2H), 3.61 - 3.32 (m, 16H), 3.21 (br, 4H), 3.17 - 3.10 (m, 1H), 3.02 - 2.95 (m, 1H), 3.01 (br, 2H), 2.93 (s, 3H), 1.84 (br, 4H), 1.26 - 1.25 (m, 1H), 1.19 - 1.15 (m, 2H), 1.13 - 1.10 (m, 1H), 1.02 - 0.99 (m, 2H). HRMS (ESI) m/z 814.3093 [M + K]⁺, calcd. for [C₃₋H₅₋FKN₇O₁₁]⁺, 814.3189.

2,2’2”-(10-(2-(6-(3-Carboxy-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinolin-7-yl)octahydro-1H-pyrrolo[3,4-b]pyridin-1-yl)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triy]triacetic acid (4f)

Compound 4f, 0.14 g was obtained, powdery light yellow solid, yield 85%. 1H NMR (300 MHz, CD3OD), δ 8.80 (s, 1H), 7.69 - 7.64 (m, 1H), 4.56 - 4.51 (m, 1H), 4.15 (br, 3H), 3.97 - 3.90 (m, 8H), 3.65 (br, 4H), 3.52 - 3.45 (m, 8H), 3.38 - 3.35 (m, 2H), 3.08 (br, 4H), 2.87 (br, 1H), 2.35 (br, 1H), 1.88 (br, 4H), 1.64 - 1.59 (m, 2H), 1.34 - 1.30 (m, 2H), 1.21 - 1.16 (m, 2H), 1.10 - 1.06 (m, 1H), 0.93 - 0.87 (m, 1H). HRMS (ESI) m/z 705.2896 [M + K]⁺, calcd. for [C₃₂H₄₁FKN₆O₁₁]⁺, 705.2896.

2,2’2”-(10-(2-(1-(6-Carboxy-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinolin-10-yl)cylopropyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-trial [triacetic acid (4g)

Compound 4g, 0.13 g was obtained, powdery light yellow solid, yield 81%. 1H NMR (300 MHz, CD3OD), δ 8.79 (s, 1H), 7.91 (s, 1H), 7.53 (d, J = 10 Hz, 1H), 4.75 (br, 1H), 4.56 (br, 1H), 4.47 (br, 1H), 3.49 - 3.40 (m, 8H), 3.32 - 3.17 (m, 8H), 3.21 - 3.15 (m, 8H), 1.33 (br, 2H), 1.26 (s, 3H), 1.17 - 1.10 (m, 2H). HRMS (ESI) m/z 705.2880 [M + H]⁺, calcd. for [C₃₋H₅₋FKN₇O₁₁]⁺, 705.2880. HRMS (ESI) m/z 743.2354 [M + H]⁺, calcd. for [C₃₋H₅₋FKN₇O₁₁]⁺, 743.2354.

Biological activity

MIC and MBC determinations in vitro

MRSA (clinical isolates), P. aeruginosa (clinical isolates) and E. coli (clinical isolates) were used in this experiment, which was provided by the People’s Liberation Army 304 Hospital. The antimicrobial activities of these newly synthesized compounds were evaluated in vitro against MRSA, P. aeruginosa and E. coli, which are the representative of Gram-positive bacteria strains and Gram-negative bacterial strains. The standard two folds serial dilution method in 96-well micro-test plates was employed according to the National Committee for Clinical Laboratory Standards (NCCLS) [44].

MRSA interaction with compound 4c, as an example of screening method, was described as following in detail. The experiments were performed in 96-well flat bottom plates in a sterile workbench. Firstly, a rough screening outlined an antibacterial concentration range, then a series of 4c solutions in different concentrations were prepared by two folds serial dilution method respectively. The final concentration of the MRSA, logarithmic growth phase bacteria, was diluted to 10⁶ colony-forming units/mL (CFU/mL). Then, 180 μL of 4c solution in the above serial different concentrations and 20 μL of MRSA were added to each well and mixed, respectively. Three sets of independent experiments were performed. Finally, the samples of plates were incubated in the dark at 37 °C and CFU was evaluated after 24 h. The turbidity degree of the bacterial solution was eye observed. The minimum inhibitory concentration (MIC) is defined as the first drug concentration with reduced turbidity, clear and transparent solution. Then, 100 μL of the different gradient concentration drug and bacteria mixed solution in the 96-well micro-test plate were separately aspirated, and the mixture was uniformly coated on LB solid medium, and further incubated at 37 °C in the dark, and evaluated after 24 h. The number of colonies less than or equal to 5 is used as the minimum bactericidal concentration (MBC).

The ADMET prediction

The ADMET (absorption, distribution, metabolism, excretion and toxicity) properties of the synthesized compounds were calculated using in-silico tools and web-based applications. Hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), number of rotatable bonds (RBN), water accessible surface area (ASA), rule of five and total polar surface area (ASA_P) were calculated by MOE ver 2008.10 (Chemical Computing Group, Montreal, Canada). While, CPY2D6 inhibition (CYP2D6), Plasma protein binding (PPB), Carcino-nogenicity Mouse (Carcino_M) and hERG inhibition (hERG) were calculated via PreADMET ver 2.0 (Yonsei University, Seoul, Korea, https://preadmet.bmdrc.kr).

Docking study

A molecular docking study was performed using Glide modules of Schrödinger suite 2009 (Schrödinger LLC, NewYork) [45]. The X-ray structure of DNA gyrase complex with ciprofloxacin (PDB ID: 2XCT) and DNA topoisomerase IV with a novel fluoroquinolone (PDB ID: 4KPF) were retrieved from the protein data bank (PDB). Before docking, initial proteins were refined using protein preparation wizard to assign bond orders, add hydrogen atoms, treat metal ions, predict side chains and remove all water molecules [46, 47]. Subsequently, the proteins were

© Springer
optimized by reorienting the hydrogen bond network and energy minimized using OPLS2005 force field [48]. Receptor grid was generated around the co-crystallized ligands with the center coordinates 2XCT (x = 2.6951, y = 44.4391, z = 68.1055) and 4KPF (x = -40.3970, y = 78.2317, z = -10.9810), respectively. The size of the grid box was set to 20 Å and Vander Waals radius of receptor atoms was scaled to 1.00 with partial atomic charge less than 0.25. All the synthesized quinolones were constructed using the Maestro workspace build panel and prepared using the Ligprep application to generate variations on the ionization states, tautomers, stereochemistry and ring conformations. Then the molecular geometry of the structures was energy minimized using the OPLS2005 force field. With the default parameters, docking calculations were performed using Standard Precision (SP) mode [49]. Docking score, glide energy and glide ligand efficiency were together used to determine the best binding pose for each ligand. The Pose Viewer panel [50] was applied for visualization and distance measurement of the H-bond contacts between the ligands and active site resides of model proteins.

Atomic force microscope assay of sample preparation

The prepared bacterial suspension was diluted to 10^7 CFU/mL with LB liquid medium and was mixed with the drug solution in an equal volume so that the concentration of 1c and 4c was 10 μmol/L respectively. The mixing solution was incubated at 37 °C for 30 min. The blank control group was also established at the same time. After that, the sample was centrifuged at 9000 RPM for 5 min and the supernatant was discarded. The precipitate was washed twice with physiological saline and centrifuged at 9000 RPM for 5 min. Finally, the bacterial samples were dropped onto the surface of the mica plate (about 0.5 cm²), dried naturally at ambient temperature, then scanned by an atomic force microscope (Veeco Multi Mode 8/ B0021, Veeco German).

Cytotoxicity assay on the L-02, A549 and MCF-7 cells

According to reports in the literature [51, 52], it is very meaningful to study compound 4c for the toxicity of human normal liver cells (L-02) and tumor cells (A549 and MCF-7). Here, L-02 cell was used as an example of the cytotoxicity assay in vitro [53]. L-02 cells in the log phase at the density 5 x 10^3 cells/well were collected and cultured in complete DMEM at 37 °C under 5% CO2 incubator for 24 h. After different concentrations of 1c and 4c were added to each well, L-02 cells were cultured for 24 h at 37 °C under 5% CO2. Then the medium was poured off before 100 μL of serum-free medium and 20 μL of MTS were added to each well, and the mixture was incubated for 1 h at 37 °C. Finally, the cell viability was measured by a microplate reader (Thermo, Varioskan Flash 3001, USA) at a wavelength of 490 nm, and calculated cell survival rate according to the following formula:

\[
\text{Cell survival rate(%) = } (\frac{\text{OD}_{\text{test}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}}) \times 100\%
\]

The difference between each group was compared with one-way variance analysis and comparison of cell absorbance values was used in repeated measurement analysis of variance. p < 0.05 indicated that the difference was statistically significant.

Acknowledgements The authors gratefully acknowledge Chinese Academy of Medical Sciences & Peking Union Medical College information technology center for affording the Schrödinger suite 2009 and MOE ver 2008.10 software. This study was supported by the national Mega-project for Innovative Drugs (2019ZX09721001-006-001), the CAMS Innovation Fund for Medical Sciences (2019-12M-1-005/2021-12M-1-052) and the Tianjin Key Technology R&D Program (20YFZCSY00570).

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Feng LS, Liu ML, Wang S, Chai Y, Lv K, Shan GZ, et al. Synthesis of naphthyridine derivatives containing 8-alkoxyiminoo-1,6-dizaspiro[3.4]octane scaffolds. Tetrahedron 2011;67:8264–70.
2. Andriole VT. The quinolones: Past, present, and future. Clin Infect Dis. 2005;41:S113.
3. Mitscher LA. Bacterial topoisomerase inhibitors: Quinolone and pyridone antibacterial agents. Chem Rev. 2005;105:559–92.
4. Zhang GF, Zhang S, Pan B, Liu X, Feng LS. 4-Quinolone derivatives and their activities against Gram positive pathogens. Eur J Med Chem. 2018;143:710–23.
5. Wan ZL, Yun C, Liu ML, Guo HY, Sun LY. Synthesis and in vitro antibacterial activity of 7-(4-alkoxyiminoo-3-methyl-3-methylaminoepiperidin-1-yl)quinolones. Acta Pharm Sin. 2010;45:860–8.
6. Hu YQ, Zhang S, Xu Z, Lv ZS, Liu ML, Feng LS. 4-Quinolone hybrids and their antibacterial activities. Eur J Med Chem. 2017;13:335–45.
7. Ancieri G, Griffith E, Gruenwald G, Heyd A, O’Brien B, Screen P, et al. A survey of clinical experience with ciprofloxacin, a new quinolone antimicrobial. J Clin Pharm. 2015;28:179–89.
8. Chu DT. Section review anti-infectives: The future role of quinolones. Expert Opin Ther Pat. 1996;6:711–37.
9. Dalhoff A. Antiviral, antifungal, and antiparasitic activities of fluoroquinolones optimized for treatment of bacterial infections: A puzzling paradox or a logical consequence of their mode of action? Eur J Clin Microbiol Infect Dis. 2015;34:661–8.
10. Florindo C, Costa A, Matos C, Nunes SL, Matias AN, Duarte CMM, et al. Novel organic salts based on fluoroquinolone drugs: Synthesis, bioavailability and toxicological profiles. Int J Pharm. 2014;469:179–89.
48. Harder E, Damm W, Maple J, Wu C, Reboul M, Xiang JY, et al. OPLS3: A force field providing broad coverage of drug-like small molecules and proteins. J Chem Theory Comput. 2015;12:281–96.

49. Mangiatordi GF, Trisciuzzi D, Alberga D, Denora N, Iacobazzi RM, Gadaleta D, et al. Novel chemotypes targeting tubulin at the colchicine binding site and unbiasing P-glycoprotein. Eur J Med Chem. 2017;139:792–803.

50. Miliutina M, Ejaz SA, Khan SU, Iaroshenko VO, Villinger A, Iqbal J, et al. Synthesis, alkaline phosphatase inhibition studies and molecular docking of novel derivatives of 4-quinolones. Eur J Med Chem. 2017;126:408–20.

51. Alim I, Yuto K. Anti-methicillin-resistant Staphylococcus aureus (MRSA) activity of MC21-B, an antibacterial compound produced by the marine bacterium Pseudoalteromonas phenolica O-BC30T. Int J Antimicrob Agents. 2009;34:131–5.

52. Wang W, Liao Y, Tang C, Huang X, Luo Z, Chen J, et al. Cytotoxic and antibacterial compounds from the coral-derived fungus Aspergillus tritici SP2-8-1. Mar Drugs. 2017;15:348.

53. Xiao Y, Xue R, You T, Li X, Pei F, Wang X, et al. Gadolinium-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid conjugate of arabinogalactan as a potential liver-targeting magnetic resonance imaging contrast agent. Carbohydr Res. 2014;395:9–14.