Discovery of Inhibitors Targeting Protein-Protein Interaction between Bacterial RNA Polymerase and NusG as Novel Antimicrobials

Yingbo Zheng,[†,#] Cheuk Hei Kan,[‡,#] Tsz Fung Tsang,[‡] Yanpeng Liu,[†] Tiankuang Liu,[†] Man Wai Tsang,[‡] Long Yin Lam,[†] Xiao Yang,[,‡] Cong Ma*[,†]*

[†]State Key Laboratory of Chemical Biology and Drug Discovery, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Kowloon, Hong Kong SAR, China

[‡]Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong SAR, China

ABSTRACT: Bacterial RNA polymerase (RNAP), the core enzyme responsible for bacterial transcription, requires the NusG factor for efficient transcription elongation and termination. As the primary binding site for NusG, the RNAP clamp-helix (CH) domain represents a potential protein-protein interaction (PPI) target for novel antimicrobial agent design and discovery. In this study, we designed a pharmacophore model based on the essential amino acids of the CH for binding to NusG, such as R270, R278, and R281 (*Escherichia coli* numbering), and identified a hit compound with mild antimicrobial activity. Subsequent rational design and synthesis of this hit compound led to improved antimicrobial activity against *Streptococcus pneumoniae*, with the minimum inhibitory concentration (MIC) reduced

from 128 to 1 μg/mL. Additional characterization of the antimicrobial activity, inhibitory activity against RNAP-NusG interaction, and cell-based transcription and fluorescent assays of the optimized compounds demonstrated their potential for further lead optimization.

INTRODUCTION

Bacterial transcription is an essential biological process in prokaryotic organisms, encompassing the initiation, elongation, and termination stages. It involves the synthesis of an RNA molecule complementary to the DNA template strand of the bacterial genome, orchestrated by the primary enzyme RNA polymerase (RNAP).[1] The RNA product further serves as a template for ensuing protein synthesis, shaping the cellular physiology and metabolic activities of the bacteria.

A group of small proteins called transcription factors are essential for regulating the transcription steps, such as σ, NusB, NusE, and NusG.[2] NusG is one of the essential bacterial transcription factors, forming a critical and ubiquitous component of bacterial transcription machinery, with determinable roles in both transcription elongation and termination processes.[3] Several studies have revealed that NusG augments the overall efficiency and regulation of transcription by bridging communications between the elongating RNAP and downstream elements or factors.[4] In terms of transcription termination, NusG has been shown to interact and facilitate the function of the termination factor Rho.[5,6] Additionally, NusG is found to be involved in the orchestration of transcription-translation coupling, thereby safeguarding the integrity of the newly synthesized mRNA molecule.[7]

The structure of NusG highlights a two-domain architecture, with an N-terminal domain (NTD) and a C-terminal Kyrpides-Ouzounis-Woese (KOW) domain. Intricate structural analyses elucidated that the NTD of NusG adopts a beta-barrel fold, while the C-terminal KOW domain

is represented by a three-stranded beta-sheet flanked by two alpha-helices on one side. Indeed, NusG interacts directly with the RNAP primarily via the NTD. Structural studies have revealed that the association between NusG and RNAP occurs predominantly at the β' subunit of the RNAP, specifically on the clamp-helix (CH) region (**Fig. 1A** left).[8] This conformational structure facilitates the NusG-RNAP protein-protein interaction (PPI) and brings about a movement in the "clamp" region to establish a stable elongation complex, thus aiding in securing the transcription bubble and effectively enhancing the transcriptive engagement of RNAP with the DNA template.[9]

In addition, the homologous protein of NusG, known as Spt5 in archaea and eukaryotes, is the only conserved transcription factor found among all three domains of life. Unlike bacteria, where NusG acts as a monomeric transcription factor, in archaea and eukaryotes, Spt5 forms a heterodimeric complex with Spt4 via the NTD.[10] This complex couples RNA processing and chromatin modification to transcription elongation.[11] In humans, the Spt4-Spt5 complex is called DSIF (DRB-sensitivity-inducing factor), and it regulates the processivity of RNA Polymerase II (Pol II) and activates transcription.[12] Despite the shared roles, sequences and structures of NusG homologs across all domains of life, NusG theoretically represents a viable target for inhibiting bacterial growth without affecting human cells. This is because the binding site and amino acid sequence we have selected for drug design are only conserved in bacteria exclusively **(Fig. 1B)**, making it practically a selective target for antibacterial drug discovery.

In summary, bacterial transcription is a pivotal biological process, fundamental to gene expression in prokaryotic organisms, while NusG serves as an indispensable modulator, furnishing the means to enhance efficiency during transcription while also facilitating sequence-specific termination of the process. Considering the important role of NusG in bacterial transcription, we designed and synthesized a series of inhibitors targeting RNAP-

NusG PPI in this study and validated their target specificity and antimicrobial activity.

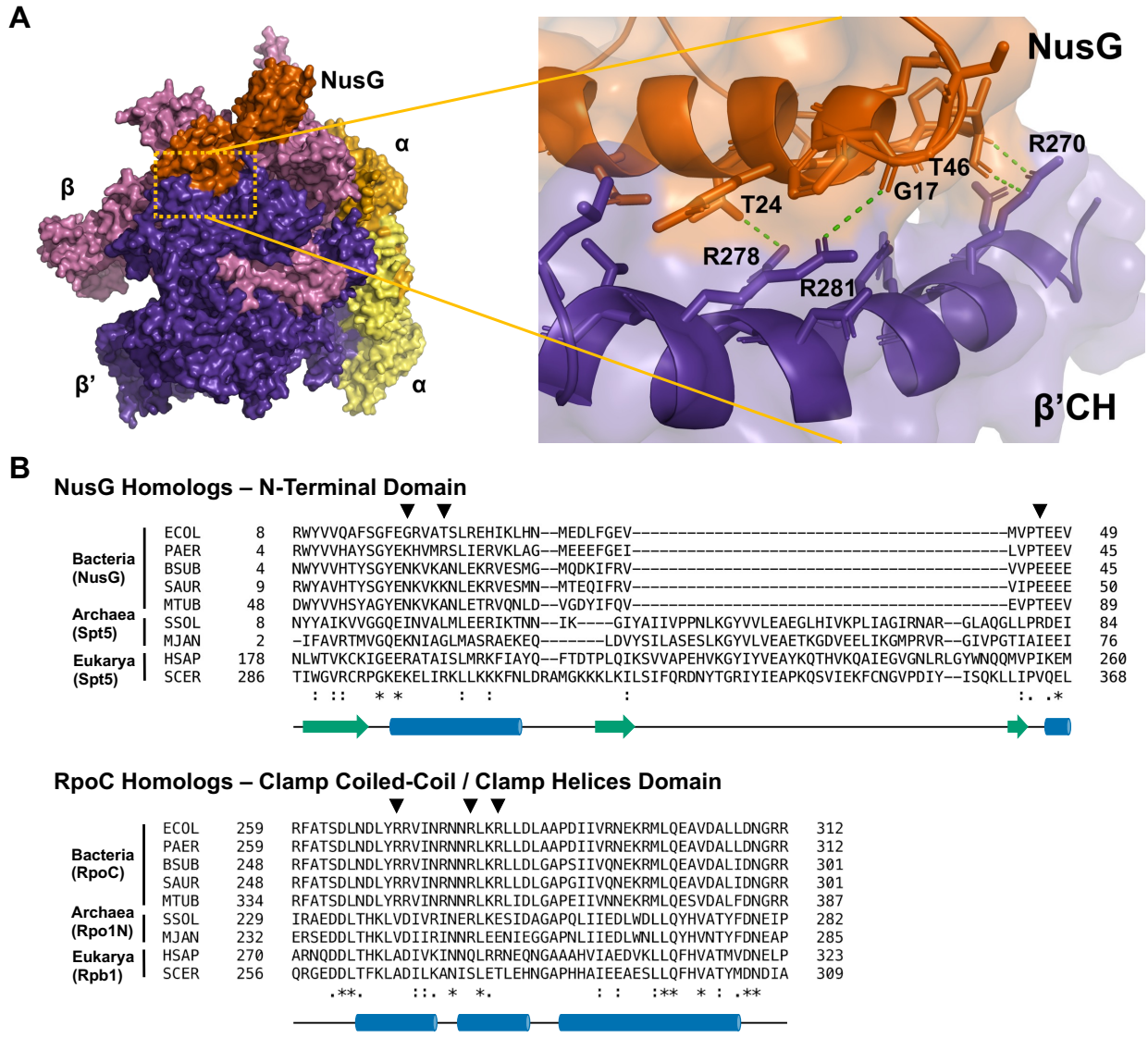


Fig. 1. Structural and sequence analysis of *Escherichia coli* RNA polymerase core enzyme and NusG. **(A)** Crystal structure of *E. coli* RNA polymerase core enzyme (subunits *ααββ'*) bound to NusG (PDB: 5TBZ).[13] Left: the overall structure of a bacterial transcription complex, highlighting the key components of the core enzyme (Blue: RNAP β' subunit; Reddish orange: NusG). Right: the key hydrogen bond interactions between NusG and the β'CH region. **(B)** Multiple sequence alignment of selected clamp coiled-coil or clamp helices regions in the RpoC (RNAP β' subunit) homologs and the N-Terminal Domain of NusG homologs from

Bacteria, Archaea, and Eukarya. The alignment was conducted using MAFFT (provided by European Bioinformatics Institute).[14, 15] Amino acids responsible for hydrogen bond formation are indicated by ▼ symbols. The alignment reveals that the highlighted arginine residues in β'CH domain are conserved within the Bacteria domain but not in the Archaea and Eukarya domains. Secondary structure elements predicted by JPred[16] are depicted as green arrows (beta-strands) and blue rods (alpha-helices), while loops are indicated by solid black lines. The UniProt codes and the abbreviation of species can be found in the Supporting Information.

RESULTS AND DISCUSSION

Design and *in silico* screening

The X-ray crystal structure of *E. coli* NusG in complex with RNAP (PDB: 5TBZ[13]) was used to generate the pharmacophore model. Studies showed that the binding of NusG to β'CH is an important interaction site in RNAP-NusG PPI,[13] in which NusG interacts with β'CH at the site in close proximity to or even overlaps with σ.[13, 17, 18] NusG uses the carbonyl groups of G17, T24 and T46 in the main chain for binding to the guanine residues of R281, R278, and R270 of β'CH (**Fig. 1A** right). Our previous study has shown that the three arginine residues are critical for RNAP binding to σ, the essential transcription initiation factor.[19] We considered these hydrogen bonding interactions also important for the interaction for the NusG function in transcription and thus selected G17, T24 and T46 of NusG to create the pharmacophore model, supplemented by exclusive areas to avoid steric clashes.

Discovery Studio 2016 was used to create the pharmacophore model, and the mini-Maybridge drug-like compound library was virtually screened. 57 compounds were identified among which 10 compounds were selected after checking the active conformation. All these 10 compounds were tested for antimicrobial activity. One hit compound, **AW00783**, demonstrated mild antimicrobial activity against *Streptococcus pneumoniae* and *Staphylococcus aureus*. It is a linear compound containing two terminal aromatic rings with a secondary hydroxy group in the middle (**Fig. 2** left). Although the binding area of β'CH is very similar to our previous study on sigmacidins as inhibitors targeting RNAP-σ PPI,[20] we intended to study the structure-activity relationship of **AW00783** in this study. If the antimicrobial activity can be improved, the entirely different structure of **AW00783** from sigmacidins or other marketed antibiotics will have the potential for development to a new class of antimicrobials.

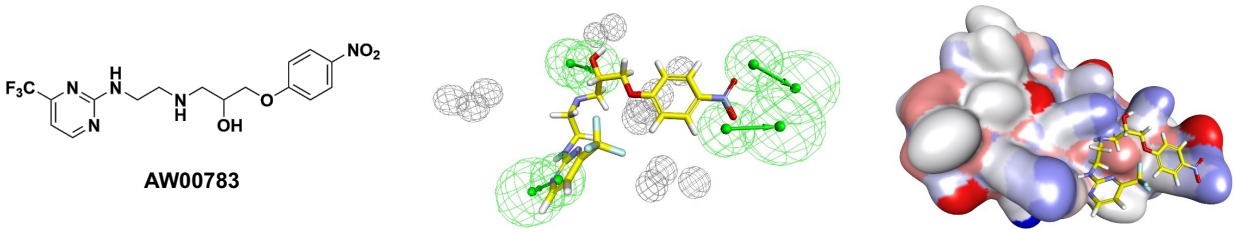


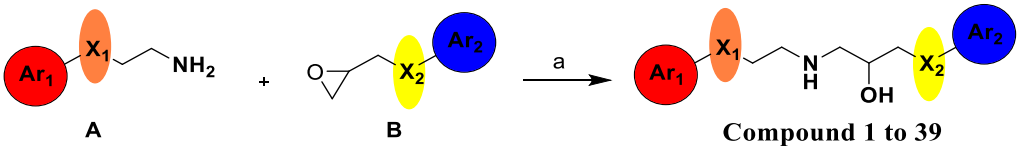
Fig. 2. Left: **AW00783** structure; middle, **AW00783** docking to the pharmacophore model (Green spheres: hydrogen bond donor; Gray spheres: exclusive areas); Right: **AW00783** docking to β'CH (Surface view).

Structure-Activity Relationship Studies

According to the docking model of **AW00783** fitting to the pharmacophore model (**Fig. 2** right), we decided first to modify the two terminal aryl rings by changing diverse common and available substituents in pharmaceuticals, followed by isosteric variations in the middle linear chain, especially the heteroatoms that can affect the conformation of molecular structures.

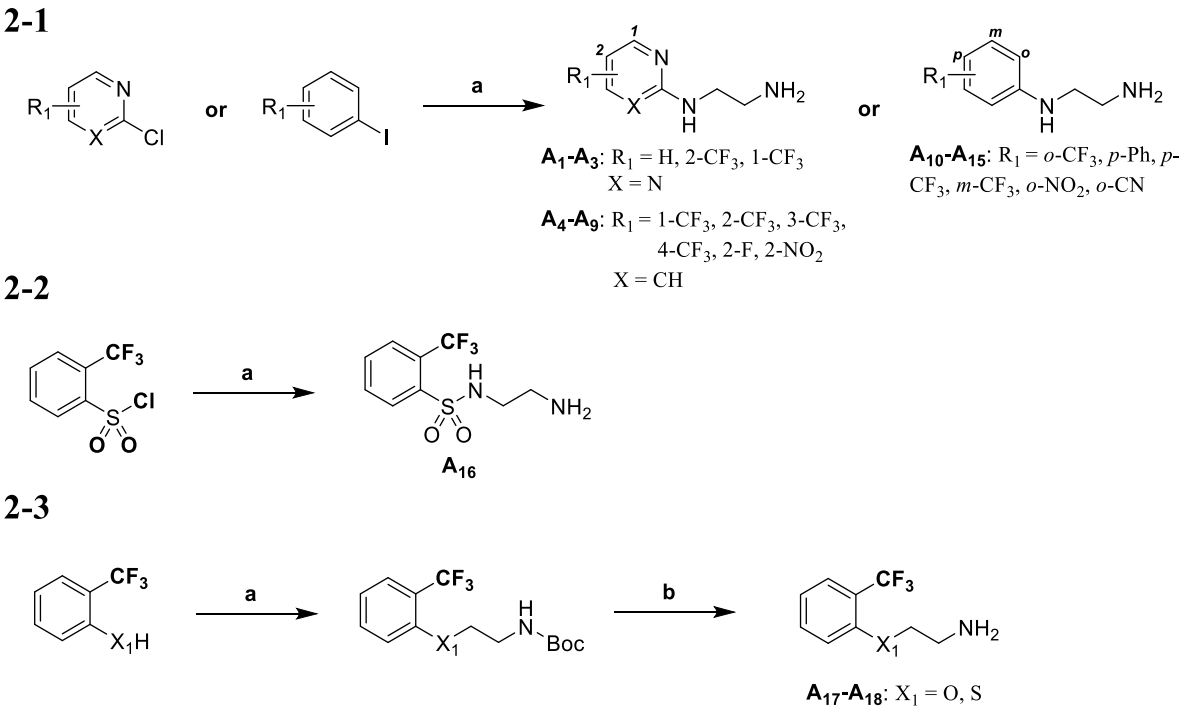
Scheme 1 depicts the general synthetic procedure for the synthesis of target compounds **1-39**, which were obtained by nucleophilic substitution reactions between specific amines **A** and epoxides **B**, respectively. Four series of amine substrates **A** were separately prepared through substitution reactions with diverse aromatic rings Ar_1 and amine side chains (**Scheme 2**). Commercially available chloroarenes reacted with ethylenediamine to form diamines **A1-A15**. For the synthesis of sulfonamide **A16**, a nucleophilic acyl substitution reaction was carried out to form the sulfonamide structure. Ether **A17** and thioether **A18** were synthesized by a nucleophilic substitution using phenol and thiophenol, respectively, followed by deprotection of the BOC group.

Scheme 1. Synthetic Route to Compounds **1-39**[a].



[a]Reagents and conditions: (a) N,N-diisopropylethylamine (DIPEA), EtOH, reflux, 8 h.

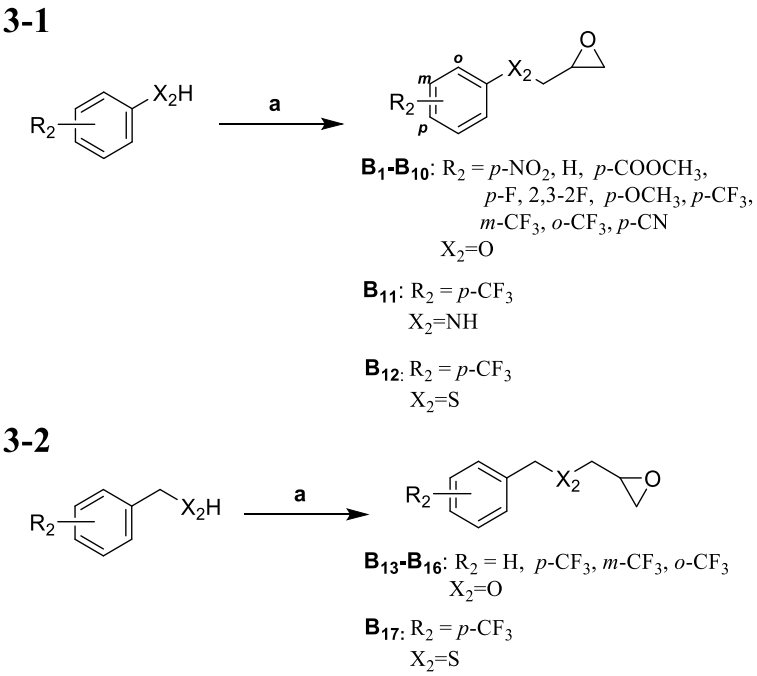
Scheme 2. Synthetic Route to Substrates **A1**-**A18**[a].



[a]Reagents and conditions: (a) for **A1**-**A9**: ethylenediamine monohydrate, K_2CO_3, THF, reflux, overnight; for **A10**-**A15**: ethylenediamine monohydrate, CuCl, Cs_2CO_3, DMSO, 120 °C, 8 h; for **A16**: ethylenediamine monohydrate, triethylamine (Et_3N), DCM, rt, overnight; for **A17**-**A18**: 2-(Boc-amino)ethyl bromide, K_2CO_3, DMF, 65 °C, 8 h; (b) CF_3COOH, DCM, reflux, 8 h.

The epoxide substrates **B** were prepared through nucleophilic substitution reactions of substituted phenols, aniline, thiophenol, benzyl alcohols and mercaptan with epichlorohydrin (**Scheme 3**). To synthesize amine **B11**, the weak nucleophile *para*-trifluoromethylaniline preferably reacted with the epoxide group of epichlorohydrin. In the presence of a substoichiometric amount of zinc salt, the intermediate 1-chloro-3-((4-(trifluoromethyl)phenyl)amino)propan-2-ol was obtained first. Subsequently, intramolecular nucleophilic substitution reaction took place to provide the desired compound.

Scheme 3. Synthetic Route to Substrates **B1**-**B17**[a].



[a]Reagents and conditions: (a) for **B1**-**B10**: epichlorohydrin, KI, Cs_2CO_3, DMF, 80 °C, overnight; for **B11**: (i) epichlorohydrin, $Zn(SO_3CF_3)_2$, $CHCl_3$, 60 °C, 12 h; (ii) KI, MeCN, reflux, 6 h; for **B12**: epichlorohydrin, K_2CO_3, MeCN, reflux, 8 h; for **B13**-**B16**: epichlorohydrin, TBAB, 1M NaOH aqueous solution, rt, 18 h; for **B17**: epichlorohydrin, KOH, water : dioxane = 1: 1, rt, 8 h.

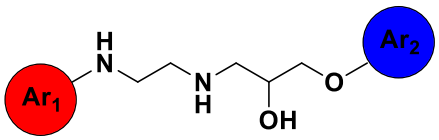
A panel of bacteria was used for the antimicrobial activity evaluation on compounds **1-39**, including eight pathogens from the "WHO priority pathogens list, 2024".[21] The panel consisted of four Gram-positive strains: *Enterococcus faecalis* ATCC® 19433, *S. aureus* ATCC® 25923 and 29213, *S. pneumonia* ATCC® 49619; and five Gram-negative ones: *Klebsiella pneumoniae* ATCC® 700603, *Acinetobacter baumannii* ATCC® 19606, *Pseudomonas aeruginosa* ATCC® 27853, *Enterobacter cloacae* ATCC® 13047*, and E. coli* ATCC® 25922. To determine the minimum inhibitory concentration (MIC) of the tested compounds, a broth microdilution assay was conducted following the guidelines established by the Clinical & Laboratory Standards Institute (CLSI).[22] The hit compound **AW00783** exhibited mild antimicrobial activity against *S. aureus* and *S. pneumoniae*, with MICs ranged between 128 to 256 μg/mL.

As shown in **Table 1**, modifications were initially carried out on the two terminal aromatic rings of the hit compound. Compound **1**, lacking substitution groups on Ar_1, exhibited no antimicrobial activities. This result suggests that the presence of the trifluoromethyl group on Ar_1 of **AW00783** might contribute to the interaction with R281 rather than the nitrogen atom of pyrimidine.[23] This hypothesis was validated by the moderate activities of compound **8** containing a trifluoromethyl group on the benzene ring as Ar_1 which lacks nitrogen of pyrimidine. Furthermore, compounds **2** to **6**, with a trifluoromethyl substitution on the pyridine ring at different positions, indicated that the relative position of the trifluoromethyl group on the aromatic ring was also influential to antimicrobial activity. Among these derivatives, **4** and **8** exhibited promising activity and were selected for further modifications. Simultaneously, the evaluation of derivatives with different substitution groups on Ar_2 demonstrated that trifluoromethyl remained the preferred choice compared to other hydrogen bond acceptors, as shown in **Table 1**. The unimproved activities of compounds **10** to **13**, with alternative hydrogen bond acceptors, suggested that other substituents might not be compatible for binding to R270. Although the nitro group contributed to both hydrogen bonding and electrostatic interactions,

it exhibited a weaker affinity for the β'CH compared to the trifluoromethyl group, as observed in the binding model of **AW00783**. Additionally, three compounds **4**, **8** and **14** demonstrated some antimicrobial activities against Gram-negative bacteria.

Further modifications were conducted on the substitution group of Ar_1 when it was substituted with trifluoromethyl on Ar_2. Compounds **17** and **19** with with trifluoromethyl at the *para*- and *ortho*-positions of the benzene ring in Ar_1 demonstrated the greatest activity in this group of analogues, particularly **19**. This compound exhibited the most potent antimicrobial activity against Gram-positive bacteria, particularly with a MIC value of 4 μg/mL against *S. pneumoniae*. It also possessed improved activity against Gram-negative bacteria such as *A. baumannii*, *E. cloacae* and *E. coli*, with MICs around 16 μg/mL (**Table 1**). In contrast, when the cyano group, known for its role as a hydrogen bond acceptor and commonly used in rational drug design, was substituted for the same position, antimicrobial activities of compound **21** dropped. Meanwhile, modifications of the CF_3 position of Ar_2 from compound **19** to **22** and **23** did not yield more promising derivatives. This finding suggests that the trifluoromethyl group acting as a hydrogen bond acceptor at *para*- or *ortho*-position in Ar_1 and *para*-position in Ar_2 is the optimal substitution on the terminal aromatic rings.

Table 1: Antimicrobial activity of the hit Compound and Compounds **1-24** (MIC μg/mL).



No	Structure		MIC (μg/mL)								
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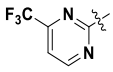
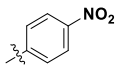
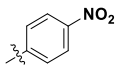
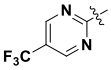
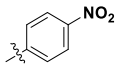
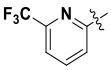
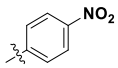
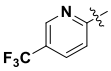
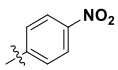
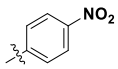
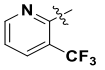
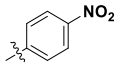
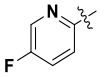
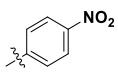
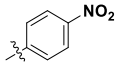
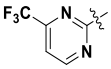
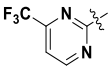
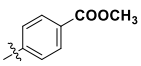
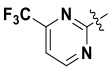
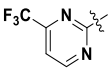
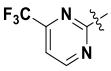
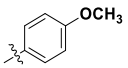
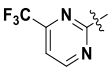
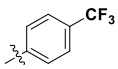
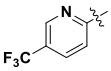
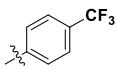
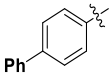
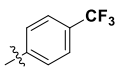
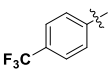
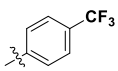
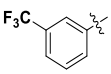
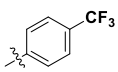
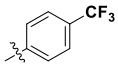
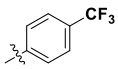
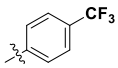
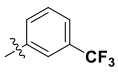
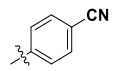
AW00783			256	256	128	128	>256	>256	>256	>256	>256

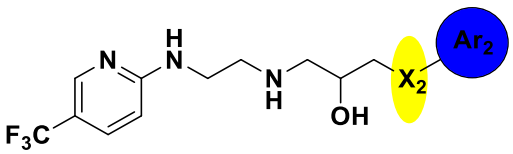
1			>256	>256	>256	>256	>256	>256	>256	>256	>256
2			>256	256	256	128	>256	>256	>256	>256	>256
3			128	128	64	32	>256	256	>256	256	256
4			128	32	32	64	>256	128	>256	128	128
5			128	128	128	64	>256	>256	>256	256	>256
6			256	128	128	64	>256	>256	>256	>256	>256
7			>256	>256	>256	>256	>256	>256	>256	>256	>256
8			128	64	64	64	>256	128	>256	128	128
9			>256	>256	>256	128	>256	>256	>256	>256	>256
10			>256	>256	>256	256	>256	>256	>256	>256	>256
11			>256	>256	256	128	>256	>256	>256	>256	>256
12			256	256	256	64	>256	256	>256	256	128
13			>256	>256	>256	256	>256	>256	>256	>256	>256
14			64	64	64	32	>256	128	>256	>256	>256
15			64	64	32	16	>256	64	>256	>256	64
16			>256	>256	>256	>256	>256	>256	>256	>256	>256
17			32	64	32	8	>256	256	>256	256	128
18			>256	>256	>256	>256	>256	>256	>256	>256	>256
19			16	8	16	4	>256	16	>256	32	16
20			128	128	128	32	>256	>256	>256	128	>256

21			128	128	128	64	>256	128	>256	128	16
22			16	32	32	16	>256	32	>256	32	32
23			32	64	32	32	>256	64	>256	64	64
24			>256	>256	128	128	>256	>256	>256	>256	>256

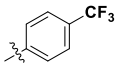
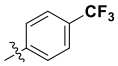
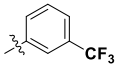
EFAE: E. faecalis ATCC® 19433, SAUR[a]: S. aureus ATCC® 25923, SAUR[b]: S. aureus ATCC® 29213, SPNE: S. pneumonia ATCC® 49619, KPNE: K. pneumoniae ATCC® 700603, ABAU: A. baumannii ATCC® 19606, PAER: P. aeruginosa ATCC® 27853, ECLO: E. cloacae ATCC® 13047, ECOL: E. coli ATCC® 25922.

In addition to the modifications on Ar_1 and Ar_2, we also investigated the length of the X_2 linker. Compound **25** with X_2 as NH slightly improved the activity compared to **15**. As indicated in **Table 2**, one methylene group was inserted between the oxygen and Ar_2. Compared to phenyl ether **17**, benzyl ether **27** showed 2-fold superior activity against *A. baumannii* and comparable activity against other Gram-positive and Gram-negative bacteria. In addition, modification of Ar_2 by changing substituted positions of CF_3 led to activity change (**27-29**), showing that *para*-substitution was favored as a similar trend to previously synthesized analogues (**19, 22, 23**). While deletion of CF_3 diminished the activity (**26**).

Table 2: Antimicrobial activity of the Compounds **25-29** (MIC μg/mL).



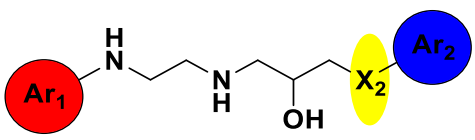
No	Structure	MIC (μg/mL)

	X_2	Ar_2	EFAE	SAUR[a]	SAUR[b]	SPNE	KPNE	ABAU	PAER	ECLO	ECOL
25	NH		16	32	32	16	>256	32	>256	64	64
26	OCH_2		256	256	128	128	>256	256	>256	256	256
27	OCH_2		32	32	32	16	>256	64	>256	128	128
28	OCH_2		64	64	32	32	>256	128	>256	256	64
29	OCH_2		128	64	64	64	>256	128	>256	128	128

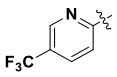
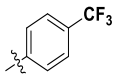
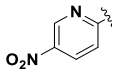
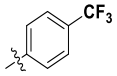
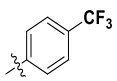
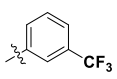
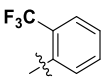
EFAE: E. faecalis ATCC® 19433, SAUR[a]: S. aureus ATCC® 25923, SAUR[b]: S. aureus ATCC® 29213, SPNE: S. pneumonia ATCC® 49619, KPNE: K. pneumoniae ATCC® 700603, ABAU: A. baumannii ATCC® 19606, PAER: P. aeruginosa ATCC® 27853, ECLO: E. cloacae ATCC® 13047, ECOL: E. coli ATCC® 25922.

When we changed X_2 from methylene ether to thioether. Compound **30** showed superior antimicrobial activity to **15**, exhibiting the best antimicrobial activities among the pyridine derivatives. This improved activity against both Gram-positive and Gram-negative bacteria was comparable to compounds **19**, as shown in **Table 3**. Other modifications of CF_3 positions on Ar_1 (**32-34**) while maintaining X_2 as methylene ether showed similar effects to the activity as **27-29**.

Table 3: Antimicrobial activity of the Compounds **30-34** (MIC μg/mL).



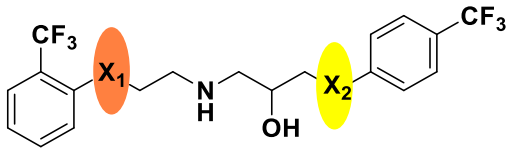
No	Structure	MIC (μg/mL)

	Ar_1	Ar_2	X_2	EFAE	SAUR[a]	SAUR[b]	SPNE	KPNE	ABAU	PAER	ECLO	ECOL
30			SCH_2	32	16	8	16	>256	32	>256	64	32
31			OCH_2	128	128	64	64	256	128	>256	128	128
32			OCH_2	64	64	64	32	>256	64	>256	128	64
33			OCH_2	>256	>256	128	128	>256	>256	>256	>256	>256
34			OCH_2	>256	>256	128	128	>256	>256	>256	>256	>256

EFAE: E. faecalis ATCC® 19433, SAUR[a]: S. aureus ATCC® 25923, SAUR[b]: S. aureus ATCC® 29213, SPNE: S. pneumonia ATCC® 49619, KPNE: K. pneumoniae ATCC® 700603, ABAU: A. baumannii ATCC® 19606, PAER: P. aeruginosa ATCC® 27853, ECLO: E. cloacae ATCC® 13047, ECOL: E. coli ATCC® 25922.

With the structure and activity data in hand, we envisaged that isosteric change in the middle chain may further improve antimicrobial activity. By respectively maintaining Ar_1 and Ar_2 as *ortho*- and *para*-CF_3, isosteric modifications on X_1 and X_2 were conducted. As shown in **Table 4**, when X_1 was changed from N in compound **19** to O or S, the antimicrobial activity of **36** or **37** against both Gram-positive and Gram-negative bacteria was retained, except the activity of **37** against *E. cloacae.* Similar to compound **30**, compound **38** with X_2 changed from O to S demonstrated the greatest activity against Gram-positive bacteria, especially *S. pneumoniae* with an MIC value of 1 μg/mL. When both X_1 and X_2 changed to S, **39** maintained antimicrobial activity compared to compound **37** against Gram-positive bacteria.

Table 4: Antimicrobial activity of the Compounds **35-39** (MIC μg/mL).



No	Structure		MIC (μg/mL)								
	X_1	X_2	EFAE	SAUR[a]	SAUR[b]	SPNE	KPNE	ABAU	PAER	ECLO	ECOL
35	SO_2NH	O	64	64	64	32	>256	64	>256	128	64
36	O	O	16	16	16	8	>256	16	>256	32	16
37	S	O	8	16	8	4	>256	32	>256	128	16
38	NH	S	8	4	4	1	>256	>256	>256	32	64
39	S	S	8	8	8	4	>256	>256	>256	>256	32

EFAE: E. faecalis ATCC® 19433, SAUR[a]: S. aureus ATCC® 25923, SAUR[b]: S. aureus ATCC® 29213, SPNE: S. pneumonia ATCC® 49619, KPNE: K. pneumoniae ATCC® 700603, ABAU: A. baumannii ATCC® 19606, PAER: P. aeruginosa ATCC® 27853, ECLO: E. cloacae ATCC® 13047, ECOL: E. coli ATCC® 25922.

A structure-activity relationship (SAR) can be summarized as follows (**Fig. 3**): the left aryl group can be heteroaryl rings such as pyridine or pyrimidine (Y and/or Z = N), but a benzene ring is favored. The substituent R_1 on the left aryl ring prefers electron-withdrawing groups, such as CF_3, at the *ortho* or *para* position. The left linker X_1 improves antimicrobial activity when O or sulfonamide is replaced by NH or S. Although the substituents R_2 on the right aryl ring show a similar preference to R_1, superior antimicrobial activity is obtained when the right linker X_1 is S rather than O or other isosteres. The SAR demonstrates that this series of compounds does not possess symmetric pharmacophores.

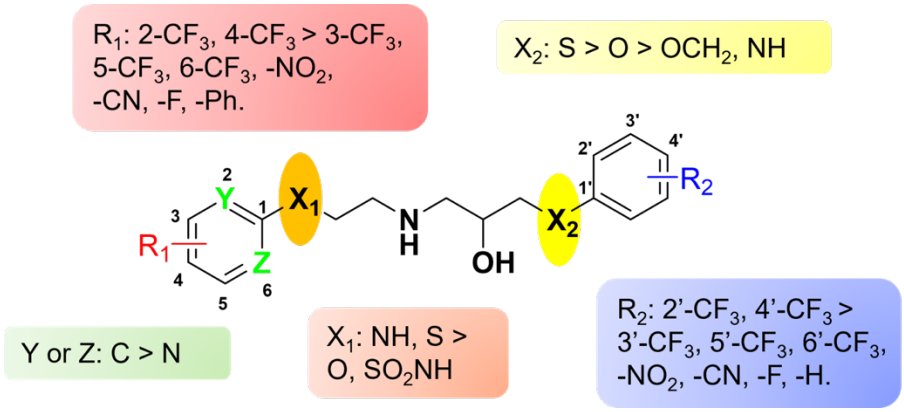


Fig. 3. SAR of the synthesized inhibitor compounds as antimicrobials

Antimicrobial Activity against Representative Bacterial Pathogens

After evaluating the preliminary antimicrobial activity of our compounds, we further screened the selected compounds against a panel of clinically relevant pathogens to validate their clinical prospect. Among the tested pathogens, *S. pneumoniae* exhibited particular susceptibility to our compound series, prompting us to expand our antimicrobial activity testing to include groups A and B *Streptococci*: *Streptococcus pyogens* (Group A *Streptococcus*), known to cause strep throat, localized skin infection, and necrotizing fasciitis,[24] and *Streptococcus agalactiae* (Group B *Streptococcus*), responsible for neonatal infections.[25] In addition, we evaluated the effects of our compounds on clinically significant Gram-positive pathogens, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. To further validate the antimicrobial activity of our compounds against *S. pneumoniae*, we included several clinical isolates of this bacterium denoted as CUHK-X01-04. The identities of these isolates have been confirmed using Bruker MALDI Biotyper® and are listed in **Table S2**.

The results in **Table 5** demonstrate the promising antimicrobial activity of our compounds against clinically challenging pathogens, with MICs ranging from 2 μg/mL to 16 μg/mL. Notably, compound **38** presented robust antimicrobial activity against *S. saprophyticus*, with a

MIC comparable to vancomycin (2 μg/mL). Furthermore, all the other compounds showed antimicrobial activity against the listed pathogens, with MICs comparable to those of other Gram-positive bacteria shown in **Tables 1-4**.

Table 5: Antimicrobial activity (MIC μg/mL) evaluation of derivatives against representative pathogenic Gram-positive bacteria.

Compound	SEPI	SSAP	SPYO	SAGA
19	16	8	16	16
36	8	8	8	8
38	8	2	8	16
39	4	4	8	8
Van	2	2	0.5	1
Cip	0.5	1	1	4
Oxa	0.25	2	0.125	1
Gen	0.125	≤0.0625	32	64

SEPI: S. epidermidis ATCC® 12228, SSAP: S. saprophyticus ATCC® 15305, SPYO: S. pyogenes (group A Streptococcus) ATCC® 19615, SAGA: S. agalactiae (group B Streptococcus) ATCC® 12386. Van: Vancomycin, Cip: Ciprofloxacin, Oxa: Oxacillin, Gen: Gentamicin.

Table 6 provides further insights into the inhibitory potential of our compounds against *S. pneumoniae*, with MICs as low as 1 μg/mL. Of particular, compound **38** displayed a broad spectrum of antimicrobial activity against all the tested strains. In conclusion, our findings indicate that all investigated compounds possess promising antimicrobial activity against Gram-positive bacteria, particularly *S. pneumoniae*.

Table 6: Antimicrobial activity (MIC; μg/mL) evaluation of derivatives against an array of *S. pneumoniae* strains, including clinical isolates.

Compound	SPNE[a]	SPNE[b]	SPNE[c]	CUHK-X01	CUHK-X02	CUHK-X03	CUHK-X04
19	4	4	16	16	8	16	8
36	8	8	16	16	16	8	8
38	1	2	4	1	4	8	2
39	4	4	8	8	8	4	8
Van	1	0.5	1	0.5	1	1	0.5
Cip	2	1	2	2	2	4	4
Oxa	2	16	8	≤0.0625	4	0.125	64
Gen	32	8	16	32	64	64	32

SPNE[a]: S. pneumoniae ATCC® 49619, SPNE[b]: S. pneumoniae strain TCH8431 (HM-145), SPNE[c]: S. pneumoniae strain NP112 (NR-19213). Van: Vancomycin, Cip: Ciprofloxacin, Oxa: Oxacillin, Gen: Gentamicin.

Time-Kill Kinetics

The time-kill kinetic assay, performed in accordance with the guidelines set by the CLSI,[26] was employed to assess the *in vitro* activity of the antimicrobial agent against a specific bacterial strain within a defined timeframe. In this study, the time-kill kinetics of compound **38** were measured against *S. aureus* subtypes, ATCC® 25923 (**Fig. 4A**) and community-associated methicillin-resistant *S. aureus* (CA-MRSA) strain USA300 (**Fig. 4B**) in liquid culture to simplify the experimental requirement for testing against *S. pneumoniae*. Notably, treatment with compound **38** at 1× MIC demonstrated growth inhibition after 2 hours, followed by a gradual reduction in bacterial count with prolonged treatment at 4 and 6 hours. Moreover, when higher concentrations of compound **38** (4× and 16× MIC) were used in the experiment, both subtypes of *S. aureus* were completely and efficiently eradicated below the theoretical level of detection (200 CFU/mL) over 6 hours. Specifically, it reduced bacterial counts below the

detectable threshold within 2 hours for *S. aureus* ATCC® 25923, while the USA300 strain required 4 hours to achieve the same level of bacterial elimination. Overall, our findings indicate that compound **38** exhibits growth inhibition of bacteria at 1× MIC, while higher compound concentrations can effectively eliminate bacteria below the detectable threshold. This ability to eliminate, rather than merely inhibit, the bacterial growth makes it particularly effective for treating patients with life-threatening infections, such as endocarditis, meningitis, osteomyelitis, and neutropenia,[27] or those who are immunocompromised.[28]

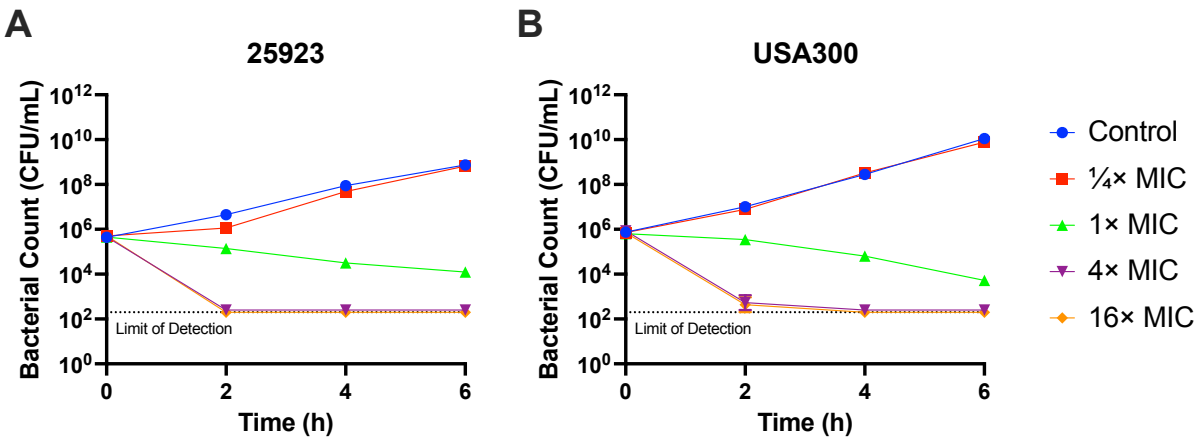


Fig. 4. The effect of compound **38** on the time-kill kinetics of **(A)** *S. aureus* ATCC® 25923 and **(B)** CA-MRSA strain USA300 at ¼×, 1×, 4× and 16× MIC in cation-adjusted Mueller-Hinton broth (CA-MHB) media. Experiments were performed in triplicates. 25923: *S. aureus* ATCC® 25923; USA300: CA-MRSA strain USA300.

NusG-β'CH PPI Inhibition

To validate the disruptive effect of compound **38** on the interaction between β'CH and NusG from *Bacillus subtilis* strain 168, we employed an in-house developed competitive protein complement assay[29] to determine the 50% inhibition concentration (IC_{50}) of the compound. In

the assay, NusG and β'CH were fused with two complementation fragments of Nano-Luc luciferase. When a specific inhibitor targeting the NusG-β'CH PPI was introduced to the reaction, the efficient reformation of the natural luciferase complex was hindered. Consequently, the catalytic reaction of the reconstituted luciferase was inhibited, leading to changes in luminescence levels. These changes served as a measure of inhibitory activity exhibited by the tested compound. As depicted in **Fig. 5**, compound **38** demonstrated inhibitory activity against the NusG-β'CH PPI, with an IC_{50} value of 142 ± 34 μM. This protein complement assay confirms that compound **38** disrupts the interaction between NusG and β'CH. Notably, when compared to the hit compound **AW00783**, which has an IC_{50} value of 666 ± 53 μM (**Fig. S2A**), compound **38** demonstrates superior inhibition of the NusG-β'CH interaction.

In addition, the inhibition of NusG-β'CH interaction has been benchmarked to a peptide equivalent to the native residues 19 – 34 (EGRVATSLREHIKLHN) of the *E. coli* NusG NTD. This fragment was termed NusG-NTD in the competitive protein complement assay. Compared with compound **38**, a similar decrease in the luminescence signal was detected in the presence of the non-tagged NusG-NTD, suggesting the PPI inhibitory effect (**Fig. S2B**). Our data implied that the decline of luminescence resulted from specific PPI inhibition. Despite having no antimicrobial activity, likely due to poor cell permeability, the peptide exhibited an IC_{50} value of 195 ± 45 μM, which is comparable to compound **38**. These results suggested that compound **38** specifically inhibited the interaction between NusG-β'CH *in vitro* as designed, and therefore very likely to result in its antimicrobial activity.

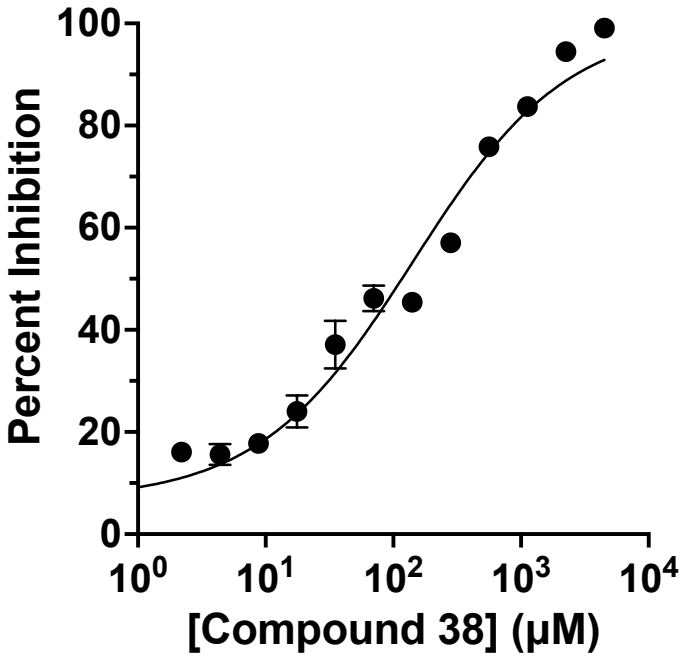


Fig. 5. Inhibition of the interaction between RNAP β'CH and NusG by compound **38** measured by the protein complement assay. The results are presented as $n = 3$, with variability shown as ±SE from one representative experiment. Technical repeats were conducted at least twice with similar results.

Cell-Based Transcription Assay

To investigate the effects of compound **38** on cellular RNA synthesis, a plasmid-based reporter system was adapted. This reporter system had previously demonstrated the essential role of NusG in bacterial cells.[30] A previously established plasmid, pSL103 encoded a reporter gene *cat* with the presence of a regulatory Rho-dependent terminator upstream in the promoter region (**Fig. 6A**). In this study, pSL103 was transformed into the *E. coli* K12 strain, which was susceptible to compound **38** with a MIC of 16 μg/mL. Compared to the untreated pSL103 transformants, exposure to compound **38** at ⅛× and ¼× of the MIC led to a significant increase in the transcription levels of the *cat* gene (**Fig. 6B**). This observation suggests that compound

38 improves the readthrough of the Rho-dependent terminator in the pSL103 construct. Given that NusG is widely known to promote Rho-dependent termination, and previous literature has indicated that depletion of NusG caused similar deleterious effects on pSL103 transformants,[30,31] it can be inferred that compound **38** may inhibit the function of NusG in bacterial cells.

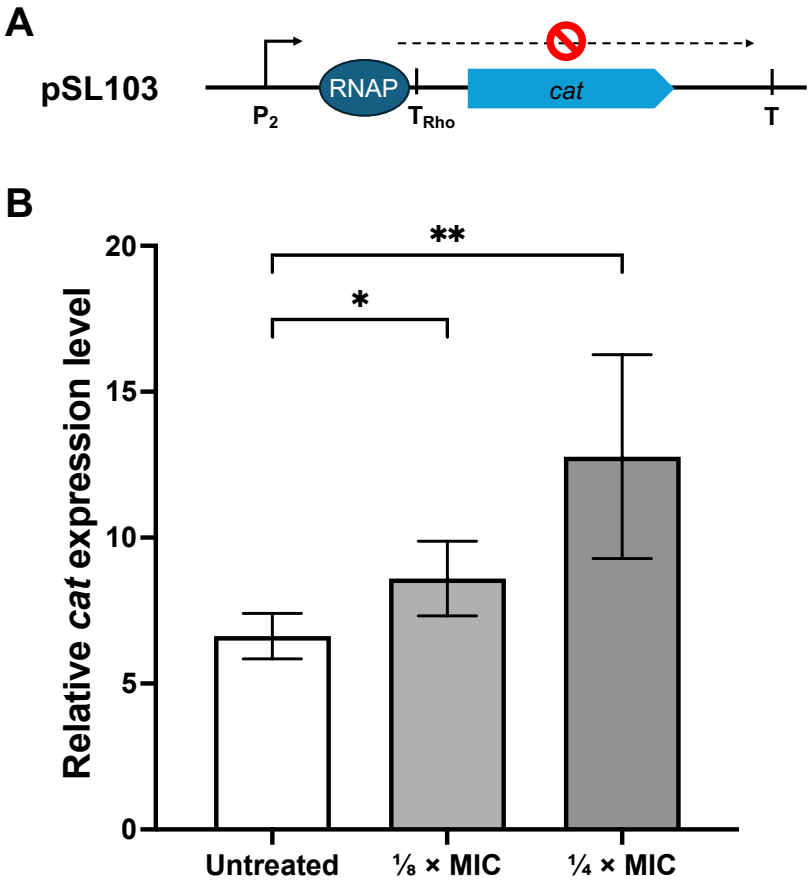


Fig. 6. Cell-based transcription assay that showed compound effects against NusG function. **(A)** Scheme of the plasmid pSL103 used. **(B)** Relative *cat* expression levels in pSL103 transformants treated differently. Technical repeats were performed to ensure data reproducibility. Statistical significance between treatment groups and untreated control was assessed using unpaired *t*-tests, with $p<0.05$ denoted by ∗ and $p<0.01$ denoted by ∗∗.

Epifluorescence Microscopy

To confirm that compound **38** had a mechanism of action consistent as the transcription inhibitor targeting the NusG-β'CH interaction, we conducted bacterial cytological profiling using a panel of reporter strains of *B. subtilis*. These strains expressed green fluorescent protein (GFP)-tagged proteins involved in various cellular processes, including transcription (transcription factor NusG[32] and RNA polymerase β'subunit (RpoC),[32] translation (ribosome subunit RpsB),[33] and cell wall synthesis coordination (MreB).[34] The cells were treated with either control antibiotics or compound **38** at 1× MIC and observed using epifluorescence microscopy after a 15-minute incubation period.

The analysis of the reporter strains revealed that compound **38** exerted an interference on bacterial transcription, as indicated by the delocalization of RpoC **(Fig. 7A)** and NusG **(Fig. 7B)**. The observed delocalization effects induced by compound **38** closely resembled those observed with rifampicin, a commercially available antibiotic binding to RNAP that targets transcription processes. When RpsB-GFP was visualized, both rifampicin and compound **38** displayed a similar delocalization effect **(Fig. 7C)**. This result aligns with the findings of a previous study,[35] where treating *B. subtilis* with rifampicin caused an expansion of the nucleoid, resulting in a diffuse structure extending throughout the entire cell. Furthermore, protein synthesis inhibitors such as tetracycline and chloramphenicol were shown to extend RpsB from the cell poles towards the mid-cell, as observed in **Fig. 7C**.

Regarding the subcellular localization of MreB, the cell membrane-targeting antibiotic oxacillin caused the loss of this protein (**Fig. 7D**). However, the subcellular localization of MreB was also subtly affected by rifampicin and compound **38** treatments, as the treated cells did not align with the untreated control cells. Given that both rifampicin and our compound can disrupt transcription, the synthesis of MreB and other proteins may have been affected,

leading to the observed changes in MreB localization. Overall, the bacterial cytological profile of compound **38** closely resembles that of rifampicin, suggesting that compound **38** primarily targets transcription, which functions by disrupting the interaction between NusG and RNA polymerase.

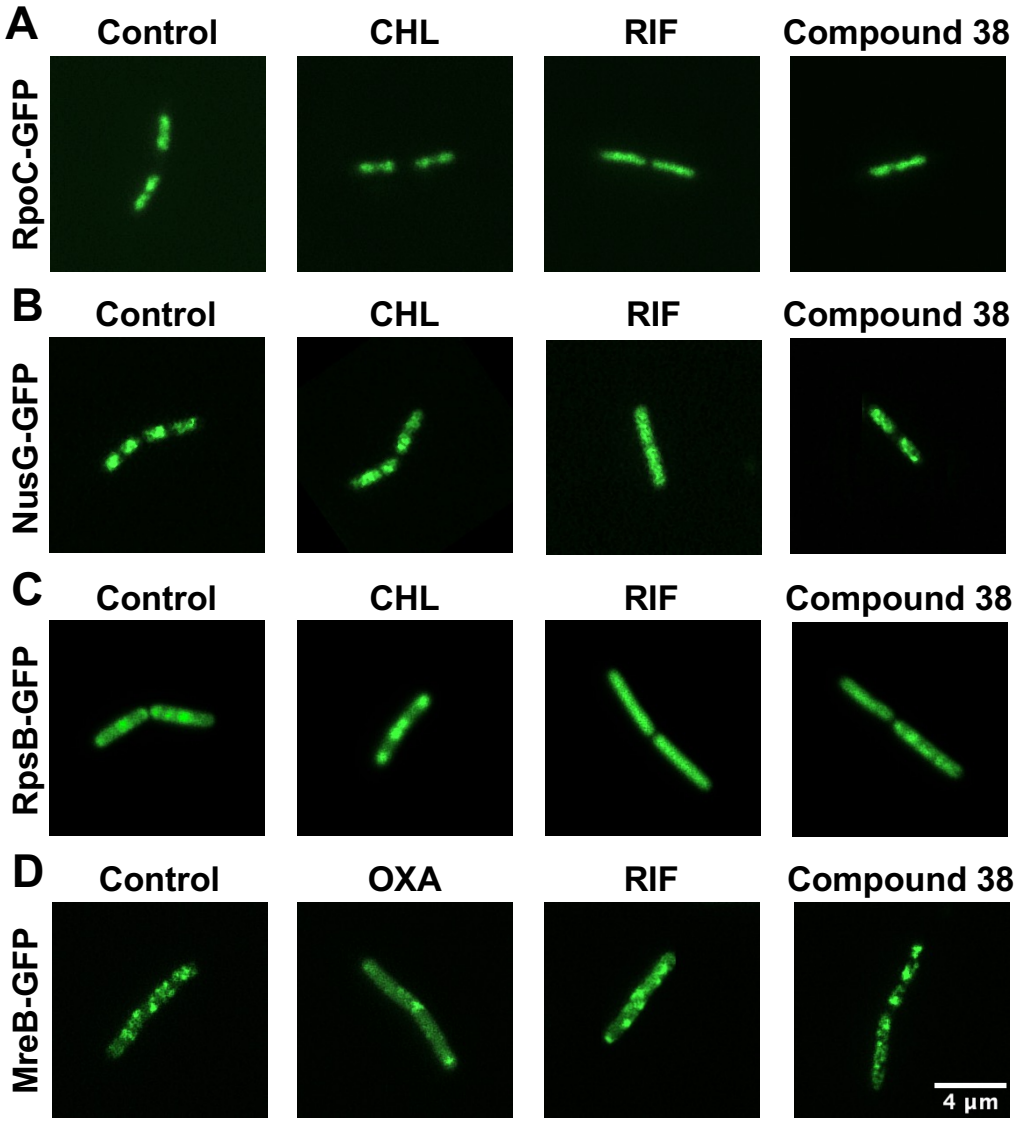


Fig. 7. Epifluorescence microscopy of *B. subtilis* with GFP tagged on **(A)** RNAP β'CH (RpoC), **(B)** transcription factor NusG, **(C)** ribosome RpsB, and **(D)** cell membrane coordination MreB. The untreated control (first panel) demonstrates no significant alteration in localization, whereas the antibiotics-treated cells show a distinct pattern of delocalization. Upon treatment

with 1× MIC of compound **38** (fourth panel), cells exhibit a delocalization, with the morphology resembles that observed in rifampicin-treated cells (third panel). Overall, compound **38** showed a similar bacterial cytological profile to rifampicin. CHL: chloramphenicol, RIF: rifampicin, OXA: oxacillin. Scale bar: 4 μm.

In silico ADME evaluation

To evaluate the drug-like properties of synthesized compounds, Schrödinger Maestro was used to predict absorption, distribution, metabolism and excretion (ADME) of the four compounds (**19**, **36**, **38**, and **39**) with the greatest antimicrobial activity in this research. Various pharmacokinetic and pharmacodynamic parameters were calculated: octanol-water partitioning coefficient (QPlogPo/w), aqueous solubility (QPlogS), binding to human serum albumin (QPlogKhsa), number of likely metabolic reactions (#metab), apparent Caco-2 cell permeability (QPPCaco), human oral absorption, and predicted IC_{50} value for blockage of HERG Kþ channels (QPloghERG).

As shown in **Table 7**, the predicted lipophilicity and aqueous solubility of all the 4 selected compounds have acceptable logP and logS. Furthermore, the blood protein binding (QPlogKhsa) and the number of likely metabolic reactions (#metab) remained within the standard ranges, suggesting the drug-like of the selected compound structures. It was suggested that the selected compounds possessed outstanding oral absorption, and low possibility of heart toxicity.

Table 7: *In silico* ADME evaluation of compounds **19**, **36**, **38**, and **39**.

Principle Descriptors	**19**	**36**	**38**	**39**	Standard Range[a]

QPlogPo/w	4.853	5.191	5.197	5.354	-2.0-6.5
QPlogS	-5.366	-5.526	-5.506	-5.074	-6.5-0.5
QPlogKhsa	0.423	0.553	0.520	0.694	-1.5-1.5
#metab	7	5	6	3	1-8
QPlogBB	0.293	0.379	0.430	0.453	-3.0-1.2
CNS	1	1	1	1	-2 (inactivity), +2 (activity)
QPPCaco	739.15	898.99	866.17	849.91	<25 poor, >500 great
(%) Human Oral Absorption	100	99.14	98.16	99.04	<25% poor, >80% high
QPlogHERG	-7.299	-7.237	-7.092	-6.276	<-5

[a] Statistics of 95% of known drugs by Qikprop of Maestro.

CONCLUSION

In the past research, we adopted structure-based drug design,[36] and successfully discovered two series of PPI inhibitors targeting RNAP-σ[37-40] and NusB-NusE PPIs[41-47] and optimized the antimicrobial activity to a level comparable to marketed antibiotics. Bacterial transcription is a valid but underutilized target.[48,49] In this study, we present the discovery and evaluation of novel analogues of inhibitors targeting bacterial RNAP and the transcription factor NusG. Although the function of NusG in transcription regulation has been extensively studied[3] and NusG has been identified to play a critical role for the fitness of rifampicin resistant *Mycobacterium tuberculosis* in a recent study,[50] this work, to the best of our knowledge, is the first reported attempt to utilize bacterial RNAP-NusG interaction as a target to design novel

PPI inhibitors that have antimicrobial activities. Through a rational design approach based on a pharmacophore model, followed by the synthesis of analogues, we successfully identified a series of compounds with inhibitory activity against the interaction between RNAP β'CH and NusG. These compounds, particularly compound **38**, exhibited potent antimicrobial activity against both Gram-positive and Gram-negative bacteria. Notably, they displayed remarkable efficacy in inhibiting the growth of *S. pneumoniae*, with a MIC as low as 1 μg/mL. The time-kill kinetics also revealed that compound **38** gradually eradicated bacteria at a concentration of 1× MIC or higher, showing a bactericidal character. Lastly, the protein-protein inhibition assay and fluorescent microscopy images confirmed that compound **38** inhibited with NusG and β'CH interaction and disrupted the subcellular localisation of the transcription complex, and an in-cell transcription assay further validated its mechanism of action. Based on these findings, we concluded that our transcription inhibitor effectively halted bacterial transcription by disrupting the interaction between β'CH and NusG, leading to the inhibition of bacterial growth. The detailed mechanism of how **38** affects NusG function in an *in vitro* transcription assay system is currently under consideration.[51] Exploring the possibility of an alternative target of **38** or its derivatives is also a worthy exercise in future directions. The efficacy, pharmacokinetics and toxicities of **38** or derivatives remained to be elucidated. This work forms the basis for the structural optimization of **38** to explore its potential as a new antimicrobial molecule targeting bacterial transcription.

Experimental Section

General

Unless otherwise stated, chemicals and reagents being utilized in synthesis were in commercial grade and required no purification. All reactions were monitored by thin-layer chromatography (TLC) on glass sheets (Silica gel F_{254}), which were visualized under UV light. Flash chromatography purification was conducted using silica gel (200-300 mesh) column. ^{1}H-NMR (400 MHz or 600 MHz), ^{13}C-NMR (100 MHz or 150 MHz) and ^{19}F-NMR (565 MHz) spectra were measured on Bruker Avance-III spectrometer with TMS as an internal standard. Chemical shifts were presented in δ (ppm) and coupling constants (J) in Hz. High resolution mass spectrometry (HRMS) spectra were measured by Agilent 6540 Liquid Chromatography-Electrospray Ionization (LC-EI) QTOF Mass Spectrometer. The purity of all product compounds tested for biological activities was >95%, determined by analytical high-performance liquid chromatography (HPLC) being performed on Waters HPLC system including 2535 quaternary gradient module, 2707 autosampler and 2998 photodiode array (PDA) detector with an XBridge C18, 4.6×100 mm, 5 mM particle size.

Synthetic procedures for Scheme 2.

General procedure for the synthesis of compound **A_1-A_{17}**. Four different procedures were used to obtain the titled compounds:

Method A: ***N*^{1}-(pyrimidin-2-yl)ethane-1,2-diamine (A_1)**. To a solution of 2-chloropyrimidine (50 mg, 0.44 mmol) and K_2CO_3 (72.4 mg, 0.52 mmol) in THF, ethylenediamine monohydrate (177.6 mL, 2.18 mmol) was added at room temperature. The mixture was stirred at reflux overnight and monitored to react completely. After cooling to room temperature and filtration,

the filtrate was concentrated and purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (50:1-20:1, with additional drops of aqueous ammonia) to provide **A_1**, pale yellow oil, 50.1 mg, 83% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.24 (d, J = 4.8 Hz, 2H), 7.24 (t, J = 5.7 Hz, 1H), 6.53 (t, J = 4.8 Hz, 1H), 3.30 (q, J = 6.2 Hz, 2H), 2.72 (t, J = 6.5 Hz, 2H).

***N*^{1}-(5-(trifluoromethyl)pyrimidin-2-yl)ethane-1,2-diamine (A_2)**. The titled compound was prepared from 2-chloro-5-(trifluoromethyl)pyrimidine (50 mg, 0.27 mmol) and ethylenediamine monohydrate (111.5 mL, 1.37 mmol) in a similar manner as described for compound **A_1**. The elution fluid of DCM/MeOH (50:1-20:1, with additional drops of aqueous ammonia) was used for chromatography. Pale yellow oil, 49.7 mg, 88% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62-8.52 (m, 2H), 8.12 (t, J = 5.7 Hz, 1H), 3.37 (s, 2H), 2.74 (d, J = 12.9 Hz, 2H).

***N*^{1}-(4-(trifluoromethyl)pyrimidin-2-yl)ethane-1,2-diamine (A_3)**. The titled compound was prepared from 2-chloro-4-(trifluoromethyl)pyrimidine (50 mg, 0.27 mmol) and ethylenediamine monohydrate (111.5 mL, 1.37 mmol) in a similar manner as described for compound **A_1**. The elution fluid of DCM/MeOH (50:1-20:1, with additional drops of aqueous ammonia) was used for chromatography. Pale yellow oil, 48.2 mg, 85% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.59 (d, J = 26.5 Hz, 1H), 7.87 (d, J = 29.7 Hz, 1H), 6.95 (d, J = 4.9 Hz, 1H), 3.58 (s, 4H).

***N*^{1}-(6-(trifluoromethyl)pyridin-2-yl)ethane-1,2-diamine (A_4)**. The titled compound was prepared from 2-chloro-6-(trifluoromethyl)pyridine (50 mg, 0.28 mmol) and ethylenediamine monohydrate (112.1 mL, 1.38 mmol) in a similar manner as described for compound **A_1**. The elution fluid of DCM/MeOH (100:1-30:1, with additional drops of aqueous ammonia) was used for chromatography. Pale yellow oil, 48.1 mg, 85% yield. ^{1}H NMR (600 MHz,

Chloroform-*d*) δ 7.52 (t, J = 7.9 Hz, 1H), 6.92 (d, J = 7.2 Hz, 1H), 6.57 (d, J = 8.5 Hz, 1H), 5.18 (s, 1H), 3.42 (q, J = 5.8 Hz, 2H), 2.97 (t, J = 5.9 Hz, 2H).

***N*^{1}-(5-(trifluoromethyl)pyridin-2-yl)ethane-1,2-diamine (A_5)**. The titled compound was prepared from 2-chloro-5-(trifluoromethyl)pyridine (50 mg, 0.28 mmol) and ethylenediamine monohydrate (112.1 mL, 1.38 mmol) in a similar manner as described for compound A_1. The elution fluid of DCM/MeOH (100:1-30:1, with additional drops of aqueous ammonia) was used for chromatography. Pale yellow oil, 47.6 mg, 84% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.34 (d, J = 2.2 Hz, 1H), 7.58 (dd, J = 8.8, 2.4 Hz, 1H), 6.46 (d, J = 8.8 Hz, 1H), 5.38 (s, 1H), 3.45 (q, J = 5.8 Hz, 2H), 2.99 (s, 2H).

***N*^{1}-(4-(trifluoromethyl)pyridin-2-yl)ethane-1,2-diamine (A_6)**. The titled compound was prepared from 2-chloro-4-(trifluoromethyl)pyridine (50 mg, 0.28 mmol) and ethylenediamine monohydrate (112.1 mL, 1.38 mmol) in a similar manner as described for compound A_1. The elution fluid of DCM/MeOH (100:1-30:1, with additional drops of aqueous ammonia) was used for chromatography. Pale yellow oil, 48.4 mg, 86% yield. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.22 (d, J = 5.3 Hz, 1H), 6.75 (dd, J = 5.3, 1.4 Hz, 1H), 6.61 (s, 1H), 5.23 (s, 1H), 3.43 (q, J = 5.8 Hz, 2H), 2.99 (t, J = 5.9 Hz, 2H).

***N*^{1}-(3-(trifluoromethyl)pyridin-2-yl)ethane-1,2-diamine (A_7)**. The titled compound was prepared from 2-chloro-3-(trifluoromethyl)pyridine (50 mg, 0.28 mmol) and ethylenediamine monohydrate (112.1 mL, 1.38 mmol) in a similar manner as described for compound A_1. The elution fluid of DCM/MeOH (100:1-30:1, with additional drops of aqueous ammonia) was used for chromatography. Pale yellow oil, 46.4 mg, 82% yield. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.28-8.24 (m, 1H), 7.66 (dd, J = 7.6, 1.7 Hz, 1H), 6.63 (dd, J = 7.5, 5.0 Hz, 1H), 5.38 (s, 1H), 3.58 (q, J = 5.7 Hz, 2H), 2.98 (t, J = 6.0 Hz, 2H).

***N*^{1}-(5-fluoropyridin-2-yl)ethane-1,2-diamine (A_8)**. The titled compound was prepared from

2-chloro-5-fluoropyridine (50 mg, 0.38 mmol) and ethylenediamine monohydrate (154.7 mL, 1.9 mmol) in a similar manner as described for compound **A_1**. The elution fluid of DCM/MeOH (100:1-30:1, with additional drops of aqueous ammonia) was used for chromatography. Pale yellow oil, 46.1 mg, 78% yield.

***N*^{1}-(5-nitropyridin-2-yl)ethane-1,2-diamine (A_9)**. The titled compound was prepared from 2-chloro-5-nitropyridine (50 mg, 0.32 mmol) and ethylenediamine monohydrate (128.3 mL, 1.58 mmol) in a similar manner as described for compound **A_1**. The elution fluid of DCM/MeOH (100:1-30:1, with additional drops of aqueous ammonia) was used for chromatography. Yellow oil, 49.2 mg, 86% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.91 (d, J = 2.8 Hz, 1H), 8.15-8.05 (m, 1H), 6.62 (d, J = 9.3 Hz, 1H), 3.50 (s, 2H), 2.85 (t, J = 6.2 Hz, 2H).

Method B: ***N*^{1}-(2-(trifluoromethyl)phenyl)ethane-1,2-diamine (A_{10})**. To a solution of 1-iodo-2-(trifluoromethyl)benzene (50 mg, 0.18 mmol), CuCl (3.6 mg, 0.038 mmol) and Cs_2CO_3 (119.8 mg, 0.37 mmol) in DMSO, ethylenediamine monohydrate (74.6 mL, 0.92 mmol) was added at room temperature. The mixture was stirred at 120 ℃ for 8 hours and monitored to react completely. After cooling to room temperature and filtration, water was added and the filtrate was extracted with ethyl acetate for three times and washed with saturated brine. The combined organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia) to provide **A_{10}**, pale yellow oil, 26.4 mg, 70% yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.46 (d, J = 7.7 Hz, 1H), 7.38 (t, J = 7.9 Hz, 1H), 6.79-6.71 (m, 2H), 4.79 (s, 1H), 3.27 (q, J = 5.7 Hz, 2H), 3.02 (t, J = 5.9 Hz, 2H).

***N*^{1}-([1,1'-biphenyl]-4-yl)ethane-1,2-diamine (A_{11})**. The titled compound was prepared from 4-iodo-1,1'-biphenyl (50 mg, 0.18 mmol) and ethylenediamine monohydrate (72.6 mL, 0.89 mmol) in a similar manner as described for compound **A_{10}**. The elution fluid of DCM/MeOH

(200:1-100:1, with additional drops of aqueous ammonia) was used for chromatography. Pale yellow oil, 28.5 mg, 75% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.26 (d, J = 1.6 Hz, 1H), 7.57-7.54 (m, 2H), 7.49-7.46 (m, 2H), 7.42 (t, J = 7.7 Hz, 2H), 7.31 (d, J = 1.3 Hz, 2H), 6.75-6.70 (m, 2H), 3.62 (q, J = 6.0 Hz, 2H), 3.40 (t, J = 5.8 Hz, 2H).

***N*^{1}-(4-(trifluoromethyl)phenyl)ethane-1,2-diamine (A$_{12}$)**. The titled compound was prepared from 1-iodo-4-(trifluoromethyl)benzene (50 mg, 0.18 mmol) and ethylenediamine monohydrate (74.8 mL, 0.92 mmol) in a similar manner as described for compound **A$_{10}$**. The elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia) was used for chromatography. Pale yellow oil, 27.1 mg, 72% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.27 (s, 1H), 7.43 (d, J = 8.4 Hz, 2H), 6.64 (d, J = 8.4 Hz, 2H), 5.89 (s, 1H), 4.50 (s, 1H), 3.61 (q, J = 6.0 Hz, 2H), 3.37 (q, J = 5.6 Hz, 2H).

***N*^{1}-(3-(trifluoromethyl)phenyl)ethane-1,2-diamine (A$_{13}$)**. The titled compound was prepared from 1-iodo-3-(trifluoromethyl)benzene (50 mg, 0.18 mmol) and ethylenediamine monohydrate (74.8 mL, 0.92 mmol) in a similar manner as described for compound **A$_{10}$**. The elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia) was used for chromatography. Pale yellow oil, 28.1 mg, 75% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.26 (d, J = 7.9 Hz, 1H), 6.95 (d, J = 7.6 Hz, 1H), 6.84 (d, J = 2.4 Hz, 1H), 6.79 (dd, J = 8.2, 2.3 Hz, 1H), 4.34 (s, 1H), 3.22 (t, J = 5.8 Hz, 2H), 3.00 (t, J = 5.8 Hz, 2H).

***N*^{1}-(2-nitrophenyl)ethane-1,2-diamine (A$_{14}$)**. The titled compound was prepared from 1-iodo-2-nitrobenzene (50 mg, 0.2 mmol) and ethylenediamine monohydrate (79.6 mL, 1 mmol) in a similar manner as described for compound **A$_{10}$**. The elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia) was used for chromatography. Yellow oil, 27.8 mg with 76% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.27 (s, 1H), 8.18 (dt, J = 8.6, 2.8 Hz, 1H), 7.44 (m, 1H), 6.88 (dd, J = 8.7, 2.6 Hz, 1H), 6.65 (m, 1H), 3.44-3.36 (m, 2H),

3.07 (td, *J* = 6.1, 3.1 Hz, 2H).

2-((2-aminoethyl)amino)benzonitrile (A_{15}). The titled compound was prepared from 2-iodobenzonitrile (50 mg, 0.22 mmol) and ethylenediamine monohydrate (86.6 mL, 1.1 mmol) in a similar manner as described for compound **A_{10}**. The elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia) was used for chromatography. Pale yellow oil, 27.6 mg with 79% yield. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.40 (td, *J* = 7.6, 1.5 Hz, 2H), 6.72-6.68 (m, 2H), 3.40 (t, *J* = 5.8 Hz, 2H), 3.07 (t, *J* = 5.8 Hz, 2H).

Method C: ***N*-(2-aminoethyl)-2-(trifluoromethyl)benzenesulfonamide (A_{16})**. To a solution of ethylenediamine monohydrate (16.7 mL, 0.2 mmol) and triethylamine (61.4 mL, 0.61 mmol) in DCM, 2-(trifluoromethyl)benzenesulfonyl chloride (50 mg, 0.2 mmol) was added dropwise in ice bath. The mixture was warmed to room temperature and stirred for overnight. After being monitored to react completely, the mixture was concentrated and purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-50:1, with additional drops of aqueous ammonia) to provide **A_{16}**, yellow oil, 50.5 mg, 92% yield. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.23-8.17 (m, 1H), 7.98 (dd, *J* = 6.9, 2.2 Hz, 1H), 7.83 (m, 2H), 3.05 (t, *J* = 6.2 Hz, 2H), 2.78 (t, *J* = 6.2 Hz, 2H).

Method D: **2-(2-(trifluoromethyl)phenoxy)ethan-1-amine (A_{17})**. To a solution of 2-(trifluoromethyl)phenol (50 mg, 0.31 mmol) and K_2CO_3 (426.3 mg, 3.08 mmol) in DMF, *tert*-butyl (2-bromoethyl)carbamate (82.9 mg, 0.37 mmol) was added at room temperature. The mixture was stirred at 65 ℃ for 8 hours and monitored to react completely. After cooling to room temperature and filtration, water was added and the filtrate was extracted with ethyl acetate for three times and washed with saturated brine. The combined organic layer was dried over anhydrous Na_2SO_4 and purified by column chromatography on silica gel with the elution fluid of Hexane/EA (10:1-5:1) to achieve *tert*-butyl (2-(2-

(trifluoromethyl)phenoxy)ethyl)carbamate, pale yellow solid, 83.6 mg, 89% yield. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.55 (m, 2H), 7.16 (d, J = 8.3 Hz, 1H), 7.06 (t, J = 7.6 Hz, 1H), 4.13 (t, J = 5.9 Hz, 2H), 3.48 (m, 2H), 1.45 (s, 9H).

To a solution of *tert*-butyl (2-(2-(trifluoromethyl)phenoxy)ethyl)carbamate (50 mg, 0.16 mmol) in DCM, TFA (110.1 mL, 1.64 mmol) was added at room temperature. The mixture was stirred at reflux for overnight and monitored to react completely. After cooling to room temperature, NaOH aqueous (1M) solution was added in ice bath to regulate the pH value ranging from 8 to 10. The mixture was extracted with DCM for three times and washed with saturated brine. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to give the titled compound **A$_{17}$**. Pale yellow oil, 32.1 mg, 96% yield. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.62 (dd, J = 7.8, 1.5 Hz, 1H), 7.56-7.52 (m, 1H), 7.11 (t, J = 7.6 Hz, 1H), 7.01 (d, J = 8.3 Hz, 1H), 4.22 (t, J = 5.0 Hz, 2H), 3.86 (q, J = 5.4 Hz, 2H).

2-((2-(trifluoromethyl)phenyl)thio)ethan-1-amine (A$_{18}$). The titled compound was prepared in an analogous fashion as described for compound **A$_{17}$**, using (trifluoromethyl)benzenethiol (50 mg, 0.28 mmol) and *tert*-butyl (2-bromoethyl)carbamate (75.5 mg, 0.34 mmol) to provide *tert*-butyl (2-((2-(trifluoromethyl)phenyl)thio)ethyl)carbamate. The elution fluid of Hexane/EA (10:1-5:1) was used for chromatography. Pale yellow solid, 58.8 mg, 65% yield. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.67 (t, J = 7.8 Hz, 2H), 7.55 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.7 Hz, 1H), 4.85 (d, J = 1.9 Hz, 1H), 3.32-3.24 (m, 2H), 3.11 (dd, J = 8.3, 6.1 Hz, 2H), 1.44 (s, 9H). Compound **A$_{18}$** was prepared from *tert*-butyl (2-((2-(trifluoromethyl)phenyl)thio)ethyl)carbamate (50 mg, 0.15 mmol) through the de-protection by trifluoroacetic acid (104.6 mL, 1.56 mmol). Pale yellow oil, 33.1 mg, 96% yield. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.67-7.63 (m, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.29 (d, J = 15.3 Hz, 1H), 3.07 (td, J = 6.3, 1.6 Hz, 2H), 2.92 (td, J = 6.3, 1.7 Hz, 2H).

Synthetic procedures for Scheme 3.

General procedure for the synthesis of compound **B_1-B_{17}**. Four different procedures were used to obtain the titled compounds:

Method A: **2-((4-nitrophenoxy)methyl)oxirane (B_1)**. The solution of 4-nitrophenol (50 mg, 0.36 mmol), Cs_2CO_3 (175.7 mg, 0.54 mmol) and KI (11.9 mg, 0.072 mmol) in DMF being stirred at room temperature for 30 minutes, 2-(chloromethyl)oxirane (84.5 mL, 1.08 mmol) was added. The solution was stirred at 80 ℃ for overnight and monitored to react completely. After cooling to room temperature and filtration, water was added and the filtrate was extracted with ethyl acetate for three times and washed with saturated brine. The combined organic layer was dried over anhydrous Na_2SO_4 and purified by column chromatography on silica gel with the elution fluid of Hexane/EA (200:1-50:1) to give the compound **B_1**, pale yellow oil, 57.9 mg, 83% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25-8.19 (m, 2H), 7.22-7.16 (m, 2H), 4.53 (dd, J = 11.5, 2.5 Hz, 1H), 4.00 (dd, J = 11.5, 6.6 Hz, 1H), 3.39 (m, 1H), 2.88 (t, J = 4.6 Hz, 1H), 2.75 (dd, J = 5.1, 2.6 Hz, 1H).

2-(phenoxymethyl)oxirane (B_2). The titled compound was prepared from phenol (50 mg, 0.53 mmol) and 2-(chloromethyl)oxirane (124.9 mL, 1.59 mmol) in a similar manner as described for compound **B_1**. The elution fluid of Hexane/EA (200:1-50:1) was used for chromatography. Pale yellow oil, 67.2 mg, 84% yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.36-7.30 (m, 2H), 7.04-6.94 (m, 3H), 4.25 (dd, J = 11.0, 3.1 Hz, 1H), 3.97 (dd, J = 11.0, 5.7 Hz, 1H), 3.38 (m, 1H), 2.92 (t, J = 4.5 Hz, 1H), 2.78 (dd, J = 5.0, 2.7 Hz, 1H).

methyl 4-(oxiran-2-ylmethoxy)benzoate (B_3). The titled compound was prepared from methyl 4-hydroxybenzoate (50 mg, 0.33 mmol) and 2-(chloromethyl)oxirane (77.2 mL, 0.99 mmol) in a similar manner as described for compound **B_1**. The elution fluid of Hexane/EA

(200:1-50:1) was used for chromatography. Pale yellow oil, 54 mg, 79% yield. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.00-7.98 (m, 2H), 6.95-6.92 (m, 2H), 4.30 (dd, J = 11.0, 3.0 Hz, 1H), 3.98 (dd, J = 11.0, 5.9 Hz, 1H), 3.88 (s, 3H), 3.38 (m, 1H), 2.93 (t, J = 4.5 Hz, 1H), 2.77 (dd, J = 4.9, 2.6 Hz, 1H).

2-((4-fluorophenoxy)methyl)oxirane (B_4). The titled compound was prepared from 4-fluorophenol (50 mg, 0.45 mmol) and 2-(chloromethyl)oxirane (104.8 mL, 1.34 mmol) in a similar manner as described for compound **B_1**. The elution fluid of Hexane/EA (200:1-50:1) was used for chromatography. Pale yellow oil, 62.6 mg, 84% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.56 (d, J = 8.3 Hz, 2H), 7.00 (d, J = 8.3 Hz, 2H), 4.32 (m, 1H), 3.98 (m, J = 11.1, 6.0, 1.9 Hz, 1H), 3.38 (m, 1H), 2.98-2.91 (m, 1H), 2.78 (m, 1H).

2-((3,4-difluorophenoxy)methyl)oxirane (B_5). The titled compound was prepared from 3,4-difluorophenol (50 mg, 0.38 mmol) and 2-(chloromethyl)oxirane (90.3 mL, 1.15 mmol) in a similar manner as described for compound **B_1**. The elution fluid of Hexane/EA (200:1-50:1) was used for chromatography. Pale yellow oil, 58.6 mg, 82% yield. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.07 (q, J = 9.3 Hz, 1H), 6.76 (m, 1H), 6.63 (m, 1H), 4.23 (dd, J = 11.0, 2.8 Hz, 1H), 3.87 (dd, J = 11.0, 5.9 Hz, 1H), 3.35 (m, 1H), 2.93 (t, J = 4.5 Hz, 1H), 2.76 (dd, J = 4.8, 2.6 Hz, 1H).

2-((4-methoxyphenoxy)methyl)oxirane (B_6). The titled compound was prepared from 4-methoxyphenol (50 mg, 0.4 mmol) and 2-(chloromethyl)oxirane (94.6 mL, 1.21 mmol) in a similar manner as described for compound **B_1**. The elution fluid of Hexane/EA (200:1-50:1) was used for chromatography. Yellow oil, 55.3 mg, 76% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.28 (t, J = 3.4 Hz, 2H), 6.92-6.87 (m, 2H), 4.23 (dd, J = 11.1, 3.1 Hz, 1H), 3.96 (dd, J = 11.1, 5.7 Hz, 1H), 3.39-3.33 (m, 1H), 2.93 (t, J = 4.5 Hz, 1H), 2.77 (dd, J = 4.9, 2.7 Hz, 1H), 2.47 (s, 3H).

2-((4-(trifluoromethyl)phenoxy)methyl)oxirane (B_7). The titled compound was prepared from 4-(trifluoromethyl)phenol (50 mg, 0.31 mmol) and 2-(chloromethyl)oxirane (72.5 mL, 0.93 mmol) in a similar manner as described for compound B_1. The elution fluid of Hexane/EA (200:1-50:1) was used for chromatography. Pale yellow oil, 57.4 mg, 85% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.60-7.49 (m, 2H), 7.04-6.93 (m, 2H), 4.30 (m, 1H), 3.94 (m, 1H), 3.35 (m, 1H), 2.90 (dd, J = 5.7, 3.3 Hz, 1H), 2.75 (m, 1H).

2-((3-(trifluoromethyl)phenoxy)methyl)oxirane (B_8). The titled compound was prepared from 3-(trifluoromethyl)phenol (50 mg, 0.31 mmol) and 2-(chloromethyl)oxirane (72.5 mL, 0.93 mmol) in a similar manner as described for compound B_1. The elution fluid of Hexane/EA (200:1-50:1) was used for chromatography. Pale yellow oil, 55 mg, 82% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.41 (t, J = 8.0 Hz, 1H), 7.25 (s, 1H), 7.17 (d, J = 2.1 Hz, 1H), 7.12 (dd, J = 8.3, 2.5 Hz, 1H), 4.31 (dt, J = 10.9, 2.2 Hz, 1H), 3.97 (m, 1H), 3.39 (m, 1H), 2.94 (td, J = 4.5, 1.4 Hz, 1H), 2.79 (dt, J = 4.4, 2.0 Hz, 1H).

2-((2-(trifluoromethyl)phenoxy)methyl)oxirane (B_9). The titled compound was prepared from 2-(trifluoromethyl)phenol (50 mg, 0.31 mmol) and 2-(chloromethyl)oxirane (72.5 mL, 0.93 mmol) in a similar manner as described for compound B_1. The elution fluid of Hexane/EA (200:1-50:1) was used for chromatography. Pale yellow oil, 56.2 mg, 84% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.59 (dd, J = 7.6, 1.6 Hz, 1H), 7.50 (td, J = 8.0, 1.6 Hz, 1H), 7.05 (t, J = 8.4 Hz, 2H), 4.35 (dd, J = 11.2, 2.9 Hz, 1H), 4.12 (dd, J = 11.2, 5.0 Hz, 1H), 3.39 (m, 1H), 2.92 (t, J = 4.6 Hz, 1H), 2.85 (dd, J = 5.0, 2.6 Hz, 1H).

4-(oxiran-2-ylmethoxy)benzonitrile (B_{10}). The titled compound was prepared from 4-hydroxybenzonitrile (50 mg, 0.42 mmol) and 2-(chloromethyl)oxirane (98.6 mL, 1.26 mmol) in a similar manner as described for compound B_1. The elution fluid of Hexane/EA (200:1-50:1) was used for chromatography. Pale yellow oil, 60.9 mg, 83% yield. ^{1}H NMR (400 MHz,

Chloroform-*d*) δ 7.63-7.54 (m, 2H), 7.02-6.95 (m, 2H), 4.34 (dd, J = 11.1, 2.8 Hz, 1H), 3.96 (dd, J = 11.2, 5.9 Hz, 1H), 3.37 (m, 1H), 2.93 (t, J = 4.5 Hz, 1H), 2.77 (dd, J = 4.8, 2.6 Hz, 1H).

Method B: ***N*-(oxiran-2-ylmethyl)-4-(trifluoromethyl)aniline ($\mathbf{B_{11}}$)**. The solution of 4-(trifluoromethyl)aniline (50 mg, 0.31 mmol) and $Zn(SO_3CF_3)_2$ (13.3 mg, 0.062 mmol) in $CHCl_3$, 2-(chloromethyl)oxirane (24.3 mL, 0.31 mmol) was added dropwise in ice bath. After warming to room temperature, the solution was stirred at 60 ℃ for 12 hours and monitored to react completely. After cooling to room temperature and filtration, water was added and the filtrate was extracted with ethyl acetate for three times and washed with saturated brine. The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated to achieve the crude product. The solution of 1-chloro-3-((4-(trifluoromethyl)phenyl)amino)propan-2-ol (67.3 mg, 0.27 mmol) and KI (8 mg, 0.05 mmol) in MeCN was stirred under reflux for 6 hours. and monitored to react completely. After cooling to room temperature and filtration, water was added and the filtrate was extracted with ethyl acetate for three times and washed with saturated brine. The combined organic layer was dried over anhydrous Na_2SO_4 and purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1) to give compound $\mathbf{B_{11}}$. Yellow oil, 42.5 mg, 74% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.44 (d, J = 8.5 Hz, 2H), 6.67 (d, J = 8.4 Hz, 2H), 4.23 (s, 1H), 3.63 (m, 1H), 3.35-3.18 (m, 2H), 2.86 (t, J = 4.3 Hz, 1H), 2.71 (dd, J = 4.8, 2.6 Hz, 1H).

Method C: **2-(((4-(trifluoromethyl)phenyl)thio)methyl)oxirane ($\mathbf{B_{12}}$)**. The solution of 4-(trifluoromethyl)benzenethiol (50 mg, 0.28 mmol) and K_2CO_3 (58.2 mg, 0.42 mmol) in acetonitrile, 2-(chloromethyl)oxirane (43.9 mL, 0.56 mmol) was added at room temperature. The solution was stirred under reflux for 8 hours and monitored to react completely. After cooling to room temperature and filtration, water was added and the filtrate was extracted with ethyl acetate for three times and washed with saturated brine. The combined organic layer was

dried over anhydrous Na_2SO_4 and purified by column chromatography on silica gel with the elution fluid of Hexane/EA (200:1-50:1) to give compound **B_{12}**. Yellow oil, 53.8 mg, 82% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.55 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 8.3 Hz, 2H), 3.25-3.18 (m, 2H), 3.12 (dd, J = 15.5, 6.5 Hz, 1H), 2.86-2.82 (m, 1H), 2.64 (dd, J = 4.9, 2.3 Hz, 1H).

Method D: **2-((benzyloxy)methyl)oxirane (B_{13})**. The solution of 2-(chloromethyl)oxirane (43.4 mL, 0.56 mmol) and TBAB (16.3 mg, 0.023 mmol) in 50% NaOH aqueous solution, benzyl alcohol (50 mg, 0.46 mmol) was added dropwise in ice bath. The solution was warmed to room temperature and stirred for 18 hours and monitored to react completely. The reaction was quenched with iced water and extracted with ethyl acetate for three times and washed with saturated brine. The combined organic layer was dried over anhydrous Na_2SO_4 and purified by column chromatography on silica gel with the elution fluid of Hexane/EA (200:1-100:1) to provide compound **B_{13}**. Pale yellow oil, 68.1 mg, 90% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.44 – 7.31 (m, 4H), 7.31 – 7.26 (m, 1H), 4.52 (d, J = 1.8 Hz, 2H), 3.75 (dd, J = 11.5, 2.7 Hz, 1H), 3.29 (dd, J = 11.5, 6.4 Hz, 1H), 3.15 (ddt, J = 6.8, 4.2, 2.7 Hz, 1H), 2.76 – 2.71 (m, 1H), 2.56 (dd, J = 5.1, 2.7 Hz, 1H).

2-(((4-(trifluoromethyl)benzyl)oxy)methyl)oxirane (B_{14}). The titled compound was prepared from 2-(chloromethyl)oxirane (26.7 mL, 0.34 mmol) and (4-(trifluoromethyl)phenyl)methanol (50 mg, 0.28 mmol) in a similar manner as described for compound **B_{13}**. The elution fluid of Hexane/EA (200:1-50:1) was used for chromatography. Pale yellow oil, 59.5 mg, 90% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.56 (d, J = 8.5 Hz, 2H), 7.00 (d, J = 8.5 Hz, 2H), 4.31 (dd, J = 11.1, 2.8 Hz, 1H), 3.99-3.91 (m, 1H), 3.38 (m, 1H), 2.93 (t, J = 4.5 Hz, 1H), 2.78 (dd, J = 4.9, 2.6 Hz, 1H).

2-(((3-(trifluoromethyl)benzyl)oxy)methyl)oxirane (B_{15}). The titled compound was prepared from 2-(chloromethyl)oxirane (26.7 mL, 0.34 mmol) and (3-

(trifluoromethyl)phenyl)methanol (50 mg, 0.28 mmol) in a similar manner as described for compound **B_{13}**. The elution fluid of Hexane/EA (200:1-50:1) was used for chromatography. Pale yellow oil, 57.7 mg, 88% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.56 (tt, J = 24.8, 5.9 Hz, 4H), 4.66 (m, 2H), 3.86 (m, 1H), 3.47 (dt, J = 11.4, 5.7 Hz, 1H), 3.23 (m, 1H), 2.84 (q, J = 5.1 Hz, 1H), 2.65 (dt, J = 5.1, 2.7 Hz, 1H).

2-(((2-(trifluoromethyl)benzyl)oxy)methyl)oxirane (B_{16}). The titled compound was prepared from 2-(chloromethyl)oxirane (26.7 mL, 0.34 mmol) and (2-(trifluoromethyl)phenyl)methanol (50 mg, 0.28 mmol) in a similar manner as described for compound **B_{13}**. The elution fluid of Hexane/EA (200:1-50:1) was used for chromatography. Pale yellow oil, 60.1 mg, 91% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.78-7.55 (m, 3H), 7.40 (t, J = 7.7 Hz, 1H), 4.85-4.73 (m, 2H), 3.86 (dd, J = 11.3, 3.0 Hz, 1H), 3.52 (dd, J = 11.4, 5.9 Hz, 1H), 3.25 (m, 1H), 2.85 (t, J = 4.6 Hz, 1H), 2.68 (dd, J = 5.0, 2.7 Hz, 1H).

Method D: **2-(((4-(trifluoromethyl)benzyl)thio)methyl)oxirane (B_{17})**. The solution of 2-(chloromethyl)oxirane (61.1 mL, 0.78 mmol) and KOH (43.8 mg, 0.78 mmol) in dioxane and water (1:1), (4-(trifluoromethyl)phenyl)methanethiol (50 mg, 0.26 mmol) was added dropwise in ice bath. The solution was warmed to room temperature and stirred for 8 hours and monitored to react completely. The mixture was extracted with ethyl acetate for three times and washed with saturated brine. The combined organic layer was dried over anhydrous Na_2SO_4 and purified by column chromatography on silica gel with the elution fluid of Hexane/EA (200:1-100:1) to provide compound **B_{17}**. Yellow oil, 52.8 mg, 82% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.60 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 8.0 Hz, 2H), 3.93-3.82 (m, 2H), 3.14 (m, 1H), 2.79 (t, J = 4.4 Hz, 1H), 2.66-2.51 (m, 3H).

Synthetic procedures for target compounds

General procedure for the Synthesis of Compounds **1** to **39**. To a solution of selected compound **A_1**-**A_{17}** (1 equiv) and compounds **B_1**-**B_{17}** (1 equiv) in EtOH, DIPEA (2 equiv) was added. The solution was stirred at reflux for 8 hours and monitored to react completely. After warming to room temperature, the mixture was concentrated and purified by column chromatography to provide the titled compounds.

1-(4-nitrophenoxy)-3-((2-(pyrimidin-2-ylamino)ethyl)amino)propan-2-ol (1). The titled compound was prepared from **A_1** (30 mg, 0.22 mmol) and **B_1** (42.4 mg, 0.22 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 52.9 mg, 73% yield, mp 144-146℃. ^{1}H NMR (400 MHz, Chloroform-*d*) *d* 8.27 (d, *J* = 4.8 Hz, 2H), 8.23-8.18 (m, 2H), 7.00-6.95 (m, 2H), 6.55 (dd, *J* = 5.4, 4.3 Hz, 1H), 5.46 (s, 1H), 4.11-4.08 (m, 3H), 3.57 (q, *J* = 5.8 Hz, 2H), 2.97-2.91 (m, 3H), 2.86-2.80 (m, 1H); ^{13}C NMR (101 MHz, Chloroform-*d*) *d* 163.6, 162.5, 158.1, 141.8, 125.9, 114.6, 110.8, 71.1, 68.2, 51.3, 49.1, 41.2. HRMS(ESI): calcd for $C_{15}H_{19}N_5O_4$, $[M + H]^+$ 334.1515; found, 334.1514. HPLC purity: 97.06%.

1-(4-nitrophenoxy)-3-((2-((5-(trifluoromethyl)pyrimidin-2-yl)amino)ethyl)amino)propan-2-ol (2). The titled compound was prepared from **A_2** (30 mg, 0.15 mmol) and **B_1** (28.4 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Yellow solid, 41.5 mg, 71% yield, mp 133-134℃. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 8.47 (s, 2H), 8.25-8.17 (m, 2H), 7.01-6.95 (m, 2H), 6.01 (s, 1H), 4.14-4.07 (m, 3H), 3.62 (q, *J* = 5.8 Hz, 2H), 2.95 (m, 3H), 2.84 (dd, *J* = 12.3, 7.3 Hz, 1H), 2.39 (s, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) *δ* 163.5, 163.4, 155.8, 155.8, 155.8, 155.7, 141.8, 125.9, 125.3, 114.5, 114.2, 71.0, 68.3, 51.3, 48.6, 41.2. ^{19}F NMR (565 MHz, DMSO-d_6) δ -59.26. HRMS(ESI): calcd for $C_{16}H_{18}F_3N_5O_4$, $[M + H]^+$ 402.1389; found, 402.1382. HPLC purity: 97.06%.

1-(4-nitrophenoxy)-3-((2-((6-(trifluoromethyl)pyridin-2-yl)amino)ethyl)amino)propan-2-ol (3). The titled compound was prepared from **A_4** (30 mg, 0.15 mmol) and **B_1** (28.5 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Yellow solid, 38.9 mg, 66% yield, mp 110-111℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.18 (d, *J* = 8.9 Hz, 2H), 7.50 (t, *J* = 7.9 Hz, 1H), 6.94 (dd, *J* = 15.7, 8.1 Hz, 3H), 6.54 (d, *J* = 8.5 Hz, 1H), 5.16 (t, *J* = 5.6 Hz, 1H), 4.09 (d, *J* = 7.0 Hz, 3H), 3.48 (q, *J* = 5.8 Hz, 2H), 2.93 (dd, *J* = 9.6, 4.4 Hz, 3H), 2.82 (dd, *J* = 12.3, 7.2 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.6, 158.6, 146.6, 146.3, 141.7, 138.1, 125.9, 123.0, 120.3, 114.5, 110.3, 109.1, 109.1, 71.1, 68.2, 51.3, 48.8, 41.5. ^{19}F NMR (565 MHz, DMSO-d_6) δ -67.29. HRMS(ESI): calcd for $C_{17}H_{19}F_3N_4O_4$, $[M + H]^+$ 401.1437; found, 401.1431. HPLC purity: 98.85%.

1-(4-nitrophenoxy)-3-((2-((5-(trifluoromethyl)pyridin-2-yl)amino)ethyl)amino)propan-2-ol (4). The titled compound was prepared from **A_5** (30 mg, 0.15 mmol) and **B_1** (28.5 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Yellow solid, 37.7 mg, 65% yield, mp 131-132℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.29 (s, 1H), 8.15 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 8.8 Hz, 1H), 6.93 (d, *J* = 8.8 Hz, 2H), 6.42 (d, *J* = 8.8 Hz, 1H), 5.59 (t, *J* = 5.7 Hz, 1H), 4.19-4.04 (m, 3H), 3.49 (q, *J* = 5.3, 4.8 Hz, 2H), 2.97-2.89 (m, 3H), 2.83 (dd, *J* = 12.3, 7.9 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.6, 160.3, 145.9, 145.8, 141.7, 134.4, 125.9, 123.2, 115.6, 115.3, 114.5, 106.6, 71.2, 68.3, 51.5, 48.6, 41.4. ^{19}F NMR (565 MHz, DMSO-d_6) δ -59.18. HRMS(ESI): calcd for $C_{17}H_{19}F_3N_4O_4$, $[M + H]^+$ 401.1437; found, 401.1433. HPLC purity: 96.36%.

1-(4-nitrophenoxy)-3-((2-((4-(trifluoromethyl)pyridin-2-yl)amino)ethyl)amino)propan-2-ol (5). The titled compound was prepared from **A_6** (30 mg, 0.15 mmol) and **B_1** (28.5 mg, 0.15

mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Yellow solid, 42.1 mg, 72% yield, mp 120-122℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.25-8.16 (m, 3H), 6.97 (dd, *J* = 9.0, 1.5 Hz, 2H), 6.75 (d, *J* = 5.3 Hz, 1H), 6.58 (s, 1H), 5.15 (s, 1H), 4.14-4.07 (m, 3H), 3.49 (q, *J* = 5.7 Hz, 2H), 3.00-2.91 (m, 3H), 2.84 (dd, *J* = 12.4, 7.1 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.5, 158.9, 149.4, 141.8, 139.8, 139.5, 125.9, 124.4, 121.6, 114.5, 108.2, 108.2, 71.0, 68.3, 51.4, 48.7, 41.7. ^{19}F NMR (565 MHz, DMSO-d_6) δ -64.04. HRMS(ESI): calcd for $C_{17}H_{19}F_3N_4O_4$, $[M + H]^+$ 401.1437; found, 401.1431. HPLC purity: 98.44%.

1-(4-nitrophenoxy)-3-((2-((3-(trifluoromethyl)pyridin-2-yl)amino)ethyl)amino)propan-2-ol (6). The titled compound was prepared from **A$_7$** (30 mg, 0.15 mmol) and **B$_1$** (28.5 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Yellow solid, 40.1 mg, 69% yield, mp 115-117℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.28-8.15 (m, 3H), 7.65 (d, *J* = 7.6 Hz, 1H), 7.00-6.93 (m, 2H), 6.62 (dd, *J* = 7.6, 5.0 Hz, 1H), 5.41 (d, *J* = 5.4 Hz, 1H), 4.11 (q, *J* = 3.9 Hz, 3H), 3.63 (q, *J* = 5.6 Hz, 2H), 2.97 (m, 3H), 2.84 (dd, *J* = 12.3, 6.5 Hz, 1H), 2.57(s, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.6, 154.7, 151.6, 141.7, 135.2, 135.1, 135.1, 135.0, 125.9, 125.8, 123.1, 120.4, 114.5, 111.5, 108.8, 108.5, 108.2, 71.0, 68.3, 51.1, 48.6, 40.9. ^{19}F NMR (565 MHz, DMSO-d_6) δ -62.80. HRMS(ESI): calcd for $C_{17}H_{19}F_3N_4O_4$, $[M + H]^+$ 401.1437; found, 401.143. HPLC purity: 98.7%.

1-((2-((5-fluoropyridin-2-yl)amino)ethyl)amino)-3-(4-nitrophenoxy)propan-2-ol (7). The titled compound was prepared from **A$_8$** (30 mg, 0.19 mmol) and **B$_1$** (37.7 mg, 0.19 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Yellow solid, 47.1 mg, 70% yield, mp 139-141℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.25-8.13 (m, 4H), 7.01-6.94 (m, 2H), 5.50 (t, *J*

= 5.7 Hz, 1H), 4.09 (s, 3H), 3.53 (q, *J* = 5.8 Hz, 2H), 2.96-2.90 (m, 3H), 2.82 (dd, *J* = 12.3, 6.8 Hz, 1H), 2.30 (s, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) *δ* 163.6, 159.5, 153.4, 150.9, 145.7, 145.4, 141.8, 125.9, 114.5, 71.1, 68.2, 51.3, 48.9, 41.8. ^{19}F NMR (565 MHz, DMSO-d_6) δ -157.53. HRMS(ESI): calcd for $C_{15}H_{18}FN_5O_4$, $[M + H]^+$ 352.1469; found, 352.1478. HPLC purity: 95.72%.

1-(4-nitrophenoxy)-3-((2-((2-(trifluoromethyl)phenyl)amino)ethyl)amino)propan-2-ol (8). The titled compound was prepared from **A_{10}** (30 mg, 0.15 mmol) and **B_1** (28.7 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). Yellow solid, 42.8 mg, 73% yield, mp 102-103℃. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 8.25-8.17 (m, 2H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.01-6.95 (m, 2H), 6.76 (dd, *J* = 8.2, 5.2 Hz, 2H), 4.91 (s, 1H), 4.13 (s, 3H), 3.30 (m, 2H), 3.08-2.94 (m, 3H), 2.89-2.82 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) *δ* 163.6, 145.7, 145.7, 141.8, 133.2, 129.3, 126.8, 126.7, 126.7, 126.6, 125.9, 123.9, 121.2, 116.1, 114.5, 114.0, 113.7, 113.4, 113.1, 111.9, 71.0, 68.5, 51.0, 48.0, 42.8. ^{19}F NMR (565 MHz, DMSO-d_6) δ -61.42. HRMS(ESI): calcd for $C_{18}H_{20}F_3N_3O_4$, $[M + H]^+$ 400.1484; found, 400.1491. HPLC purity: 95.37%.

1-phenoxy-3-((2-((4-(trifluoromethyl)pyrimidin-2-yl)amino)ethyl)amino)propan-2-ol (9) [48]. The titled compound was prepared from **A_3** (30 mg, 0.15 mmol) and **B_2** (21.9 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 38.6 mg, 75% yield, mp 113-115℃. ^{1}H NMR (400 MHz, Chloroform-*d*) *d* 8.44 (s, 1H), 7.27 (d, *J* = 7.6 Hz, 2H), 6.99-6.84 (m, 3H), 6.77 (s, 1H), 6.53 (s, 1H), 4.15 (s, 1H), 3.98 (s, 2H), 3.59 (s, 2H), 2.96-2.80 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) *d* 162.5, 160.4, 158.6, 156.9, 156.5, 156.2, 155.8, 129.5, 121.9, 121.1, 119.2, 116.4, 114.5, 105.3, 70.6, 68.7, 51.8, 48.6, 41.1. ^{19}F NMR (565

MHz, Chloroform-*d*) δ -70.83. HRMS(ESI): calcd for $C_{16}H_{19}F_3N_4O_2$, $[M + H]^+$ 357.1538; found, 357.153. HPLC purity: 97.91%.

methyl 4-(2-hydroxy-3-((2-((4-(trifluoromethyl)pyrimidin-2-yl)amino)ethyl) amino)propoxy)benzoate (10). The titled compound was prepared from **A_3** (30 mg, 0.15 mmol) and **B_3** (30.3 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 41.4 mg, 68% yield, mp 124-125°C. ^{1}H NMR (400 MHz, Chloroform-*d*) *d* 8.40 (s, 1H), 7.91 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 6.74 (d, *J* = 4.9 Hz, 1H), 6.50 (t, *J* = 5.9 Hz, 1H), 4.12 (m, 1H), 4.02-3.95 (m, 2H), 3.84 (s, 3H), 3.56 (q, *J* = 5.8 Hz, 4H), 2.92-2.83 (m, 3H), 2.79 (dd, *J* = 12.1, 8.1 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) *d* 166.7, 162.5, 162.3, 160.3, 156.8, 156.5, 156.1, 131.5, 122.8, 121.8, 119.1, 114.0, 105.4, 105.3, 105.3, 70.7, 68.4, 51.8, 51.6, 48.6, 41.0. ^{19}F NMR (565 MHz, Chloroform-*d*) δ -70.83. HRMS(ESI): calcd for $C_{18}H_{21}F_3N_4O_4$, $[M + H]^+$ 415.1593; found, 415.1586. HPLC purity: 98.5%.

1-(4-fluorophenoxy)-3-((2-((4-(trifluoromethyl)pyrimidin-2-yl)amino)ethyl)amino)propan-2-ol (11). The titled compound was prepared from **A_3** (30 mg, 0.15 mmol) and **B_4** (24.5 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 39.4 mg, 72% yield, mp 116-117°C. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 8.46 (s, 1H), 6.96 (t, *J* = 8.4 Hz, 2H), 6.90-6.72 (m, 3H), 6.17 (s, 1H), 4.15-4.04 (m, 1H), 3.95 (d, *J* = 5.2 Hz, 2H), 3.60 (q, *J* = 6.0 Hz, 2H), 3.10-2.52 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) *δ* 162.5, 160.4, 158.6, 157.0, 156.6, 156.2, 155.9, 154.7, 154.7, 121.9, 119.1, 116.4, 115.9, 115.7, 115.6, 115.5, 105.5, 105.5, 71.2, 68.6, 51.6, 48.6, 41.1. ^{19}F NMR (565 MHz, Chloroform-*d*) δ -70.82, -123.52. HRMS(ESI): calcd for $C_{16}H_{18}F_4N_4O_2$, $[M + H]^+$ 375.1444; found, 375.1438. HPLC purity: 98.74%.

1-(3,4-difluorophenoxy)-3-((2-((4-(trifluoromethyl)pyrimidin-2-yl)amino)ethyl)amino)propan-2-ol (12). The titled compound was prepared from **A_3** (30 mg, 0.15 mmol) and **B_5** (27.1 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 42.2 mg, 73% yield, mp 109-111℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.46 (d, J = 4.9 Hz, 1H), 7.04 (q, J = 9.4 Hz, 1H), 6.81 (d, J = 4.9 Hz, 1H), 6.72 (m, 1H), 6.60 (dd, J = 8.9, 4.2 Hz, 1H), 6.18 (t, J = 5.8 Hz, 1H), 4.09 (m, 1H), 3.93 (d, J = 5.1 Hz, 2H), 3.60 (q, J = 5.8 Hz, 2H), 2.95-2.70 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 162.5, 160.4, 157.0, 156.6, 156.2, 154.9, 154.9, 154.9, 154.8, 151.7, 151.6, 149.2, 149.1, 146.4, 146.3, 144.0, 143.9, 124.6, 121.8, 119.1, 117.3, 117.3, 117.1, 117.1, 116.4, 109.8, 109.8, 109.8, 109.7, 105.6, 105.5, 105.5, 104.3, 104.1, 71.3, 68.4, 51.5, 48.6, 41.1. ^{19}F NMR (565 MHz, Chloroform-*d*) δ -70.84, -135.30 (d, J = 21.4 Hz), -147.81 (d, J = 21.5 Hz). HRMS(ESI): calcd for $C_{16}H_{17}F_5N_4O_2$, $[M + H]^+$ 393.135; found, 393.1343. HPLC purity: 98.98%.

1-(4-methoxyphenoxy)-3-((2-((4-(trifluoromethyl)pyrimidin-2-yl)amino)ethyl)amino)propan-2-ol (13) [49]. The titled compound was prepared from **A_3** (30 mg, 0.15 mmol) and **B_6** (26.2 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 40.1 mg, 71% yield, mp 116-117℃. ^{1}H NMR (400 MHz, Chloroform-*d*) *d* 8.52-8.41 (m, 1H), 6.82 (m, 5H), 6.14 (s, 1H), 4.09 (m, 1H), 3.94 (d, J = 5.2 Hz, 2H), 3.77 (s, 3H), 3.59 (q, J = 5.8 Hz, 2H), 2.95-2.87 (m, 3H), 2.81 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) *d* 162.5, 160.4, 156.6, 156.2, 154.1, 152.7, 121.9, 119.1, 115.5, 114.7, 105.5, 105.5, 71.3, 68.8, 55.7, 51.7, 48.6, 41.2. ^{19}F NMR (565 MHz, Chloroform-*d*) δ -70.83. HRMS(ESI): calcd for $C_{17}H_{21}F_3N_4O_3$, $[M + H]^+$ 387.1644; found, 387.1644. HPLC purity: 99.02%.

1-(4-(trifluoromethyl)phenoxy)-3-((2-((4-(trifluoromethyl)pyrimidin-2-yl)amino)ethyl)amino)propan-2-ol (14). The titled compound was prepared from **A_3** (30 mg, 0.15 mmol) and **B_7** (31.8 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 41.5 mg, 67% yield, mp 105-107℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.46 (s, 1H), 7.53 (d, *J* = 8.6 Hz, 2H), 6.96 (d, *J* = 8.6 Hz, 2H), 6.81 (d, *J* = 4.9 Hz, 1H), 6.20 (t, *J* = 5.7 Hz, 1H), 4.14 (m, 1H), 4.03 (d, *J* = 5.1 Hz, 2H), 3.61 (q, *J* = 5.7 Hz, 2H), 3.08-2.74 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 162.5, 161.0, 160.4, 156.6, 156.2, 127.0, 126.9, 126.9, 126.9, 125.7, 123.4, 123.1, 123.0, 121.8, 119.1, 114.5, 105.6, 105.5, 70.6, 68.4, 51.5, 48.7, 41.1. ^{19}F NMR (565 MHz, Chloroform-*d*) δ -61.54, -70.84. HRMS(ESI): calcd for $C_{17}H_{18}F_6N_4O_2$, $[M + H]^+$ 425.1412; found, 425.1406. HPLC purity: 96.47%.

1-(4-(trifluoromethyl)phenoxy)-3-((2-((5-(trifluoromethyl)pyridin-2-yl)amino)ethyl)amino)propan-2-ol (15). The titled compound was prepared from **A_5** (30 mg, 0.15 mmol) and **B_7** (31.9 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 42.2 mg, 69% yield, mp 118-120℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.36-8.27 (m, 1H), 7.55 (t, *J* = 8.7 Hz, 3H), 6.96 (d, *J* = 8.5 Hz, 2H), 6.41 (d, *J* = 8.8 Hz, 1H), 5.28 (s, 1H), 4.10 (m, 1H), 4.06-4.00 (m, 2H), 3.49 (q, *J* = 5.7 Hz, 2H), 2.97-2.89 (m, 3H), 2.82 (dd, *J* = 12.2, 7.7 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.0, 160.3, 146.1, 146.1, 146.0, 146.0, 134.4, 134.4, 134.3, 130.9, 127.0, 127.0, 126.9, 126.9, 125.9, 125.7, 123.5, 123.2, 123.0, 115.8, 115.4, 114.5, 106.6, 70.5, 68.5, 51.4, 48.6, 41.5. ^{19}F NMR (565 MHz, DMSO-d_6) δ -59.20, -59.77. HRMS(ESI): calcd for $C_{18}H_{19}F_6N_3O_2$, $[M + H]^+$ 424.146; found, 424.1454. HPLC purity: 99.4%.

1-((2-([1,1'-biphenyl]-4-ylamino)ethyl)amino)-3-(4-(trifluoromethyl)phenoxy)propan-2-

ol (16). The titled compound was prepared from **A_{11}** (30 mg, 0.14 mmol) and **B_7** (30.8 mg, 0.14 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 42.2 mg, 71% yield, mp 149-151°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.56 (d, J = 8.1 Hz, 4H), 7.47 (d, J = 8.2 Hz, 2H), 7.43 (d, J = 7.6 Hz, 2H), 7.29 (d, J = 8.2 Hz, 1H), 6.99 (d, J = 8.4 Hz, 2H), 6.73 (d, J = 8.3 Hz, 2H), 4.16-4.03 (m, 4H), 3.33 (q, J = 5.1 Hz, 2H), 3.01-2.91 (m, 3H), 2.85 (dd, J = 12.2, 7.5 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.0, 147.6, 141.1, 130.6, 128.7, 128.0, 127.0, 127.0, 127.0, 126.9, 126.3, 126.1, 125.7, 123.5, 123.1, 123.0, 114.5, 113.2, 70.6, 68.5, 51.5, 48.8, 43.7. ^{19}F NMR (565 MHz, DMSO-d_6) δ -59.72. HRMS(ESI): calcd for $C_{24}H_{25}F_3N_2O_2$, $[M + H]^+$ 431.1946; found, 431.1939. HPLC purity: 99.02%.

1-(4-(trifluoromethyl)phenoxy)-3-((2-((4-(trifluoromethyl)phenyl)amino)ethyl)amino)propan-2-ol (17). The titled compound was prepared from **A_{12}** (30 mg, 0.15 mmol) and **B_7** (32.1 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 42.2 mg, 69% yield, mp 108-109°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.54 (d, J = 8.5 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 6.61 (d, J = 8.3 Hz, 2H), 4.47 (t, J = 5.5 Hz, 1H), 4.12 (m, 1H), 4.06-3.98 (m, 2H), 3.28 (q, J = 5.5 Hz, 2H), 2.97-2.87 (m, 3H), 2.82 (dd, J = 12.2, 7.8 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.9, 150.7, 128.4, 127.0, 127.0, 127.0, 126.9, 126.7, 126.6, 126.6, 126.6, 126.3, 125.7, 123.6, 123.5, 123.2, 123.0, 119.1, 118.7, 114.5, 111.9, 70.5, 68.5, 51.5, 48.4, 43.0. ^{19}F NMR (565 MHz, DMSO-d_6) δ -58.81, -59.75. HRMS(ESI): calcd for $C_{19}H_{20}F_6N_2O_2$, $[M + H]^+$ 423.1507; found, 423.1503. HPLC purity: 97.3%.

1-(4-(trifluoromethyl)phenoxy)-3-((2-((3-(trifluoromethyl)phenyl)amino)ethyl)amino)propan-2-ol (18). The titled compound was

prepared from **A_{13}** (30 mg, 0.15 mmol) and **B_7** (32.1 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 44.3 mg, 71% yield, mp 89-91℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.57 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 4.4 Hz, 1H), 6.98 (t, *J* = 8.8 Hz, 3H), 6.88-6.74 (m, 2H), 4.29 (s, 1H), 4.17-4.02 (m, 3H), 3.30 (s, 2H), 3.06-2.81 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.0, 148.4, 131.8, 131.4, 131.1, 129.6, 128.4, 127.0, 127.0, 126.9, 126.9, 125.7, 123.5, 123.2, 123.0, 116.0, 114.5, 114.0, 113.9, 113.9, 113.9, 108.9, 108.9, 108.9, 70.5, 68.5, 51.4, 48.5, 43.3. ^{19}F NMR (565 MHz, DMSO-d_6) δ -59.74, -61.33. HRMS(ESI): calcd for $C_{19}H_{20}F_6N_2O_2$, $[M + H]^+$ 423.1507; found, 423.1520. HPLC purity: 98.83%.

1-(4-(trifluoromethyl)phenoxy)-3-((2-((2-(trifluoromethyl)phenyl)amino)ethyl)amino)propan-2-ol (19). The titled compound was prepared from **A_{10}** (30 mg, 0.15 mmol) and **B_7** (32.1 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 41.3 mg, 66% yield, mp 65-66℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.56 (d, *J* = 8.5 Hz, 2H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 6.99 (d, *J* = 8.5 Hz, 2H), 6.76 (d, *J* = 7.8 Hz, 2H), 4.93 (d, *J* = 5.2 Hz, 1H), 4.15-4.05 (m, 3H), 3.30 (q, *J* = 6.0 Hz, 2H), 3.07-2.93 (m, 3H), 2.85 (dd, *J* = 12.3, 6.6 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.0, 145.7, 133.1, 127.0, 126.9, 116.1, 114.5, 111.9, 70.5, 68.7, 51.2, 48.1, 42.9. ^{19}F NMR (565 MHz, DMSO-d_6) δ -59.75, -61.46. HRMS(ESI): calcd for $C_{19}H_{20}F_6N_2O_2$, $[M + H]^+$ 423.1507; found, 423.1503. HPLC purity: 98.13%.

1-((2-((2-nitrophenyl)amino)ethyl)amino)-3-(4-(trifluoromethyl)phenoxy)propan-2-ol (20). The titled compound was prepared from **A_{14}** (30 mg, 0.17 mmol) and **B_7** (36.1 mg, 0.17 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH

(200:1-100:1, with additional drops of aqueous ammonia). Yellow solid, 41.9 mg, 64% yield, mp 97-99℃. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.40 (d, J = 5.1 Hz, 1H), 8.19 (dd, J = 8.6, 1.5 Hz, 1H), 7.55 (d, J = 8.4 Hz, 2H), 7.45 (m, 1H), 7.01 (d, J = 8.5 Hz, 2H), 6.86 (d, J = 8.6 Hz, 1H), 6.67 (m, 1H), 4.12 (m, 3H), 3.44 (q, J = 5.9 Hz, 2H), 3.13-2.97 (m, 3H), 2.88 (dd, J = 12.2, 6.2 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.0, 145.4, 136.2, 132.1, 127.0, 127.0, 126.9, 126.9, 125.7, 123.4, 123.1, 123.0, 115.4, 114.5, 113.8, 70.4, 68.7, 51.1, 47.9, 42.4. ^{19}F NMR (565 MHz, DMSO-d_6) δ -59.74. HRMS(ESI): calcd for $C_{18}H_{20}F_3N_3O_4$, $[M + H]^+$ 400.1484; found, 400.1490. HPLC purity: 99.68%.

2-((2-((2-hydroxy-3-(4-(trifluoromethyl)phenoxy)propyl)amino)ethyl)amino)benzonitrile (21). The titled compound was prepared from **A$_{15}$** (30 mg, 0.19 mmol) and **B$_7$** (40.6 mg, 0.19 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 47.4 mg, 66% yield, mp 157-158℃. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.56 (d, J = 8.5 Hz, 2H), 7.40 (m, 2H), 7.02 (d, J = 8.5 Hz, 2H), 6.73-6.67 (m, 2H), 5.13 (d, J = 5.9 Hz, 1H), 4.12 (m, 3H), 3.33 (m, 2H), 3.06-2.95 (m, 3H), 2.86 (dd, J = 12.2, 6.5 Hz, 1H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 161.0, 150.4, 134.3, 132.8, 127.0, 127.0, 126.9, 126.9, 125.3, 123.5, 123.4, 123.2, 118.0, 116.7, 114.6, 110.8, 96.0, 70.5, 68.8, 51.2, 48.1, 42.7. HRMS(ESI): calcd for $C_{19}H_{20}F_3N_3O_2$, $[M + H]^+$ 380.1586; found, 380.1593. HPLC purity: 98.38%.

1-(3-(trifluoromethyl)phenoxy)-3-((2-((2-(trifluoromethyl)phenyl)amino)ethyl)amino)propan-2-ol (22). The titled compound was prepared from **A$_{10}$** (30 mg, 0.15 mmol) and **B$_8$** (32.1 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 42.4 mg, 69% yield, mp 119-121℃. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.49-7.44 (m, 1H), 7.40 (m, 2H), 7.27 (d, J = 20.1 Hz, 1H),

7.17 (t, *J* = 2.0 Hz, 1H), 7.10 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.78-6.73 (m, 2H), 4.93 (s, 1H), 4.15-4.05 (m, 3H), 3.31 (m, 2H), 3.06-2.94 (m, 3H), 2.85 (dd, *J* = 12.2, 6.9 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) *δ* 158.7, 145.7, 133.1, 132.1, 131.7, 130.0, 127.9, 126.8, 126.7, 126.6, 126.6, 125.2, 123.9, 122.5, 121.2, 117.9, 117.8, 117.8, 117.7, 116.0, 114.0, 113.7, 113.5, 111.9, 111.4, 111.4, 111.4, 111.3, 70.6, 68.7, 51.2, 48.1, 42.9. ^{19}F NMR (565 MHz, DMSO-d_6) δ -61.18, -61.52. HRMS(ESI): calcd for $C_{19}H_{20}F_6N_3O_2$, $[M + H]^+$ 423.1507; found, 423.1518. HPLC purity: 96.63%.

1-(2-(trifluoromethyl)phenoxy)-3-((2-((2-(trifluoromethyl)phenyl)amino)ethyl)amino)propan-2-ol (23). The titled compound was prepared from **A$_{10}$** (30 mg, 0.15 mmol) and **B$_9$** (32.1 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 40.6 mg, 65% yield, mp 78-80℃. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 7.59 (d, *J* = 7.7 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.08-7.00 (m, 2H), 6.74 (t, *J* = 8.3 Hz, 2H), 4.96 (s, 1H), 4.14 (d, *J* = 2.5 Hz, 3H), 3.29 (q, *J* = 5.3 Hz, 2H), 3.04-2.94 (m, 3H), 2.90 (dd, *J* = 10.9, 4.1 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) *δ* 156.4, 145.8, 133.4, 133.1, 129.3, 127.1, 127.1, 127.0, 127.0, 126.7, 126.6, 126.6, 126.5, 125.1, 123.9, 122.4, 120.5, 118.9, 118.6, 115.9, 113.7, 113.4, 113.1, 112.9, 111.9, 71.0, 68.6, 51.0, 48.1, 42.8. ^{19}F NMR (565 MHz, DMSO-d_6) δ -60.89, -61.55. HRMS(ESI): calcd for $C_{19}H_{20}F_6N_3O_2$, $[M + H]^+$ 423.1507; found, 423.1509. HPLC purity: 96.79%.

4-(2-hydroxy-3-((2-((2-(trifluoromethyl)phenyl)amino)ethyl)amino)propoxy)benzonitrile (24). The titled compound was prepared from **A$_{10}$** (30 mg, 0.15 mmol) and **B$_{10}$** (25.7 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 42.1 mg, 75% yield,

mp 128-130℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.63-7.57 (m, 2H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.00-6.95 (m, 2H), 6.79-6.72 (m, 2H), 4.91 (s, 1H), 4.15-4.06 (m, 3H), 3.34-3.25 (m, 2H), 3.07-2.93 (m, 3H), 2.84 (dd, *J* = 12.3, 6.3 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.8, 145.7, 145.7, 134.0, 133.2, 126.8, 126.7, 126.7, 126.6, 123.9, 119.1, 116.1, 115.2, 113.7, 113.4, 111.9, 104.4, 70.6, 68.5, 51.1, 48.1, 42.9. ^{19}F NMR (565 MHz, DMSO-d_6) δ -61.43. HRMS(ESI): calcd for $C_{19}H_{20}F_3N_3O_2$, $[M + H]^+$ 380.1586; found, 380.1590. HPLC purity: 96.25%.

1-((4-(trifluoromethyl)phenyl)amino)-3-((2-((5-(trifluoromethyl)pyridin-2-yl)amino)ethyl)amino)propan-2-ol (25). The titled compound was prepared from **A_5** (30 mg, 0.15 mmol) and **B_{11}** (31.8 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 36.2 mg, 59% yield, mp 130-132℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.32 (s, 1H), 7.59 (d, *J* = 8.8 Hz, 1H), 7.40 (d, *J* = 8.3 Hz, 2H), 6.62 (d, *J* = 8.3 Hz, 2H), 6.42 (d, *J* = 8.9 Hz, 1H), 5.40 (t, *J* = 5.7 Hz, 1H), 4.58 (s, 1H), 3.94 (m, 1H), 3.48 (q, *J* = 5.8 Hz, 2H), 3.30 (dd, *J* = 12.9, 3.7 Hz, 1H), 3.13 (dd, *J* = 12.8, 7.1 Hz, 1H), 2.99-2.39 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.3, 150.7, 146.0, 146.0, 145.9, 134.5, 134.4, 128.9, 126.7, 126.6, 126.6, 126.6, 126.2, 125.9, 123.6, 123.2, 119.3, 119.0, 118.6, 115.9, 115.5, 115.2, 112.1, 106.5, 68.3, 52.8, 52.8, 48.7, 47.3, 47.3, 41.6. ^{19}F NMR (565 MHz, Chloroform-*d*) δ -61.05, -61.18. HRMS(ESI): calcd for $C_{18}H_{21}F_6N_4O$, $[M + H]^+$ 423.1620; found, 423.1614. HPLC purity: 98.89%.

1-(benzyloxy)-3-((2-((5-(trifluoromethyl)pyridin-2-yl)amino)ethyl)amino)propan-2-ol (26). The titled compound was prepared from **A_5** (30 mg, 0.15 mmol) and **B_{13}** (24 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 39.9 mg, 73% yield,

mp 64-69°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 (d, J = 2.1 Hz, 1H), 7.57 (dd, J = 8.9, 2.4 Hz, 1H), 7.41-7.29 (m, 5H), 6.42 (d, J = 8.8 Hz, 1H), 5.42 (s, 1H), 4.57 (s, 2H), 3.94 (m, 1H), 3.57-3.44 (m, 4H), 2.91 (t, J = 5.8 Hz, 2H), 2.75 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.3, 146.0, 146.0, 145.9, 137.8, 134.3, 134.2, 134.2, 134.2, 128.5, 127.9, 127.8, 125.9, 123.3, 115.6, 115.3, 106.8, 73.5, 72.7, 69.0, 51.7, 48.5, 41.3. HRMS(ESI): calcd for $C_{18}H_{22}F_3N_3O_2$, $[M + H]^+$ 370.1742; found, 370.1734. HPLC purity: 95.09%.

1-((4-(trifluoromethyl)benzyl)oxy)-3-((2-((5-(trifluoromethyl)pyridin-2-yl)amino)ethyl)amino)propan-2-ol (27). The titled compound was prepared from **A_5** (30 mg, 0.15 mmol) and **B_{14}** (33.9 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 37.1 mg, 59% yield, mp 90-91°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.32 (d, J = 2.3 Hz, 1H), 7.65-7.53 (m, 3H), 7.44 (d, J = 8.0 Hz, 2H), 6.41 (d, J = 8.8 Hz, 1H), 5.50 (t, J = 5.5 Hz, 1H), 4.61 (s, 2H), 3.95 (m, 1H), 3.58-3.49 (m, 2H), 3.45 (q, J = 5.7 Hz, 2H), 2.90 (t, J = 5.8 Hz, 2H), 2.80-2.69 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.3, 146.0, 145.9, 142.0, 134.3, 134.3, 130.1, 129.8, 127.6, 125.9, 125.4, 125.4, 125.4, 125.3, 123.2, 122.7, 115.6, 115.2, 106.6, 73.2, 72.6, 69.1, 51.7, 48.5, 41.4. ^{19}F NMR (565 MHz, Chloroform-*d*) δ -61.16, -62.53. HRMS(ESI): calcd for $C_{19}H_{21}F_6N_3O_2$, $[M + H]^+$ 438.1616; found, 438.1612. HPLC purity: 97.4%.

1-((3-(trifluoromethyl)benzyl)oxy)-3-((2-((5-(trifluoromethyl)pyridin-2-yl)amino)ethyl)amino)propan-2-ol (28). The titled compound was prepared from **A_5** (30 mg, 0.15 mmol) and **B_{15}** (33.9 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 33.8 mg, 54% yield, mp 66-67°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.33 (d, J = 2.3 Hz, 1H), 7.61-7.46 (m, 5H), 6.42 (d, J = 8.8 Hz, 1H), 5.36 (s, 1H), 4.62 (s, 2H), 3.95

(m, 1H), 3.59-3.52 (m, 2H), 3.47 (q, *J* = 5.7 Hz, 2H), 2.92 (t, *J* = 5.8 Hz, 2H), 2.83-2.69 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.3, 139.0, 130.8, 128.9, 124.6, 124.2, 115.3, 73.1, 72.7, 69.1, 51.7, 48.5, 41.4. HRMS(ESI): calcd for $C_{19}H_{21}F_6N_3O_2$, $[M + H]^+$ 438.1616; found, 438.1629. HPLC purity: 95.18%

1-((2-(trifluoromethyl)benzyl)oxy)-3-((2-((5-(trifluoromethyl)pyridin-2-yl)amino)ethyl)amino)propan-2-ol (29). The titled compound was prepared from **A$_5$** (30 mg, 0.15 mmol) and **B$_{16}$** (33.9 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Pale yellow solid, 34.2 mg, 54% yield, mp 71-73℃. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.33-8.30 (m, 1H), 7.66 (t, *J* = 7.6 Hz, 2H), 7.58-7.53 (m, 2H), 7.40 (t, *J* = 7.7 Hz, 1H), 6.42 (d, *J* = 8.8 Hz, 1H), 5.49 (s, 1H), 4.74 (s, 2H), 3.97 (m, 1H), 3.59 (dd, *J* = 9.6, 3.9 Hz, 1H), 3.55 (dd, *J* = 9.6, 6.3 Hz, 1H), 3.48-3.44 (m, 2H), 2.91 (t, *J* = 5.8 Hz, 2H), 2.79 (dd, *J* = 12.2, 3.7 Hz, 1H), 2.74 (dd, *J* = 12.2, 8.0 Hz, 1H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 160.3, 146.0, 146.0, 146.0, 145.9, 136.5, 134.3, 134.2, 134.2, 134.2, 132.0, 129.1, 128.0, 127.8, 127.6, 127.4, 127.3, 127.0, 125.9, 125.9, 125.9, 125.8, 125.5, 125.2, 123.7, 123.4, 121.9, 121.6, 115.7, 115.5, 115.2, 115.0, 73.4, 69.5, 69.1, 51.7, 48.5, 41.4. ^{19}F NMR (565 MHz, Chloroform-d) δ -60.07, -62.64. HRMS(ESI): calcd for $C_{19}H_{21}F_6N_3O_2$, $[M + H]^+$ 438.1616; found, 438.1628. HPLC purity: 95.9%

1-((4-(trifluoromethyl)benzyl)thio)-3-((2-((5-(trifluoromethyl)pyridin-2-yl)amino)ethyl)amino)propan-2-ol (30). The titled compound was prepared from **A$_5$** (30 mg, 0.15 mmol) and **B$_{17}$** (36.3 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Yellow solid, 43.7 mg, 66% yield, mp 64-66℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.38-8.28 (m, 1H), 7.58 (dd, *J* = 8.6, 3.2 Hz, 3H), 7.45 (d, *J* = 8.0 Hz, 2H), 6.43 (d, *J* = 8.8 Hz, 1H),

5.38 (s, 1H), 3.81 (s, 3H), 3.46 (q, *J* = 5.7 Hz, 2H), 2.89 (m, 2H), 2.78 (dd, *J* = 12.1, 3.3 Hz, 1H), 2.65-2.51 (m, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) *δ* 160.3, 146.1, 146.0, 146.0, 146.0, 142.3, 134.4, 134.3, 134.3, 134.3, 129.6, 129.3, 129.2, 125.9, 125.6, 125.5, 125.5, 125.5, 125.4, 123.2, 122.7, 115.7, 115.3, 106.6, 68.7, 53.9, 48.5, 41.5, 36.3, 36.2. ^{19}F NMR (565 MHz, Chloroform-d) δ -61.16, -62.48. HRMS(ESI): calcd for $C_{19}H_{21}F_6N_3OS$, $[M + H]^+$ 454.1388; found, 454.1385. HPLC purity: 97.15%.

1-((2-((5-nitropyridin-2-yl)amino)ethyl)amino)-3-((4-(trifluoromethyl)benzyl)oxy)propan-2-ol (31). The titled compound was prepared from **A$_9$** (30 mg, 0.16 mmol) and **B$_{14}$** (38.2 mg, 0.16 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). Yellow solid, 43.1 mg, 70% yield, mp 80-81℃. ^{1}H NMR (600 MHz, Chloroform-*d*) *δ* 9.02 (d, *J* = 2.7 Hz, 1H), 8.18 (d, *J* = 9.1 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 7.9 Hz, 2H), 6.38 (d, *J* = 9.2 Hz, 1H), 5.95 (s, 1H), 4.63 (s, 2H), 3.96 (m, 1H), 3.59-3.50 (m, 4H), 2.95 (t, *J* = 5.8 Hz, 2H), 2.81 (dd, *J* = 12.2, 3.7 Hz, 1H), 2.74 (dd, *J* = 12.2, 7.9 Hz, 1H). ^{13}C NMR (151 MHz, Chloroform-*d*) *δ* 161.1, 147.0, 141.9, 135.8, 130.2, 129.9, 127.6, 125.5, 125.4, 125.4, 125.4, 125.0, 123.2, 121.4, 73.1, 72.7, 69.2, 51.6, 48.2, 41.5. ^{19}F NMR (565 MHz, Chloroform-*d*) δ -62.52. HRMS(ESI): calcd for $C_{18}H_{21}F_3N_4O_4$, $[M + H]^+$ 415.1593; found, 415.1601. HPLC purity: 98.35%.

1-((4-(trifluoromethyl)benzyl)oxy)-3-((2-((2-(trifluoromethyl)phenyl)amino)ethyl)amino)propan-2-ol (32). The titled compound was prepared from **A$_{10}$** (30 mg, 0.15 mmol) and **B$_{14}$** (34.1 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 39.9 mg, 62% yield, mp 67-69℃. ^{1}H NMR (600 MHz, Chloroform-*d*) *δ* 7.61 (d, *J* = 8.0 Hz, 2H), 7.46 (d, *J* = 7.9 Hz, 3H), 7.41-7.36 (m,

1H), 6.75 (d, *J* = 7.9 Hz, 2H), 4.94 (d, *J* = 5.4 Hz, 1H), 4.62 (s, 2H), 3.94 (m, 1H), 3.56 (m, 2H), 3.27 (q, *J* = 5.6 Hz, 2H), 3.00-2.92 (m, 2H), 2.81 (m, 1H), 2.73 (m, 1H). ^{13}C NMR (151 MHz, Chloroform-*d*) *δ* 145.8, 142.1, 133.1, 120.0, 129.8, 127.6, 126.7, 126.7, 126.6, 126.6, 126.1, 125.4, 125.4, 125.4, 125.3, 125.0, 124.3, 123.2, 115.9, 113.6, 113.4, 111.9, 73.1, 72.6, 69.3, 51.4, 48.0, 42.9. ^{19}F NMR (565 MHz, Chloroform-*d*) δ -62.51, -62.58. HRMS(ESI): calcd for $C_{20}H_{22}F_6N_2O_2$, $[M + H]^+$ 437.1664; found, 437.1669. HPLC purity: 95.05%.

1-((3-(trifluoromethyl)benzyl)oxy)-3-((2-((2-(trifluoromethyl)phenyl)amino)ethyl)amino)propan-2-ol (33). The titled compound was prepared from **A$_{10}$** (30 mg, 0.15 mmol) and **B$_{15}$** (34.1 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 38.1 mg, 59% yield, mp 77-79°C. ^{1}H NMR (600 MHz, Chloroform-*d*) *δ* 7.62 (s, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.49-7.44 (m, 2H), 7.40-7.36 (m, 1H), 6.74 (t, *J* = 8.1 Hz, 2H), 4.94 (t, *J* = 5.1 Hz, 1H), 4.61 (s, 2H), 3.94 (m, 1H), 3.57 (m, 2H), 3.26 (q, *J* = 5.7 Hz, 2H), 2.96 (m, 2H), 2.81 (dd, *J* = 12.1, 4.1 Hz, 1H), 2.72 (dd, *J* = 12.1, 7.3 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) *δ* 145.8, 145.8, 145.8, 139.1, 133.1, 130.8, 130.6, 128.9, 128.8, 126.7, 126.6, 126.6, 126.5, 125.5, 124.6, 124.5, 124.5, 124.5, 124.4, 124.3, 124.3, 124.2, 124.2, 123.9, 122.8, 115.9, 113.9, 113.7, 113.6, 113.4, 113.3, 113.1, 111.9, 111.8, 73.1, 72.7, 69.2, 51.4, 48.0, 42.8. ^{19}F NMR (565 MHz, Chloroform-d) δ -62.61, -62.62. HRMS(ESI): calcd for $C_{20}H_{22}F_6N_2O_2$, $[M + H]^+$ 437.1664; found, 437.1664. HPLC purity: 98.51%.

1-((2-(trifluoromethyl)benzyl)oxy)-3-((2-((2-(trifluoromethyl)phenyl)amino)ethyl)amino)propan-2-ol (34). The titled compound was prepared from **A$_{10}$** (30 mg, 0.15 mmol) and **B$_{16}$** (34.1 mg, 0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with

additional drops of aqueous ammonia). White solid, 42.9 mg, 68% yield, mp 64-65°C. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.67 (d, J = 7.9 Hz, 2H), 7.56 (t, J = 7.6 Hz, 1H), 7.42 (m, 3H), 6.74 (t, J = 8.1 Hz, 2H), 4.94 (d, J = 5.3 Hz, 1H), 4.76 (s, 2H), 3.96 (m, 1H), 3.60 (m, 2H), 3.27 (q, J = 5.6 Hz, 2H), 2.97 (m, 2H), 2.86-2.71 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 145.8, 145.8, 145.8, 136.7, 136.7, 133.1, 132.0, 129.3, 129.1, 128.4, 128.2, 127.9, 127.6, 127.3, 126.7, 126.7, 126.6, 126.6, 126.5, 125.9, 125.9, 125.8, 125.8, 125.7, 123.9, 123.0, 120.2, 115.9, 114.0, 113.7, 113.4, 113.1, 111.9, 73.3, 69.5, 69.2, 51.5, 48.0, 42.9. ^{19}F NMR (565 MHz, Chloroform-d) δ -60.07, -62.64. HRMS(ESI): calcd for $C_{20}H_{22}F_6N_2O_2$, $[M + H]^+$ 437.1664; found, 437.1663. HPLC purity: 99.4%.

***N*-(2-((2-hydroxy-3-(4-(trifluoromethyl)phenoxy)propyl)amino)ethyl)-2-(trifluoromethyl)benzenesulfonamide (35)**. The titled compound was prepared from **A_{16}** (30 mg, 0.11 mmol) and **B_7** (24.4 mg, 0.11 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (100:1-50:1, with additional drops of aqueous ammonia). White solid, 37.7 mg, 69% yield, mp 95-97°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.11 (dd, J = 8.1, 3.0 Hz, 1H), 7.98 (dd, J = 8.1, 2.6 Hz, 1H), 7.89 (t, J = 7.7 Hz, 1H), 7.83 (t, J = 7.7 Hz, 1H), 7.63 (dd, J = 9.0, 2.8 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 5.06 (s, 1H), 4.02 (m, 1H), 3.93 (m, 1H), 3.84 (m, 1H), 2.99-2.93 (m, 2H), 2.61 (m, 3H), 2.54 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.0, 140.0, 133.7, 133.7, 133.2, 133.2, 130.4, 128.9, 128.9, 128.8, 128.8, 127.4, 127.3, 127.3, 127.3, 126.7, 126.5, 126.5, 126.1, 125.9, 124.3, 124.1, 122.5, 121.8, 121.5, 121.3, 121.3, 115.4, 71.4, 68.5, 52.3, 49.2, 43.3. ^{19}F NMR (565 MHz, Chloroform-d) δ -57.95, -61.54. HRMS(ESI): calcd for $C_{19}H_{20}F_6N_2O_4S$, $[M + H]^+$ 487.1126; found, 487.1132. HPLC purity: 99.23%.

1-(4-(trifluoromethyl)phenoxy)-3-((2-(2-(trifluoromethyl)phenoxy)ethyl)amino)propan-2-ol (36). The titled compound was prepared from **A_{17}** (30 mg, 0.15 mmol) and **B_7** (31.9 mg,

0.15 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 37.4 mg, 60% yield, mp 83-85℃. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.62 (dd, J = 16.9, 8.2 Hz, 4H), 7.27 (d, J = 8.4 Hz, 1H), 7.10 (t, J = 8.2 Hz, 3H), 5.09 (d, J = 5.0 Hz, 1H), 4.16 (t, J = 5.6 Hz, 2H), 4.04 (dd, J = 9.8, 4.5 Hz, 1H), 3.96 (dd, J = 9.8, 6.1 Hz, 1H), 3.90 (t, J = 5.9 Hz, 1H), 2.94 (t, J = 5.6 Hz, 2H), 2.76 (dd, J = 12.0, 4.8 Hz, 1H), 2.68 (dd, J = 11.9, 6.7 Hz, 1H). ^{113}C NMR (151 MHz, DMSO-d_6) δ 162.0, 156.9, 134.7, 130.1, 127.4, 127.4, 127.3, 127.3, 127.1, 127.1, 127.1, 126.0, 125.2, 124.2, 123.4, 121.5, 121.3, 120.7, 117.8, 117.6, 117.4, 115.4, 114.1, 71.4, 69.0, 68.5, 52.5, 48.6. ^{19}F NMR (565 MHz, Chloroform-d) δ -61.51, -62.26. HRMS(ESI): calcd for $C_{19}H_{19}F_6NO_3$, $[M + H]^+$ 424.1347; found, 424.1355. HPLC purity: 95.69%.

1-(4-(trifluoromethyl)phenoxy)-3-((2-((2-(trifluoromethyl)phenyl)thio)ethyl)amino)propan-2-ol (37). The titled compound was prepared from **A$_{18}$** (30 mg, 0.14 mmol) and **B$_7$** (29.6 mg, 0.14 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 34.5 mg, 58% yield, mp 77-78℃. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.68 (d, J = 7.9 Hz, 1H), 7.59-7.46 (m, 4H), 7.33 (t, J = 7.6 Hz, 1H), 6.99 (d, J = 8.4 Hz, 2H), 4.08-4.01 (m, 3H), 3.17 (m, 2H), 2.99-2.87 (m, 3H), 2.83-2.76 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-d) δ 161.1, 135.4, 132.0, 131.6, 130.6, 130.3, 127.0, 127.0, 127.0, 126.9, 126.9, 126.9, 126.3, 125.7, 125.1, 123.4, 123.1, 123.0, 122.4, 120.3, 114.5, 70.5, 68.2, 51.1, 47.9, 35.2. HRMS(ESI): calcd for $C_{19}H_{19}F_6NO_2S$, $[M + H]^+$ 440.1119; found, 440.1120. HPLC purity: 98.46%.

1-((2-((2-(trifluoromethyl)phenyl)amino)ethyl)amino)-3-((4-(trifluoromethyl)phenyl)thio)propan-2-ol (38). The titled compound was prepared from **A$_{10}$** (30 mg, 0.15 mmol) and **B$_{12}$** (34.4 mg, 0.15 mmol), purified by column chromatography on

silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 34.3 mg, 71% yield, mp 65-66℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.53 (d, J = 8.2 Hz, 2H), 7.42 (m, 4H), 6.75 (dd, J = 8.1, 5.4 Hz, 2H), 4.87 (s, 1H), 3.86 (m, 1H), 3.34-3.22 (m, 2H), 3.20-3.09 (m, 2H), 3.03-2.88 (m, 3H), 2.72 (dd, J = 12.2, 7.5 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 145.7, 145.7, 141.3, 133.1, 128.4, 128.1, 128.0, 127.7, 126.8, 126.7, 126.7, 126.6, 126.6, 125.8, 125.8, 125.7, 125.7, 125.4, 123.9, 122.7, 116.1, 113.7, 113.4, 111.9, 68.5, 53.4, 48.0, 42.9, 37.7. ^{19}F NMR (565 MHz, Chloroform-d) δ -62.48, -62.55. HRMS(ESI): calcd for $C_{19}H_{20}F_6N_2OS$, $[M + H]^+$ 439.1279; found, 439.1279. HPLC purity: 96.13%.

1-((4-(trifluoromethyl)phenyl)thio)-3-((2-((2-(trifluoromethyl)phenyl)thio)ethyl)amino)propan-2-ol (39). The titled compound was prepared from **A_{18}** (30 mg, 0.14 mmol) and **B_{12}** (31.8 mg, 0.14 mmol), purified by column chromatography on silica gel with the elution fluid of DCM/MeOH (200:1-100:1, with additional drops of aqueous ammonia). White solid, 46.8 mg, 66% yield, mp 73-75℃. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.70 -7.67 (m, 1H), 7.54 (t, J = 8.6 Hz, 3H), 7.49 (t, J = 7.7 Hz, 1H), 7.43 (d, J = 8.2 Hz, 2H), 7.33 (t, J = 7.6 Hz, 1H), 3.82 (m, 1H), 3.18-3.10 (m, 4H), 2.93-2.84 (m, 3H), 2.65 (dd, J = 12.2, 8.1 Hz, 1H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 141.5, 135.4, 132.0, 131.6, 130.6, 130.4, 127.9, 127.9, 127.7, 127.5, 127.0, 127.0, 126.9, 126.9, 126.3, 125.8, 125.8, 125.7, 125.7, 125.0, 124.6, 123.2, 122.8, 68.2, 53.3, 47.8, 37.5, 35.2. ^{19}F NMR (565 MHz, Chloroform-d) δ -60.78, -62.46. HRMS(ESI): calcd for $C_{19}H_{19}F_6NOS_2$, $[M + H]^+$ 456.0890; found, 456.0893. HPLC purity: 95.63%.

Pharmacophore design and virtual screening

Pharmacophore design and virtual screening were performed as described previously.[36,37] Using the published crystal structure of *E. coli* NusG in complex with RNAP (PDB: 5TBZ),[13]

NusG residues identified as being involved in intermolecular bonding with RNAP CH were chosen and then marked as a pharmacophore feature using the "add query feature" in Discovery Studio 2016. The pharmacophore model was used for *in silico* screening of the mini-Maybridge compound library.

Determination of MIC

The antimicrobial activity of the compounds was determined by broth microdilution according to CLSI guidelines. [22] The test medium was cation-adjusted Mueller-Hinton broth (CA-MHB), if not specified elsewhere, or brain heart infusion (BHI) broth for *Streptococci*. Serial two-fold dilutions of the tested compound were performed for the tested chemicals, starting from 256 μg/mL to 0.25 μg/mL, and the bacterial cell inoculum was adjusted to approximately 5×10^5 CFU per mL. Results were taken after 20 hours of incubation at 37 °C. The MIC was defined as the lowest concentration of antibiotic with no visible growth. Experiments were performed in duplicates.

Plasmid Construction, Protein Overproduction and Purification

All cloning experiments were performed using the *E. coli* strain DH5α. The plasmids and primers used are listed in **Tables S5** and **S6**, respectively.

The *nusG* gene was amplified from *B. subtilis* 168 colonies using the primers LgBiTNusGF and LgBiTNusGR (**Table S6**). The PCR amplicons were digested with *Acc*65I and *Eco*RI, and subsequently ligated into the pCU202 plasmid (**Table S5**), resulting in the plasmid pCU287.

The overexpression plasmids pCU253 or pCU287 were transformed into *E. coli* BL21 (DE3). The transformants were cultured in auto-induction medium (Formedium) at room temperature for 48 hours before harvesting. The cell pellets were resuspended in lysis buffer (20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 8.0) mixed with B-PER Complete

Bacterial Protein Extraction Reagent (ThermoFisher) at a 1:1 volume ratio. After sonication, the lysate was clarified by centrifugation at 8,000 × *g* for 1 hour at 4 °C. The supernatant was loaded onto a 1 mL HisTrap HP column (Cytiva) pre-equilibrated with 10 mL of lysis buffer. Non-specific binding was removed by washing with 10 mL of wash buffer (20 mM sodium phosphate, 500 mM NaCl, 40 mM imidazole, pH 8.0). The his-tagged proteins were eluted with elution buffer (20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 8.0). Fractions containing the target proteins were buffer exchanged into PBS with 30% glycerol for storage at -80 °C.

Binding Inhibition Assay

Previously established protocols were used for inhibitor testing.[29] Briefly, C-SmBiT-CH (pCU253; 250 nM in PBS) was added to 96-well plates and then mixed with 20 μL of compound or peptide at desired concentrations. The mixture was incubated for 10 minutes at 37 °C. N-LgBiT-NusG (pCU287; 250 nM in PBS) was then added to each well, followed by incubation for 10 minutes at 37 °C. After the final incubation step, an equal volume of Promega Nano-Glo® Luciferase Assay Substrate (Promega) was added to the reaction mixture. The emitted luminescence was measured using a Synergy H1 plate reader (Agilent). The experiment was performed in triplicates with technical repeats for consistent results.

Time-Kill Kinetics

The dose- and time-dependent antimicrobial effects of compounds on *S. aureus* strains under aerobic conditions were assessed by adapting from relevant CLSI guidelines.[26] *S. aureus* cells were suspended to approximately 1.5 × 10^6 CFU/mL (colony forming unit per mL) at log growth phase in CA-MHB broth supplemented with varying concentrations of compounds (i.e., ¼×, 1×, 4×, and 16× MICs). As an untreated control, bacteria were incubated in CA-MHB

broth without the compounds. The cultures were grown at 37 °C with shaking at 180 rpm. At defined time intervals (i.e., 0, 2, 4, 6 h), 20 μL samples were taken from each treatment group, followed by a 10-fold serial dilution in sterile phosphate-buffered saline (PBS). From each dilution, 5 μL samples were spotted on Columbia blood agar plates. The plates were then incubated at 37 °C overnight, after which the number of viable bacteria in each sample was counted and expressed as CFU/mL. The entire experiment was performed in triplicate.

Cell-Based Transcription Assay

E. coli K12 was transformed with the reporter plasmids pSL102 or pSL103. The transformants were cultured in LB (Lennox) broth supplemented with 100 μg/mL ampicillin until OD_{600} = 0.6. Compound **38** was then added at ⅛ × or ¼ × MIC and the cultures were incubated at 37 °C for 30 minutes. The harvested cell pellets were stored at -80 °C until further use.

The *E. coli* cells were lysed in 1× TE (Tris-EDTA) buffer containing 1.5 mg/mL lysozyme, and the cellular total RNA was extracted using the RNeasy Mini Kit (Qiagen). The purified RNA was used as templates for complementary DNA (cDNA) synthesis using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), followed by cDNA quantification with the Qubit ssDNA Assay Kit (Invitrogen) and Qubit 4 Fluorometer (ThermoFisher). The primers for detecting the reporter gene *cat* and the housekeeping gene *gyrB* are listed in **Table S6**.

The primers and cDNA templates were diluted as recommended by the manufacturer's protocol of the 2× PowerUp™ SYBR™ Green Master Mix (Applied Biosystems). For each 10 μL reaction loaded in a 0.1 mL-sized MicroAmp® Fast 96-Well Reaction Plate (Applied Biosystems), 5 μL of the 2× master mix, 1 μL of both forward and reverse primers, and 3 μL of cDNA template were mixed. For the no-template control (NTC) reactions, the cDNA template was replaced by an equal volume of nuclease-free water. The loaded plate was sealed

with MicroAmp™ Optical Adhesive Film (Applied Biosystems) and placed onto a StepOnePlus™ Real-Time PCR System (Applied Biosystems), where 40-cycle reactions were performed. The experiments were carried out in triplicates, and the data were analyzed using the ΔCT method. The statistical significance was determined by GraphPad Prism using the unpaired *t*-test and presented as $p \leq 0.05$ (∗) and ≤0.01 (∗∗).

Epifluorescence Microscopy

Various *B. subtilis* strains were cultivated overnight on selective LB (Lennox) agar plate at 37 °C. Single colony was picked and allowed to grow in LB medium with selective antibiotic and xylose at 37 °C with shaking at 180 rpm overnight. The culture was then diluted to OD_{600} = 0.05 and allowed to grow until OD_{600} = 0.50 at 37 °C with agitation. Control antibiotics and compound at 1× MIC were then added to the culture, followed by further incubation at 37 °C with shaking for 30 minutes. Afterwards, 2.5 μL of cell culture was placed onto 1.2% freshly prepared agarose plate and covered with a coverslip prior to imaging. Fluorescence images were captured using a Nikon Eclipse Ti2-E Live-cell Fluorescence Imaging System equipped with a 100×/1.45 oil objective, and the GFP (green fluorescent protein) signal was visualized using a FITC (fluorescein isothiocyanate) (525/50) filter.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org.

Additional figures and tables, ^{1}H NMR, ^{13}C NMR, and HPLC traces of compounds **1**−**39** (PDF)

Molecular formula strings (CSV)

3D model of **AW00783** docked at RNAP β'CH (PDB)

Corresponding Authors

Cong Ma; Email: cong.ma@polyu.edu.hk

Xiao Yang; Email: xiaoyang@cuhk.edu.hk

Author Contributions

# Y.Z. and C.H.K. contributed equally.

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Abbreviations Used

BHI, brain heart infusion; CA-MHB, cation-adjusted Mueller-Hinton broth; CA-MRSA, community-associated methicillin-resistant *Staphylococcus aureus*; CFU, colony forming unit; CH, clamp-helix; CLSI, Clinical & Laboratory Standards Institute; DIPEA, *N,N*-diisopropylethylamine; DSIF, DRB-sensitivity-inducing factor; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; HPLC, high-performance liquid chromatography; HRMS, high resolution mass spectrometry; IC_{50}, 50% inhibition concentration; KOW, Kyrpides-Ouzounis-Woese; LC-EI, liquid chromatography-electrospray ionization; MIC, minimum inhibitory concentration; NTC, no-template control; NTD, N-terminal domain; PBS, phosphate-buffered saline; PCA, protein complement assay; PDA, photodiode array; Pol II, eukaryotic RNA polymerase II; PPI, protein-protein interaction; RNAP, bacterial RNA polymerase; SAR, structure-activity relationship; TE: tris-EDTA; TLC, thin-layer chromatography

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