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Structural basis of quinolone derivatives, inhibition of type I and II topoisomerases and inquiry into the relevance of bioactivity in odd or even branches with molecular docking study

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The structural modification of quinolone derivatives has been a hot spot in recent years, especially the modification of the N-1 position, which is the part that this article focuses on. In this paper, series of synthesized quinoline quaternary ammonium salts with odd and even carbon number alkyl groups in N-1 position were used to explain the influence of the alkyl side chain on activity. With respect to all the recently synthesized twenty products, the biological activity results exhibited significant antitumor and antibacterial activity with obvious differences in the target alkylidene substituted compounds and the antibacterial activities apparently had the prominent odd-carbon number predominance. Compound 8-((4-(benzylxoy)phenyl)amino)-7-(ethoxy carbonyl)-5-propyl-[1,3]dioxolo[4,5-g]quinolin-5-ium (4d) was found to be the most potent derivative with IC_{50} values of 4 ± 0.88, 4 ± 0.42, 14±1.96, and 32±3.66 against A-549, Hela, SGC-7901, and L-02 cells, respectively, stronger than the positive control 5-FU and MTX. Furthermore, it had the most potent bacterial inhibitory activity of MIC value against E. coli (ATCC 25921) and Staphylococcus aureus (ATCC 8739) at 3.125 nmol mL^{-1}. With respect to molecular simulations, in order to illustrate the possible mechanism of the difference between the series of compounds in the even or odd carbon chain alkylidene substitution, this paper simulated the conceivable mode and explained the main interactions. Finally, we could find that the position and proportion of hydrogen bonds and other interactions in each series were regarded as the main reasons for this difference in activity.

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1. Introduction

As an important antibacterial agent, quinolone has been widely concerned by human beings. As a fully synthetic drug, quinolone drugs have also played a great role in the field of antibacterial and anticancer [1–3]. Since the first synthetic quinolone drug was put into clinical practice in 1962, this family has a history of nearly 60 years, and it has indeed become a veteran role of antibacterial drugs [4–8]. The most primitive discoveries often give people infinite enlightenment, guide future generations to design and synthesize based on this drug and have had very successful cases, but also stimulated experts to explore their molecular level mechanism of action [9–14]. For the design and development of any pharmacy, relevant internal mechanism research is often indispensable [15–25]. As of now, it is well known that the target of quinolone drugs is topoisomerase, and the target of antibacterial action is topoisomerase II. The main mechanism of its anticancer activity has recently been discovered as an inhibitor of topoisomerase I [5,26,27]. DNA topoisomerase plays an important role in the regulation of DNA transcription, replication and gene expression. Studies have found that DNA topoisomerases in cancer cells exhibit high levels of expression independent of other factors. Quinolone derivatives exhibit cytotoxicity by intercalating DNA and thus interfere with the DNA replication process, and have achieved initial success in drug design. For example, some anticancer agents based on topoisomerase I have been launched in the early 21st century. Belotecan hydrochloride, led by Chong KunDang, has shown outstanding advantages for the treatment of non-small cell lung cancer and ovarian cancer. The latter irinotecan hydrochloride developed by Yakult and Pfizer has emerged in the fight against the small cell lung cancer, gastric cancer and solid cancer. Amaridine is another marketed anticancer drug targeted at topoisomerase II, which is used in the treatment of leukemia. [28–31] After the exploration of experts, quinolones exert their biological activities by inhibiting the formation of bacterial DNA topoisomerase. The successful exploration of
structural modifications are mainly inclined to increase antibacterial activity. In recent years, more and more quinoline drugs have been used in the development of anticancer drugs, such as dini. The common modification group is an alkyl substituent, and the salt-forming structure modification at the N-1 position also shows a good effect on the modification (Scheme 1). Studies have shown that the antibacterial activity of quinolone may be determined by two factors, one is the drug's ability to inhibit the target enzyme DNA gyrase, the other is the transport effect or post-rotation process [50–57]. Therefore, it may lead to erroneous conclusions based on the traditional minimum inhibitory concentration (MIC) without considering target enzyme factors [58–62]. This paper reports the effects of the systematic changes of R1 salt-forming groups (20 compounds) on the inhibitory effects of bacterial and cancer cell growth. At the same time, the structure-activity relationship including the N-1 position is defined as far as possible synthesis of the best compounds and their activity as long as docking study of DNA topoisomerase.

2.1. Hydrocarbyl substitution

The quinolone structural modifications that generally occurred at the N-1 position are mainly ethyl and cyclopropyl. Studies have found that bulky alkyl substituents have stronger antibacterial activity. 1 and 8 position can form a ring at the same time and it was also a common structural modification.

2.2. Quaternary ammonium salt modification

With the development of synthetic drugs, in order to improve the water solubility of drugs, the introduction of iodoalkyl at the N-1 position to form a quaternary ammonium salt structure has also become a new research direction. The parent body with antibacterial activity is modified by the quaternary ammonium salt structure to improve the water solubility of the target product. After this modification, the oil-water partition coefficient is improved in a reasonable direction.

3. Effects of salt formation at N-1 on biological activity

The introduction of alkyl chains through haloalkyl can achieve high efficiency and convenience [63,64]. In this paper, iodomethane, iodopropane, and iodoisopropanol were used to grow and modify the N-1 chain to form salts, and a series of quinoline ammonium salts with odd carbon number chains were obtained (Scheme 2). The quaternary ammonium salt with even representative carbon chain is mainly synthesized from the derivatives of iodoethane and quinoline.

4. Methods and methodology

4.1. Biological activity

4.1.1. Antibacterial activity test

The synthesized MIC values of final compounds 1–20 were evaluated for their antibacterial activity against E. coli (ATCC 29213) and Staphylococcus aureus (ATCC 8739). The DMSO solution of test compounds were added to the culture medium to obtain final concentrations of 1–50 nmol mL⁻¹. A standardized suspension of the test bacterium was inoculated and incubated for 24–48 h at 37 °C, then the minimal inhibitory concentrations (MIC values) were calculated. In order to avoid random errors and ensure the accuracy of the experiment, we choose ciprofloxacin and amoxicillin as the controls and performed triple parallel experiments.
4.1.2. Cytotoxicity activity test
The human lung cancer cell line (A549), human Hela cell line (HeLa), human gastric cancer cell line (SGC-7901), and normal liver cell line (L-02) were used to evaluate the anti-proliferation of compounds in five cell lines active. Anticancer drugs were jointly tested for 5-FU and MTX as positive controls. Dissolved in DMSO and then performed activity tests.

4.2. Docking simulations
AutoDock Vina 1.1.2. and MGLTools software were used to perform docking simulations. In the present study, the 3D crystal structure of human topoisomerase I and S. aureus DNA gyrase (Topo II) reported in Protein Data Bank (PDB) were used as the receptor for docking studies (PDB ID: 1TL8, 2XCT). The co-crystallized

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**Scheme 1.** Examples of common substitutions at the N-1 position of quinolone.

**Scheme 2.** Schematic diagram of overall design of synthesized active derivatives.
structures of the target proteins were downloaded from the PDB and prepared for docking using the docking program AutoDockVina 1.1.2. and MGLTools. The docking result was analyzed and optimized by Pymol 1.5.6.

5. Result and discussion

5.1. Chemistry

We firstly synthesized series of ethyl-8-chloro-[1,3]dioxol-4,5-gquinoline-7-carboxylate intermediate. Then it was found that the synthetic route shown in Scheme 2, adapted from some related synthetic manipulations, could serve as a suitable approach for the desired correspondence compounds. Subsequent synthetic studies revealed that the novel 7-(ethoxycarbonyl)-8-(aryl)amino)-[1,3]dioxol-4,5-gquinolin-5-iom iodide derivatives synthesized according to Scheme 3 could be obtained in moderate yield. The iodination of ethyl-8-(aryl)-[1,3]dioxol-4,5-gquinoline-7-carboxylate derivatives with an excess of iodomethane or iodopropane under reflux provided the target compounds (Scheme 3). Finally, we elucidated the bioactivity of target compounds from molecular docking study as having anticancer and antitumor activities.

5.2. The spectroscopic property of tested compounds

Ultraviolet-visible (UV–vis) spectra were recorded on a UV-2550 spectrophotometer using a 1 cm path length quartz cuvette at room temperature. Spectroscopic evaluation was performed in DMSO solvent at the concentration of 5 mM. It was found that the compounds in each series have spectral images with almost uniform absorption wavelengths, which may be caused by similar conjugated environments. Therefore, the compound in which methyl iodide is substituted as the representative is shown in Fig. 2. From the result, it is with characteristic benzene ring band that absorption in the wavelength range of 230–270 nm. The absorption peaks of the 1–3 series of spectra substituted with a benzene ring at the 4-position are located at 358 nm. The compound 4b with bisbenzene ring substitution has a peak at 380 nm that is different from several other products, which may be caused by the existence of a new conjugated environment between the C-4 benzyloxybenzene structure.

5.3. Evaluation of antibacterial activity in vitro

The antibacterial activity test was also possessed against E. coli (ATCC 29213) and Staphylococcus aureus (ATCC 8739) as shown in Table 1 and Fig. 3. Among the tested compounds 1a-4e with the different 4-position N-phenyl substituent on quinoline possessed the changed dual activity against all cancer cell and strains tested.

In the respective series where each 4-position is the same, a parallel comparison under the condition against S. aureus (ATCC 8739) found that the compounds 1b, 1e, 2b, 2e, 3b, 3e showed lower IC50 values. Compounds 3b and 4b showed 8 to 4 times the antibacterial activity compared to the reference variable, which was likely to be due to the salt-forming modification of the compound at N-1 position. This may be because the 1-position is substituted with an odd-chain alkyl chain. It is worth mentioning that

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Scheme 1. The basic route for the synthesis of the target compound to be tested. Reagents and conditions: i) Phenethylamine, K2CO3, ACN, 80 °C, 8 h. ii) 3,4-Dimethoxyphenethylamine, K2CO3, ACN, 80 °C, 8 h. iii) Benzylamine, K2CO3, ACN, 80 °C, 8 h. iv) 4-Benzoyloxaniline hydrochloride, K2CO3, ACN, 80 °C, 8 h. v) R-I (Iodomethane (1b), Iodoethane (1c), 1-Iodopropane (1d), 2-Iodopropane (1e)), ACN, reflux, 2 h. vi) Reflux, Dimethoxyphenethylamine, 4d (R-I (Iodomethane (2b), Iodoethane (2c), 1-Iodopropane (2d), 2-Iodopropane (2e)), ACN, reflux, 2 h. vii) Reflux, Iodopropane (3a). viii) R-I (Iodomethane (3b), Iodoethane (3c), 1-Iodopropane (3d), 2-Iodopropane (3e)), ACN, reflux, 2 h. viii) R-I (Iodomethane (4b), Iodoethane (4c), 1-Iodopropane (4d), 2-Iodopropane (4e)), ACN, reflux, 2 h.
the even-numbered branch substitution in series 3 gave compound 3c the worst activity in this group at 25 nmol mL\(^{-1}\), which extremely may be due to the iodoethyl group substitution modification at the N-1 position. For series 4, when substituted by benzylxoyphenethylamine, the four alkyl substitutions all show the same activity intensity at 3.125 nmol mL\(^{-1}\) far exceeds the reference drug concentration of 25 nmol mL\(^{-1}\) in Amoxicillin and 12.5 nmol mL\(^{-1}\) in Ciprofloxacin of saccharin is probably because the aromatic hydrophobic structure of the large group at position 4 has a greater influence on antibacterial activity, temporarily exceeding the effect of the alkyl side chain. An overview of the overall biological activity presents an activity advantage of odd carbon number substitution.

At the same time, the ClogP value of the compound reflects the water solubility of the drug, which has special significance for whether the drug can penetrate the cell membrane and enter the cell to play a role. Compounds tend to be more water-soluble after salt formation, but this is also affected by the size of the salt-forming side chain groups. We can find that the volume of the iodoalkyl group with a 1-position substituent exhibits different ClogP values. For example, the formation of the quaternary ammonium salt of compound 1b (methyl iodine, logP = 2.27) increases the water solubility, which decreases slightly with the increase in branching until the effect of products 1e (isopropyl iodine, logP = 3.13) was worse than the raw material 1a (unsustituted, logP = 2.53), which also led the difference in activity.

### 5.4. Evaluation of cytotoxicity activity in vitro

The conversion of a 4-position carbonyl group to a hydrophobic aromatic amine group to obtain target compounds, and most of these structures contained a hydrophobic group, and then
quaternized ammonium salt at the N-1 position to form the final product. IC_{50} were assessed for their anti-proliferative activity in above mentioned five cell lines by MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) assay (Fig. 4, Table 2). Compared with the positive control 5-FU and MTX, all 20 compounds showed superior anticancer activity. It is worth mentioning that the substitution of even-numbered carbon chains into salts shows unusual anticancer activity in each series against five cell lines. As shown in the activity experiments (Table 2), the anticancer activity (IC_{50}) of the products with even carbon chain substitution (such as compounds 1-4c) were slightly inferior to other products with odd carbon chain in the same series of structurally modified quaternary ammonium salts at the 4-position. That is, among the quinoline compounds with same substituent at the 4-position, the anticell proliferation activity of the derivative substituted with the iodopropyl at the 1-position was weaker than that of the iodomethyl, iodopropyl, iodopropyl substituted products. For example, compounds 4d of 4 ± 0.42–14±1.96 and 4e of 5 ± 0.64–17±0.41 were 2–3 times more active than 4c of 7 ± 0.48–29±5.79 against five cell lines.

5.5. Analysis of docking result

Molecular docking is an intuitive method that initially simulates the binding of compounds to sites of action. These tight interactions allow it to bind strongly to Topoisomerase II, which is considered a target for quinoline antibacterial effects. Therefore, the combination model clarifies the reason for the obvious efficacy of these compounds from another perspective. It was of interest to elucidate the mechanism by which the synthesized active compounds persuaded their antibacterial activities (Figs. 5–8). The co-crystallized structures of the target proteins of S. aureus were downloaded from the PDB bank and prepared for docking by AutoDockVina 1.1.2. and MGLTools (PDB ID: 2XCT). In the analysis of the molecule simulation results of antibacterial activity, in order to control the variables, we selected series 1 (1a-1e) and series 3 (3a-3e) with the same phenylethylamine substituent at the 4-position substituent for parallel comparison within each group (Figs. 5, 7). The 3D ligand interactions of the compound 1b inhibitor with protein displayed affinity score −9.9 Kcal mol^{-1} and mainly showed two hydrogen bond acceptor interactions between N at 4-position of quinoline ring and bases DA 13 and DT 8 and ionic interactions between oxygen of the carbonyl group and amino acid residues D 437 and R 458. It also displayed Ar-H interaction with bases DT 10 and DA 13 along with π-π stacks between benzene substituent at 4-position and base DT 8 and DG 9, in addition to other hydrophobic interactions (Fig. 5a). Whereas the binding energies of compounds 1c and 1d were inferior to those of the same series of compounds, which are −8.7 and −8.9 Kcal mol^{-1}, respectively. There were no hydrogen bond interactions in compound 1c binding mode. It exhibited Ar-Pi interactions between benzene ring of quinoline ring and base DT 10, with pyridine ring.
Fig. 4. Cytotoxic concentration (IC_{50}) of tested compounds 1–20 against (a) human lung cancer cell line (A549); (b) human hela cell line (HeLa); (c) human gastric carcinoma cell line (SGC-7901); (d) normal hepatocyte cell line (L02) by the MTT assay.

| Compounds | IC_{50} A549 | IC_{50} Hela | IC_{50} SGC-7901 | IC_{50} L02 |
|-----------|-------------|-------------|-----------------|-------------|
| 1a        | 31±6.25     | 73±6.55     | >120            | >200        |
| 1b        | 54±5.39     | 36±0.98     | 89±1.80         | 139±13.60   |
| 1c        | 82±2.49     | 57±2.77     | 84±7.49         | 140±10.35   |
| 1d        | 55±4.38     | 44±3.95     | 89±2.89         | 127±9.58    |
| 1e        | 57±14.24    | 51±2.94     | 73±4.18         | 131±8.08    |
| 2a        | 94±6.63     | 73±4.83     | >120            | >200        |
| 2b        | >120        | >120        | 114±1.81        | >200        |
| 2c        | >120        | >120        | >120            | >200        |
| 2d        | >120        | >120        | >120            | >200        |
| 2e        | >120        | >120        | >120            | >200        |
| 3a        | 46±18.96    | >120        | >120            | >200        |
| 3b        | 49±5.42     | 30±2.06     | 74±4.99         | 125±9.67    |
| 3c        | 59±7.95     | 35±0.87     | 110±2.52        | 131±7.10    |
| 3d        | 38±7.32     | 39±1.82     | 92±2.76         | 121±15.18   |
| 3e        | 90±3.21     | 68±4.96     | >120            | >200        |
| 4a        | 17±0.95     | >120        | >120            | >200        |
| 4b        | 47±8.39     | 45±14.13    | 85±11.62        | 124±8.45    |
| 4c        | 12±1.48     | 7±0.48      | 29±5.79         | 13±1.41     |
| 4d        | 4±0.88      | 4±0.42      | 14±1.96         | 32±3.66     |
| 4e        | 5±0.64      | 7±1.05      | 17±0.41         | 54±4.88     |
| 5-FU      | 98±0.72     | 109±2.81    | 113±1.07        | >200        |
| MTX       | 60±9.14     | 107±1.97    | 84±7.47         | 185±13.10   |

and base DG 9 and base DA 13, with benzene ring substituent at 8-position and base DC 12. It also displayed ionic interactions between benzene ring and R 458 with between pyridine ring and amino acid residue E 477 in addition to π-π interaction of N-phenyl ring with base DT 8 (Fig. 5b). Compound 1d exhibited hydrogen bond donor interaction between N at 4-position and base DT 10. However, it displayed a similar low affinity score (−8.9 Kcal mol⁻¹) to docking mode of 1c. It revealed three Ar-H interactions with bases DT 10, DA 13 and DT 8, besides π-π interactions between quinolone core and bases DG 9 and DC 12 in addition to ionic interaction of N-phenyl ring with amino acid residues K 417, D 437, R 458, respectively (Fig. 5c). In contrast, 1e with an odd carbon number chain at N-1 position showed excellent inhibitory activity against S. aureus with affinity score −9.4 Kcal mol⁻¹. Its 3D ligand interaction revealed one hydrogen bond donor between the imine N and amino acid residue D 437 in addition to π-H.
interactions of the quinoline ring also with base DT 10, DG 9, and DA 13, respectively, besides another π-H interaction between benzene ring introduced at position 8 with amino acid residue F 1123. And the spatial position of the compound at the binding site was very appropriate which would greatly be the cause of its good interactions and desired potency (Fig. 5d).

When the quinoline 4-position substituent is N-benzyl (3a-e), it is also fascinating to examine compounds with a long odd or even-numbered carbon substituent at 4-position of quinolone core. The docking mode of compound 3b had an affinity score at $-9.8$ Kcal mol$^{-1}$ (Fig. 7a). In the combined mode, the π-π interactions that occupied a large proportion between quinoline ring and bases DG 9, DC 12, and DA 13, respectively, played a major role. Moreover, π-π stacks also existed between benzene ring introduced at N-1 position and base DT 8. There was also a hydrogen bond receptor interaction between oxygen atom in dioxamol and base DC 12, which plays a role in determining the conformation direction of the entire structure in addition some other ion interactions and
van der Waals forces. As for the compound 3c substituted with an even ethyl chain at the N-1 position, it showed a weaker effect consistent with the antibacterial activity (Fig. 7b). The oxygen atom at the ‘bottom’ of the compound is some 2.9 Å away from the DNA amine N of base DC 12, indicating that the oxygen atom is acting as an only O hydrogen-bond receptor. In this model, π−π interactions between compound and DNA bases takes up the main part of the force. In addition, there are other ionic and hydrophobic forces between the compound and the protein chain. According to the above, in the absence of CH−π fixation, it also lacks one hydrogen bond effect than the odd-chain-substituted compounds in the same series, which may prove its SAR simulation mechanism. Whereas the corresponding compounds 3d, 3e perform well in biological activity, and the docking results contain two hydrogen
bonding interactions, which may also be the main reason why these two odd-chain substituted compounds have outstanding activity (Fig. 7c, d). In addition, in compound 3d, there are \( \pi \cdot H \) interactions between the benzene ring and the bases DT 8 and DA 13, respectively, and this force also exists between the 8-substituted benzene ring base DT 10. Besides some other ionic bonds. The action of compound 3e also complements the antibacterial activity results, with the acceptor hydrogen bond between R 458 and carbonyl oxygen and the 4-position N and D 437 donor hydrogen bonds. Moreover, there are other CH-\( \pi \) interactions, which are between the quinoline parent ring and the bases DT 8, DA 13, DC 12 and DG 9, severally. Through the above, these molecular docking analyses find the most potent 2XCT inhibitors mechanism or mode for the prevention and treatment of infections caused by S. aureus (Table 3).

We also analyzed the anticancer activity and docked to simulate its mechanism of action. The target of its action is topoisomerase I, so the proteins we downloaded from the PDB database were processed after docking (PDBID: 1TL8). The docking found that the compounds with alkyl substituents were very close to the docking position and direction. In order to facilitate the explanation of the effect of the action force, we selected series 1 (compounds 1b-1e) for comparison and explanation (Fig. 9, Table 4). Compound 1b showed pleasant docking parameters, with a docking binding energy of \(-10.2 \text{ Kcal mol}^{-1}\), the highest in this series, which also masterly proved that the introduction of the quaternary ammonium salt iodomethyl side chain greatly improves the anticancer activity of the compound. There are multiple \( \pi \cdot \pi \) stacking interactions between the pyridine ring of the compound and the base DT 105, between TPC 11 and the quinoline ring in the parent ring, and between the parent benzene ring and the base DA 113. At the same time, there are five ionic \( \pi \) bonds of the compound with amino acid Q 633, amino acid H 632, amino acid R 488, amino acid R 364 and amino acid K 532, respectively. In addition, there are some hydrophobic effects, for example, the N-benzyl substituent and the amino acid I 535 are tightly connected by the hydrophobic bond. These forces are the main representative forces of molecular simulation in this series. In contrast, compound 1d with odd carbon number has almost the same spatial arrangement and spatial force as compound 1b with affinity at \(-9.7 \text{ Kcal mol}^{-1}\). In addition to the lack of I535 hydrophobic bond, the simulation results are almost identical, which may be one of the reasons why its biological activity is slightly lower than that of 1b (stronger than other in series 1). The affinity of compound 1e with odd alkyl substituent chain is \(-9.6 \text{ Kcal mol}^{-1}\), which is almost weaker than that of compound 1c (\(-9.8 \text{ Kcal mol}^{-1}\)). However, it was found that there is a reasonable hydrogen bond between TPC 11 and carbonyl oxygen except for some necessary \( \pi \cdot \pi \) stacks. Finally, we analyzed the binding mode of compound 1c and find that it was quite different in this group. First, its spatial distribution is very different from other similar compounds, and the amino acid distribution around it is also scarcely visible to the naked eye. Secondly, the binding of compound 1c with 1TL8 mainly depends on the \( \pi \cdot \pi \) interactions between the pyridine and benzene ring of the parent core and TPC 11, between the base DT 10 and the pyridine ring, and between the N-benzyl substituent and the base DG 112, which seemed to be the same as the former compounds. However, the ion-\( \pi \) bonds that play the role of spatial positioning were very rare, only of com-

### Table 3
Docking residues around the active pocket against *S. aureus* (PDBID: 2XCT).

| Entry | Affinity score ([kcal mol]{-1}) | Interaction with receptor | MIC |
|-------|---------------------------------|---------------------------|-----|
| (PDBID 2XCT) |                                |                           |     |
| 1b    | \(-9.9\)                        | DC12 DA13                 |     |
| 1c    | \(-8.7\)                        | DA13 DG9 DT10             |     |
| 1d    | \(-8.9\)                        | DT8                       |     |
| 1e    | \(-9.4\)                        | D437                      |     |
| 2b    | \(-9.8\)                        | G459 0437                 |     |
| 2c    | \(-8.7\)                        | D437                      |     |
| 2d    | \(-9.3\)                        | DT8                       |     |
| 2e    | \(-9.8\)                        | DG9 R458 K417             |     |
| 3b    | \(-9.3\)                        | DC12 DG9                 |     |
| 3c    | \(-9.3\)                        | DC12                      |     |
| 3d    | \(-9.0\)                        | D437                      |     |
| 3e    | \(-9.2\)                        | R458 DG9                  |     |
| 4b    | \(-9.9\)                        | D437                      |     |
| 4c    | \(-9.2\)                        | DA13 F1123                |     |
| 4d    | \(-8.8\)                        | DA13 DG9                  |     |
| 4e    | \(-8.9\)                        | R1122 DG9 D437            |     |

### Table 4
Docking residues around the active pocket against *Human topoisomerase I* (PDBID: 1TL8).

| PDBID | Entry | Affinity score ([kcal mol]{-1}) | Interaction with receptor |
|-------|-------|---------------------------------|---------------------------|
| 1TL8  | 1b    | \(-10.2\)                       | TPC11 DA13 DT10 Q633 H632 R488 R364 K532 1535 |
|       | 1c    | \(-9.8\)                        | TPC11 DT10 DG12 K532 K425 |
|       | 1d    | \(-9.7\)                        | TPC11 DA13 DT10 Q633 H632 R488 R364 |
|       | 1e    | \(-9.6\)                        | TPC11 DT10 DA112 K425 K532 |
Fig. 9. The 3D binding mode of derivatives 1b-e in the binding site of Human topoisomerase I (PDB ID: 1TL8). (a) Docking of compound 1b; (b) Docking compound 1c; (c) Docking of compound 1d; (d) Docking of compound 1e. ([H] Hydrogen Bonds are shown in pink and the yellow represents [π] π-π accumulation. Green and gray represent Ion-Pi interaction and Hydrophobic bonds involved in amino acid bonding, respectively).}

Fig. 10. The 3D binding mode of derivatives 4d in the binding site of Human topoisomerase I (PDB ID: 1TL8). ([H] Hydrogen Bonds are shown in pink and the yellow represents [π] π-π accumulation. CH-Pi interaction involved in amino acid bonding is colored by marine).}

Pound with amino acid K 532 and amino acid K 425 have cation–Pi action, respectively, which may also be the reason for their weak action. And may also be listed as the reason for its poor anticancer activity to 1b in the aspect of molecular docking.

According to the anticancer activity data, the activity of compound 4d in the odd chain is the most prominent, so it is necessary to carry out targeted analysis of the compound. The docking of 4d displayed he binding affinity of −9.3 Kcal mol⁻¹ (Figs. 10, 11). In the binding site of the enzyme displayed mainly hydrogen bond acceptor interactions through the oxygen of benzodioxoles with TPC11, it also existed between the carbonyl oxygen and base DA113, in addition between the oxygen of arylamine and amino acid residue K 425, and hydrogen bond donor interaction between the N at the 4-position and base DG 112. The compound also displayed π-π interactions of arylamine-substituted group with DG 112, and CH-π interactions of arylamine-substituted group and base DA 113. It also displayed other hydrophobic and cation interactions. According to the above analysis, even if the activities of the odd or even alkyl side chains were different, it is undoubted that the substituted compounds have outstanding antitumor activity. The interactions and affinity scores of the compounds suggest that the target compounds can served as an antitumor drug against cancer cells.
Fig. 11. Orthogonal views in two-dimensional spatial structure of 1T18–4d fusion protein. The active site of compound 4d (light blue, sticks) in DNA protein. Molecular figures generated with Pymol 1.5.6 shown in publication type.

6. Conclusion

A summary of the common substitutions of several quinoline at the N-1 position was explored, and at the same time, the biological activity of the 20 compounds we synthesized with the odd and even carbon numbers of the alkyl side chain of the quaternary ammonium salt at the N-1 position were performed to explore the structure-activity relationship. The present study describes the design and bioactivities of twenty new quinoline derivatives as promising antimicrobial and antitumor agents based on DNA gyrase and DNA Top I. Among the synthesized derivatives, twelve compounds (1b, 1e, 2b, 2e, 3b, 3d, 3e, 4a, 4b, 4c, 4d, and 4e) showed promising antibacterial activities against tested E. coli and S. aureus bacterial strains more potent than amoxicillin and ciprofloxacin as reference. In the results of antitumor activity, compound 4d showed very high antitumor activity, no matter in A549, SCC-7901, or HeLa cell line and become the most ideal dual active product. SAR study pointed out the importance of the 4-position substituted with hydrophobic phenyl rings and the N-1 position substituted with iodized salt to the antibacterial potency. Besides, the substitution of alkyl side chains with improved water solubility is a necessary condition for obtaining the best antibacterial and antitumor activity. In actual experiments, the clog P value emphasizes its effect on the increase in the antibacterial activity of the quaternized ammonium products. In addition, docking study that substitution with odd carbon chain are essential for optimal antibacterial activities than even carbon chain (1c, 2c, 3c) and also discern the possible mechanism for their antibacterial potency. It revealed strong binding interactions in DNA gyrase site on odd carbon chain and weak binding on even carbon chain. Antitumor activity docking in DNA Top I also revealed the possible mechanism of odd and even alkyl side chain activity, showing a clear difference in action. The study of N-1 substitution could lay a foundation for the follow-up study.

Declaration of Competing Interest

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.

CRediT authorship contribution statement

Yilin Wang: Conceptualization, Software. Fuyan Xiao: Data curation, Writing - original draft. Guofan Jin: Methodology, Supervision, Writing - review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2020.128869.

References

[1] S.S. Bhagwat, M. Nandanwar, A. Kansagara, A. Patel, S. Takalkar, R. Chavan, H. Periasamy, R. Yeole, P.K. Deshpande, S. Bhaviar, A. Bhatia, J. Ahdal, R. Jain, M. Patel, Levonadifloxacin, a novel broad-spectrum anti-MRSA benziquinoline quinoline agent: review of current evidence, Drug Des. Dev. Therapy 13 (2019) 4351–4365, doi:10.2147/ddt.s229882.
[2] M. Neame, C. King, A. Riordan, A. Iyer, R. Kneen, I. Sinha, D.B. Hawcutt, Seizures and quinoline antibiotics in children; a systematic review of adverse events, Eur. J. Pediatr. Pharm. Sci. Pract. 27 (2020) 60–64, doi:10.1116/ ejpdmpharm-2018-001805.
[3] D.C. Vieira, W.G. Lima, M.C. de Paiva, Plasmid-mediated quinolone resistance (PMQR) among Enterobacteriaceae in Latin America: a systematic review, Mol. Biol. Rep. 47 (2020) 1471–1483, doi:10.1007/s11033-019-05220-9.
[4] U. Acaroz, R. Dietrich, M. Krauer, E. Maertlbauer, Development of a generic enzyme-immunoassay for the detection of fluoro(quinolone)-Residues in Food-stuffs Based on a Highly Sensitive Monoclonal Antibody, Food Anal. Methods 13 (2020) 780–792, doi:10.1007/s12161-019-01695-1.
[5] S.L. Badshah, A. Ullah, New developments in non-quinolone-based antibiotics for the inhibition of bacterial gyrase and topoisomerase IV, Eur. J. Med. Chem. 152 (2018) 393–400, doi:10.1016/j.ejmech.2018.04.059.
[6] F. Gao, P. Wang, H. Yang, Q. Miao, L. Ma, G. Lu. Recent developments of quinoline-based derivatives and their activities against Escherichia coli, Eur. J. Med. Chem. 157 (2018) 1223–1248, doi:10.1016/j.ejmech.2018.08.095.
[7] Q. Song, Z. Xu, H. Gao, D. Zhang, Overview of the development of quinoline resistance in Salmonella species in China, 2005-2016, Infect Drug Resist 11 (2018) 267–274, doi:10.2147/iddr.s157460.
[8] I.V. Ukrainets, Y.V. Mospanova, N.L. Berezyankova, A.A. Davidenko, Modification of the Benzene Moiety of the Quinoline Nucleus of 4-Hydroxy-6,7-dimethoxy-2-oxo-N-(pyrind-3-ylmethyl)-1,2-dihydroquinoline-3-carboxamide as an Attempt to Enhance Its Analogic Activity, Khimiko-farmatsevtsiiskii zhurnal 52 (2018) 9–12, doi:10.3906/0023–1134-2018-52-10-8–12.
[9] A. Li, J.W. Schertzler, X. Yong, Molecular conformation affects the interaction of the Pseudomonas quinolone signal with the bacterial outer membrane, J. Biol. Chem. 294 (2019) 1089–1094, doi:10.1074/jbc.A118.006844.
[10] H. Naziz, G. Sains, S.R. Ansari, R. Ertelkin, H. Haas, E. Deziel, D.A. Stevens. Novel intermolecular molecular interaction: pseudomomas aeruginosa Quinolone Signal (PQS) modulates Aspergillus fumigatus response to iron, Microbiol.-Sgm 166 (2020) 44–55, doi:10.1099/mic.000835.
[11] H.M. Patel, D.F. Rajani, M.G. Sharma, H.G. Bhat, Synthesis, molecular docking and biological evaluation of mannich products based on thiophene nucleus using ionic liquid, Lett Drug Des. Discov. 16 (2019) 119, doi:10.2174/15701808156680502123743.
[12] H.M. Patel, K.D. Patel, H.D. Patel, Facile synthesis and biological evaluation of new mannich products as potential antibacterial, antifungal and antituberculosis agents: molecular docking study, Curr. Bioact. Compd. 13 (2017) 47–58, doi:10.2174/15734072126616601657145130.
[13] H.M. Patel, Synthesis of new mannich products bearing quinoline nucleus using reusable ionic liquid and antitubercular evaluation, Green Sustain. Chem. 5 (2015) 137, doi:10.4236/gsc.2015.54017.
[14] H.M. Patel, Current bioactive compounds synthesis, characterization and microbial studies of novel mannich products using multicomponent reactions, 14 (2018) 278–288. 10.2174/157340721666805021464716.
[15] Z. Ali, H. Ma, M.T. Rashid, A. Wali, S. Younas, Preliminary study to evaluate the phytochemicals and phytochemical properties in red and black date’s vinegar, Food Sci. Nutr. 7 (2019) 1796–1805, doi:10.1002/fsn3.1009.
[16] H. Cui, M. Bai, Y. Sun, M.A.-S. Abdel-Samie, L. Lin, Antibacterial activity and mechanism of Chuzhou chrysanthemum essential oil, J. Funct. Foods 48 (2018) 159–166, doi:10.1016/j.jff.2018.07.021.
and Iran, cycl. L. 823–830, of derivatives J. Nat. Prod. 2019 e13294, doi: 10.1021/acs.jnpb.9b00518.

D.M. Patel, R.M. Vala, M.G. Sharma, D.P. Rajani, H.M. Patel. A practical green view to the functionalized 1,2,4-triazole-3,5-dibqunin-8(1H)-one scaffolds using group-assisted (3H)-tritium-donner para AChE-hydrolysis
test, Chem. Sci. Commun. 4 (2019) 1031–1041, doi: 10.1039/s08030605.

D.M. Patel, M.G. Sharma, R.M. Vala, I. Lagunes, A. Puerta, J.M. Padron, D.R. Pati-

nani, H.M. Patel. Hydroxyl allyl ammonium ionic liquid assisted green and one-

pot telescopic C–O bond formation, their chemical and pharmacological evaluation, Bioorg. Chem. 86 (2019) 137–150, doi: 10.1016/j.bioorg.2019.01.029.

H. Cui, C. Zhang, C. Li, L. Lin, Antibacterial mechanism of oregano essential oil. J. Food. Eng. 2018 1412–1413, doi: 10.1016/j.jfoodeng.2017.11.040.

Y. Feng, Y. Zhu, J. Wan, X. Yang, C.K. Firempong, J. Yu, X. Hu. Enhanced bioavailability, reduced irritation and increased hypolipidemic activity of self-assembled capsaicin produg nanoparticles, J. Funct. Foods 44 (2018) 137–145, doi: 10.1016/j.jff.2018.02.033.

E. Fokum, H.M. Zabed, Q. Guo, J. Yun, M. Yang, H. Pang, Y. An, W. Li, X. Qi. Metallic engineering of bacterial strains using CRISPR/Cas9 systems for biosynthesis of value-added products, Food Biosci. 28 (2019) 125–132, doi: 10.1016/j.fbis.2019.01.003.

R. Niu, Y. Yang, Y. Wang, S. Luo, C. Zhang, Y. Wang. Development and character-

ization of an immunoaffinity column for the detection of dafneloxacin residues in milk samples, Food Sci. Technol. 39 (2019) 500–506, doi: 10.15406/fst.2019.39.01.04517.

Y. Jin, J.-H. Yuan, Q. Zhuang, Y. Zhang, S. Wu. Effect of broccoli ex-
tracts on proliferation and apoptosis in the colon SW620 Cells, Int. J. Agric. Biol. 22 (2019) 769–773, doi: 10.17749/ijab.15.1128.

Z. Zhang. G.K. Mahanu, R. Castoria, Q. Yang, M.T. Apilaya. Recent develop-
ments in the enhancement of the postsynaptic blockers with unconven-
tional chemicals compounds, Trends Food Sci. Technol. 78 (2018) 180–187, doi: 10.1016/j.tifs.2018.06.002.

W.-D. Cai, W.-P. Qiu, Z.-B. Ding, L.-X. Wu., J.-K. Yan. Conformational and rhe-

gological properties of a quaternary ammonium salt of curdin, Food Chem. 280 (2019) 130–138, doi: 10.1016/j.foodchem.2018.12.059.

X.-M. Chen, Z. Ma, D.D. Kitts. Effects of processing method and age of leaves on phytochemical profiling and bioactivity of coffee leaves, Food Chem. 249 (2019) 143–153, doi: 10.1016/j.foodchem.2019.01.077.

M. Elena Diaz-Casado, J.L. Quiles, E. Barriocanal-Casado, P. Gonzalez-Garcia, M. Battino, L.C. Lopez, A. Varela-Lopez. The paradox of coenyzme Q10 in ag-

ing, Nutrients 11 (2019) 1–12, doi: 10.3390/nu11020457.

L. Jiang, D. Wei, K. Zeng, J. Shao, F. Zhu, D. Du. An enhanced direct competitive immunoassay for the detection of kanamycin and tobramycin in milk using multi-enzyme–particle–amplification, Food Anal. Methods 11 (2018) 2066–2075, doi: 10.1007/s12161-018-1185-5.

R. Osae, C. Zhou, W. Tchabo, B. Xu, E. Bonah, E.A. Aleyongere, H. Ma. Optimiza-
tion of osmocorrelation pretreatment of ginger (Zingiber officinale Rosc) using response surface methodology: effect on antioxidant activity, enzyme in-
hibitory, phenolic content, and physical properties, J. Food Proc. Process. 42 (2019) e13218, doi: 10.1002/jfpp.13218.

R. Osae, C. Zhou, B. Xu, W. Tchabo, H.E. Tahir, A.T. Mustapha, H. Ma. Effects of ultrasound, escomic dehydration, and osmocorrelation pretreatments on bioac-
tivity compounds, chemical composition, antioxidant activity, color, and antiox-
tioxidant activity of dried ginger slices, J. Food. Biochem. 43 (2019) e12382, doi: 10.1111/jfbi.12382.

X. Ren, Q. Liang, H. Ma. Effects of sweeping frequency ultrasound pretreatment on the hydrolysis of zingiberone in ginger, Ind. Eng. Chem. Res. 58 (2019) 4020–4027, doi: 10.1021/acs.iecr.9b02373.

F. Sarpong, P. Qting-Darko, M.K. Gbuly, L.P. Amenofor, M.T. Rashid, C. Zhou. Comparative study of enzymes inactivation and pigmentation of apple (Malus domestica) slices by selected fungi during low temperature storage, J. Food Biochem. 42 (2018) e12681, doi: 10.1111/jfs.12681.

F. Sarpong, X. Yu, C. Zhou, H. Yang, B.B. Utoezinjwa, J. Bai, B. Wu, H. Ma. Influence of anti-browning agent pretreatment on drying kinetics, enzymes inactivation and other qualities of dried banana (Musa spp.) under relative humidity-convective air dryer, J. Food. Meas. Characterization 12 (2018) 1229–1241, doi: 10.1016/j.jfmc.2018.01.002.

L. Wang, W. Li, Y. Liu, W. Zhu, J. Han, Y. Wang, N. Green separation of brome-
lain in food sample with high retention of enzyme activity using recyclable aqueous two-phase system containing a new synthesized thermo-responsive copolymer and salt, Food Chem. 262 (2019) 48–57, doi: 10.1016/j.foodchem.2019.01.005.

C. Zhang, H. Cui, Y. Han, F. Yu, X. Shi. Development of a biomimetic enzyme-linked immunosorbent assay based on molecularly imprinted polymers on paper for the detection of carbyl, Food Chem. 240 (2018) 893–897, doi: 10.1016/j.foodchem.2017.07.090.

Y.-Q. He, C.-Y. Ma, Y. Pan, L.-J. Yin, J. Zhou, Y. Duan, H. Zhang, H. Ma. Bioavail-
ability of corn gluten meal hydrolysates and their effects on the immune sys-
tem in turkeys, Czech J. Food Sci. 36 (2018) 1–7, doi: 10.17221/32/2017-cjfs.

X. Jin, Q. Wang, X. Yang, M. Guo, W. Li, J. Shi, M. Adu-Frimpong, X. X.

W. Deng, J. Yu. Chemical characterisation and hypolipidemic effects of two purified Puertos eringyi polysaccharides, Int. J. Food Sci. Technol. 53 (2018) 2298–2307, doi: 10.1111/ijfs.14601.
[62] X. Ren, Q. Liang, X. Zhang, T. Hou, S. Li, H. Ma, Stability and antioxidant activities of corn protein hydrolysates under simulated gastrointestinal digestion, Cereal Chem. 95 (2018) 760–769, doi: 10.1002/cche.10092.

[63] G. Jin, F. Xiao, Z. Li, X. Qi, L. Zhao, X. Sun, Design, synthesis, and dual evaluation of quinoline and quinolinium iodide salt derivatives as potential anticancer and antibacterial agents, ChemMedChem 15 (2020) 600–609, doi: 10.1002/cmdc.202000002.

[64] G. Jin, Z. Li, F. Xiao, X. Qi, X. Sun, Optimization of activity localization of quinoline derivatives: design, synthesis, and dual evaluation of biological activity for potential antitumor and antibacterial agents, Bioorg. Chem. 99 (2020) 103837, doi: 10.1016/j.bioorg.2020.103837.