Design, Synthesis and Antibacterial Activities of Triazole-pyrimidine Derivatives as SecA Inhibitors

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Research Article

Keywords: SecA inhibitor, small molecule, antimicrobial, target, drug-resistant

Posted Date: May 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-476874/v1>

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Abstract

SecA, a key component of bacterial Sec-dependent secretion pathway, is an attractive target for exploring novel antimicrobials. Along this line, we reported optimization of a hit bistriazole (SCA 21) which has been previously identified as a SecA inhibitor. Herein we describe the synthesis of some novel triazole-pyrimidine derivative by structural modification of SCA 21. Some of them have been evaluated for their antimicrobial activity against *Escherichia coli* NR698 (a leaky mutant), *Staphylococcus aureus* and *Bacillus anthracis* Sterne.

Introduction

Bacterial pathogens' infectious diseases have become a serious clinical issue in recent years because of the emergence and spread of drug resistance.^[1] Thus, the development of novel antibacterial agents, especially those with a new drug target and/or with the capability to overcome drug resistance is an urgent need.^[2] In bacterial cells, more than 30% of proteins become functional after being transported outside the cytoplasm. Trans-membrane movement of most of these proteins is *via* the Sec pathway (i.e. secretion pathway). SecA constitute a key enzyme of the bacterial protein secretion (Sec) pathways. SecA ATPase, one of the essential components of the Sec machinery provides a major pathway to aid translocation of cytosol proteins across or into the cytoplasmic membrane.^[3–9] SecA is considered as an attractive target by researchers in order to find novel antibacterial drugs because it is a conserved and essential protein in all bacteria and absent in humans.^[10, 11] Several studies carried out have shown that inhibition of SecA can lead to bacteriostatic and bactericidal effects^[12]. From this perspective, we are interested working in targeting SecA, which is a critical protein secretion machinery essential for bacterial survival.^[2, 11, 13] Currently, small organic molecules described in the literature as SecA inhibitors mainly include Rose Bengal,^[14] bithiouracil,^[15] bistriazole^[16] and their derivatives,^[17] thiazolo[4,5-d]pyrimidine derivatives^[18] and others.^[19–22] In order to find new SecA inhibitors, it is worthwhile to explore the chemistry space of known inhibitor to increase structural diversity.

Compounds containing triazole or pyrimidine own a wide range of biological activities including antibacterial,^[23, 24] antifungal,^[25, 26] anti-tubercular,^[27] and antiviral,^[28, 29] among others^[30, 31]. Due to their excellent biological activities, herein we reported the design, synthesis and antibacterial activities of some compounds containing both triazole and pyrimidine motifs (Schemes 1 and 2), modified from compound **1** (SCA 21) in Fig. 1.

The results and implications in guiding future work in this area are described below.

Results And Discussion

Chemistry

The compound in Figure 1 has been identified as SecA inhibitor for further optimization in earlier work [32].

To enhance the potency, we began to simplify the structure by dissecting the lead compound in half and removing part A. Then by changing the methyl to an alkylamine which have been biotinylated, we hypothesize that these compounds, due to the presence of several nitrogen atoms, would have the ability to form more hydrogen bonding interactions with the target protein SecA, and would be beneficial in enhancing antibacterial activity. Also, by changing the methyl to an azidopentyl, we think that could improve the potency because sodium azide is a well-known SecA inhibitor [33]. The general strategy of analogue design is shown in Figure 2.

Compounds synthesis began by reacting commercially available benzoyl chloride with hydrazine carbothioamide, followed by self-condensation of 2 in the presence of 5% sodium hydroxide under reflux conditions to afford 3 [34] (Scheme 1). Compounds 7 and 15 were prepared by following published procedures in Scheme 2 [35] and Scheme 4 [36]. Compounds 8, 9, 17 and 18 were synthesized by reaction of intermediate 7 and 15 with 2,4,6-trichloro pyrimidine at room temperature under weakly basic conditions [37]. Compounds 10 and 19 were obtained by reaction of 3 with respectively 8 and 17 in the presence of potassium carbonate in acetonitrile. Then compounds 10 were deprotonated in trifluoroacetic acid at room temperature to give compounds 11 [38]. The reaction of 11 with 12 gave compounds 13a, b *via* a nucleophilic attack by unprotonated amine on the ester group, followed by an amide bond formation and release of N-hydroxysuccinimide (Scheme 3) [39]. SCA 107 was synthesized and tested in our earlier work [32].

Biological evaluation

The newly synthesized compounds 11a (SCA 257), 11b (SCA 255), 13b (SCA 256) and 19 (SCA 259) were first screened for their *in vitro* antibacterial activity using a truncated version of *E. coli* SecA, EcSecAN68, at 25 μ M and at 50 μ M. There were three compounds (SCA255, SCA256 and SCA259) that showed greater than 50% inhibition at 50 μ M, but only one compound (SCA259) showed more than 50% inhibition at 25 μ M, as shown in Figure 2. SCA 259 showed more potent inhibition against EcSecAN68 than SCA107, which was one of the best triazole-pyrimidine inhibitors from our previous study with IC_{50} at 30 μ M against EcSecAN68 [32]. SCA 259 was then evaluated further at various concentrations to allow the determination of IC_{50} value at 5.9 μ M. Thus, SCA 259 was more potent than the lead compound SCA21 with IC_{50} at 18 μ M against EcSecAN68 [32]. These results suggested that the azide pentyl group is beneficial for potent inhibitory activity. The biotinylated compound SCA 256 was more active than compound SCA 255 with the amine group.

Compounds SCA 257 and SCA 259 were also evaluated for their antimicrobial activities against *B. anthracis* Sterne, *S. aureus* 6538 and *E. coli* NR698. The results are shown in Table 1. It showed that SCA 259 has potent inhibitory activity against the three tested bacterial strains, higher than that of the lead

compound SCA 21^[32]. Compound SCA 259 showed MIC at 3.1 μ M against *B. anthracis* Sterne, at 1.6 μ M against *S. aureus* 6538 and at 12.5 μ M against *E. coli* NR698, which are comparable to some of our best compounds in this class.

Table 1

Bacteriostatic effects of SecA inhibitors

	MIC (μ M)		
<i>Strains:</i>	SCA257	SCA259	SCA21 ^[32]
<i>B. anthracis</i> Sterne	100	3.1	6.25
<i>S. aureus</i> 6538	50	1.6	12.5
<i>E. coli</i> NR698	>100	12.5	25

Overall, the results indicate very useful information for researchers interested in designing small molecules SecA inhibitors for improved potency.

Conclusion

Some novel triazole-pyrimidine derivatives were designed, synthesized and evaluated as SecA inhibitors. Compound SCA 259 with azide pentyl group was the most potent analogue developed from this study. This compound expressed better inhibitory activity against SecA ATPase than that of the known inhibitor SCA 21.

Experimental

All chemical reagents and solvents used were of reagent grade or purified using standard methods. TLC analyses were conducted on silica gel plates (Sorbent Silica G UV254). Column chromatography was carried out on flash silica gel (Sorbent 230–400 mesh). NMR spectra were recorded at ¹H (400 MHz) and ¹³C (100 MHz) on a Bruker instrument. Coupling constants (*J*) and chemical shifts (δ) are given in hertz and ppm respectively, using TMS (¹H NMR) and solvents (¹³C NMR) as internal standards.

General procedure for the synthesis of 8a, b

To a solution of 7 (1.04 mmol) in acetonitrile (5 mL) was added K₂CO₃ (434 mg, 3.14 mmol) and 2,4,6-trichloropyrimidine (230 mg, 1.25 mmol) and the mixture was stirred at room temperature. Upon completion, the resulting mixture was diluted with ethyl acetate and then washed with water and brine. The organic layer was dried over anhydrous Na₂SO₄, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt) of the residue gave 8.

tert-butyl (2-((4,6-dichloropyrimidin-2-yl)thio)ethyl)carbamate 8a (287 mg, 85%)

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.81 (s, 1H), 7.09 (s, 1H), 3.24 (d, *J* = 2.6 Hz, 4H), 1.36 (s, 9H); ¹³C NMR (DMSO-*d*₆): δ 173.1, 164.0, 155.9, 118.2, 79.5, 40.1, 32.2, 28.4.

tert-butyl (5-((4,6-dichloropyrimidin-2-yl)thio)pentyl)carbamate 8b (331 mg, 87%).

¹H NMR (400 MHz, CDCl₃-*d*) δ 7.10 (s, 1H), 4.52 (s, 1H), 3.19 (t, *J* = 7.3 Hz, 2H), 3.16 – 3.05 (m, 2H), 1.73 (q, *J* = 7.3 Hz, 2H), 1.51 (d, *J* = 52.9 Hz, 14H); ¹³C NMR (CDCl₃-*d*): δ 173.1, 164.0, 155.9, 118.2, 79.5, 40.3, 36.7, 29.9, 29.2, 28.4, 25.6.

General procedure for the synthesis of 10a, b

To a solution of 3 (98 mg, 0.31 mmol) in acetonitrile (5 mL) was added K₂CO₃ (86.5 mg, 0.62 mmol) and compound 8 (0.20 mmol) at room temperature. The mixture was stirred at same temperature for 8 h. The resulting mixture was diluted with ethyl acetate and then washed with water and brine. The organic layer was dried over anhydrous Na₂SO₄, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt) of the residue gave 10.

tert-butyl (2-((4-((5-(3,5-bis(trifluoromethyl)phenyl)-4H-1,2,4-triazol-3-yl)thio)-6-chloropyrimidin-2-yl)thio)ethyl)carbamate 10a (100 mg, 83%).

¹H NMR (400 MHz, CDCl₃-*d*) δ 8.59 (s, 2H), 7.92 (s, 1H), 7.23 (s, 1H), 4.96 (s, 1H), 3.47 (t, *J* = 6.4 Hz, 2H), 3.35 (t, *J* = 6.5 Hz, 2H), 1.45 (s, 9H); ¹³C NMR (CDCl₃-*d*): δ 170.9, 170.1, 159.5, 159.4, 155.8, 148.5, 132.2, 132.2, 131.9, 131.6, 126.0, 124.6, 122.4, 121.91, 113.2, 79.0, 38.6, 28.5, 26.4.

tert-butyl (5-((4-((5-(3,5-bis(trifluoromethyl)phenyl)-4H-1,2,4-triazol-3-yl)thio)-6-chloropyrimidin-2-yl)thio)pentyl)carbamate 10b (103 mg, 80%).

¹H NMR (400 MHz, CDCl₃-*d*) δ 8.64 (s, 2H), 7.96 (s, 1H), 7.07 (s, 1H), 4.66 (s, 1H), 3.15 (m, 4H), 1.75 (m, 2H), 1.57 (m, 4H), 1.47 (s, 9H); ¹³C NMR (CDCl₃-*d*) δ 173.6, 172.9, 160.2, 159.6, 155.8, 149.9, 135.0, 132.2, 131.9, 126.7, 125.9, 123.2, 122.6, 113.6, 77.4, 39.7, 30.1, 28.9, 28.5, 27.5, 26.1.

General procedure for the synthesis of 11a, b

10 (0.13 mmol) and trifluoroacetic acid (2.64mg, 0.26 mmol) was stirred at room temperature until no further gas was released. Diethyl ether (5 ml) was added into the clear solution and stirred. After the solid came out, the mixture was suction filtered. The solid was washed by diethyl ether and dried in a vacuum. The solid was dissolved in water and 10 % NaOH was added drop by drop into the solution to get white crystals in an ice bath. After suction filtration, the crystals were washed with water and dried in a vacuum to obtain 11.

2-((4-((5-(3,5-bis(trifluoromethyl)phenyl)-4H-1,2,4-triazol-3-yl)thio)-6-chloropyrimidin-2-yl)thio)ethanamine 11a (50 mg, 0.1 mmol).

^1H NMR (MeOD- d_4): δ 8.64 (s, 2H), 8.07 (s, 1H), 7.08 (s, 1H), 3.45 (t, J = 6.4Hz, 2H), 3.29 (t, J = 6.8 Hz, 2H); ^{13}C NMR (MeOD- d_4): δ 170.96, 170.10, 159.51, 159.39, 148.47, 132.25, 132.17, 131.92, 131.58, 126.00, 124.62, 122.43, 121.91, 113.19, 38.63, 26.40. HRMS (ESI): Calc. for $\text{C}_{16}\text{H}_{10}\text{ClF}_6\text{N}_6\text{S}_2$ $[\text{M}-\text{H}^+]$: 499.0001; found: 499.0010.

5-((4-((5-(3,5-bis(trifluoromethyl)phenyl)-4H-1,2,4-triazol-3-yl)thio)-6-chloropyrimidin-2-yl)thio)pentan-1-amine 11b (64 mg, 0.12 mmol), 74%.

^1H NMR (MeOD- d_4): δ 8.64 (s, 2 H), 8.02 (s, 1H), 6.84 (s, 1H), 3.36 (s, 1H), 3.10 (t, J = 7.6Hz, 2H), 2.92 (t, J = 7.6Hz, 2H), 1.69 (m, 4H), 1.47 (m, 2H); ^{13}C NMR (MeOD- d_4) δ 173.62, 172.93, 160.24, 159.62, 149.97, 135.04, 132.25, 131.92, 126.74, 125.89, 123.18, 122.59, 113.59, 39.66, 30.14, 28.91, 27.49, 26.10. HRMS (ESI): Calc. for $\text{C}_{19}\text{H}_{16}\text{ClF}_6\text{N}_6\text{S}_2$ $[\text{M}-\text{H}^+]$: 541.0471; found: 541.0486.

General procedure for the synthesis of 13a, b

To a solution of 11 (0.09 mmol) in anhydrous DMF (3 mL) and triethylamine (34 μ l, 0.24 mmol) was added compound 12 (28 mg, 0.08 mmol). After stirring for 10 h, the reaction solution was concentrated in vacuo at 49 °C. The resulting residue was dissolved in 10 mL of ethyl acetate and washed with 5 mL of a saturated sodium bicarbonate solution. The organic layer was separated and dried over sodium sulfate and concentrated. The crude product was purified by column chromatography (MeOH : DCM, 3:7) to afford compound 13.

N-(2-((4-((5-(3,5-bis(trifluoromethyl)phenyl)-4H-1,2,4-triazol-3-yl)thio)-6-chloropyrimidin-2-yl)thio)ethyl)-5-((3aR,4R,6aS)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl) pentanamide 13a (96 mg, 73%).

^1H NMR (DMSO- d_6): δ 8.61 (s, 2H), 8.31 (s, 1H), 8.03 (t, J = 5.2 Hz, 1H), 7.39 (s, 1H), 6.40 (s, 1H), 6.35 (s, 1H), 4.30 (m, 1H), 4.12 (m, 1H), 3.18 (m, 2H), 3.07 (m, 2H), 2.85 (dd, J = 5.2 Hz, 1H), 2.60 (d, J = 12.4 Hz, 1H), 2.00 (m, 2H), 1.59 (m, 1H), 1.43 (m, 3H), 1.25 (m, 3H); ^{13}C NMR (DMSO- d_6) δ 173.47, 172.79, 169.03, 163.19, 158.96, 131.83, 131.50, 126.92, 124.86, 124.10, 122.15, 113.63, 61.48, 59.67, 55.84, 40.29, 37.77, 35.51, 29.57, 28.58, 28.44, 25.59. HRMS (ESI): Calc. for $\text{C}_{26}\text{H}_{25}\text{ClF}_6\text{N}_8\text{O}_2\text{S}_3$ [M^+]: 727.0934; found: 727.0930.

N-(5-((4-((5-(3, 5-Bis(trifluoromethyl) phenyl)-4H-1, 2, 4-triazol-3-yl) thio)-6- chloropyrimidin-2-yl) thio) pentyl)-5-((3aR, 4R, 6aS)-2-oxohexahydro-1H-thieno[3, 4- d]imidazol-4-yl) pentanamide 13b (51 mg, 71%).

^1H -NMR (DMSO- d_6): δ 8.61 (s, 2H), 8.32 (s, 1H), 7.75 (t, J = 5.2 Hz, 1H), 7.32 (s, 1H), 6.43 (s, 1H), 6.36 (s, 1H), 4.29 (m, 1H), 4.12 (m, 1H), 3.10 (m, 3H), 2.97 (m, 2H), 2.80 (dd, J = 5.2 Hz, 1H), 2.70 (d, J = 12.4 Hz, 1H), 2.03 (t, J = 7.2 Hz, 2H), 1.59 (m, 3H), 1.43 (m, 3H), 1.23 – 1.36 (m, 7H); ^{13}C -NMR (DMSO- d_6) δ 173.7, 172.3, 168.7, 163.2, 159.0, 131.8, 131.5, 130.9, 126.8, 124.8, 124.0, 122.1, 113.5, 61.5, 59.6, 55.9, 40.2, 38.5, 35.6, 29.6, 28.9, 28.6, 28.4, 28.2, 25.9, 25.7. HRMS-ESI: Calc. for $\text{C}_{29}\text{H}_{32}\text{ClF}_6\text{N}_8\text{O}_2\text{S}_3$ [M^+]: 769.1403; found: 769.1390.

2-((5-Azidopentyl) thio)-4, 6-dichloropyrimidine 17

To a solution of 7 (152 mg, 1.04 mmol) in acetonitrile (5 mL) was added K_2CO_3 (434 mg, 3.14 mmol) and compound 10 (230 mg, 1.25 mmol) at room temperature. The mixture was stirred at same temperature for 8 h. The resulting mixture was diluted with ethyl acetate and then washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 , filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 9:1) of the residue gave 17 (310 mg, 84%). ^1H NMR (CDCl_3 - d): δ 7.18 (s, 1H), 3.23 (t, J = 6.8 Hz, 2H), 3.23 (t, J = 7.2 Hz, 2H), 1.76 (m, 2H), 1.66 (m, 2H), 1.55 (m, 2H); ^{13}C NMR (CDCl_3 - d): δ 174.8, 159.9, 159.8, 116.5, 51.1, 29.7, 28.2, 25.7.

2-((5-Azidopentyl) thio)-4-((5-(3, 5-bis(trifluoromethyl) phenyl)-4H-1, 2, 4-triazol-3-yl) thio)-6-chloro pyrimidine 19 (99 mg, 83%).

To a solution of 3 (98 mg, 0.31 mmol) in acetonitrile (5 mL) was added K_2CO_3 (86.5 mg, 0.62 mmol) and compound 17 (61 mg, 0.20 mmol) at room temperature. The mixture was stirred at same temperature for 8 h. The resulting mixture was diluted with ethyl acetate and then washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 , filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 7:3) of the residue gave ^{19}H -NMR ($CDCl_3-d$): δ 13.01 (s, 1H), 8.61 (s, 2H), 7.94 (s, 1H), 7.17 (s, 1H), 3.34 (t, J = 6.4 Hz, 2H), 3.25 (t, J = 7.2 Hz, 2H), 1.80 (m, 2H), 1.66 (m, 2H), 1.58 (m, 2H); ^{13}C -NMR ($CDCl_3-d$) δ 173.7, 163.4, 159.6, 147.2, 132.3, 132.0, 126.4, 124.5, 123.1, 121.8, 118.8, 115.5, 113.9, 51.1, 29.7, 28.3, 25.7. HRMS-ESI: Calc. for $C_{19}H_{14}ClF_6N_8S_2$ $[M-H^+]$: 567.0376; found: 567.0371.

ATPase assays:

Inhibition on ATPase activity of EcSecAN68 was determined by malachite green colorimetric assay as previously described^[17]. IC_{50} is defined as the concentration of the compound that inhibits 50% of ATPase activity.

Bacteriostatic effect:

Bacteriostatic effects were evaluated at 37°C in 96-well microtiter plates as previously described^[17]. Minimum inhibitory concentration (MIC) is the lowest concentration of compounds at which bacterial cells were not able to grow at tested condition.

Declarations

Acknowledgments

We acknowledge Binghe Wang and Phang-Cheng Tai respectively for the synthesis and the biological evaluation in their labs in Georgia State University. FB was a visiting scholar at GSU when conducting the lab research with the partial financial support from the Islamic Development Bank (IDB) under a merit scholarship programme. Part of the research was conducted with the partial financial support of the National Institute of Health awarded to PCT and BW (AI104168).

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Figures

Figure 1

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