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Research paper

Free radical rearrangement synthesis and microbiological evaluation of novel 2-sulfoether-4-quinolone scaffolds as potential antibacterial agents

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ABSTRACT

To develop novel antibacterial agents, 2-sulfoether-4-quinolone scaffolds were synthesized by a free radical process and evaluated for their antibacterial abilities. Excellent activities against Gram-positive bacteria were observed, among which compounds **3m**, **3n**, **3p** and **3t** possessed the lowest MICs against both *S. aureus* and *B. cereus* (0.8 μM and 1.61 μM, respectively). The structure-activity relationship (SAR) showed that: (i) the antibacterial activity was related to the substituent, such as 2-SCH_3 = 2-SCH_2CH_3 > 2-$S(=O)CH_3$ > 2-OH, 8-Br > 7-Br > 6-Br; (ii) -CF_3 increased the antibacterial activity; (iii) the di-substituted group performed the better activity. The DNA supercoiling inhibitory analysis confirmed their fluoroquinolone characters. The docking showed that compound **3n** was nicely bound into the DNA-gryase complex *via* extensive interactions, including conventional hydrogen bonds, halogen bonds and hydrophobic interactions. The microscopy analysis of compound **3n** against *S. aureus* exhibited the damages on the cell wall construction, which may facilitate the penetration into Gram-positive bacteria.

1. Introduction

The treatment of infections is facing a major problem caused by antimicrobial resistance (AMR) of the pathogen bacteria, and sometimes even worse, for instance, *Mycobacterium tuberculosis* and *Staphylococcus aureus* have a strong vitality and the rapid multi-antibiotics-resistance (MDR) development capability [1–3]. In contrast, new antibacterial agents are currently being developed at a much slower pace than the growing need. With diminishing available pharmaceutical options, to more effectively counter the threat of AMR, the number of innovative antibiotics must be increased. It could be addressed by identifying new mechanisms, origins and distribution of AMR through the combination of computational modeling and advanced biological tools [4], or by generating new chemicals that can extend the life of existing antibiotics [5,6].

The nitrogen-containing heterocycles are important building blocks in the development of medicinal chemistry [7–9], among of which the quinolones are one of the most early studied and modified [10]. The bacterial type II topoisomerase, including DNA gyrase (also named topoisomerase II) and topoisomerase IV, is the major target of quinolones. Both enzymes are able to remove positive or negative supercoiling. DNA gyrase prefer to perform the intramolecular reactions to resolve chromosomal DNA supercoils, while the topoisomerase IV is prone to the intermolecular reactions, facilitating the decatenation of the linked DNA molecules [11,12]. Thus, both enzymes are the lethal targets of quinolones. As entirely synthetic drugs, the therapeutic 4-quinolone have been studied and modified extensively, resulting four generations with enhanced activity against gastrointestinal infections, respiratory and urinary tract diseases [13–15]. The most efficient approach to seek for novel 4-quinolone antibacterial agents is to synthesize new analogues or modify existing drugs, in combination with the analysis and improvement of the pharmacokinetics and metabolic properties [9,10,16–20]. Since the modification based on 2-substituted-4-quinolone are still not fully investigated [21], which

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prompt us to further understand this type of scaffold.

The quinolone structural motifs are pharmacologically active substances for drug discovery since many of them have been shown to exhibit excellent biological and pharmacological activities [22,23], such as antiparasitic [24–26], antimicrobial [27,28], antiviral [29] and antitumor activity [30,31]. Furthermore, the heterocyclic compounds with nitrogen and sulfur atoms have been identified to possess the most comprehensive spectrum of biological activities [32,33], for which we put our attempts on the synthesis of new heterocyclic derivatives combining both known active quinolone and sulfur atom in one molecular frame. The 2-substituted-4-quinolone was obtained by a free radical process and a series of 2-methylthioquinolin-4(1H)-one derivatives were synthesized to explore the effects of 2-substituents group on antibacterial activity (Fig. 1). Most of the compounds possessed excellent antibacterial activities against Gram-positive bacteria, among which the most potent compound **3n** with 2-SCH_3 and CF_3 di-substitution was used to study the preliminary mechanism of antibacterial activity against *S. aureus*.

2. Results and discussion

2.1. Chemistry

Twenty 2-methylthioquinolin-4(1H)-ones (**3a–3t**, as shown in Table 1) were obtained from its corresponding acetoacetanilide (**1**) (Scheme 1) according to the literature method [33,34]. The intermediate 2-(bis(methylthio)methylene)-3-oxo-N-phenylbutanamide (**2**) were synthesized by the nucleophilic reaction of active methylene group of acetoacetanilide (**1**) with carbon bisulfide in the present of K_2CO_3 and subsequent methylation with dimethyl sulfate. The thermal cyclization of compound **2** gave the title compound **3** in *o*-dichlorobenzene.

Surprisingly, the cyclization doesn't produce 4-methylthio-2-quinolone, but forms 2-methylthio-4-quinolone. The cyclization could be a free radical process (Scheme 2), that is, the themolysis of compound **2** generates a radical intermediate (**2a**) by removing methanethiol, and accompanying with the cleavage of C-N bond, and then the electrophilic addition of alkene free radical to nitrogen-atom produces an intermediate **2b**. Finally, the title compound **3** was obtained from the rearrangement reaction of ortho-H-migration and carbonyl addition of the intermediate **2b**. This offers an efficient synthetic way to this kind of 2-thiosubstituted-4-quinolone.

Further, compounds **3a–3t** were characterized by NMR spectra, high resolution mass spectra (HRMS) and X-ray crystallography. The 2-methylthio-4-quinolone core for each molecule was readily confirmed by observation of the C-atom signals at δ(C) 172–175 C(4-C=O), 153–159 C(2), while the δ(C) of 2-carbonyl C-atom signal is no more than 170 for the 4-methylthio-2-quinolone core [34,35].

To observe the effect of 2-methylthio of 4-quinolone on the antibacterial activity, the oxidation product **4** was obtained from the quinolone **3a** using hydrogen peroxide in AcOH. Further, the 2-methylthio of quinolone **3a** was hydrolyzed in 50% sulfuric acid to afford the hydrolysis product **5** (Scheme 3).

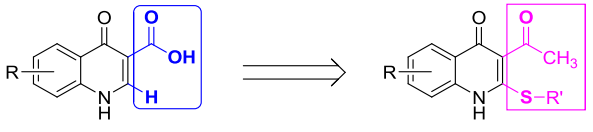
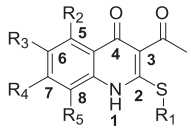


Fig. 1. The general design strategy in this study.

Table 1
The chemical structures of compounds **3a–3t**, **4** and **5**.



Compound	R_1	R_2	R_3	R_4	R_5
3a	SCH_3	H	CF_3	H	H
3b	SCH_3	H	H	CF_3	H
3c	SCH_3	H	H	H	Cl
3d	SCH_3	H	H	H	CH_3
3e	SCH_3	H	CH_3	H	H
3f	SCH_3	H	H	H	OCH_3
3g	SCH_3	H	OCH_3	H	H
3h	SCH_3	H	H	H	Br
3i	SCH_3	H	H	Br	H
3j	SCH_3	H	Br	H	H
3k	SCH_2CH_3	H	CF_3	H	H
3l	SCH_3	H	OCF_3	H	H
3m	SCH_3	CF_3	H	H	Cl
3n	SCH_3	CF_3	H	H	CF_3
3o	SCH_3	CF_3	H	H	OCH_3
3p	SCH_3	H	CN	CF_3	H
3q	SCH_3	CF_3	H	H	F
3r	SCH_3	H	NO_2	H	Cl
3s	SCH_3	H	H	H	H
3t	SCH_3	H	CF_3	H	Cl
4	S=OCH_3	H	CF_3	H	H
5	OH	H	CF_3	H	H

2.2. Crystal structure analysis

The structure of compound **3a** was determined by X-ray crystallography. Crystal data of **3a**: Clear light colorless crystals obtained from EtOH/H_2O, yield, 68%; mp 155–157 °C; $C_{13}H_9F_3NO_2S\cdot H_2O$, $M = 318.29$, Monoclinic, space group $P1\ 21/n\ 1$; $a = 4.6964(6)$, $b = 16.416(2)$, $c = 18.198(2)$ (Å); $\alpha = 90$, $\beta = 94.200(13)$, $\gamma = 90$, $V = 1399.2(3)$ Å^3, $T = 293.48(10)$ K, $Z = 4$, $Dc = 1.511$ g/cm^3, $F(000) = 652$, Reflections collected/Independent reflections = 2743/1436, Data/restraints/parameters = 2743/1/195, Goodness of fit on $F^2 = 1.045$, Fine, $R_1 = 0.0480$, $wR(F^2) = 0.1248$.

Crystal data of **3j**: Clear light colorless crystals obtained from EtOH, yield, 57%; mp 173–174 °C; $C_{12}H_{10}BrNO_2S\cdot CH_3CH_2OH$, $M = 358.25$, Triclinic, space group $P\ -1$; $a = 4.4810(4)$, $b = 9.7061(16)$, $c = 17.5903(19)$ (Å); $\alpha = 101.267(11)$, $\beta = 92.790(8)$, $\gamma = 102.661(11)$, $V = 728.71(16)$ Å^3, $T = 293(2)$ K, $Z = 2$, $Dc = 1.633$ g/cm^3, $F(000) = 364$, Reflections collected/Independent reflections = 4986/2650, Data/restraints/parameters = 2650/1/185, Goodness of fit on $F^2 = 1.040$, Fine, $R_1 = 0.0367$, $wR(F^2) = 0.1022$.

The structures of compounds **3a** and **3j** were determined using MoKα radiation ($\lambda = 0.71073$ Å), which are both 4-quinolones rather than 2-quinolones as shown in Fig. 2. The 2-substituted group is methylthio, which is consistent with it in reference [35]. Crystallographic data (excluding structure factors) for the structure had been deposited with the Cambridge Crystallographic Data Center as supplementary publication Nos. CCDC 1581290 and CCDC 1821733 for compounds **3a** and **3j**, respectively.

2.3. Biological activity

In order to determine the antimicrobial potential of the synthetic quinolone derivatives, they were initially tested against a