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Fante Bamba, Jinshan Jin, Phang C. Tai* and Binghe Wang*

Synthesis and biological evaluation of novel 4-oxo-5-cyano thiouracil derivatives as SecA inhibitors

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Abstract: The continuous emergence of drug-resistant strains of bacteria poses an urgent risk to human health and dictates the need for new antimicrobials. Along this line, we have been working on developing inhibitors of SecA, a key component of the bacterial Sec-dependent secretion machinery. Herein, we describe the synthesis and antimicrobial evaluation of 6-oxo-1,6-dihydropyrimidine-5-carbonitrile derivatives as potential SecA inhibitors.

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

Introduction

Bacterial infection remains a serious threat to human health, largely because of the continuous emergence of drug-resistance strains of bacteria [1, 2]. To address this concern, both the World Health Organization (WHO) [3] and the US Center for Disease Control (CDC) [4] issued calls for urgent actions. Therefore, there has been much effort in search of new antimicrobials. Especially desirable are efforts toward identifying and inhibiting new targets and/or new mechanisms of actions. Along this line, we have been working on developing SecA inhibitors as a way to achieve antimicrobial activities. SecA is a key component of the bacterial protein secretion (Sec) pathways. It is an ATPase-driven "motor," which couples the hydrolysis of ATP to the stepwise translocation of preproteins across the bacterial cytoplasmic membrane [5-8]. Because SecA is a conserved and essential protein in all bacteria and is absent in humans, it is considered as a promising antibacterial drug target. Various SecA inhibitors (Figure 1) have been described in the literature [9]. Prior to our recent efforts, sodium azide was known as a SecA inhibitor and was reported to possess antibacterial activity with IC_{50} of 1mM. The first natural product inhibitor of SecA, CJ-21058 [10] isolated from an unidentified fungus, showed antibacterial activity against Gram-positive bacteria by inhibiting the SecA translocation ATPase activity. Additional examples include pannomycin [11], thiazolo[4,5-d]pyrimidine derivatives [12], and others [13, 14]. During our studies on developing small molecule SecA inhibitors, we reported a structure-based virtual screening approach for the discovery of small-molecule inhibitors of the intrinsic ATPase activity of SecA [15]. Later on, we described optimization work [16] and inhibitors of different structural scaffolds [17, 18]. Among all the efforts, we reported 4-oxo-5-cyano thiouracil derivatives as SecA inhibitors [19]. Herein, we describe our efforts in optimizing a hit (Figure 1), a 4-oxo-5-cyano thiouracil, and evaluation of new analogs for antibacterial activities. The work is based on the general structural scaffold of the lead with the aim of searching for the surrounding chemical space [16]. Below, we describe the results and implications in guiding future work in this area.

***Corresponding authors: Phang C. Tai and Binghe Wang,** Departments of Chemistry and Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, Georgia 30303, USA, e-mail: wang@gsu.edu
Fante Bamba, Departments of Chemistry, Georgia State University, Atlanta, Georgia 30303, USA; Laboratoire de Chimie Organique et des Substances Naturelles, Université Félix Houphouët-Boigny, 22 Bp 582 Abidjan 22, COTE D'IVOIRE
Jinshan Jin, Departments of Biology, Georgia State University, Atlanta, Georgia 30303, USA

Results and discussion

Chemistry

Based on our earlier work [19], the compound in Figure 1 was the lead compound for further exploration of the chemical space that could inhibit SecA. In this respect, we focused our attention on the *para* position of the phenyl

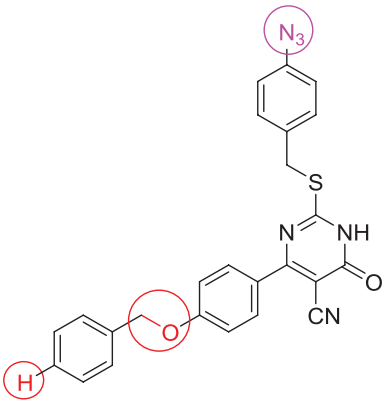
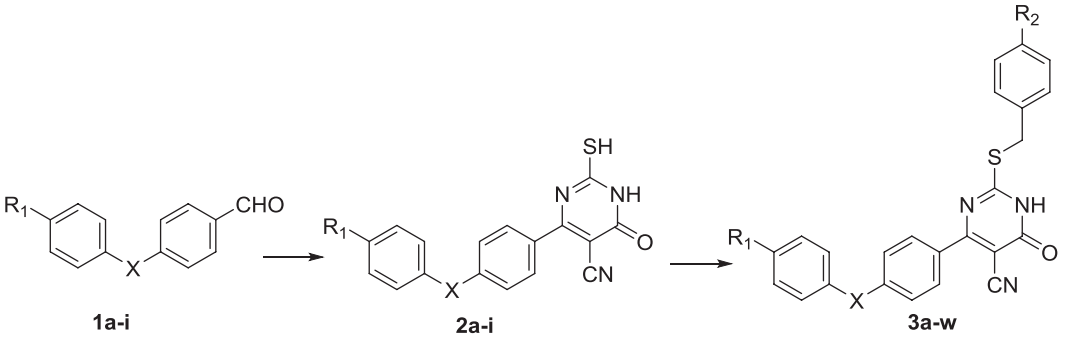


Figure 1 Lead compound for the synthesis of novel SecA inhibitors.

group, *para* position of the benzylthio group and the linker between the biphenyl rings. Specifically, we designed compounds with various substituents such as F-, Me-, MeO- on the phenyl group at position 4; linkers such as -CH_2O-, -O-, or -HN-CO- between the two phenyl rings, and substituents such as -N_3, -COOMe, or -CF_3 at the 4-position of the benzylthioether moiety. Such variations led to compounds **3a-w**.

The synthetic route of the compounds **3a-w** is outlined in Scheme 1. Compounds **1a-e** were prepared by *O*-benzylation of 4-hydroxybenzaldehyde using the appropriate aryl halides under basic conditions [20]. The corresponding 4-formylbenzoic acid was coupled with the



1a. R_1 = H, X = -CH_2-O-
1b. R_1 = Me, X = -CH_2-O-
1c. R_1 = MeO, X = -CH_2-O-
1d. R_1 = F, X = -CH_2-O-
1e. R_1 = CF_3, X = -CH_2-O-
1f. R_1 = H, X = -O-
1g. R_1 = H, X = -HN-CO-
1h. R_1 = Cl, X = -HN-CO-
1i. R_1 = Me, X = -HN-CO-

2a. R_1 = H, X = -CH_2-O-
2b. R_1 = Me, X = -CH_2-O-
2c. R_1 = MeO, X = -CH_2-O-
2d. R_1 = F, X = -CH_2-O-
2e. R_1 = CF_3, X = -CH_2-O-
2f. R_1 = H, X = -O-
2g. R_1 = H, X = -HN-CO-
2h. R_1 = Cl, X = -HN-CO-
2i. R_1 = Me, X = -HN-CO-

3a. SCA-242 R_2 = -CF_3, R_1 = H, X = -CH_2-O-
3b. SCA-224 R_2 = -N_3, R_1 = Me, X = -CH_2-O-
3c. SCA-234 R_2 = -COOMe, R_1 = Me, X = -CH_2-O-
3d. SCA-235 R_2 = -N_3, R_1 = MeO, X = -CH_2-O-
3e. SCA-229 R_2 = -COOMe, R_1 = MeO, X = -CH_2-O-
3f. SCA-246 R_2 = -CF_3, R_1 = MeO, X = -CH_2-O-
3g. SCA-228 R_2 = -N_3, R_1 = F, X = -CH_2-O-
3h. SCA-236 R_2 = -COOMe, R_1 = F, X = -CH_2-O-
3i. SCA-244 R_2 = -CF_3, R_1 = F, X = -CH_2-O-
3j. SCA-231 R_2 = -N_3, R_1 = -CF_3, X = -CH_2-O-
3k. SCA-237 R_2 = -COOMe, R_1 = -CF_3, X = -CH_2-O-
3l. SCA-243 R_2 = -CF_3, R_1 =-CF_3, X = -CH_2-O-
3m. SCA-241 R_2 = -CF_3, R_1 = H, X = -O-
3n. SCA-273 R_2 = -N_3, R1 = H, X = -HN-CO-
3o. SCA-275 R_2 = -COOMe, R1 = H, X = -HN-CO-
3p. SCA-267 R_2 =-CF_3, R1 = H, X = -HN-CO-
3q. SCA-274 R_2 = -N_3, R_1 = Cl, X = -HN-CO-
3r. SCA-268 R_2 = -COOMe, R_1 = Cl, X = -HN-CO-
3s. SCA-269 R_2 = -CF_3, R_1 = Cl, X = -HN-CO-
3t. SCA-276 R_2 = -N_3, R_1 = Me, X = -HN-CO-
3u. SCA-271 R_2 = -COOMe, R_1 = Me, X = -HN-CO-
3v. SCA-272 R_2 = -CF_3, R_1 = Me, X = -HN-CO-
3w. SCA-226 R_2 = -COOMe, R_1 = H, X = -O-

Reagents and conditions: **1a-e)** HO-Ph-*p*-CHO, Br-CH_2-Ph-*p*-R_1, K_2CO_3, DMF, 65-97%; **1f)** F-Ph-*p*-CHO, Ph-OH, Cs_2CO_3, DMF, 80 °C; **1g-i)** HO_2C-Ph-*p*-CHO, H_2N-Ph-*p*-R_1, isobutylchloroformate, Et_3N, CH_2Cl_2, 0°C - rt, 49-83%; **2a-i)** Thiourea, NC-$CH_2COOCH_2CH_3$, piperidine, CH_3CH_2OH, reflux, overnight, 36-42%; **3a-w)** *p*-R_2-Ph-CH_2-Br, CH_3CN, rt, overnight, 47-71%.

Scheme 1 Syntheses of compounds **3a-w.**

respective phenyl/aryl amines using isobutyl chloroformate in the presence of triethylamine to provide amide of type **1g-h** by published procedure [21]. Following the methods for obtaining the core scaffold previously [16], compounds **2a-I** were obtained by condensation of an appropriate aldehyde with ethyl cyanoacetate and thiourea in the presence of piperidine. This was followed by the *S*-benzylation in the presence of potassium carbonate in acetonitrile to obtain compounds **3a-w**.

Biological evaluation

The activities of the synthesized compounds were evaluated in a MIC assay using established procedures using a mutant of *Escherichia. coli* with a compromised outer membrane [19]. It was found that most of the compounds were not active at concentrations as high as 250 μM. Three compounds showed MIC at about 20 μM (**3i, l, w**), and one at about 8 μM (**3m**), which is comparable to some of our best compounds in this class [22]. In addition, select compounds were also tested against Gram-positive bacteria *Bacillus anthracis* (Sterne strain). With the limited data set, it seems that the potency against *B. anthracis* was comparable with or higher than that of *E. coli*. For example, the MIC of **3w** was 25 μM against *E. coli* and 12.5 μM against *B. anthracis*. By examining the details, it seems that a CF_3 group at the R_2 position is beneficial, especially if the central linker is $-CH_2$-O-. With a -O- linker, it seems that an ester group also offers a good outcome. Overall, the results are very useful information for those who might be interested in SecA inhibitor for both improved potencies and for avoiding chemical space that would not be productive.

Conclusion

We have described the design, syntheses and biological evaluation of a novel structural class of small-molecule SecA inhibitors with μM inhibition. A CF_3 group at the R_2 position and a diaryl ether linker seem to be beneficial for improved potency of the lead compound.

Experimental

All chemical reagents and solvents used were 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 ^{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 (^{1}H NMR) and solvents (^{13}C NMR) as internal standards.

General procedure for the synthesis of (2a-i): Our previously published [24] procedure was followed. Briefly, to a solution of ethanol (25 mL) and appropriate aldehyde (RCHO, 5 mmol) was added ethyl cyanoacetate (0.5 mL, 5 mmol), thiourea (0.38 g, 5 mmol) and piperidine (1.0 mL, 10 mmol). The mixture was heated under reflux overnight and then cooled to room temperature. The precipitate was dissolved in 0.5 M NaOH (20 mL) and washed with ethyl acetate (15 ml ×3). Then the aqueous solution was acidified to pH ~ 2 by slow addition of 1 M HCl. This caused the product to precipitate, which was then filtered using vacuum filtration.

General procedure for the synthesis of (3a-w): To a solution of 4-(4-(benzyloxy)phenyl)-2-mercapto-6-oxo-1,6-dihydropyrimidine-5-carbonitrile derivatives (1.36 mmol) in CH_3CN (10 ml), K_2CO_3 (6.79 mmol) was added and the resulting mixture was stirred for 10-15 min. To this was added the appropriate (bromomethyl)phenyl derivatives (1.22 mmol) and the reaction was stirred at room temperature for 16-18h. Upon disappearance of the starting material, the solvent was removed *in vacuo*. The dried residue was washed by H_2O (pH = 9-10, 20 mL × 2) and brine (15 ml × 2) followed by product extraction in ethyl acetate (20 ml). The solvent was evaporated *in vacuo* to obtain crude product, which was purified using silica gel column chromatography.

2-mercapto-4-(4-((4-methylbenzyl)oxy)phenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2b)

Yield 37%; white solid; ^{1}H NMR (DMSO-d_6): δ 13.16 (bs, 1H), 12.86 (s, 1 H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.1 Hz, 2H), 7.22 (d, *J* = 7.6 Hz, 2H), 7.16 (d, *J* = 8.4 Hz, 2H), 5.16 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 177.5, 161.7, 159.7, 137.8, 133.9, 131.2, 129.5, 128.4, 122.8, 116.1, 115.1, 89.5, 69.9, 21.3. **HRMS (ESI) (m/z):** Calcd. for $C_{19}H_{14}N_3O_2S$, $[M-H]^+$: 348.0803; found: 348.0803.

2-mercapto-4-(4-((4-methoxybenzyl)oxy)phenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2c)

Yield 42%; white solid; ^{1}H NMR (DMSO-d_6): δ 13.15 (bs, 1H), 12.85 (s, 1H), 7.66 (d, *J* = 8.8 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.4 Hz, 2H), 6.96 (d, *J* = 8.4 Hz, 2H), 5.12 (s, 2H),

3.76 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 177.4, 161.7, 161.6, 159.6, 159.6, 131.2, 130.1, 128.7, 22.7, 116.1, 115.0, 114.3, 89.4, 69.7, 55.5. **HRMS (ESI) (m/z):** Calcd. for $C_{19}H_{14}N_3O_3S$, $[M-H]^+$: 364.0750; found: 364.0753.

4-(4-((4-fluorobenzyl)oxy)phenyl)-2-mercapto-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2d)

Yield 40%; white solid; ^{1}H NMR (DMSO-d_6): δ 13.17 (bs, 1H), 12.84 (s, 1H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.54 (m, 2H), 7.24 (t, *J* = 8.8 Hz, 2H), 7.18 (d, *J* = 8.8 Hz, 2H), 5.19 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 177.4, 163.6, 161.4, 159.5, 133.2, 131.1, 130.6, 115.8, 115.6, 114.9, 89.1, 69.2. **HRMS (ESI) (m/z):** Calcd. for $C_{18}H_{12}FN_3O_2S$, $[M-H]^+$: 352.0551; found: 352.0556.

2-mercapto-6-oxo-4-(4-((4-(trifluoromethyl)benzyl)oxy)phenyl)-1,6-dihydropyrimidine-5-carbonitrile (2e)

Yield 36%; white solid; ^{1}H NMR (DMSO-d_6): δ 13.18 (bs, 1H), 12.73 (s, 1H), 7.79 (d, *J* = 8.0 Hz, 4H), 7.70 (m, 4H), 7.19 (d, *J* = 8.8 Hz, 2H), 5.34 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 177.8, 161.9, 161.2, 159.9, 141.9, 131.2, 129,1, 128.7, 128.6, 126.0, 125.8, 125.8, 123.6, 123.3, 116.3, 115.0, 89.2, 69.0. **HRMS (ESI) (m/z):** Calcd. for $C_{19}H_{11}F_3N_3O_2S$, $[M-H]^+$: 402.0519; found: 402.0522.

4-(5-cyano-2-mercapto-6-oxo-1,6-dihydropyrimidin-4-yl)-N-phenylbenzamide (2g)

Yield 42%; white solid; ^{1}H NMR (DMSO-d_6): δ 12.32 (s, 1H), 10.41 (s, 1H), 8.07 (d, *J* = 8.4 Hz, 2H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.10 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (DMSO-d_6): δ 180.1, 167.6, 165.3, 163.9, 160.9, 141.1, 139.4, 137.5, 136.6, 129.5, 129.1, 129.0, 128.2, 128.0, 124.3, 120.9, 120.8, 118.6, 117.0, 88.8. **HRMS (ESI) (m/z):** Calcd. for $C_{18}H_{11}N_4O_2S$, $[M-H]^+$: 347.0597; found: 347.0599.

N-(4-chlorophenyl)-4-(5-cyano-2-mercapto-6-oxo-1,6-dihydropyrimidin-4-yl)benzamide (2h)

Yield 38%; white solid; ^{1}H NMR (DMSO-d_6): δ 11.76 (s, 1H), 10.52 (s, 1H), 8.03 (d, *J* = 8.0 Hz, 2H), 7.86 (m, 4H), 7.42 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR (DMSO-d_6): δ 183.1, 166.6, 165.5, 162.7, 140.3, 138.5, 136.3, 128.9, 128.7, 127.9, 122.4, 118.8, 86.2. **HRMS (ESI) (m/z):** Calcd. for $C_{18}H_{10}ClN_4O_2S$, $[M-H]^+$: 381.0208; found: 381.0209.

4-(5-cyano-2-mercapto-6-oxo-1,6-dihydropyrimidin-4-yl)-N-(p-tolyl)benzamide (2i)

Yield 34%; white solid; ^{1}H NMR (DMSO-d_6): δ 11.68 (s, 1H), 10.29 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 2H), 7.86 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.4Hz, 2H); ^{13}C NMR (DMSO-d_6): δ 183.6, 167.0, 165.2, 162.7, 140.8, 137.0, 136.6, 133.2, 129.4, 128.6, 127.7, 120.9, 119.1, 86.0, 20.9. **HRMS (ESI) (m/z):** Calcd. for $C_{19}H_{13}N_4O_2S$, $[M-H]^+$: 361.0754; found: 361.0763.

4-(4-(benzyloxy)phenyl)-6-oxo-2-((4-(trifluoromethyl)benzyl)thio)-1,6-dihydropyrimidine-5-carbonitrile (3a)

Yield 54%; white solid; ^{1}H NMR (DMSO-d_6): δ 7.93 (d, *J* = 8.4 Hz, 2H), 7.66 (qd, *J* = 8.0 Hz, 4H), 7.48 (d, *J* = 7.2 Hz, 2H), 7.41 (t, *J* = 7.2 Hz, 1H), 7.37(m, 1H), 7.18 (d, *J* = 8.4 Hz, 2H), 5.22 (s, 2H), 4.61 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 166.7, 165.5, 161.6, 142.5, 136.9, 131.2, 130.1, 128.9, 128.4, 128.3, 127.9, 126.0, 125.8, 125.7, 123.3, 116.9, 115.2, 91.9, 69.9, 33.8. **HRMS (ESI) (m/z):** Calcd. for $C_{26}H_{17}F_3N_3O_2S$, $[M-H]^+$: 492.0994; found: 492.0998.

2-((4-azidobenzyl)thio)-4-(4-((4-methylbenzyl)oxy)phenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (3b)

Yield 61%; white solid; ^{1}H NMR (DMSO-d_6): δ 8.00 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.20 (t, *J* = 8.8 Hz, 4H), 7.07 (d, *J* = 8.4 Hz, 2H), 5.17 (s, 2H), 4.54 (s, 2H), 2.31 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 166.8, 165.4, 161.8, 139.0, 137.7, 133.9, 133.9, 131.2, 131.1, 129.5, 128.3, 127.7, 119.6, 116.7, 115.3, 92.0, 69.9, 34.0, 21.2. **HRMS (ESI) (m/z):** Calcd. for $C_{26}H_{19}N_6O_2S$, $[M-H]^+$: 479.1290; found: 479.1300.

methyl4-(((5-cyano-4-(4-((4-methylbenzyl)oxy)phenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)methyl)benzoate (3c)

Yield 55%; white solid; ^{1}H NMR (DMSO-d_6): δ 7.94 (d, *J* = 8.0 Hz, 2H), 7.90 (d, *J* = 8.0 Hz, 2H), 7.57 (d, *J* = 7.6 Hz, 2H), 7.36 (d, *J* = 7.6 Hz, 2H), 7.21 (d, *J* = 7.6 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 5.17 (s, 2H), 4.60 (s, 2H), 3.83 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 166.8, 166.4, 165.5, 161.7, 137.7, 133.9, 131.2, 129.8, 129.7, 129.5, 129.0, 128.4, 127.8, 115.2, 91.8, 69.9, 52.5, 34.1, 21.2. **HRMS (ESI) (m/z):** Calcd. for $C_{28}H_{22}N_3O_4S$, $[M-H]^+$: 496.1331; found: 496.1337.

2-((4-azidobenzyl)thio)-4-(4-((4-methoxybenzyl)oxy) phenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (3d)

Yield 50%; white solid; ^{1}H NMR (DMSO-d_6): δ 8.00 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.07 (d, *J* = 8.4 Hz, 2H), 6.96 (d, *J* = 8.4 Hz, 2H), 5.14 (s, 2H), 4.54 (s, 2H), 3.76 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 168.1, 166.6, 165.3, 161.9, 161.7, 159.6, 139.0, 133.9, 131.2, 131.1, 130.1, 128.8, 127.7, 119.7, 116.7, 115.3, 115.0, 114.3, 92.0, 69.8, 55.5, 34.0. **HRMS (ESI) (m/z):** Calcd. for $C_{26}H_{19}N_6O_3S$, $[M-H]^+$: 495.1239; found: 495.1249.

Methyl4-(((5-cyano-4-(4-((4-methoxybenzyl)oxy)phenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)methyl)benzoate (3e)

Yield 49%; white solid; ^{1}H NMR (DMSO-d_6): δ 7.95 (d, *J* = 8.8 Hz, 2H), 7.90 (d, *J* = 8.0 Hz, 4H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 6.96 (d, *J* = 8.4 Hz, 2H), 5.13 (s, 2H), 4.60 (s, 2H), 3.83 (s, 3H), 3.76 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 166.6, 166.3, 165.2, 161.8, 159.6, 142.9, 131.2, 130.1, 128.8, 129.7, 129.1, 128.8, 127.6, 115.1, 114.3, 92.0, 69.8, 55.5, 52.6, 34.1. **HRMS (ESI) (m/z):** Calcd. for $C_{28}H_{22}N_3O_5S$, $[M-H]^+$: 512.1280; found: 522.1299.

4-(4-((4-methoxybenzyl)oxy)phenyl)-6-oxo-2-((4-(trifluoromethyl)benzyl)thio)-1,6-dihydropyrimidine-5-carbonitrile (3f)

Yield 54%; white solid; ^{1}H NMR (DMSO-d_6): δ 7.92 (d, *J* = 8.4 Hz, 2H), 7.66 (qd, *J* = 8.0 Hz, 4H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 5.12 (s, 2H), 4.60 (s, 2H), 3.73 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 166.6, 161.7, 159.6, 142.6, 131.3, 130.1, 128.8, 128.5, 128.2, 127.9, 126.0, 125.8, 125.7, 117.1, 115.2, 114.3, 91.7, 69.7, 55.5, 30.9. **HRMS (ESI) (m/z):** Calcd. for $C_{27}H_{19}F_3N3O_3$, $[M-H]^+$: 522.1099; found: 522.1090.

2-((4-azidobenzyl)thio)-4-(4-((4-fluorobenzyl)oxy) phenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (3g)

Yield 47%; white solid; ^{1}H NMR (DMSO-d_6): δ 8.00 (d, *J* = 8.0 Hz, 2H), 7.54 (t, *J* = 7.2 Hz, 4H), 7.46 (d, *J* = 7.6 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.8 Hz, 2H), 7.07 (d, *J* = 7.6 Hz, 2H), 5.21 (s, 2H), 4.54 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 166.6, 165.5, 163.5, 161.8, 161.6, 161.1, 139.1, 133.9, 133.2, 133.1, 131.3, 131.1, 130.6, 130.5, 127.9, 119.6, 116.7, 115.9, 115.6, 115.3, 115.1, 92.0, 69.3, 34.0. **HRMS (ESI) (m/z):** Calcd. for $C_{25}H_{16}FN_6O_2S$, $[M-H]^+$: 483.1039; found: 483.1031.

Methyl 4-(((5-cyano-4-(4-((4-fluorobenzyl)oxy)phenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)thio)methyl)benzoate (3h)

Yield 63%; white solid; ^{1}H NMR (DMSO-d_6): δ 7.94 (d, *J* = 8.8 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 4H), 7.52 - 7.58 (m, 4H), 7.25 (t, *J* = 8.8 Hz, 2H), 7.17 (d, *J* = 8.8 Hz, 2H), 5.20 (s, 2H), 4.58 (s, 2H), 3.84 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 166.6, 166.4, 163.5, 161.4, 143.4, 133.2, 131.1, 130.6, 130.5, 129.7, 129.7, 129.0, 128.2, 117.3, 115.8, 115.6, 115.1, 90.6, 69.2, 52.5, 34.1. **HRMS (ESI) (m/z):** Calcd. for $C_{27}H_{19}FN_3O_4S$, $[M-H]^+$: 500.1080; found:500.1093.

4-(4-((4-fluorobenzyl)oxy)phenyl)-6-oxo-2-((4-(trifluoromethyl)benzyl)thio)-1,6-dihydropyrimidine-5-carbonitrile (3i)

Yield 66%; white solid; ^{1}H NMR (DMSO-d_6): δ 7.92 (d, *J* = 8.0 Hz, 2H), 7.94 (qd, *J* = 8.0 Hz, 4H), 7.53 (t, *J* = 6.4 Hz, 2H), 7.23 (t, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 5.19 (s, 2H), 4.58 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 166.7, 166.2, 163.5, 161.4, 161.1, 142.8, 133.2, 132.5, 131.1, 130.6, 130.5, 130.1, 128.2, 125.7, 117.3, 115.8, 115.6, 115.1, 90.6, 69.2, 33.8. **HRMS (ESI) (m/z):** Calcd. for $C_{26}H_{16}F_4N_3O_2S$, $[M-H]^+$: 510.0899; found: 510.0896.

2-((4-azidobenzyl)thio)-6-oxo-4-(4-((4-(trifluoromethyl) benzyl)oxy)phenyl)-1,6-dihydropyrimidine-5-carbonitrile (3j)

Yield 71%; white solid; ^{1}H NMR (DMSO-d_6): δ 8.01 (d, *J* = 8.8 Hz, 2H), 7.79 (d, *J* = 8.0 Hz, 2H), 7.70 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 7.06 (d, *J* = 8.4 Hz, 2H), 5.36 (s, 2H), 4.53 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 161.4, 141.8, 139.0, 133.9, 131.3, 131.1, 129.1, 128.6, 128.1, 125.8, 119.6, 116.7, 115.3, 115.1, 92.1, 69.0, 34.0. **HRMS (ESI) (m/z):** Calcd. for $C_{26}H_{16}F_3N_6O_2S$, $[M-H]^+$: 533.1008; found: 533.1001.

Methyl 4-(((5-cyano-6-oxo-4-(4-((4-(trifluoromethyl) benzyl)oxy)phenyl)-1,6-dihydropyrimidin-2-yl)thio) methyl)benzoate (3k)

Yield 52%; white solid; ^{1}H NMR (DMSO-d_6): δ 7.96 (d, *J* = 8.0 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.79 (d, *J* = 7.6 Hz, 2H), 7.70 (d, *J* = 7.6 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 5.76 (s, 2H), 4.60 (s, 2H), 3.83 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 166.4, 161.4, 142.9, 141.9, 131.3, 129.7,

129.7, 129.1, 128.6, 125.8, 125.8, 115.3, 92.1, 69.0, 55.3, 52.5, 34.1. **HRMS (ESI) (m/z):** Calcd. for $C_{28}H_{19}F_3N_3O_4S$, $[M-H]^+$: 550.1048; found: 550.1039.

6-oxo-4-(4-((4-(trifluoromethyl)benzyl)oxy)phenyl)-2-((4-(trifluoromethyl)benzyl)thio)-1,6-dihydropyrimidine-5-carbonitrile (3l)

Yield 68%; white solid; ^{1}H NMR (DMSO-d_6): δ7.94 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 8.0 Hz, 2H), 7.70 (d, J = 8.0 Hz, 2H), 7.66 (qd, J = 8.4 Hz, 4H), 7.20 (d, J = 8.8 Hz, 2H), 5.35 (s, 2H), 4.61 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 166.7, 165.3, 161.4, 142.4, 141.8, 131.3, 130.1, 129.1, 128.7, 128.2, 128.0, 125.8, 125.8, 125.7, 123.3, 116.7, 115.2, 92.2, 69.0, 33.9. **HRMS (ESI) (m/z):** Calcd. for $C_{27}H_{17}F_6N_3O_2S$, $[M-H]^+$: 560.0867; found: 560.0870.

6-oxo-4-(4-phenoxyphenyl)-2-((4-(trifluoromethyl) benzyl)thio)-1,6-dihydropyrimidine-5-carbonitrile (3m)

Yield 84%; white solid; ^{1}H NMR (DMSO-d_6): δ 7.93 (d, J = 8.0 Hz, 2H), 7.66 (qd, J = 8.0 Hz, 4H), 7.47 (t, J = 7.2 Hz, 2H), 7.24 (t, J = 7.2 Hz, 1H), 7.14 (d, J = 7.6 Hz, 2H), 7.10 (d, J = 7.6 Hz, 2H), 4.58 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 166.6, 166.3, 162.8, 160.4, 155.6, 142.7, 131.4, 130.7, 130.2, 130.1, 128.4, 128.1, 126.0, 125.8, 125.7, 125.1, 123.3, 120.3, 117.7, 117.0, 90.3, 33.8. **HRMS (ESI) (m/z):** Calcd. for $C_{25}H_{15}F_3N_3O_2S$, $[M-H]^+$: 478.0837; found: 478.0828.

4-(2-((4-azidobenzyl)thio)-5-cyano-6-oxo-1,6-dihydropyrimidin-4-yl)-N-phenylbenzamide (3n)

Yield 65%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.41 (s, 1H), 8.08 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.37 (t, J = 8.0 Hz, 2H), 7.12 (t, J = 7.6 Hz, 1H), 7.07 (d, J = 8.0 Hz, 2H), 4.48 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 167.8, 167.1, 165.5, 163.0, 139.4, 138.8, 137.8, 134.6, 131.1, 129.1, 128.2, 124.6, 120.8, 119.6, 117.0, 93.4, 43.0. **HRMS (ESI) (m/z):** Calcd. for $C_{25}H_{16}N_7O_2S$, $[M-H]^+$: 478.1086; found: 478.1099.

Methyl4-(((5-cyano-6-oxo-4-(4-(phenylcarbamoyl) phenyl)-1,6-dihydropyrimidin-2-yl)thio)methyl)benzoate (3o)

Yield 54%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.41 (s, 1H), 8.08 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 8.4 Hz, 2H), 7.91 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 8.0 Hz, 2H), 7.37 (t, J = 8.0 Hz, 2H), 7.12 (t, J = 7.6 Hz, 1H), 4.57 (s, 2H), 3.83 (s, 3 H); ^{13}C NMR (DMSO-d_6): δ 167.7, 167.0, 166.4, 163.6, 143.4, 139.4, 138.8, 137.7, 129.7, 129.1, 129.0, 128.9, 128.2, 124.4, 120.8, 117.0, 93.4, 52.5, 34.1. **HRMS (ESI) (m/z):** Calcd. for $C_{27}H_{19}N_4O_4S$, $[M-H]^+$: 495.1127; found: 495.1132.

4-(5-cyano-6-oxo-2-((4-(trifluoromethyl)benzyl)thio)-1,6-dihydropyrimidin-4-yl)-N-phenylbenzamide (3p)

Yield 48%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.41 (s, 1H), 8.08 (d, J = 8.0 Hz, 2H), 7.97 (d, J = 8.4 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 7.67 (qd, J = 8.0 Hz, 4H), 7.37 (t, J = 8.0 Hz, 2H), 7.12 (t, J = 7.6 Hz, 1H), 4.56 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 167.7, 167.0, 165.3, 164.1, 143.0, 139.4, 138.9, 137.6, 130.1, 129.1, 129.0, 128.2, 125.7, 124.3, 123.3, 120.8, 117.1, 93.4, 33.9. **HRMS (ESI) (m/z):** Calcd. for $C_{26}H_{16}F_3N_4O_2S$, $[M-H]^+$: 505.0946; found: 505.0941.

4-(2-((4-azidobenzyl)thio)-5-cyano-6-oxo-1,6-dihydropyrimidin-4-yl)-N-(4-chlorophenyl)benzamide (3q)

Yield 18%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.56 (s, 1H), 8.07 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 8.0 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.8 Hz, 2H), 7.07 (d, J = 8.4 Hz, 2H), 4.44 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 168.5, 166.9, 165.4, 164.7, 139.3, 138.7, 138.5, 137.1, 134.9, 133.6, 131.0, 129.0, 128.2, 127.8, 127.1, 125.5, 123.9, 122.3, 119.7, 119.5, 117.5, 113.8, 111.7, 92.7, 33.9. **HRMS (ESI) (m/z):** Calcd. for $C_{25}H_{15}ClN_7O_2S$, $[M-H]^+$: 512.0696; found: 512.0698.

Methyl4-(((4-(4-((4-chlorophenyl)carbamoyl)phenyl)-5-cyano-6-oxo-1,6-dihydropyrimidin-2-yl)thio)methyl) benzoate (3r)

Yield 31%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.55 (s, 1H), 8.08 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 8.4 Hz, 2H), 7.90 (d, J = 8.0 Hz, 2H), 7.84 (d, J = 8.8 Hz, 2H), 7.57 (d, J = 8.0 Hz, 2H), 7.43 (d, J = 8.8 Hz, 2H), 4.57 (s, 2H), 3.84 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 167.6, 166.9, 166.4, 165.4, 163.7, 143.5, 139.0, 138.4, 137.3, 129.7, 129.3, 129.2, 129.1, 129.0, 128.9, 128.2, 127.9, 122.3, 117.0, 93.3, 52.58, 34.1. **HRMS (ESI) (m/z):** Calcd. for $C_{27}H_{18}ClN_4O_4S$, $[M-H]^+$: 529.0737; found: 529.0734.

N-(4-chlorophenyl)-4-(5-cyano-6-oxo-2-((4-(trifluoromethyl)benzyl)thio)-1,6-dihydropyrimidin-4-yl) benzamide (3s)

Yield 17%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.55 (s, 1H), 8.05 (d, J = 8.0 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 7.84

(d, *J* = 8.8 Hz, 2H), 7.67 (qd, *J* = 8.0 Hz, 4H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.8Hz, 2H), 4.52 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 168.2, 166.9, 165.4, 143.1, 139.1, 138.4, 137.30, 130.1, 129.0,128.2, 127.9, 127.1, 126.0, 125.8, 125.7, 122.3, 117.2, 93.1, 33.8. **HRMS (ESI) (m/z):** Calcd. for $C_{26}H_{15}ClF_3N_4O_2S$, $[M-H]^+$: 539.0556; found: 539.0541.

4-(2-((4-azidobenzyl)thio)-5-cyano-6-oxo-1,6-dihydropyrimidin-4-yl)-N-(p-tolyl)benzamide (3t)

Yield 51%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.34 (s, 1H), 8.08 (d, *J* = 8.4 Hz, 2H), 8.03 (d, *J* = 8.4 Hz, 2H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.07 (d, *J* = 8.0 Hz, 2H), 4.51 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 167.1, 165.2, 138.9, 137.8, 136.7, 134.6, 133.5, 129.5, 129.0, 128.2, 120.8, 120.0, 119.6, 116.7, 93.4, 34.0, 20.9. **HRMS (ESI) (m/z):** Calcd. for $C_{26}H_{18}N_7O_2S$, $[M-H]^+$: 492.1243; found: 492.1250.

Methyl-4-(((5-cyano-6-oxo-4-(4-(p-tolylcarbamoyl) phenyl)-1,6-dihydropyrimidin-2-yl)thio)methyl)benzoate (3u)

Yield 62%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.34 (s, 1H), 8.07 (d, *J* = 8.0 Hz, 2H), 7.98 (d, *J* = 8.0 Hz, 2H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 4.58 (s, 2H), 3.83 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 167.5, 167.0, 166.4, 165.1, 143.5, 138.7, 137.7, 136.9, 133.3, 132.1, 129.9, 129.7, 129.5, 129.0, 128.9, 128.1, 117.0, 93.3, 52.5, 34.1, 20.9. **HRMS (ESI) (m/z):** Calcd. for $C_{28}H_{21}N_4O_4S$, $[M-H]^+$: 509.1278; found: 509.1277.

4-(5-cyano-6-oxo-2-((4-(trifluoromethyl)benzyl)thio)-1,6-dihydropyrimidin-4-yl)-N-(p-tolyl)benzamide (3v)

Yield 45%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.34 (s, 1H), 8.07 (d, *J* = 8.4 Hz, 2H), 7.97 (d, *J* = 8.4 Hz, 2H), 7.64 – 7.71 (m, 6H), 7.16 (d, *J* = 8.4 Hz, 2H), 4.59 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 167.1, 167.0, 165.1, 162.8, 142.7, 138.5, 137.8, 136.9, 133.3, 130.2, 129.4, 129,0, 128.5, 128.2, 125.7, 123.5, 120.8, 116.7, 93.7, 33.9, 20.9. **HRMS (ESI) (m/z):** Calcd. for $C_{27}H_{18}F_3N_4O_2S$, $[M-H]^+$: 519.1108; found: 519.1094.

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