MedChemComm

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/medchemcomm

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Design, Synthesis, Antibacterial Evaluation of Novel Azolylthioether Quinolones as MRSA DNA Intercalators†

Ling Zhang, Kannekanti Vijaya Kumar†, Syed Rasheed†, Shao-Lin Zhang, Rong-Xia Geng, Cheng-He Zhou*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A series of azolylthioether quinolones were synthesized and characterized by NMR, IR, MS and HRMS spectra. All the newly prepared compounds were screened for their antimicrobial activities. Bioactive assay manifested that most of the azolylthioether quinolones exhibited good antimicrobial activities. Especially imidazolylthioether quinolone **4e** displayed remarkable anti-MRSA and anti-*P. aeruginosa* efficacies with low MIC values of 0.25 μg/mL, even superior to reference drugs. They induced bacterial resistance more slowly than clinical drugs. Molecular docking study indicated strong binding interaction of compound **4e** with topoisomerase IV-DNA complex, which correlated with the inhibitory effect. The preliminarily interactive investigation of compound **4e** with genomic DNA isolated from MRSA bacteria revealed that compound **4e** could intercalate into MRSA DNA through copper ion bridge to form a steady **4e**–Cu^{2+}–DNA ternary complex which might further block DNA replication to exert the powerful bioactivities.

1. Introduction

Azolylthioethers are an important class of structural fragments for bioactivity in a number of natural and synthetic biologically active compounds. Lots of azolylthioether derivatives have been prevalent drugs such as anticancer imidazoylthioether Azathioprine, antibacterial imidazoylthioether Cefodizime, antibacterial tetrazolylthioether Cefmenoxime and herbicidal tetrazolylthioether Azimsulfuron and so on (Figure 1). [1] This kind of compounds exerts their function by transforming thioether group into thiol moiety, thus playing an essential role in the process of bacterial inactivation. [2] Ectogenesis thiol compounds could not only maintain the functional and structural integrity of many important proteins in biology, regulate the rate of intra-cellular oxidoreductive species, but also participate in regulation of various physiological functions through different mechanism. Moreover, the sulfur moiety as an electron-rich center is able to improve lipophilicity and modulate electron density of the azole ring, thereby influencing the transmembrane diffusion ability to the anticipant targets, as well as the interaction with hydrogen bond donors of the organism. [3] Recently, a large number of azolylthioether derivatives as antimicrobial agents with medicinal value are being actively exploited. [4-6] Our previous work reported structurally simple triazolylthioether derivative **WQP** with strong antimicrobial efficacy against *E. typhosa* (MIC = 16 μg/mL) and *P. aeruginosa* (MIC = 16 μg/mL), which was comparable to the reference drug Chloromycin. [7] All above have strongly pointed out spacious potentiality of azolylthioether compounds as medicinal agents.

Quinolones are the most important synthetic antibacterial agents with large potentiality in urinary tract, respiratory and bone joint infections as well as sexually transmitted diseases, prostatitis, pneumonia and acute bronchitis. [8,9] However, its increasingly serious drug resistance caused by the prevalently clinical use and the limited activity against clinically important Gram-positive pathogens have attracted a large amount of effort towards further researches of quinolones to develop new more effective antibacterial agents with broader antimicrobial spectrum and better therapeutic index. [10,11] Recent studies of quinolone antibacterial agents showed that the basic group at the C–7 position is the most adaptable site for chemical change and an area that greatly influences their potency, spectrum and safety. [12] Much work has been directing toward the development of new quinolones with the goal of further extending the antibacterial spectrum and overcoming the drug-resistance.

Azole ethanol fragment was revealed to be highly beneficial for antimicrobial potency. Numerous clincal drugs containing azole ethanol fragments such as imidazolyl ethanols (e.g. Metronidazole, Secnidazole and Ornidazole) and triazolyl

Key Laboratory of Applied Chemistry of Chongqing Municipality, Institute of Bioorganic & Medicinal Chemistry, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China.
† *Postdoctoral fellow from Indian Institute of Chemical Technology (IICT), India*
* *Corresponding authors; E-mail: zhouch@swu.edu.cn (C. H. Zhou); Tel.: +86-23-68254967; Fax: +86-23-68254967*
† Electronic supplementary information (ESI) available: Experimental procedures for our prepared compounds and bioassay procedures; Spectral data for the prepared compounds

ethanols (e.g. Fluconazole, Voriconazole) have been applied in clinic for many years to treant microbial infections. Therefore, such fragments were widely employed in the exploit of new drug molecules. They have become useful medicinal fragments in drug discovery and the introduction was revealed to be able to improve the bioactivity of target compounds. [13] Moreover, linkage of a hydroxyl group to the C–7 side chain of quinolones was revealed to led to increased *in vitro* and *in vivo* antibacterial activity. [14] Reasonably, the introduction of azolyl ethanol into quinolone ring might be an effective strategy to overcome quinolone-resistance and increase biological activity.

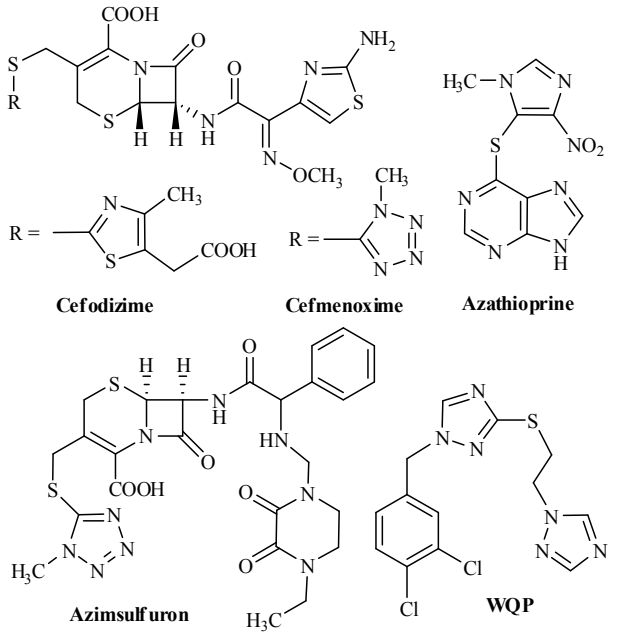


Figure 1 Structures of some azolylthioether-based bioactive compounds.

However, to the best of our knowledge, azolylthioether ethanol modified quinolones have been seldom reported. Thus, importantly antimicrobial tetrazolyl thioether fragment and some other azolyl thioethers derived from imidazole-thiol and triazole-thiol were incorporated into target molecules in order to explore the effect of azoyl thioethers on antimicrobial activity, which were expected to have large roles in the treatment of disease-caused bacteria and fungi. Meanwhile, some new triazole-thiols were also prepared to investigate the effects of the thiol substituent in azole ring on antibacterial and antifungal activities. In view of the above observations, a series of novel azolylthioether ethanol modified quinolones were designed (Figure 2) and prepared from commercial quinolones and 2-(chloromethyl)oxirane according to the synthetic routes shown in Scheme 1. The phenyl moieties bearing chloro group were introduced into target compounds due to the reason that they could usually improve the pharmacological properties by enhancing the rate of absorption and transport of drugs *in vivo*. [15]

The antibacterial and antifungal potency for all newly synthesized compounds were evaluated *in vitro* against four Gram-positive bacteria including Methicillin-Resistant *Staphylococcus aureus* N315 (MRSA), four Gram-negative bacteria and five fungi. The preliminary antimicrobial mechanism was investigated by evaluating the interactions of the prepared highly active compound with MRSA DNA.

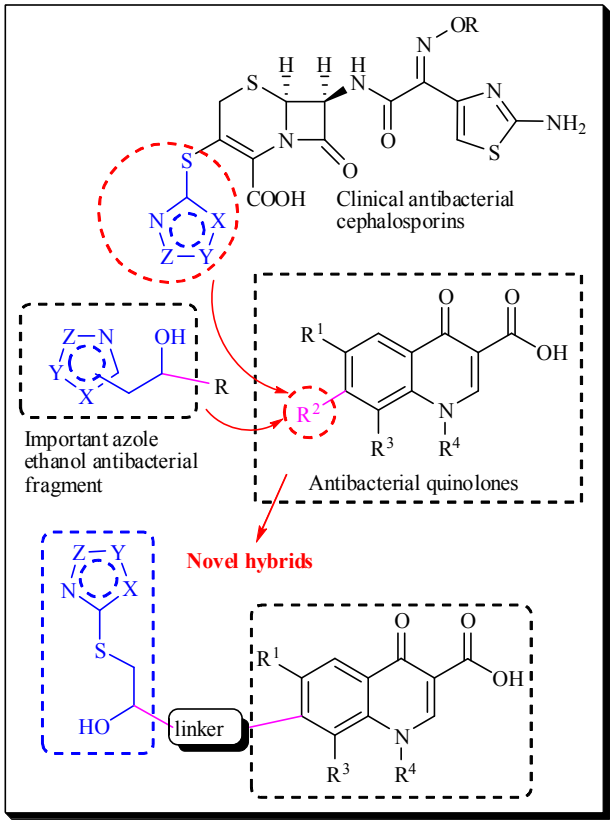


Figure 2. Design of novel azolylthioether quinolones.

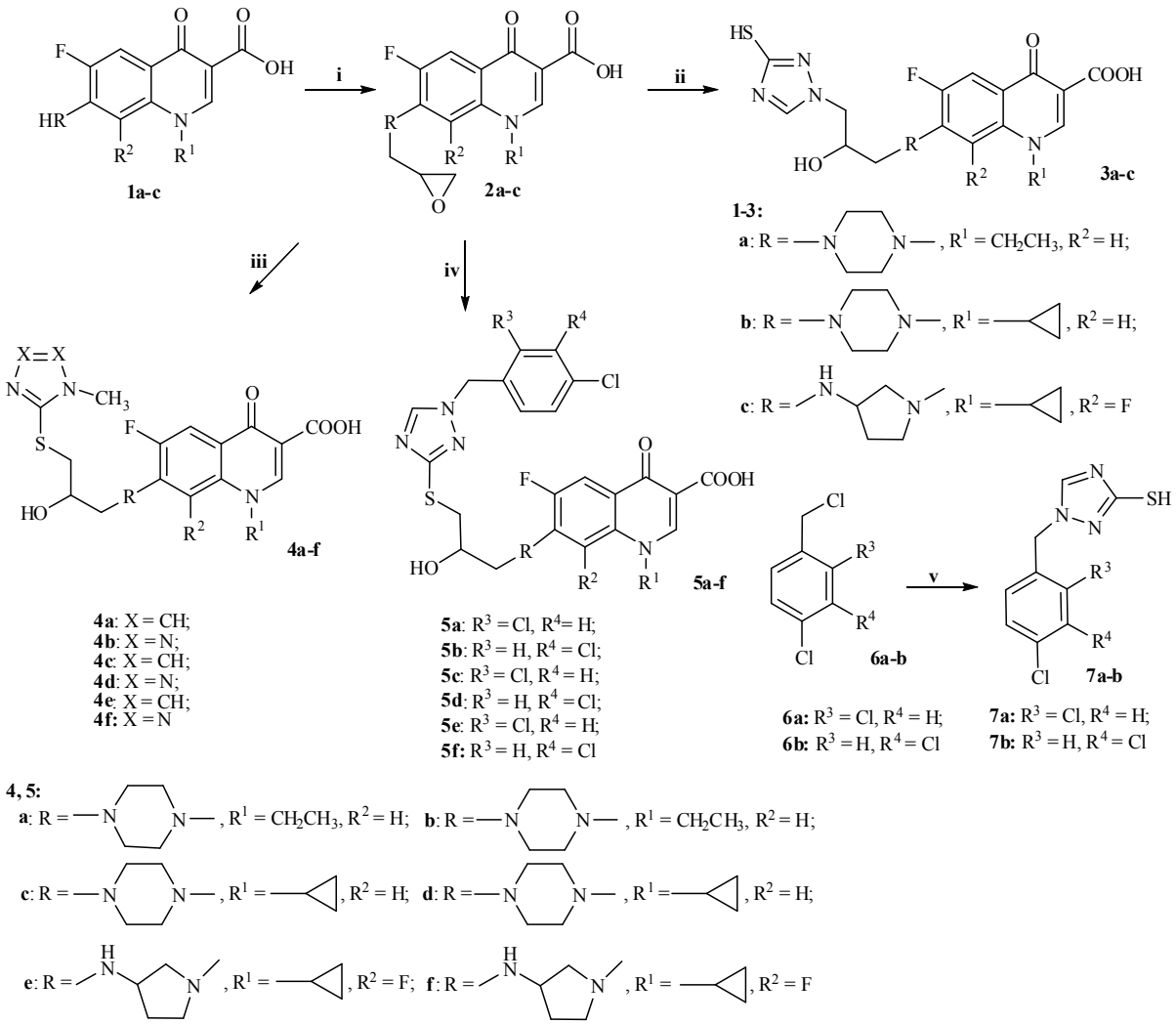
2. Chemistry

The target azolylthioether quinolones were synthesized according to the synthetic route outlined in Scheme 1. Intermediates **2a–c** were prepared in the yields of 52.4–67.4% by *N*–alkylation of commercially available quinolones with 2-(chloromethyl)oxirane and then treatment with formic acid to adjust the pH value to 5.5–6.5. Commercially convenient azoles in acetonitrile were reacted with compounds **2a–c** respectively at 75 ºC in the presence of potassium carbonate as base to produce the target quinolone azoles **3a–c**, **4a–f** and **5a–f** in 20.5–35.4% yields through simple treatment with formic acid to adjust the pH value to 5.5–6.5. The intermediates **7a–b** were prepared by the reaction of thiosemicarbazide with halobenzyl halides **6a–b** *via* a multi-component procedure without isolation of intermediates in satisfactory yields (82.3% and 84.1%) when dissolved in ethanol in the absence of base. [16] All the new compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR, MS and HRMS spectra (Supporting Information 1).

3. Results and discussion

3.1. Antimicrobial activities

The obtained results as depicted in Table 1 revealed that azolylthioether quinolones **3a–c**, **4a–f** and **5a–f** could effectively inhibit the growth of all the tested bacterial and fungi strains with MIC values of 0.25–256 μg/mL. Excitingly, some prepared compounds were even more active than the reference drugs. Particularly, compounds **4e**, **5d** and **5f** showed broad antimicrobial spectrum and excellent antibacterial activities in comparison with other compounds.



Scheme 1 Synthetic routes of azolylthioether quinolones. Reagents and conditions: (i) 2-(chloromethyl)oxirane, CH_3CN, rt, 24 h; HCOOH, rt, 1 h; (ii) triazole-thiol, K_2CO_3, acetonitrile, 70 ºC, 20 h; HCOOH, rt, 1 h; (iii) azole-thiols, K_2CO_3, acetonitrile, 70 ºC, 20 h; HCOOH, rt, 1 h; (iv) triazole-thiols **7a–b**, K_2CO_3, acetonitrile, 70 ºC, 20 h; HCOOH, rt, 1 h; (v) $NH_2NHCSNH_2$, CH_3CH_2OH, 40 ºC; HCOOH, H_2SO_4, H_2O, 100 ºC.

Table 1 showed the significant effects of the types of azole rings on biological activity. Among them, imidazolylthioether functionalized compound **4e** gave the best antibacterial activity against *P. aeruginosa* and MRSA with MIC values of 0.25 μg/mL which was more active than Clinafloxacin (MIC = 1 μg/mL). The substitution of imidazolylthioether with thiol-triazole or tetrazolylthioether group which yielded compounds **3a–c**, **4b**, **4d** and **4f** resulted in relatively lower activity towards the tested strains. However, compounds **5d** and **5f** bearing benzyltriazole-thioether groups displayed fairly good antibacterial activities with the MIC values in the range of 0.25–32 μg/mL in comparison with reference drugs Chloramphenicol (MIC = 8–32 μg/mL) and superior to respective Norfloxacin (MIC = 1–8 μg/mL), Ciprofloxacin (MIC = 1 μg/mL) and Clinafloxacin (MIC = 0.5–1 μg/mL). It indicated that introduction of phenyl group on thiol-triazole derivatives seemed to be favorable for their antimicrobial efficacy. These results revealed that existence of azolylthioether moiety especially imidazolylthioether in this series of quinolone derivatives should be of special importance in the antibacterial profiles.

Excitedly, the target compounds **4e**, **5d** and **5f** exhibited good biological activity against MRSA with quite low MIC values of 0.25–1 μg/mL which were more active than Chloramphenicol (MIC = 16 μg/mL). Especially, quinolone imidazolylthioether **4e** (MIC = 0.25 μg/mL) exerted superior anti-MRSA activity to Norfloxacin (MIC = 2 μg/mL), Ciprofloxacin (MIC = 1 μg/mL) and Clinafloxacin (MIC = 1 μg/mL). These implied that this new type of quinolone azoles should have the large potentiality to be developed as anti-MRSA agents.

It was also noteworthy that compound **4e** demonstrated effective anti-*P. aeruginosa* activity with MIC value of 0.25 μg/mL, which was 64-fold more potent than the reference drug Chloramphenicol and 4-fold more active than Clinafloxacin. Meanwhile, triazolylthioether **5f** showed low MIC values of 1 μg/mL against *P. aeruginosa*. Notably, bacterium *E. typhosa* was also more sensitive to target compounds **4e** (MIC = 0.5 μg/mL), **3c** (MIC = 0.5 μg/mL), **5d** (MIC = 0.5 μg/mL) and **5f** (MIC = 0.5 μg/mL) than Chloramphenicol (MIC = 32 μg/mL), Norfloxacin

(MIC = 1 μg/mL), Ciprofloxacin (MIC = 1 μg/mL) and Clinafloxacin (MIC = 1 μg/mL), but only weak inhibitory activity was observed for compounds **3a**, **4a**–**b**, **5a**–**b** and **7a**–**b**.

Table 1. *In vitro* antimicrobial data as MIC (μg/mL)[a, b, c] for compounds **2–7**

Compds	Fungi					Gram-positive bacteria				Gram-negative bacteria			
	C. utilis	*A. flavus*	*S. cervisea*	*C. albicans*	*C. mycoderma*	*M. luteus*	MRSA	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*	*B. proteus*	*E. typhosa*
2a	8	1	1	4	1	8	2	8	64	16	2	4	16
2b	2	1	1	2	1	2	1	4	8	2	1	4	2
2c	4	0.5	0.25	2	2	0.25	2	0.5	0.25	0.5	0.5	0.25	1
3a	64	512	128	128	128	64	64	128	128	64	64	128	64
3b	128	64	64	8	32	16	16	2	4	4	8	4	1
3c	4	8	8	2	8	2	4	0.5	0.5	4	1	1	0.5
4a	64	256	128	16	64	32	64	16	16	64	8	64	16
4b	64	64	128	32	64	64	128	128	32	128	8	128	32
4c	128	64	32	4	16	4	2	2	4	8	8	2	1
4d	256	8	8	8	16	8	8	8	8	16	8	4	2
4e	32	32	16	0.5	1	0.5	0.25	0.5	0.5	0.25	1	0.5	0.5
4f	128	1	1	2	1	1	2	2	1	1	1	1	1
5a	64	4	16	64	64	64	2	2	64	16	8	4	32
5b	128	16	32	64	128	32	8	4	128	64	16	8	64
5c	4	16	64	32	16	2	1	1	2	4	2	2	1
5d	2	16	32	16	8	1	1	0.5	0.5	2	1	0.5	0.5
5e	16	64	64	16	4	4	8	2	0.5	1	1	0.5	1
5f	8	8	16	0.5	1	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5
7a	512	256	512	512	128	128	128	512	512	256	256	512	256
7b	512	512	512	512	256	256	128	256	512	512	256	512	512
A	no	no	no	no	no	8	16	8	32	16	16	32	32
B	no	no	no	no	no	2	2	2	1	4	2	8	1
C	no	no	no	no	no	1	1	1	1	1	1	1	1
D	no	no	no	no	no	0.5	1	0.5	0.5	1	1	0.5	1
E	8	256	16	1	4	no	no	no	no	no	no	no	no

[a] Minimum inhibitory concentrations were determined by micro broth dilution method for microdilution plates.

[b] MRSA, Methicillin-Resistant *Staphylococcus aureus* N315; *S. aureus, Staphylococcus aureus* ATCC25923; *B. subtilis, Bacillus subtilis* ATCC6633; *M. luteus, Micrococcus luteus* ATCC4698; *E. coli, Escherichia coli* JM109; *P. aeruginosa*, *Pseudomonas aeruginosa* ATCC27853; *B. proteus*, *Bacillus proteus* ATCC13315; *E. typhosa, Eberthella typhosa* ATCC14028; *C. utilis, Candida utilis* ATCC9950; *A. flavus, Aspergillus flavus* ATCC204304; *S. cervisea, Sacchromycese cervisea* ATCC9763; *C. albicans, Candida albicans* ATCC10231; *C. mycoderma*, *Candida mycoderma* ATCC9888.

[c] **A** = Chloromycin, **B** = Norfloxacin, **C** = Ciprofloxacin, **D** = Clinafloxacin, **E** = Fluconazole.

The intermediates **2a**–**c** with oxiran-2-ylmethyl groups at C–7 position of quinolones, as shown in Table 1, also displayed moderate to good activities against all the tested bacterial strains in comparison with reference drugs. Particularly, compound **2c** gave the better antimicrobial efficiencies with MIC values of 0.25 μg/mL against *M. luteus* and *B. subtilis*, than the corresponding reference drug Clinafloxacin (MIC = 0.5 μg/mL). For Gram-negative *P. aeruginosa*, *E. coli* and *B. proteus* strains, compound **2c** (MIC = 0.5, 0.5 and 0.25 μg/mL, respectively) displayed stronger inhibitory activity than Clinafloxacin (MIC = 1, 1 and 0.5 μg/mL, respectively). The *in vitro* antifungal evaluation also revealed that the intermediates **2a**–**c** exhibited generally good antifungal activities against most of the tested fungal strains in comparison with those of quinolone imidazoles.

3.2 Development of resistance to compound 5a

Considering the high-level resistance of Norfloxacin to MRSA strains, [16,17] the representative compound **5a** was further selected to investigate the developing rate of bacterial resistance. We exposed a standard strain of MRSA towards increasing concentrations of compound **5a** from sub-MIC for sustained passages and determined the MIC values of compound **5a** for each passage of MRSA. After 25 passages of growth in the presence of 2 μg/mL of compound **5a**, bacterial resistance did not increase any further, and the MIC value was only 4 μg/mL. By contrast, MRSA quickly developed resistance to Norfloxacin, showing resistance to the original MIC (2 μg/mL) after 6 passages. This assay indicated that bacteria MRSA do not develop resistance against compound **5a** as easily as they do against Norfloxacin. [18]

3.3 Molecular modeling

To rationalize the observed antibacterial activity and understand the possible mechanism of the target azolylthioether quinolones, a flexible ligand receptor docking investigation was undertaken. The crystal structure data (topoisomerase IV-DNA complex) were obtained from the protein data bank, which was representative target to investigate the antibacterial mechanism of quinolones. [19] Target compound **4e** was selected to dock with the topoisomerase IV-DNA complex.

According to the docking evaluation, imidazolylthioether quinolone **4e** possessed good total score (8.60) and showed high binding energy (49.0 kJ/mol) against topoisomerase IV-DNA complex. Method validation and interaction of compound **4e** with topoisomerase IV-DNA receptor were shown in Figures 3(i) and 3(ii). The docking result of compound **4e** with topoisomerase IV-DNA complex might rationalize the possible antibacterial mechanism. The carboxyl group of this molecule was in close

vicinity to the residue SER-79 of the topoisomerase IV-DNA complex. The hydroxyl group in the 7-position of quinolone ring was in close proximity to the residue ARG-456, which indicated the necessity of hydroxyl group for the increased bioactivity. The molecule **4e** could also form hydrogen bonds with ASN-473 and GLU-474 of topoisomerase IV-DNA complex through the nitrogen atom of imidazolylthioether moiety. Furthermore, compound **4e** could intercalate into superhelical DNA of the enzyme-DNA complex (Supporting Information: Figure S3). This cooperative binding might be beneficial to stabilize the quinolone-DNA-enzyme complex, which might be responsible for the strong inhibitory efficacy of compound **4e** against MRSA.

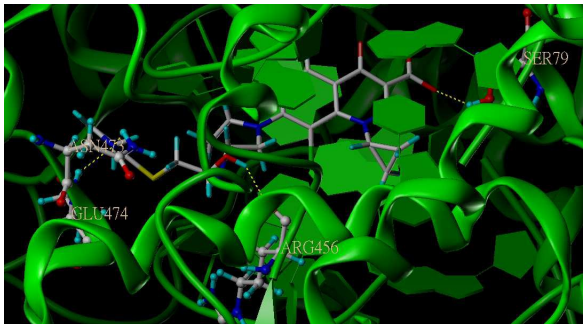


Figure 3(i) Three-dimensional conformation of compound **4e** docked in topoisomerase IV-DNA complex (i)

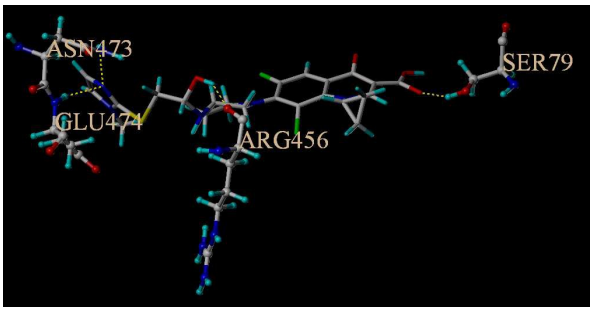


Figure 3(ii) Three-dimensional conformation of compound **4e** docked in topoisomerase IV-DNA complex (ii)

3.4 Interactions with MRSA DNA

The highly active potency of the prepared compounds against MRSA encouraged us to explore the possible antimicrobial action mechanism. Therefore, we isolated the DNA from MRSA bacteria by lyses, digestion, precipitation, and concentration four-step process (Supporting Information 3). The interactive studies of the high bioactive compound **4e** with MRSA DNA on molecular level were carried out *in vitro* by UV–vis and fluorescence spectroscopic methods.

3.4.1 Absorption spectra of DNA in the presence of compound 4e

With a fixed concentration of DNA, UV–vis absorption spectra were recorded with the increasing amount of compound **4e**. As shown in Figure 4, UV–vis spectra displayed that the maximum absorption peak of DNA at 260 nm exhibited proportional increase and slight red shift with the increasing concentration of compound **4e**. Meanwhile, the phenomenon indicated that the measured value of the simply sum of free DNA and free compound **4e** was a little greater than the absorption value of **4e**–DNA complex. This meant that a weak hypochromic effect existed between DNA and compound **4e**. Moreover the intercalation of the aromatic chromophore of compound **4e** into the helix and the strong overlap of π-π* states of the large π-conjugated system with the electronic states of DNA bases were consistent with the observed spectral changes. [20,21]

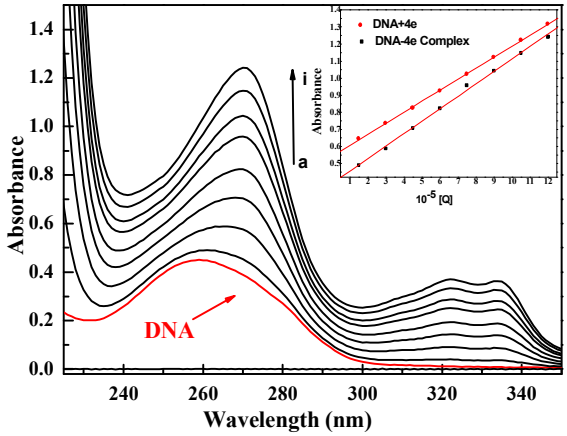


Figure 4 UV absorption spectra of DNA with different concentrations of compound **4e** (pH = 7.4, T = 290 K). Inset: comparison of absorption at 260 nm between the **4e**–DNA complex and the sum values of free DNA and free compound **4e**. *c*(DNA) = 3.56 × 10^{-5} mol/L, and *c*(compound **4e**) = 0–1.2 × 10^{-5} mol/L for curves *a*-*i* respectively at increment 0.15 × 10^{-5}.

3.4.2 Iodide quenching experiments

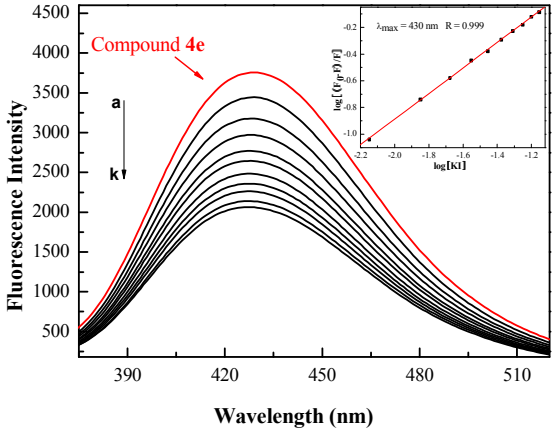


Figure 5 Fluorescence spectra of compound **4e** with increasing concentration of KI. *c*(compound **4e**) = 2.0 × 10^{-6} mol/L; *c*(KI)/(7.0 × 10^{-3} mol/L), *a*–*k*: from 0.0 to 3.0 at increments of 0.3; red line shows the fluorescence spectrum of compound **4e** only; T = 290 K, λ_{ex} = 280 nm. Inset: Stern–Volmer plot of the fluorescence titration data of compound **4e**.

Steady-state emission quenching experiments using KI as quencher might provide further information about the binding of complexes with DNA. In the presence of DNA, the Stern–Volmer plots were changed. This could be explained by repulsions of the highly negative I^- ion from the DNA polyanion backbone which hinders access of I^- ion to the DNA bound complexes. A larger slope for the Stern–Volmer curve parallels poorer protection and low binding. KI fluorescence quenching curves of compound **4e** with and without DNA were given in Figure 5 and Figure 6.

The composition of the binary complex can be deduced from the following equations. [22]

$$M + nL = ML_n \quad (1)$$

$$\log[(F_0/F)-1] = \log K_a + n\log[M] \quad (2)$$

Where M is the quencher, L is the drug molecule with a fluorophore, ML_n is binary complex whose resultant constant is K_a. A plot of log[(F_0/F)-1] versus log[M] will give straight line with a slope of n and y-axis intercept logKa.

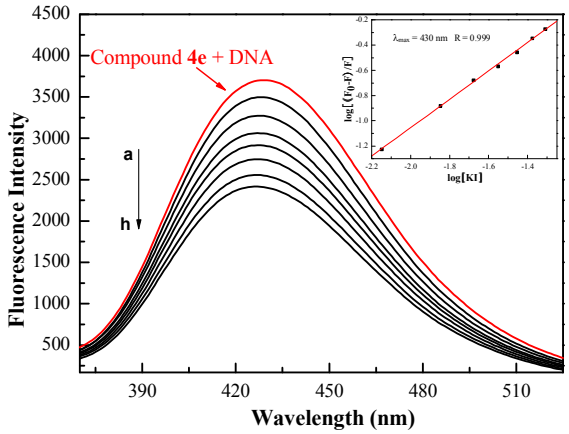


Figure 6 Fluorescence spectra of compound **4e** and DNA system with increasing concentration of KI. *c*(compound **4e**) = 2.0 × 10^{-6} mol/L; *c*(DNA) = 3.3 × 10^{-6} mol/L; *c*(KI)/(7.0 × 10^{-3} mol/L), *a*–*h*: from 0.0 to 2.1 at increments of 0.3; red line shows the emission spectrum of compound **4e** and DNA system only; T = 290 K, λ_{ex} = 280 nm. Inset: Stern–Volmer plot of the fluorescence titration data of compound **4e** and DNA system.

As shown in Figure 5 and Figure 6, the addition of KI resulted in extensive quenching of the fluorescence intensity. The quenching data were plotted according to the Equation 2 and the slopes were calculated by the linear least–squares method. The observed quenching constants were 10.59 L/mol (R = 0.999, SD = 0.007) and 15.74 L/mol (R = 0.999, SD = 0.016) without and with MRSA DNA, respectively. The quenching of the compound **4e** fluorescence was in fact enhanced when the compound **4e** was bound to the DNA helix. Ka values were used to deduce the interaction mode of the fluorescence probe with DNA. Higher binding constants should correspond to the better protection by the DNA and a stronger inhibition of quenching by anionic species. Thus, we can conclude that compound **4e** with DNA was not of intercalation nature, but electrostatic interaction with the anionic sugar phosphate backbone of DNA. [23]

3.4.3 The influence of Cu^{2+} ion on fluorescence properties of compound 4e

The interaction between compound **4e** and Cu^{2+} ion was investigated (Supporting Information 3: Figure S4) and it showed that compound **4e** had very intense intrinsic fluorescence in aqueous solution at $\lambda_{ex}/\lambda_{em}$ = 280/430 nm. The fluorescence intensity of 6.0×10^{-6} mol/L compound **4e** solution at 430 nm dropped regularly with the increasing concentration of copper(II) ion. This may be ascribed to a strong interaction between compound **4e** and copper(II) ion.

The data (Supporting Information 3: Figure S5) were well fitted to Equation 2 and the slope was 1.23, the correlation coefficient was 3.52, respectively. The result showed that compound **4e** could form a stable 1:1 complex with Cu^{2+} ion.

3.4.4 The influence of MRSA DNA on fluorescence properties of compound 4e in the presence of Cu^{2+} ion

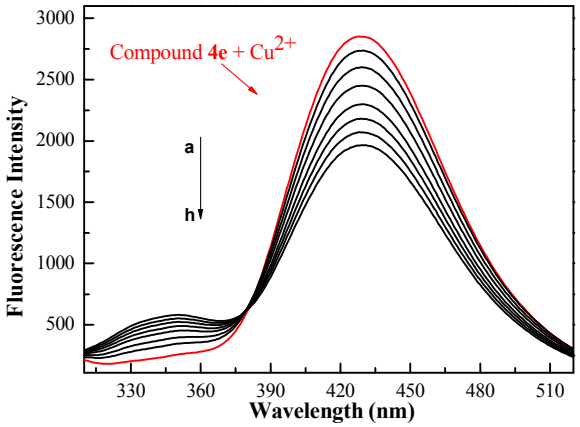


Figure 7 Fluorescence spectra of compound **4e** and Cu^{2+} system with increasing concentration of DNA, λ_{ex} = 280 nm: *c*(compound **4e**) = 6.0 × 10^{-6} mol/L, *c*(Cu^{2+})/(1.0 × 10^{-5} mol/L), *c*(DNA)/(3.3 × 10^{-6} mol/L), *a*–h: from 0.0 to 0.7 at increments of 0.1; red line shows the emission spectrum of compound **4e** in the presence of Cu^{2+} ion only; T = 290 K, λ_{ex} = 280 nm.

After many attempts, compound **4e** was discovered to be able to unwind the DNA double helix in the presence of appropriate amount of Cu^{2+} ion, and the amount was almost equivalent with intracellular quantity in bacteria. The fluorescent variation of compound **4e** in the presence of constant concentration of Cu^{2+} ion was monitored during titration with different concentrations of MRSA DNA (Figure 7). As shown in Figure 7, when DNA solution was added, the fluorescence intensity of compound **4e** was quenched remarkably, and the quenching degree was aggravated with the increasing of the concentration of DNA in aqueous solution. The result implied that there was competition between the DNA and Cu^{2+} ion when metal ion coexisted with DNA in solution of compound **4e**, and it might suggest the formation of a ternary complex among DNA, compound **4e** and Cu^{2+} ion.

The data (Supporting Information 3: Figure S6) were well fitted to Equation 2 and the slope was 1.13, the correlation coefficient was 8.0×10^4, respectively. The result indicated that compound **4e** in the presence of Cu^{2+} ion can form a stable 1:1 complex with DNA.

The interactive mode deduced from the above fluorescence experimental results indicated that a ground-state binary complex was formed between compound **4e** and Cu^{2+} ion, and a ground-state ternary complex also can be formed among Cu^{2+} ion, compound **4e** and DNA.

4. Conclusion

In conclusion, a class of novel azolylthioether quinolones were successfully synthesized *via* a convenient and efficient synthetic route. All the newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, MS, IR and HRMS spectra. The biological results revealed that some target compounds exhibited good antibacterial and antifungal activities against most of the tested strains, specially against MRSA even superior to reference drugs. They induced bacterial resistance more slowly than clinical drugs. Molecular docking study indicated strong binding interaction of compound **4e** with topoisomerase IV-DNA complex, which correlated with the inhibitory effect. The interactive investigations of compound **4e**, genomic DNA isolated from MRSA bacteria and Cu^{2+} ion indicated a possible

action mechanism. Compound **4e** formed a binary complex with Cu^{2+} ion initially, then formed complex with DNA by a intercalated binding form, and finally released Cu^{2+} ion to form interaction between compound **4e** and DNA, which might further block DNA replication to exert their powerful antimicrobial activities. All these results should suggest a promising starting point to optimize the structures of azolylthioether quinolones to access potent antimicrobial agents. Further researches, including the *in vivo* bioactive evaluation along with the toxicity investigation, the action mechanism by use of gel electrophoresis, as well as their corresponding metal complexes and salts (hydrochloride, nitrate, acetate and lactate) are now in progress. All these will be discussed in the future paper.

Acknowledgments

This work was partially supported by National Natural Science Foundation of China [(No. 21172181, 21372186), the Research Fund for International Young Scientists from International (Regional) Cooperation and Exchange Program NSFC (No. 81350110523, 81450110094)], the Key Program of Natural Science Foundation of Chongqing (CSTC2012jjB10026), the Specialized Research Fund for the Doctoral Program of Higher Education of China (SRFDP 20110182110007) and Chongqing Special Foundation for Postdoctoral Research Proposal (Xm2014127).

Notes and References

1 (a) L. Zhang, X. M. Peng, G. L. V. Damu; R. X. Geng and C. H. Zhou, *Med. Res. Rev.*, 2014, **34**, 340–437; (b) X. M. Peng, G. X. Cai and C. H. Zhou, *Curr. Top. Med. Chem.*, 2013, **13**, 1963–2010; (c) L. L. Dai, S. F. Cui, G. L. V. Damu and C. H. Zhou, *Chin. J. Org. Chem.*, 2013, **33**, 224–244 (in Chinese). (d) Y. L. Luo, B. Kishore, K. V. Kumar, C. H. Zhou and G. X. Cai, *Sci. China: Chem*., 2015, **58**, 483–494.

2 S. Y. Liau , D. C. Read, W. J. Pugh, J. R. Furr, and A. D. Russell, *Lett. Appl. Microbiol.*, 1997, **25**, 279–283.

3 T. Uno, H. Kondo, Y. Inoue, Y. Kawahata, M. Sotomura, K. Iuchi and G. Tsukamoto, *J. Med. Chem.,* 1990, **33**, 2929–2932.

4 (a) B. P. Mallikarjuna, B. S. Sastry, G. V. S. Kumar, Y. Rajendraprasad, S. M. Chandrashekar and K. Sathisha, *Eur. J. Med. Chem.*, 2009, **44**, 4739–4746; (b) G. Bonanomi, S. Braggio, A. M. Capelli, A. Checchia, R. D. Fabio, C. Marchioro, L. Tarsi, G. Tedesco, S. Terreni, A. Worby, C. Heibreder and F. Micheli, *ChemMedChem*, 2010, **5**, 705–715; (c) G. V. S. Kumar, Y. Rajendraprasad, B. P. Mallikarjuna, S. M. Chandrashekar and C. Kistayya, *Eur. J. Med. Chem*., 2010, **45**, 2063–2074.

5 (a) S. L. Zhang, G. L. V. Damu, L. Zhang, R. X. Geng and C. H. Zhou, *Eur. J. Med. Chem.,* 2012, **55**, 164–175; (b) F. F. Zhang, L. L. Gan and C. H. Zhou, *Bioorg. Med. Chem. Lett*., 2010, **20**, 1881–1884; (c) Y. Y. Zhang and C. H. Zhou, *Bioorg. Med. Chem. Lett*., 2011, **21**, 4349–4352; (d) S. F. Cui, Y. Wang, J. S. Lv, G. L. V. Damu and C. H. Zhou, *Sci. Sin.: Chim.,* 2012, **42**, 1105–1131 (in Chinese); (e) Y. Shi and C. H. Zhou, *Bioorg. Med. Chem. Lett*., 2011, **21**, 956–960; (f) C. H. Zhou, L. L. Gan, Y. Y. Zhang, F. F. Zhang, G. Z. Wang, L. Jin and R. X. Geng, *Sci. China, Ser B: Chem*., 2009, **52**, 415–458; (g) S. L. Zhang, J. J. Chang, G. L. V. Damu, B. Fang, X. D. Zhou, R. X. Geng and C. H. Zhou, *Bioorg. Med. Chem. Lett*., 2013, **23**, 1008–1012; (h) C. H. Zhou and Y. Wang, *Curr. Med. Chem.*, 2012, **19**, 239–280; (i) S. L. Zhang, J. J. Chang, G. L. V. Damu, R. X. Geng and C. H. Zhou, *Med. Chem. Commun*., 2013, **4**, 839–846.

6 Q. P. Wang, J. Q. Zhang, G. L. V. Damu, K. Wan, H. Z. Zhang and C. H. Zhou, *Sci. China: Chem*., 2012, **55**, 2134–2153.

7 D. J. S. Prasad, M. Ashok, P. Karegoudar, B. Poojary, B. S. Holla and N. S. Kumari, *Eur. J. Med. Chem.*, 2009, **44**, 551–557.

8 (a) A. Tamilselvi and G. Mugesh, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 3692–3697; (b) Z. Z. Li, Z. Gu, K. Yin, R. Zhang, Q. Deng and J. N. Xiang, *Eur. J. Med. Chem.*, 2009, **44**, 4716–4720.

9 (a) C. Mugnaini, S. Pasquini and F. Corelli, *Curr. Med. Chem.*, 2009, **16**, 1746–1767; (b) A. Ahmed and M. Daneshtalab, *J. Pharm. Pharm. Sci.,* 2012, **15**, 52–72; (c) V. Uivarosi, *Molecules*, 2013, **18**, 11153–11197.

10 K. J. Aldred, R. J. Kerns and N. Osheroff, *Biochemistry*, 2014, **53**, 1565–1574.

11 (a) J. A. Wiles, B. J. Bradbury and M. J. Pucci, *Expert Opin. Ther. Patents*, 2010, **20**, 1295–1319; (b) N. Hidemasa, S. N. Koko and N. Norihisa, *Inter. J. Antimicrobial Agents*, 2014, **43**, 478–479. (c) S. F. Cui, L. P. Peng, H. Z. Zhang, S. Rasheed, K. V. Kumar and C. H. Zhou, *Eur. J. Med. Chem*., 2014, **86**, 318–334; (d) F. W. Zhou, H. S. Lei, L. Fan, L. Jiang, J. Liu, X. M. Peng, X. R. Xu, L. Chen, C. H. Zhou, Y. Y. Zou, C. P. Liu, Z. Q. He and D. C. Yang, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 1912–1917; (e) S. F. Cui, Y. Ren, S. L. Zhang, X. M. Peng, G. L. V. Damu, R. X. Geng and C. H. Zhou, *Bioorg. Med. Chem. Lett*., 2013, **23**, 3267–3272.

12 (a) H. Y. Han, L. Chen, X. R. Xu, L. Fan, Y. Yang and D. C. Yang, *Sci. Sin.*: *Chim*., 2011, **41**, 461–473; (b) A. Foroumadi, R. Ashraf-Askari, M. H. Moshafi, S. Emami and A. Zeynali, *Pharmazie.*, 2003, **58**, 432–434; (c) Z. Dang, Y. S. Yang, R. Y. Ji and S. H. Zhang, *Bioorg. Med. Chem. Lett*., 2007, **17**, 4523–4526.

13 G. L. V. Damu, S. F. Cui, X. M. Peng, Q. M. Wen, G. X. Cai and C. H. Zhou, *Bioorg. Med. Chem. Lett*. 2014, **24**, 3605–3608.

14 Y. Wang, G. L. V. Damu, J. S. Lv, R. X. Geng, D. C. Yang and C. H. Zhou, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 5363–5366.

15 (a) X. L. Wang, K. Wan and C. H. Zhou, *Eur. J. Med. Chem.*, 2010, **45**, 4631–4639; (b) L. L. Gan, B. Fang and C. H. Zhou, *Bull. Korean Chem. Soc.*, 2010, **31**, 3684–3692; (c) K. Wan and C. H. Zhou, *Bull. Korean Chem. Soc.*, 2010, **31**, 2003–2010.

16 C. Davidovich, A. Bashan, T. Auerbach-Nevo, R. D. Yaggie, R. R. Gontarek and A. Yonath, *Proc. Natl. Acad. Sci. USA.,* 2007, **104**, 4291–4296.

17 Y. Zhao, Y. Tian, Y. Cui, W. Liu, W. Ma and X. Jiang, *J. Am. Chem. Soc.*, 2010, **132**, 12349–12356.

18 I. Chopra, *J Antimicrob. Chemother.,* 2007, **59**, 587–590.

19 B. D. Bax, P. F. Chan, D. S. Eggleston, A. Fosberry, D. R. Gentry, F. Gorrec, I. Giordano, M. M. Hann, A. Hennessy, M. Hibbs, J. Huang, E. Jones, J. Jones, K. K. Brown, C. J. Lewis, E. W. May, M. R. Saunders, O. Singh, C. Spitzfaden, C. Shen, A. Shillings, A. F. Theobald, A. Wohlkonig, N. D. Pearson, M. N. Gwynn, *Nature*, 2010, **466**, 935–940.

20 X. L. Li and Y. J. Hu, *Biomacromolecules*, 2012, **13**, 873–880.

21 G. W. Zhang, P. Fu, L. Wang and M. M. Hu, *J. Agric. Food Chem.*, 2011, **59**, 8944–8952.

22 G. W. Song, Y. He and Z. X. Cai, *J. Fluoresc.*, 2004, **14**, 705–710.

23 L. S. Lerman, *J. Mol. Biol.,* 1961, **3**, 18–30.

A series of azolylthioether quinolones were synthesized and characterized by NMR, IR, MS and HRMS spectra. All the newly prepared compounds were screened for their antimicrobial activities. Bioactive assay manifested that most of the azolylthioether quinolones exhibited good antimicrobial activities. Especially imidazolylthioether quinolone **4e** displayed remarkable anti-MRSA and anti-*P. aeruginosa* efficacies with low MIC values of 0.25 μg/mL, even superior to reference drugs. They induced bacterial resistance more slowly than clinical drugs. Molecular docking study indicated strong binding interaction of compound **4e** with topoisomerase IV-DNA complex, which correlated with the inhibitory effect. The preliminarily interactive investigation of compound **4e** with genomic DNA isolated from MRSA bacteria revealed that compound **4e** could intercalate into MRSA DNA through copper ion bridge to form a steady **4e**–Cu^{2+}–DNA ternary complex which might further block DNA replication to exert the powerful bioactivities.