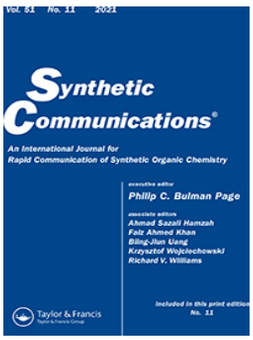

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Synthesis, antibacterial activities and molecular docking study of thiouracil derivatives containing oxadiazole moiety

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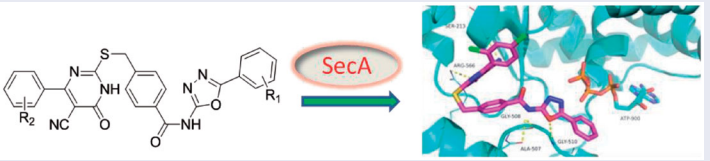
ABSTRACT

A series of novel thiouracil derivatives **9** containing an oxadiazole moiety have been synthesized by structural modification of a lead SecA inhibitor, **2**. These compounds have been evaluated for their antibacterial activities against *Bacillus amyloliquefaciens*, *Staphylococcus aureus* and *Bacillus subtilis*. Among them, compounds **9g** and **9n** exhibited promising antibacterial activities against the tested strains. Compound **9g** was also tested for its inhibitory activities against SecA ATPase, and the IC_{50} value of compound **9g** was 19.9 μg/mL, lower than that of compound **2** (20.8 μg/mL). Molecular docking work indicates that compound **9g** likely occupies the pocket formed by SecA IRA2 and NBD domain.

GRAPHICAL ABSTRACT



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Antibacterial activities; thiouracil; molecular docking; SecA inhibitor

Introduction

With the widespread use of antibiotics, bacterial resistance is increasingly becoming a global public health issue.[1–4] Therefore, new types of antibacterial drugs are in urgent need. Traditionally, the search for new antibacterial agents often takes on the following approaches: (1) modifying the known antibacterial drugs; (2) searching for novel targets for drug discovery work; (3) developing new methods for the treatment of bacterial infection.[5–7] Along these lines, we have been interested in inhibiting the bacterial key protein in secretory pathway (Sec pathway) as a way to achieve antibacterial effects.

The secretory pathway is an important way of transmembrane transport for the proteins in bacteria, and more than 1/3 proteins were transported outside of the cytoplasm to become functional. SecA is one of the key enzymes in the Sec machinery, which

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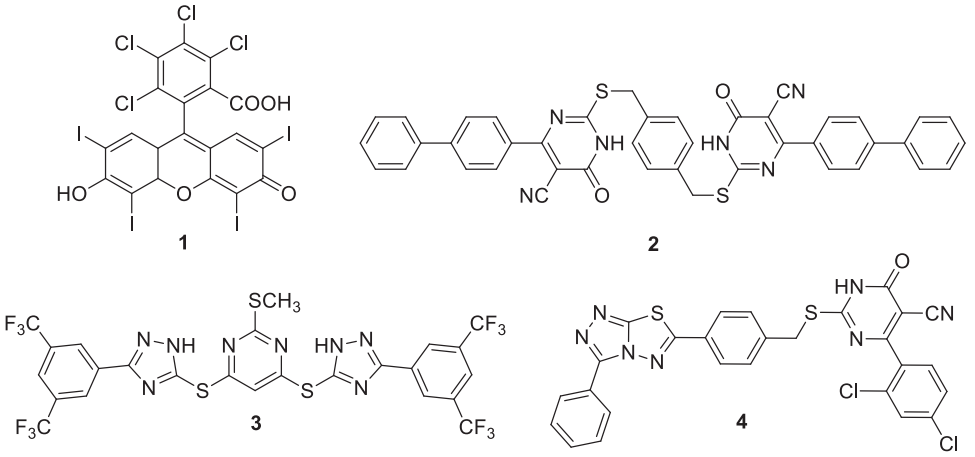


Figure 1. Chemical structures of compounds **1–4**.

provides the energy for the Sec pathway by hydrolysis of the ATP.[8,9] In addition, SecA is conserved and essential for bacterial and does not exist in the human. SecA which is a peripheral membrane protein should be accessible to the small molecule compounds.[10–14] Therefore, drug developing with SecA as a target has drawn much attention and it is very promising to find the next generation of antibacterial drugs. Known small organic molecules that can inhibit the SecA include Rose Bengal **1**, bisthiouracil **2**, and bistriazole derivative **3** (Figure 1).[15–17] In order to develop novel SecA inhibitors as antibacterial agents, and to better understand the SAR and the biological mechanism of SecA, it is worthwhile to explore the chemistry space of known inhibitor to increase structural diversity.

Recently, with the SecA as target, we adopt the active group splicing method to modify the structure of SecA inhibitors bisthiouracil **2**. There are more N and O heteroatoms in thiouracil which seems to produce more hydrogen bonding with the target. Thiouracil derivatives show a wide range of biological activities, such as antibacterial activity and anticancer activity.[18] The acyl thiourea and triazolo-thiadiazole moieties were introduced to the compound **2** to produce a series of thiouracil derivatives containing the acyl thiourea or triazolo-thiadiazole moieties. Some of these compounds have high SecA inhibitory activity and antibacterial activity,[19] among which the compound **4** (Figure 1) showed high antibacterial and SecA inhibitor activities (its IC_{50} value against SecA was 9.7 μg/mL which was obviously lower than that of the lead compound **2**).

Oxadiazole is an important nitrogen-containing heterocyclic pharmacophore, and their derivatives have many biological activities, such as anti-cancer,[20,21] anti-inflammatory,[22,23] anti-bacterial and anti-fungal effects.[24,25] According to the method of "combinatorial optimization," herein, we would like to report the design and synthesis of a series of compounds **9**, containing oxadiazole (Figure 2), modified from the parent compound **2**. We hypothesize that the oxadiazole scaffolds could mimic the thiouracil to have the capability to form more hydrogen bonding interaction with the target protein SecA, and would be beneficial to improve the antibacterial activity.

Results and discussion

Chemistry

The synthetic route of the desired thiouracil derivatives containing an oxadiazole moiety **9** are shown in Scheme 1. The synthesis started with aromatic aldehydes reacting with semicarbazide to give compound **5**, which was converted to compound **6** upon reaction with bromine. And this compound and 4-(chloromethyl)benzoyl chloride were dissolved in dry tetrahydrofuran and reacted at room temperature to obtain intermediate **7**. The elimination of hydrogen chloride in the reaction process will increase the acidity of the reaction system, protonation the amino group in compound **6**, and reduce the reaction activity. Therefore, in order to make the reaction smoothly, basic substances (such as

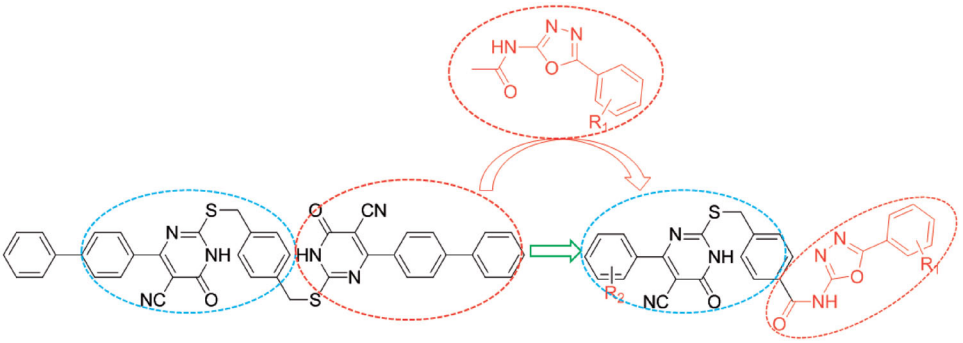
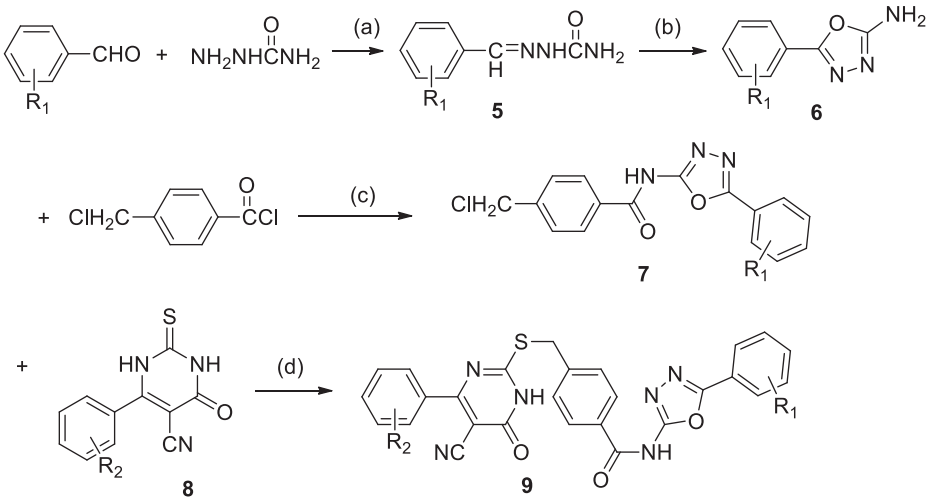


Figure 2. Design strategy for target compounds **9**.



Scheme 1. Synthesis of compounds **9**. Conditions and reagents: (a) water/ethanol reflux; (b) acetic acid/sodium acetate, bromine, r. t.; (c) THF, TEA, r.t.; (d) K_2CO_3, acetonitrile, reflux.

Table 1. *In vitro* anti-bacterial activity of compounds **9a–o**.

			MIC (μg/mL)		
Compounds	R1	R2	*Bacillus amyloliquefaciens*	*Staphylococcus aureus*	*Bacillus subtilis*
9a	H	H	100	100	50
9b	H	4-CH_3	>100	100	100
9c	H	4-OCH_3	>100	>100	100
9d	H	4-Ph	100	>100	50
9e	H	2-Cl	50	50	25
9f	H	4-Cl	50	50	50
9g	H	2,4-diCl	25	6.25	6.25
9h	2-Cl	H	100	50	100
9i	2-Cl	4-CH_3	50	100	100
9j	2-Cl	4-OCH_3	100	50	50
9k	2-Cl	4-Ph	50	25	50
9l	2-Cl	2-Cl	50	25	25
9m	2-Cl	4-Cl	50	50	25
9n	2-Cl	2,4-diCl	50	12.5	12.5
9o	2-Cl	2,6-diCl	50	25	50
2			12.5	25	50
Norfloxacin			25	25	25

triethylamine) are added as acid binding agent. The aromatic aldehyde reacted with ethyl cyanoacetate and thiourea to give compound **8** with the 50–75% yield.[12] The substituents on the benzene ring of aromatic aldehydes have a great influence on the yield. For example, the yield of aromatic aldehydes with 4-ph in the benzene ring with higher steric hindrance is lower. The compound **8** further on reaction with thiouracil **7** in acetonitrile under reflux gave the target **9**. The structures of all the new compounds were characterized by NMR, MS, and elemental analysis.

Taking the structure of compound **9a** as an example, in the NMR spectra, the single peak at the chemical shift δ 4.313 is the proton signal peak of CH_2 between the sulfur atom and the benzene ring, and the other are the proton signal peak on the benzene ring.

Biological assay

In vitro antibacterial activity

The *in vitro* antibacterial activities of the new compounds **9a–o** were tested and the tested strains included *Bacillus amyloliquefaciens*, *Bacillus subtilis* and *Staphylococcus aureus*. Norfloxacin and the lead compound **2** (Figure 1) were selected as control (the results are shown in Table 1). The results show that some of the compounds, such as **9e**, **9g**, **9k**, **9l**, **9m** and **9n** have strong and selective antibacterial activities. Among them, compound **9g** had the strongest inhibitions, and MIC values against the three tested bacteria were 25, 12.5, 12.5 μg/mL respectively, which were similar with that of norfloxacin, or even lower. In addition, the MIC of compounds **9e**, **9l** and **9m** against *Bacillus subtilis* were 25 μg/mL and that of **9n** was 12.5 μg/mL. Their inhibitory activities against *Bacillus subtilis* were superior to that of compound **2**. The MIC of compounds **9k**, **9l** and **9o** against *Staphylococcus aureus* were 25 μg/mL and that of **9n** was 12.5 μg/mL, which showed strong inhibition against *Staphylococcus aureus*, similar with that of the compound **2** and norfloxacin. The anti *Bacillus amyloliquefaciens* activities of other compounds in compound **9a–o** were generally lower than that of compound **2** and

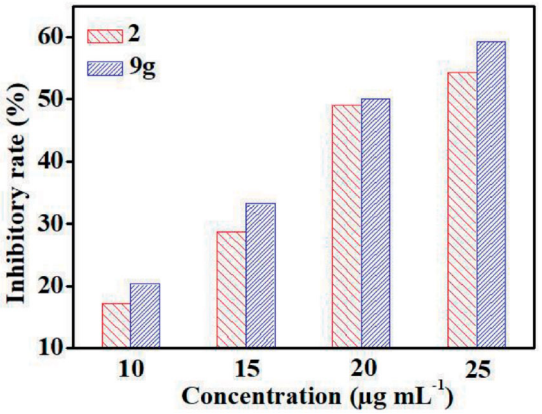


Figure 3. SecA Inhibitory activities of **9g** and **2**.

norfloxacin, except **9g**. When R_2 is 2,4-diCl, compounds **9g** and **9n** have more higher antibacterial activities than the others, but there are CH_3 or OCH_3 on the phenyl near the thiouracil the new compounds have poor activities which indicated that when there are more Cl in R_2 the newly synthesized have more stronger activities. It seems that the introduction of Cl atoms on the marginal phenyls would be helpful for the activities.

SecA inhibitory activity test

The most potent compounds **9g** were evaluated for their inhibitory activities against SecA ATPase sourced from *BL21.19 [secA(am) supFtstrp (am)zch::Tn10recA::catclp A::kan]*. The results indicated that compound **9g** showed significant enzyme inhibitory activity as shown in Figure 3. For example, the inhibition by **9g** was (50.1 ± 1.7)% at 20 μg/mL and (59.3 ± 1.6)% at 25 μg/mL respectively, which were slightly higher than those of compound **2** at the same concentrations. Compound **9 g** was studied for its 50% inhibitory concentration (IC_{50}), and the IC_{50} value was 19.9 μg/mL, which was lower than that of compound **2** (20.8 μg/mL). It seems that both compounds **9 g** and **2** have antibacterial activities as SecA inhibitors.

Computational modeling

To achieve some initial insights into the binding interactions,[26–28] the newly synthesized compound **9g** was docked into the SecA crystal structure. Two possible binding pockets were selected: the ATPase ATP-binding site and the pocket, which is formed by IRA2 and NBD domain, as proposed before.[18,19] 20 outputs were generated and the docking scores were 3.78–5.61 at the ATP-binding site and 5.00–6.67 at the pocket. Thus, it is reasonable to suggest that the new inhibitor binds at a similar position as the lead structure. The interactions are shown in Figure 4: molecule **9g** generated hydrogen-bond interactions with SER213, ALA507, GLY508, GLY510 and ARG566.

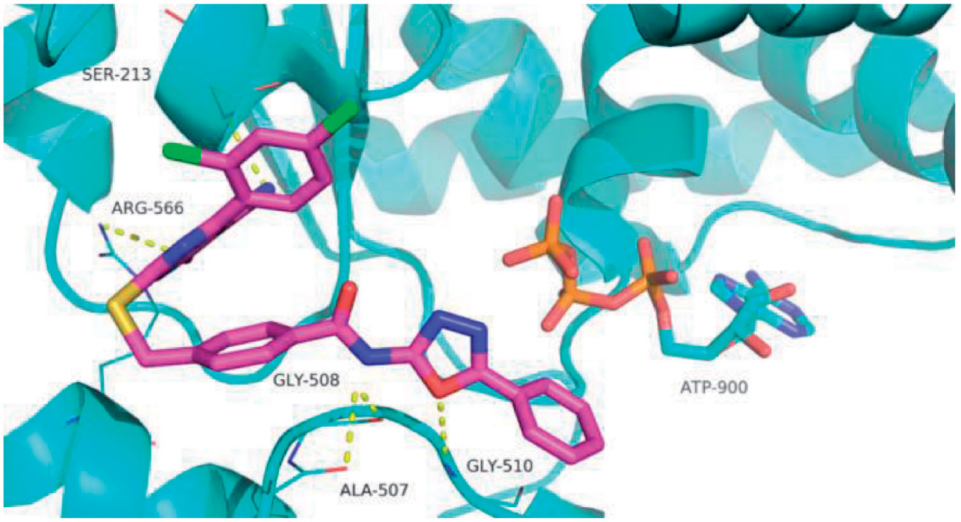


Figure 4. The proposed docking conformation of the compound **9g** (pink sticks). Protein structures are shown in cyan sticks and ribbons. The molecule ATP is shown in cyan sticks.

Conclusions

A series of thiouracil derivatives containing oxadiazole **9** were designed, synthesized and evaluated for their antibacterial activities. Some of the compounds **9** have strong antibacterial activities. Additionally, compound **9g** expressed stronger inhibitory activity against the SecA ATPase than the known inhibitor **2**, suggesting that both compounds are antibacterial agents as SecA inhibitors. Molecular simulation showed that compound **9g** likely binds to a pocket formed by SecA IRA2 and NBD domain.

Experimental

Column chromatography was carried out on flash silica gel (300–800 mesh). TLC analysis was conducted on silica gel plates (Silica G UV254). Melting points were measured in an open capillary on a SGW X-4 melting point apparatus and are uncorrected. Element analysis was performed using a Heraeus (CHNO, rapid) elemental analyzer. NMR spectra were recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C on a Bruker instrument. Chemical shifts (δ values) and coupling constants (*J* values) are given in ppm and hertz, respectively, using TMS (^{1}H NMR) as internal standard. ESI-MS spectra were determined using an Agilent G6300 ion Trap mass spectrometer, and signals were recorded in *m/z*. Absorption spectra were recorded on a FLUOstar Omega Microplate Reader.

General procedures for preparation of compounds 9

To a solution of the aldehyde (RCHO, 10 mmol), ethyl cyanoacetate (1.0 mL, 10 mmol), and thiourea (0.76 g, 10 mmol) in absolute ethanol (50 mL) was added piperidine (2.0 mL, 20 mmol); the mixture was refluxed overnight and then cooled to room

temperature. The precipitate was dissolved in 0.5 M NaOH (20 mL) and washed with ethyl acetate (10 mL × 3). The aqueous solution was acidified to pH 2 by slow addition of 1 M HCl. Then the product precipitated (crystallized) out to give compound **8**.[18,19]

Semicarbazide (5 mmol) and water (50 mL) were added into a 250 mL flask and stirred to dissolve. Then aromatic aldehyde (5 mmol) and ethanol (20 mL) were added and the mixture was stirred under reflux reaction for 3 h to give crude **5**. This crude product was recrystallized from ethanol to give the pure product **5**. Compound **5** (3 mmol), anhydrous sodium acetate (6 mmol) and glacial acetic acid (12 mL) were added into a 100 mL flask and stirred to dissolve, then the mixture of bromine (0.6 mL) and glacial acetic acid (2 mL) were slowly added to stir at room temperature. After completion of the reaction, the mixture was poured into ice-water (100 mL) to give white precipitate, filtered and recrystallized from ethanol to give compound **6**.

4-(Chloromethyl)benzoyl chloride (2 mmol), dry tetrahydrofuran (20 mL) and trimethylamine (2 mL) were added into a 100 mL flask and stirred to dissolve. Then the compound **6** (2 mmol) was added to stir at room temperature for 12 h. After completion of the reaction, the mixture was poured into cold water (150 mL). The tetrahydrofuran dissolved in water, then the light yellow precipitate was produced, filtered and dried to give compound **7**.

A solution of compound **8** (1.1 mmol), compound **7** (1 mmol) and K_2CO_3 (3 mmol) in acetonitrile (30 mL) were heated under reflux reaction. After the completion of this reaction monitored by TLC, the solvent was removed to obtain crude **9**. Compound **9** was washed with 0.2 mol/L NaOH, then dried and separated by column chromatography ($V_{ethyl\ acetate}/V_{methanol}$ = 8:1–15:1) to obtain the target products **9**.

4-(((5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)thio)methyl)-N-(5-phenyl-1,3,4-oxadiazol-2-yl)benzamide(9a)

Light yellow powder (75%), m.p. 234.2–237.3 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 8.003–8.016(d, J = 7.8 Hz, 1H, ArH), 7.867–7.879 (d, J = 7.2 Hz, 1H, ArH), 7.750–7.760 (m, 4H, ArH), 7.497–7.510 (d, J = 7.8 Hz, 2H, ArH), 7.456–7.467 (m, 4H, ArH), 7.348–7.361 (d, J = 7.8 Hz, 2H, ArH), 4.313 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 170.31, 169.30, 167.08, 166.37, 138.68, 137.25, 129.57, 128.99, 128.88, 128.54, 128.45, 128.07, 128.01, 127.82, 126.81, 124.91, 33.75; MS (ESI) *m/z*: 505.0 ([M-H]$^+$). Anal. Calcd for $C_{27}H_{18}N_6O_3S$: C, 64.02; H, 3.58; N, 16.59. Found: C, 64.10; H, 3.70; N, 16.68.

Experimental procedures and characterization data associated with this article can be found, in the online version.

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Disclosure statement

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.

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