Advances in Biochemistry
2021; 9(4): 98-105
http://www.sciencepublishinggroup.com/j/ab
doi: 10.11648/j.ab.20210904.12
ISSN: 2329-0870 (Print); ISSN: 2329-0862 (Online)

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

Fanté Bamba*, Camara Tchambaga Etienne, Coulibali Sioménan, Akpa Sagne Jacques, Coulibaly Souleymane, Adjou Ané

Laboratoire de Constitution et Réaction de la Matière, UFR SSMT, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire

Email address:
fante_bamba1@yahoo.fr (F. Bamba)

*Corresponding author

To cite this article:
Fanté Bamba, Camara Tchambaga Etienne, Coulibali Sioménan, Akpa Sagne Jacques, Coulibaly Souleymane, Adjou Ané. Design, Synthesis and Antibacterial Activities of Triazole-Pyrimidine Derivatives as SecA Inhibitors. *Advances in Biochemistry*. Vol. 9, No. 4, 2021, pp. 98-105. doi: 10.11648/j.ab.20210904.12

Received: October 26, 2021; **Accepted**: November 12, 2021; **Published**: November 23, 2021

Abstract: Background: To highlight the magnitude of the important challenge now facing scientists, drug resistance needs exploration of novel antimicrobial agents. The identification of new and vital target in bacteria and then designing their inhibitors can be explored. Thus, targeting SecA, a central component of the bacterial general secretion system, is a promising strategy for the development of novel antimicrobials. Objective: To evaluate new compounds as SecA inhibitors synthesized by structural modification of bistriazole SCA-21. Method: A new compounds were synthesized and evaluated for antibacterial activity against *Escherichia coli* NR698 (*E. coli* a leaky mutant), *Staphylococcus aureus (S. aureus)* and *Bacillus anthracis (B. anthracis)*. Results: Some novel triazole-pyrimidine derivatives by structural modification of known SecA inhibitor SCA 21 were synthesized and their structures were confirmed by ^{1}H NMR, ^{13}C NMR and Mass spectral analysis. The synthesized compound showed antimicrobial activity against *E. coli* NR698 (a leaky mutant), *S. aureus* and *B. anthracis* Sterne. Conclusion: Five novel triazole-pyrimidine derivatives were designed, synthesized and evaluated as SecA inhibitors. At the end of this study, compound SCA 259 with azide pentyl group was found as the most potent inhibitor. It expressed better inhibitory activity against SecA ATPase than else known inhibitor SCA 21.

Keywords: Triazole-Pyrimidine, SecA Inhibitor, Small Molecule, Antimicrobial, Target, Drug-resistant

1. Introduction

Bacterial pathogens' infectious diseases became a serious clinical issue in recent years because of the emergence and spread of drug resistance [1]. To address this concern, there is an urgent need to develop new antibacterial agents, preferably those with new mechanism of actions from new drug targets to overcome drug resistance [2]. It has been shown that more than 30 % of proteins in bacterial cells became functional after translocation outside the cytoplasm. Most of the proteins' trans-membrane movement happens *via* the Sec pathway (i.e. secretion pathway). SecA constitutes a key enzyme of the bacterial protein secretion (Sec) pathways. A major route to help proteins translocation from cytosol across or into the cytoplasmic membrane is provided by SecA ATPase, one of the central components of the Sec transport system [3-9]. SecA is considered as an attractive target by researchers in order to find novel antibacterial drugs because it is a highly conserved and essential protein present in all bacteria and absent in humans [10, 11]. Several studies carried out showed that inhibition of SecA could lead to bacteriostatic and bactericidal effects [12]. From this perspective, our goal as scientists became to work in the field of targeting SecA, which is a critical protein secretion machinery indispensable for bacterial survival [2, 11, 13]. Currently, most of the small organic molecules reported in the literature as SecA inhibitors essentially include bisthiouracil [14], Rose Bengal [15], bistriazole [16] and their derivatives [17], thiazolo[4,5-d]pyrimidine derivatives [18] and others 19-22]. To go further than what is already known about SecA small molecules, we will explore the chemistry space to increase structural diversity in their scaffold for the development of new SecA inhibitors.

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 report the design, synthesis and antibacterial activities of some compounds containing both triazole and pyrimidine motifs (Figure 2), modified from compound SCA 21 in Figure 1.

The results and implications for guidance future research in this area are described below.

2. Experimental

All chemical reagents and solvents were from Sigma Aldrich and used without further purification 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 ^{1}H (400 MHz) and ^{13}C (100 MHz) on a Bruker instrument. Coupling constants (*J*) and chemical shifts (δ) are given in hertz and ppm respectively, using TMS as internal standards.

2.1. General Procedure for the Synthesis of 8a, b

To *tert*-butyl (2-mercaptoethyl)carbamate derivatives *7* (1.04 mmol) in 5 mL of CH_3CN, K_2CO_3 (434 mg, 3.14 mmol) and 2,4,6-trichloropyrimidine (230 mg, 1.25 mmol) were added and the mixture was stirred at room temperature. Upon completion, the solvent was removed under reduced pressure. The crude residue 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 the solvent was evaporated *in vacuo*. The residue was purified using silica gel column chromatography (hexane/AcOEt) to yield *8*.

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

^{1}H 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); ^{13}C 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%)

^{1}H NMR (400 MHz, $CDCl_3$-*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, 13H); ^{13}C NMR ($CDCl_3$-*d*): δ 173.1, 164.0, 155.9, 118.2, 79.5, 40.3, 36.7, 29.9, 29.2, 28.4, 25.6.

2.2. General Procedure for the Synthesis of 10a, b

To a solution of 5-(3,5-bis(trifluoromethyl)phenyl)-4*H*-1,2,4-triazole-3-thiol *3* (98 mg, 0.31 mmol) in 5 mL of CH_3CN, K_2CO_3 (86.5 mg, 0.62 mmol) and compound *8* (0.20 mmol) were added at room temperature. The mixture was stirred at same temperature for 8 h. Upon disappearance of the starting material, CH_3CN was eliminated under reduced pressure. The resulting residue was diluted with ethyl acetate and then washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and filtered. After removing the solvent *in vacuo,* the residue was purified by flash chromatography eluting with (hexane/AcOEt) to yield *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%).

^{1}H NMR (400 MHz, $CDCl_3$-*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); ^{13}C NMR ($CDCl_3$-*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%).

^{1}H NMR (400 MHz, $CDCl_3$-*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); ^{13}C NMR ($CDCl_3$-*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.

2.3. General Procedure for the Synthesis of 11a, b

10 (0.13 mmol) was dissolved in mixture of CH_2Cl_2 and TFA (1:1) at room temperature. The reaction mixture was stirred at same temperature until starting material disappeared. The reaction was monitored by Thin Layer-Chromatography (TLC). The reaction mixture was quenched with KOH aqueous solution (2M). The organic phase was separated, washed with water and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum to afford *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, 76%).

^{1}H 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 $C_{16}H_{10}ClF_6N_6S_2$ [M-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, 74%).

^{1}H NMR (MeOD-d_4): δ 8.64 (s, 2 H), 8.02 (s, 1H), 6.84 (s, 1H), 3.37 (s, 1H), 3.11 (t, *J* = 7.6Hz, 2H), 2.92 (t, *J* = 7.6Hz, 2H), 1.70 (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 $C_{19}H_{16}ClF_6N_6S_2$ [M-H^+]: 541.0471; found: 541.0486.

2.4. General Procedure for the Synthesis of 13a, b

To a solution of *11* (0.09 mmol) in 3 mL of anhydrous DMF and triethylamine (34 µl, 0.24 mmol), was added compound *12* (28 mg, 0.08 mmol). The resulting mixture was stirred at room temperature for 10 h. The reaction mixture was diluted with water, extracted with ethyl acetate and

brine. The combined organic layers were dried over sodium sulfate, filtered and evaporated *in vacuio*. The crude product was purified by flash chromatography on silica gel using (MeOH: DCM, 3:7) to afford compound *13*.

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

^{1}H NMR (DMSO-d_6): δ 8.61 (s, 2H), 8.31 (s, 1H), 8.03 (t, *J* = 5.2Hz, 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 $C_{26}H_{25}ClF_6N_8O_2S_3$ [M^+]: 727.0934; found: 727.0930.

N-(5-((4-((5-(3, 5-Bis(trifluoromethyl) phenyl)-4*H*-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%). ^{1}H-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 $C_{29}H_{32}ClF_6N_8O_2S_3$ [M+H^+]: 769.1403; found: 769.1390.

2.5. Procedure Synthesis for the 2-((5-Azidopentyl) thio)-4, 6-dichloropyrimidine 17

K_2CO_3 (434 mg, 3.14 mmol) and 2,4,6-trichloropyrimidine (230 mg, 1.25 mmol) were added to a solution of compound *15* (152 mg, 1.04 mmol) in acetonitrile (5 mL) then the resulting mixture was stirred at room temperature. Upon completion, the solvent was removed under reduced pressure. The crude residue 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 the solvent was evaporated *in vacuo*. Flash chromatography using (hexane/AcOEt = 9:1) of the residue afforded *17* (310 mg, 84%). ^{1}H NMR ($CDCl_3$- *d*): δ 7.13 (s, 1H), 3.33 (t, J = 6.8 Hz, 2H), 3.23 (t, J = 7.2 Hz, 2H), 1.78 (m, 2H), 1.67 (m, 2H), 1.55 (m, 2H); ^{13}C NMR ($CDCl_3$-d): δ 174.0, 161.9, 115.5, 51.3, 31.3, 28.3, 26.3, 25.8.

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 CH_3CN (5 mL) was added K_2CO_3 (86.5 mg, 0.62 mmol) and compound *17* (61 mg, 0.20 mmol) then the resulting mixture was stirred at room temperature. Upon completion, the solvent was removed under reduced pressure. The crude residue 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 the solvent was evaporated *in vacuo*. The crude residue was purified using silica gel column chromatography (hexane/AcOEt = 7:3) to yield *19*. ^{1}H-NMR ($CDCl_3$-*d*): δ 13.01 (s, 1H), 8.62 (s, 2 H), 7.94 (s, 1H), 7.17 (s, 1H), 3.35 (t, J = 6.4 Hz, 2H), 3.25 (t, J = 7.2 Hz, 2H), 1.81 (m, 2H), 1.67 (m, 2H), 1.58 (m, 2H); ^{13}C-NMR ($CDCl_3$-*d*): δ 173.8, 163.4, 159.7, 147.2, 132.4, 132.0, 126.4, 124.5, 123.1, 121.8, 118.9, 115.5, 113.9, 51.2, 29.8, 28.3, 25.8. 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 microtitier 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.

3. Results and Discussion

3.1. Chemistry

The compound SCA 21 in Figure 1 was identified as SecA inhibitor for further optimization in earlier work [32].

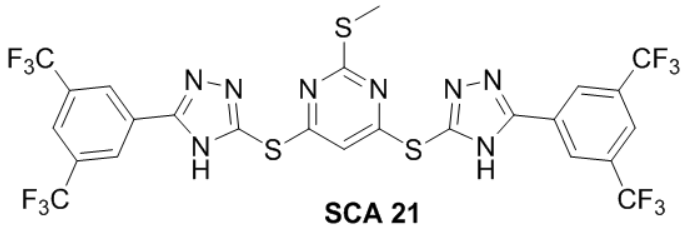


Figure 1. *Lead compound for the synthesis of novel SecA inhibitors.*

To enhance SCA 21's potency, we began to simplify the structure by dissecting the lead compound in half and removing part *B*. Then, by changing the methyl group to an alkylamine moiety which has been biotinylated, we hypothesized 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. We expected that it will be able to increase the antibacterial activity or at least it should be more beneficial in terms of activity. Also, by changing the

methyl to an azidopentyl, we thought that could improve the potency because sodium azide is a well-known SecA inhibitor [33]. The general strategy of analogue design was showed in Figure 2.

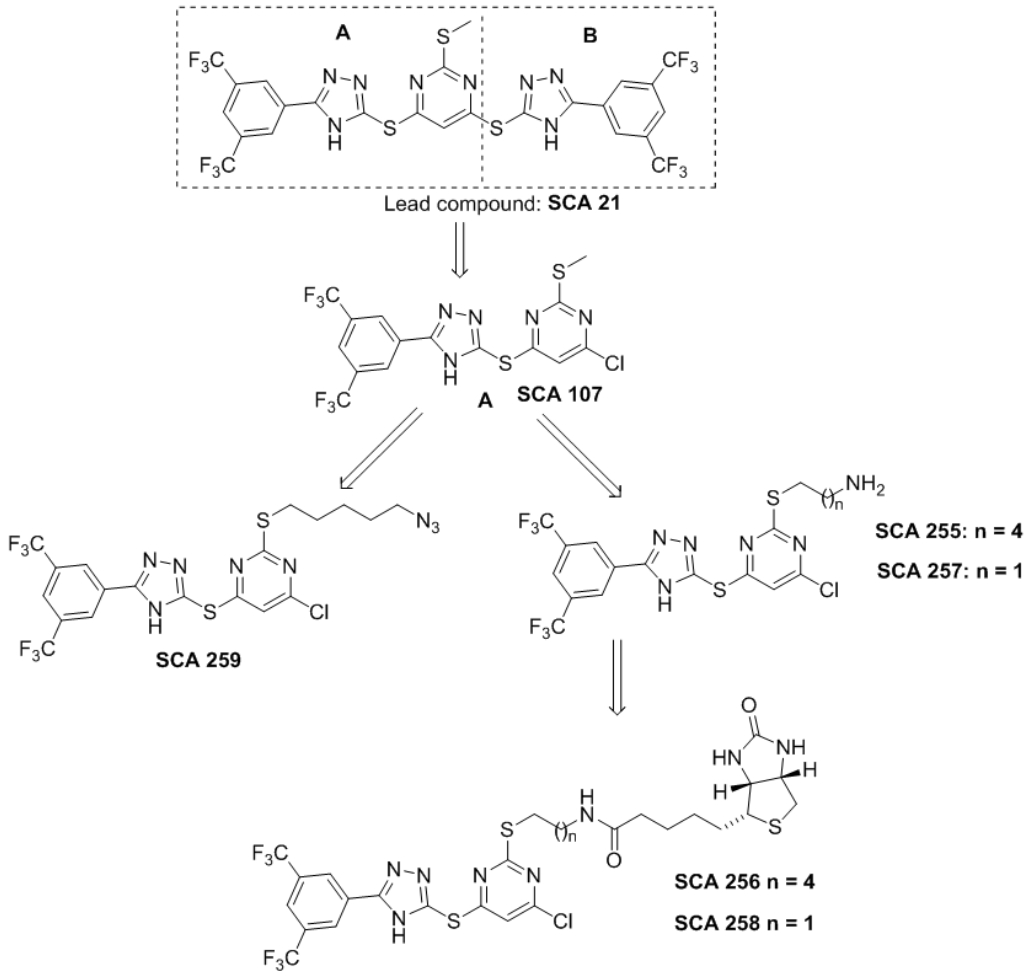
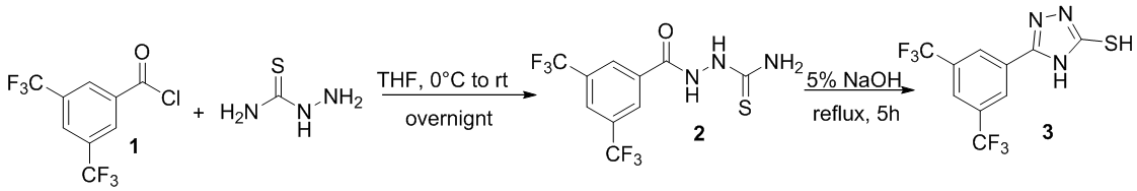


Figure 2. *Optimization of the lead compound for the synthesis of novel SecA inhibitors.*

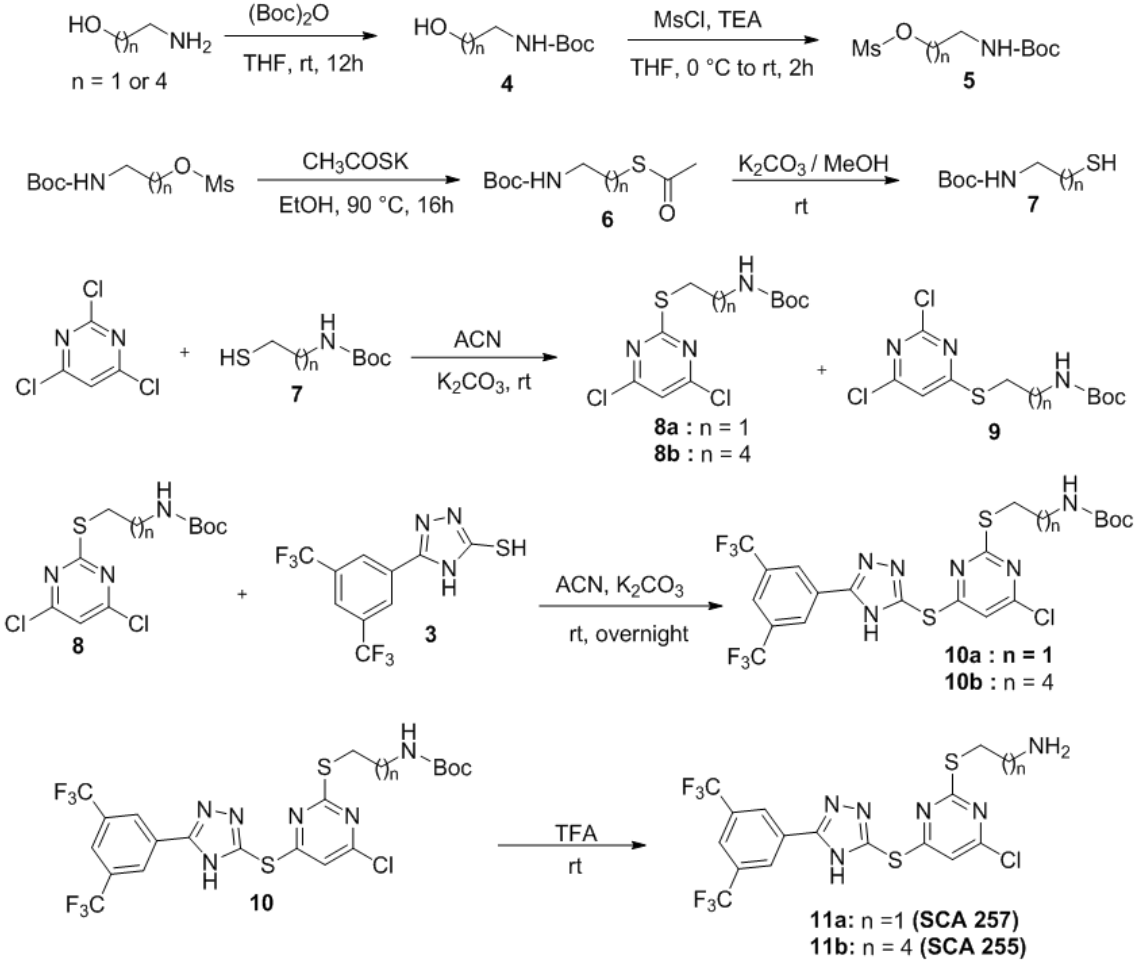
The desired compounds synthesis began from commercially available 3,5-Bis(trifluoromethyl) benzoyl chloride *1* by reaction with hydrazine carbothioamide in tetrahydrofuran at room temperature overnight. The resulting compound *2* refluxed in 5% sodium hydroxide solution by self-condensation affording *3* [34] (Scheme 1). Compounds *7* and *15* were prepared following earlier reported procedures respectively in Scheme 2 [35] and Scheme 4 [36]. Compounds *8*, *9*, *17* and *18* were synthesized by reaction of compounds *7* and *15* with 2,4,6-trichloro pyrimidine in CH_3CN at room temperature in the presence of potassium carbonate [37]. Compounds *10* and *19* were obtained by reaction of *3* with respectively *8* and *17* in acetonitrile under weakly basic conditions (Schemes 2 and 4). Then, compounds *10* were deprotected with 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 the free amine on the ester group, followed by an amide bond formation and a release of N-hydroxysuccinimide (Scheme 3) [39]. SCA 107 was synthesized and tested in our earlier work [32].



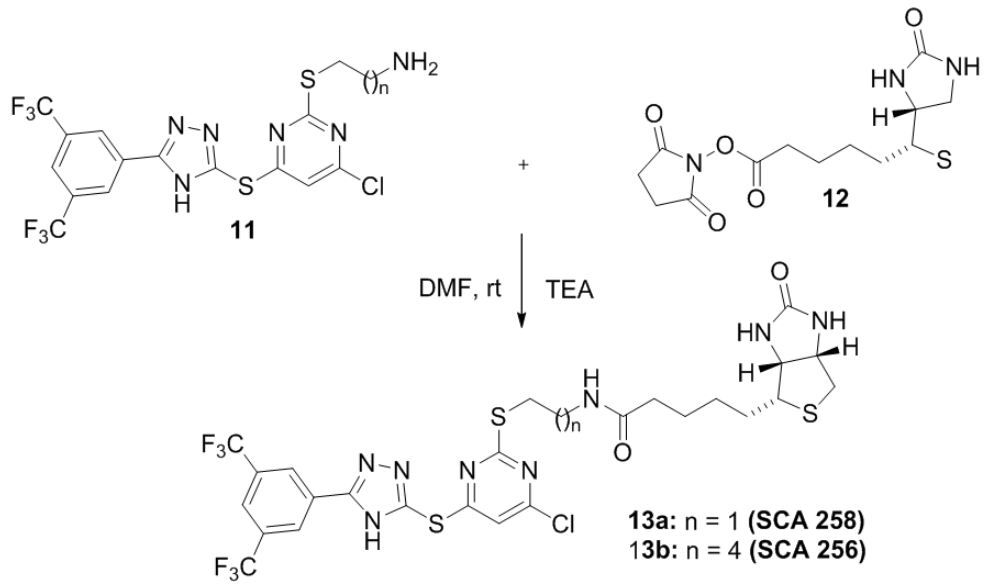
Scheme 1. *Synthesis of compound 3.*

The structures of the synthesized compounds were established by ^{1}H NMR, ^{13}C NMR and mass spectrometry. In their ^{1}H NMR spectra, the singlet signal at 13.01 ppm was assigned to the NH of the triazole group even if this signal

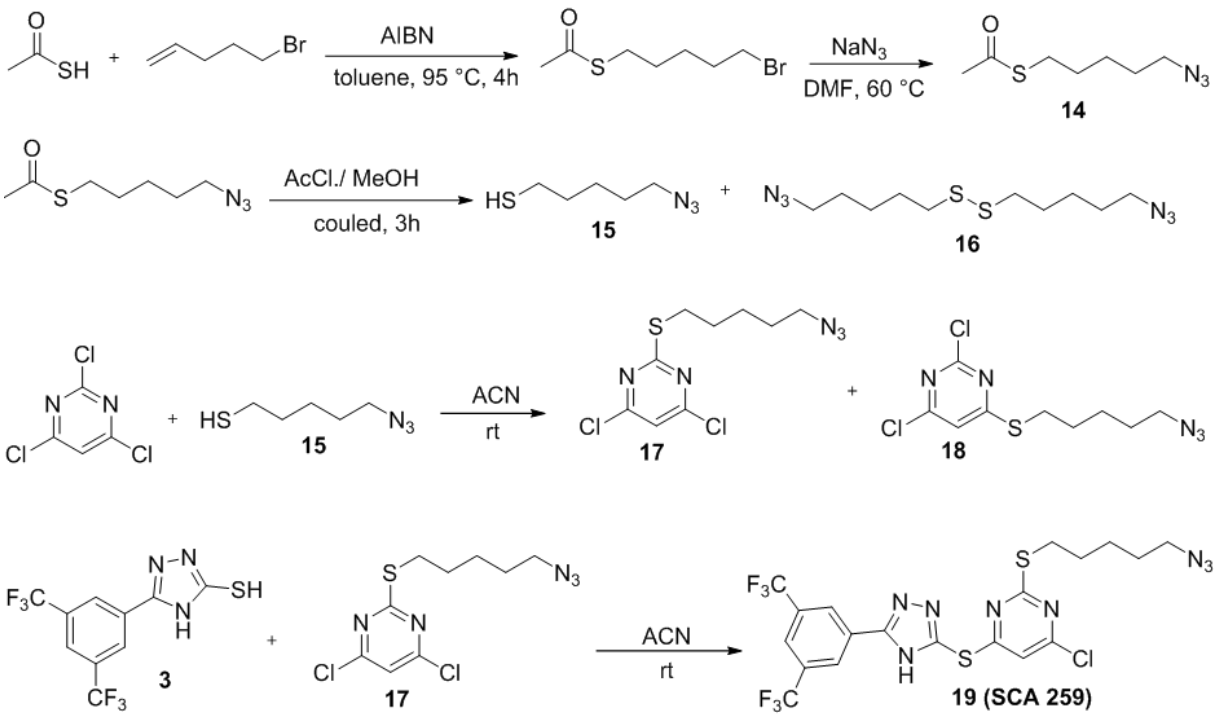
was not observed in all compounds could be due to the exchange with residual deuterared water (D_2O). In their ^{13}C NMR spectra, the signal around 172 ppm confirmed the presence of the amidine groups. The peaks at 174 and 168 ppm were assigned to the C=O in compounds *13a, b*. Characteristic signals resonated from 51 to 25 ppm were assigned to the aliphatic carbons for the compound *19* and appeared from 38 to 25 ppm for compounds *13a, b*. Hight resolution mass spectral analysis results were in accordance with the calculated values.



Scheme 2. *Synthesis of compounds 11a, b.*



Scheme 3. *Synthesis of compounds 13a, b.*



Scheme 4. *Synthesis of compound 19.*

3.2. 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 µM and at 50 µM. Three compounds (SCA255, SCA256 and SCA259) showed inhibition greater than 50% at 50 µM, but only one compound (SCA259) showed more than 50% inhibition at 25 µM, as shown in Figure 3. In addition, 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 µM against EcSecAN68 [32]. SCA 259 was then further evaluated at various concentrations to allow the determination of IC_{50} value at 5.9 µM. Thus, SCA 259 was more potent than the lead compound SCA21 with IC_{50} at 18 µM against EcSecAN68 [32]. These results suggested that the azide pentyl group was beneficial for potent inhibitory activity. The biotinylated compound SCA 256 was more active than compound SCA 255 with the amine group. Thus, even if the biotinylated compound SCA 256 was not more active than our earlier best triazole-pyrimidine SCA 107, the biotinylation was beneficial to enhance the antibacterial activity of SCA 255.

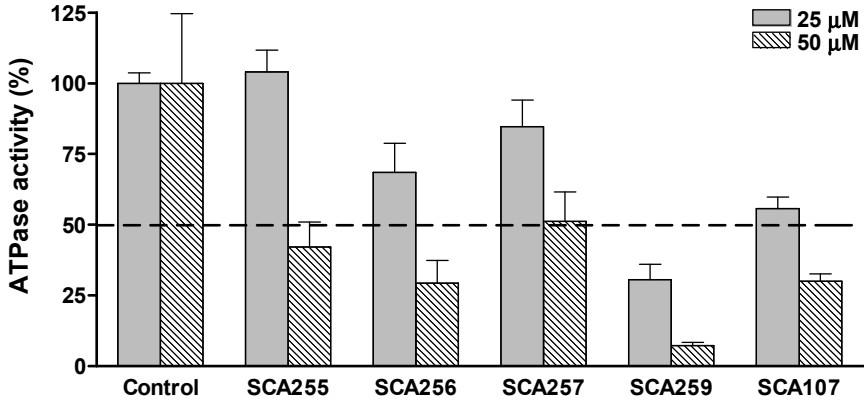


Figure 3. *Screening for inhibition of the ATPase activity of EcSecAN68.*

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

Table 1. *Bacteriostatic effects of SecA inhibitors.*

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

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

4. Conclusion

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

Acknowledgements

We wish to thank Binghe Wang and Phang-Cheng Tai respectively for helpful synthesis and biological evaluation in their labs in Georgia State University (GSU). Jin Jinshan provided helpful for biological activities.

References

[1] Rice LB (2009) The clinical consequences of antimicrobial resistance. Curr Opin Microbiol 12 (5): 476-481.

[2] Smets D, Loos MS, Karamanou S, Economou A (2019) Protein Transport Across the Bacterial Plasma Membrane by the Sec Pathway. Protein J 38 (3): 262-273.

[3] Kusters I, Driessen AJ (2011) SecA, a remarkable nanomachine. Cell Mol life Sci 68: 2053-2066.

[4] Hsieh YH, Zhang H, Lin BR, Cui N, Na B, Yang H, Jiang C, Sui SF, Tai PC (2011) SecA Alone Can Promote Protein Translocation and Ion Channel Activity: SecYEG increases efficiency and signal peptide specificity. J Biol Chem 286: 44702-44709.

[5] Banerjee T, Zheng Z, Abolafia J, Harper S, Oliver D (2017) The SecA protein deeply penetrates into the SecYEG channel during insertion, contacting most channel transmembrane helices and periplasmic regions. J Biol Chem 292: 19693-19707.

[6] Findik BT, Smith VF, Randall LL. (2018) Penetration into membrane of amino-terminal region of SecA when associated with SecYEG in active complexes. Protein Sci 27: 681-691.

[7] Cranford-Smith T, Huber D (2018) The way is the goal: how SecA transports proteins across the cytoplasmic membrane in bacteria. FEMS Microbiol Lett 365.

[8] Ma C, Wu X, Sun D, Park E, Catipovic MA, Rapoport TA, Gao N, Li L (2019) Structure of the substrate-engaged SecA-SecY protein translocation machine. Nat Commun 10: 2872.

[9] Gupta R, Toptygin D, Kaiser CM (2020) The SecA motor generates mechanical force during protein translocation. Nat Commun 11: 3802.

[10] Rao CVS, De Waelheyns E, Economou A, Anné J (2014) Antibiotic targeting of the bacterial secretory pathway. Biochim Biophys Acta 1843 (8): 1762-1783.

[11] Chaudhary AS, Chen W, Jin J, Tai PC, Wang B (2015) SecA: a potential antimicrobial target. Future Med Chem 7 (8): 989-1007.

[12] Jin J, Hsieh YH, Chaudhary AS, Cui J, Houghton JE, Sui SF, Wang B, Tai PC (2018) SecA inhibitors as potential antimicrobial agents: differential actions on SecA-only and SecA-SecYEG protein-conducting channels. FEMS Microbiol Lett 365 (15): fny 145.

[13] Catipovic MA, Bauer BW, Loparo JJ, Rapoport TA (2019) Protein translocation by the SecA ATPase occurs by a power-stroke mechanism. EMBO J 38 (9): e101140.

[14] Chen W, Huang YJ, Gundala SR, Yang H, Li M, Tai PC, Wang B (2010) The first low microM SecA inhibitors. Bioorg Med Chem 18 (4): 1617-1625.

[15] Cui J, Jin J, Hsieh YH, Yang H, Ke B, Damera K, Tai PC, Wang B (2013) Design, Synthesis and Biological Evaluation of Rose Bengal Analogues as SecA Inhibitors. ChemMedChem 8 (8): 1384-1393.

[16] Li M, Huang YJ, Tai PC, Wang B (2008) Discovery of the first SecA inhibitors using structure-based virtual screening. Biochem Biophys Res Commun 368 (4): 839-845.

[17] Chaudhary AS, Jin J, Chen W, Tai PC, Wang B (2015) Design, syntheses and evaluation of 4-oxo-5-cyano thiouracils as SecA inhibitors. Bioorg Med Chem 23: 105-117.

[18] Jang MY, De Jonghe S, Segers K, Anné J, Herdewijn P (2011) Synthesis of novel 5-amino-thiazolo[4,5-d]pyrimidines as *E. coli* and *S. aureus* SecA inhibitors. Bioorg Med Chem 19 (1): 702-714.

[19] Akula N, Trivedi P, Han FQ, Wang N (2012) Identification of small molecule inhibitors against SecA of *Candidatus Liberibacter* asiaticus by structure based design. Eur J Med Chem 54: 919-924.

[20] Akula N, Zheng H, Han FQ, Wang N (2011) Discovery of novel SecA inhibitors of *Candidatus Liberibacter* asiaticus by structure based design. Bioorg Med Chem Lett 21 (14): 4183-4188.

[21] Bamba F, Jin J, Chaudhary AS, Tai PC, Wang B (2021) Design, synthesis, and biological evaluation of pyrimidine analogs as SecA inhibitors. Med Chem Res 30: 1334-1340.

[22] Bamba F, Jin J, Tai PC, Wang B (2020) Synthesis and biological evaluation of novel 4-oxo-5-cyano thiouracil derivatives as SecA inhibitors. Heterocyc Commun 26: 76-83.

[23] Eswaran S, Adhikari AV, Shetty NS (2009) Synthesis and antimicrobial activities of novel quinoline derivatives carrying 1,2,4-triazole moiety. Eur J Med Chem 44: 4637-4647.

[24] Schneider P, Hawser S, Islam K (2003) Iclaprim, a novel diaminopyrimidine with potent activity on trimethoprim sensitive and resistant bacteria. Bioorg Med Chem Lett 13: 4217-4221.

[25] Barot KP, Manna KS, Ghate MD (2017) Design, synthesis and antimicrobial activities of some novel 1,3,4-thiadiazole, 1,2,4-triazole-5-thione and 1,3-thiazolan-4-one derivatives of benzimidazole. Journal of Saudi Chemical Society 21: S35-S43.

[26] Smith J, Andes D (2008) Therapeutic drug monitoring of antifungals: pharmacokinetic and pharmacodynamic considerations. Therapeutic drug monitoring 30: 167-172.

[27] Afreen F, Chakraborty R, Thakur A (2015) Synthesis of a triazole derivative and evaluation of their antituberculer activity. Int J Pharmaceut Chem 5: 343-349.

[28] Pandey V, Tusi Z, Tusi S, Joshi M (2012) Synthesis and biological evaluation of some novel 5-[(3-aralkyl amido/imidoalkyl) phenyl]-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines as antiviral agents. ISRN Org Chem 2012: 760517. https://doi.org/10.5402/2012/760517.

[29] De Clercq E (2004) Antiviral drugs in current clinical use. J Clin Virol 30: 115-133.

[30] Sharma V, Chitranshi N, Agarwal AK (2014) Significance and biological importance of pyrimidine in the microbial World. Int J Med Chem 2014: 202784. doi: 10.1155/2014/202784.

[31] Kaur P, Chawla A (2017) 1,2,4-triazole: a review of pharmacological activities. Int Res J Pharm 8: 10-29.

[32] Cui J, Jin J, Chaudhary AS, Hsieh YH, Zhang H, Dai C, Damera K, Chen W, Tai PC, Wang B (2016) Design, synthesis and evaluation of triazole-pyrimidine analogues as SecA inhibitors. ChemMedChem. 11 (1): 43-56. doi: 10.1002/cmdc.201500447.

[33] Nakane A, Takamatsu H, Oguro A, Sadaie Y, Nakamura K, Yamane K (1995) Acquisition of azide-resistance by elevated SecA ATPase activity confers azide-resistance upon cell growth and protein translocation in Bacillus subtilis. Microbiology 141: 113-21.

[34] Plech T, Wujec M, Siwek A, Kosikowska U, Malm A (2011) Synthesis and antimicrobial activity of thiosemicarbazides, s-triazoles and their Mannich bases bearing 3-chlorophenyl moiety. Eur J Med Chem 46 (1): 241-248.

[35] Leysen D, Defert O, Kaval N, Blom P, Boland S (2011) Heterocyclic amides as rock inhibitors. WO2011107608A1.

[36] Sommer WJ, Weck M (2007) Facile functionalization of gold nanoparticles via microwave-assisted 1,3 dipolar cycloaddition. Langmuir 23 (24): 11991-11995. doi: 10.1021/la7018742.

[37] Patil SS, Jadhav RP, Patil AA, Patil SV, Bobade VD (2010) J Chem Pharm Res 2: 38 – 51.

[38] Fresno N, Macías-González M, Torres-Zaguirre A, Romero-Cuevas M, Sanz-Camacho P, Elguero J, Pavón J, Rodríguez de Fonseca F, Goya P, Pérez-Fernández R (2015) Novel Oxazolidinone-Based Peroxisome Proliferator Activated Receptor Agonists: Molecular Modeling, Synthesis, and Biological Evaluation. J Med. Chem 58 (16): 6639–6652.

[39] Li K, Chen Y, Li S, Nguyen HG, Niu Z, You S, Mello CM, Lu X, Wang Q (2010) Chemical Modification of M13 Bacteriophage and Its Application in Cancer Cell Imaging. Bioconjugate Chem 21 (7): 1369-1377.