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N- 取代苯基 -5- 取代苯基 -3H-1,2,4- 三唑 -3- 硫酮衍生物的合成及抗菌活性研究

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摘要 目的 以苯甲酸为原料，经 4 步反应合成一系列 N- 取代苯基 -5- 取代苯基 -3H-1,2,4- 三氮唑 -3- 硫酮化合物并研究其抗菌活性。**方法** 基于课题组前期对新型潜在三唑类抗菌化合物 6h 的作用机制研究，筛选多个侧链基团，使用乙醇和碳酸钠作溶剂改善最后一步反应条件，通过硅胶柱色谱分离纯化目标化合物，合成一系列 1,2,4- 三唑类化合物并采用质谱 (MS) 和 ^{1}H NMR、^{13}C NMR 进行结构表征。通过琼脂扩散法初步筛选所有化合物对肺炎克雷伯菌、金黄色葡萄球菌和铜绿假单胞菌 3 种常见菌株的抗菌活性，并通过微量稀释法进一步测定它们的最小抑菌浓度 (MIC 值)。**结果** 合成 17 个含有卤代苯基和其他侧链基团的目标化合物，其 MS 以及核磁共振谱图数据表明所有化合物结构正确。抗菌活性初步筛选可知化合物 6**a**、6**b**、6**d**、6**f**、6**g**、6**h**、6**k**、6**m** 和 6**p** 等 9 个化合物具有不错的抑菌能力，其 MIC 测试结果表明，大部分化合物对所测菌株的 MIC 值在 25~100μg/mL 范围内。尤其是化合物 6**h** 和 6**k** 对肺炎克雷伯菌的 MIC 值达到 25μg/mL，抑菌活性与对照药物氨苄西林相当。**结论** 在前期作用机制研究基础上，通过对构效关系的阐述，发现一些侧链片段如间位卤代苯基或对位卤代苯基、三氟甲基苯基等具有吸电子基团的苯基、吡啶基等对 1,2,4- 三唑类衍生物的抗菌活性有明显增强作用，证实侧链基团与受体蛋白形成特异性协调作用和氢键作用从而发挥衍生物的抗菌活性。

关键词：1,2,4- 三唑 -3- 硫酮衍生物；抗菌活性；构效关系
中图分类号：R978.1 **文献标志码**：A

Synthesis and antimicrobial study of novel 4,5-disubstituted aryl-2,4-dihydro-3H-1,2,4-triazole-3-thoxone derivatives

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Abstract Objective To synthesize a series of 4,5-disubstituted aryl-2,4-dihydro-3H-1,2,4-triazole-3-thione derivatives and study their antibacterial activity. **Methods** A novel triazole derivate antibacterial compound 6**h** was discovered as a lead compound. The binding capacity of the active site of 6h was further analyzed in detail

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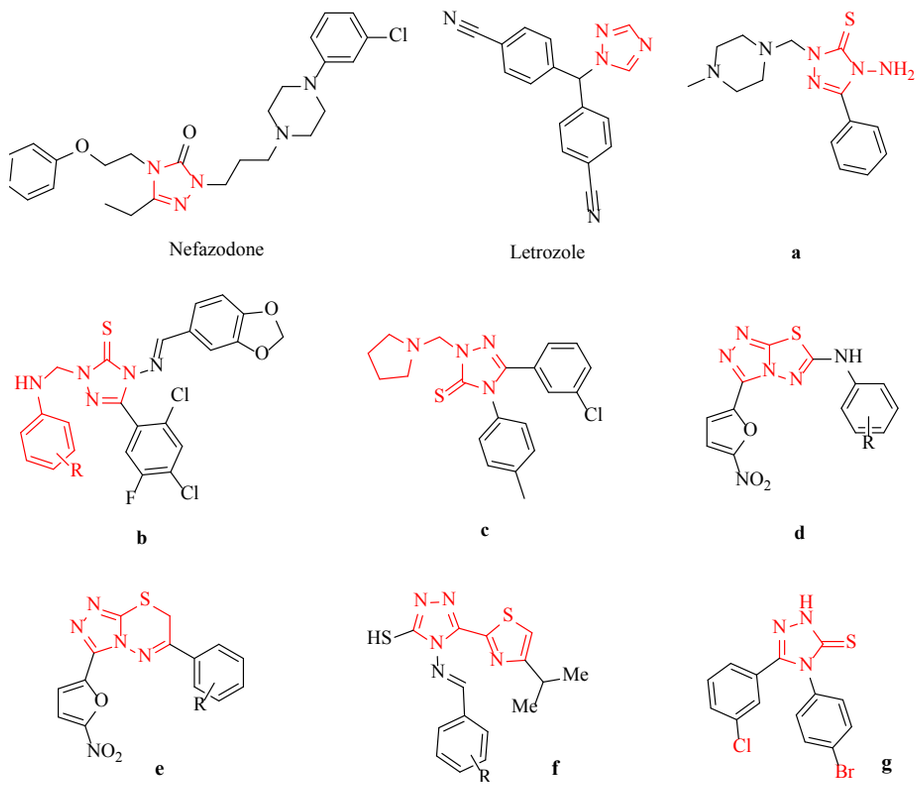
with the target protein by molecular docking experiments in silico. With the assistance of computer-aided design, multiple side chain fragments were high-throughput screened to select the optimal candidate with the triazole core. The 17 4,5-disubstituted aryl-2,4-dihydro-3H-1,2,4-triazole-3-thoxone derivatives were designed and synthesized, together with the characterization by ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was determined by the microdilution method. **Results** The MS and NMR spectra data of the compounds indicated that the newly synthesized compounds were structurally correct. The nine compounds (6**a**, 6**b**, 6**d**, 6**f**, 6**g**, 6**h**, 6**k**, 6**m** and 6**p**) initially screened have antibacterial ability. The MIC values of the above compounds were further tested, and the results showed that most of the compounds of MIC value were in the range of 25~100μg/mL. In particular, the MIC values of compounds 6**h** and 6**k** against *Klebsiella pneumoniae* reached 25μg/mL, and the antibacterial activity was comparable to that of the control drug ampicillin. **Conclusion** A detailed analysis of the structure-activity relationship revealed that some side chain fragments, such as meta or para substituted phenyl, electron-withdrawing trifluoromethyl phenyl, nitrogen-containing pyridine have significant effects on improving the antimicrobial activity of 1,2,4-triazole derivatives.

1 Introduction

Over the past decades, the incidence and mortality of microbial infections has increased significantly due to the widespread abuse of broad-spectrum antibiotics, the extensive use of immunosuppressants in the clinic, organ transplantation and traumatic treatments. It is one of the leading complications associated with cancer and immunodeficiency diseases[1-2]. Therefore, development of highly effective antibacterial drugs are imminent. The 1,2,4-triazole core group is a sort of chemical structural nucleuses, exhibiting a wide range of biological activities, such as anxiolytic, antidepressant[3], antitumor[4], antituberculosis[5-6], anti-inflammatory[7], anticonvulsant[8],cardiovascular treatment[9] and etc. In clinical trials, azole compounds are also antibacterial-like compounds with good pharmacological effects and prominent antibacterial activities, contained in many clinical antibacterial drugs, such as nefazone, letrozole (Fig. 1), Voriconazole (Fig. 2), and etc[10]. At present, most of the antibacterial drugs with 1,2,4-triazole as the mother nucleus are hampered by their intrinsic toxic effect with strong drug resistances[11]. Therefore, it is extremely necessary to develop novel structure of azole antibacterial drugs.

In the last few years, the structural modification on 1,2,4-triazole core group has drawn great attentions[12]. Through comprehensive literature investigations, it is well known that the bioactivity diversity of 1,2,4-triazole derivatives is related to the substituent groups in the side chains[13]. Nitrogen-containing groups play an important role in the antibacterial activities of 1,2,4-triazole derivatives. The antimicrobial activity of 1,2,4-triazole derivatives can be improved by introducing amino[14-15], schiff base[16], hydrazine[17], aniline, pyrrole[18] and other nitrogen-containing groups into the 1,2,4-triazole ring in order to form a bridging structure. For example, N-methylpiperazine-substituted triazolethione mannich compound **a** (Fig. 1) demonstrates a strong antibacterial activity[19]. Aniline-derived triazolethione schiff base compound **b** and tetrahydropyrrolyl schiff base-derived triazolethione compound **c** have a broad spectrum of highly effective antifungal activity[20-21]. It provides strong evidences that nitrogen-containing groups enhances the antibacterial activity of 1,2,4-triazole derivatives. On the other hand, bibliographic retrieval shows that the conjugation of heterocyclic pyridazine, pyrimidine, and pyrazine into 1,2,4-triazole rings forming a double heterocyclic or multi heterocyclic frameworks, which are beneficial to the antitumor activity of 1,2,4-triazole derivatives[22]. For instance, triazolothiazole **d** or triazolothiazine **e** skeleton structure have potential anti-tumor activity[23-24]. In addition, derivatives **f** obtained by the introduction of a thiazole ring to thiatriazole can be used as antitubercular agents[5-6].

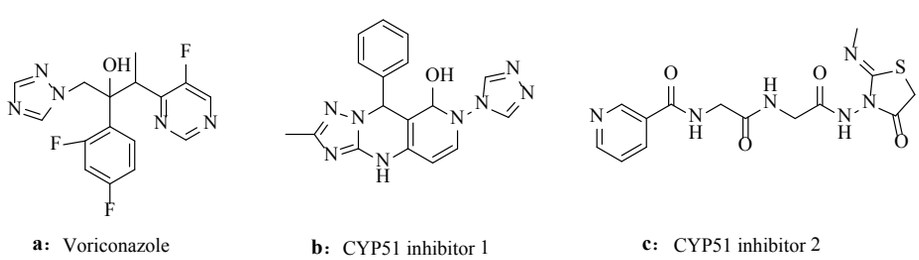
At present, antibacterial drugs with 1,2,4-triazole as the active pharmacodynamic group are developed clinically, acting as the inhibitor to cytochrome p-450-dependent 14α-sterol demethylase (CYP51), thereby blocking the synthesis of ergosterol to achieve the antibacterial effect[25]. In the molecular docking study, CYP51 inhibitors are mainly divided into two parts: the parent nucleus nitrogen-containing group and the



a：4- 氨基 -2-((4- 甲基哌嗪基) 甲基)-5- 苯基 -3H-1, 2, 4- 三唑 -3- 硫酮；**b**：苯胺衍生的三唑硫酮席夫碱化合物；**c**：5-(3- 氯苯基)-2-(吡咯烷基甲基)-4-(对甲苯基) -1, 2, 4- 三唑 -3- 硫酮；**d**: 3-(5- 硝基呋喃 -2- 基)-N- 苯基 -[1, 2, 4] 三唑并 [3, 4-b][1, 3, 4] 噻二唑 -6- 胺化合物与乙烷 (1:1)；**e**: 3-(5- 硝基呋喃 -2- 基)-6- 苯基 -7H-[1, 2, 4] 三唑并 [3, 4-b][1, 3, 4] 噻二嗪；**f**: (E)-4-(苯亚甲基氨基)-5-(4- 异丙基吡啶 -2- 基)-1, 2, 4- 三唑 -3- 硫醇；**g**: 4-(4- 溴苯基)-5-(3- 氯苯基)-1, 2, 4- 三唑 -3- 硫酮；奈法唑酮和来曲唑

图 1　部分已报道的 1,2,4- 三唑 -3- 硫酮衍生物的化学结构

Fig. 1　Partially reported structure of 1,2,4-triazole-3-thione derivatives



a：伏立康唑：具有二氟苯酚侧链的 CYP51 抑制剂；**b**：CYP51 抑制剂 1：具有芳香环苯侧链的 CYP51 抑制剂；**c**：CYP51 抑制剂 2：具有酰胺侧链的 CYP51 抑制剂

图 2　含有不同侧链的 CYP51 抑制剂

Fig. 2　CYP51 inhibitors with different side chains

side chain fragments. Nitrogen atoms on the nucleus of triazoles can coordinate with heme ferroporphyrin in the CYP51 active region, forming hydrogen bond interactions with the CYP51 active site. According to the molecular simulation docking study[26](Fig. 3), the triazole ring in Voriconazole molecule forms hydrophobic cleavage interaction between CYP51 receptor amino acid residues such as HIS374, SER375, ILF376, LEU503, PHE504 and ILE373, forming non-bonding interaction with other residual amino acids and groups such as hydroxyl. The further verification was that nitrogen-containing parent nucleus can form relatively strong coordination with CYP51. Other than the nitrogen-containing core group, other side chain fragments are also important parts of the molecular docking process. The hydrophobic amino acid residues LEU121, TYR132, PRO230, GLY303, LEU376 forms hydrophobic pockets, while the aromatic side chains of the derivatives (Fig. 2) can be placed directly into the hydrophobic pocket. The aromatic side chain also interacts with the alkali amino acid residue HIS377(MIC=32μg/mL,

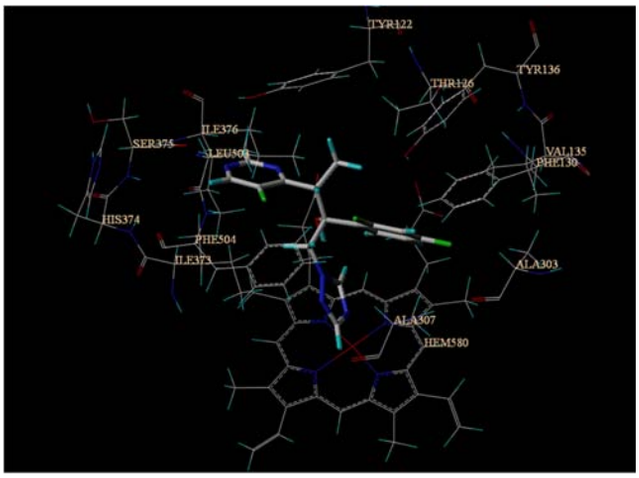


图 3　伏立康唑与 CYP51 活性位点结合模式 [26]

Fig. 3　Computed voriconazole binding to the active site of CYP51[26]

C.alb. Strain100)[27]. The amide side chains of CYP51 inhibitors form two hydrogen bonds with TYR132, in addition to demonstrating hydrophobic effects with THR188, PHE126 and PHE233 (MIC=30μg/mL, *C.alb*)[28]. As a side chain, difluorophenol can insert into a hydrophobic pocket in ALA303, ALA307, VAL135, PHE130, TYR136, HEM580, forming hydrogen bonds with ALA307(MIC=0.0625mg/mL, *C.alb*)[26]. In summary, the nitrogenous groups in the parent nucleus interact with heme and interact with hydrogen bond receptors in the cavity at the bottom of the receptors. The spatial structure of the side chain groups determines the manners in which the inhibitor binds to various amino acid residues of CYP51. The side chains interact with the hydrophobic amino acid residues of the receptors, forming a non-bonding effect. Therefore, the binding capacity of the side chain to the hydrophobic amino acid is tunable in order to further study how the hydrophobic crack acts on the antifungal activities.

The 1,2,4-triazole schiff base compounds are prominent after the introduction of halogen and aromatic ring groups, in particular anti-inflammatory, anti-bacterial, and etc[29]. Many studies have confirmed that the introduction of different haloaryl groups and substituted benzene rings into the pharmacophore structure can effectively regulate the physical and chemical properties of the compounds for the enhancement of antibacterial activity[20]. It was confirmed that the derivative **g** (Fig. 1) was obtained by partially introducing a halogenated phenyl group and a substituted aryl group at the 4-,5-position of 1,2,4-triazole-3-thione, showing a certain degree of antimicrobial activity[30]. In view of this, it is worthwhile that different halophenyl and aryl groups are introduced into the 1,2,4-triazole core group to improve the antimicrobial activity of compounds. Moreover, nitrogenous heterocyclic groups play important roles in the biological activities of 1,2,4-triazole compounds. However, the conjugation of pyridine group to the 1,2,4-triazole ring has not been investigated yet. Here, we intend to introduce the pyridine group into the 1,2,4-triazole ring on the premise of introducing halophenyl group to explore its potential antimicrobial activity. Moreover, the trifluoromethyl-substituted heterocyclic compounds are important in modern medicinal chemistry[31]. We tried to introduce the three fluoromethyl substituted phenyl group into the 1,2,4-triazole core group to increase the antimicrobial activity of the derivatives.

With the help of computer-aided drug design function, we discovered and synthesized a potential new triazole antibacterial compound 6**h**. The compound 6**h** was used for molecular docking with CYP51 to study its ability to bind to the active site of the CYP51 receptor protein. By analyzing the results of the molecular docking studies, we expect to discover more pharmacophores, which can improve the biological activity of compounds. Using compound 6**h** as a lead compound, we screened a number of halogenated phenyl groups, aryl groups, pyridyl groups and trifluoromethylphenyl groups by computer-aided function, based on the principle of pharmacophore splicing, with the connection of triazole skeleton. The synthesis of 17 4,5-disubstituted phenyl-1,2,4-triazole-3-thione compounds was performed. The antibacterial activities of these compounds against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were validated, while their structure-activity relationship was analyzed in detail.

2　**Results**

2.1　Molecular docking

We carried out molecular docking between compound 6**h** and CYP51 protein (PDB: 6AY4) to

study their binding mode (Fig. 4). The two benzene ring side chains of compound 6**h** molecule vertically formed *L*-type molecular conformation with the parent nuclear triazepine, and the chlorine atoms on the two benzene rings played an important role in binding to the active pockets of the target protein molecule. It can be seen that the active pocket of the target protein has a small opening, which has strong selectivity for small molecules, and molecules with larger molecular weight are not easy to enter the pocket. The amino acid residues VAL468, LEU358, MET360, ILE361 of the acceptor molecule form a hydrophobic pocket, and the 2-chlorophenyl side chain is combined with the hydrophobic pocket. Meanwhile, the 2-substituted chlorine atom of the benzene ring forms a hydrogen bond with the hydroxyl group on the TYR120 amino acid residue. Another benzene side chain can be directly inserted into a hydrophobic pocket composed of amino acid residues THR297, GLY294, TYR120, PHE423, CYS430. The 3-position chlorine atom on the benzene ring forms a hydrogen bond with the carbonyl atom on the MET360 amino acid residue. The parent nuclear nitrogen-containing heterocycle of compound 6**h** forms a coordinated and non-bonding interaction with the bottom cavity of the receptors.

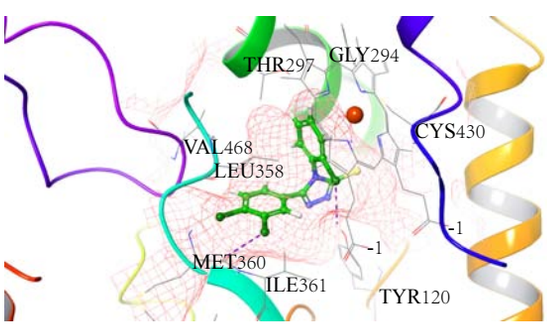


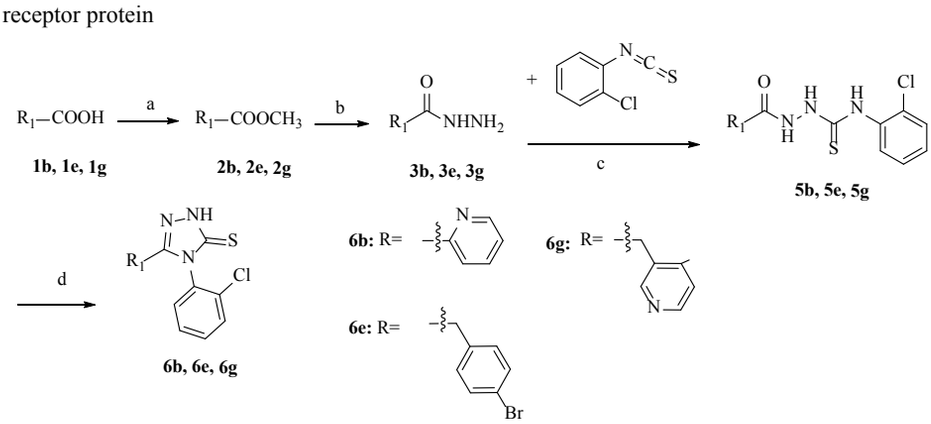
图 4　化合物 6h 与 CYP51 受体蛋白的分子对接模型

Fig. 4　Molecular docking mode of compound 6h with CYP51 receptor protein

2.2　Chemistry

The 4-(2-chlorophenyl)-4,5-dihydro-3H-1,2,4-triazole-3-thione pyridyl derivatives and hydrocarbyl derivatives described in this experiment were synthesized as shown in Fig. 5. Compound 1**(b**, **e**, **g)** was esterified in methanol solution to be transformed as compound 2**(b**, **e**, **g)**, in addition with substitution reaction of excessive hydrazine hydrate to transform as compound 3**(b**, **e**, **g)**. Compounds 3**(b**, **e**, **g)** and 1-chloro-2-isothio cyanatobenzene were together performed with condensation reactions under anhydrous ethanol to produce compound 5**(b, e, g)**, with further reaction to obtain compound 6**(b, e, g)** in EtOH solution. According to this synthetic route, compounds 6**b,** 6**e,** 6**g** can be synthesized sequentially. All synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR and MS.

The 4-(2-chlorophenyl)-5-halogenated phenyl-4,5-dihydro-3H-1,2,4-triazole-3-thione derivatives, 4-(3-chlorophenyl)-5-Halogenated phenyl-4,5-dihydro-3H-1, 2,4-triazole-3-thione and 5-(2-fluorophenyl)-4-(m-tolyl)- 4,5-dihydro-3H-1,2,4-triazole-3-thione described in this experiment were synthesized as shown in Fig. 6. Halobenzoic acid (compound 1**a~m**) was esterified in methanol solution to be transformed as methyl

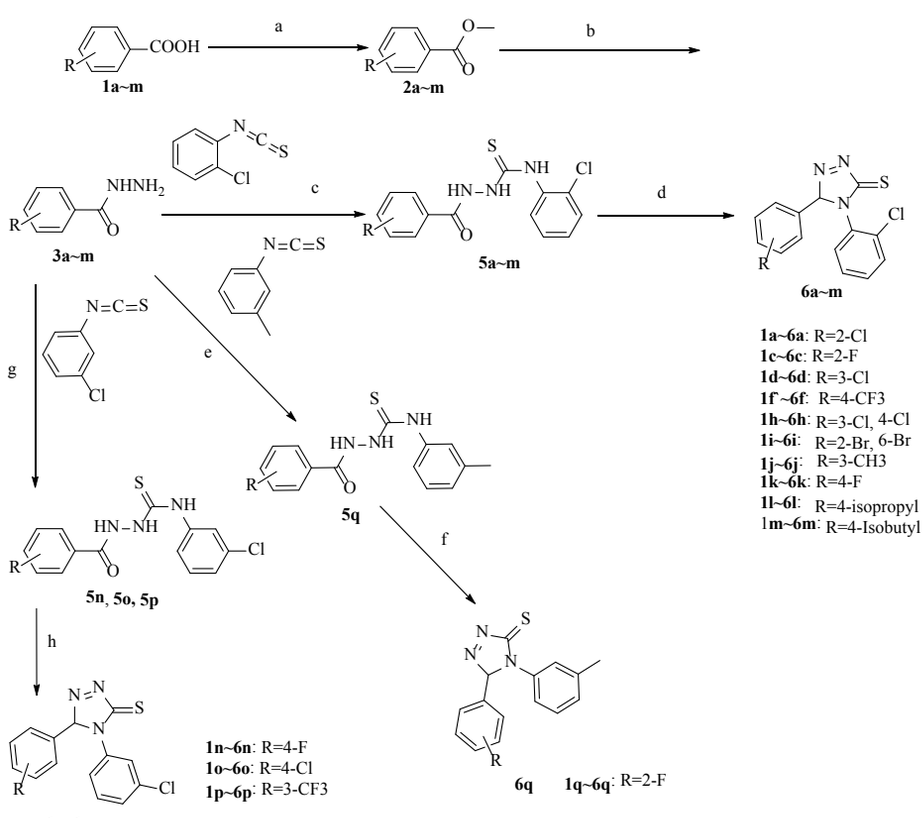


(a)：甲醇，浓硫酸，90℃，5h；(b)：无水乙醇，水合肼，90℃，3h；(c)：无水乙醇，90℃，3h；(d)：碳酸钠，乙醇，105℃，2h

图 5　4-(2- 氯苯基)-5- 取代基 -1,2,4- 三唑 -3- 硫酮的吡啶衍生物和烃基衍生物的合成

Fig. 5　Synthesis of 4-(2-chlorophenyl)-5-substituent -3H-1,2,4- triazole-3-thione pyridyl derivatives and hydrocarbyl derivatives 6**b**, 6**e**, 6**g**

halobenzoate (compound 2**a~m**), in addition with substitution reaction of excessive hydrazine hydrate to transform as halogenated benzoyl hydrazide (compound 3**a~m**). Compounds 3**a~m** and 1-chloro-2-isothiocyanato benzene were together performed with condensation reactions under anhydrous ethanol to produce 2-halobenzene-N-(2-chlorophenyl) hydrazine-1-carbothio amide (compound 5**a~m**), with further reaction to obtain 4-(2-chlorophenyl)-5-halogenated phenyl-4,5–dihydro- 3H-1,2,4-triazole-3-thione (compound 6**a~m**) in EtOH solution. According to this synthetic route, compound 6**a**, 6**c**, 6**d**, 6**f**, 6**h**, 6**i**, 6**j**, 6**k**, 6**l**, 6**m** can be synthesized sequentially. Halobenzoic acid(Compound 1**n**, 1**o**, 1**p**) was esterified in methanol solution to be transformed as methyl halobenzoate (compound 2**n**, 2**o**, 2**p**), in addition with substitution reaction of excessive hydrazine hydrate to transform as *Halogenated benzoyl* hydrazide (compound 3**n**, 3**o**, 3**p**). Compounds 3**n**, 3**o**, 3**p** and 1-chloro-3-isothiocyanato benzene were together performed with condensation reactions under anhydrous ethanol to produce 2-halobenzene-N-(3-chlorophenyl) hydrazine-1-carbothioamide (compound 5**n**, 5**o**, 5**p**), with further reaction to obtain 4-(3-chlorophenyl)-5-haloge nated phenyl-4,5-dihydro-3H-1,2,4-triazole-3-thione (compound 6**n**, 6**o**, 6**p**) in EtOH solution. According to this synthetic route, compound 6**n**, 6**o**, 6**p** can be synthesized sequentially. 2-fluorobenzoic acid (compound 1**q**) was esterified in methanol solution to be transformed as methyl 2-fluorobenzoate (compound 2**q**), in addition with substitution reaction of excessive hydrazine hydrate to transform as 2-fluorobenzohydrazide (compound 3**q**). Compounds 3**q** and 1-isothiocyanato-3-methylbenzene were together performed with condensation reactions under anhydrous ethanol to produce N-(3-chlorophenyl)-2-(2-fluorobenzoyl) hydra zine-1-carbothioamide (compound 5**q**), with further



试剂与条件：(a)：甲醇，浓硫酸，90℃，5h；(b)：无水乙醇，水合肼，90℃，3h；(c)：无水乙醇，90℃，3h；(d)：碳酸钠，乙醇，105℃，2h；(e)：乙醇，90℃，3h；(f)：碳酸钠，乙醇，105℃，2h；(g)：乙醇，90℃，3h；(h)：碳酸钠，乙醇，105℃，2h

图 6　4,5- 二取代苯基 -3H-1,2,4- 三唑 -3- 硫酮化合物 (6**a**, 6**c**, 6**d**, 6**f**, 6**h**, 6**i**, 6**j**, 6**k**, 6**l**, 6**m**, 6**n**, 6**o**, 6**p**, 6**q**) 的合成

Fig. 6　Synthesis of 4,5-disubstituted aryl-2,4-dihydro-3H-1,2,4-triazole-3-thione compounds 6**a**, 6**c**, 6**d**, 6**f**, 6**h**, 6**i**, 6**j**, 6**k**, 6**l**, 6**m**, 6**n**, 6**o**, 6**p** and 6**q**

reacttion to obtain 5-(2-fluorophenyl)-4-(m-tolyl)-4,5-dihydro-3H-1,2,4-triazole-3-thione (compound 6**q**) in EtOH solution. All synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR and MS.

2.3 Antimicrobial evaluation

Based on the activity data measured in Tab. 1, it can be seen that most of the target compounds exhibited varying degrees of inhibitory activity on these three fungal strains and changed with the degrees of substituents. Among them, compound 6**h** shows a good inhibitory effect against the three fungal strains while the MIC values are reached 25, 25, and 50μg/mL as well as its antimicrobial capacity was comparable to that of the control drug ampicillin. Compounds 6**b**, 6**d**, 6**k** and 6**m** also showed good inhibitory activity against the three fungal strains, indicating that it have broad-spectrum antibacterial activity. Compounds 6**a**, 6**f**, 6**g** and 6**p** also showed some degree of antibacterial activity. Compounds 6**c**, 6**e**, 6**i**, 6**j**, 6**l**, 6**n**, 6**o** and 6**q** had no antibacterial activity.

3 Discussions

We obtained 17 target compounds by introducing different substituents at the 4-/5-position of the triazole ring. When the 4-position substituent of the triazole ring is 2-chlorobenzyl, different substituents are introduced on the 5-position benzene ring respectively to obtain target compounds 6**a~m**. Here, we replaced the 5-position benzene ring with hydrocarbon group or pyridyl group to obtain compounds 6**e**, 6**b**, 6**g**. When we placed 3-chlorophenyl at the 4-position of the triazole ring and introduced different substituents at the 5-position benzene ring, the derivatives 6**n**, 6**o**, 6**p** were obtained. When the 4-position substituent of the triazole ring is 3-toluene group, the 2-fluorine group is introduced at the 5-position benzene ring to obtain compound 6**q**. According to the antibacterial activity data of the target compound 6**a~q**, we validated that these compounds exhibited different degrees of antibacterial effect against different strains. Therefore, we speculate that the antibacterial activity of the target compounds 6**a~q** is related to the type and location of substituents. The target compounds with the same concentration were applied to three different experimental strains, and the results showed that the antimicrobial activity of the target compounds were successively as following: 6**h**> 6**d**> 6**m**>6**k**>6**b**>6**f**>6**p**>6**a**>6**g**>6**c**>6**e**>6**i**>6**j**>6**l**>6**n**>6**o**>6**q**. The structure-activity relationship is concluded below.

(1) The antibacterial activity of the compound obtained by connecting 2-chlorphenyl instead of 3-chlorphenyl to the 4-position of the triazole ring was excellent, such as 6**k** (4-position: 2-chlorophenyl) > 6**n** (4-position: 3-chlorophenyl). According to the research results of the mechanism of action in the early stage of the experiment, it is precisely because the 2-position chlorine atom on this side chain firmly combines with the hydroxyl group on the amino acid residue of the receptor protein in the form of hydrogen bond and the antibacterial activity of the compound is enhanced.

(2) When the 4-position substituent is constant, the compound in which the 5-position of the triazole ring is directly bond to the aromatic benzene ring, which is stronger than the compound directly bonded to the hydrocarbon group, such as 6**d** (5-position: 3-chlorophenyl) > 6**e** (5-position: 4-bromophenyl).

(3) When the 4-position substituent is constant,

Tab.1 Antifungal activities of the target compounds 6**a~q** *in vitro* [MIC/(μg/mL)]

表 1 目标化合物 6**a~q** 的体外抗菌活性 [MIC/(μg/mL)]

Compound No.	*Klebsiella planticola* MTCC 530	*Staphylococcus aureus* MLS-16 MTCC 2940	*Pseudomonas aeruginosa* MTCC 2453
6a	＞400	＞400	100
6b	100	100	＞400
6c	＞400	＞400	＞400
6d	25	50	50
6e	＞400	＞400	＞400
6f	100	100	＞400
6g	200	＞400	＞400
6h	25	25	50
6i	＞400	＞400	＞400
6j	＞400	＞400	＞400
6k	50	100	100
6l	＞400	＞400	＞400
6m	50	50	100
6n	＞800	＞800	＞800
6o	＞800	＞800	＞800
6p	＞400	50	＞400
6q	＞800	＞800	＞800
Ampicillin	6.25	6.25	3.125

the antibacterial activity of the compound in which the 5-position substituent of the triazole ring is nitrogen heterocycle, which is stronger than the 5-position substituent group is hydrocarbon group, such as 6**b** (5-position: 2-pyridyl) > 6**g** (5-position: 4-chloropyridin -3-yl)methyl).

(4) When the 4-position substituent of the triazole ring is certain and 5-position group is aromatic benzene ring, the property and position of substituent R on the benzene ring will affect the antibacterial activity of the target compounds. When the position of substituent R is certain, the antibacterial activity of the compound whose substituent R is electron-withdrawing group is stronger than that of electron-donating group, such as 6**f** (R: 4-trifluoromethyl) > 6**l** (R: 4-isopropyl), 6**d** (R: 3-chloro) > 6**j** (R: 3-methyl). When the types of substituent R are the same, the ability of the position of the substituent R to enhance the antibacterial activity of compound is as following: Meta-substituent > para substituent > ortho substituent, such as 6**d** (R: 3-chloro) > 6**a** (R: 2-chloro), 6**k** (R: 4-fluorine) > 6**c** (R: 2-fluorine), and the more substituents on the benzene ring, the stronger of the antibacterial effect of the compound, such as 6h (R: 3,4-dichloro) > 6**d** (R: 3-chloro). When the substituent R is a hydrocarbon group and the position is constant, the compound having substituent R with a large hindered group has a stronger antibacterial activity than R with a small hindered group, such as 6**m** (R: 4-tert-butyl) > 6**l** (R: 4-isopropyl).

(5) The antibacterial activity of the compound having the substituent R with strong electron withdrawing group is superior to the substituent R with weak electron withdrawing group, such as 6**p** (R: 3-trifluoromethyl) > 6**n** (R: 4-fluorine), 6**o** (R: 4-chloro).

(6) The 3-methylphenyl at the 4-position of the triazole ring does not enhance the antibacterial activity of compound, for example, compound 6**q** has no antibacterial activity. At present, our laboratory is carrying out other related structural modifications of the triazole ring.

4 Synthesis method and process

4.1 General information

All chemical reagents were purchased from commercial suppliers, without further purification. The reaction was monitored by thin layer chromatography (TLC) on the pre-coated silica gel plate. The ^{1}H NMR and ^{13}C NMR spectra were performed on a Bruker Avance 400 NMR spectrometer in DMSO-d_6 with tetramethylsilane (TMS) as an internal reference. The MS spectra were obtained on the Waters UPLC/Q-TOF- MS instrument. Molecular docking experiments were completed on Schrödinger Maestro. Other instruments also include X-4 precision micro-melting point measuring instrument (Beijing Fukai Instrument Co., LTD.), Agilent HPLC 1260 (HP, USA), McMahon Turbidimetric Tube, and S1000 Colony Counter (Guangdong Huankai Microbiological Technology Co., LTD.).

4.2 Molecular docking experimental method

In the molecular docking study, the X-ray crystal structures of CYP51 bound with ligand obtained from the RCSB Protein Data Bank (PDB), and its PDB ID is 6AY4[32]. The protein was added to hydrogen, deleting waters, filling the missing loops and was further refined by energy minimization and optimization by prepared with the Protein Preparation Wizard of Schrödinger Maestro (Protein Preparation Wizard, Schrödinger, LLC, New York, NY, 2016) used to bind to ligand molecules. The ligand molecule were prepared with LigPrep module of Schrödinger suite (LigPrep, Schrödinger, LLC, New York, NY, 2016), and the parameters were chosen as follows: OPLS3 force field, Generate possible states at target pH=(7.0±2.0) using Epik, Generate all combinations in stereoisomers computation, the remaining parameters kept the default settings. After that, the treated ligand molecule were docked towards CYP51 protein grid (6AY4) using the tool Glide at Extra Precision (XP) level, the best pose of each inhibitor was selected out based on their highest docking score and good binding mode.

4.3 Antibacterial activity evaluation method

The antibacterial activity of the synthesized compounds against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was initially screened by the agar diffusion method.

The MIC values of those compounds after preliminary screening are measured by the microdilution method. The compound 1.6mg was weighed, and the concentration was aseptically distilled (containing 5% dimethyl sulfoxide as a cosolvent). The 1.6mg/mL solution was obtained by 2 times dilution. The specific operation process is as follows: Prepare 10 sterile test tubes; 0.5mL distilled water was added into each sterile tube, and 10 tubes were successively numbered 1~10. Take 0.5mL solution of the test substance concentration of 1.6mg/mL into the No.1 test tube, mix it, and then pipette 0.5mL of the solution into the No.2 test tube. Dilute to the No. 9 tube in this way, then discard the excess 0.5mL of the solution and discard it. The No. 10 tube is used as a control tube. Thus, 10 different concentrations of the solution to be tested were obtained, and the drug concentrations of the 1~10 tubes were 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125μg/mL and 0, respectively. The strains were inoculated on an ordinary agar plate, placed in a thermostatic chamber at 35℃ for 24h, and the strains were transferred to test tubes using inoculation loops, and diluted into the bactericidal solution having a concentration of 10^6cells/mL by adding physiological saline. The standard bacteria solution was evenly coated on the M-H's agar medium with aseptic cotton swab, and then punched out with a sterile round glass tube having a diameter of 6mm. The prepared compound solutions with different concentration gradients were added to the wells respectively, and the culture dishes were placed in a 35℃ incubator for 24h. The minimum concentration of the drug solution for obtaining the sterile growth hole is the MIC value.

4.4 General method

The preparation of the compounds 6**a~q** was accomplished by the series of steps described below for the preparation of 6**a**.

4.4.1 General procedure for synthesis of compounds 2**a**

Weigh 3mmol o-chlorobenzoic acid in the reaction flask, add 20mL of methanol to dissolve, then add 0.95mL concentrated sulfuric acid catalysis, 90 ℃ reflux reaction, the reaction process was followed by TLC, and the reaction takes about 5h to complete. Adding sodium bicarbonate solution and neutralizing the sulfuric acid in the reaction system, adding a large amount of water dispersion reaction system, extracting with ethyl acetate (10mL×8 times), washing with saturated saline, drying with anhydrous sodium sulfate, recovering the solvent to get light yellow Solid powder 2**a**, conversion rate of 100%.

4.4.2 General procedure for synthesis of compounds 3**a**

To the reaction flask followed by the addition of 10mL of anhydrous ethanol, 0.2mL of hydrazine hydrate and the previous step obtained 2-chlorobenzoate, 90 ℃ reflux reaction 3h, compound 3**a** is obtained after completion of the reaction, the conversion rate of 100%.

4.4.3 General procedure for synthesis of compounds 5**a**

The 2-chlorobenzohydrazide (Compound 3**a**) obtained in the previous step was dissolved in 15mL of absolute ethanol, and then added to the another raw material in an amount of the same substance, 2-chlorophenyl thiocyanate, refluxing at 90 ℃ . The progress of the reaction was followed by thin layer chromatography,and the reaction was complete after about 3h. After being placed, the white solid was precipitated in the solution. After filtration, washed with anhydrous alcohol, white powder 5a after drying, the conversion rate of 100%.

4.4.4 General procedure for synthesis of compounds 6**a**

About 25mL of 95% ethanol solution, 0.3mmol Na_2CO_3 and the white solid powder obtained in the previous step were added to the reaction flask, and the reaction was completed by refluxing at 105 ℃ for 2h. The reaction was stopped and diluted hydrochloric acid was added dropwise until the solution was neutral. The solution was extracted by ethyl acetate in fractions (10mL×8times) and purified by silica gel column chromatography (cyclohexane: ethyl acetate=9:1~11:1) to obtain a white solid of 6**a**, with a conversion of 80%.^{1}H NMR (400MHz, DMSO-d_6) δ 14.36(s, 1H, NH), 7.64(dd, *J*=6.2, 3.6Hz, 1H, ArH), 7.55(dd, *J*=11.4, 6.2Hz, 3H, ArH), 7.50~7.42(m, 3H, ArH), 7.36(t, *J*=7.6Hz, 1H,ArH). ^{13}C NMR(101MHz, DMSO-d_6) δ 168.24,148.34 ,133.15,132.78,132.42,131.70,131.62,130.98,130.07,129.79, 128.01,127.14,124.55; MS(*m/z*):322, found 322[M]$^+$. m.p. 172~173℃ .

Compound 6**b~q** was synthesized in the same method as compound 6**a**: 4-(2-chlorophenyl)-5-(pyridin-2-yl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**b**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.41(s, 1H, NH), 8.25(d, *J*=4.6Hz, 1H, ArH), 7.94(dt, *J*=15.4, 7.8Hz, 2H, ArH), 7.60(d, *J*=7.2Hz, 1H, ArH), 7.50(dt, *J*=15.4, 6.8Hz, 3H, ArH), 7.44~7.36(m, 1HArH). ^{13}C NMR(101MHz, DMSO) δ 169.31, 149.00, 148.83, 144.80, 137.49, 133.44, 131.62, 131.08, 130.79, 129.54, 127.79, 125.13, 122.75. MS(*m/z*): 288, found 287[M]$^-$.m.p. 168~169℃ .

4-(2-chlorophenyl)-5-(2-fluorophenyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**c**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.37(s, 1H, NH), 7.62(t, *J*=4.8Hz, 1H, ArH), 7.61~7.44(m, 3H, ArH), 7.43(d, *J*=7.6Hz, 1H, ArH), 7.26(dd, *J*=17.8, 8.6Hz, 2H, ArH). ^{13}C NMR(101MHz, DMSO) δ 168.47, 160.58, 158.09, 146.35, 133.59, 133.51, 131.68, 131.61, 131.55, 131.20, 130.03, 128.10, 124.69, 124.66, 116.20, 115.99, 113.55; MS(*m/z*): 305, found 304[M]$^-$. m.p. 177~179℃ .

4-(2-chlorophenyl)-5-(3-chlorophenyl)-2, 4-dihydro-3H-1,2,4-triazole-3-thione(6**d**). H NMR(400MHz, DMSO-d_6) δ 14.35(s, 1H, NH), 7.79~7.72(m, 1H, ArH), 7.72~7.64(m, 1H, ArH), 7.62~7.55(m, 2H, ArH), 7.53(d, *J*=8.2Hz, 1H, ArH), 7.41(t, *J*=8.0Hz, 1H, ArH), 7.36(s, 1H, ArH), 7.29(d, *J*=7.8Hz, 1H, ArH). ^{13}C NMR(101MHz, DMSO-d$_6$) δ 168.86, 148.97, 133.31, 131.99, 131.86, 131.75, 131.62, 130.78, 130.60, 130.30, 128.57, 127.40, 127.16, 126.09; MS(*m/z*): 322, found 320[M]$^-$. m.p. 174~175℃ .

5-(4-bromobenzyl)-4-(2-chlorophenyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**e**). ^{1}H NMR(400MHz, DMSO-d_6) δ 13.92(s, 1H), 7.61(dd, *J*=25.8, 7.8Hz, 2H), 7.48(t, *J*=7.8Hz, 1H), 7.40(s, 2H), 7.38(s, 1H), 6.90(d, *J*=8.0Hz, 2H), 3.36(s, 1H). ^{13}C NMR(101MHz, DMSO-d_6) δ 168.01, 150.55, 133.36, 132.03, 131.77, 131.08, 130.95, 130.78, 120.16, 30.76; MS(*m/z*): 381, found 380[M]$^-$. m.p. 179~180℃ .

4-(2-chlorophenyl)-5-(4-(trifluoromethyl) phenyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**f**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.40(s, 1H), 7.86~7.76(m, 2H), 7.74~7.64(m, 3H), 7.63~7.57(m, 2H), 7.55(s, 1H). ^{13}C NMR(101MHz, DMSO-d_6) δ 168.92, 148.94, 132.03, 131.88, 131.67, 131.58, 131.48, 128.59, 127.29, 126.47; MS (*m/z*): 355, found 354[M]$^-$. m.p. 169~170℃ .

4-(2-chlorophenyl)-5-((4-chloropyridin-3-yl) methyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**g**). ^{1}H NMR(400MHz, DMSO-d_6) δ 13.94(s, 1H), 7.96(d, *J*=2.4Hz, 1H), 7.66(d, *J*=8.6Hz, 1H), 7.63~7.56(m, 1H), 7.57~7.48(m, 3H), 7.40(d, *J*=8.2Hz, 1H). ^{13}C NMR(101MHz, DMSO-d_6) δ 168.09, 150.15, 149.90, 148.94, 131.14, 130.66, 130.25, 129.42, 128.48, 123.87, 27.89; MS(*m/z*): 337, found 335[M]$^-$. m.p. 173~175℃ .

4-(2-chlorophenyl)-5-(3, 4-dichlorophenyl)-2, 4-dihydro-3H-1,2,4-triazole-3-thione(6**h**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.42(s, 1H), 7.83~7.74(m, 1H), 7.69(d, *J*=8.6Hz, 2H), 7.60(dd, *J*=6.3, 3.1Hz, 2H), 7.53(d, *J*=2.0Hz, 1H), 7.29(dd, *J*=8.4, 2.0Hz, 1H). ^{13}C NMR(101MHz, DMSO-d_6) δ 168.94, 148.21, 133.64, 132.10, 131.60, 131.53, 130.97(d, *J*=9.3Hz), 130.36, 129.19, 128.63, 125.92; MS(*m/z*): 357, found 356[M]$^-$. m.p. 178~179℃ .

4-(2-chlorophenyl)-5-(2, 6-dibromophenyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**i**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.44(s, 1H), 7.98(d, *J*=2.6Hz, 1H), 7.79(t, *J*=3.8Hz, 1H), 7.75~7.68(m, 1H), 7.62(tt, *J*=4.0, 1.9Hz, 2H), 7.48(d, *J*=2.2Hz, 2H). ^{13}C NMR(101MHz, DMSO-d$_6$) δ 169.43, 148.07, 136.03, 132.66, 132.39, 131.98, 131.95, 130.84, 129.63, 129.43, 129.12, 123.19, 55.38; MS(*m/z*): 445, found 444[M]$^-$. m.p. 181~182℃ .

4-(2-chlorophenyl)-5-(m-tolyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**j**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.22(s, 1H), 7.68(dd, *J*=16.0, 7.1Hz, 2H), 7.56(dd, *J*=7.6, 3.3Hz, 2H), 7.29~7.17(m, 3H), 7.05(d, *J*=7.2Hz, 1H), 2.22(s, 3H, CH3). ^{13}C NMR(101MHz, DMSO-d_6) δ 168.62, 150.35, 138.11, 132.16, 131.83, 131.80, 131.76, 131.27, 130.22, 128.56, 128.44, 128.06, 125.44, 124.39, 20.78; MS(*m/z*): 302, found 301[M]$^-$. m.p. 165~167℃ .

4-(2-chlorophenyl)-5-(4-fluorophenyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**k**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.27(s, 1H), 7.76~7.71(m, 1H), 7.69~7.64(m, 1H), 7.57(dd, *J*=6.4, 3.0Hz, 2H), 7.44~7.36(m, 2H), 7.24(t, *J*=8.8Hz, 2H).^{13}C NMR(101MHz, DMSO-d_6) δ 168.67, 161.89, 149.55,

131.88, 131.85, 131.69, 130.28, 130.12, 130.03, 128.53, 122.09, 122.06, 116.11, 115.90; MS(*m/z*): 305, found 304[M]$^-$. m.p. 175~177℃.

4-(2-chlorophenyl)-5-(4-isopropylphenyl)-2, 4-dihydro-3H-1,2,4-triazole-3-thione(6**l**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.19(s, 1H), 7.68(d, *J*=7.4Hz, 2H), 7.57(t, *J*=6.8Hz, 2H), 7.25(s, 4H), 2.84(q, *J*=7.0Hz, 1H), 1.14(d, *J*=7.0Hz, 6H). ^{13}C NMR(101MHz, DMSO-d_6) δ 168.62, 151.15, 150.20, 132.26, 131.90, 131.82, 131.78, 130.28, 128.53, 127.29, 126.74, 123.04, 33.15, 23.42, 23.39; MS(*m/z*): 329, found 328[M]$^-$. m.p. 170~171℃.

5-(4-(tert-butyl)phenyl)-4-(2-chlorophenyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**m**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.21(s, 1H), 7.71~7.63(m, 2H), 7.63~7.51(m, 2H), 7.38(dd, *J*=8.4, 2.6Hz, 2H), 7.27(dd, *J*=8.6, 2.6Hz, 2H), 1.43~0.95(m, 9H).^{13}C NMR(101MHz, DMSO-d_6) δ 169.11, 153.91, 150.57, 132.77, 132.42, 132.37, 132.24, 130.82, 129.09, 127.45, 126.15, 123.17, 35.05, 31.22; MS(*m/z*): 343, found 342[M]$^-$. m.p. 177~178℃.

4-(3-chlorophenyl)-5-(4-fluorophenyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**n**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.19(s, 1H), 7.63(t, *J*=2.0Hz, 1H), 7.61~7.47(m, 2H), 7.45~7.35(m, 2H), 7.34(dt, *J*=7.8, 1.4Hz, 1H), 7.30~7.19(m, 2H).^{13}C NMR(101MHz, DMSO-d_6) δ 168.42, 163.01(d, *J*=248.9Hz), 149.66, 135.64, 133.20, 132.88~128.96(m), 129.57, 128.98, 127.70, 122.18(d, *J*=3.3Hz), 115.78(d, *J*=22.1Hz); MS(*m/z*): 305, found 304[M]$^-$. m.p. 173~174℃.

4-(3-chlorophenyl)-5-(4-chlorophenyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**o**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.23(s, 1H), 7.63(t, *J*=2.0Hz, 1H), 7.58(ddd, *J*=8.2, 2.2, 1.2Hz, 1H), 7.56~7.43(m, 3H), 7.41~7.30(m, 3H).^{13}C NMR(101MHz, DMSO-d_6) δ 168.55, 149.52, 135.58, 135.30, 133.23, 130.87, 130.16, 129.62, 124.49; MS(*m/z*): 322, found 321[M]$^-$. m.p. 186~187℃.

4-(3-chlorophenyl)-5-(3-(trifluoromethyl) phenyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**p**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.31(s, 1H), 7.82(dt, *J*=7.2, 2.2Hz, 1H), 7.74~7.59(m, 3H), 7.63~7.56(m, 2H), 7.60~7.49(m, 1H), 7.39(dt, *J*=7.8, 1.6Hz, 1H).^{13}C NMR(101MHz, DMSO-d_6) δ 168.68, 149.13, 135.49, 133.28, 132.33, 130.89, 129.92, 129.67, 128.98, 127.74, 127.01, 126.64, 124.97; MS(*m/z*): 355, found 354[M]$^-$. m.p. 178~179℃.

5-(2-fluorophenyl)-4-(m-tolyl)-2, 4-dihydro-3H-1, 2, 4-triazole-3-thione(6**q**). ^{1}H NMR(400MHz, DMSO-d_6) δ 14.28(s, 1H), 7.53(dddd, *J*=13.8, 7.6, 6.4, 1.8Hz, 2H), 7.32~7.23(m, 2H), 7.23~7.18(m, 2H), 7.14(d, *J*=2.0Hz, 1H), 7.06(dt, *J*=8.0, 1.4Hz, 1H), 2.25(s, 3H). ^{13}C NMR(101MHz, DMSO-d_6) δ 168.19, 159.31(d, *J*=250.2Hz), 146.60, 138.41, 133.70, 133.32(d, *J*=8.4Hz), 132.05(d, *J*=1.7Hz), 129.84, 128.71, 128.27, 124.91, 115.95, 115.75, 114.02(d, *J*=14.4Hz), 20.66; MS(*m/z*): 285, found 284[M]$^+$. m.p. 174~176℃.

5 Conclusions

In this paper, the antibacterial activity of 17 4, 5-disubstituted phenyl-3H-1,2,4-triazole-3-thione compounds was tested *in vitro*. Among them, the target compounds with good antibacterial activity were 6**h** and 6**k**, which is worthy of further research to develop into lead compounds. Based on the above analysis and discussion of the structure-activity relationship of the target compounds, the following conclusions can be drawn. First, 2-chlorobenzyl at the 4-position of the triazole ring is more conducive to enhance the antibacterial action of compound than 3-chlorobenzyl. Thus, we do not recommend introducing 3-methylphenyl into the 4-position of the triazole ring. Second, when the 4-substituent is certain, the antibacterial activity of compound will be enhanced by directly connecting the aromatic benzene ring or the nitrogen heterocycle instead of the hydrocarbon group to the triazole ring. In addition, when the triazole ring 4-position substituent and 5-position aromatic benzene ring are determined, the properties and locations of substituents on the benzene ring enhance the antibacterial activity of compounds as following: electron-withdrawing group > electron- donating group, strong electron-withdrawing group > weak electron-withdrawing group, meta substituent > para substituent > ortho substituent. The effects of these groups can be superimposed on each other, that is, the more these

groups are introduced, the stronger the antibacterial activity of compound. Finally, the effect of the steric hindrance of substituent R on the antibacterial activity is as following: large steric hindrance group > small steric hindrance group.

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