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Discovery of Antibacterials That Inhibit Bacterial RNA Polymerase Interactions with Sigma Factors

Jiqing Ye, Adrian Jun Chu, Rachel Harper, Shu Ting Chan, Tsun Lam Shek, Yufeng Zhang, Margaret Ip, Mariya Sambir, Irina Artsimovitch, Zhong Zuo,* Xiao Yang,* and Cong Ma*

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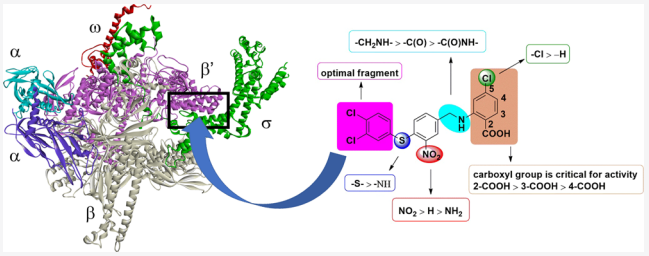
ABSTRACT: Formation of a bacterial RNA polymerase (RNAP) holoenzyme by a catalytic core RNAP and a sigma (σ) initiation factor is essential for bacterial viability. As the primary binding site for the housekeeping σ factors, the RNAP clamp helix domain represents an attractive target for novel antimicrobial agent discovery. Previously, we designed a pharmacophore model based on the essential amino acids of the clamp helix, such as R278, R281, and I291 (*Escherichia coli* numbering), and identified hit compounds with antimicrobial activity that interfered with the core−σ interactions. In this work, we rationally designed and synthesized a class of triaryl derivatives of one hit compound and succeeded in drastically improving the antimicrobial activity against *Streptococcus pneumoniae*, with the minimum inhibitory concentration reduced from 256 to 1 μg/mL. Additional characterization of antimicrobial activity, inhibition of transcription, in vitro pharmacological properties, and cytotoxicity of the optimized compounds demonstrated their potential for further development.



INTRODUCTION

Infectious diseases caused by bacteria have re-emerged to become a crisis for human health and the global economy.[1] Bacterial strains resistant to all current antibiotics have been reported.[2] Therefore, there is an urgent need for structurally novel antimicrobial agents with targets and mechanisms distinct from those of the existing drugs. Structure-based drug design and synthesis have been adapted to identify novel antimicrobial agents by exploring novel biological targets,[3] and the synthetic antimicrobial agents have several advantages over antibiotics developed from natural products: structural simplicity, amenability to efficient synthetic modifications, and a lack of innate resistance mechanisms.[4]

Protein−protein interactions (PPIs) have been increasingly recognized as valuable targets for drug design.[5] Many bacterial transcription factors directly bind to RNA polymerase (RNAP) and regulate all steps in RNA synthesis.[6] Among these, initiation sigma factors are essential for transcription. They associate with the core RNAP (Figure 1A) to form a holoenzyme (Figure 1B),[7,8] the only RNAP state that is able to specifically recognize a promoter and initiate transcription.[9] Among several domains of RNAP that form an extensive interface with σ,[8,10,11] the β' subunit clamp helix (CH) domain is thought to play the crucial role.[12] The primary interaction occurs between the β'CH and the region 2.2 of σ ($\sigma_{2.2}$) (Figure 1C).[13] The housekeeping σ factor (σ^{70} in Gram-negative *Escherichia coli*; σ^{A} in Gram-positive *Bacillus subtilis*) is highly conserved and essential for bacterial viability.[14,15] Thus, the holoenzyme formation has been considered as a potential target for novel antimicrobial discovery. André et al. used an affinity assay for high-throughput screening to search for hit compounds capable of inhibiting interactions between *E. coli* RNAP and σ^{70} and identified the SB series of compounds as a result.[16] By aligning the SB series to other compounds with unknown or diverse binding sites on RNAP, Hinsberger et al. designed a pharmacophore model and identified anthranilic acid derivatives that inhibited *E. coli* holoenzyme formation.[17] However, the exact binding sites of SB series and anthranilic acid derivatives are still unknown.

In order to identify RNAP-σ inhibitors, we developed a pharmacophore model based on a homology model of *B. subtilis* RNAP-σ^{A}.[19] We first used a house-made bis-indole compound library and identified hit compounds by native gel electrophoresis. The inhibitory mechanism was assessed by isothermal calorimetry titration and in vitro and cell-based transcription assays;[20] however, the compounds' inadequate antimicrobial activity and solubility issues limited further development of these indole derivatives. In pursuit of an alternative strategy, we analyzed the major binding interface of

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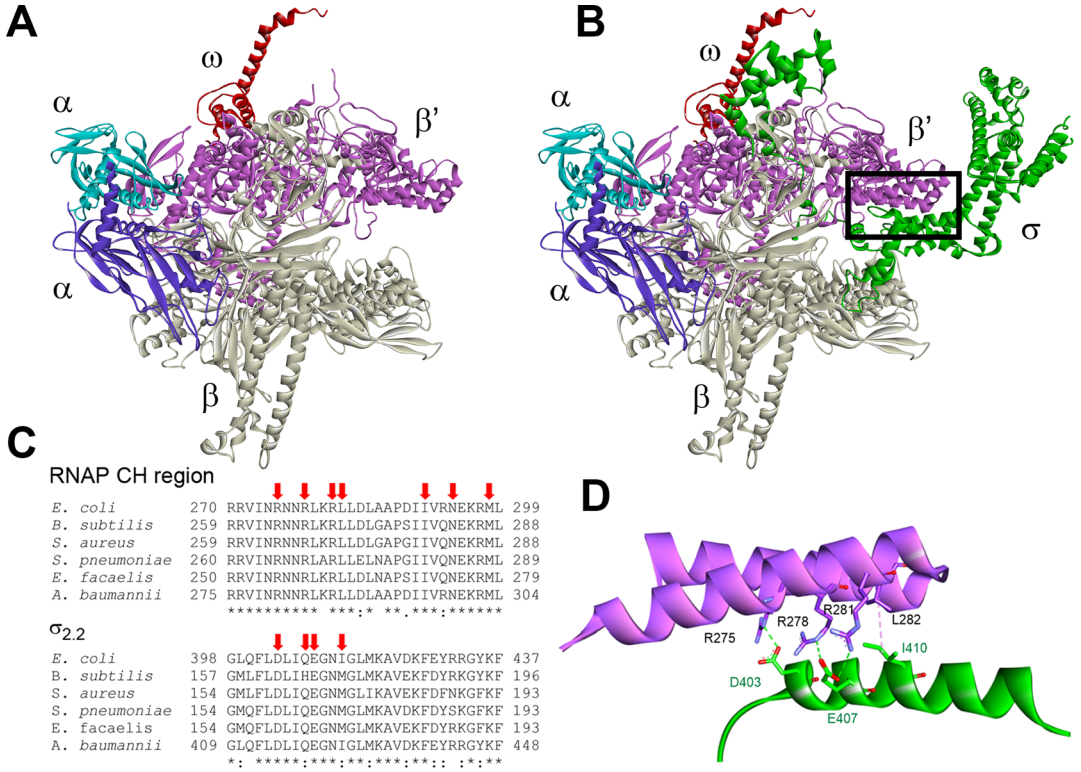

Figure 1. (A) *E. coli* RNAP core enzyme (subunits $\alpha\alpha\beta\beta'\omega$) crystal structure (PDB 4LJZ with σ^{70} not shown);[18] (B) *E. coli* RNAP σ^{70} holoenzyme crystal structure (PDB 4LJZ); (C) amino acid sequence alignments of the β'CH region and $\sigma_{2.2}$ of representative bacteria (red arrows indicate amino acids important for binding);[21] (D) key contacts between the *E. coli* β'CH and $\sigma_{2.2}$.

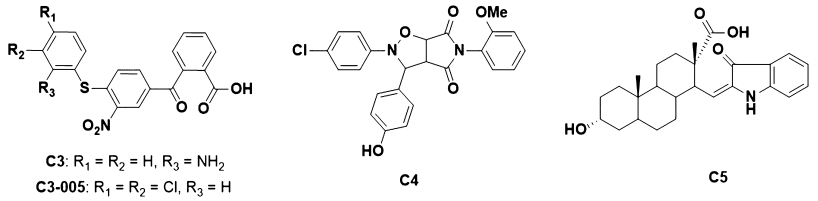

Figure 2. Structures of hit compounds **C3**, **C4**, **C5**, and **C3-005**.

B. subtilis RNAP-σ^A holoenzyme, focusing on critical amino acid residues identified by functional assays. The β' subunits with single alanine substitutions of these residues have been tested. The results showed that R278A or R281A proteins completely lost the affinity to σ^A, implicating spatially proximate R278 and R281 as critical determinants on the σ/β' interface;[19] other important β'CH residues have also been identified in this analysis (Figure 1D).

According to the chemical and spatial properties of these residues, a new pharmacophore model was developed and employed for *in silico* screening of Maybridge Mini HitFinder, a drug-like compound library composed by 2000 diverse compounds.[21] Seven compounds have been selected from top 25 hits by comparing docking models and energy-minimized conformations. Subsequent functional studies revealed that some of these seven compounds (**C3**, **C4**, and **C5**) inhibited RNAP holoenzyme formation and RNA synthesis in vitro (Figure 2).[21] In addition, fluorescence microscopy demonstrated that the hit compound **C5** altered the RNAP localization in the cell.

Recently, we found that another hit compound, **C3**, possessed mild antimicrobial activity against *Streptococcus pneumoniae*.[22] Interestingly, the derivative **C3-005**, in which we have simply modified the aminobenzene ring of **C3** to dichlorobenzene, demonstrated a dramatic activity improvement against *S. pneumoniae* from a minimum inhibitory concentration (MIC) of 256 μg/mL to 8 μg/mL and significantly attenuated *S. pneumoniae* toxin production.[22]

Encouraged by the drug-like structure, synthetic efficiency, and modification potential of the **C3** series of compounds, we pursued systematic structural optimization. Although **C3-005** demonstrated superior antimicrobial activity than **C3**, we still preferred to employ the latter as the starting point for a comprehensive derivative design, synthesis, and antimicrobial activity assessment. The structure–activity relationship (SAR) was discussed, and the pharmacological properties such as hemolytic activity and cell permeability were also evaluated.

RESULTS

Derivative Design. The bioactive hit compound **C3** 2-(4-((2-aminophenyl)thio)-3-nitrobenzoyl)benzoic acid was labeled as compound **1** and used as the lead in this study. It was fitted into the pharmacophore model with the aminophenyl group binding to I291 (*E. coli* numbering) (Figure 3, **1**), nitrobenzoyl group to L282, and benzoic acid to R278 or R281 of the β'CH region.[22] We planned to sequentially modify

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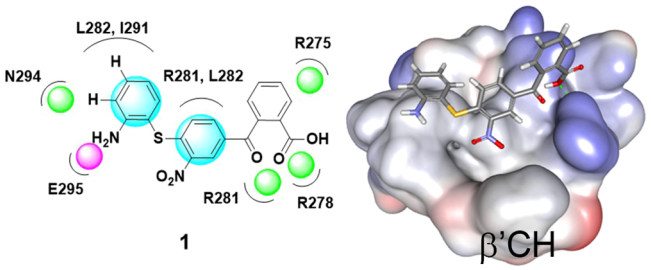


Figure 3. Docking model of **1** to β'CH (PDB 4LJZ).[18]

the left benzene ring of compound **1**, bridging atom S, middle nitro group, carbonyl linker between middle and right benzene rings, and substitution on the right aromatic group, as these groups are either involved in binding to the CH domain or play key roles in molecular conformation. The functional groups of compound **1** may be optimized by replacement with isosteres or bioisosteres to better fit in the binding site, and the prediction could be assessed by the biological activities.

Chemical Synthesis and Antimicrobial Activity against *S. pneumoniae*. Scheme 1 depicts the general procedure for the synthesis of hit compound **1** and its derivatives **2**−**32** with modifications on the left benzene ring. Commercially available 2-(4-chloro-3-nitrobenzoyl) benzoic acid **1a** reacted with methanol in the presence of thionyl chloride formed methyl benzoate **1b**.[23] Subsequently, substitutions of chloride **1b** by thiophenol or aniline derivatives, piperidine, and morpholine generated benzoate **1c**−**32c**.[24] The methyl esters were then hydrolyzed to yield compounds **1**−**32**.

The antimicrobial activities of compounds **1**−**32** were evaluated according to the Clinical & Laboratory Standards Institute (CLSI) guidelines against *S. pneumoniae* ATCC 49619.[25] The hit compound **1** demonstrated mild antimicrobial activity with a minimum inhibitory concentration (MIC) of 256 μg/mL against *S. pneumoniae*.[22]

As shown in Table 1, most of modifications of substitution on the left benzene ring (**1**−**3**, **5**−**15**) maintained or improved the activity against *S. pneumoniae*. The best result was obtained in the presence of 3,4-dichlorobenzene (**13**), with an MIC of 8 μg/mL. The replacement of benzene ring by heteroaromatic rings did not improve the antimicrobial activity (**4**, **16**). When the thioether linker was switched to amine (**17**−**28**), methylamine (**29**−**30**), piperidine (**31**), or morpholine (**32**), the antimicrobial activity was reduced. For example, both compounds **1** and **17** possess the same 2-amino substitution on the left benzene ring; **17** with the amine linker lost activity (MIC > 256 μg/mL), whereas **1** with the thioether linker showed an MIC of 256 μg/mL. Another example is compound **23**. With the amine linker, the compound had an MIC of 64 μg/mL, significantly inferior to compound **13** (MIC 8 μg/mL) with the thioether linker when both compounds possess the same 3,4-dichloro substituent on the left benzene ring. The calculated log *P* (clog *P*) values of compounds varied from 0.9 (**28**) to 6.1 (**11**). Compound **13** with the greatest activity possesses a relatively high clog *P* value of 5.9, suggesting a more hydrophobic character than most of other derivatives, which may lead to greater membrane permeability. However, the comparison of **13** to **11** on antimicrobial activity (MIC 8 and 32 μg/mL, respectively) showed that the substitution of the dichloro group at 3,4-position rather than 2,4-position was more important, even the latter has a higher clog *P* value (Table 1).

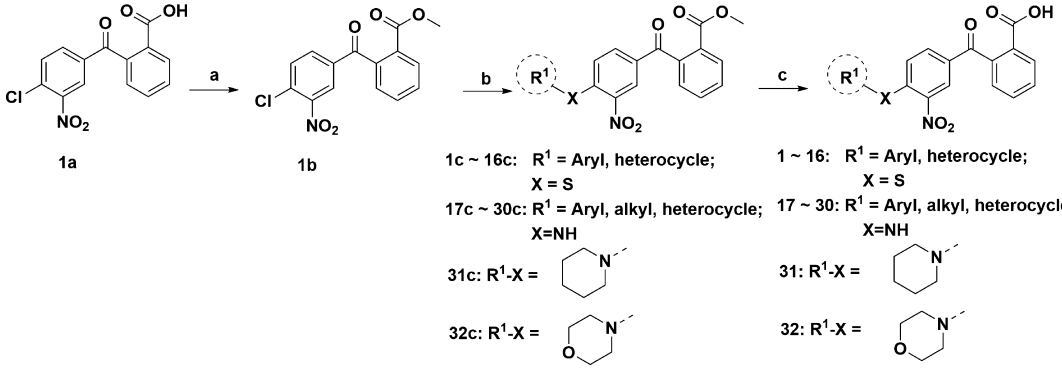
Next, we tested the requirement for the nitro group on the middle benzene ring. Compound **33** was prepared from bromobenzene. Friedel−Crafts acylation of phthalic anhydride with bromobenzene reacted at the para-position to generate **33b**,[26] followed by esterification to give **33c**. Palladium-catalyzed C−S bond formation of **33c** with 3,4-dichlorobenzenethiol provided **33d**,[27] which was hydrolyzed to provide compound **33** (Scheme 2).

The synthesis of compound **34** was accomplished, as shown in Scheme 3. Intermediate **13c** obtained in the previous experiment was reduced by Fe/NH_4Cl to provide **34b**, which was then hydrolyzed to give **34**.

Compounds **33**−**34** were evaluated for their antimicrobial activities. As compared to nitro **13**, the deletion of the nitro group slightly decreased the antimicrobial activity (**33**). Conversion of nitro to amine (**34**) additionally reduced the activity, while the clog *P* values were maintained at 5.2−6.1. Therefore, we decided to keep the nitro group on the middle benzene ring unmodified for further derivative synthesis (Table 2).

Compounds **35**−**37**, **41**, and **42** were synthesized, as shown in Scheme 4. In this series of compounds, we intended to investigate the amide linker replacing the carbonyl group of the hit compound **1** by maintaining the quasiplanar structure of the middle and right benzene rings through the conjugated system. As the linker length was extended to contain one more N atom, we synthesized the derivatives bearing benzoic acid at

Scheme 1. Synthetic Route to Compounds 1−32[a]



[a]Reagents and conditions: (a) $SOCl_2$, MeOH, reflux; (b) for **1c**−**16c**: thiophenols, NaOAc, EtOH, reflux; for **17c**−**31c**: amines, N,N-diisopropylethylamine (DIPEA), DMF, 130 °C or $Pd(OAc)_2$, xantphos, THF, 100 °C; (c) (i) THF/H_2O (v/v = 2:1), NaOH, rt; (ii) 2 N HCl.

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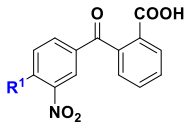
Table 1. Antimicrobial Activity[a] and cLog *P* of Compounds 1−32[b]



No	R[1]	MIC (μg/mL)	cLogP	No	R[1]	MIC (μg/mL)	cLogP
1 (C3)		256	3.39	17		>256	3.09
2		128	4.14	18		128	4.82
3		128	4.77	19		>256	3.28
4		>256	3.67	20		256	5.07
5		64	3.39	21		256	6.14
6		128	3.39	22		>256	2.87
7		32	4.96	23		64	5.67
8		256	3.64	24		>256	4.26
9		32	5.13	25		256	4.32
10		64	4.39	26		>256	4.39
11		32	6.06	27		>256	3.10
12		32	5.62	28		>256	0.93
13 (C3-005)		8	5.94	29		256	3.94
14		256	4.07	30		256	4.08
15		128	4.90	31		256	3.31
16		256	4.30	32		>256	1.93

[a]Antimicrobial activity against *S. pneumoniae* ATCC 29619. [b]**1**, **5**, **6**, **10**, and **13** were previously reported.[22]

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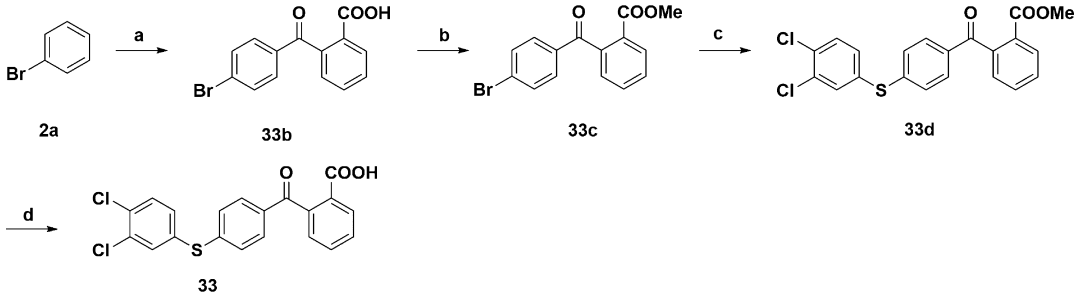
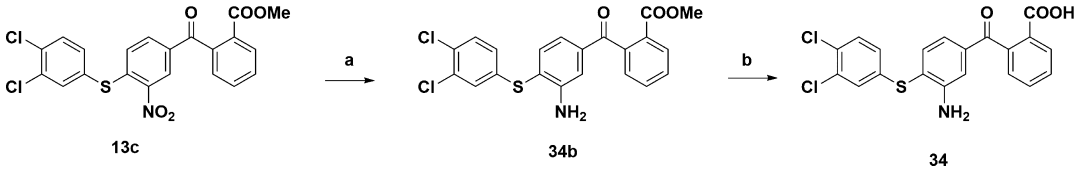
Scheme 2. Synthetic Route to Compound 33[a]



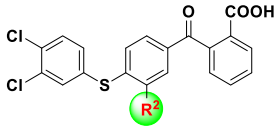
[a]Reagents and conditions: (a) phthalic anhydride, $AlCl_3$, toluene, reflux; (b) $SOCl_2$, MeOH, reflux; (c) 3,4-dichlorobenzenethiol, $Pd_2(dba)_3$, xantphos, DIPEA, dioxane, reflux; (d) (i) THF/H_2O (v/v = 2:1), NaOH, rt; (ii) 2 N HCl.

Scheme 3. Synthetic Route to Compound 34[a]



[a]Reagents and conditions: (a) Fe, NH_4Cl, EtOH/H_2O (v/v = 4:1), reflux; (b) (i) THF/H_2O (v:v = 2:1), NaOH, rt; (ii) 2 N HCl.

Table 2. Antimicrobial Activity[a] and cLog *P* of Compounds 33−34



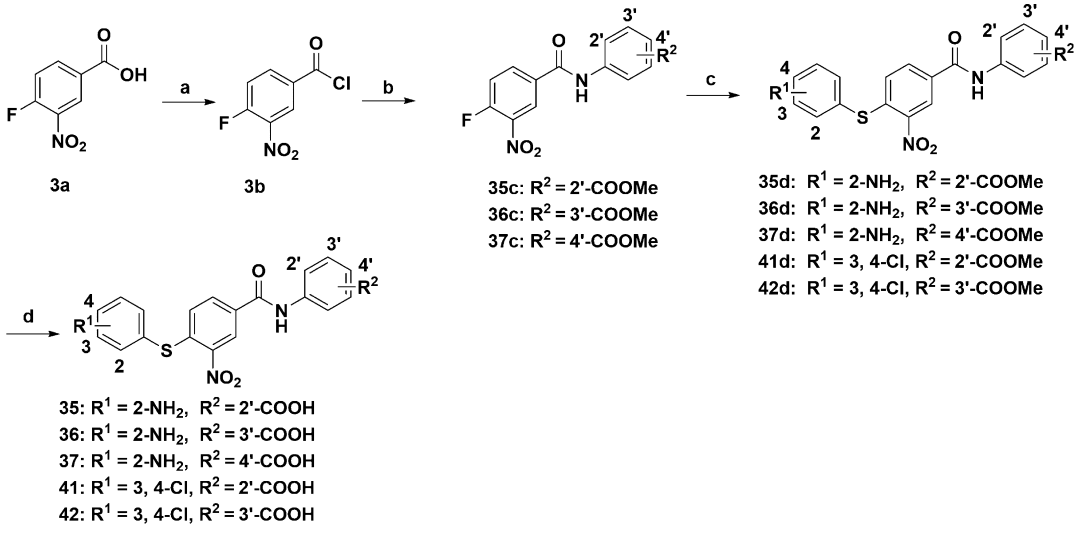
no	R^2	MIC (μg/mL)	clog *P*
33	−H	16	6.09
34	$-NH_2$	32	5.23

[a]Antimicrobial activity against *S. pneumoniae* ATCC 29619.

the ortho-(2′-), meta-(3′-), or para-(4′-) position of amide to study the SAR by adapting the extension strategy, and assess whether the amide linker can be accommodated by changing the carboxylic acid position on the right benzene ring. 4-Fluoro-3-nitrobenzoic acid **3a** was converted to benzoyl chloride **3b**, followed by amide synthesis to provide **35c**−**37c**. Substitution of aryl fluoride and hydrolysis of methyl ester group on the right benzene ring afforded the target compounds **35**−**37**, **41**, and **42**.

Derivatives with an amine linker were also synthesized to examine whether a flexible conformation is preferred. Compounds **38**−**40**, **43**−**44**, **46**−**51**, and **53**−**57** were synthesized from 4-fluorobenzaldehyde **3a**. Nitration of **3a** furnished **3b**, which was subjected to reductive amination with aniline derivatives to give **38c**−**40c**, **46c**−**51c**, and **53c**−**57c**.[28,29] Using various substituted thiophenols, substitution of

Scheme 4. Synthetic Route to Compounds 35−37, 41, and 42[a]



[a]Reagents and conditions: (a) oxalyl chloride, DMF, dichloromethane (DCM), rt; (b) anilines, tetraethylammonium, DCM, 0 °C; (c) thiophenols, NaOAc, EtOH, reflux; (d) (i) THF/H_2O (v/v = 2:1), NaOH, rt; (ii) 2 N HCl.

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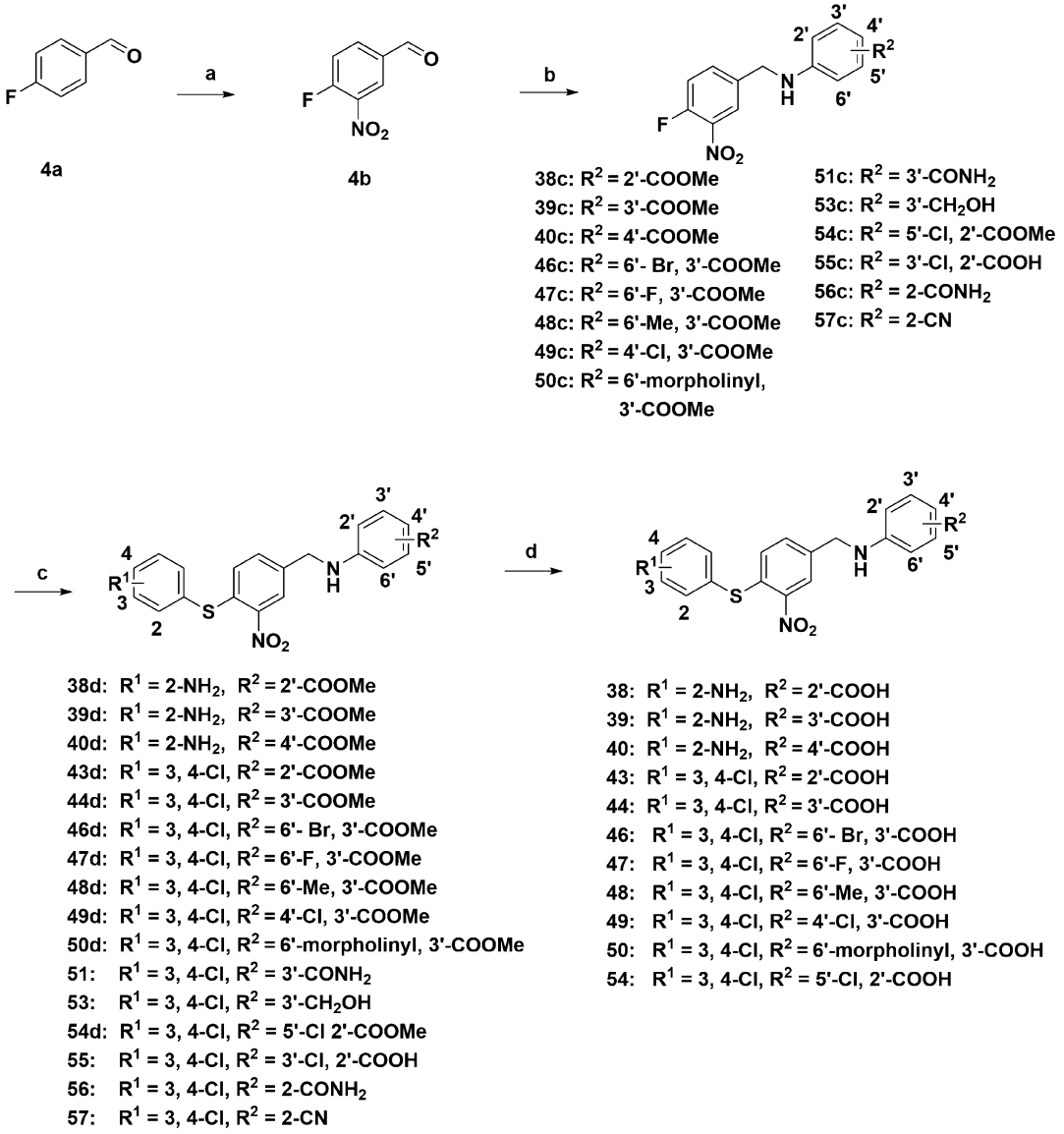
Scheme 5. Synthetic Route to Compounds 38−40, 43−44, 46−51, and 53−57[a]



[a]Reagents and conditions: (a) HNO_3, H_2SO_4, 0 °C; (b) anilines, $NaBH_4$, EtOH/$NaBH(OAc)_3$, DCM, rt; (c) thiophenols, NaOAc, EtOH, reflux; (d) (i) THF/H_2O (v/v = 2:1), NaOH, rt, or dioxane/H_2O (v/v = 2:1), NaOH, 50 °C; (ii) 2 N HCl.

fluoride afforded **38d−40d**, **43d−44d**, **46d−50d**, **51**, **53**, **54d**, and **55−57**. After hydrolysis of methyl esters, compounds **38−40**, **43−44**, **46−50**, and **54** were obtained (Scheme 5).

Compounds **45** and **52** were prepared through the following steps: **4b** underwent the substitution with 3, 4-dichlorobenzenethiol to generate intermediate **45c**, followed by reduction of aldehyde to give alcohol **45d**. The substitution of hydroxyl using PBr_3 provided bromide **45e**, followed by substitution of bromide by a substituted aniline to furnish **45f**. The hydrolysis of the methyl ester group provided the final product **45**. Starting from the same intermediate **45c**, reductive amination afforded sulfonamide **52** (Scheme 6).

As shown in Table 3, when the carbonyl linker of compound **1** was replaced by amide, the antimicrobial activity increased fourfold (**35**). The benzoic acid substitution moved from ortho- to meta- or para-position of amide reduced the activity (**36**, **37**). Replacement of the amide linker with flexible methylamine did not alter the activity (**38−40**). Interestingly, the 3,4-dichlorobenzene ring proved to be superior to 2-aminobenzene for antimicrobial activity when the carbonyl linker was present. In contrast, the derivative with both 3,4-dichlorobenzene ring and amide linker lost activity completely (**41**), and changing the substitution position of benzoic acid barely improved the activity (**42**). The best antimicrobial activity from this series of compounds was obtained when 3,4-dichlorobenzene was combined with the methylamine linker, resulting in an MIC of 4 μg/mL (**43**). Again, *meta*-amino benzoic acid was not preferred (**44**). In summary, the appropriate structure for binding to β′CH can be concluded when comparing compounds **41−44**. These four compounds have close clog *P* values ranging from 6.4 to 7.3, but the antimicrobial activity showed a much significant difference (Table 3). 3,4-Dichloro group substituted on the left benzene ring demonstrated to be a great combination with the methylamine linker, compared to amide, while the two linkers did not show much difference when 2-amino group was present on the left benzene ring of the molecule.

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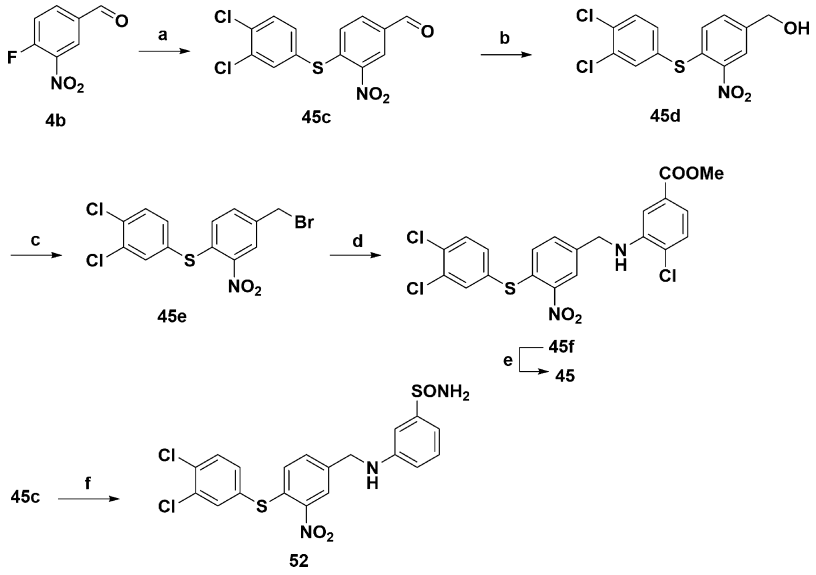
Scheme 6. Synthetic Route to Compounds 45 and 52[a]



[a]Reagents and conditions: (a) thiophenol, NaOAc, EtOH, reflux; (b) $NaBH_4$, MeOH, 0 °C to rt; (c) PBr_3, toluene, 100 °C, 1 h; (d) methyl 3-amino-4-chlorobenzoate, DIPEA, DMF, 100 °C; (e) dioxane/H_2O (v/v = 2:1), NaOH, 50 °C; (f) 3-amino benzenesulfonamide, $NaBH(OAc)_3$, CH_2Cl_2, rt, overnight.

Based on these data, we maintained both 3,4-dichlorobenzene and methylamine linker for further structure modification (Table 4). As benzoic acid is predicted to interact with β' R278 or R281 (Figure 3), we reasoned that increasing the acidity of benzoic acid by introducing electron-withdrawing groups would improve the protein−ligand interaction. Indeed, compared to compound **44**, electron-withdrawing halides (**45**, **46**) at para-position of benzoic acid increased the activity, whereas electron-donating methyl (**48**) decreased the activity. Compound **47** with *p*-fluoro substitution lost activity completely. This anomalous phenomenon needs to be investigated further and could be due to an impaired delivery to the target. Changing benzoic acid to amide (**51**, **56**), sulfonamide (**52**), alcohol (**53**), or nitrile (**57**) at 2- or 3-position of the amine linker led to a loss of the antimicrobial activity. The best result was obtained when 5-Cl-2-benzoic acid is present (**54**), with MIC of 1 μg/mL comparable to the "last resort" antibiotic vancomycin (Table S1). Note that the activity of **54** increased fourfold compared to **43**, which may also be partially contributed by increased bacterial membrane permeability of **54** with a clog *P* value of 8.2. However, **45** and **46** with halide substitution at para-position of benzoic acid only exhibited twofold improvement on antimicrobial activity of **44**.

In-Depth Biological Investigation. *Antimicrobial Activity against Gram-Positive Pathogens.* Representative compounds **13**, **44**, **45**, **54**, and **55** were tested against a representative panel of Gram-positive pathogens (Figure 4). Compared with the reported compound **13**,[22] **54** showed greatly improved antimicrobial activity against *S. pneumoniae*, as well as both groups A and B streptococci: *Streptococcus pyogenes* (group A streptococcus, GAS) responsible for serious throat and skin infections[30] and *Streptococcus agalactiae* (group B streptococcus, GBS) responsible for severe conditions in neonates.[31] Although a panel of Gram-negative pathogens was also tested (Table S1), most of the compounds were ineffective against Gram-negative pathogens (MIC > 256 μg/mL), probably because of poor outer membrane permeability and/or vigorous drug efflux.

Time-Kill Kinetics. The dose- and time-dependent relationship between antimicrobial activity and bacterial growth can be assessed by the time-kill assay. **54** was added to the liquid culture of *S. aureus* and *S. agalactiae* at various concentrations, and its antimicrobial profiles in both pathogens were assessed by constructing the time-kill curves. *S. aureus* and *S. agalactiae* (GBS) were cultured in liquid media with agitation in accordance with the CLSI guidelines.[32] **54** was largely bacteriostatic over the course of 6 h against *S. aureus* and GBS even at 16 MIC with no significant decrease in CFU counts (Figure 5A,B).

Time-kill curves were also constructed by adding **54** to *S. pneumoniae* at various concentrations and measuring the in vitro antimicrobial activity over time. *S. pneumoniae* cultures were raised in liquid media with agitation in the presence of 5% CO_2.[33] **54** was largely bacteriostatic at 1 MIC, while increasing to 4 and 16 MICs yielded no further bactericidal effects over time after an initial sharp drop in the cell count (Figure 5C). Compared to trends in *S. aureus* and GBS, **54** appears to be able to elicit stronger bacteriostatic activity (ca. 2-fold $\log_{10}$ decrease) at higher concentrations in *S. pneumoniae*.

Central Metabolism. One of the characteristics of an effective antibiotic is the arrest of bacterial respiration.[34] The ATP production of *S. pneumoniae* cells in the presence of **54** at various concentrations was monitored alongside the time-kill kinetics using the same experimental setup. A significant decrease in *S. pneumoniae* ATP production rate was observed at 1/4 MIC of **54**, and increasing concentrations of the compound further impacted cellular respiration (Figure 5D). This mirrored previously reported trends of the transcription inhibitor rifampicin where antimicrobial efficacy is inversely correlated to bacterial respiration rates.[33,35]

Toxin Secretion. A hallmark of *S. pneumoniae* virulence is the release of its exotoxin, pneumolysin, into the extracellular milieu. Nonlytic antibiotics, such as rifampicin and macrolides,

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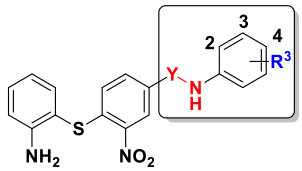
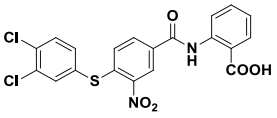
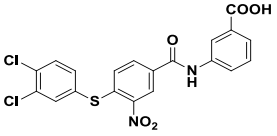
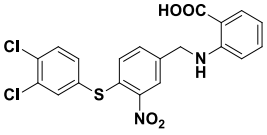
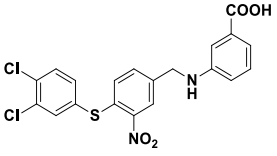
Table 3. Antimicrobial Activity[a] and cLog *P* of Compounds 35−44



No.	Structure		MIC (μg/mL)	cLogP
	Y	R^3		
35		2-COOH	64	4.47
36		3-COOH	128	3.84
37		4-COOH	256	3.84
38		2-COOH	64	4.78
39		3-COOH	64	4.15
40		4-COOH	256	4.15
41			>256	7.01
42			256	6.38
43			4	7.34
44			8	6.71

[a]Antimicrobial activity against *S. pneumoniae* ATCC 29619.

have been shown to repress pneumolysin release at sub-MIC levels.[36−38] In this study, we explored the effects of **54** on pneumolysin secretion. Overnight cultures of *S. pneumoniae* in the brain−heart infusion (BHI) medium were exposed to 1/2 and 1/4 of the predetermined MICs of the test compound **54**, the nonlytic transcription inhibitor rifampicin, the bacteriolytic drug ceftriaxone, and drug-free control. Western blot analysis was performed using the supernatant harvested from the overnight cultures following centrifugation.

The control drugs performed as previously described in the literature with ceftriaxone treatment increased, while rifampicin suppressed the release of pneumolysin (Figure 6).[39] A substantial decrease in pneumolysin levels was observed in **54**-treated cultures compared to the drug-free control (Figure 6). This suggests that **54** inhibits *S. pneumoniae* growth in a bacteriostatic mechanism with the suppression of the exotoxin release.

Cytotoxicity. The representative derivatives **13**, **43**, **45**, **46**, **54**, and **55** with improved antimicrobial activity were subjected to cytotoxic evaluations.[40] Human HepG2 liver cancer and A549 lung carcinoma cell lines were chosen to examine the potential toxicity to liver cells, where drugs are normally metabolized, and to lung cells, the primary site of *S. pneumoniae* infection. The results demonstrated that all tested compounds possess very low toxicity to these two human cancer cell lines with 50% cytotoxic concentration (CC_{50}) values >70 μM (Table 5). With the improvement of antimicrobial activity, the derivatives expressed increased the therapeutic index value up to 57 (**43** to A549), suggesting that

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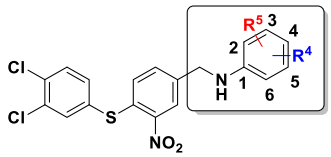
Table 4. Antimicrobial Activity[a] and cLog *P* of Compounds 45−57



no	R^4	R^5	MIC (μg/mL)	clog *P*
45	6-Cl	3-COOH	4	7.56
46	6-Br	3-COOH	4	7.71
47	6-F	3-COOH	>256	6.99
48	6-CH_3	3-COOH	16	7.21
49	4-Cl	3-COOH	256	6.96
50	6-morpholin-1-yl	3-COOH	16	6.23
51	H	3-$CONH_2$	>256	5.48
52	H	3-SO_2NH_2	64	5.16
53	H	3-CH_2OH	>256	5.58
54	5-Cl	2-COOH	1	8.19
55	3-Cl	2-COOH	8	7.59
56	H	2-$CONH_2$	>256	5.88
57	H	2-CN	>256	6.76

[a]Antimicrobial activity against *S. pneumoniae* ATCC 29619.

Compd.	EFAE	SAUR[a]	SAUR[b]	SPNE	SEPI	SSAP	SPYO	SAGA
1	256	>256	>256	256	>256	256	256	>256
13	32	16	16	8	16	32	16	16
43	8	8	4	4	4	4	4	4
44	8	8	8	8	8	8	8	8
45	4	8	8	4	4	4	4	4
54	4	4	4	1	2	2	1	2
55	8	8	8	8	4	8	2	4
VAN	1	2	1	1	2	1	1	1
RIF	4	0.01	0.03	0.0625	0.03	0.03	0.03	0.03

Figure 4. Antimicrobial activity (MIC μg/mL) of selected compounds against clinically important Gram-positive pathogens. EFAE: *Enterococcus faecalis* ATCC 19433, SAUR[a]: *Staphylococcus aureus* ATCC 25923, SAUR[b]: *S. aureus* ATCC 29213, SPNE: *S. pneumoniae* ATCC 49619, SEPI: *Streptococcus epidermidis* ATCC 12228, SSAP: *Streptococcus saprophyticus* ATCC 15305, SPYO: *S. pyogenes* ATCC 19615, SAGA: *S. agalactiae* ATCC 12386, VAN: vancomycin, RIF: rifampicin.

the compounds are generally safe when assessed at the cellular level.

In Vitro Pharmacological Evaluation. *Hemolytic Property.* Three compounds **13**, **43**, and **45** were chosen to assess their hemolytic property. As an important pharmacological parameter, the hemolytic property determines whether compounds can be used in animal studies without the destruction of blood cells.[41,42] As shown in Figure 7, all three compounds demonstrated low hemolytic potential at all tested concentrations (0.1, 1, and 10 μg/mL). The hemolytic values of these compounds were lower than the suggested nonhemolytic cutoff value (<10%), suggesting very low toxic potential to human blood cells.

Caco-2 Cell Permeability. The human intestinal epithelial cancer cell line Caco-2 was used to determine the permeability of compounds **13** and **43**.[41,42] As shown in Table 6, compounds **13** and **43** displayed excellent human intestinal epithelial cell permeability, which is superior to 1×10^{-5} cm/s as the standard of great intestinal epithelial cell permeability. The result indicated that the potential of oral availability could be expected because of excellent gut absorption.

Study of the Mechanism of Action. *β′CH−σ[A] PPI Inhibition.* With the antimicrobial activity, cytotoxicity, and in vitro pharmacological data in hand, we intended to assess the molecular mechanism of this series of diaryl benzoic acid derivatives. Several representative compounds with various antimicrobial activities were tested for inhibition of the interaction between β′CH−σ using our previously established protein complementation assay (Figure S1).[43] As shown in Table 7, all of the tested compounds were able to disrupt CH−σ interactions, in contrast to no-drug control.[21,43] These results demonstrate that structural modifications maintained the inhibitory activity against CH−σ binding in vitro, and the inhibitory activity approximately matched the trend of antimicrobial activity of compounds. Compound **54** was not able to be tested in this experiment because of insolubility under the testing conditions, which can be reflected by its high clog *P* value. Nevertheless, the inhibitory activity of **54** was examined in the following transcription assay because of the relatively low concentrations used.

In Vitro Transcription Initiation Assay. We next tested compounds **13** and **54** for their ability to inhibit RNAP−σ interactions using an in vitro transcription assay. We preincubated the *E. coli* core RNAP with various concentrations of the compounds [or dimethyl sulfoxide (DMSO)] and then added the σ^{70} subunit together with a DNA template containing a model bacteriophage λ P_R promoter and nucleotide substrates to produce a 26-mer radiolabeled RNA (Figure 8). We observed that although both compounds inhibited transcription, **54** exhibited much more potent inhibition. These results support the proposed mechanism of action of these derivatives via the inhibition of the CH−σ interactions. They also show that, at least for these two representative derivatives, structural modifications leading to stronger inhibitory effects on transcription in a purified in vitro system also support superior antibacterial activity (Figure 4).

Confocal Microscopy. As previously reported,[21] we examined the effects of the benzoic acid compounds on cellular RNAP localization using fluorescence microscopy. In *B. subtilis* BS1048 strain, the *rpoC* gene is fused to *gfp* to produce the green fluorescent protein (GFP)-tagged β′ subunit of RNAP from the chromosomal locus. The fluorescently labeled RNAP localizes to the centrally localized nucleoid, as expected (Figure 9 Ctrl).[21] The antimicrobial activity of compound **54** against *B. subtilis* was measured with MIC 4 μg/mL prior to the microscopic assay. When **54** was added at 1/2 MIC, delocalization of the fluorescence from the nucleoid could be observed as compared to the control cells (Figure 9 1/2 MIC), suggesting that chromosomes became decondensed. As the concentration of **54** increased, diffusion of the fluorescent signal into the cytosolic region became more apparent (Figure 9 1 MIC and 2 MIC). These results suggest that **54** inhibits transcription initiation in the cell, triggering the diffusion of core RNAP, which cannot bind to promoters and engage in transcription from the nucleoid.

Cell Content Quantification of the Major Macromolecules. The levels of the major macromolecules in *S. aureus* ATCC 29213 cells were analyzed after the treatment of **54**, in comparison with a known transcription inhibitor rifampicin. **54** and rifampicin were added to *S. aureus* cells in liquid culture at 1/4, and 1/8 MIC levels at the late lagging phase (OD_{600} = 0.2), and cells were harvested during the mid-exponential growth (OD_{600} = 0.6). As shown in Figure 10A, the total levels of DNA remained mostly unaffected by the treatments

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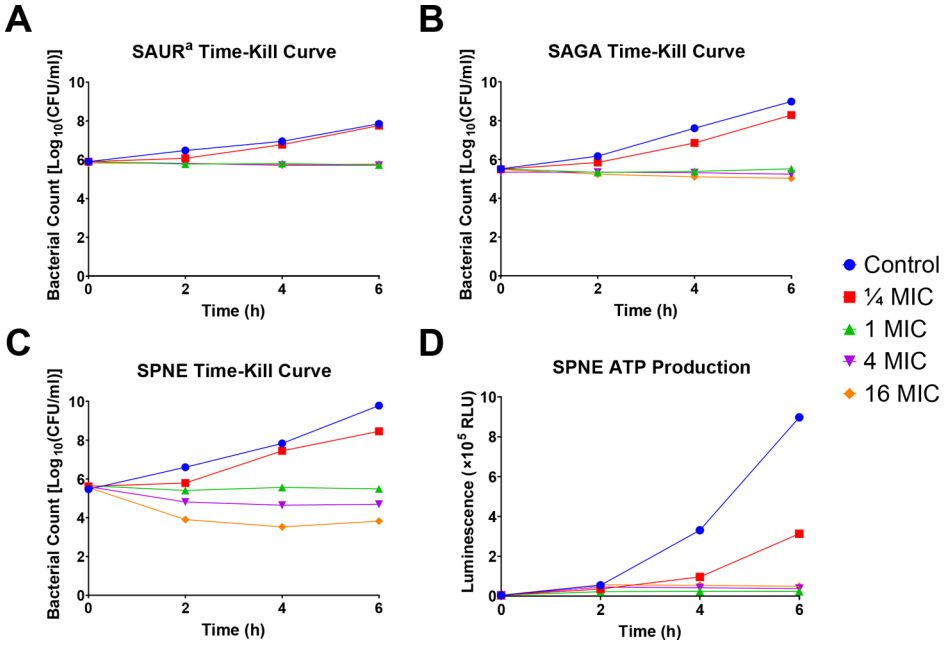

Figure 5. Effects of **54** on the time-kill kinetics of (A) *S. aureus* and (B) *S. agalactiae*. The effects of **54** on (C) the time-kill kinetics and (D) ATP production of *S. pneumoniae* when challenged at 1/4, 1, 4, and 16 MIC.

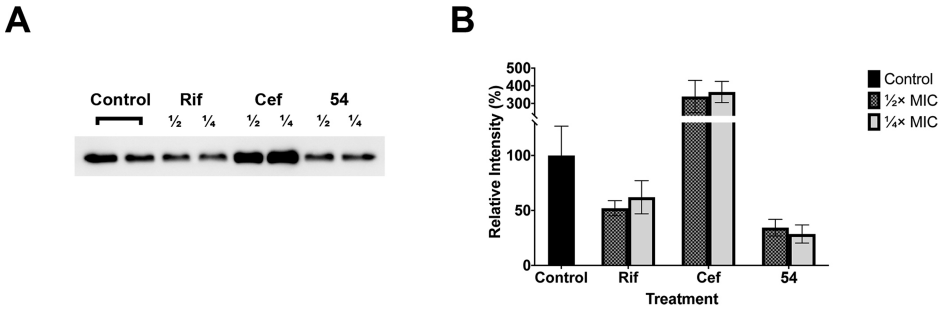

Figure 6. Effect of **54** on the expression of *S. pneumoniae* exotoxin. (A) Representative Western blot showing levels of pneumolysin in the culture supernatant following overnight incubation of *S. pneumoniae* at 1/2 and 1/4 MICs of **54**, rifampicin (Rif), and ceftriaxone (Cef) and (B) relative intensity of the bands at 1/2 (checker bars) and 1/4 (light gray bars) MICs of the treatments normalized against the untreated control group (solid black bar).

Table 5. Cytotoxicity of Representative Compounds

	CC_{50} (μM)		therapeutic index	
no.	HepG2	A549	HepG2	A549
13	63.8 ± 9.3	76.7 ± 11.4	7	9
43	170.1 ± 15.4	252.2 ± 13.3	38	57
45	110.3 ± 12.4	116.3 ± 10.8	27	28
46	92.6 ± 12.6	67.7 ± 11.3	24	18
54	74.4 ± 12.5	75.7 ± 12.5	36	37
55	97.7 ± 13.1	93.3 ± 13.0	12	11

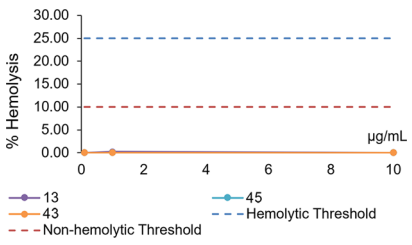

Figure 7. In vitro hemolysis study of compounds **13**, **43**, and **45**.

Table 6. Caco-2 Cell Permeability of Compounds 13 and 43

no.	P_{app} average (n = 4) (cm/s)
13	$3.50 \pm 0.62 \times 10^{-5}$
43	$3.62 \pm 0.60 \times 10^{-5}$

Table 7. Inhibition of CH−σ Interactions by Representative Compounds

no.	MIC (μg/mL)	IC_{50} (μM)	no.	MIC (μg/mL)	IC_{50} (μM)
33	16	67.0 ± 4.7	**44**	8	42.9 ± 2.5
37	256	97.0 ± 9.6	**45**	4	15.0 ± 0.7
43	4	11.6 ± 0.6	**55**	8	25.1 ± 1.5

compared to the no-drug control. Total RNA levels were reduced by rifampicin, which was consistent with its known mechanism and previous observations.[40] Treatment with **54** also resulted in a reduction in the total level of RNA in the staphylococcal cells (Figure 10B). Together with our observations that **54** inhibits transcription in vitro (Figure 8) and releases RNAP from the nucleoid (Figure 9), this result argues that **54** inhibits transcription in target bacterial cells.

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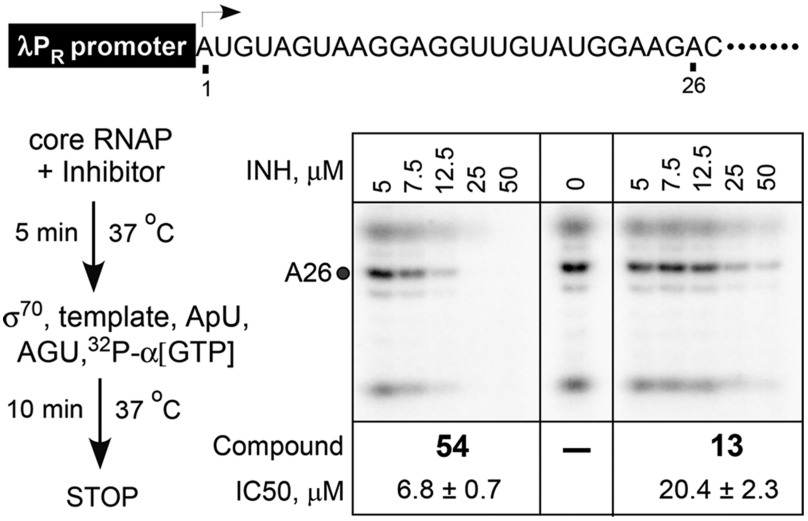

Figure 8. In vitro assay of RNA synthesis inhibition by compounds **13** and **54**, as compared to the DMSO control (0 sample in the middle). IC_{50} values were determined from three independent experiments. A representative gel is shown.

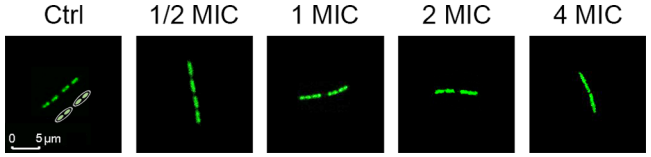

Figure 9. Confocal microscopy of *B. subtilis* cells monitoring RNAP-GFP fluorescence. **54** was added to the culture at various MIC levels.

Reduction in total protein levels in *S. aureus* ATCC 29213 was also observed following the treatment with **54** (Figure 10C), as would be expected as a consequence of the reduced RNA synthesis.

DISCUSSION

The inhibition of transcription is lethal in all cells. Bacterial transcription is an established antimicrobial target, with rifampicin and fidaxomicin as approved antibiotics in the market. Other inhibitors of transcription, most of which target RNAP, have been used extensively in mechanistic studies of the transcription cycle.[6,44] Importantly, even though human RNAPs are structurally similar to their bacterial homologues, sequence similarity is limited to a few key regions and inhibitors of bacterial and human RNAP do not exhibit cross-domain effects. Therefore, bacterial transcription represents a valid potential target for antibiotic discovery.[3]

In this article, we examined a series of triaryl derivatives of a previously identified hit compound **1**, which inhibits the formation of the RNAP holoenzyme.[21] These derivatives were obtained through pharmacophore-based rational design and synthesis. We then carried out a systematic evaluation of the compound series and found that a general diaryl benzoic acid structure is required for the antimicrobial activity. This structure also possesses desirable drug-like properties, as shown by cytotoxicity and in vitro pharmacological assays. Finally, we reported an improved, more potent derivative **54** of this class of diaryl benzoic acid antimicrobials with enhanced antimicrobial effects against *S. pneumoniae* with the MIC improved to 1 μg/mL and other Gram-positive pathogens at 1−2 μg/mL, comparable to vancomycin (Table S1).

SAR Summary. Based on the antimicrobial activity against *S. pneumoniae*, several important points could be drawn from the SAR of this series of triaryl compounds (Figure 11): 3,4-dichlorobenzene is preferred over other substituted benzene or pyridine as the left aryl ring, probably because of increased binding to β′CH L282 and I291 (Figure 3);[18] the compounds with left thioether linker have superior activity to amine; the nitro group on the middle aryl ring can be deleted with slightly reduced activity because the middle benzene ring maintains the affinity with L282; the right methylamine linker is favored over carbonyl or amide when the 3,4-dichloro group substituted on the left benzene ring; 2-benzoic acid as the right aryl ring with

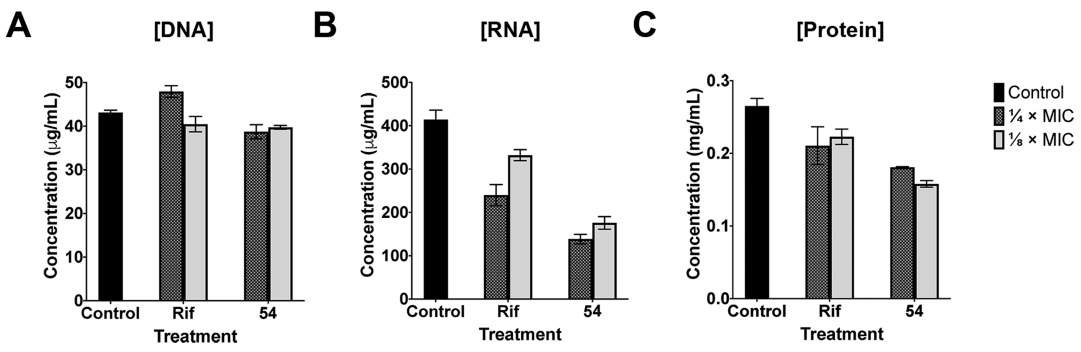

Figure 10. Effects of **54** on the levels of total (A) DNA, (B) RNA, and (C) protein of *S. aureus* ATCC 29213 when challenged at 1/4 × (checkered bars) and 1/8 × (light gray bars) MICs compared to rifampicin (Rif) and the no-drug control (solid black bars).

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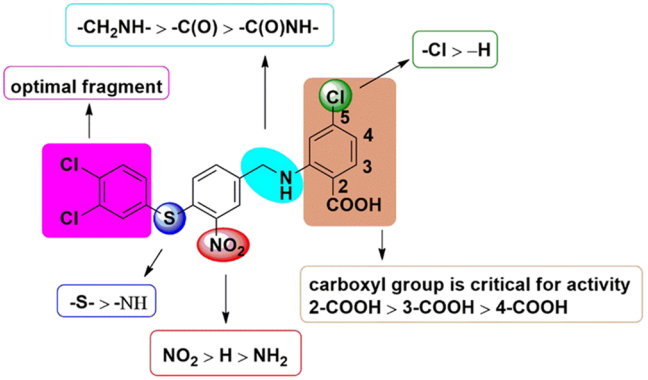

Figure 11. SAR of tested compounds **1**–**57**

an electron-withdrawing group at the para-position exhibits the greatest activity, possibly because of the improved ionic binding interaction with R278 and R281.

The mechanism of **54** was characterized using various methods including in vitro transcription assay, fluorescent microscopy, and macromolecular content quantification. We show that **54** suppresses bacterial transcription by inhibiting the formation of bacterial RNAP holoenzyme. We also demonstrate that **54** is largely bacteriostatic but can arrest cellular ATP production in *S. pneumoniae* similar to the transcription inhibitor rifampicin. **54** was also able to decrease the level of secreted pneumococcal virulence factor pneumolysin. The suppression is very likely to happen at the transcription level, and the effect of **54** on the transcriptome of *S. pneumoniae* cells is currently under investigation.

Although we demonstrated that the class of diaryl benzoic acid antimicrobials could inhibit bacterial RNAP holoenzyme formation using both in vitro and cellular assays, the possibility of other cellular targets should not be neglected, and any off-target effects of the compounds need to be investigated in further studies. Detailed structural information of the diaryl benzoic acid antimicrobials in complex with their potential target protein will reveal critical contacts at the interface, information necessary for rational optimization of the lead. We are currently pursuing structural studies of the β'CH fragment bound to representative diaryl benzoic acid compounds. Finally, the demonstrated in vitro inhibitory properties of these compounds need to be assessed in vivo. Analysis of the efficacy of these compounds in treating Gram-positive infections in small animals is currently underway in our laboratory.

CONCLUSIONS

In summary, these diaryl benzoic acid antimicrobials reported in this study possess good antimicrobial activities, low cytotoxicity, and drug-like in vitro pharmacological properties. Therefore, they are promising candidates for further development as novel antimicrobial agents. Our approach continues to pave the way for a solid platform of novel antimicrobial discovery, with a focus on bacterial transcription as a drug target. In addition to their potential future therapeutic value, this series of compounds could be developed as chemical probes for studies of σ factor-mediated transcription regulation.

EXPERIMENTAL SECTION

General. Starting materials and regents, unless otherwise stated, were of commercial grade and were used without further purification. All reactions were monitored by thin-layer chromatography (TLC) on glass sheets (Silica gel F_{254}) which can be visualized under UV light. Flash chromatography was carried out using silica gel (200–300 mesh). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured on a Bruker AVANCE-III spectrometer with TMS as an internal standard. Chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz. High-resolution mass spectrometry (MS) spectra were measured using a QTOF-2 micromass spectrometer by electron spray ionization. The purity of all of the compounds tested for biological activities was >95%, determined by analytical HPLC (Agilent 1260 Infinity) on a reverse-phase column (Agilent ZORBAX Eclipse plus C18, 4.6 × 100 mm, 5 μm particle size) with acetonitrile/water as the eluent.[45] Compounds reported were screened for PAINS.

Synthetic Procedures for Scheme 1. *General Procedure for the Synthesis of Compounds **1c**–**16c**.* To a flask were added compound **1b** (64 mg, 0.2 mmol), benzenethiol (1.2 equiv, 0.24 mmol), NaOAc (82 mg, 1 mmol), and EtOH 5 mL. The mixture was stirred at reflux for 4 h. After cooling to room temperature, the precipitate was filtered, washed with EtOH and water successively, and dried to give the titled compounds. Alternatively, water was added, and the aqueous solution was extracted by EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated, and purified by chromatography to provide the titled compounds.

Methyl 2-(4-chloro-3-nitrobenzoyl)benzoate (**1b**), methyl 2-(4-((2-aminophenyl)thio)-3-nitrobenzoyl)benzoate (**1c**), methyl 2-(4-((3-aminophenyl)thio)-3-nitrobenzoyl)benzoate (**5c**), methyl 2-(4-((4-aminophenyl)thio)-3-nitrobenzoyl)benzoate (**6c**), methyl 2-(4-((2-amino-4-chlorophenyl)thio)-3-nitrobenzoyl)benzoate (**10c**), and methyl 2-(4-((3,4-dichlorophenyl)thio)-3-nitrobenzoyl)benzoate (**13c**) were synthesized following the previously published procedures.[22]

*Methyl 2-(4-((2-Methoxyphenyl)thio)-3-nitrobenzoyl)benzoate (**2c**).* Pale yellow solid, 70 mg, 71% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (d, J = 1.7 Hz, 1H), 8.09 (d, J = 7.8 Hz, 1H), 7.76 (dd, J = 8.5, 1.8 Hz, 1H), 7.68 (td, J = 7.5 Hz, 0.8 Hz, 1H), 7.61 (dd, J = 12.3, 4.7 Hz, 2H), 7.56 (td, J = 7.9 Hz, 1.2 Hz, 1H), 7.38 (d, J = 7.2 Hz, 1H), 7.09 (t, J = 7.6 Hz, 1H), 7.06 (d, J = 8.3 Hz, 1H), 6.89 (d, J = 8.6 Hz, 1H), 3.82 (s, 3H), 3.73 (s, 3H).

*Methyl 2-(4-((2-Fluorophenyl)thio)-3-nitrobenzoyl)benzoate (**3c**).* Yellow solid, 70 mg, 84% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (d, J = 1.6 Hz, 1H), 8.10 (d, J = 7.5 Hz, 1H), 7.79 (dd, J = 8.6, 1.7 Hz, 1H), 7.75–7.55 (m, 4H), 7.38 (d, J = 7.3 Hz, 1H), 7.33 (d, J = 7.4 Hz, 1H), 7.27 (m, 1H), 6.92 (d, J = 8.3 Hz, 1H), 3.74 (s, 3H).

*Methyl 2-(3-Nitro-4-(pyridin-2-ylthio)benzoyl)benzoate (**4c**).* Pale yellow solid, 70 mg, 89% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (d, J = 4.1 Hz, 1H), 8.46 (d, J = 0.8 Hz, 1H), 8.09 (d, J = 7.7 Hz, 1H), 7.79 (t, J = 7.7 Hz, 2H), 7.68 (t, J = 7.4 Hz, 1H), 7.62 (m, 2H), 7.43–7.33 (m, 2H), 7.24 (d, J = 8.5 Hz, 1H), 3.73 (s, 3H).

*Methyl 2-(4-((3-(Isopropylamino)phenyl)thio)-3-nitrobenzoyl)-benzoate (**7c**).* Purified by chromatography using hexane/EtOAc (10:1). Yellow solid, 65 mg, 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51 (d, J = 1.5 Hz, 1H), 8.10 (d, J = 7.8 Hz, 1H), 7.77 (dd, J = 8.6, 1.7 Hz, 1H), 7.68 (t, J = 7.4 Hz, 1H), 7.61 (t, J = 7.2 Hz, 1H), 7.36 (d, J = 7.3 Hz, 1H), 7.26 (t, J = 7.9 Hz, 1H), 7.08 (d, J = 8.6 Hz, 1H), 6.86 (d, J = 7.5 Hz, 1H), 6.77 (s, 1H), 6.70 (dd, J = 8.2, 1.7 Hz, 1H), 3.74 (s, 3H), 3.69 (s, 1H), 3.64 (d, J = 6.2 Hz, 1H), 1.24 (d, J = 6.0 Hz, 6H).

*Methyl 2-(4-((3-Acetamidophenyl)thio)-3-nitrobenzoyl)benzoate (**8c**).* Yellow solid, 45 mg, 50% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.20 (s, 1H), 8.36 (s, 1H), 8.04 (d, J = 7.7 Hz, 1H), 7.95 (s, 1H), 7.79 (m, 2H), 7.72 (m, 2H), 7.51 (t, J = 7.1 Hz, 2H), 7.32 (d, J = 7.6 Hz, 1H), 7.01 (d, J = 8.6 Hz, 1H), 3.65 (s, 3H), 2.06 (s, 3H).

*Methyl 2-(4-((3-Benzamidophenyl)thio)-3-nitrobenzoyl)-benzoate (**9c**).* Yellow solid, 57 mg, 56% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (s, 1H), 8.10 (d, J = 7.7 Hz, 1H), 7.95 (s, 1H),

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7.89 (d, J = 7.9 Hz, 4H), 7.78 (d, J = 8.6 Hz, 1H), 7.68 (t, J = 7.3 Hz, 1H), 7.61 (m, 2H), 7.57−7.48 (m, 3H), 7.38 (m, 2H), 7.02 (d, J = 8.6 Hz, 1H), 3.74 (s, 3H).

*Methyl 2-(4-((2,4-Dichlorophenyl)thio)-3-nitrobenzoyl)benzoate (**11c**).* Yellow solid, 60 mg, 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (d, J = 1.2 Hz, 1H), 8.10 (d, J = 7.7 Hz, 1H), 7.81 (dd, J = 8.4, 1.3 Hz, 1H), 7.74−7.58 (m, 4H), 7.41 (dd, J = 8.3, 2.0 Hz, 1H), 7.38 (d, J = 7.5 Hz, 1H), 6.81 (d, J = 8.6 Hz, 1H), 3.74 (s, 3H).

*Methyl 2-(4-((2,4-Dimethylphenyl)thio)-3-nitrobenzoyl)benzoate (**12c**).* Yellow solid, 68 mg, 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (d, J = 1.7 Hz, 1H), 8.08 (d, J = 7.7 Hz, 1H), 7.71 (dd, J = 8.6, 1.7 Hz, 1H), 7.66 (dd, J = 7.3, 0.8 Hz, 1H), 7.61 (d, J = 7.4 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.36 (d, J = 7.2 Hz, 1H), 7.23 (s, 1H), 7.13 (d, J = 7.7 Hz, 1H), 6.78 (d, J = 8.6 Hz, 1H), 3.73 (s, 3H), 2.40 (s, 3H), 2.32 (s, 3H).

*Methyl 2-(4-((4-(Diethylcarbamoyl)-2-nitrophenyl)thio)-3-nitrobenzoyl)benzoate (**14c**).* Yellow solid, 37 mg, 34% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.47 (d, J = 1.8 Hz, 1H), 8.13 (d, J = 8.2 Hz, 2H), 7.89 (dd, J = 8.4, 1.8 Hz, 1H), 7.72 (td, J = 7.5, 1.0 Hz, 1H), 7.68−7.62 (m, 2H), 7.58 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 7.4 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 3.77 (s, 3H), 3.59 (s, 2H), 3.32 (s, 2H), 1.29 (d, J = 3.8 Hz, 6H).

*Methyl 2-(4-((2-Methyl-6-nitrophenyl)thio)-3-nitrobenzoyl)-benzoate (**15c**).* Yellow solid, 72 mg, 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (d, J = 1.8 Hz, 1H), 8.10 (d, J = 7.7 Hz, 1H), 7.82 (dd, J = 8.5, 1.7 Hz, 1H), 7.74−7.67 (m, 2H), 7.66−7.59 (m, 3H), 7.40 (d, J = 7.3 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 3.74 (s, 3H), 2.47 (s, 3H).

*Methyl 2-(4-((2-Methylfuran-3-yl)thio)-3-nitrobenzoyl)benzoate (**16c**).* Yellow solid, 45 mg, 57% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.54 (d, J = 1.7 Hz, 1H), 8.11 (d, J = 7.7 Hz, 1H), 7.86 (dd, J = 8.6, 1.7 Hz, 1H), 7.70 (td, J = 7.4, 0.9 Hz, 1H), 7.63 (td, J = 7.7, 1.0 Hz, 1H), 7.49 (d, J = 1.8 Hz, 1H), 7.37 (d, J = 7.2 Hz, 1H), 7.16 (d, J = 8.6 Hz, 1H), 6.39 (d, J = 1.7 Hz, 1H), 3.75 (s, 3H), 2.38 (s, 3H).

*General Procedure for the Synthesis of **17c**−**32c**.* Two different procedures were used to obtain the titled compounds:

Method A: To a flask were added compound **1b** (64 mg, 0.2 mmol), amine (2 equiv, 0.4 mmol), DIPEA (99 μL, 0.6 mmol), and dimethylformamide (DMF) (2 mL). The mixture was heated to reflux for 6 h. After cooling to room temperature, water was added, and the aqueous solution was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated, and purified by chromatography to provide the titled compounds.

Method B: An oven-dried Schlenk tube was charged with compound **1b** (64 mg, 0.3 mmol), amine (1.1 equiv, 0.33 mmol), $Pd(OAc)_2$ (0.09 mmol, 20.2 mg), Xantphos (0.09 mmol, 52.1 mg), Cs_2CO_3 (0.9 mmol, 293 mg), and tetrahydrofuran (THF) (5 mL) and purged with nitrogen. The reaction mixture was stirred at 100 °C for 12 h and then cooled to room temperature. Water was added, and the aqueous phase was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated, and purified by chromatography to provide the titled compounds.

*Methyl 2-(4-((2-Aminophenyl)amino)-3-nitrobenzoyl)benzoate (**17c**).* Method A, purified by chromatography using hexane/EtOAc (8:1), orange solid, 45 mg, 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.45 (s, 1H), 8.51 (d, J = 1.8 Hz, 1H), 8.10 (d, J = 7.7 Hz, 1H), 7.93 (dd, J = 9.0, 1.5 Hz, 1H), 7.67 (t, J = 7.0 Hz, 1H), 7.60 (t, J = 7.2 Hz, 1H), 7.36 (d, J = 7.3 Hz, 1H), 7.22 (t, J = 7.7 Hz, 1H), 7.14 (d, J = 7.7 Hz, 1H), 6.92−6.79 (m, 3H), 3.83 (s, 2H), 3.77 (s, 3H).

*Methyl 2-(3-Nitro-4-(m-tolylamino)benzoyl)benzoate (**18c**).* Method A, purified by chromatography using hexane/EtOAc (10:1), orange solid, 60 mg, 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.87 (s, 1H), 8.47 (d, J = 2.0 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 7.95 (dd, J = 9.0, 1.8 Hz, 1H), 7.68 (td, J = 7.5, 1.0 Hz, 1H), 7.61 (td, J = 7.5, 0.9 Hz, 1H), 7.37 (m, 2H), 7.22 (d, J = 9.1 Hz, 1H), 7.14 (m, 3H), 3.78 (s, 3H), 2.42 (s, 3H).

*Methyl 2-(4-((3-(Hydroxymethyl)phenyl)amino)-3-nitrobenzoyl)-benzoate (**19c**).* Method A, purified by chromatography using hexane/EtOAc (10:1), orange solid, 57 mg, 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.89 (s, 1H), 8.48 (d, J = 1.9 Hz, 1H), 8.11 (d, J = 7.7 Hz, 1H), 7.95 (dd, J = 9.1, 1.7 Hz, 1H), 7.68 (t, J = 7.2, 1H), 7.61 (t, J = 8.2 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.38 (d, J = 7.4 Hz, 1H), 7.36−7.31 (m, 2H), 7.24 (m, 2H), 4.77 (d, J = 5.4 Hz, 2H), 3.77 (s, 3H).

*Methyl 2-(4-((3-Chlorophenyl)amino)-3-nitrobenzoyl)benzoate (**20c**).* Method B, purified by chromatography using hexane/EtOAc (10:1), yellow solid, 77 mg, 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.80 (s, 1H), 8.48 (d, J = 1.9 Hz, 1H), 8.09 (d, J = 7.8 Hz, 1H), 7.96 (dd, J = 9.0, 1.8 Hz, 1H), 7.67 (t, J = 7.4 Hz, 1H), 7.59 (t, J = 7.7 Hz, 1H), 7.42−7.35 (m, 2H), 7.33−7.27 (m, 2H), 7.21 (t, J = 9.6 Hz, 2H), 3.76 (s, 3H).

*Methyl 2-(4-((3-(tert-Butyl)phenyl)amino)-3-nitrobenzoyl)-benzoate (**21c**).* Method A, purified by chromatography using hexane/EtOAc (10:1), orange solid, 74 mg, 86% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.92 (s, 1H), 8.47 (d, J = 1.9 Hz, 1H), 8.11 (d, J = 7.3 Hz, 1H), 7.96 (dd, J = 9.1, 1.7 Hz, 1H), 7.68 (td, J = 7.5, 1.0 Hz, 1H), 7.61 (td, J = 7.7, 1.1 Hz, 1H), 7.44−7.35 (m, 3H), 7.31 (s, 1H), 7.21 (d, J = 9.1 Hz, 1H), 7.14 (d, J = 7.4 Hz, 1H), 3.78 (s, 3H), 1.37 (s, 9H).

*Methyl 2-(4-((3-Carbamoylphenyl)amino)-3-nitrobenzoyl)-benzoate (**22c**).* Method A, purified by chromatography using hexane/EtOAc (8:1), orange solid, 80 mg, 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.90 (s, 1H), 8.52 (d, J = 1.9 Hz, 1H), 8.12 (d, J = 7.7 Hz, 1H), 7.96 (dd, J = 9.0, 1.8 Hz, 1H), 7.83 (s, 1H), 7.72 (d, J = 7.4 Hz, 1H), 7.68 (dd, J = 7.4, 1.0 Hz, 1H), 7.63 (dd, J = 7.7, 1.1 Hz, 1H), 7.61−7.55 (m, 1H), 7.50 (d, J = 8.6 Hz, 1H), 7.39 (dd, J = 7.7, 0.7 Hz, 1H), 7.23 (d, J = 9.0 Hz, 1H), 3.78 (s, 3H).

*Methyl 2-(4-((3,4-Dichlorophenyl)amino)-3-nitrobenzoyl)-benzoate (**23c**).* Method B, purified by chromatography using hexane/EtOAc (10:1), yellow solid, 50 mg, 37% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.77 (s, 1H), 8.50 (d, J = 1.9 Hz, 1H), 8.14−8.10 (m, 1H), 8.02−7.97 (m, 1H), 7.70 (t, J = 7.2 Hz, 1H), 7.62 (t, J = 7.3 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.46 (d, J = 2.4 Hz, 1H), 7.38 (d, J = 7.4 Hz, 1H), 7.22 (d, J = 9.0 Hz, 1H), 7.19 (dd, J = 8.6, 2.4 Hz, 1H), 3.78 (s, 3H).

*Methyl 2-(4-((4-Methoxyphenyl)amino)-3-nitrobenzoyl)-benzoate (**24c**).* Method A, purified by chromatography using hexane/EtOAc (8:1), orange solid, 61 mg, 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.79 (s, 1H), 8.45 (s, 1H), 8.11 (d, J = 7.7 Hz, 1H), 7.93 (d, J = 9.1 Hz, 1H), 7.68 (t, J = 7.5 Hz, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.38 (d, J = 7.3 Hz, 1H), 7.23 (d, J = 7.9 Hz, 2H), 7.03 (m, 3H), 3.88 (s, 3H), 3.77 (s, 3H).

*Methyl 2-(3-Nitro-4-(phenylamino)benzoyl)benzoate (**25c**).* Method A, purified by chromatography using hexane/EtOAc (8:1), orange solid, 70 mg, 93% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.90 (s, 1H), 8.48 (d, J = 1.9 Hz, 1H), 8.11 (d, J = 7.6 Hz, 1H), 7.95 (dd, J = 9.1, 1.8 Hz, 1H), 7.68 (td, J = 7.3, 0.7 Hz, 1H), 7.61 (td, J = 7.4, 0.8 Hz, 1H), 7.49 (t, J = 7.7 Hz, 2H), 7.41−7.29 (m, 4H), 7.21 (d, J = 9.1 Hz, 1H), 3.77 (s, 3H).

*Methyl 2-(3-Nitro-4-(quinolin-8-ylamino)benzoyl)benzoate (**26c**).* Method A, purified by chromatography using hexane/EtOAc (8:1), dark yellow solid, 50 mg, 58% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.35 (s, 1H), 8.98 (dd, J = 4.2, 1.6 Hz, 1H), 8.53 (d, J = 2.1 Hz, 1H), 8.24 (dd, J = 8.3, 1.5 Hz, 1H), 8.13 (d, J = 7.8 Hz, 1H), 8.07 (dd, J = 9.0, 1.9 Hz, 1H), 7.85 (d, J = 8.9 Hz, 2H), 7.70 (td, J = 7.4, 1.1 Hz, 1H), 7.68−7.61 (m, 2H), 7.59 (d, J = 7.7 Hz, 1H), 7.55 (dd, J = 8.3, 4.2 Hz, 1H), 7.45−7.41 (m, 1H), 3.77 (s, 3H).

*Methyl 2-(3-Nitro-4-(thiazol-2-ylamino)benzoyl)benzoate (**27c**).* Method B, purified by chromatography using hexane/EtOAc (10:1), yellow solid, 72 mg, 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.95 (s, 1H), 8.83 (d, J = 9.1 Hz, 1H), 8.60 (d, J = 2.0 Hz, 1H), 8.17−8.05 (m, 2H), 7.70 (td, J = 7.6, 0.9 Hz, 1H), 7.63 (td, J = 7.7, 1.0 Hz, 1H), 7.51 (d, J = 3.6 Hz, 1H), 7.40 (d, J = 7.4 Hz, 1H), 7.02 (d, J = 3.6 Hz, 1H), 3.75 (s, 3H).

*Methyl 2-(4-((2-Aminocyclohexyl)amino)-3-nitrobenzoyl)-benzoate (**28c**).* Method A, purified by chromatography using hexane/EtOAc (5:1), yellow solid, 55 mg, 72%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (d, J = 8.3 Hz, 1H), 8.41 (d, J = 1.9 Hz, 1H), 8.10 (d, J = 7.7 Hz, 1H), 8.04 (d, J = 9.1 Hz, 1H), 7.66 (d, J = 7.3 Hz, 1H), 7.60 (d, J = 7.7 Hz, 1H), 7.36 (d, J = 7.4 Hz, 1H), 7.10 (d, J =

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9.2 Hz, 1H), 3.76 (s, 3H), 3.38 (qd, J = 9.5, 3.9 Hz, 1H), 2.83 (td, J = 9.7, 3.9 Hz, 1H), 2.13 (d, J = 12.4 Hz, 1H), 2.03 (d, J = 10.7 Hz, 1H), 1.83 (s, 2H), 1.44−1.31 (m, 4H).

*Methyl 2-(4-(Benzylamino)-3-nitrobenzoyl)benzoate (**29c**).* Method A, purified by chromatography using hexane/EtOAc (8:1), orange solid, 50 mg, 64% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.82 (t, J = 5.6 Hz, 1H), 8.48 (d, J = 1.9 Hz, 1H), 8.10 (d, J = 7.8 Hz, 1H), 7.99 (dd, J = 9.0, 1.5 Hz, 1H), 7.67 (td, J = 7.4, 0.8 Hz, 1H), 7.60 (td, J = 7.7, 0.9 Hz, 1H), 7.45−7.38 (m, 2H), 7.36 (d, J = 6.9 Hz, 4H), 6.93 (d, J = 9.0 Hz, 1H), 4.63 (d, J = 5.6 Hz, 2H), 3.75 (s, 3H).

*Methyl 2-(4-((2-Fluorobenzyl)amino)-3-nitrobenzoyl)benzoate (**30c**).* Method A, purified by chromatography using hexane/EtOAc (8:1), orange solid, 53 mg, 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.79 (t, J = 4.2 Hz, 1H), 8.48 (d, J = 1.8 Hz, 1H), 8.10 (d, J = 7.8 Hz, 1H), 8.02 (dd, J = 9.0, 1.6 Hz, 1H), 7.67 (t, J = 7.4 Hz, 1H), 7.60 (t, J = 7.1 Hz, 1H), 7.40−7.31 (m, 3H), 7.16 (m, 2H), 6.95 (d, J = 9.1 Hz, 1H), 4.69 (d, J = 5.8 Hz, 2H), 3.75 (s, 3H).

*Methyl 2-(3-Nitro-4-(piperidin-1-yl)benzoyl)benzoate (**31c**).* Method A, purified by chromatography using hexane/EtOAc (15:1), yellow solid, 70 mg, 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, J = 7.6 Hz, 1H), 8.05 (d, J = 2.0 Hz, 1H), 7.90 (dd, J = 8.8, 2.0 Hz, 1H), 7.66 (t, J = 7.2 Hz, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.36 (d, J = 7.4 Hz, 1H), 7.08 (d, J = 8.9 Hz, 1H), 3.75 (s, 3H), 3.22−3.16 (m, 4H), 1.79−1.66 (m, 6H).

*Methyl 2-(4-Morpholino-3-nitrobenzoyl)benzoate (**32c**).* Method A, purified by chromatography using hexane/EtOAc (8:1), yellow solid, 60 mg, 81% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14−8.06 (m, 2H), 7.94 (dd, J = 8.7, 2.0 Hz, 1H), 7.68 (td, J = 7.5, 1.0 Hz, 1H), 7.61 (td, J = 7.5, 0.9 Hz, 1H), 7.41−7.33 (m, 1H), 7.10 (d, J = 8.8 Hz, 1H), 3.90−3.82 (m, 4H), 3.76 (s, 3H), 3.24−3.16 (m, 4H).

*General Procedure for the Synthesis of Compounds **1**−**32**.* The methyl esters of compounds **1c**−**32c** were hydrolyzed with 1 M NaOH solution in THF (1:1) at room temperature overnight. The mixture was then diluted with a small amount of water and washed twice with CH_2Cl_2. The aqueous solution was acidified by the addition of 2 M HCl. The precipitate was collected by filtration and washed with water to afford the titled compounds. If the compound was not pure at this stage of the procedure, it was purified by silica gel column chromatography.

2-(4-((2-Aminophenyl)thio)-3-nitrobenzoyl)benzoic acid (**1**), 2-(4-((3-aminophenyl)thio)-3-nitrobenzoyl)benzoic acid (**5**), 2-(4-((4-aminophenyl)thio)-3-nitrobenzoyl)benzoic acid (**6**), 2-(4-((2-amino-4-chlorophenyl)thio)-3-nitrobenzoyl)benzoic acid (**10**), and 2-(4-((3,4-dichlorophenyl)thio)-3-nitrobenzoyl)benzoic acid (**13**) were synthesized following the previously published procedures.[22]

*2-(4-((2-Methoxyphenyl)thio)-3-nitrobenzoyl)benzoic Acid (**2**).* The titled compound was prepared from the hydrolysis of **2c** (70 mg, 0.16 mmol) in 1 N NaOH (0.8 mL) and THF (0.8 mL). Yellow solid, 60 mg, 92% yield, mp 209−211 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.31 (s, 1H), 8.34 (d, J = 1.7 Hz, 1H), 8.01 (d, J = 7.5 Hz, 1H), 7.75 (dd, J = 13.3, 7.2 Hz, 2H), 7.69 (m, 1H), 7.64 (m, 2H), 7.47 (d, J = 7.3 Hz, 1H), 7.26 (d, J = 8.2 Hz, 1H), 7.13 (t, J = 7.3 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 3.77 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.5, 167.3, 160.5, 144.4, 143.3, 140.8, 137.7, 134.6, 133.8, 133.5, 133.3, 130.7, 130.3, 128.4, 127.8, 126.0, 122.4, 116.4, 113.2, 56.5. HRMS (ESI): calcd for $C_{21}H_{14}NO_6S$, $[M - H]^-$ 408.0547; found, 408.0539. HPLC purity: 97.04%.

*2-(4-((2-Fluorophenyl)thio)-3-nitrobenzoyl)benzoic Acid (**3**).* The titled compound was prepared from the hydrolysis of **3c** (60 mg, 0.15 mmol) in 1 N NaOH (0.7 mL) and THF (0.7 mL). Yellow solid, 43 mg, 72% yield, mp 175−176 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.34 (s, 1H), 8.39 (s, 1H), 8.02 (d, J = 7.5 Hz, 1H), 7.76 (t, J = 7.6 Hz, 3H), 7.70 (dd, J = 13.9, 6.6 Hz, 2H), 7.49 (t, J = 8.3 Hz, 2H), 7.42 (t, J = 7.4 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.4, 168.2 (d, J = 8.1 Hz), 162.8 (d, J = 248.5 Hz), 144.5, 140.7, 139.9 (d, J = 3.0 Hz), 138.4 (d, J = 10.1 Hz), 138.2, 137.2 (d, J = 3.0 Hz), 134.5 (d, J = 8.1 Hz), 133.8, 130.2, 129.8, 129.5, 128.0, 126.9 (d, J = 3.0 Hz), 126.3, 125.4, 117.5 (d, J = 22.2 Hz), 116.7 (d, J = 19.2 Hz). HRMS (ESI): calcd for $C_{20}H_{11}FNO_5S$, $[M - H]^-$ 396.0347; found, 396.0345. HPLC purity: 99.25%.

*2-(3-Nitro-4-(pyridin-2-ylthio)benzoyl)benzoic Acid (**4**).* The titled compound was prepared from the hydrolysis of **4c** (70 mg, 0.18 mmol) in 1 N NaOH (0.9 mL) and THF (0.9 mL). Yellow solid, 40 mg, 58% yield, mp 205−206 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.74 (s, 1H), 8.62 (d, J = 4.0 Hz, 1H), 8.31 (d, J = 1.7 Hz, 1H), 8.02 (d, J = 7.5 Hz, 1H), 7.92 (t, J = 7.0 Hz, 1H), 7.77 (m, 2H), 7.70 (m, 2H), 7.49 (m, 2H), 7.37 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.5, 167.3, 153.6, 151.6, 147.1, 140.7, 139.1, 138.8, 136.0, 133.3, 132.0, 130.8, 130.5, 130.1, 128.8, 128.8, 127.9, 125.4, 124.5. HRMS (ESI): calcd for $C_{19}H_{11}N_2O_5S$, $[M - H]^-$ 379.0394; found, 379.0388. HPLC purity: 100.00%.

*2-(4-((3-(Isopropylamino)phenyl)thio)-3-nitrobenzoyl)benzoic Acid (**7**).* The titled compound was prepared from the hydrolysis of **7c** (65 mg, 0.14 mmol) in 1 N NaOH (0.7 mL) and THF (0.7 mL). Yellow solid, 40 mg, 66% yield, mp 130−132 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.32 (s, 1H), 8.31 (s, 1H), 8.01 (d, J = 7.5 Hz, 1H), 7.80 (d, J = 8.7 Hz, 1H), 7.75 (d, J = 7.5 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 7.46 (d, J = 7.3 Hz, 1H), 7.25 (t, J = 7.7 Hz, 1H), 7.08 (d, J = 8.5 Hz, 1H), 6.78−6.69 (m, 3H), 5.86 (s, 1H), 3.53 (m, 1H), 1.12 (d, J = 6.2 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.5, 167.3, 150.3, 144.8, 144.3, 140.8, 134.4, 133.6, 133.2, 131.5, 130.7, 130.5, 130.2, 129.5, 128.8, 127.9, 126.0, 121.8, 118.6, 114.9, 43.4, 22.7. HRMS (ESI): calcd for $C_{23}H_{19}N_2O_5S$, $[M - H]^-$ 435.102; found, 435.1022. HPLC purity: 97.60%.

*2-(4-((3-Acetamidophenyl)thio)-3-nitrobenzoyl)benzoic Acid (**8**).* The titled compound was prepared from the hydrolysis of **8c** (45 mg, 0.1 mmol) in 1 N NaOH (0.5 mL) and THF (0.5 mL). Yellow solid, 32 mg, 73% yield, mp 144−146 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.33 (s, 1H), 10.21 (s, 1H), 8.34 (s, 1H), 8.01 (d, J = 7.5 Hz, 1H), 7.94 (s, 1H), 7.80−7.65 (m, 4H), 7.51 (t, J = 7.9 Hz, 1H), 7.46 (d, J = 7.3 Hz, 1H), 7.31 (d, J = 7.5 Hz, 1H), 6.99 (d, J = 8.5 Hz, 1H), 2.05 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.4, 169.2, 167.3, 144.5, 143.7, 141.6, 141.0, 134.9, 133.8, 133.3, 131.4, 130.7, 130.2, 130.0, 129.7, 128.9, 127.7, 125.8, 125.5, 121.4, 24.5. HRMS (ESI): calcd for $C_{22}H_{15}N_2O_6S$, $[M - H]^-$ 435.0656; found, 435.0651. HPLC purity: 96.56%.

*2-(4-((3-Benzamidophenyl)thio)-3-nitrobenzoyl)benzoic Acid (**9**).* The titled compound was prepared from the hydrolysis of **9c** (57 mg, 0.1 mmol) in 1 N NaOH (0.5 mL) and THF (0.5 mL). Yellow solid, 31 mg, 57% yield, mp 144−145 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.31 (s, 1H), 10.49 (s, 1H), 8.35 (d, J = 1.6 Hz, 1H), 8.15 (s, 1H), 8.01 (d, J = 7.8 Hz, 2H), 7.95 (d, J = 7.3 Hz, 2H), 7.79 (d, J = 9.3 Hz, 1H), 7.75 (d, J = 7.2 Hz, 1H), 7.69 (t, J = 7.2 Hz, 1H), 7.62 (d, J = 7.3 Hz, 1H), 7.60−7.51 (m, 3H), 7.47 (d, J = 7.1 Hz, 1H), 7.39 (d, J = 7.5 Hz, 1H), 7.06 (d, J = 8.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.5, 167.2, 166.3, 144.6, 143.8, 141.5, 140.7, 135.0, 134.8, 133.9, 133.3, 132.3, 131.4, 130.9, 130.8, 130.5, 130.1, 129.7, 129.0, 128.9, 128.2, 127.8, 126.9, 125.9, 122.7. HRMS (ESI): calcd for $C_{27}H_{17}N_2O_6S$, $[M - H]^-$ 497.0813; found, 497.0805. HPLC purity: 95.26%.

*2-(4-((2,4-Dichlorophenyl)thio)-3-nitrobenzoyl)benzoic Acid (**11**).* The titled compound was prepared from the hydrolysis of **11c** (60 mg, 0.13 mmol) in 1 N NaOH (0.6 mL) and THF (0.6 mL). Yellow solid, 52 mg, 89% yield, mp 260−262 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.40 (S, 1H), 8.39 (d, J = 10.3 Hz, 1H), 8.01 (dd, J = 18.5, 10.5 Hz, 2H), 7.87 (dd, J = 11.7, 8.3 Hz, 1H), 7.82−7.60 (m, 4H), 7.49 (t, J = 8.9 Hz, 1H), 6.92 (t, J = 10.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.4, 167.2, 144.7, 141.1, 140.7, 140.3, 139.8, 137.5, 135.3, 134.2, 133.4, 131.1, 130.8, 130.3, 130.0, 129.8, 128.7, 127.7, 126.0. HRMS (ESI): calcd for $C_{20}H_{10}Cl_2NO_5S$, $[M - H]^-$ 445.9662; found, 445.9656. HPLC purity: 98.13%.

*2-(4-((2,4-Dimethylphenyl)thio)-3-nitrobenzoyl)benzoic Acid (**12**).* The titled compound was prepared from the hydrolysis of **12c** (68 mg, 0.16 mmol) in 1 N NaOH (0.8 mL) and THF (0.8 mL). Yellow solid, 38 mg, 58% yield, mp 240−242 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.34 (s, 1H), 8.00 (d, J = 7.5 Hz, 1H), 7.72 (dd, J = 6.0, 2.7 Hz, 2H), 7.66 (t, J = 7.4 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.44 (d, J = 7.3 Hz, 1H), 7.32 (s, 1H), 7.20 (d, J = 7.6 Hz, 1H), 6.77 (d, J = 8.5 Hz, 1H), 2.36 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.5, 144.5, 143.4, 143.0, 141.8, 141.1, 137.2, 134.8,

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133.8, 133.0, 132.8, 130.8, 130.6, 130.2, 129.2, 127.9, 127.6, 126.1, 125.1, 21.3, 20.4. HRMS (ESI): calcd for $C_{22}H_{16}NO_5S$, $[M - H]^-$ 406.0755; found, 406.0747. HPLC purity: 99.01%.

*2-(4-((4-(Diethylcarbamoyl)-2-nitrophenyl)thio)-3-nitrobenzoyl)-benzoic Acid (**14**).* The titled compound was prepared from the hydrolysis of **14c** (37 mg, 0.07 mmol) in 1 N NaOH (0.4 mL) and THF (0.4 mL). Yellow solid, 25 mg, 69% yield, mp 129−130 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.40 (s, 1H), 8.37 (d, J = 1.7 Hz, 1H), 8.19 (s, 1H), 8.03 (d, J = 7.0 Hz, 1H), 7.86−7.62 (m, 5H), 7.51 (d, J = 7.5 Hz, 1H), 7.37 (d, J = 8.2 Hz, 1H), 3.46 (s, 2H), 3.23 (s, 2H), 1.17 (s, 3H), 1.08 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.5, 167.3, 151.2, 147.2, 140.6, 139.8, 138.5, 136.8, 136.5, 134.0, 133.4, 132.6, 132.2, 130.9, 130.5, 130.2, 127.9, 127.6, 125.5, 123.8, 43.4, 14.4, 13.1. HRMS (ESI): calcd for $C_{25}H_{20}N_3O_8S$, $[M - H]^-$ 522.0977; found, 522.0960. HPLC purity: 97.21%.

*2-(4-((2-Methyl-6-nitrophenyl)thio)-3-nitrobenzoyl)benzoic Acid (**15**).* The titled compound was prepared from the hydrolysis of **15c** (70 mg, 0.15 mmol) in 1 N NaOH (0.8 mL) and THF (0.8 mL). Yellow solid, 50 mg, 76% yield, mp 269−271 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.40 (s, 1H), 8.41 (d, J = 1.6 Hz, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 6.9 Hz, 1H), 7.79 (m, 4H), 7.69 (t, J = 7.4 Hz, 1H), 7.50 (d, J = 7.3 Hz, 1H), 6.86 (d, J = 8.5 Hz, 1H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.4, 167.3, 155.6, 146.5, 144.9, 140.7, 135.4, 135.3, 134.3, 133.4, 133.0, 130.8, 130.3, 130.0, 128.4, 127.8, 126.0, 122.7, 120.8, 20.9. HRMS (ESI): calcd for $C_{21}H_{13}N_2O_7S$, $[M - H]^-$ 437.0449; found, 437.0443. HPLC purity: 99.56%.

*2-(4-((2-Methylfuran-3-yl)thio)-3-nitrobenzoyl)benzoic Acid (**16**).* The titled compound was prepared from the hydrolysis of **16c** (45 mg, 0.11 mmol) in 1 N NaOH (0.5 mL) and THF (0.5 mL). Yellow solid, 31 mg, 73% yield, mp 198−199 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.45 (s, 1H), 8.35 (s, 1H), 8.02 (d, J = 7.5 Hz, 1H), 7.84 (d, J = 1.3 Hz, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.77 (t, J = 7.3 Hz, 1H), 7.70 (t, J = 7.4 Hz, 1H), 7.47 (d, J = 7.3 Hz, 1H), 7.17 (d, J = 8.5 Hz, 1H), 6.58 (s, 1H), 2.33 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.5, 167.3, 158.8, 144.7, 143.8, 143.2, 140.8, 134.85, 133.84, 133.4, 130.8, 130.4, 130.1, 128.4, 127.8, 126.1, 115.3, 105.7, 12.0. HRMS (ESI): calcd for $C_{19}H_{12}NO_6S$, $[M - H]^-$ 382.0391; found, 382.0387. HPLC purity: 98.13%.

*2-(4-((2-Aminophenyl)amino)-3-nitrobenzoyl)benzoic Acid (**17**).* The titled compound was prepared from the hydrolysis of **17c** (40 mg, 0.1 mmol) in 1 N NaOH (0.5 mL) and THF (0.5 mL). Dark yellow solid, 34 mg, 90% yield, mp 201−202 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.46 (s, 1H), 8.20 (d, J = 1.9 Hz, 1H), 7.98 (d, J = 7.4 Hz, 1H), 7.76 (dd, J = 9.0, 1.6 Hz, 1H), 7.65 (dt, J = 22.1, 7.1 Hz, 2H), 7.34 (d, J = 7.2 Hz, 1H), 7.14−7.04 (m, 2H), 6.82 (d, J = 7.7 Hz, 1H), 6.70−6.59 (m, 2H), 5.21 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.3, 151.4, 145.2, 143.4, 137.7, 134.6, 133.4, 133.3, 132.9, 130.6, 130.3, 130.1, 127.9, 127.6, 126.1, 117.3, 115.7, 109.0. HRMS (ESI): calcd for $C_{20}H_{14}N_3O_5$, $[M - H]^-$ 376.0939; found, 376.0936. HPLC purity: 95.02%.

*2-(3-Nitro-4-(m-tolylamino)benzoyl)benzoic Acid (**18**).* The titled compound was prepared from the hydrolysis of **18c** (60 mg, 0.15 mmol) in 1 N NaOH (0.8 mL) and THF (0.8 mL). Dark yellow solid, 38 mg, 66% yield, mp 90−92 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.23 (s, 1H), 9.87 (s, 1H), 8.23 (d, J = 1.9 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.78 (d, J = 10.4 Hz, 1H), 7.73 (d, J = 7.3 Hz, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.43 (d, J = 7.4 Hz, 1H), 7.37 (t, J = 7.7 Hz, 1H), 7.22−7.10 (m, 4H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.8, 167.4, 145.9, 141.2, 139.7, 138.4, 135.6, 135.5, 132.9, 132.2, 130.4, 130.3, 123.0, 128.7, 127.8, 127.5, 126.6, 126.1, 122.7, 117.0, 21.4. HRMS (ESI): calcd for $C_{21}H_{15}N_2O_5$, $[M - H]^-$ 375.0986; found, 375.0985. HPLC purity: 97.89%.

*2-(4-((3-(Hydroxymethyl)phenyl)amino)-3-nitrobenzoyl)benzoic Acid (**19**).* The titled compound was prepared from the hydrolysis of **19c** (50 mg, 0.12 mmol) in 1 N NaOH (0.6 mL) and THF (0.6 mL). Dark yellow solid, 36 mg, 75% yield, mp 132−134 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.22 (s, 1H), 9.90 (s, 1H), 8.24 (d, J = 1.3 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.76 (m, 2H), 7.67 (t, J = 7.4 Hz, 1H), 7.43 (t, J = 7.0 Hz, 2H), 7.32 (s, 1H), 7.25 (t, J = 7.1 Hz, 2H), 7.17 (d, J = 9.1 Hz, 1H), 5.28 (s, 1H), 4.54 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 167.3, 145.9, 144.9, 141.2, 138.4, 135.5, 133.0, 132.3, 130.5, 130.4, 130.3, 129.9, 128.8, 127.9, 126.6, 124.7, 123.8, 123.3, 117.0, 62.9. HRMS (ESI): calcd for $C_{21}H_{15}N_2O_6$, $[M - H]^-$ 391.0936; found, 391.0932. HPLC purity: 95.83%.

*2-(4-((3-Chlorophenyl)amino)-3-nitrobenzoyl)benzoic Acid (**20**).* The titled compound was prepared from the hydrolysis of **20c** (70 mg, 0.18 mmol) in 1 N NaOH (0.9 mL) and THF (0.9 mL). Yellow solid, 45 mg, 63% yield, mp 159−161 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.24 (s, 1H), 9.85 (s, 1H), 8.24 (d, J = 1.8 Hz, 1H), 8.01 (d, J = 7.5 Hz, 1H), 7.78 (s, 1H), 7.74 (s, 1H), 7.67 (s, 1H), 7.54−7.45 (m, 2H), 7.42 (d, J = 7.3 Hz, 1H), 7.40−7.30 (m, 2H), 7.23 (d, J = 9.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 167.4, 144.9, 141.2, 140.5, 135.6, 134.2, 133.2, 132.9, 131.6, 130.6, 130.4, 130.3, 128.4, 127.7, 127.5, 126.2, 125.2, 123.7, 117.5. HRMS (ESI): calcd for $C_{20}H_{12}ClN_2O_5$, $[M - H]^-$ 395.044; found, 395.0434. HPLC purity: 96.87%.

*2-(4-((3-(tert-Butyl)phenyl)amino)-3-nitrobenzoyl)benzoic Acid (**21**).* The titled compound was prepared from the hydrolysis of **21c** (70 mg, 0.16 mmol) in 1 N NaOH (0.8 mL) and THF (0.8 mL). Yellow solid, 56 mg, 84% yield, mp 110−112 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.22 (s, 1H), 9.91 (s, 1H), 8.24 (s, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.77−7.70 (m, 1H), 7.67 (t, J = 7.4 Hz, 1H), 7.46−7.31 (m, 4H), 7.19 (d, J = 7.3 Hz, 1H), 7.15 (d, J = 9.1 Hz, 1H), 1.30 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 167.4, 153.0, 145.9, 141.3, 138.1, 135.5, 132.8, 132.2, 130.8, 130.4, 130.3, 129.7, 128.7, 127.8, 126.6, 123.7, 122.8, 122.6, 116.9, 35.0, 31.5. HRMS (ESI): calcd for $C_{24}H_{21}N_2O_5$, $[M - H]^-$ 417.1456; found, 417.1455. HPLC purity: 98.35%.

*2-(4-((3-Carbamoylphenyl)amino)-3-nitrobenzoyl)benzoic Acid (**22**).* The titled compound was prepared from the hydrolysis of **22c** (70 mg, 0.17 mmol) in 1 N NaOH (0.8 mL) and THF (0.8 mL). Yellow solid, 43 mg, 62% yield, mp 139−141 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.21 (s, 1H), 9.95 (s, 1H), 8.25 (d, J = 1.6 Hz, 1H), 8.02 (s, 1H), 8.01 (d, J = 7.5 Hz, 1H), 7.86 (s, 1H), 7.80 (d, J = 6.3 Hz, 2H), 7.75 (t, J = 7.6 Hz, 1H), 7.67 (t, J = 7.4 Hz, 1H), 7.60−7.50 (m, 2H), 7.49−7.39 (m, 2H), 7.17 (d, J = 9.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 167.6, 167.3, 145.6, 141.2, 138.8, 136.2, 135.6, 133.0, 132.6, 130.5, 130.4, 130.1, 128.6, 128.4, 127.8, 126.9, 125.6, 124.6, 117.2. HRMS (ESI): calcd for $C_{21}H_{14}N_3O_6$, $[M - H]^-$ 404.0888; found, 404.0882. HPLC purity: 96.05%.

*2-(4-((3,4-Dichlorophenyl)amino)-3-nitrobenzoyl)benzoic Acid (**23**).* The titled compound was prepared from the hydrolysis of **23c** (45 mg, 0.1 mmol) in 1 N NaOH (0.5 mL) and THF (0.5 mL). Yellow solid, 30 mg, 70% yield, mp 115−117 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.24 (s, 1H), 9.84 (s, 1H), 8.24 (s, 1H), 8.02 (d, J = 7.3 Hz, 1H), 7.85−7.63 (m, 5H), 7.43 (d, J = 7.3 Hz, 1H), 7.38 (d, J = 7.5 Hz, 1H), 7.27 (d, J = 9.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.8, 167.3, 144.5, 141.1, 139.4, 135.6, 135.5, 133.8, 132.9, 132.8, 132.3, 131.7, 130.4, 128.2, 128.1, 127.9, 127.8, 126.8, 125.1, 117.8. HRMS (ESI): calcd for $C_{20}H_{11}Cl_2N_2O_5$, $[M - H]^-$ 429.0051; found, 429.0041. HPLC purity: 95.09%.

*2-(4-((4-Methoxyphenyl)amino)-3-nitrobenzoyl)benzoic Acid (**24**).* The titled compound was prepared from the hydrolysis of **24c** (61 mg, 0.15 mmol) in 1 N NaOH (0.8 mL) and THF (0.8 mL). Yellow solid, 42 mg, 71% yield, mp 225−226 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.17 (s, 1H), 9.87 (s, 1H), 8.22 (s, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 9.8 Hz, 1H), 7.73 (d, J = 7.2 Hz, 1H), 7.67 (t, J = 7.4 Hz, 1H), 7.42 (d, J = 7.3 Hz, 1H), 7.29 (d, J = 8.7 Hz, 2H), 7.05 (d, J = 8.7 Hz, 2H), 6.99 (d, J = 9.1 Hz, 1H), 3.80 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.8, 167.3, 158.4, 147.0, 141.3, 135.4, 132.9, 131.5, 130.9, 130.4, 130.3, 128.9, 128.0, 127.8, 126.0, 116.7, 115.4, 55.8. HRMS (ESI): calcd for $C_{21}H_{15}N_2O_6$, $[M - H]^-$ 391.0936; found, 391.0929. HPLC purity: 98.67%.

*2-(3-Nitro-4-(phenylamino)benzoyl)benzoic Acid (**25**).* The titled compound was prepared from the hydrolysis of **25c** (70 mg, 0.18 mmol) in 1 N NaOH (0.9 mL) and THF (0.9 mL). Yellow solid, 55 mg, 82% yield, mp 184−186 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.28 (s, 1H), 9.90 (s, 1H), 8.23 (d, J = 1.7 Hz, 1H), 8.01 (d, J = 7.6

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Hz, 1H), 7.78 (dd, J = 9.0, 1.5 Hz, 1H), 7.73 (t, J = 7.4 Hz, 1H), 7.66 (t, J = 7.3 Hz, 1H), 7.48 (t, J = 7.7 Hz, 2H), 7.41 (d, J = 7.4 Hz, 1H), 7.38 (d, J = 7.6 Hz, 2H), 7.31 (t, J = 7.3 Hz, 1H), 7.15 (d, J = 9.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 167.5, 145.8, 141.3, 138.6, 135.5, 132.8, 132.3, 130.8, 130.4, 130.2, 130.2, 128.7, 127.7, 126.8, 126.8, 125.7, 116.9. HRMS (ESI): calcd for $C_{20}H_{13}N_2O_5$, $[M - H]^-$ 361.083; found, 361.0828. HPLC purity: 100.00%.

*2-(3-Nitro-4-(quinolin-8-ylamino)benzoyl)benzoic Acid (**26**).* The titled compound was prepared from the hydrolysis of **26c** (50 mg, 0.12 mmol) in 1 N NaOH (0.6 mL) and THF (0.6 mL), deep yellow solid, 36 mg, yield 73%, mp 151−153 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.17 (s, 1H), 8.97 (s, 1H), 8.46 (d, J = 7.4 Hz, 1H), 8.31 (s, 1H), 8.01 (d, J = 4.5 Hz, 1H), 7.91 (d, J = 6.6 Hz, 1H), 7.83 (s, 2H), 7.76 (d, J = 7.5 Hz, 1H), 7.70−7.52 (m, 4H), 7.29 (d, J = 4.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.2, 142.5, 141.1, 140.3, 137.2, 135.8, 135.3, 134.3, 131.0, 130.2, 129.6, 129.4, 129.1, 128.0, 127.3, 126.9, 123.5, 123.06, 117.5, 117.2. HRMS (ESI): calcd for $C_{23}H_{14}N_3O_5$, $[M - H]^-$ 412.0939; found, 412.0937. HPLC purity: 96.02%.

*2-(3-Nitro-4-(thiazol-2-ylamino)benzoyl)benzoic Acid (**27**).* The titled compound was prepared from the hydrolysis of **27c** (60 mg, 0.16 mmol) in 1 N NaOH (0.8 mL) and THF (0.8 mL). Yellow solid, 39 mg, 66% yield, mp 99−100 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.23 (s, 1H), 10.78 (s, 1H), 8.44 (d, J = 8.4 Hz, 1H), 8.18 (s, 1H), 8.03 (d, J = 7.6 Hz, 1H), 7.92 (d, J = 8.8 Hz, 1H), 7.77 (t, J = 7.3 Hz, 1H), 7.70 (t, J = 7.3 Hz, 1H), 7.46 (m, 2H), 7.30 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.1, 167.3, 161.9, 141.0, 140.3, 139.2, 135.9, 135.2, 133.1, 130.5, 130.5, 130.3, 129.7, 127.9, 127.1, 120.4, 114.5. HRMS (ESI): calcd for $C_{17}H_{10}N_3O_5S$, $[M - H]^-$ 368.0347; found, 368.0343. HPLC purity: 98.63%.

*2-(4-((2-Aminocyclohexyl)amino)-3-nitrobenzoyl)benzoic Acid (**28**).* The titled compound was prepared from the hydrolysis of **28c** (50 mg, 0.13 mmol) in 1 N NaOH (0.6 mL) and THF (0.6 mL). The purification by chromatography used DCM/MeOH (10:1). Yellow solid, 33 mg, 68% yield, decomposition temperature 265 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.15 (m, 2H), 7.94 (dd, J = 5.5, 3.4 Hz, 1H), 7.62 (d, J = 0.5 Hz, 1H), 7.47 (dd, J = 5.4, 3.3 Hz, 2H), 7.17 (d, J = 9.2 Hz, 1H), 7.12 (dd, J = 5.3, 3.3 Hz, 1H), 3.94 (m, 1H), 3.20 (m, 1H), 1.96−1.80 (m, 2H), 1.65 (d, J = 10.3 Hz, 2H), 1.55 (m, 1H), 1.43 (m, 1H), 1.38−1.10 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 195.5, 169.5, 147.0, 141.3, 138.7, 135.6, 131.1, 123.0, 129.6, 128.8, 128.2, 126.7, 126.2, 115.1, 55.2, 53.7, 31.6, 31.1, 24.4, 23.9. HRMS (ESI): calcd for $C_{20}H_{20}N_3O_5$, $[M - H]^-$ 382.1408; found, 382.1406. HPLC purity: 98.62%.

*2-(4-(Benzylamino)-3-nitrobenzoyl)benzoic Acid (**29**).* The titled compound was prepared from the hydrolysis of **29c** (50 mg, 0.13 mmol) in 1 N NaOH (0.6 mL) and THF (0.6 mL). Yellow solid, 35 mg, 73% yield, mp 88−90 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.18 (s, 1H), 9.18 (t, J = 5.8 Hz, 1H), 8.22 (d, J = 1.7 Hz, 1H), 7.99 (d, J = 7.5 Hz, 1H), 7.71 (t, J = 7.1 Hz, 2H), 7.65 (t, J = 7.4 Hz, 1H), 7.42−7.32 (m, 5H), 7.27 (t, J = 6.7 Hz, 1H), 7.03 (d, J = 9.2 Hz, 1H), 4.71 (d, J = 6.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 167.4, 147.7, 141.37, 138.3, 135.6, 132.8, 131.1, 130.4, 130.4, 130.2, 129.1, 128.9, 127.8, 127.7, 127.4, 125.0, 115.7, 46.3. HRMS (ESI): calcd for $C_{21}H_{15}N_2O_5$, $[M - H]^-$ 375.0986; found, 375.0982. HPLC purity: 99.55%.

*2-(4-((2-Fluorobenzyl)amino)-3-nitrobenzoyl)benzoic Acid (**30**).* The titled compound was prepared from the hydrolysis of **30c** (50 mg, 0.13 mmol) in 1 N NaOH (0.6 mL) and THF (0.6 mL). Yellow solid, 35 mg, 73% yield, mp 100−102 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.18 (s, 1H), 9.06 (t, J = 5.9 Hz, 1H), 8.24 (d, J = 1.9 Hz, 1H), 7.99 (d, J = 7.5 Hz, 1H), 7.73 (dd, J = 18.3, 8.4 Hz, 2H), 7.65 (t, J = 7.2 Hz, 1H), 7.36 (m, 3H), 7.28−7.21 (m, 1H), 7.17 (t, J = 7.4 Hz, 1H), 7.03 (d, J = 9.1 Hz, 1H), 4.77 (d, J = 6.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 167.4, 160.6 (d, J = 245.4 Hz), 147.6, 141.3, 135.8, 132.8, 131.2, 130.3 (d, J = 17.2 Hz), 129.8 (d, J = 8.1 Hz), 129.3 (d, J = 4.2 Hz), 128.9, 127.8, 125.2, 125.13, 125.10, 125.0, 124.9, 115.9 (d, J = 21.2 Hz), 115.4, 40.5 (d, J = 4.0 Hz). HRMS (ESI): calcd for $C_{21}H_{14}FN_2O_5$, $[M - H]^-$ 393.0892; found, 393.0890. HPLC purity: 99.58%.

*2-(3-Nitro-4-(piperidin-1-yl)benzoyl)benzoic Acid (**31**).* The titled compound was prepared from the hydrolysis of **31c** (70 mg, 0.19 mmol) in 1 N NaOH (1 mL) and THF (1 mL). Yellow solid, 58 mg, 86% yield, mp 191−193 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.23 (s, 1H), 8.00 (d, J = 7.6 Hz, 1H), 7.93 (d, J = 2.0 Hz, 1H), 7.72 (t, J = 7.0 Hz, 1H), 7.70−7.67 (m, 1H), 7.65 (t, J = 5.5 Hz, 1H), 7.41 (d, J = 7.3 Hz, 1H), 7.31 (d, J = 8.9 Hz, 1H), 3.14 (s, 4H), 1.60 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 167.4, 148.9, 141.4, 138.8, 134.0, 132.8, 130.5, 130.34, 130.26, 128.0, 127.8, 127.5, 120.4, 51.7, 25.6, 23.7. HRMS (ESI): calcd for $C_{19}H_{17}N_2O_5$, $[M - H]^-$ 353.1143; found, 353.1140. HPLC purity: 99.21%.

*2-(4-Morpholino-3-nitrobenzoyl)benzoic Acid (**32**).* The titled compound was prepared from the hydrolysis of **32c** (60 mg, 0.17 mmol) in 1 N NaOH (0.8 mL) and THF (0.8 mL), yellow solid, 42 mg, 69% yield, mp 192−193 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.23 (s, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.98 (d, J = 1.8 Hz, 1H), 7.78−7.70 (m, 2H), 7.67 (t, J = 7.5 Hz, 1H), 7.43 (d, J = 7.3 Hz, 1H), 7.35 (d, J = 8.9 Hz, 1H), 3.70 (m, 4H), 3.17 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.1, 167.3, 148.3, 141.2, 139.4, 134.2, 133.3, 130.4, 130.2, 128.5, 127.8, 120.6, 66.2, 50.9. HRMS (ESI): calcd for $C_{18}H_{15}N_2O_6$, $[M - H]^-$ 355.0936; found, 355.0933. HPLC purity: 100.00%.

Synthetic Procedures for Scheme 2. *2-(4-Bromobenzoyl)-benzoic Acid (**33b**).* To a suspension of the phthalic anhydride (2 g, 13.5 mmol) in bromobenzene (11.38 mL, 108 mmol) was added aluminum chloride (2.7 g, 20.3 mmol). The mixture was stirred at 90 °C for 2 h and then cooled to room temperature. The reaction mixture was poured onto crushed ice, followed by conc. HCl (5 mL), the mixture was extracted with DCM and basified with 10% Na_2CO_3 solution for product extraction into the aqueous phase. The aqueous phase was acidified to pH 3 with conc. HCl. The resulting precipitate was collected by filtration and dried in vacuo. **33b** was obtained as a white solid without further purification, 3.5 g, 85% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.25 (s, 1H), 8.00 (d, J = 7.5 Hz, 1H), 7.79−7.60 (m, 4H), 7.54 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 7.5 Hz, 1H).

*Methyl 2-(4-Bromobenzoyl)benzoate (**33c**).* To a solution of compound **33b** (500 mg) in MeOH (10 mL) was added $SOCl_2$ (10 drops). The mixture was heated to reflux and stirred overnight. The reaction mixture was cooled down to room temperature, and the white solid precipitate was collected and dried to provide compound **33c** as a white solid, 450 mg, 86% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, J = 7.7 Hz, 1H), 7.69−7.63 (m, 1H), 7.63−7.55 (m, 5H), 7.39 (d, J = 7.6 Hz, 1H), 3.67 (s, 3H).

*Methyl 2-(4-((3,4-Dichlorophenyl)thio)benzoyl)benzoate (**33d**).* To a round-bottom flask were added compound **33c** (64 mg, 02 mmol), i-Pr_2NEt (66 μL, 0.4 mmol), and dry 1,4-dioxane. The mixture was degassed and filled with nitrogen (3 cycles). $Pd_2(dba)_3$ (18 mg, 0.02 mmol), xantphos (23 mg, 0.04 mmol), and 3,4-dichlorothiophenol (26 μL, 0.2 mmol) were added, and then, the mixture was degassed twice more. The mixture was stirred at reflux overnight. The reaction mixture was cooled down to room temperature, filtered, and concentrated. The crude product was purified by flash column chromatography on silica gel using hexane/EtOAc (10:1) to afford thioether **33d** as a yellow solid, 65 mg, 77.9% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (d, J = 7.5 Hz, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.65 (dd, J = 7.5, 1.0 Hz, 1H), 7.59 (td, J = 7.6, 0.9 Hz, 1H), 7.55 (d, J = 2.1 Hz, 1H), 7.46 (d, J = 8.3 Hz, 1H), 7.43−7.38 (m, 1H), 7.30 (d, J = 2.1 Hz, 1H), 7.26 (d, J = 8.5 Hz, 2H), 3.69 (s, 3H).

*2-(4-((3,4-Dichlorophenyl)thio)benzoyl)benzoic Acid (**33**).* To a solution of **33d** (42 mg, 0.1 mmol) in dioxane (0.5 mL) was added 1 N NaOH (0.5 mL) at room temperature. The mixture was heated to 50 °C. After stirring for 12 h, the mixture was cooled down to room temperature and diluted with a small amount of water. The mixture was washed with DCM, and the aqueous phase was collected and acidified with 1 N HCl solution. The precipitate was filtered and dried to provide compound **33** as a gray solid, 20 mg, 50% yield. mp 164−166 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.22 (s, 1H), 7.99 (d, J = 7.6 Hz, 1H), 7.72 (dd, J = 12.2, 7.3 Hz, 3H), 7.66 (t, J = 7.5 Hz,

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1H), 7.59 (d, J = 8.2 Hz, 2H), 7.42 (d, J = 6.7 Hz, 2H), 7.37 (d, J = 8.3 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.0, 167.3, 141.6, 135.9, 134.2, 133.6, 133.0, 132.7, 132.3, 132.0, 130.3, 129.2, 127.8. HRMS (ESI): calcd for $C_{20}H_{11}Cl_2O_3S$, $[M - H]^-$ 400.9811; found, 400.9806. HPLC purity 95.07%.

Synthetic Procedures for Scheme 3. *Methyl 2-(3-Amino-4-((3,4-dichlorophenyl)thio)benzoyl)benzoate (**34b**).* **13c** (46 mg, 0.1 mmol), iron powder (28 mg, 0.5 mmol), and saturated ammonium chloride solution (1 mL) and EtOH (4 mL) were added to a round-bottom flask and heated to reflux for 1 h. The reaction mixture was cooled down to room temperature and filtered. The precipitate was washed with ethyl acetate, and the combined solution was then washed twice with NaCl solution. The organic phase was dried with anhydrous Na_2SO_4 and concentrated to provide **34b** (35 mg) as yellow jelly which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, J = 7.7 Hz, 1H), 7.67 (t, J = 7.3 Hz, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.45 (dd, J = 7.5, 4.0 Hz, 2H), 7.31 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 1.5 Hz, 1H), 7.17 (d, J = 2.1 Hz, 1H), 7.02 (dd, J = 8.0, 1.6 Hz, 1H), 6.95 (dd, J = 8.4, 2.1 Hz, 1H), 4.40 (s, 2H), 3.70 (s, 3H).

*2-(3-Amino-4-((3,4-dichlorophenyl)thio)benzoyl)benzoic Acid (**34**).* To a mixture of **34b** (35 mg, 0.08 mmol) in dioxane (0.5 mL) was added 1 N NaOH solution (0.5 mL). The mixture was heated to 50 °C. After stirring for 12 h, the mixture was cooled down to room temperature and diluted with a small amount of water. The mixture was washed with DCM, and the aqueous phase was collected and acidified by 1 N HCl solution. The precipitate was filtered and dried to afford compound **34** as a yellow solid, 26 mg, 62% yield. mp 188−190 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.17 (s, 1H), 7.98 (d, J = 7.5 Hz, 1H), 7.71 (t, J = 7.2 Hz, 1H), 7.64 (t, J = 7.3 Hz, 1H), 7.55 (d, J = 8.5 Hz, 1H), 7.40 (d, J = 7.9 Hz, 2H), 7.32 (d, J = 1.9 Hz, 1H), 7.12 (d, J = 1.1 Hz, 1H), 7.02 (dd, J = 8.5, 2.0 Hz, 1H), 6.80 (dd, J = 7.9, 1.3 Hz, 1H), 5.73 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.5, 150.5, 142.3, 140.0, 137.3, 137.0, 132.7, 132.1, 131.5, 130.8, 130.1, 128.9, 128.7, 127.6, 117.0, 116.1, 115.6. HRMS (ESI): calcd for $C_{20}H_{12}Cl_2NO_3S$, $[M - H]^-$ 415.992; found, 415.9923. HPLC purity 95.38%.

Synthetic Procedures for Scheme 4. *Methyl 2-(4-Fluoro-3-nitrobenzamido)benzoate (**35c**).* To a solution of 4-fluoro-3-nitrobenzoic acid **3a** (185 mg, 1 mmol) in dry CH_2Cl_2 (5 mL) were added oxalyl chloride (2 mmol) and five drops of dry DMF under a nitrogen atmosphere. The solution was stirred at room temperature overnight. The solution was concentrated to remove the excess of oxalyl chloride. The resulting acyl chloride solution was then added dropwise to a solution of methyl 2-aminobenzoate (1 mmol) and Et_3N (0.279 mL, 2 mmol) in dry CH_2Cl_2 (5 mL) under a nitrogen atmosphere, and the mixture was stirred at room temperature overnight. The solution was then quenched with water and extracted with CH_2Cl_2. The organic phase was washed with 1 M HCl and brine successively, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified via silica gel column using hexane/EtOAc (8:1) to provide **35c**; yellow solid, 190 mg, 60%, yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.30 (s, 1H), 8.88 (d, J = 8.4 Hz, 1H), 8.81 (d, J = 6.6 Hz, 1H), 8.34 (d, J = 5.6 Hz, 1H), 8.14 (d, J = 7.8 Hz, 1H), 7.67 (t, J = 7.6 Hz, 1H), 7.49 (t, J = 9.2 Hz, 1H), 7.21 (t, J = 7.5 Hz, 1H), 4.02 (s, 3H).

*Methyl 3-(4-Fluoro-3-nitrobenzamido)benzoate (**36c**).* The titled compound was prepared by condensing methyl 3-aminobenzoate and 4-fluoro-3-nitrobenzoic acid in a similar manner as described for compound **35c**. An eluent of hexane/EtOAc (8:1) was used for chromatography. Yellow solid, 200 mg, 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (dd, J = 6.8, 2.1 Hz, 1H), 8.30−8.24 (m, 1H), 8.19 (s, 1H), 8.07 (s, 1H), 8.03 (d, J = 8.2 Hz, 1H), 7.90 (d, J = 7.7 Hz, 1H), 7.55−7.44 (m, 2H), 3.95 (s, 3H).

*Methyl 4-(4-Fluoro-3-nitrobenzamido)benzoate (**37c**).* The titled compound was prepared by condensing methyl 4-aminobenzoate and 4-fluoro-3-nitrobenzoic acid in a similar manner as described for compound **35c**. An eluent of hexane/EtOAc (8:1) was used for chromatography. Yellow solid, 130 mg, 41% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (d, J = 4.9 Hz, 1H), 8.32−8.22 (m, 1H), 8.12 (d, J = 8.6 Hz, 2H), 7.77 (d, J = 8.6 Hz, 2H), 7.49 (t, J = 9.3 Hz, 1H), 3.95 (s, 3H).

*Methyl 3-(4-((2-Aminophenyl)thio)-3-nitrobenzamido)benzoate (**35d**).* To a flask were added **35c** (64 mg, 0.2 mmol), 2-aminobenzenethiol (30 mg, 0.24 mmol), NaOAc (82 mg, 1 mmol), and EtOH (5 mL). The mixture was stirred at room temperature overnight. Water was added, and then, the solution was extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and purified by chromatography using hexane/EtOAc (10:1) to provide **35d** as a pale yellow solid, 45 mg, 53% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, 2H), 7.94 (d, J = 8.0 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.36−7.29 (m, 2H), 6.83 (td, J = 8.0, 1.8 Hz, 3H), 6.65 (t, J = 7.5 Hz, 1H), 6.53 (d, J = 8.2 Hz, 1H), 4.49 (d, J = 5.7 Hz, 2H), 4.30 (s, 2H), 3.89 (s, 3H).

*Methyl 3-(4-((2-Aminophenyl)thio)-3-nitrobenzamido)benzoate (**36d**).* To a flask were added **36c** (64 mg, 0.2 mmol), 2-aminobenzenethiol (30 mg, 0.24 mmol), NaOAc (82 mg, 1 mmol), and EtOH (5 mL). The mixture was stirred at room temperature overnight. Water was added, and then the aqueous solution was extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and purified by chromatography using hexane/EtOAc (8:1) to provide **36d** as a yellow solid, 75 mg, 89% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.57 (s, 1H), 8.86 (s, 1H), 8.13 (s, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.6 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.43 (t, J = 7.9 Hz, 2H), 7.34 (t, J = 7.7 Hz, 1H), 6.95 (d, J = 8.5 Hz, 1H), 6.84 (m, 2H), 4.39 (s, 2H), 3.91 (s, 3H).

*Methyl 4-(4-((2-Aminophenyl)thio)-3-nitrobenzamido)benzoate (**37d**).* To a flask were added **37c** (64 mg, 0.2 mmol), 2-aminobenzenethiol (30 mg, 0.24 mmol), NaOAc (82 mg, 1 mmol), and EtOH (5 mL). The mixture was stirred at room temperature overnight. Water was added, and then, the aqueous solution was extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and purified by chromatography using hexane/EtOAc (8:1) to provide **37d** as a yellow solid, 60 mg, 71% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.75 (s, 1H), 8.86 (d, J = 1.5 Hz, 1H), 8.09 (dd, J = 8.5, 1.6 Hz, 1H), 7.95 (dd, J = 22.2, 8.8 Hz, 4H), 7.39 (d, J = 7.6 Hz, 1H), 7.30 (t, J = 7.3 Hz, 1H), 6.91 (d, J = 8.6 Hz, 1H), 6.86 (d, J = 8.1 Hz, 1H), 6.68 (t, J = 7.2 Hz, 1H), 5.62 (s, 2H), 3.84 (s, 3H).

*Methyl 2-(4-((3,4-Dichlorophenyl)thio)-3-nitrobenzamido)-benzoate (**41d**).* To a flask were added **35c** (64 mg, 0.2 mmol), 3, 4-dichlorobenzenethiol (43 mg, 0.24 mmol), NaOAc (82 mg, 1 mmol), and EtOH (5 mL). The mixture was stirred at room temperature overnight. Water was added, and then, the solution was extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and purified by chromatography using hexane/EtOAc (8:1) to provide **41d** as a yellow solid, 83 mg, 87% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.24 (s, 1H), 8.98 (d, J = 1.6 Hz, 1H), 8.89 (d, J = 8.5 Hz, 1H), 8.13 (d, J = 7.9 Hz, 1H), 8.04 (dd, J = 8.5, 1.8 Hz, 1H), 7.76 (d, J = 1.8 Hz, 1H), 7.65 (t, J = 8.6 Hz, 2H), 7.48 (dd, J = 8.2, 1.8 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.04 (d, J = 8.5 Hz, 1H), 4.00 (s, 3H).

*Methyl 3-(4-((3,4-Dichlorophenyl)thio)-3-nitrobenzamido)-benzoate (**42d**).* To a flask were added **36c** (64 mg, 0.2 mmol), 3, 4-dichlorobenzenethiol (43 mg, 0.24 mmol), NaOAc (82 mg, 1 mmol), and EtOH (5 mL). The mixture was stirred at room temperature overnight. Water was added, and then, the solution was extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and purified by chromatography using hexane/EtOAc (8:1) to provide **42d** as a yellow solid, 72 mg, 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.76 (d, J = 1.9 Hz, 1H), 8.19 (t, J = 2.0 Hz, 1H), 8.03 (m, 2H), 7.98 (dd, J = 8.5, 2.0 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.74 (d, J = 2.0 Hz, 1H), 7.64 (d, J = 8.3 Hz, 1H), 7.53−7.45 (m, 2H), 7.03 (d, J = 8.5 Hz, 1H), 3.95 (s, 3H).

*General Procedure for the Synthesis of Compounds **35**−**37**, **41**, and **42**.* The methyl esters of the titled compounds (**35d**−**37d**, **41d**, and **42d**) were hydrolyzed with 1 M NaOH in THF (1:1) at room temperature overnight. The mixture was then diluted with a small amount of water and washed with CH_2Cl_2. The organic phase was

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acidified by the addition of 2 M HCl. The precipitate was filtered and washed with water to afford the titled compounds.

*2-(4-((2-Aminophenyl)thio)-3-nitrobenzamido)benzoic Acid (**35**).* The titled compound was prepared from the hydrolysis of **35d** (42 mg, 0.1 mmol) in 1 N NaOH (0.5 mL) and THF (0.5 mL). Yellow solid, 30 mg, 73% yield, mp 259−261 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.86 (s, 1H), 12.27 (s, 1H), 8.81 (s, 1H), 8.61 (d, J = 8.3 Hz, 1H), 8.06 (d, J = 7.2 Hz, 2H), 7.68 (t, J = 7.5 Hz, 1H), 7.39 (d, J = 7.4 Hz, 1H), 7.30 (t, J = 7.4 Hz, 1H), 7.24 (t, J = 7.5 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 6.85 (d, J = 8.1 Hz, 1H), 6.68 (t, J = 7.3 Hz, 1H), 5.65 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.5, 162.7, 151.3, 145.3, 142.3, 141.0, 137.7, 134.7, 132.9, 132.2, 131.71, 131.69, 128.0, 124.9, 123.8, 120.6, 117.6, 117.3, 115.7, 109.2. HRMS (ESI): calcd for $C_{20}H_{14}N_3O_5S$, $[M - H]^-$ 408.0660; found, 408.0656. HPLC purity: 99.30%.

*3-(4-((2-Aminophenyl)thio)-3-nitrobenzamido)benzoic Acid (**36**).* The titled compound was prepared from the hydrolysis of **36d** (42 mg, 0.1 mmol) in 1 N NaOH (0.5 mL) and THF (0.5 mL). Yellow solid, 35 mg, 85% yield, mp 251−252 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.02 (s, 1H), 10.64 (s, 1H), 8.89 (d, J = 1.7 Hz, 1H), 8.39 (s, 1H), 8.10 (dd, J = 8.5, 1.7 Hz, 1H), 8.05 (d, J = 7.9 Hz, 1H), 7.70 (d, J = 7.7 Hz, 1H), 7.50 (t, J = 7.9 Hz, 1H), 7.39 (dd, J = 7.6, 1.0 Hz, 1H), 7.33−7.26 (m, 1H), 6.88 (dd, J = 19.5, 8.3 Hz, 2H), 6.68 (t, J = 7.1 Hz, 1H), 5.63 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.7, 163.6, 151.3, 145.2, 141.6, 139.4, 137.7, 133.1, 132.8, 131.8, 129.4, 127.4, 125.7, 125.2, 124.8, 121.7, 117.3, 115.7, 109.4. HRMS (ESI): calcd for $C_{20}H_{14}N_3O_5S$, $[M - H]^-$ 408.0660; found, 408.0653. HPLC purity: 98.48%.

*4-(4-((2-Aminophenyl)thio)-3-nitrobenzamido)benzoic Acid (**37**).* The titled compound was prepared from the hydrolysis of **37d** (42 mg, 0.1 mmol) in 1 N NaOH (0.5 mL) and THF (0.5 mL). Yellow solid, 33 mg, 82.5% yield, mp 247−249 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.79 (s, 1H), 10.73 (s, 1H), 8.86 (s, 1H), 8.09 (d, J = 7.7 Hz, 1H), 7.95 (m, 2H), 7.90 (m, 2H), 7.39 (m, 1H), 7.30 (m, 1H), 6.90 (m, 1H), 6.85 (m, 1H), 6.67 (m, 1H), 5.63 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.4, 163.9, 151.3, 145.2, 143.3, 141.8, 137.7, 133.1, 132.8, 131.8, 130.7, 127.4, 126.3, 125.8, 120.1, 117.3, 115.7, 109.4. HRMS (ESI): calcd for $C_{20}H_{14}N_3O_5S$, $[M - H]^-$ 408.0660; found, 408.0652. HPLC purity: 95.33%.

*2-(4-((3,4-Dichlorophenyl)thio)-3-nitrobenzamido)benzoic Acid (**41**).* The titled compound was prepared from the hydrolysis of **41d** (48 mg, 0.1 mmol) in 1 N NaOH (0.5 mL) and THF (0.5 mL). Yellow solid, 27 mg, 58.3% yield, mp 279−281 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.78 (s, 1H), 8.79 (s, 1H), 8.61 (d, J = 8.3 Hz, 1H), 8.11 (d, J = 8.5 Hz, 1H), 8.06 (d, J = 7.8 Hz, 1H), 8.03 (d, J = 1.8 Hz, 1H), 7.85 (d, J = 8.3 Hz, 1H), 7.71−7.59 (m, 2H), 7.21 (dd, J = 14.5, 8.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.5, 162.3, 145.1, 141.4, 140.9, 137.2, 135.9, 134.3, 134.1, 134.0, 133.3, 132.85, 132.80, 132.7, 131.7, 131.1, 129.8, 124.8, 123.7, 120.4. HRMS (ESI): calcd for $C_{20}H_{11}Cl_2N_2O_5S$, $[M - H]^-$ 460.9771; found, 460.9767. HPLC purity: 95.02%.

*3-(4-((3,4-Dichlorophenyl)thio)-3-nitrobenzamido)benzoic Acid (**42**).* The titled compound was prepared from the hydrolysis of **42d** (48 mg, 0.1 mmol) in 1 N NaOH (0.5 mL) and THF (0.5 mL). Yellow solid, 40 mg, 86.3% yield, mp 280−282 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.70 (s, 1H), 8.89 (s, 1H), 8.38 (s, 1H), 8.15 (d, J = 8.1 Hz, 1H), 8.04 (d, J = 7.6 Hz, 1H), 8.00 (s, 1H), 7.83 (d, J = 8.1 Hz, 1H), 7.70 (d, J = 7.3 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.6, 163.4, 145.0, 140.9, 139.4, 137.1, 135.9, 134.2, 133.8, 133.3, 132.8, 132.7, 131.9, 131.3, 129.4, 129.3, 125.4, 125.3, 124.9, 121.7. HRMS (ESI): calcd for $C_{20}H_{11}Cl_2N_2O_5S$, $[M - H]^-$ 460.9771; found, 460.9766. HPLC purity: 96.76%.

Synthetic Procedures for Scheme 5. *4-Fluoro-3-nitrobenzaldehyde (**4b**).* 4-Fluorobenzaldehyde **4a** (1.24 g, 10 mmol) was slowly added dropwise to a solution of H_2SO_4 (8 mL) and HNO_3 (1 mL) at 0 − 5 °C. The temperature was kept under 5 °C. After the addition was completed, the solution was warmed to room temperature over 1 h. The reaction mixture was poured into ice; the precipitate was filtered, washed with ice water, and dried to provide compound **4b**; white solid, 1.55 g, 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.06 (s, 1H), 8.61 (dd, J = 7.0, 1.6 Hz, 1H), 8.22 (ddd, J = 8.3, 4.0, 1.9 Hz, 1H), 7.57−7.45 (m, 1H).

*Methyl 2-((4-Fluoro-3-nitrobenzyl)amino)benzoate (**38c**).* In a dried round-bottom flask, **4b** (169 mg, 1 mmol) and methyl 2-aminobenzoate (151 mg, 1 mmol) were dissolved in CH_2Cl_2 (10 mL) and stirred at room temperature for 30 min. Then, the reaction was cooled down to 0 °C, and $NaBH(OAc)_3$ (636 mg, 3 mmol) was added in three portions over 1 h. The reaction was allowed to stir at room temperature overnight and quenched with a saturated aqueous solution of sodium bicarbonate. The mixture was extracted with CH_2Cl_2. The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography using hexane/EtOAc (10:1) to give the titled compound; yellow solid, 155 mg, yield 51%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (s, 1H), 8.05 (d, J = 6.8 Hz, 1H), 7.97 (d, J = 7.9 Hz, 1H), 7.64 (m, 1H), 7.35−7.23 (m, 2H), 6.68 (t, J = 7.4 Hz, 1H), 6.51 (d, J = 6.9 Hz, 1H), 4.53 (d, J = 5.0 Hz, 2H), 3.91 (s, 3H).

*Methyl 3-((4-Fluoro-3-nitrobenzyl)amino)benzoate (**39c**).* The titled compound was prepared from **4b** (169 mg, 1 mmol) and methyl 3-aminobenzoate (151 mg, 1 mmol) in a similar manner as described for compound **38c**. An eluent of hexane/EtOAc (10:1) was used for chromatography. Yellow solid, 220 mg, 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (dd, J = 7.0, 2.0 Hz, 1H), 7.64 (m, 1H), 7.42 (d, J = 7.7 Hz, 1H), 7.28−7.25 (m, 2H), 7.22 (d, J = 7.8 Hz, 1H), 6.76 (dd, J = 8.1, 2.3 Hz, 1H), 4.45 (d, J = 5.5 Hz, 2H), 3.88 (s, 3H).

*Methyl 4-((4-Fluoro-3-nitrobenzyl)amino)benzoate (**40c**).* The titled compound was prepared from **4b** (169 mg, 1 mmol) and methyl 4-aminobenzoate (151 mg, 1 mmol) in a similar manner as described for compound **38c**. An eluent of hexane/EtOAc (10:1) was used for chromatography. Yellow solid, 125 mg, yield 41%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, J = 6.8 Hz, 1H), 7.88 (d, J = 8.5 Hz, 2H), 7.67−7.60 (m, 1H), 7.30−7.25 (m, 1H), 6.58 (d, J = 8.6 Hz, 2H), 4.70 (s, 1H), 4.49 (d, J = 5.7 Hz, 2H), 3.87 (s, 3H).

*Methyl 4-Bromo-3-((4-fluoro-3-nitrobenzyl)amino)benzoate (**46c**).* In a dried round-bottom flask, 4-fluoro-3-nitrobenzaldehyde **4b**, (85 mg, 0.5 mmol), methyl 3-amino-4-bromobenzoate (115 mg, 0.5 mmol), and EtOH (5 mL) were stirred at room temperature for 1 h. Then, the reaction was cooled down to 0 °C, and $NaBH_4$ (32 mg, 1 mmol) was added in two portions over 30 min. The reaction was allowed to stir at room temperature overnight (or until completion monitored by TLC). The reaction was quenched with a saturated aqueous solution of NH_4Cl. After extraction with CH_2Cl_2, the combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography using hexane/EtOAc (10:1) to give the titled compound; yellow solid, 100 mg, 52% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, J = 6.3 Hz, 1H), 7.65 (s, 1H), 7.54 (d, J = 7.9 Hz, 1H), 7.30 (t, J = 7.7 Hz, 2H), 7.18 (s, 1H), 4.99 (s, 1H), 4.54 (d, J = 5.0 Hz, 2H), 3.86 (s, 3H).

*Methyl 4-Fluoro-3-((4-fluoro-3-nitrobenzyl)amino)benzoate (**47c**).* The titled compound was prepared in an analogous fashion as described for compound **46c** using 4-fluoro-3-nitrobenzaldehyde **4b** (85 mg, 0.5 mmol) and methyl 3-amino-4-fluorobenzoate (85 mg, 0.5 mmol) through the reductive amination reaction. Yellow solid, 97 mg, 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, J = 6.4 Hz, 1H), 7.67 (d, J = 7.7 Hz, 1H), 7.49−7.39 (m, 1H), 7.33 (d, J = 9.8 Hz, 1H), 7.28−7.24 (m, 1H), 7.13−7.02 (m, 1H), 4.52 (s, 3H), 3.88 (s, 3H).

*Methyl 3-((4-Fluoro-3-nitrobenzyl)amino)-4-methylbenzoate (**48c**).* The titled compound was prepared in an analogous fashion as described for compound **46c** using 4-fluoro-3-nitrobenzaldehyde **4b** (85 mg, 0.5 mmol) and methyl 3-amino-4-methylbenzoate (84 mg, 0.5 mmol) through the reductive amination reaction. An eluent of hexane/EtOAc (10:1) was used for chromatography. Yellow solid, 73 mg, 46% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (d, J = 6.9 Hz, 1H), 7.71−7.64 (m, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.32−7.26 (m, 1H), 7.17 (s, 2H), 4.52 (d, J = 4.4 Hz, 2H), 3.86 (s, 3H), 2.27 (s, 3H).

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*Methyl 2-Chloro-5-((4-fluoro-3-nitrobenzyl)amino)benzoate (**49c**).* The titled compound was prepared in an analogous fashion as described for compound **46c** using 4-fluoro-3-nitrobenzaldehyde **4b** (85 mg, 0.5 mmol) and methyl 5-amino-2-chlorobenzoate (93 mg, 0.5 mmol) through the reductive amination reaction. An eluent of hexane/EtOAc (10:1) was used for chromatography. Yellow solid, 150 mg, 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (d, J = 6.6 Hz, 1H), 7.68−7.59 (m, 1H), 7.31 (d, J = 10.2 Hz, 1H), 7.24 (d, J = 8.7 Hz, 1H), 7.05 (d, J = 2.6 Hz, 1H), 6.64 (dd, J = 8.6, 2.7 Hz, 1H), 4.44 (d, J = 5.3 Hz, 2H), 4.37 (s, 1H), 3.92 (s, 3H).

*Methyl 3-((4-Fluoro-3-nitrobenzyl)amino)-4-morpholinobenzoate (**50c**).* The titled compound was prepared in an analogous fashion as described for compound **46c** using 4-fluoro-3-nitrobenzaldehyde **4b** (85 mg, 0.5 mmol) and methyl 3-amino-4-morpholinobenzoate (118 mg, 0.5 mmol) through the reductive amination reaction. An eluent of hexane/EtOAc (6:1) was used for chromatography. Yellow solid, 88 mg, 45% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (d, J = 7.1 Hz, 1H), 7.69−7.59 (m, 1H), 7.49 (dd, J = 8.1, 1.6 Hz, 1H), 7.31 (d, J = 10.4 Hz, 1H), 7.18 (d, J = 1.4 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 5.19 (t, J = 5.2 Hz, 1H), 4.49 (d, J = 5.6 Hz, 2H), 3.93−3.86 (m, 4H), 3.86 (s, 3H), 3.04−2.95 (m, 4H).

*3-((4-Fluoro-3-nitrobenzyl)amino)benzamide (**51c**).* The titled compound was prepared in an analogous fashion as described for compound **38c** using 4-fluoro-3-nitrobenzaldehyde **4b** (85 mg, 0.5 mmol) and 3-aminobenzamide (68 mg, 0.5 mmol) through the reductive amination reaction. An eluent of hexane/EtOAc (15:1) was used for chromatography. Yellow solid, 65 mg, 45% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.13 (d, J = 6.7 Hz, 1H), 7.76 (s, 2H), 7.63−7.48 (m, 1H), 7.17 (s, 1H), 7.14−6.96 (m, 3H), 6.71 (d, J = 7.3 Hz, 1H), 6.57 (s, 1H), 4.40 (s, 2H).

*(3-((4-Fluoro-3-nitrobenzyl)amino)phenyl)methanol (**53c**).* The titled compound was prepared in an analogous fashion as described for compound **38c** using 4-fluoro-3-nitrobenzaldehyde **4b** (85 mg, 0.5 mmol) and (3-aminophenyl)methanol (62 mg, 0.5 mmol) through the reductive amination reaction. An eluent of hexane/EtOAc (8:1) was used for chromatography. Yellow solid, 88 mg, 64% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, J = 6.7 Hz, 1H), 7.71−7.61 (m, 1H), 7.28 (t, J = 9.5 Hz, 1H), 7.18 (t, J = 7.8 Hz, 1H), 6.76 (d, J = 7.4 Hz, 1H), 6.65 (s, 1H), 6.52 (d, J = 7.9 Hz, 1H), 4.62 (s, 2H), 4.44 (s, 2H), 4.29 (s, 1H), 1.62 (s, 1H).

*Methyl 4-Chloro-2-((4-fluoro-3-nitrobenzyl)amino)benzoate (**54c**).* In a dried round-bottom flask, 4-fluoro-3-nitrobenzaldehyde **4b** (85 mg, 0.5 mmol), methyl 2-amino-4-chlorobenzoate (93 mg, 0.5 mmol), and EtOH (1 mL) were stirred at room temperature for 24 h. The solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (3 mL). $NaBH(OAc)_3$ (316 mg, 1.5 mmol) was added in three portions over 1 h, and the mixture was stirred at room temperature overnight. The reaction was quenched with a saturated aqueous solution of NH_4Cl and extracted with CH_2Cl_2. The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography using hexane/EtOAc (10:1) to give the titled compound. Yellow solid, white solid, 30 mg, yield 18%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1H), 8.05 (dd, J = 6.8, 1.8 Hz, 1H), 7.90 (d, J = 8.5 Hz, 1H), 7.68−7.59 (m, 1H), 7.35−7.29 (m, 1H), 6.65 (dd, J = 8.6, 1.8 Hz, 1H), 6.51 (d, J = 1.6 Hz, 1H), 4.51 (d, J = 5.8 Hz, 2H), 3.90 (s, 3H).

*2-Chloro-6-((4-fluoro-3-nitrobenzyl)amino)benzoic Acid (**55c**).* In a dried round-bottom flask, 4-fluoro-3-nitrobenzaldehyde **4b** (85 mg, 0.5 mmol), 2-amino-6-chlorobenzoic acid (86 mg, 0.5 mmol), and EtOH (5 mL) were stirred at room for 24 h. $NaBH(OAc)_3$ (316 mg, 1.5 mmol) was added in three portions over 1 h, and the mixture was stirred at room temperature for 2 days. The reaction was quenched with an excess amount of saturated aqueous solution of $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2. The organic layers were acidified, and the precipitate was filtered and dried to provide the titled compound as a gray solid, 120 mg, yield 74%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09−8.00 (m, 1H), 7.62 (dd, J = 4.6, 3.4 Hz, 1H), 7.31 (d, J = 10.4 Hz, 1H), 7.17 (t, J = 8.2 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 6.43 (d, J = 8.5 Hz, 1H), 4.51 (s, 2H).

*2-((4-Fluoro-3-nitrobenzyl)amino)benzamide (**56c**).* The titled compound was prepared in an analogous fashion as described for compound **46c** using 4-fluoro-3-nitrobenzaldehyde **4b** (85 mg, 0.5 mmol) and 2-aminobenzamide (68 mg, 0.5 mmol) through the reductive amination reaction. An eluent of hexane/EtOAc (8:1) was used for chromatography. Yellow solid, 100 mg, 69% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (s, 1H), 8.05 (d, J = 6.8 Hz, 1H), 7.69−7.61 (m, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.27 (t, J = 8.9 Hz, 1H), 6.68 (t, J = 7.5 Hz, 1H), 6.51 (d, J = 8.4 Hz, 1H), 5.73 (m, 2H), 4.51 (d, J = 5.8 Hz, 2H).

*2-((4-Fluoro-3-nitrobenzyl)amino)benzonitrile (**57c**).* The titled compound was prepared in an analogous fashion as described for compound **46c** using 4-fluoro-3-nitrobenzaldehyde (**4b**, 85 mg, 0.5 mmol) and 2-aminobenzonitrile (59 mg, 0.5 mmol) through the reductive amination reaction. An eluent of hexane/EtOAc (10:1) was used for chromatography. Yellow solid, 92 mg, 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (t, J = 4.7 Hz, 1H), 8.06 (dd, J = 6.9, 1.8 Hz, 1H), 7.70−7.58 (m, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.33−7.23 (m, 1H), 6.68 (t, J = 7.5 Hz, 1H), 6.51 (d, J = 8.4 Hz, 1H), 5.77 (s, 2H), 4.51 (d, J = 5.9 Hz, 2H).

*Methyl 2-((4-((2-Aminophenyl)thio)-3-nitrobenzyl)amino)-benzoate (**38d**).* To a flask were added methyl 2-((4-fluoro-3-nitrobenzyl)amino)benzoate (**38c**, 61 mg, 0.2 mmol), 2-aminobenzenethiol (30 mg, 0.24 mmol), NaOAc (82 mg, 1 mmol), and EtOH 2 mL. The mixture was stirred at reflux for 4 h. After cooling to room temperature, the precipitate was filtered, washed with EtOH and water successively, and dried to provide compound **38d** as a yellow solid, 70 mg, 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, 2H), 7.95 (d, J = 7.9 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.36−7.29 (m, 2H), 6.85−6.82 (m, 3H), 6.65 (t, J = 7.5 Hz, 1H), 6.53 (d, J = 8.4 Hz, 1H), 4.48 (d, J = 5.7 Hz, 2H), 4.30 (s, 2H), 3.89 (s, 3H).

*Methyl 3-((4-((2-Aminophenyl)thio)-3-nitrobenzyl)amino)-benzoate (**39d**).* The titled compound was prepared from methyl 3-((4-fluoro-3-nitrobenzyl)amino)benzoate (**39c**, 64 mg, 0.2 mg) and 2-aminobenzenethiol (30 mg, 0.24 mmol) in a similar manner as described for compound **38d**. Yellow solid, 66 mg, 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1H), 7.46 (d, J = 7.6 Hz, 1H), 7.40 (t, J = 8.2 Hz, 2H), 7.34 (t, J = 7.7 Hz, 1H), 7.28 (s, 1H), 7.23 (t, J = 7.9 Hz, 1H), 6.89−6.79 (m, 3H), 6.76 (dd, J = 8.0, 2.1 Hz, 1H), 4.42 (d, J = 5.3 Hz, 2H), 4.31 (s, 2H), 3.89 (s, 3H).

*Methyl 4-((4-((2-Aminophenyl)thio)-3-nitrobenzyl)amino)-benzoate (**40d**).* The titled compound was prepared from methyl 4-((4-fluoro-3-nitrobenzyl)amino)benzoate (**40c**, 61 mg, 0.2 mmol) and 2-aminobenzenethiol (30 mg, 0.24 mmol) in a similar manner as described for compound **38d**. Yellow solid, 66 mg, 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, 1H), 7.86 (d, J = 8.5 Hz, 2H), 7.46 (d, J = 7.6 Hz, 1H), 7.35 (m, 2H), 6.84 (m, 3H), 6.56 (d, J = 8.6 Hz, 2H), 4.60 (t, J = 5.1 Hz, 1H), 4.44 (d, J = 5.6 Hz, 2H), 4.30 (s, 2H), 3.86 (s, 3H).

*Methyl 2-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzoate (**43d**).* The titled compound was prepared from methyl 2-((4-fluoro-3-nitrobenzyl)amino)benzoate (**38c**, 64 mg, 0.2 mg) and 3,4-dichlorobenzenethiol (43 mg, 0.24 mmol) in a similar manner as described for compound **38d**. Yellow solid, 72 mg, 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (t, J = 5.5 Hz, 1H), 8.23 (s, 1H), 7.96 (dd, J = 8.0, 1.4 Hz, 1H), 7.69 (d, J = 2.0 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.46−7.38 (m, 2H), 7.35−7.29 (m, 1H), 6.87 (d, J = 8.4 Hz, 1H), 6.67 (t, J = 7.5 Hz, 1H), 6.52 (d, J = 8.4 Hz, 1H), 4.51 (d, J = 5.9 Hz, 2H), 3.90 (s, 3H).

*Methyl 3-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzoate (**44d**).* The titled compound was prepared from methyl 3-((4-fluoro-3-nitrobenzyl)amino)benzoate (**39c**, 64 mg, 0.2 mg) and 3,4-dichlorobenzenethiol (43 mg, 0.24 mmol) in a similar manner as described for compound **38d**. Yellow solid, 76 mg, 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.68 (d, J = 1.9 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.49−7.35 (m, 4H), 7.23 (t, J = 7.9 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 6.76 (dd, J = 8.0, 2.1 Hz, 1H), 4.44 (d, J = 5.7 Hz, 2H), 4.38 (d, J = 5.6 Hz, 1H), 3.89 (s, 3H).

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*Methyl 4-Bromo-3-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)-amino)benzoate (**46d**).* The titled compound was prepared from methyl 4-bromo-3-((4-fluoro-3-nitrobenzyl)amino)benzoate **46c** (77 mg, 0.2 mmol) and 3,4-dichlorobenzenethiol (43 mg, 0.24 mmol) in a similar manner as described for compound **38d**. Yellow solid, 97 mg, 89% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, 1H), 7.71 (d, J = 1.9 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.54 (d, J = 8.2 Hz, 1H), 7.43 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 1.7 Hz, 1H), 7.19 (d, J = 1.5 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 4.93 (t, J = 5.6 Hz, 1H), 4.53 (d, J = 5.9 Hz, 2H), 3.88 (s, 3H).

*Methyl 3-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-4-fluorobenzoate (**47d**).* The titled compound was prepared from methyl 4-fluoro-3-((4-fluoro-3-nitrobenzyl)amino)benzoate **47c** (100 mg, 0.31 mmol) and 3,4-dichlorobenzenethiol (66.7 mg, 0.37 mmol) in a similar manner as described for compound **38d**. Yellow solid, 82 mg, 55% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, 1H), 7.70 (s, 1H), 7.57 (dd, J = 8.2, 1.8 Hz, 1H), 7.43 (m, 3H), 7.31−7.25 (m, 1H), 7.12−6.99 (m, 1H), 6.90 (dd, J = 8.3, 1.6 Hz, 1H), 4.49 (s, 3H), 3.88 (s, 3H).

*Methyl 3-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-4-methylbenzoate (**48d**).* The titled compound was prepared from methyl 3-((4-fluoro-3-nitrobenzyl)amino)-4-methylbenzoate **48c** (63 mg, 0.2 mmol) and 3,4-dichlorobenzenethiol (43 mg, 0.24 mmol) in a similar manner as described for compound **38d**. Yellow solid, 58 mg, 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (s, 1H), 7.70 (d, J = 1.7 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.43 (m, 3H), 7.20−7.13 (m, 2H), 6.89 (d, J = 8.4 Hz, 1H), 4.51 (d, J = 5.7 Hz, 2H), 4.05 (t, J = 5.4 Hz, 1H), 3.87 (s, 3H), 2.25 (s, 3H).

*Methyl 2-Chloro-5-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)-amino)benzoate (**49d**).* The titled compound was prepared from methyl 2-chloro-5-((4-fluoro-3-nitrobenzyl)amino)benzoate **49c** (67 mg, 0.2 mmol) and 3,4-dichlorobenzenethiol (43 mg, 0.24 mmol) in a similar manner as described for compound **38d**. Yellow solid, 73 mg, 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 0.9 Hz, 1H), 7.69 (d, J = 1.9 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.41 (td, J = 7.9, 1.7 Hz, 2H), 7.22 (d, J = 8.7 Hz, 1H), 7.04 (d, J = 2.9 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.62 (dd, J = 8.7, 2.9 Hz, 1H), 4.41 (d, J = 5.7 Hz, 2H), 4.32 (t, J = 5.6 Hz, 1H), 3.92 (s, 3H).

*Methyl 3-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-4-morpholinobenzoate (**50d**).* The titled compound was prepared from methyl 3-((4-fluoro-3-nitrobenzyl)amino)-4-morpholinobenzoate **50c** (78 mg, 0.2 mmol) and 3,4-dichlorobenzenethiol (43 mg, 0.24 mmol) in a similar manner as described for compound **38d**. Yellow solid, 49 mg, 45% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.71 (s, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.42 (t, J = 8.4 Hz, 2H), 7.19 (s, 1H), 7.07 (d, J = 8.1 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 5.16 (t, J = 5.6 Hz, 1H), 4.47 (d, J = 5.7 Hz, 2H), 3.88 (m, 7H), 3.10−2.91 (m, 4H).

*Methyl 4-Chloro-2-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)-amino)benzoate (**54d**).* The titled compound was prepared from methyl 4-chloro-2-((4-fluoro-3-nitrobenzyl)amino)benzoate **54c** (30 mg, 0.09 mmol) and 3,4-dichlorobenzenethiol (18 mg, 0.1 mmol) in a similar manner as described for compound **38d**. Yellow solid, 23 mg, 44% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (t, J = 5.4 Hz, 1H), 8.23 (s, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.71 (d, J = 1.9 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.43 (dd, J = 8.4, 2.0 Hz, 2H), 6.89 (d, J = 8.4 Hz, 1H), 6.64 (dd, J = 8.5, 1.8 Hz, 1H), 6.51 (d, J = 1.5 Hz, 1H), 4.48 (d, J = 5.8 Hz, 2H), 3.89 (s, 3H).

*General Procedure for the Synthesis of Compounds **38**−**40**, **43**−**44**, **46**−**51**, and **54**−**57**.* The methyl esters of the intermediates (**38d**−**40d**, **43d**−**44d**, **46d**−**50d**, and **54d**) were hydrolyzed with 1 M NaOH in dioxane (1:1) at 50 °C overnight. The mixture was then diluted with a small amount of water and washed with CH_2Cl_2. The solution was acidified by the addition of 2 M HCl. The precipitate was filtered, washed with water, and dried to afford the titled compounds.

*2-((4-((2-Aminophenyl)thio)-3-nitrobenzyl)amino)benzoic Acid (**38**).* The titled compound was prepared from the hydrolysis of methyl 2-((4-((2-aminophenyl)thio)-3-nitrobenzyl)amino)benzoate **38d** (41 mg, 0.1 mmol) in 1 M NaOH (0.3 mL) and dioxane (1 mL) at 50 °C overnight. Yellow solid, 21 mg, 53% yield, mp 201−203 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.77 (s, 1H), 8.38 (s, 1H), 8.22 (s, 1H), 7.80 (d, J = 7.5 Hz, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.33 (d, J = 7.4 Hz, 1H), 7.27 (dd, J = 16.9, 8.2 Hz, 2H), 6.82 (d, J = 8.0 Hz, 1H), 6.74 (d, J = 8.3 Hz, 1H), 6.63 (t, J = 7.5 Hz, 2H), 6.57 (t, J = 7.4 Hz, 1H), 5.53 (s, 2H), 4.52 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.4, 151.4, 150.7, 145.4, 138.3, 137.9, 135.9, 134.8, 133.4, 132.6, 132.2, 127.7, 124.6, 117.2, 115.6, 115.3, 112.1, 111.3, 110.1, 44.8. HRMS (ESI): calcd for $C_{20}H_{16}N_3O_4S$, $[M - H]^-$ 394.0867; found, 394.0862. HPLC purity: 99.32%.

*3-((4-((2-Aminophenyl)thio)-3-nitrobenzyl)amino)benzoic Acid (**39**).* The titled compound was prepared from the hydrolysis of methyl 3-((4-((2-aminophenyl)thio)-3-nitrobenzyl)amino)benzoate **39d** (41 mg, 0.1 mmol) in 1 M NaOH (0.3 mL) and dioxane (1 mL) at 50 °C overnight. Yellow solid, 27 mg, 68% yield, mp 105−107 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.66 (s, 1H), 8.25 (s, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 7.5 Hz, 1H), 7.26 (t, J = 7.6 Hz, 1H), 7.20−7.10 (m, 3H), 6.82 (d, J = 8.0 Hz, 1H), 6.75 (m, 2H), 6.63 (m, 2H), 5.53 (s, 2H), 4.34 (d, J = 5.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.3, 151.4, 148.7, 145.4, 138.7, 137.9, 135.8, 133.5, 132.6, 132.1, 129.5, 127.3, 124.6, 117.6, 117.2, 116.7, 115.6, 113.6, 110.1, 45.4. HRMS (ESI): calcd for $C_{20}H_{16}N_3O_4S$, $[M - H]^-$ 394.0867; found, 394.0863. HPLC purity: 97.18%.

*4-((4-((2-Aminophenyl)thio)-3-nitrobenzyl)amino)benzoic Acid (**40**).* The titled compound was prepared from the hydrolysis of methyl 4-((4-((2-aminophenyl)thio)-3-nitrobenzyl)amino)benzoate **40d** (41 mg, 0.1 mmol) in 1 M NaOH (0.3 mL) and dioxane (1 mL) at 50 °C overnight. Yellow solid, 33 mg, 84% yield, decomposition temperature 250 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.04 (s, 1H), 8.23 (s, 1H), 7.64 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.2 Hz, 1H), 7.33 (d, J = 7.3 Hz, 1H), 7.26 (t, J = 7.6 Hz, 1H), 7.13 (t, J = 5.9 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 6.73 (d, J = 8.4 Hz, 1H), 6.64 (t, J = 7.3 Hz, 1H), 6.59 (d, J = 8.6 Hz, 2H), 5.52 (s, 2H), 4.38 (d, J = 5.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.2, 151.4, 145.4, 138.3, 137.9, 135.8, 133.6, 132.6, 131.6, 127.4, 124.7, 117.2, 115.6, 111.7, 110.1, 45.0. HRMS (ESI): calcd for $C_{20}H_{16}N_3O_4S$, $[M - H]^-$ 394.0867; found, 394.0863. HPLC purity: 96.24%.

*2-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)benzoic Acid (**43**).* The titled compound was prepared from the hydrolysis of methyl 2-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzoate **43d** (46 mg, 0.1 mmol) in 1 M NaOH (0.3 mL) and dioxane (1 mL) at 50 °C overnight. Yellow solid, 22 mg, 49% yield, mp 171−173 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.56 (s, 1H), 8.17 (s, 1H), 7.91 (s, 1H), 7.84 (d, J = 7.3 Hz, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.56 (t, J = 5.7 Hz, 2H), 7.06 (t, J = 7.1 Hz, 1H), 7.00 (d, J = 8.1 Hz, 1H), 6.50−6.34 (m, 2H), 4.46 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 150.1, 146.0, 140.8, 136.5, 135.3, 133.8, 133.6, 133.5, 133.0, 132.6, 132.4, 132.0, 129.9, 124.2, 118.6, 114.7, 110.9, 45.2. HRMS (ESI): calcd for $C_{20}H_{13}Cl_2N_2O_4S$, $[M - H]^-$ 446.9979; found, 446.9968. HPLC purity: 96.26%.

*3-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)benzoic Acid (**44**).* The titled compound was prepared from the hydrolysis of methyl 3-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzoate **44d** (46 mg, 0.1 mmol) in 1 M NaOH (0.3 mL) and dioxane (1 mL) at 50 °C overnight. Yellow solid, 25 mg, 56% yield, mp 224−226 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23 (s, 1H), 7.92 (d, J = 1.9 Hz, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.60 (dd, J = 8.4, 1.2 Hz, 1H), 7.56 (dd, J = 8.3, 2.0 Hz, 1H), 7.16 (s, 1H), 7.13 (m, 2H), 7.01 (d, J = 8.3 Hz, 1H), 6.78−6.71 (m, 1H), 6.63 (t, J = 5.9 Hz, 1H), 4.38 (d, J = 5.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 148.4, 145.9, 140.5, 136.6, 135.3, 134.0, 133.8, 133.5, 133.0, 132.6, 132.3, 129.9, 129.1, 124.3, 117.9, 115.9, 113.7, 45.5. HRMS (ESI): calcd for $C_{20}H_{13}Cl_2N_2O_4S$, $[M - H]^-$ 446.9979; found, 446.9975. HPLC purity: 95.35%.

*4-Bromo-3-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzoic Acid (**46**).* The titled compound was prepared from the hydrolysis of methyl 4-bromo-3-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)benzoate **46d** (40 mg, 0.07 mmol) in 1 M NaOH (0.3 mL) and dioxane (1.0 mL) at 50 °C overnight. Yellow solid, 35 mg, 90% yield, mp 231−233 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22 (s, 1H), 7.92 (d, J = 1.8 Hz, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.62−7.52 (m, 2H), 7.49 (d, J = 8.0 Hz, 1H), 7.07 (d, J =

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8.3 Hz, 1H), 7.01 (d, J = 8.1 Hz, 2H), 6.26 (s, 1H), 4.51 (d, J = 5.9 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.4, 145.8, 144.8, 139.7, 136.7, 135.4, 134.4, 133.6, 133.5, 133.0, 132.8, 132.6, 132.2, 129.9, 124.1, 118.9, 113.1, 112.2, 45.4. HRMS (ESI): calcd for $C_{20}H_{12}BrCl_2N_2O_4S$, $[M - H]^-$ 524.9084; found, 524.9079. HPLC purity: 99.36%.

*3-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-4-fluorobenzoic Acid (**47**).* The titled compound was prepared from the hydrolysis of methyl 3-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)-4-fluorobenzoate **47d** (30 mg, 0.06 mmol) in 1 M NaOH (0.2 mL) and dioxane (0.6 mL) at 50 °C overnight. Yellow solid, 21 mg, 75% mg, mp 237−239 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.24 (s, 1H), 7.91 (s, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.60 (d, J = 8.2 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.18 (m, 1H), 7.10 (m, 2H), 7.00 (d, J = 8.3 Hz, 1H), 6.52 (s, 1H), 4.45 (d, J = 5.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.3, 153.7 (d, J = 245.4 Hz), 145.8, 134, 136.6, 136.2, 136.1, 135.4, 134.3, 133.64, 133.57, 133.0, 132.6, 132.2, 129.9, 124.2, 118.4 (d, J = 8.0 Hz), 114.8 (d, J = 18.2 Hz), 113.2 (d, J = 4.0 Hz), 45.0. HRMS (ESI): calcd for $C_{20}H_{12}Cl_2FN_2O_4S$, $[M - H]^-$ 464.9884; found, 464.9872. HPLC purity: 100.00%.

*3-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-4-methylbenzoic Acid (**48**).* The titled compound was prepared from the hydrolysis of methyl 3-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)-4-methylbenzoate **48d** (42 mg, 0.09 mmol) in 1 M NaOH (0.3 mL) and dioxane (1.0 mL) at 50 °C overnight. Yellow solid, 27 mg, 65% yield, mp 222−224 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23 (s, 1H), 7.91 (s, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.58 (m, 2H), 7.09 (m, 2H), 6.99 (d, J = 8.3 Hz, 1H), 6.91 (s, 1H), 5.95 (s, 1H), 4.45 (d, J = 5.1 Hz, 2H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.2, 145.94, 145.87, 140.58, 136.6, 135.4, 134.0, 133.6, 133.6, 133.0, 132.6, 132.3, 130.2, 129.9, 127.3, 124.2, 118.1, 110.2, 45.5, 18.4. HRMS (ESI): calcd for $C_{21}H_{15}Cl_2N_2O_4S$, $[M - H]^-$ 461.0135; found, 461.0128. HPLC purity: 98.78%.

*2-Chloro-5-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzoic Acid (**49**).* The titled compound was prepared from the hydrolysis of methyl 2-chloro-5-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)benzoate **49d** (30 mg, 0.06 mmol) in 1 M NaOH (0.2 mL) and dioxane (0.6 mL) at 50 °C overnight. Yellow solid, 22 mg, 76% yield, mp 148−150 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (d, J = 1.0 Hz, 1H), 7.92 (d, J = 2.0 Hz, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.64−7.47 (m, 2H), 7.00 (d, J = 8.3 Hz, 1H), 6.93 (d, J = 8.5 Hz, 1H), 6.62 (s, 1H), 6.50−6.34 (m, 2H), 4.31 (d, J = 6.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 146.9, 146.8, 145.8, 140.5, 136.6, 135.4, 134.1, 133.8, 133.6, 133.0, 132.6, 132.3, 123.0, 129.8, 124.2, 117.4, 113.4, 112.9, 45.5. HRMS (ESI): calcd for $C_{20}H_{12}Cl_3N_2O_4S$, $[M - H]^-$ 480.9589; found, 480.9589. HPLC purity: 98.50%.

*3-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-4-morpholinobenzoic Acid (**50**).* The titled compound was prepared from the hydrolysis of methyl 3-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)-4-morpholinobenzoate **50d** (35 mg, 0.06 mmol) in 1 M NaOH (0.2 mL) and dioxane (0.6 mL) at 50 °C overnight. Yellow solid, 22 mg, 65% yield, mp 147−149 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.45 (s, 1H), 8.22 (s, 1H), 7.92 (s, 1H), 7.77 (d, J = 8.2 Hz, 1H), 7.56 (t, J = 5.5 Hz, 2H), 7.24 (d, J = 7.7 Hz, 1H), 7.02 (dd, J = 12.0, 8.4 Hz, 2H), 6.92 (s, 1H), 5.94 (t, J = 5.2 Hz, 1H), 4.48 (d, J = 5.1 Hz, 2H), 3.82 (s, 4H), 2.90 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.3, 145.8, 141.4, 141.3, 140.9, 136.7, 135.5, 134.1, 133.6, 133.4, 133.0, 132.6, 132.2, 129.8, 123.9, 118.9, 118.7, 111.3, 67.0, 51.6, 45.9. HRMS (ESI): calcd for $C_{24}H_{20}Cl_2N_3O_5S$, $[M - H]^-$ 532.0506; found, 532.0499. HPLC purity: 95.73%.

*3-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzamide (**51**).* The titled compound was prepared from 3-((4-fluoro-3-nitrobenzyl)amino)benzamide **51c** (58 mg, 0.2 mmol) and 3,4-dichlorobenzenethiol (51 mg, 0.3 mmol) in a similar manner as described for compound **38d**. Yellow solid, 40 mg, 45% yield, mp 158−160 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.23 (s, 1H), 7.91 (s, 1H), 7.77 (d, J = 8.3 Hz, 2H), 7.60 (dd, J = 8.4, 1.2 Hz, 1H), 7.56 (dd, J = 8.3, 1.8 Hz, 1H), 7.18 (s, 1H), 7.10 (t, J = 7.7 Hz, 1H), 7.08−7.02 (m, 2H), 7.01 (d, J = 8.3 Hz, 1H), 6.70 (d, J = 7.4 Hz, 1H), 6.55 (t, J = 6.1 Hz, 1H), 4.39 (d, J = 6.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.9, 148.5, 145.8, 140.5, 136.6, 135.7, 135.4, 134.1, 133.9, 133.6, 133.0, 132.6, 132.3, 129.8, 129.2, 124.3, 115.8, 115.6, 111.8, 45.3. HRMS (ESI): calcd for $C_{20}H_{14}Cl_2N_3O_3S$, $[M - H]^-$ 446.0138; found, 446.0129. HPLC purity: 100.00%.

*(3-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)phenyl)-methanol (**53**).* The titled compound was prepared from (3-((4-fluoro-3-nitrobenzyl)amino)phenyl)methanol **53c** (85 mg, 0.30 mmol) and 3,4-dichlorobenzenethiol (77 mg, 0.45 mmol) in a similar manner as described for compound **38d**. Yellow solid, 100 mg, 77% yield, mp 118−120 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, J = 0.9 Hz, 1H), 7.67 (d, J = 2.0 Hz, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.41 (td, J = 8.2, 1.8 Hz, 2H), 7.15 (t, J = 7.8 Hz, 1H), 6.87 (d, J = 8.4 Hz, 1H), 6.73 (d, J = 7.5 Hz, 1H), 6.63 (s, 1H), 6.50 (dd, J = 8.0, 2.0 Hz, 1H), 4.60 (d, J = 5.9 Hz, 2H), 4.41 (d, J = 5.8 Hz, 2H), 4.23 (t, J = 5.4 Hz, 1H), 1.60 (t, J = 6.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.5, 145.5, 142.3, 138.5, 136.9, 136.1, 134.7, 134.6, 134.0, 132.5, 131.9, 131.5, 129.6, 128.9, 124.3, 116.8, 112.1, 111.4, 65.4, 46.8. HRMS (ESI): calcd for $C_{20}H_{15}Cl_2N_2O_3S$, $[M - H]^-$ 433.0186; found, 433.0184. HPLC purity: 96.19%.

*4-Chloro-2-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzoic Acid (**54**).* The titled compound was prepared from the hydrolysis of methyl 4-chloro-2-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)benzoate **54d** (30 mg, 0.06 mmol) in 1 M NaOH (0.2 mL) and dioxane (0.8 mL) at 50 °C overnight. Yellow solid, 20 mg, 69% yield, mp 220−222 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.95 (s, 1H), 8.54 (s, 1H), 8.23 (s, 1H), 7.93 (d, J = 1.8 Hz, 1H), 7.79 (m, 2H), 7.58 (m, 2H), 7.02 (d, J = 8.4 Hz, 1H), 6.69 (d, J = 1.3 Hz, 1H), 6.60 (dd, J = 8.5, 1.6 Hz, 1H), 4.59 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.9, 151.5, 145.8, 139.4, 139.2, 136.7, 135.5, 134.6, 134.0, 133.6, 133.0, 132.6, 132.2, 130.0, 124.4, 115.3, 111.3, 110.9, 44.6. HRMS (ESI): calcd for $C_{20}H_{12}Cl_3N_2O_4S$, $[M - H]^-$ 480.9589; found, 480.9579. HPLC purity: 95.83%.

*2-Chloro-6-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzoic Acid (**55**).* The titled compound was prepared from 2-chloro-6-((4-fluoro-3-nitrobenzyl)amino)benzoic acid **55c** (120 mg, 0.5 mmol) and 3,4-dichlorobenzenethiol (107 mg, 0.6 mmol) in a similar manner as described for compound **38d**. Yellow solid, 90 mg, 37% yield, mp 204−206 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.47 (s, 1H), 8.21 (s, 1H), 7.91 (d, J = 1.9 Hz, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.56 (d, J = 8.3 Hz, 2H), 7.09 (t, J = 8.2 Hz, 1H), 7.00 (d, J = 8.3 Hz, 1H), 6.65 (d, J = 7.8 Hz, 1H), 6.47 (d, J = 8.4 Hz, 1H), 4.46 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.0, 147.2, 145.8, 139.8, 136.6, 135.4, 134.3, 133.6, 133.0, 132.6, 132.2, 131.8, 131.6, 129.8, 124.3, 118.7, 117.4, 110.4, 45.2. HRMS (ESI): calcd for $C_{20}H_{12}Cl_3N_2O_4S$, $[M - H]^-$ 480.9589; found, 480.9579. HPLC purity: 95.12%.

*2-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzamide (**56**).* The titled compound was prepared from 2-((4-fluoro-3-nitrobenzyl)amino)benzamide **56c** (58 mg, 0.20 mmol) and 3,4-dichlorobenzenethiol (43 mg, 0.24 mmol) in a similar manner as described for compound **38d**. Yellow solid, 37 mg, 41% yield, mp 184−186 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.68 (t, J = 6.0 Hz, 1H), 8.19 (s, 1H), 7.92 (d, J = 1.9 Hz, 1H), 7.88 (s, 1H, C(=O)N<u>H</u>), 7.78 (d, J = 8.3 Hz, 1H), 7.62 (d, J = 7.3 Hz, 1H), 7.60−7.54 (m, 2H), 7.22 (s, C(=O)N<u>H</u> 1H), 7.19 (t, J = 7.7 Hz, 1H), 7.01 (d, J = 8.3 Hz, 1H), 6.62−6.50 (m, 2H), 4.49 (d, J = 6.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 149.5, 145.8, 140.2, 136.6, 135.4, 134.3, 133.7, 133.6, 133.04, 132.98, 132.6, 132.3, 130.0, 129.6, 124.2, 115.2, 115.1, 112.0, 45.0. HRMS (ESI): calcd for $C_{20}H_{16}Cl_2N_3O_3S$, $[M + H]^+$ 448.0284; found, 448.0276. HPLC purity: 100.00%.

*2-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzonitrile (**57**).* The titled compound was prepared from 2-((4-fluoro-3-nitrobenzyl)amino)benzonitrile **57c** (54 mg, 0.20 mmol) and 3,4-dichlorobenzenethiol (43 mg, 0.24 mmol) in a similar manner as described for compound **38d**. Yellow solid, 50 mg, 58% yield, mp 173−175 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (s, 1H), 7.70 (d, J = 1.8 Hz, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.50−7.39 (m, 3H), 7.36 (t, J = 7.8 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 6.76 (t, J = 7.5 Hz, 1H), 6.54 (d, J = 8.5 Hz, 1H), 5.13 (t, J = 5.3 Hz, 1H), 4.51 (d, J = 5.8 Hz, 2H).

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^{13}C NMR (100 MHz, $CDCl_3$): δ 149.3, 145.4, 137.0, 137.0, 136.6, 134.9, 134.7, 134.4, 134.1, 133.0, 132.1, 131.9, 131.2, 129.1, 124.3, 117.8, 117.6, 110.9, 96.6, 46.2. HRMS (ESI): calcd for $C_{20}H_{12}Cl_2N_3O_2S$, $[M - H]^-$ 429.0106; found, 428.0033. HPLC purity: 100.00%.

Synthetic Procedures for Scheme 6. *4-((3,4-Dichlorophenyl)-thio)-3-nitrobenzaldehyde (**45c**).* The titled compound was prepared from 4-fluoro-3-nitrobenzaldehyde **4b** (169 mg, 1.0 mmol) and 3,4-dichlorobenzenethiol (214 mg, 1.2 mmol) in a similar manner as described for compound **38d**. Yellow solid, 246 mg, 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.02 (s, 1H), 8.75 (d, J = 1.4 Hz, 1H), 7.90 (dd, J = 8.5, 1.5 Hz, 1H), 7.74 (d, J = 1.9 Hz, 1H), 7.64 (d, J = 8.3 Hz, 1H), 7.46 (dd, J = 8.2, 1.9 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H).

*(4-((3,4-Dichlorophenyl)thio)-3-nitrophenyl)methanol (**45d**).* In a round-bottom flask was added 4-((3,4-dichlorophenyl)thio)-3-nitrobenzaldehyde **45c** (100 mg, 0.3 mmol) in MeOH (5 mL) and cooled to 0 °C with an ice-water bath. $NaBH_4$ (23 mg, 0.6 mmol) was added, and the reaction mixture was allowed to stir at room temperature for another 1 h. The reaction was quenched with NH_4Cl solution and extracted with EtOAc. The organic phase was combined, washed with brine, dried with anhydrous Na_2SO_4, concentrated, and purified by column chromatography using hexane/EtOAc (8:1) to provide compound **45d** as a pale yellow solid, 87 mg, 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (s, 1H), 7.69 (d, J = 2.0 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.43 (td, J = 8.2, 1.8 Hz, 2H), 6.92 (d, J = 8.4 Hz, 1H), 4.77 (d, J = 5.7 Hz, 2H).

*(4-(Bromomethyl)-2-nitrophenyl)(3,4-dichlorophenyl)sulfane (**45e**).* Phosphorus tribromide (20 μL, 0.22 mmol) was added to a stirred solution of (4-((3,4-dichlorophenyl)thio)-3-nitrophenyl)-methanol **45d** (60 mg, 0.18 mmol) in toluene (2 mL) at 40 °C. The reaction was stirred at 100 °C for 30 min. The reaction was cooled to room temperature, extracted with EtOAc, washed with water and brine, dried with anhydrous Na_2SO_4, concentrated, and purified by column chromatography using hexane/EtOAc (15:1) to provide the titled compound as a yellow solid, 58 mg, 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (d, J = 1.8 Hz, 1H), 7.71 (d, J = 2.0 Hz, 1H), 7.59 (d, J = 8.3 Hz, 1H), 7.44 (ddd, J = 8.2, 4.2, 2.0 Hz, 2H), 6.88 (d, J = 8.4 Hz, 1H), 4.49 (s, 2H).

*Methyl 4-Chloro-3-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)-amino)benzoate (**45f**).* To a flask were added (4-(bromomethyl)-2-nitrophenyl) (3,4-dichlorophenyl)sulfane (**45e**) (118 mg, 0.3 mmol), methyl 3-amino-4-chlorobenzoate (56 mg, 0.3 mmol), DIPEA (149 μL, 0.9 mmol), and DMF (2 mL). The reaction was stirred at 100 °C for 4 h. The reaction mixture was cooled to room temperature and quenched with water and extracted with EtOAc. The organic phase was combined, washed with brine, dried with anhydrous Na_2SO_4, concentrated, and purified by column chromatography using hexane/EtOAc (10:1) to provide the titled compound as a yellow solid, 32 mg, 21% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.25 (d, J = 1.2 Hz, 1H), 7.91 (d, J = 1.9 Hz, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.59 (d, J = 7.6 Hz, 1H), 7.57 (dd, J = 8.4, 2.0 Hz, 1H), 7.42 (d, J = 8.2 Hz, 1H), 7.16 (dd, J = 8.2, 1.6 Hz, 1H), 7.06 (d, J = 1.6 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.61 (t, J = 6.1 Hz, 1H), 4.53 (d, J = 6.1 Hz, 2H), 3.76 (s, 3H).

*4-Chloro-3-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzoic Acid (**45**).* The titled compound was prepared from the hydrolysis of methyl 4-chloro-3-((4-((3,4-dichlorophenyl)thio)-3-nitrobenzyl)amino)benzoate **45f** (30 mg, 0.06 mmol) in 1 M NaOH (0.2 mL) and dioxane (0.6 mL) at 50 °C overnight. Yellow solid, 17 mg, 59% yield, mp 196–198 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21 (s, 1H), 7.92 (s, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.57 (d, J = 8.2 Hz, 2H), 7.28 (d, J = 8.1 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.07 (s, 1H), 6.99 (d, J = 8.3 Hz, 1H), 6.34 (s, 1H), 4.49 (d, J = 5.9 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.7, 145.8, 143.8, 139.7, 136.6, 135.4, 134.4, 133.6, 133.5, 133.0, 132.6, 132.2, 131.7, 123.0, 129.6, 124.2, 122.7, 118.3, 112.0, 45.1. HRMS (ESI): calcd for $C_{20}H_{12}Cl_3N_2O_4S$, $[M - H]^-$ 480.9589; found, 480.9577. HPLC purity: 97.49%.

*3-((4-((3,4-Dichlorophenyl)thio)-3-nitrobenzyl)amino)-benzenesulfonamide (**52**).* In a dried round-bottom flask, 4-((3,4-dichlorophenyl)thio)-3-nitrobenzaldehyde **45c** (50 mg, 0.15 mmol), 3-aminobenzenesulfonamide (23 mg, 0.15 mmol), and CH_2Cl_2 (5 mL) were stirred at room temperature for 1 h. The reaction was cooled down to 0 °C, and $NaBH(OAc)_3$ (95 mg, 0.45 mmol) was added in two portions over 1 min. The reaction was allowed to stir at room temperature overnight. The reaction was quenched with a saturated solution of NH_4Cl and extracted with CH_2Cl_2. The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography using hexane/EtOAc (15:1) to give the titled compound, yellow solid, 21 mg, 29% yield, mp 191–193 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.24 (s, 1H), 7.91 (s, 1H), 7.79 (d, J = 8.3 Hz, 1H), 7.58 (dd, J = 15.2, 8.4 Hz, 2H), 7.21 (t, J = 8.0 Hz, 1H), 7.17 (s, 2H), 7.05 (s, 1H), 7.00 (dd, J = 11.9, 8.4 Hz, 2H), 6.87 (t, J = 5.7 Hz, 1H), 6.71 (d, J = 7.8 Hz, 1H), 4.39 (d, J = 5.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 148.8, 145.8, 145.3, 134.0, 136.6, 135.4, 134.3, 133.9, 133.6, 133.0, 132.6, 132.3, 130.0, 129.9, 124.4, 115.2, 113.6, 109.7, 45.3. HRMS (ESI): calcd for $C_{19}H_{14}Cl_2N_3O_4S_2$, $[M - H]^-$ 481.9808; found, 481.9801. HPLC purity: 97.55%.

Bacterial Strains and Antibiotics. The bacterial strains used in this study for microdilution assay were as follows: *E. faecalis* ATCC 19433, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, *S. pyogenes* ATCC 19615, *S. agalactiae* ATCC 12386, *S. epidermidis* ATCC 12228, *S. saprophyticus* ATCC 15305 (The American Type Culture Collection, Manassas, Virginia, United States). The antibiotic controls were purchased from Sigma-Aldrich (St. Louis, Missouri, United States).

Determination of MIC. The antimicrobial activity of the compounds was determined by broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[25] The test medium was cation-adjusted Mueller–Hinton broth (MHB) if not specified elsewhere or BHI as indicated in the main text. Serial twofold dilutions of the tested compound were performed for the tested chemicals starting from 256 to 0.5 μg/mL, and the bacterial cell inoculum was adjusted to approximately 5×10^5 CFU per mL. Results were taken after 20 h of incubation at 37 °C. The MIC was defined as the lowest concentration of antibiotic with no visible growth. Experiments were performed in at least duplicates.

Cytotoxicity Assay. Human HepG2 liver cancer and A549 lung carcinoma cell lines were used in this study. The cells were seeded at 2.5×10^5 per well. After 24 h of incubation, the tested compounds will be added in a twofold serial dilution ranging from 1.562 to 50 μg/mL. The plates were incubated at 37 °C. At 48 h and 72 h after adding the compound, the MTT assay was performed as described previously.[40] 5-Fluorouracil was used as the positive control, and DMSO was used as the negative control.

Hemolysis Assay. To separate erythrocytes from human whole blood, 35 mL of human whole blood was centrifuged at 500*g*, 4 °C for 5 min. After centrifugation, the upper layers of plasma and buffy coat were aspirated by a micropipette. NaCl solution (150 mM) was added to the packed erythrocytes and filled up to the original volume mark, and the solution was gently shaken to resuspend the erythrocytes. The centrifugation process was repeated with the same condition, and after centrifugation, the upper layers were aspirated and discarded, followed by the resuspension of erythrocytes using phosphate-buffered saline (PBS) (pH 7.4). A 40× diluted erythrocyte suspension was prepared by adding 1 mL of the final suspension into 39 mL of PBS (pH 7.4). Compounds with a final concentration of 0.1, 1 and 10 μg/mL were incubated at 37 °C with 2% blood solution in PBS (pH 7.4) for 45 min. The 40× diluted erythrocyte suspension (1600 μL) was added to 400 μL of the drug solution in PBS (with 0.5% DMSO). The mixture was vortexed gently for 10 s and inverted several times to ensure a thorough mixing. The prepared mixture was transferred to 96-well microplates for incubation (200 μL per well, n = 6). Positive control was Triton X-100, a known hemolytic agent, with a final concentration of 1% v/v in PBS. Negative control is the vehicle which is PBS (pH 7.4). After incubation, the reactions were terminated by centrifuging the samples 10 min at 500*g*, 4 °C, to pellet the remaining erythrocytes and erythrocyte ghosts. The supernatant (100 μL) from

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each well was transferred to a new 96-well plate, and the absorbance of the supernatant was measured at 540 nm, which is the hemoglobin absorbance peak, with CLARIOstar microplate reader (BMG Labtech, Offenburg, Germany). % of lysis was calculated with the following equation

$$\%\ \text{of lysis} = \frac{A_{\text{sample}} - A_{-\text{ve control}}}{A_{+\text{ve control}} - A_{-\text{ve control}}}$$

Caco-2 Permeability Assay. Caco-2 cells were cultured at 37 °C in Dulbecco's modified Eagle medium with 10% fetal bovine serum, 1% nonessential amino acids, 100 U/mL penicillin, and 100 μg/mL streptomycin in an atmosphere of 5% CO_2 and 90% relative humidity. The cells were passaged after 90% confluence using trypsin−ethylenediaminetetraacetic acid (EDTA) and plated at a 1:5 ratio in 75 cm^2 flasks. The cells (passage number: 45) were seeded at a density of 60,000 cells/cm^2 on polycarbonate membranes of Transwells (12 mm inner diameter, 0.4 μm pore size, 1 cm^2, Corning, NY, USA). The medium was changed the day after seeding and every second day thereafter [apical (AP) volume 0.5 mL and BL volume 1.5 mL]. The Caco-2 cell monolayers were used 21−28 days after seeding. Transepithelial electrical resistance (TEER) was measured to ensure monolayer integrity. Cell monolayers with TEER values less than 165 Ω cm^2 were discarded in transport experiments. Transport studies involved only AP to basolateral (BL) direction. Cell monolayers were preincubated with transport buffer solution (PBS +) for 20 min at 37 °C. To study absorptive transport, the donor (AP) compartment buffer was replaced with 0.5 mL of transport buffer containing 10 μg/mL compounds. The pH in both AP and BL compartments was maintained at 7.4 for all transport studies. Concentration of compounds in the receiver (BL) compartment was monitored as a function of time in the linear region of transport and under sink condition (which is the receiver concentration is <10% of the donor concentration). At each sampling time point, 100 μL of the sample was drawn from the receiver compartment, and a same volume of blank buffer was added into the receiver compartment. The concentration of compounds in sample solution was determined using liquid chromatography−MS (LC−MS)/MS.

Binding Inhibition Assay. Previously established protocols were used for inhibitor testing.[43] Protein overproduction and purification were performed as detailed previously.[43] N-SmBiT-CH (40 μL, 2.5 μM in PBS) was added to 96-well plates and then mixed with 20 μL of compound at desired concentrations. The mixture was incubated for 10 min at 37 °C. C-LgBiT-σ^A (40 μL, 2.5 μM in PBS) was then added to each well, followed by incubation for 10 min at 37 °C. After the final incubation step, an equal volume of Promega Nano-Glo Luciferase Assay Substrate was added to the reaction mixture. Luminescence emitted was measured using a Victor X3 Multilabel plate reader. The experiment was performed in triplicate with technical repeats.

In Vitro Transcription Assay. Linear templates containing the λ P_R promoter followed by an initial 26 nt C-less transcribed region were made by PCR and purified via a QIAquick PCR purification kit (Qiagen, Valencia, CA). *E. coli* core RNAP (200 nM) was incubated with 1/10 volume of each compound (or DMSO) in TGA2 (20 mM Tris-acetate, 20 mM Na-acetate, 2 mM Mg-acetate, 5% glycerol, 1 mM dithiothreitol, 0.1 mM EDTA, pH 7.9) for 10 min at 37 °C. An equal volume of mix containing σ^{70} (200 nM), DNA template (200 nM), ApU (100 μM), 5 μM UTP and ATP, and 1 μM GTP (plus 10 μCi [α^{32}P]-GTP, 3000 Ci/mmol) in TGA2 was added, followed by 10 min incubation at 37 °C. Reactions were quenched by the addition of an equal volume of STOP buffer (10 M urea, 60 mM EDTA, 45 mM Tris-borate; pH 8.3). Samples were heated for 2 min at 95 °C and separated by electrophoresis in denaturing 9% acrylamide (19:1) gels (7 M urea, 0.5× TBE). The gels were dried and RNA products were visualized using FLA9000 phosphorimaging system and ImageQuant Software. In vitro transcription assays were carried out in triplicates and averaged.

Confocal Microscopy. *B. subtilis* strain BS1048 (RNAP β′-GFP) was grown on an LB agar plate. A single colony was incubated in an LB medium at 37 °C until OD_{600} ∼ 0.6. The compound at 1/4, 1/2, 1, 2, and 4 MIC was then added to the culture and allowed to incubate for further 15 min. The cell culture (2.5 μL) was placed onto 1.2% freshly made agarose plate and covered with a coverslip prior to imaging. A Leica TCS SPE confocal microscope equipped with a 63×/1.3 oil objective and mercury metal halide bulb was used to capture the fluorescence images. The fluorescence images were processed with LAS X software.[41,42]

Quantitation of Major Macromolecules. To assess the effects elicited by varying concentrations of treatment compounds on total DNA, RNA, and protein levels during cell growth, a master culture of *S. aureus* ATCC 29213 was first inoculated at OD_{600} 0.1 and allowed to grow to OD_{600} 0.2 (early log phase) at 37 °C with agitation at 200 rpm. Upon reaching this stage, the cultures were divided into aliquots, where compounds and drugs were added at their corresponding 1/4 and 1/8 MICs, complete with an untreated control culture. The samples were then harvested upon reaching OD_{600} 0.6 (the mid-log phase of *S. aureus*) at 3 mL. Other samples were harvested at volumes normalized to OD_{600} 0.6, before being pelleted at 5000*g* for 5 min at 4 °C, and the supernatant discarded. Total DNA, RNA, and protein were extracted and purified using the AllPrep Bacterial DNA/RNA/Protein Kit (Qiagen) following the manufacturer's guidelines. DNA and RNA levels were measured with a Qubit DNA BR Assay Kit (Invitrogen) and Qubit RNA BR Assay Kit (Invitrogen) coupled with a Qubit 4 Fluorometer (Thermo Fisher), while proteins levels were determined using an Pierce BCA Protein Assay Kit (Thermo Fisher). The experiment was performed in triplicates.

Time-Kill Kinetic Assay. *S. aureus*, *S. agalactiae,* and *S. pneumoniae* cells were suspended at ∼1.5 × 10^6 CFU/mL at log phase in MHB or BHI (for *Streptococcus* spp.) media containing test compounds at defined concentrations. Untreated controls were also included. The setup was cultured at 37 °C with agitation at 200 rpm (supplemented with 5% CO_2 for *Streptococcus* spp.). At times 0, 2, 4, and 6 h, 20 μL of samples was taken from each treatment group and serially diluted in 10-folds. The number of viable bacterial cells in each sample was quantified and expressed as CFU/mL following overnight incubation at 37 °C with 5% CO_2. The experiment was performed in triplicates.

Assessing ATP Production. Log phase *S. pneumoniae* cells (∼1.5 × 10^6 CFU/mL) were suspended in BHI medium with test compounds at various concentrations. An untreated control group was also included with test compounds in medium. The setup was grown in 37 °C with agitation at 200 rpm with 5% CO_2, with 100 μL samples being taken at time points 0, 2, 4, and 6 h, respectively, for each treatment group. The ATP production level was quantified using the BacTiter-Glo Microbial Cell Viability Assay Kit (Promega, Madison, Wisconsin, United States) following the manufacturer's protocol. The experiment was performed in triplicates.

***S. pneumoniae* Toxin Release.** *S. pneumoniae* cells were cultured overnight without agitation on 96-well round-bottomed plates with or without the addition of serially diluted concentrations of test compounds. As positive controls, antibiotics were also added at serial twofold dilutions from 2 to 0.002 μg/mL for rifampicin and ceftriaxone. Each of the corresponding 1/2 and 1/4 MIC values was determined following 16−20 h of incubation. The plates were then centrifuged at 3000*g* for 3 min, and the supernatant transferred to a fresh plate from the wells designated as challenged by 1/2 and 1/4 MIC compounds, control drugs, and untreated control. The supernatants were stored in −20 °C until use in the Western blot assay.

Western Blot. Samples were separated in polyacrylamide gels at 80 V for 15 min and then at 150 V for 1 h. Bands were then transferred to a PVDF membrane at 110 V for 1 h, followed by blocking with 5% nonfat milk in TBST buffer for 1 h and overnight incubation with 1:1000 rabbit polyclonal anti-pneumolysin primary antibody (ab71811, Abcam, Cambridge, United Kingdom) at 4 °C with agitation. TBST-washing cycles were performed before and after 1 h of incubation with 1:5000 goat polyclonal anti-rabbit HRP-conjugated secondary antibody (ab97051, Abcam, Cambridge, United Kingdom) at room temperature with agitation. Blots were developed

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with Bio-Rad Clarity Western ECL substrates and imaged in a Bio-Rad ChemiDoc Touch system in chemiluminescence mode (Bio-Rad, Hercules, California, United States). The experiment was performed in triplicates and representative data presented.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c00520.

MIC (μg/mL) of compounds **1**−**57** against representative Gram-positive and -negative pathogenic bacteria; inhibitory activity of representative compounds against the RNAP β'CH−σ^A PPI in the protein complementation assay; and ^{1}H, ^{13}C NMR, and HPLC spectra (PDF)

Molecular formula strings and biological data (CSV)

PDB coordinates for the computational model (PDB)

■ AUTHOR INFORMATION

Corresponding Authors

Zhong Zuo − *School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, New Territories, Hong Kong SAR, People's Republic of China*; orcid.org/0000-0002-6976-6157; Phone: +852 3943 6862; Email: joanzuo@cuhk.edu.hk

Xiao Yang − *Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, New Territories, Hong Kong SAR, People's Republic of China*; Phone: +852 3505 2573; Email: xiaoyang@cuhk.edu.hk

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, People's Republic of China*; orcid.org/0000-0001-9245-0356; Phone: +852 3400 8810; Email: cong.ma@polyu.edu.hk

Authors

Jiqing Ye − *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, People's Republic of China*

Adrian Jun Chu − *Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, New Territories, Hong Kong SAR, People's Republic of China*

Rachel Harper − *Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, New Territories, Hong Kong SAR, People's Republic of China*

Shu Ting Chan − *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, People's Republic of China*

Tsun Lam Shek − *School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, New Territories, Hong Kong SAR, People's Republic of China*

Yufeng Zhang − *School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, New Territories, Hong Kong SAR, People's Republic of China*

Margaret Ip − *Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, New Territories, Hong Kong SAR, People's Republic of China*

Mariya Sambir − *Department of Microbiology and the Center for RNA Biology, The Ohio State University, Columbus, Ohio 43210, United States*

Irina Artsimovitch − *Department of Microbiology and the Center for RNA Biology, The Ohio State University, Columbus, Ohio 43210, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.0c00520

Author Contributions

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Notes

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■ ABBREVIATIONS

RNAP, RNA polymerase; CH, clamp helix; PPI, protein−protein interaction; MIC, minimum inhibitory concentration; SAR, structure−activity relationship; CLSI, Clinical & Laboratory Standards Institute; GFP, green fluorescent protein;; CFU, colony-forming unit; BHI, brain−heart infusion medium

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