Scientific paper

Exploration of the Chemical Space of Novel Naphthalene-Sulfonamide and Anthranilic Acid-Based Inhibitors of Penicillin-Binding Proteins

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Abstract

Penicillin-binding proteins are a well established, validated and still a very promising target for the design and development of new antibacterial agents. Based on our previous discovery of several noncovalent small-molecule inhibitor hits for resistant PBPs we decided to additionally explore the chemical space around these compounds. In order to clarify their structure-activity relationships for PBP inhibition two new series of compounds were synthesized, characterized and evaluated biochemically: the derivatives of anthranilic acid and naphthalene-sulfonamide derivatives. The target compounds were tested for their inhibitory activities on three different transpeptidases: PBP2a from methicillin-resistant *Staphylococcus aureus* (MRSA) strains, PBP5fm from *Enterococcus faecium* strains, and PBP1b from *Streptococcus pneumoniae* strains. The most promising results for both of these series of compounds were obtained against the PBP2a enzyme with the IC_{50} values in the micromolar range. Although these results do not represent a significant breakthrough in the field of noncovalent PBP inhibitors, they do provide useful structure-activity relationship data, and thus a more solid basis for the design of potent and noncovalent inhibitors of resistant PBPs.

Keywords: Penicillin-binding proteins, penicillin-resistance, noncovalent inhibitors, optimization, anthranilic acid

1. Introduction

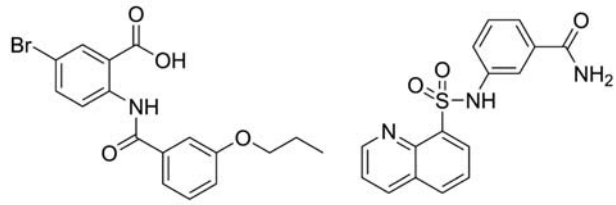
The fast development of antibacterial agents in the second half of the 20th century resulted in remarkable advances for humanity. The rate of mortality caused by infectious diseases has decreased dramatically, and it began to appear that we were the winners in the battle against pathogens**.** However, the appearance of multidrug resistant strains of pathogenic bacteria has become a serious medical issue in modern healthcare. The incidence of so-called 'superbugs' (organisms that are resistant to most of the clinically used antibiotics) is rapidly increasing. In 2004, more than 70% of pathogenic bacteria were estimated to be resistant to at least one of the currently available antibiotics.[1–3] Approximately 20 new antibiotics were approved from 2000 to 2010, of which just three had novel mechanisms of action: the lipopeptide daptomycin, oxazolidinone linezolid, and retapamulin that belongs to the natural class of compounds called pleuromutilins. Therefore, there is a serious and, unfortunately, unmet need for new antibacterial agents to treat these increasing levels of drug-resistant infections.[4]

Inhibition of bacterial cell-wall synthesis is a well-known mechanism of action of various antibiotics. In the synthesis of peptidoglycan, which is a major component of the cell wall, many enzymes are involved, and all of

them are potential targets for antibacterial agents. The last two steps of peptidoglycan biosynthesis are particularly attractive targets for potential antibacterial compounds, as they take place on the external surface of the cytoplasmic membrane and are therefore readily accesible. The transglycosylation and transpeptidation reactions are catalyzed by penicillin-binding proteins (PBPs). Bacteria have multiple PBPs that are generally membrane bound. PBPs can be divided into two classes: high molecular mass (HMM) and low molecular mass (LMM) PBPs. The bifunctional (class A) HMM PBPs catalyze both polymerization of the Glc*N*Ac-Mur*N*Ac chains and cross-linking of the adjacent stem peptide, while the monofunctional (class B) enzymes only catalyze transpeptidation (for excellent reviews, see[5,6]). Inhibition of HMM PBPs leads to cell death. The LMM PBPs are sometimes referred to as the class C PBPs;[5] their inhibition is generally not lethal, and it leads to a change in the cell-wall cross-linking pattern.[5,6]

Transpeptidation activities of PBPs are inhibited by β-lactam antibiotics that act as covalent inhibitors (including penicillins, cephalosporins, monobactams, and carbapenems). Once a PBP is acylated by a β-lactam antibiotic, it can no longer catalyze hydrolysis of the covalent acyl-enzyme intermediate, and it is inactivated; peptidoglycan transpeptidation can no longer occur, which results in bacterial death. However, bacteria have developed resistance to β-lactams by mechanisms that include: the production of β-lactamases, which can hydrolyze the β-lactams; the use of antibiotic efflux pumps, which can actively secrete these compounds from the periplasm of Gram-negative bacteria; and the production of β-lactam-insensitive PBPs. This last arises from the altered structure of the transpeptidase domain; therefore, covalent complex formation between the enzyme and β-lactams is hindered, which results in less effective antibiotics. The most representative examples are the highly mutated PBP2x from *Streptococcus pneumoniae*, the acquired low-affinity additionnal PBP in the case of PBP2a from methicillin-resistant *Staphylococcus aureus* (MRSA), and the overproduced low-affinity PBP5fm from *Enterococcus faecium*.[5,7] One of the possibilities to overcome this intrinsic poor acylation efficiency of resistant PBPs is to move away from the classical β-lactam scaffold, and thus to design new noncovalent compounds that bind tightly to the active site without acylation. Noncovalent inhibitors will not require the unfavorable conformational changes in the active site of resistant PBPs that are required for acylation, and they will also not be susceptible to β-lactamases.[5,8] To date, only a few noncovalent inhibitors of resistant PBPs have been described, and these have shown only medium potency.[9,10] The discovery of true potent noncovalent inhibitors of resistant PBPs thus remains a highly demanding and challenging task for medicinal chemistry. Such molecules will either kill the bacteria directly, or will make them more susceptible to existing antibiotics.

Recently, we discovered several noncovalent small-molecule inhibitor hits for resistant PBPs (*e.g.*, Figure 1, compounds **a, b**).[11] As the synthetic preparation of new derivatives is relatively straightforward, a wide variety of possible structural modifications is possible to explore the chemical space of these compounds. Therefore, we decided to use these two compounds as a basis for further studies in the field of noncovalent inhibitors of PBPs from penicillin-resistant bacterial strains.



a, IC_{50} (PBP2a) = 230 μM **b**, IC_{50} (PBP5fm) = 930 μM

Figure 1. Structural formulae of two previously published PBP inhibitors.[11]

2. Chemistry

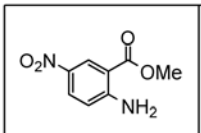
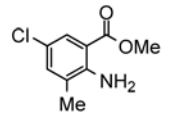
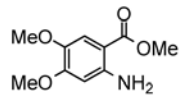
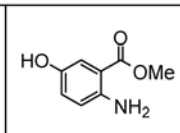
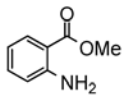
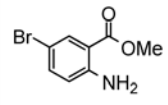
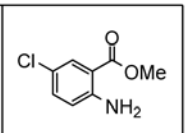
The previously published series of compounds was very limited, and thus it was not very representative.[11] We wanted to additionally explore the chemical space around these compounds, and consequently to clarify their structure-activity relationships for PBP inhibition. Therefore, two new series of compounds were synthesized: the derivatives of anthranilic acid and naphthalene-sulfonamide derivatives. All in all, 19 anthranilic-acid-based compounds and 15 naphthalene-sulfonamide-containing compounds were prepared, characterized and evaluated biochemically.

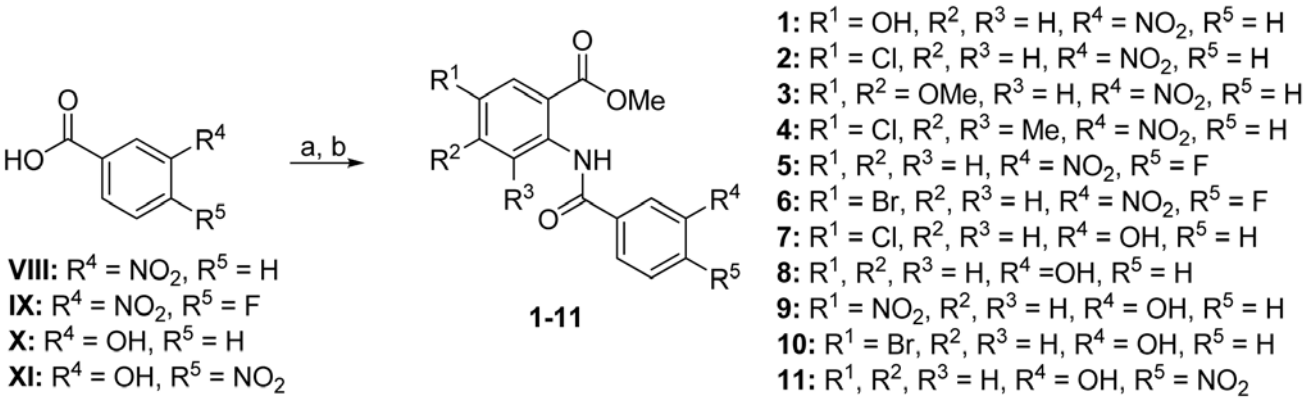
Substituted derivatives of methyl anthranilate **I–IV** were obtained from unprotected anthranilic acids with the use of $SOCl_2$ in MeOH, while derivatives **V–VII** were purchased from Sigma Aldrich and used in the subsequent reactions without purification (Table 1).

Very successful amide bond formation was achieved by *in-situ* transformation of the carboxylic group of benzoic acid derivatives **VIII–X** into acid chlorides using $SOCl_2$, followed by the addition of the corresponding *C*-protected anthranilic acid derivative **I–VII** into the reaction mixture (Scheme 1, compounds **1–11**). Compounds **10** and **11** were additionally derivatized afterwards, via formation of an alkyl ether on the OH group at the *meta* position with respect to the carboxamide moiety (Scheme 2).

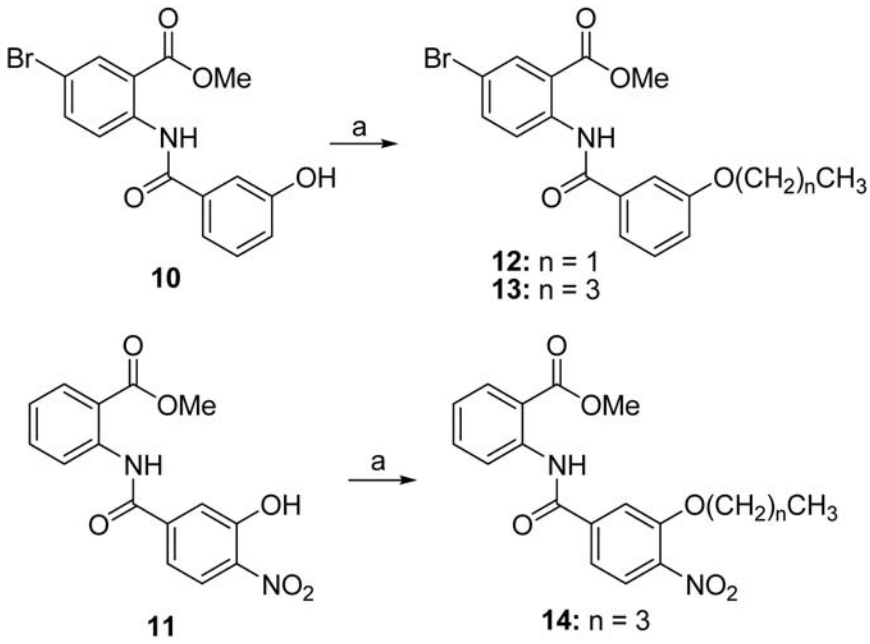
As shown in Scheme 3, esters **1–10** and **12–24** were deprotected with alkaline hydrolysis to yield the target compounds **15–27**. Moreover, compounds with an aromatic NO_2 group (**15–17**, **20**, **23**, **27**) were reduced into their corresponding amines by catalytic hydrogenation (**28–33**). As expected, when catalytic hydrogenation was performed to reduce the nitro group of compounds **16** and

Table 1. Structural formulae of the substituted methyl anthranilate derivatives.

						
I	II	III	IV	V	VI	VII



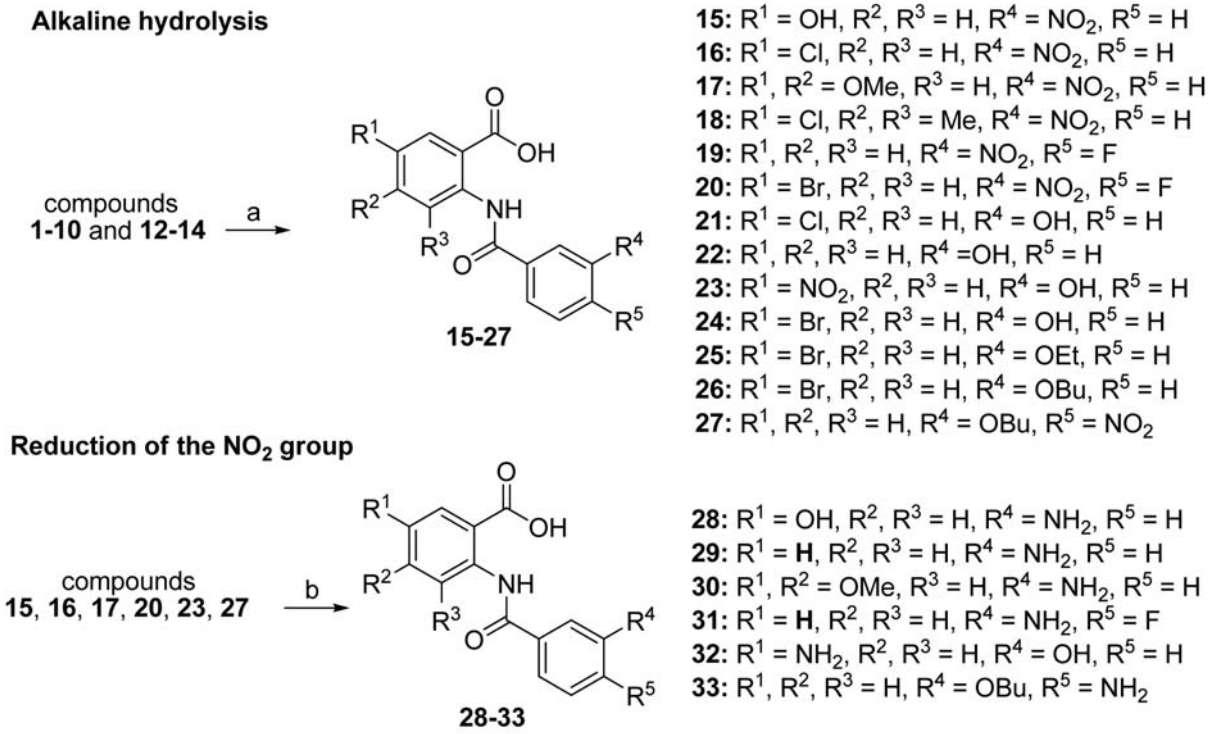
Scheme 1. Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2, py, 45 °C; (b) corresponding methyl anthranilate derivatives **I–VII** (Table 1), toluene, reflux.



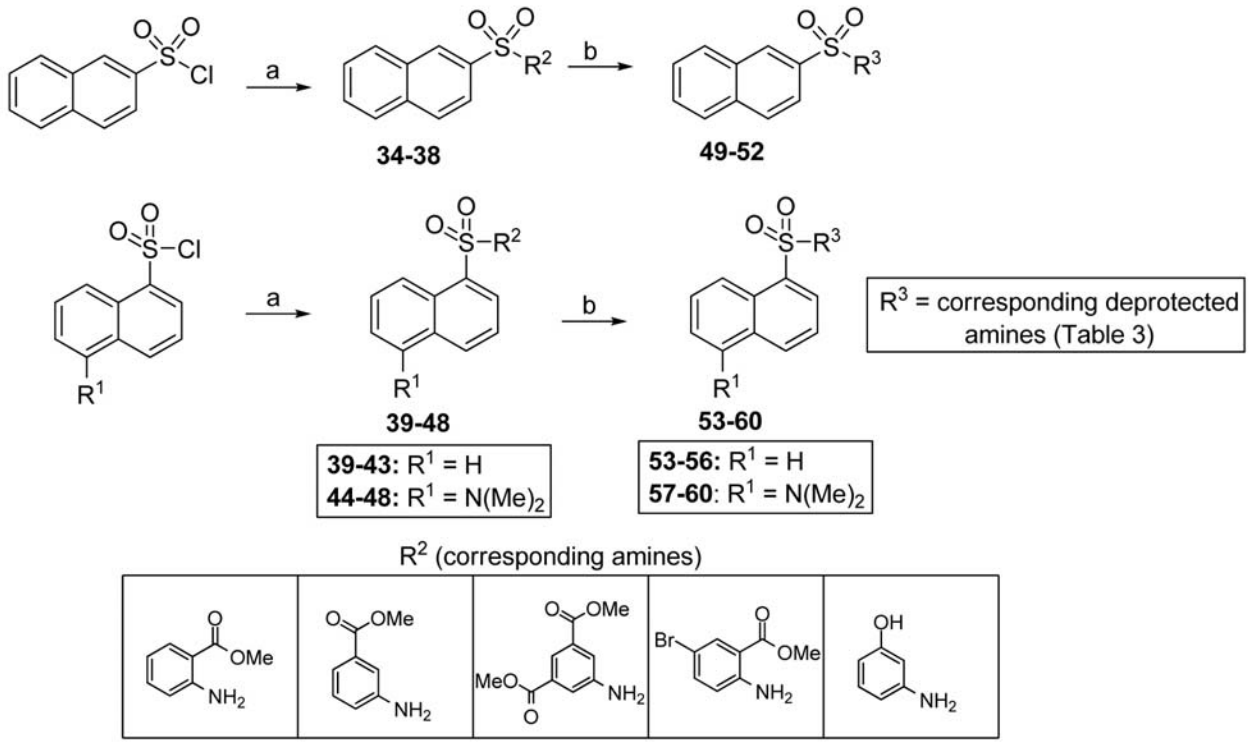
Scheme 2. Reagents and conditions: (a) $Br(CH_2)_nCH_3$, K_2CO_3, DMF, 50 °C.

20, the concomitant dehalogenation of a halogen at the *meta* position with respect to the carboxyl group occurred, to yield compounds **29** and **31**, respectively.[12,13] The removal and replacement of aromatic halogen substituents by hydrogen under conditions of hydrogenation over transition metal catalysts presumably involves intermediates formed by oxidative addition to the metal active catalyst, followed by reductive elimination.[14]

The naphthalene-sulfonamide derivatives were synthesized as shown in Scheme 4. Variously substitu-



Scheme 3. Reagents and conditions: (a) 1 M NaOH, dioxane/THF, room temp; (b) H_2, Pd/C, MeOH, room temp.



Scheme 4. Reagents and conditions: (a) corresponding amine, py, CH_2Cl_2, room temp; (b) 1 M NaOH, dioxane, room temp.

ted sulfonyl chlorides were successfully reacted with different aromatic amine derivatives, using pyridine as a base, to yield the sulfonamides **34**–**48**. When necessary, the methyl esters were hydrolyzed under alkaline conditions to the carboxylic acids **49**–**60** in the next stage.

3. Results and Discussion

3. 1. Inhibitory Activities

The target compounds were tested for their inhibitory activities on three different transpeptidases: PBP2a from MRSA strain, PBP5fm from *E. faecium* strain, and PBP1b from *S. pneumoniae strain* (Tables 2 and 3). The first two enzymes are representatives of the resistant types of transpeptidases. The results were determined as residual activities of the enzymes in the presence of 1 mM of each compound, and also as IC_{50} values for the most active derivatives. The most promising results for both of these series of compounds were obtained against the PBP2a enzyme. The majority of compounds were much weaker PBP1b inhibitors, and none of the compounds were active against the PBP5fm enzyme.

Table 2. PBP inhibitory potencies of anthranilic acid derivatives.[a]

Compound	RA[b] of PBP2a (%)	RA[b] of PBP5fm (%)	RA[b] of PBP1b (%)
15	77	85	92
16	8 **IC_{50} = 534 μM**	86	82
17	84	96	106
18	94	92	116
19	88	84	108[c]
20	9 **IC_{50} = 685 μM**	81	99[d]
21	48	88	99
22	94	84	86
23	64	96	100
24	50 **IC_{50} = 1 mM**	86	102
25	5 **IC_{50} = 490 μM**	83	108
26	2 **IC_{50} = 352 μM**	85	112[d]
27	6 **IC_{50} = 520 μM**	70	91
28	79	86	113
29	83	81	96
30	83	84	80
31	66	85	95
32	67	82	105[c]
33	51 **IC_{50} = 1 mM**	81	97

[a] Data are means of three independent experiments, each performed in duplicate. The standard deviations were within ±10% of these means.
[b] Except where noted otherwise, the residual activities (RAs) were determined at 1 mM of each compound.
[c] RAs determined at 100 μM of each compound.
[d] RAs determined at 200 μM of each compound.

3. 1. 1. Anthranilic-Acid-Based Derivatives

In our exploration of the chemical space of newly synthesized anthranilic-acid-based derivatives, and to determine the influences of the different substitution patterns on both of the phenyl rings, several functionalities were introduced onto both of these rings (Scheme 3, Table 2). As we previously established that on the phenyl rings the *meta* position with respect to both the carboxyl moiety and the carboxamide moiety is the most influential for PBP2a inhibition, we focused most of our attention on the exploration of possible substituents on these positions.

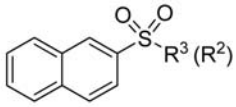
Unfortunately, most of the new substituents did not improve the PBP2a inhibitory activity of the compounds in comparison with the initial hit **a** (Figure 1). However, valuable structural data can be obtained on the basis of the results of the enzymatic evaluation of these new compounds. For instance, we have once again confirmed the importance of a large bromine atom at the *meta* position with respect to the carboxyl group, to maintain moderate PBP2a inhibition (compounds **20**, **24**–**26**). The absence of Br at the *meta* position completely abrogated the inhibitory activity (compare the residual activities between compounds **19** and **20,** or **22** and **24**, Table 2). Also, the introduction of a methoxy, nitro or amino group at the *meta* position led to reduced inhibition of PBP2a (*e.g.* compounds **17**, **23** and **32**).

It was cleary seen that the length of the alkyloxy chain positioned *meta* with respect to the carboxamide moiety has an important role in PBP2a inhibition. The compound with a butoxy fragment showed better inhibitory activity when compared with its ethoxy analog (compounds **25** and **26**; IC_{50} values of 490 μM and 352 μM, respectively). Moreover, when the chain was removed, the activity was significantly decreased (compound **24**; IC_{50} = 1 mM). It also appears that additional elongation of the chain is not desirable, as the inhibitory activity of the propoxy analog **a** (IC_{50} = 230 μM) was slightly better than for compound **26** with the butoxy chain.

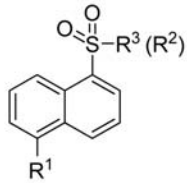
Also, when the nitro group at the *meta* position with respect to the carboxamide moiety was accompanied by Cl or Br at the appropriate position on the second phenyl ring, this led to compounds with desired inhibitory properties (compounds **16** and **20**; IC_{50} values of 534 μM and 685 μM, respectively). The compounds without the halogen atom (compound **19**) or with a hydroxy group (compound **15**) positioned *meta* with respect to the carboxyl group did not show inhibitory activity against PBP2a. The only compound that showed desirable properties and lacked the halogen at the appropriate position was compound **27** (IC_{50} = 520 μM), which had an additional NO_2 moiety positioned *para* with respect to the carboxamide group.

To better understand the binding mode of these compounds, a docking study was performed. The most active compound from this series, compound **26,** formed several interactions with the active site. Oxygen from the

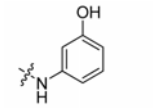
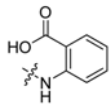
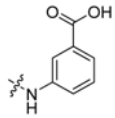
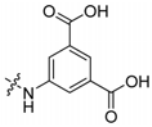
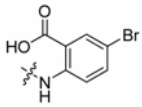
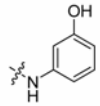
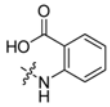
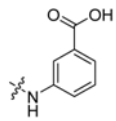
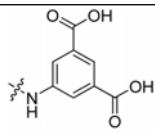
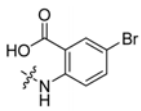
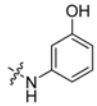
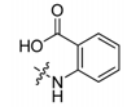
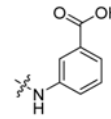
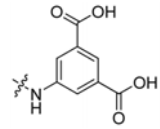
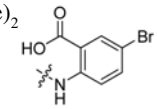
Table 3: PBP inhibitory potencies of the naphthalene-sulfonamide derivatives.[a]



38, 49-52



43, 48, 53-60

Compound	R^1	R^2 or R^3	RA[b] of PBP2a (%)	RA[b] of PBP5fm (%)	RA[b] of PBP1b (%)
38	–		98	89	98[c]
49	–		11 **IC_{50} = 425 µM**	100	97
50	–		100	97	100
51	–		90	100	112
52	–		7 **IC_{50} = 80 µM**	100	40
43	H		92	100	131
53	H		30 **IC_{50} = 740 µM**	92	89
54	H		92	100	111
55	H		100	100	98
56	H		14 **IC_{50} = 297 µM**	100	5 **IC_{50} = 540 µM**
48	N(Me)		96	100	99[d]
57	$N(Me)_2$		5 **IC_{50} = 536 µM**	92	77
58	$N(Me)_2$		84	89	61
59	$N(Me)_2$		99	99	104
60	$N(Me)_2$		4 **IC_{50} = 245 µM**	86	1 **IC_{50} = 416 µM**

[a] Data are means of three independent experiments, each performed in duplicate. The standard deviations were within ±10% of these means.
[b] Except where noted otherwise, the residual activities (RAs) were determined at 1 mM of each compound.
[c] RA determined at 100 µM of compound.
[d] RA determined at 200 µM of compound.

butoxy group formed H-bonds with Thr444 and Asn464, the amide oxygen formed an H-bond with Thr600, and the carboxylic acid group formed either H-bonds with Ser403 and Thr600 or electrostatic interactions with Lys406 (Figure 2). There were additional π–π interactions between the aromatic rings and Tyr446 (not shown, for clarity). However, the benefits of the bromine atom cannot be rationalized from these docking studies. A survey of the literature leads us to hypothesize that the Br is involved in the formation of a halogen bond,[15] although this effect could not be confirmed *in silico* because the docking programs

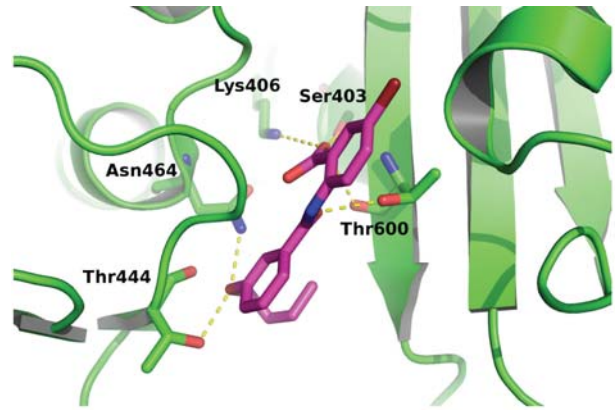


Figure 2. Docked conformation of compound **26** (magenta) into the PBP2a active site. The relevant amino-acid residues are shown as green sticks.

do not include a term for halogen bonds in their scoring functions.

3. 1. 2. Naphthalene-Sulfonamide-Based Derivatives

Better results were obtained with the second series of compounds, the naphthalene-sulfonamide derivatives. Similar to what was seen above, the best results were obtained against the PBP2a enzyme. The most potent of this series was compound **52**, with an IC_{50} of 80 μM against PBP2a. Moreover, as well as inhibition of PBP2a, two of the compounds showed moderate PBP1b inhibition (Table 3). In contrast to the starting compound **b**, none of the compounds from this series inhibited PBP5fm.

When comparing the results presented in Table 3, some structure-activity relationship data can be extracted. For instance, it appears that the carboxyl group on the phenyl ring at the *ortho* position with respect to the sulfonamide group was beneficial for the inhibition of PBP2a. Moreover, the best inhibitors additionally had the bromine atom positioned *meta* with respect to the carboxyl moiety (compounds **52**, **56** and **60**; IC_{50} values of 80 μM, 297 μM and 245 μM, respectively). The importance of the bromine atom for improvement of the inhibitory activity is undisputable if we compare compounds **49** and **52**, where the introduction of the Br led to more than 5-fold improved inhibitory activity (IC_{50} reduced from 425 μM to 80 μM). The influence of the dimethylamino group at position 5 of the naphthalene ring was, on the other hand, less pronounced, as the IC_{50} was only slightly decreased when the $–N(CH_3)_2$ moiety was introduced (compare the IC_{50} values of compounds **53**, **57** and **56**, **60**). The better inhibitory activities of compounds **49** and **52** in comparison with **53** and **56** indicates that the position of the sulfonamide moiety on the naphthalene ring also has a role in the inhibition of PBP2a. The better inhibitors were compounds with the sulfonamide moiety at position 2 of the naphthalene ring, as the results showed from two-fold to three-fold increases in the IC_{50} values for 1-substituted naphthalene derivatives.

Compounds **56** and **60** also inhibited the PBP1b enzyme with IC_{50} values of 540 μM and 416 μM, respectively. Again, it appears that it is the 5-bromo-2-sulfonamido benzoic acid that contributes most to the binding affinity. Compounds without the bromine atom positioned *meta* with respect to the carboxylic acid (compounds **53** and **57**) did not inhibit PBP1b at all. Contrary to the PBP2a inhibition, for the inhibitory activity against PBP1b, the substitution *via* position 1 of the naphthalene ring was favorable, which is clearly seen from a comparison of the inhibitory activities of compounds **52** (residual activity, 40%) and **60** (residual acivity, 1%; IC_{50} = 416 μM). Similar to PBP2a, the positive role of the dimethylamino group for inhibition of PBP1b was not substantial, as only a slight improvement was observed.

Figure 3 shows the plausible binding mode of compound **52** in the active site of PBP2a. The FlexX program predicted 3 hydrogen bonds between the inhibitor and the active site of the enzyme. The sulfonamide oxygen atoms enabled the formation of two H-bonds: one with Thr600 and the other with Asn464. Based on the position of the sulfonamide moiety in the active site, we can postulate that it acts as a natural substrate D-Ala-D-Ala peptide bond mimetic. A third hydrogen bond can be formed between the carboxyl group of the inhibitor and the residue Asn464. This undisputedly confirms the importance of the position of the carboxyl group. All of the above-mentioned amino-acid residues are a part of the conserved motifs that are characteristic of the transpeptidase domain and that define the active site of the enzyme. Additional π-interactions were possible between the naphthalene ring and Tyr446 (not shown, for clarity). Similar to what was seen above, no obvious benefits of the bromine atom could be observed from these docking studies.

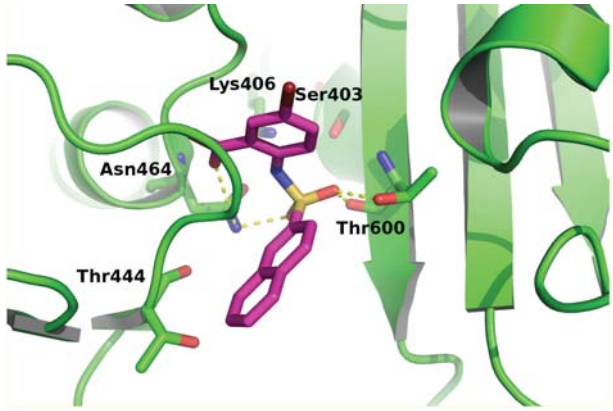


Figure 3. Docked conformation of compound **52** (magenta) into the PBP2a active site. The relevant amino-acid residues are shown as green sticks.

We also predicted the binding mode of compounds **56** and **60** (data not shown) into the active site, and compared their positions with the most potent compound (**52**). The H-bonding patterns remained the same; however, some differences in the naphthalene ring orientation were noted, which possibly contribute to the less favorable inhibition. The influence of the dimethylamino group (compound **60**) is not entirely clear, although the docking study suggested that this moiety is responsible for contact with the solvent.

3. 2. Antimicrobial Activity Evaluation

For the compounds that showed enzyme inhibitory activity, their *in-vitro* antibacterial activities were determined using a panel of different Gram-positive and Gram-negative bacterial strains. These data are given in Table 4.

Table 4. MIC determinations from the antimicrobial testing.

Bacterial strain	Compound										
	16	**20**	**25**	**26**	**27**	**49**	**52**	**53**	**56**	**57**	**60**
Escherichia coli ATCC 8739	>128	>128	>128	>128	>256	>128	>128	>128	>128	>128	>128
Proteus mirabilis ATCC 29936	>128	>128	>128	>128	>256	>128	>128	>128	>128	>128	>128
Klebsiella pneumoniae ATCC 13883	>128	>128	>128	>128	>256	>128	>128	>128	>128	>128	>128
Citrobacter freundii ATCC 8090	>128	>128	>128	>128	>256	>128	>128	>128	>128	>128	>128
Pseudomonas aeruginosa ATCC 27853	>128	>128	>128	>128	>256	>128	>128	>128	>128	>128	>128
Micrococcus luteus ATCC 9341	>128	>128	>128	32	128	>128	>128	>128	>128	>128	>128
Bacillus subtilis ATCC 6633	128	>128	64	8	128	>128	128	>128	128	>128	64
Listeria innocua ATCC 33090	128	>128	64	4	128	>128	64	>128	128	>128	64
Listeria monocytogenes ATCC 14780	128	>128	64	4	128	>128	32	>128	128	>128	64
Staphylococcus epidermidis ATCC 12228	128	>128	8	4	256	>128	128	>128	64	>128	64
Staphylococcus aureus ATCC 25923	128	>128	128	64	128	>128	128	>128	64	>128	128
Staphylococcus aureus PL1 (inducible MRSA)	128	>128	8	4	128	>128	128	>128	128	>128	128
Staphylococcus aureus ATCC 43300 (MRSA)	128	>128	8	2	128	>128	128	>128	128	128	64
Enterococcus faecalis ATCC 7937	128	>128	128	32	128	128	128	>128	128	128	64
Enterococcus faecalis ATCC 29212	128	>128	128	32	128	128	128	>128	128	128	64
Enterococcus faecium ATCC 19434	128	>128	128	64	>256	128	128	>128	128	128	128
Enterococcus hirae ATCC 9790	128	>128	128	64	128	128	128	>128	128	128	128

Most of the compounds from both of the series were unfortunately found to be weak inhibitors of bacterial growth, with minimum inhibitory concentrations (MICs) around 128 μg/mL. This might be ascribed to their poor on-target activities. Nevertheless, some compounds showed good antibacterial activities; *e.g.,* compounds **25** and **26**. This latter showed antibacterial activity against *Listeria innocua, Listeria monocytogenes, Staphylococcus epidermidis* and *Bacillus subtilis* strains, with MICs of 4 μg/mL or 8 μg/mL. Interestingly, compound **26** also prevented the growth of one strain (PL1) of the inducible methicillin-resistant *S. aureus* and the MRSA strain ATCC 43300, with MICs of 4 μg/mL and 2 μg/mL, respectively. Even more intriguing, compounds **25** and **26** prevented the growth of the two *S. aureus* strains with PBP2a, from 14-fold to 32-fold more efficiently than the *S. aureus* ATCC 25923 strain, which is sensitive to pencillin and devoid of PBP2a (Table 4). However, as the MIC values for **25** and **26** are lower than the corresponding IC_{50} values for each of these compounds, we suggest that these compounds show their antibacterial activities *in vitro* through actions on other bacterial targets, besides interacting with PBPs.

4. Conclusions

Although these synthesized compounds showed only moderate inhibitory activities (IC_{50} generally ≥100 μM), and although the data do not represent a significant breakthrough in the field of noncovalent PBP inhibitors, these data do provide useful additional structural information, and thus a more solid basis for the design of potent and noncovalent inhibitors of resistant PBPs. All of the new knowledge gathered in this field should even-

tually lead to the development of effective antibacterial agents that target a validated and very promising bacterial target. Our future plans include the synthesis of new structurally related derivatives to further improve the inhibitory potencies against PBPs, as well as the obtaining of co-crystal structures of the inhibitor-enzyme complexes that will provide an excellent basis for further rational structure-based developments of noncovalent PBP inhibitors.

5. Supporting Information

Detailed descriptions of the materials and methods and all experimental procedures used to prepare the intermediates and final compounds reported in the manuscript are available in the supporting information. Also, the enzymatic inhibition assays, antimicrobial activity evaluation and the data regarding the computational work are fully disclosed in the supplementary data.

6. Abbreviations

PBP, penicillin-binding protein; HMM, high molecular mass; LMM, low molecular mass; MRSA, methicillin-resistant *Staphylococcus aureus*

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Povzetek

Penicilin vezoči proteini (PBP) so uveljavljena, validirana ter še vedno obetavna tarča za načrtovanje in razvoj novih protimikrobnih učinkovin. Na osnovi naših, pred kratkim odkritih nekovalentnih inhibitorjev (zadetkov) PBP iz rezistentnih sevov smo se odločili dodatno raziskati kemijski prostor teh spojin. Z namenom dodobra razjasniti odnos med strukturo in delovanjem smo sintetizirali ter biokemijsko ovrednotili dve seriji spojin: derivate antranilne kisline ter naftalen-sulfonamidne derivate. Spojinam smo določili inhibitorno aktivnost na treh različnih transpeptidazah, in sicer na PBP2a iz na meticilin odpornega *Staphylococcus aureus* (MRSA), PBP5fm iz *Enterococcus faecium* (sev D63r) ter na PBP1b iz seva *Streptococcus pneumoniae*. Najbolj obetavne rezultate pri obeh serijah spojin smo dobili na encimu PBP2a z IC_{50} vrednostmi v mikromolarnem območju. Čeprav ti rezultati ne predstavljajo signifikantnega preboja na področju nekovalentnih inhibitorjev PBP, pa zagotovo nudijo uporabne podatke o odnosu med strukturo in delovanjem ter tako tudi boljšo osnovo za nadaljnje načrtovanje močnih, nekovalentnih inhibitorjev PBP iz rezistentnih bakterij.

Supporting information

Exploration of the Chemical Space of Novel Naphthalene-Sulfonamide and Anthranilic Acid-Based Inhibitors of Penicillin-Binding Proteins

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1. Materials and Methods

1. 1. Chemistry

The reagents and solvents were obtained from commercial sources (Fluka, Sigma-Aldrich, Acros Organics, Alfa Aesar, Fluorochem). The solvents were distilled before use, while the other chemicals were used as received. Analytical TLC was performed on Merck silica gel ($60F_{254}$) pre-coated plates (0.25 mm), with the compounds visualised under UV light and/or stained with the relevant reagent. Column chromatography was performed on Merck silica gel 60 (mesh 70-230), using the indicated solvents. Yields refer to the purified products, and they were not optimised. All of the melting points were determined on a Reichert hot-stage apparatus, and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance 300 DPX spectrometer at 302 K, and are reported in ppm using tetramethylsilane or solvent as internal standard (DMSO-d_6 at 2.50 ppm, $CDCl_3$ at 7.26 ppm). The coupling constants (*J*) are in Hz, and the splitting patterns are designated as: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; td, triple doublet; t, triplet; dt, double triplet; and m, multiplet. ^{13}C NMR spectra were recorded on a Bruker Avance 400 DPX spectrometer at 302 K, and are reported in ppm using solvent as internal standard (DMSO–d_6 at 39.5 ppm). Mass spectra data and high-resolution mass measurements were performed on a VG-Analytical Autospec Q mass spectrometer. The purities of all assayed compounds were determined with elemental analyes or HPLC. The analyses are indicated by the symbols of the elements, and they were within ±0.4% of the theoretical values. The purities of the compounds determined by HPLC were >95%. Elemental analyses were performed on a 240 C Perkin-Elmer C, H, N analyser. HPLC was performed on an Agilent Eclipse C18 column (4.6 × 50 mm, 5 μm), with a flow rate of 1.0 mL/min, detection at 254 nm, and an eluent system of: A = 0.1% TFA in H_2O; B = MeOH. The following gradient was applied: 0–3 min, 40% B; 3–18 min, 40% B → 80% B; 18–23 min, 80% B; 23–30 min, 80% B → 40% B. The run time was 30 min, at a temperature of 25 °C.

1. 2. Enzymatic Inhibition Assays for Low-Affinity PBP2a and PBP5fm

PBP2a from *S. aureus* ATCC 43300 and PBP5fm from *E. faecium* D63r were over-expressed and purified as described previously.[1,2] Each of the purified PBPs (2.5 μM) were first incubated with 1 mM of each of the potential inhibitors, in 100 mM phosphate buffer, 0.01% Triton X-100,[3] pH 7, for 4 h at 30 °C. Then, 25 μM fluorescein-labeled ampicillin[4] was added to detect the residual penicillin-binding activity (the residual activity). The samples were further incubated for 30 min at 37 °C in a total volume of 20 μL. Denaturation buffer was added (0.1 M Tris/HCl, pH 6.8, containing 25% glycerol, 2% SDS, 20% β-mercaptoethanol and 0.02% bromophenol blue) and the samples were heated to 100 °C for 1 min. The samples were then loaded onto a 10% SDS-acrilamide gel (10 × 7 cm), and electrophoresis was performed for 45 min at 180 V (12 mA). Detection and quantification of the residual

activities were carried out with Molecular Image FX equipment and Quantity One software (BioRad, Hercules, CA, USA). Three independent experiments, each performed in duplicate, were carried out for each inhibitor.

1. 3. Enzymatic Inhibition Assays for PBP1b

PBP1b (0.2 μM) was incubated with 1 mM of each of the potential inhibitors, in sodium phosphate buffer (pH 7.0) in the presence of 100 mM D-alanine, 0.01% Triton-X-100[3] and 0.01 mg/mL BSA, for 60 min at 25 °C. The residual activities were determined from the initial rates of hydrolysis of the thioester 2-(2-benzamidopropanoylthio) acetic acid (5 mM) used as the reporter substrate. After preincubation, the initial rate of thioester hydrolysis and of spontaneous hydrolysis ($\varepsilon[\Delta\varepsilon]_{412\ nm}$ = 13,600 M^{-1} cm^{-1}) were measured in the presence of 1 mM DTNB using a 96-well microtiter plate and a Power Wave microtiter plate reader (Bio-Tek Instruments; total volume, 150 μL). Three independent experiments, each performed in duplicate, were carried out. The activity of PBP1b in the absence of the compounds (residual activity, 100%) was measured by performing six replicates on each plate. If inhibition was detected, the residual activity was measured over a range of concentrations, from which the IC_{50} values were determined, using nonlinear regression analysis and Sigma Plot (Systat software), with the fitting of the data to the equation y = y0 + (a × b)/(b + x).

1. 4. Antibacterial Activity

Determination of the antibacterial activities was carried out on microtiter plates, in Müeller-Hinton broth and with a 200 μL final volume, following the recommended procedures of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standard Institute (CLSI).[5,6] The compounds were dissolved in Müeller-Hinton broth just before use. Inocula were prepared for each strain by resuspending isolated colonies from 18-h-cultured plates. Equivalents of 0.5 MacFarland turbidity standards (approximately 1 × 10^8 CFU/mL) were prepared in saline solution (0.085% NaCl) and then diluted 200-fold in Müeller-Hinton broth. The MICs were determined as the lowest dilution of product that showed no visual turbidity.

2. Computational

The computational analysis was performed on a workstation with 4 dual-core AMD Opteron 2.0 GHz processors, with 16 GB RAM, 4 320-GB hard drives, in RAID10 array and with nVidia GeForce 7900 graphic cards. The workstation has Fedora 7 64-bit installed. Docking was performed with FlexX 3.0 (BioSolveIT GmbH).[7] For docking into the PBP2a crystal structure, 1VQQ was used. The active site was defined as the area within 10 Å of Lys406. The maximum allowed overlap volume vas set at 20 $Å^3$. For base placement, Triangle Matching was used, and the program generated a maximum of 200 solutions per iteration, and 200 per fragmentation.

3. Experimental Procedures

3. 1. General Procedure for the Preparation of Methyl Esters I–IV

To an ice-cooled solution of an appropriate anthranilic acid (1.0 mmol) in MeOH (15 mL), $SOCl_2$ (1.78 g, 15 mmol) was slowly added, with continuous stirring. The mixture was stirred at room temperature for 2 days. After the reaction was complete (monitored by TLC), the solvent was evaporated, and saturated aqueous $NaHCO_3$ (20 mL) was added to the residue. The aqueous phase was extracted with EtOAc (3 × 20 mL), and the combined organic phases were washed with brine (2 × 20 mL), dried over Na_2SO_4, filtered, and removed under reduced pressure. The resulting residue was purified by column chromatography to provide the corresponding methyl anthranilates **I–IV**.

Methyl 2-amino-5-nitrobenzoate (I).[8]

Column chromatography (EtOAc/hexane = 1/3); Yellow crystals (needles); Yield: 56%; R_f 0.48 (EtOAc/hexane = 1/1); Mp: 166.0–167.0 °C (lit [23] 167.0–169.0 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.95 (s, 3H, CH_3), 6.52 (br s, 2H, NH_2), 6.69 (d, J = 9.2 Hz, 1H, Ar-*H*), 8.15 (dd, J = 9.2, 2.7 Hz, 1H, Ar-*H*), 8.86 (d, J = 2.7 Hz, 1H, Ar-*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 52.16, 109.20, 116.21, 128.90, 129.21, 137.24, 154.71, 167.23; HRMS (ESI) m/z calcd for $C_8H_9N_2O_4$ $[M+H]^+$ 197.0562, found 197.0562.

Methyl 2-amino-5-chloro-3-methylbenzoate (II).[9]

Column chromatography (CH_2Cl_2/MeOH = 20/1); Brown crystals (needles); Yield: 61%; R_f 0.83 (CH_2Cl_2/MeOH = 20/1); Mp: 35.5–37.5 °C (lit [24] 33.0–35.0 °C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.80 (s, 3H, CH_3), 6.59 (br s, 2H, NH_2), 7.25 (d, J = 2.3 Hz, 1H, Ar-*H*), 7.57 (d, J = 2.3 Hz, 1H, Ar-*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.22, 51.67, 110.78, 119.88, 124.89, 128.08, 134.42, 147.54, 167.97; HRMS (ESI) m/z calcd for $C_9H_{11}NO_2Cl$ $[M+H]^+$ 200.0478, found 200.0472.

Methyl 2-amino-4,5-dimethoxybenzoate (III).[10]

Column chromatography (EtOAc/hexane = 1/1); White crystals (prisms); Yield: 79%; R_f 0.43 (EtOAc/hexane = 1/1); Mp: 124.0–124.5 °C (lit [25] 127.0–129.0 °C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.54 (s, 3H, CH_3), 3.74 (s, 6H, 2 × OCH_3), 6.36 (br s, 2H, NH_2), 6.43 (s, 1H, Ar-*H*), 7.13 (s, 1H, Ar-*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 51.23, 55.65, 56.24, 99.20, 101.87, 112.37, 140.44,

147.03, 154.66, 168.08; HRMS (ESI) m/z calcd for $C_9H_{10}NO_3$ $[M+H–CH_3OH]^+$ 180.0661, found 180.0647.

Methyl 2-amino-5-hydroxybenzoate (IV).[11]

Column chromatography (EtOAc/hexane = 1/1); Pale brown crystals (prisms); Yield: 57%; R_f 0.35 (EtOAc/hexane = 1/1); Mp: 159.0–161.0 °C (lit [26] 160.0–162.0 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.76 (s, 3H, CH_3), 6.05 (br s, 2H, NH_2), 6.64 (d, J = 8.8 Hz, 1H, Ar-H), 6.80 (dd, J = 8.8, 2.9 Hz, 1H, Ar-H), 7.10 (d, J = 2.9 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 51.64, 111.05, 116.01, 118.20, 123.08, 144.97, 145.96, 168.07; HRMS (ESI) m/z calcd for $C_8H_{10}NO_3$ $[M+H]^+$ 168.0661, found 168.0653.

3. 2. General Procedure for the Preparation of Compounds 1–11.

To a solution of an appropriate carboxylic acid **VIII–X** (1.0 mmol) in CH_2Cl_2 (10 mL), pyridine (99 mg, 1.25 mmol) and $SOCl_2$ (773 mg, 6.5 mmol) were slowly added. After stirring at 45 °C for 2 h, the solvent was removed under reduced pressure. The reaction mixture was dissolved in toluene, and then the corresponding methyl anthranilate derivative **I–VII** (1.25 mmol) was added and the reaction mixture was stirred at 100 °C for 3 h. After the reaction was complete (monitored by TLC), the solvent was evaporated, then an aqueous solution of Na_2CO_3 (10%, 10 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with brine (2 × 20 mL) and dried over Na_2SO_4. The solvent was evaporated, and the pure products **1–11** were obtained by crystallization from the corresponding solvent.

Methyl 5-hydroxy-2-(3-nitrobenzamido)benzoate (1).

Crystallization from EtOH; Yellow crystals (needles); Yield: 55%; R_f 0.35 (EtOAc/hexane = 1/1); Mp: 210.5–212.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 3H, CH_3), 7.08 (dd, J = 8.9, 2.9 Hz, 1H, Ar-H), 7.35 (d, J = 2.9 Hz, 1H, Ar-H), 7.88 (t, J = 8.0 Hz, 1H, Ar-H), 7.99 (d, J = 8.9 Hz, 1H, Ar-H), 8.33–8.36 (m, 1H, Ar-H), 8.44–8.47 (m, 1H, Ar-H), 8.72 (t, J = 1.6 Hz, 1H, Ar-H), 9.80 (br s, 1H, OH), 11.08 (br s, 1H, NHCO); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.42, 116.15, 120.57, 121.54, 121.94, 124.74, 126.27, 130.27, 130.55, 133.25, 135.98, 147.91, 153.92, 162.49, 167.17; HRMS (ESI) m/z calcd for $C_{15}H_{11}N_2O_6$ $[M–H]^-$ 315.0617, found 315.0618; Anal. calcd. for $C_{15}H_{12}N_2O_6$: C, 56.96; H, 3.82; N, 8.86. Found: C, 56.92; H, 3.61; N, 8.80.

Methyl 5-chloro-2-(3-nitrobenzamido)benzoate (2).

Crystallization from EtOH; White crystals (needles); Yield: 74%; R_f 0.55 (EtOAc/hexane = 1/1); Mp: 187.0–188.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.90 (s, 3H, CH_3), 7.39 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 7.93 (t, J = 5.0 Hz, 1H, Ar-H), 8.02 (d, J = 8.4 Hz, 1H, Ar-H), 8.36–8.38 (m, 1H, Ar-H), 8.49–8.52 (m, 2H, Ar-H), 8.74 (t, J = 1.8 Hz, 1H, Ar-H), 11.64 (br s, 1H, NHCO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 52.88, 113.50, 120.40, 122.74, 123.57, 126.62, 130.10, 132.08, 132.85, 136.15, 141.35, 142.06, 148.59, 163.13, 168.60; HRMS (ESI) m/z calcd for $C_{15}H_{12}N_2O_5Cl$ $[M+H]^+$ 335.0435, found 335.0428; Anal. calcd. for $C_{15}H_{11}N_2O_5Cl$: C, 53.83; H, 3.31; N, 8.37. Found: C, 53.60; H, 2.94; N, 8.18.

Methyl 4,5-dimethoxy-2-(3-nitrobenzamido)benzoate (3).

Crystallization from EtOAc; Fluorescent yellow crystals (needles); Yield: 69%; R_f 0.26 (EtOAc/hexane = 3/5); Mp: 189.5–192.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.82 (s, 3H, CH_3), 3.88 (s, 6H, 2 × OCH_3), 7.48 (s, 1H, Ar-H), 7.92 (t, J = 8.2 Hz, 1H, Ar-H), 8.22 (s, 1H, Ar-H), 8.35–8.38 (m, 1H, Ar-H), 8.46–8.49 (m, 1H, Ar-H), 8.73 (t, J = 2.1 Hz, 1H, Ar-H), 11.74 (br s, 1H, NHCO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 52.44, 56.04, 56.19, 103.31, 106.98, 112.01, 122.71, 126.28, 129.96, 132.60, 136.58, 137.28, 144.47, 148.57, 154.08, 162.89, 168.81; HRMS (ESI) m/z calcd for $C_{17}H_{17}N_2O_7$ $[M+H]^+$ 361.1036, found 361.1021; Anal. calcd. for $C_{17}H_{16}N_2O_7$: C, 56.67; H, 4.48; N, 7.77. Found: C, 56.86; H, 4.33; N, 7.78.

Methyl 5-chloro-3-methyl-2-(3-nitrobenzamido)benzoate (4).

Crystallization from EtOH; White crystals (needles); Yield: 50%; R_f 0.79 (EtOAc/hexane = 1/1); Mp: 153.0–155.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.30 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 7.69 (s, 2H, 2 × Ar-H), 7.86 (t, J = 8.1 Hz, 1H, Ar-H), 8.37–8.40 (m, 1H, Ar-H), 8.44–8.48 (ddd, J = 8.1, 2.1, 1.0 Hz, 1H, Ar-H), 8.77 (t, J = 2.1 Hz, 1H, Ar-H), 10.41 (br s, 1H, NHCO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.43, 52.82, 122.75, 123.89, 126.58, 128.24, 130.03, 131.31, 133.33, 135.66, 135.75, 135.84, 137.76, 148.43, 163.08, 167.18; HRMS (ESI) m/z calcd for $C_{16}H_{14}N_2O_5Cl$ $[M+H]^+$ 349.0591, found 349.0599; Anal. calcd. for $C_{16}H_{13}N_2O_5Cl$: C, 55.10; H, 3.76; N, 8.03. Found: C, 55.32; H, 3.58; N, 8.03.

Methyl 2-(4-fluoro-3-nitrobenzamido)benzoate (5).

Crystallization from EtOH; Yellow crystals (needles); Yield: 81%; R_f 0.56 (EtOAc/hexane = 1/1); Mp: 136.0–138.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.00 (s, 3H, CH_3), 7.16–7.21 (m, 1H, Ar-H), 7.43–7.49 (m, 1H, Ar-H), 7.64 (td, J = 8.6, 1.6 Hz, 1H, Ar-H), 8.12 (dd, J = 8.0, 1.6 Hz, 1H, Ar-H), 8.29–8.34 (m, 1H, Ar-H), 8.79 (dd, J = 7.2, 2.4 Hz, 1H, Ar-H), 8.86 (d, J = 8.6 Hz, 1H, Ar-H), 12.27 (br s, 1H, NHCO); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.50, 119.04, 119.24 and 119.46 (1C, $^2J_{C,F}$ = 21.6 Hz), 121.92, 124.18, 125.45 and 125.47 (1C, $^4J_{C,F}$ = 1.6 Hz), 130.54, 131.09 and 131.13 (1C, $^3J_{C,F}$ = 4.0 Hz), 133.91, 134.63 and 134.73 (1C, $^2J_{C,F}$ = 10.3 Hz), 136.79 and 136.86 (1C, $^3J_{C,F}$ = 7.7 Hz), 138.87, 155.23 and 157.89 (1C, $^1J_{C,F}$ = 265 Hz), 161.85, 167.58; HRMS (ESI) m/z calcd for $C_{15}H_{12}N_2O_5F$ $[M+H]^+$ 319.0730, found

319.0724; Anal. calcd. for $C_{15}H_{11}N_2O_5F$: C, 56.61; H, 3.48; N, 8.80. Found: C, 56.80; H, 3.24; N, 8.71.

Methyl 5-bromo-2-(4-fluoro-3-nitrobenzamido)benzoate (6).

Crystallization from EtOH; White crystals (needles); Yield: 76%; R_f 0.74 (EtOAc/hexane = 1/1); Mp: 187.5–188.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.87 (s, 3H, CH_3), 7.81–7.91 (m, 2H, Ar-*H*), 8.06 (d, J = 2.4 Hz, 1H, Ar-*H*), 8.18 (d, J = 8.9 Hz, 1H, Ar-*H*), 8.32–8.37 (m, 1H, Ar-*H*), 8.69 (dd, J = 7.2, 2.4 Hz, 1H, Ar-*H*), 11.32 (br s, 1H, N*H*CO); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.77, 115.97, 119.28 and 119.49 (1C, $^2J_{C,F}$ = 21.3 Hz), 121.87, 124.44, 125.54 and 125.56 (1C, $^4J_{C,F}$ = 1.9 Hz), 130.82 and 130.85 (1C, $^3J_{C,F}$ = 4.0 Hz), 132.64, 134.81 and 134.92 (1C, $^2J_{C,F}$ = 10.4 Hz), 136.31, 136.81 and 136.89 (1C, $^3J_{C,F}$ = 8.0 Hz), 137.76, 155.31 and 157.97 (1C, $^1J_{C,F}$ = 265 Hz), 162.03, 166.16; HRMS (ESI) m/z calcd for $C_{15}H_9N_2O_5FBr$ $[M–H]^-$ 394.9679, found 394.9688; Anal. calcd. for $C_{15}H_{10}N_2O_5FBr$: C, 45.36; H, 2.54; N, 7.05. Found: C, 45.37; H, 2.35; N, 7.00.

Methyl 5-chloro-2-(3-hydroxybenzamido)benzoate (7).

Crystallization from EtOH; Yellowish crystals (prisms); Yield: 47%; R_f 0.52 (EtOAc/hexane = 1/1); Mp: 196.0–200.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.91 (s, 3H, CH_3), 7.03 (dt, J = 6.6, 1.5 Hz, 1H, Ar-*H*), 7.35–7.40 (m, 3H, Ar-*H*), 7.75 (dd, J = 9.0, 2.7 Hz, 1H, Ar-*H*), 7.96 (d, J = 2.7 Hz, 1H, Ar-*H*), 8.57 (d, J = 9.0 Hz, 1H, Ar-*H*), 9.89 (br s, 1H, O*H*), 11.44 (br s, 1H, N*H*CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 52.85, 114.74, 116.48, 119.09, 119.48, 121.90, 127.88, 130.25, 130.58, 134.69, 135,94, 140.19, 156.31, 165.47, 168.02; HRMS (ESI) m/z calcd for $C_{15}H_{13}NO_4Cl$ $[M+H]^+$ 306.0533, found 306.0533; Anal. calcd. for $C_{15}H_{12}NO_4Cl$: C, 58.93; H, 3.96; N, 4.58. Found: C, 58.68; H, 3.71; N, 4.56.

Methyl 2-(3-hydroxybenzamido)benzoate (8).

Crystallization from EtOH; White crystals (needles); Yield: 52%; R_f 0.46 (EtOAc/hexane = 1/1); Mp: 199.0–206.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.90 (s, 3H, CH_3), 7.02–7.04 (m, 1H, Ar-*H*), 7.24 (td, J = 7.0, 1.0 Hz, 1H, Ar-*H*), 7.36–7.40 (m, 3H, Ar-*H*), 7.65–7.71 (m, 1H, Ar-*H*), 8.02 (dd, J = 8.4, 1.5 Hz, 1H, Ar-*H*), 8.60 (d, J = 8.4 Hz, 1H, Ar-*H*), 9.88 (br s, 1H, O*H*), 11.58 (br s, 1H, N*H*CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 52.54, 114.64, 115.21, 119.18, 119.29, 120.48, 122.75, 130.20, 130.98, 134.88, 136.43, 141.69, 156.11, 165.37, 169.08; HRMS (ESI) m/z calcd for $C_{15}H_{14}NO_4$ $[M+H]^+$ 272.0923, found 272.0914; Anal. calcd. for $C_{15}H_{13}NO_4$: C, 66.41; H, 4.83; N, 5.16. Found: 66.19; H, 4.57; N, 5.08.

Methyl 2-(3-hydroxybenzamido)-5-nitrobenzoate (9).

Crystallization from EtOH; Yellow crystals (prisms); Yield: 77%; R_f 0.46 (EtOAc/hexane = 1/1); Mp: 209.0–212.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.98 (s, 3H, CH_3), 7.06–7.09 (m, 1H, Ar-*H*), 7.38–7.44 (m, 3H, Ar-*H*), 8.54 (dd, J = 9.3, 2.7 Hz, 1H, Ar-*H*), 8.75 (d, J = 2.7 Hz, 1H, Ar-*H*), 8.86 (d, J = 9.3 Hz, 1H, Ar-*H*), 9.98 (br s, 1H, O*H*), 11.88 (br s, 1H, N*H*CO); ^{13}C NMR (100 MHz, DMSO-d_6): δ 53.26, 114.06, 116.64, 117.53, 119.84, 120.65, 126.14, 129.22, 130.21, 134.84, 141.49, 145.52, 157.87, 165.10, 166.63; HRMS (ESI) m/z calcd for $C_{15}H_{11}N_2O_6$ $[M–H]^-$ 315.0617, found 315.0619; HPLC purity: 95.35%, retention time: 19.14 min.

Methyl 5-bromo-2-(3-hydroxybenzamido)benzoate (10).

Crystallization from EtOH; Beige crystals (prisms); Yield: 77%; R_f 0.52 (EtOAc/hexane = 1/1); Mp: 175.0–177.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.91 (s, 3H, CH_3), 7.02–7.05 (m, 1H, Ar-*H*), 7.35–7.40 (m, 3H, Ar-*H*), 7.87 (dd, J = 9.0, 2.4 Hz, 1H, Ar-*H*), 8.09 (d, J = 2.4 Hz, 1H, Ar-*H*), 8.52 (d, J = 9.0 Hz, 1H, Ar-*H*), 9.90 (br s, 1H, O*H*), 11.45 (br s, 1H, N*H*CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 52.85, 114.72, 115.20, 116.78, 119.11, 119.49, 122.15, 130.25, 133.53, 135.94, 137.57, 140.65, 156.31, 165.47, 167.92; HRMS (ESI) m/z calcd for $C_{15}H_{11}NO_4Br$ $[M–H]^-$ 347.9871, found 347.9881; HPLC purity: 98.81%, retention time: 21.87 min.

Methyl 2-(3-hydroxy-4-nitrobenzamido)benzoate (11).

Crystallization from EtOH; Yellow crystals (prisms); Yield: 72%; R_f 0.59 (EtOAc/hexane = 1/1); Mp: 158.0–161.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.99 (s, 3H, CH_3), 7.18 (td, J = 7.2, 1.0 Hz, 1H, Ar-*H*), 7.60–7.67 (m, 2H, Ar-*H*), 7.83 (d, J = 1.8 Hz, 1H, Ar-*H*), 8.11 (dd, J = 8.2, 1.5 Hz, 1H, Ar-*H*), 8.27 (d, J = 9.0 Hz, 1H, Ar-*H*), 8.87 (dd, J = 8.2, 1.0 Hz, 1H, Ar-*H*), 10.60 (br s, 1H, O*H*), 12.20 (br s, 1H, N*H*CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 52.68, 115.32, 118.49, 119.47, 120.40, 123.40, 125.77, 131.02, 134.95, 135.05, 141.04, 143.02, 155.02, 162.93, 169.08; HRMS (ESI) m/z calcd for $C_{15}H_{13}N_2O_6$ $[M+H]^+$ 317.0774, found 317.0775; Anal. calcd. for $C_{15}H_{12}N_2O_6$: C, 56.96; H, 3.82; N, 8.86. Found: C, 57.31; H, 3.51; N, 8.89.

3. 3. General Procedure for the Preparation of Compounds 12–14.

To a solution of compounds **10** or **11** (1.0 mmol) in DMF (5 mL), K_2CO_3 (601 mg, 5.0 mmol) was added, followed by the addition of an appropriate alkyl bromide (1.4 mmol) under argon. The reaction mixture was stirred at 50 °C for 24 h. After the reaction was complete, EtOAc was added and the organic layer was washed with water (3 × 10 mL) and brine (3 × 10 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude products were purified by column chromatography to provide pure compounds **12**–**14**.

Methyl 5-bromo-2-(3-ethoxybenzamido)benzoate (12).

Column chromatography (EtOAc/hexane = 1/3); White crystals (needles); Yield: 49%; R_f 0.42 (EtOAc/he-

xane = 1/3); Mp: 122.0–123.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.37 (t, J = 6.9 Hz, 3H, OCH_2CH_3), 3.89 (s, 3H, CH_3), 4.12 (q, J = 6.9 Hz, 2H, OCH_2CH_3), 7.19–7.23 (m, 1H, Ar-*H*), 7.45–7.48 (m, 1H, Ar-*H*), 7.50–7.53 (m, 2H, Ar-*H*), 7.87 (dd, J = 8.7, 2.4 Hz, 1H, Ar-*H*), 8.08 (d, J = 2.4 Hz, 1H, Ar-*H*), 8.47 (d, J = 8.7 Hz, 1H, Ar-*H*), 11.42 (br s, 1H, N*H*CO); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.50, 52.87, 63.31, 112.97, 114.83, 118.33, 118.92, 119.53, 123.01, 130.15, 132.69, 135.43, 136.68, 139.13, 158.73, 164.56, 166.64; HRMS (ESI) m/z calcd for $C_{17}H_{17}NO_4Br$ $[M+H]^+$ 378.0341, found 378.0331. Anal. calcd. for $C_{17}H_{16}NO_4Br$: C, 53.99; H, 4.26; N, 3.70. Found: C, 53.91; H, 3.87; N, 3.68.

Methyl 5-bromo-2-(3-butoxybenzamido)benzoate (13).

Column chromatography (EtOAc/hexane = 1/3); Yellowish crystals (needles); Yield: 65%; R_f 0.50 (EtOAc/hexane = 1/3); Mp: 94.5–99.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95 (t, J = 7.2 Hz, 3H, $OCH_2CH_2CH_2CH_3$), 1.45−1.50 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.72−1.76 (m, 2H, $OCH_2CH_2CH_2CH_3$), 3.89 (s, 3H, CH_3), 4.06 (t, J = 6.4 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 7.21−7.23 (m, 1H, Ar-*H*), 7.45–7.48 (m, 1H, Ar-*H*), 7.49–7.52 (m, 2H, Ar-*H*), 7.87 (dd, J = 9.0, 2.4 Hz, 1H, Ar-*H*), 8.08 (d, J = 2.4 Hz, 1H, Ar-*H*), 8.46 (d, J = 9.0 Hz, 1H, Ar-*H*), 11.42 (br s, 1H, N*H*CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.83, 19.20, 31.18, 52.77, 67.91, 113.00, 114.95, 116.67, 118.84, 119.23, 122.09, 129.80, 133.46, 135.87, 137.49, 140.85, 159.57, 165.60, 167.85; HRMS (ESI) m/z calcd for $C_{19}H_{21}NO_4Br$ $[M+H]^+$ 406.0654, found 406.0643. Anal. calcd. for $C_{19}H_{20}NO_4Br$: C, 56.17; H, 4.96; N, 3.45. Found: C, 56.57; H, 4.69; N, 3.43.

Methyl 2-(3-butoxy-4-nitrobenzamido)benzoate (14).

Column chromatography (CH_2Cl_2); Yellowish crystals (needles); Yield: 66%; R_f 0.74 (CH_2Cl_2); Mp: 82.0–84.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95 (t, J = 7.5 Hz, 3H, $OCH_2CH_2CH_2CH_3$), 1.39−1.52 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.71−1.80 (m, 2H, $OCH_2CH_2CH_2CH_3$), 3.88 (s, 3H, CH_3), 4.28 (t, J = 6.3 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 7.31 (td, J = 7.8, 1.0 Hz, 1H, Ar-*H*), 7.62 (dd, J = 8.4, 1.5 Hz, 1H, Ar-*H*), 7.71 (td, J = 8.1, 1.5 Hz, 1H, Ar-*H*), 7.83 (d, J = 1.5 Hz, 1H), 8.01 (dd, J = 7.8, 1.5 Hz, 1H, Ar-*H*), 8.07 (d, J = 8.4 Hz, 1H, Ar-*H*), 8.38 (dd, J = 8.1, 1.0 Hz, 1H, Ar-*H*), 11.46 (br s, 1H, N*H*CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.70, 19.03, 30.82, 52.62, 69.61, 114.09, 115.23, 118.01, 120.30, 123.26, 125.78, 131.06, 134.99, 139.75, 141.24, 141.64, 152.52, 163.51, 169.17; HRMS (ESI) m/z calcd for $C_{19}H_{20}N_2O_6Na$ $[M+Na]^+$ 395.1219, found 395.1202. Anal. calcd. for $C_{19}H_{20}N_2O_6$: C, 61.28; H, 5.41; N, 7.52. Found: C, 61.47; H, 5.57; N, 7.36.

3. 4. General Procedure for the Synthesis of Compounds 15–27.

Alkaline Hydrolysis.

To a stirred solution of the protected methyl anthranilates **1**–**10** and **12**–**14** (0.5 mmol) in dioxane/THF mixture (1:1, 2 mL), 1 M NaOH (1 mL) was added, and the reaction mixture stirred until the starting material had completely reacted (monitored by TLC). The solvent was then evaporated under reduced pressure, the residue diluted with H_2O (10 mL), and washed with EtOAc (2 × 10 mL). The aqueous phase was acidified to pH 2 using 1 M HCl, and extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with brine (3 × 10 mL), then dried over Na_2SO_4, filtered, and evaporated to dryness, to provide compounds **15**–**27.** If necessary, these were additionally purified by column chromatography or crystallization.

5-Hydroxy-2-(3-nitrobenzamido)benzoic acid (15).

Yellow crystals (prisms); Yield: 86%; R_f 0.19 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 248.0–251.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.08 (dd, J = 8.9, 3.0 Hz, 1H, Ar-*H*), 7.43 (d, J = 3.0 Hz, 1H, Ar-*H*), 7.88 (t, J = 8.0 Hz, 1H, Ar-*H*), 8.32−8.37 (m, 2H, Ar-*H*), 8.44−8.48 (m, 1H, Ar-*H*), 8.73 (t, J = 1.6 Hz, 1H, Ar-*H*), 9.71 (br s, 1H, O*H*), 11.86 (br s, 1H, N*H*CO), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.68, 119.37, 120.87, 121.56, 122.60, 126.25, 130.60, 131.99, 133.19, 136.07, 147.91, 153.21, 161.87, 169.53; HRMS (ESI) m/z calcd for $C_{14}H_9N_2O_6$ $[M–H]^-$ 301.0461, found 301.0460; Anal. calcd. for $C_{14}H_{10}N_2O_6$: C, 55.63; H, 3.33; N, 9.27. Found: C, 55.42; H, 3.07; N, 9.15.

5-Chloro-2-(3-nitrobenzamido)benzoic acid (16).

Crystallization from CH_3CN; White crystals (prisms); Yield: 61%; R_f 0.67 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 257.0–259.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.34 (dd, J = 8.6, 2.2 Hz, 1H, Ar-*H*), 7.92 (t, J = 8.0 Hz, 1H, Ar-*H*), 8.07 (d, J = 8.6 Hz, 1H, Ar-*H*), 8.35–8.38 (m, 1H, Ar-*H*), 8.49 (ddd, J = 8.0, 1.8, 1.0 Hz, 1H, Ar-*H*), 8.73–8.75 (m, 2H, Ar-*H*), 12.46 (br s, 1H, N*H*CO), 14.10 (br s, 1H, COO*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 115.58, 119.32, 121.56, 123.24, 126.76, 130.76, 132.76, 133.27, 135.18, 138.58, 141.43, 147.87, 162.48, 169.37; HRMS (ESI) m/z calcd for $C_{14}H_8N_2O_5Cl$ $[M–H]^-$ 319.0122, found 319.0123; Anal. calcd. for $C_{14}H_9N_2O_5Cl$: C, 52.43; H, 2.83; N, 8.74. Found: C, 52.26; H, 2.61; N, 8.73.

4,5-Dimethoxy-2-(3-nitrobenzamido)benzoic acid (17).

Crystallization from CH_3CN/MeOH mixture (5/1); Yellow crystals (prisms); Yield: 51%; R_f 0.27 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 277.5–280.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.80 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 7.51 (s, 1H, Ar-*H*), 7.91 (t, J = 8.0 Hz, 1H, Ar-*H*), 8.36–8.38 (m, 1H, Ar-*H*), 8.42 (s, 1H, Ar-*H*), 8.46–8.50 (m, 1H, Ar-*H*), 8.73 (t, J = 1.9 Hz, 1H, Ar-*H*), 12.51 (br s, 1H, N*H*CO), resonance for COO*H* missing; ^{13}C NMR

(100 MHz, DMSO-d_6): δ 55.54, 55.58, 103.39, 112.81, 119.84, 121.52, 126.50, 130.77, 133.17, 135.88, 136.07, 144.06, 147.99, 153.03, 162.08, 169.83; HRMS (ESI) m/z calcd for $C_{16}H_{13}N_2O_7$ $[M–H]^-$ 345.0723, found 345.0718; Anal. calcd. for $C_{16}H_{14}N_2O_7$: C, 55.49; H, 4.07; N, 8.09. Found: C, 55.60; H, 3.98; N, 7.76.

5-Chloro-3-methyl-2-(3-nitrobenzamido)benzoic acid (18).

Column chromatography (CH_2Cl_2/MeOH/AcOH = 9/1/0.1); White crystals (prisms); Yield: 53%; R_f 0.34 (CH_2Cl_2/MeOH/AcOH = 9/1/0.1); Mp: 203.0–205.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.27 (s, 3H, C*H*$_3$), 7.63–7.69 (m, 2H, Ar-*H*), 7.86 (t, *J* = 8.0 Hz, 1H, Ar-*H*), 8.36–8.40 (m, 1H, Ar-*H*), 8.45 (dd, *J* = 8.0, 1.5 Hz, 1H, Ar-*H*), 8.79 (t, *J* = 2.1 Hz, 1H, Ar-*H*), 10.44 (br s, 1H, N*H*CO), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.70, 122.24, 122.94, 126.18, 127.24, 130.26, 131.06, 133.00, 133.91, 135.68, 141.05, 147.74, 156.63, 166.33, 169.22; HRMS (ESI) m/z calcd for $C_{15}H_{10}N_2O_5Cl$ $[M–H]^-$ 333.0278, found 333.0281; Anal. calcd. for $C_{15}H_{11}N_2O_5Cl$: C, 53.83; H, 3.31; N, 8.37. Found: C, 53.74; H, 3.00; N, 8.26.

2-(4-Fluoro-3-nitrobenzamido)benzoic acid (19).

Column chromatography (CH_2Cl_2/MeOH/AcOH = 9/1/0.1); Yellow crystals (prisms); Yield: 63%; R_f 0.46 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 224.0–225.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.21 (td, *J* = 7.8, 1.2 Hz, 1H, Ar-*H*), 7.31 (d, *J* = 8.7 Hz, 1H, Ar-*H*), 7.61–7.68 (m, 1H, Ar-*H*), 8.06 (dd, *J* = 7.8, 1.5 Hz, 1H, Ar-*H*), 8.10 (dd, *J* = 8.7, 2.1 Hz, 1H, Ar-*H*), 8.48 (d, *J* = 2.1 Hz, 1H, Ar-*H*), 8.62 (dd, *J* = 8.1, 1.2 Hz, 1H, Ar-*H*), 12.36 (br s, 1H, N*H*CO), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.09, 119.52 and 119.74 (1C, $^2J_{C,F}$ = 21.8 Hz), 119.88, 122.96, 125.54 and 125.57 (1C, $^4J_{C,F}$ = 1.9 Hz), 124.97, 131.12 and 131.16 (1C, $^3J_{C,F}$ = 4.2 Hz), 133.35, 133.98 and 134.08 (1C, $^2J_{C,F}$ = 10.5 Hz), 136.57 and 136.64 (1C, $^3J_{C,F}$ = 7.8 Hz), 140.78, 153.73 and 156.49 (1C, $^1J_{C,F}$ = 263 Hz), 162.41, 170.12; HRMS (ESI) m/z calcd for $C_{14}H_9N_2O_5F$ $[M–H]^-$ 303.0461, found 303.0466; HPLC purity: 99.13%, retention time: 18.04 min.

5-Bromo-2-(4-fluoro-3-nitrobenzamido)benzoic acid (20).

Crystallization from EtOAc; Yellow crystals (prisms); Yield: 42%; R_f 0.13 (CH_2Cl_2/MeOH/AcOH = 9/1/0.1); Mp: 286.0–288.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.31 (d, *J* = 8.8 Hz, 1H, Ar-*H*), 7.84 (dd, *J* = 8.8, 2.5 Hz, 1H, Ar-*H*), 8.05–8.12 (m, 2H, Ar-*H*), 8.47 (d, *J* = 2.5 Hz, 1H, Ar-*H*), 8.55 (d, *J* = 9.0 Hz, 1H, Ar-*H*), 12.09 (br s, 1H, N*H*CO), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 114.40, 118.74 and 118.95 (1C, $^2J_{C,F}$ = 21.4 Hz), 119.60, 122.00, 124.59 and 124.61 (1C, $^4J_{C,F}$ = 2.0 Hz), 124.66, 133.11 and 133.14 (1C, $^3J_{C,F}$ = 4.3 Hz), 133.38, 136.45 and 136.56 (1C, $^2J_{C,F}$ = 10.8 Hz), 136.59, 139.87 and 139.95 (1C, $^3J_{C,F}$ = 8.5 Hz), 153.83 and 156.49 (1C, $^1J_{C,F}$ = 267 Hz), 162.37, 168.74; HRMS (ESI) m/z calcd for $C_{14}H_7N_2O_5BrF$ $[M–H]^-$ 380.9619, found 380.9668; HPLC purity: 100.00%, retention time: 22.72 min.

5-Chloro-2-(3-hydroxybenzamido)benzoic acid (21).

White crystals (needles); Yield: 84%; R_f 0.38 (CH_2Cl_2/MeOH/AcOH = 9/1/0.1); Mp: 277.5–280.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.00–7.04 (m, 1H, Ar-*H*), 7.35–7.39 (m, 3H, Ar-*H*), 7.72 (dd, *J* = 9.0, 2.7 Hz, 1H, Ar-*H*), 7.99 (d, *J* = 2.7 Hz, 1H, Ar-*H*), 8.72 (d, *J* = 9.0 Hz, 1H, Ar-*H*), 9.88 (br s, 1H, O*H*), 12.05 (br s, 1H, N*H*CO), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 113.95, 117.28, 118.32, 119.25, 121.63, 126.35, 129.99, 130.31, 133.78, 135.55, 139.84, 157.77, 164.66, 168.66; HRMS (ESI) m/z calcd for $C_{14}H_9NO_4Cl$ $[M–H]^-$ 290.0220, found 290.0223; HPLC purity: 100.00%, retention time: 18.89 min.

2-(3-Hydroxybenzamido)benzoic acid (22).

White crystals (needles); Yield: 72%; R_f 0.47 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 219.0–223.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.00–7.04 (m, 1H, Ar-*H*), 7.20 (td, *J* = 7.2, 1.0 Hz, 1H, Ar-*H*), 7.35–7.39 (m, 3H, Ar-*H*), 7.62–7.69 (m, 1H, Ar-*H*), 8.06 (dd, *J* = 8.0, 1.6 Hz, 1H, Ar-*H*), 8.71 (dd, *J* = 8.4, 1.0 Hz, 1H, Ar-*H*), 9.86 (br s, 1H, O*H*), 12.11 (br s, 1H, N*H*CO), 13.82 (br s, 1H, COO*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 113.93, 116.28, 117.24, 119.10, 119.72, 122.78, 129.96, 131.21, 134.25, 135.87, 141.10, 157.76, 164.63, 169.93; HRMS (ESI) m/z calcd for $C_{14}H_{10}NO_4$ $[M–H]^-$ 256.0610, found 256.0615; HPLC purity: 100.00%, retention time: 14.34 min.

2-(3-Hydroxybenzamido)-5-nitrobenzoic acid (23).

Column chromatography (CH_2Cl_2/MeOH/AcOH = 9/1/0.1); Yellow crystals (prisms); Yield: 54%; R_f 0.27 (CH_2Cl_2/MeOH/AcOH = 9/1/0.1); Mp: 267.0–268.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.04–7.10 (m, 1H, Ar-*H*), 7.33–7.42 (m, 3H, Ar-*H*), 8.48 (dd, *J* = 9.3, 2.7 Hz, 1H, Ar-*H*), 8.80 (d, *J* = 2.7 Hz, 1H, Ar-*H*), 8.93 (d, *J* = 9.3 Hz, 1H, Ar-*H*), 9.95 (br s, 1H, O*H*), 12.80 (br s, 1H, N*H*CO), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 114.10, 116.53, 117.47, 119.75, 119.90, 126.57, 129.05, 130.12, 134.98, 141.20, 146.29, 157.86, 165.06, 168.45; HRMS (ESI) m/z calcd for $C_{14}H_9N_2O_6$ $[M–H]^-$ 301.0461, found 301.0469; HPLC purity: 99.18%, retention time: 17.00 min.

5-Bromo-2-(3-hydroxybenzamido)benzoic acid (24).

Beige crystals (needles); Yield: 70%; R_f 0.85 (CH_3CN/MeOH/H_2O = 3/1/0.1); Mp: 277.0–281.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.00–7.05 (m, 1H, Ar-*H*), 7.33–7.41 (m, 3H, Ar-*H*), 7.84 (dd, *J* = 9.0, 2.7 Hz, 1H,

Ar-*H*), 8.11 (d, *J* = 2.7 Hz, 1H, Ar-*H*), 8.64 (d, *J* = 9.0 Hz, 1H, Ar-*H*), 9.93 (br s, 1H, O*H*), 12.03 (br s, 1H, N*H*CO), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 113.94, 114.14, 117.27, 118.56, 119.26, 121.88, 129.99, 133.20, 135.54, 136.66, 140.22, 157.77, 164.66, 168.58; HRMS (ESI) m/z calcd for $C_{14}H_9NO_4Br$ $[M–H]^-$ 333.9715, found 333.9711; HPLC purity: 100.00%, retention time: 19.54 min.

5-Bromo-2-(3-ethoxybenzamido)benzoic acid (25).

White crystals (prisms); Yield: 82%; R_f 0.60 (CH_2Cl_2/MeOH/AcOH = 9/1/0.1); Mp: 226.5–229.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.37 (t, *J* = 6.9 Hz, 3H, OCH_2CH_3), 4.12 (q, *J* = 6.9 Hz, 2H, OCH_2CH_3), 7.17–7.23 (m, 1H, Ar-*H*), 7.44–7.51 (m, 3H, Ar-*H*), 7.84 (dd, *J* = 9.0, 2.4 Hz, 1H, Ar-*H*), 8.12 (d, *J* = 2.4 Hz, 1H, Ar-*H*), 8.64 (d, *J* = 9.0 Hz, 1H, Ar-*H*), 12.06 (br s, 1H, N*H*CO), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.47, 63.25, 112.60, 114.26, 118.47, 118.81, 118.98, 121.89, 130.12, 133.20, 135.57, 136.64, 140.10, 158.72, 164.40, 168.59; HRMS (ESI) m/z calcd for $C_{16}H_{15}NO_4Br$ $[M+H]^+$ 364.0184, found 364.0185; HPLC purity: 99.05%, retention time: 21.20 min.

5-Bromo-2-(3-butoxybenzamido)benzoic acid (26).

White crystals (prisms); Yield: 94%; R_f 0.65 (CH_2Cl_2/MeOH/AcOH = 9/1/0.1); Mp: 206–209.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95 (t, *J* = 7.5 Hz, 3H, $OCH_2CH_2CH_2CH_3$), 1.42–1.50 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.69–1.78 (m, 2H, $OCH_2CH_2CH_2CH_3$), 4.05 (t, *J* = 6.4 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 7.18–7.24 (m, 1H, Ar-*H*), 7.45–7.52 (m, 3H, Ar-*H*), 7.83 (dd, *J* = 9.0, 2.4 Hz, 1H, Ar-*H*), 8.12 (d, *J* = 2.4 Hz, 1H, Ar-*H*), 8.64 (d, *J* = 9.0 Hz, 1H, Ar-*H*), 12.12 (br s, 1H, N*H*CO), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.60, 18.65, 30.57, 67.32, 112.75, 114.23, 118.40, 118.88, 118.93, 121.85, 130.08, 133.21, 132.56, 136.56, 140.12, 158.90, 164.38, 168.61; HRMS (ESI) m/z calcd for $C_{18}H_{17}NO_4Br$ $[M–H]^-$ 390.0341, found 390.0342; Anal. calcd. for $C_{18}H_{18}NO_4Br$: C, 55.12; H, 4.63; N, 3.57. Found: C, 55.27; H, 4.43; N, 3.50.

2-(3-Butoxy-4-nitrobenzamido)benzoic acid (27).

White crystals (prisms); Yield: 74%; R_f 0.92 (CH_3CN/MeOH/H_2O = 3/1/0.1); Mp: 174.0–176.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95 (t, *J* = 7.5 Hz, 3H, $OCH_2CH_2CH_2CH_3$), 1.37–1.52 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.70–1.80 (m, 2H, $OCH_2CH_2CH_2CH_3$), 4.28 (t, *J* = 6.3 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 7.26 (td, *J* = 7.8, 1.0 Hz, 1H, Ar-*H*), 7.62 (dd, *J* = 8.4, 1.2 Hz, 1H, Ar-*H*), 7.69 (td, *J* = 8.1, 1.2 Hz, 1H, Ar-*H*), 7.82 (d, *J* = 1.5 Hz, 1H), 8.04–8.09 (m, 2H, Ar-*H*), 8.62 (dd, *J* = 8.1, 1.0 Hz, 1H, Ar-*H*), 12.14 (br s, 1H, N*H*CO), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.53, 18.52, 30.28, 69.14, 113.65, 118.81, 120.19, 123.58, 125.41, 131.30, 134.10, 139.63, 140.36, 141.41, 149.73, 151.23, 163.07, 169.79; HRMS (ESI) m/z calcd for $C_{18}H_{17}N_2O_6$ $[M–H]^-$ 357.1087, found 357.1084; HPLC purity: 100.00%, retention time: 19.50 min.

3. 5. General Procedure for the Synthesis of Compounds 28–33.

Reduction of Nitro Group.

To a solution of the appropriate compound **15**–**17**, **20**, **23**, or **27** (1.0 mmol) in MeOH (10 mL), 10% Pd/C was added, and the mixture was hydrogenated for 4 h at room temperature. The suspension was then filtered through a pad of celite, and washed with MeOH (10 mL). The solvent was removed under reduced pressure, yielding the pure products **28**–**33**.

2-(3-Aminobenzamido)-5-hydroxybenzoic acid (28).

Off-white solid; Yield: 72%; R_f 0.34 (CH_3CN/MeOH/H_2O = 3/1/0.1); Mp: 175.0–178.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.75 (dd, *J* = 7.5, 1.6 Hz, 1H, Ar-*H*), 6.97–7.05 (m, 2H, Ar-*H*), 7.13–7.18 (m, 2H, Ar-*H*), 7.44 (d, *J* = 2.9 Hz, 1H, Ar-*H*), 8.50 (d, *J* = 9.0 Hz, 1H, Ar-*H*), 9.46 (s, 1H, O*H*), 12.19 (s, 1H, N*H*CO), resonances for NH_2 and COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 112.44, 113.55, 116.80, 116.86, 119.18, 120.31, 121.15, 129.10, 133.28, 135.78, 149.10, 152.20, 164.67, 169.82; HRMS (ESI) m/z calcd for $C_{14}H_{11}N_2O_4$ $[M–H]^-$ 271.0719, found 271.0720; HPLC purity: 95.23%, retention time: 4.01 min.

2-(3-Aminobenzamido)benzoic acid (29).

Beige crystals (needles); Yield: 54%; R_f 0.67 (CH_3CN/MeOH/H_2O = 3/1/0.1); Mp: 224.5–227.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.79 (dd, *J* = 7.6, 1.5 Hz, 1H, Ar-*H*), 7.03–7.07 (m, 1H, Ar-*H*), 7.16–7.22 (m, 3H, Ar-*H*), 7.64 (td, *J* = 8.6, 1.6 Hz, 1H, Ar-*H*), 8.05 (dd, *J* = 7.9, 1.5 Hz, 1H, Ar-*H*), 8.70–8.74 (m, 1H, Ar-*H*), 12.07 (s, 1H, N*H*CO), resonances for NH_2 and COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 112.47, 113.54, 116.25, 117.27, 119.62, 122.55, 129.25, 131.21, 134.15, 135.34, 141.28, 149.23, 165.40, 169.93; HRMS (ESI) m/z calcd for $C_{14}H_{11}N_2O_3$ $[M–H]^-$ 255.0770, found 255.0774; HPLC purity: 95.69%, retention time: 9.11 min.

2-(3-Aminobenzamido)-4,5-dimethoxybenzoic acid (30).

The product was additionally purified by crystallization from CH_2Cl_2; Brown crystals (prisms); Yield: 41%; R_f 0.40 (CH_3CN/MeOH/H_2O = 3/1/0.1); Mp: 225.0–227.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.76 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.73–6.78 (m, 1H, Ar-*H*), 7.10–7.19 (m, 3H, Ar-*H*), 7.54 (s, 1H, Ar-*H*), 8.50 (s, 1H, Ar-*H*), 13.14 (br s, 1H, N*H*CO), resonances for NH_2 and COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.31, 55.41, 102.69, 112.58, 113.78, 113.81, 113.97, 116.65, 128.88, 136.03, 136.16, 142.90, 148.91, 153.83, 164.70, 169.67; HRMS (ESI) m/z calcd for $C_{16}H_{15}N_2O_5$

[M–H]$^-$ 315.0981, found 315.0984; Anal. calcd. for $C_{16}H_{16}N_2O_5$: C, 60.75; H, 5.10; N, 8.86. Found: C, 60.76; H, 5.42; N, 8.60.

2-(3-Amino-4-fluorobenzamido)benzoic acid (31).

Brown solid; Yield: 77%; R_f 0.36 (CH_2Cl_2/MeOH/AcOH = 9/1/0.1); Mp: 270.0–272.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.97–7.03 (m, 1H, Ar-*H*), 7.18 (td, *J* = 7.8, 1.2 Hz, 1H, Ar-*H*), 7.47–7.55 (m, 1H, Ar-*H*), 7.61–7.68 (m, 2H, Ar-*H*), 8.04 (dd, *J* = 7.8, 1.5 Hz, 1H, Ar-*H*), 8.68 (d, *J* = 8.5 Hz, 1H, Ar-*H*), 12.01 (br s, 1H, N*H*CO), resonances for NH_2 and COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 114.91 and 114.99 (1C, $^3J_{C,F}$ = 7.5 Hz), 115.85 and 116.04 (1C, $^2J_{C,F}$ = 19.5 Hz), 116.20 and 116.27 (1C, $^3J_{C,F}$ = 6.4 Hz), 119.67, 122.50, 125.62, 130.62, 130.94 and 130.97 (1C, $^4J_{C,F}$ = 2.8 Hz), 134.27, 136.91 and 137.04 (1C, $^2J_{C,F}$ = 13.3 Hz), 141.36, 151.37 and 153.79 (1C, $^1J_{C,F}$ = 242 Hz), 164.16, 169.95; HRMS (ESI) m/z calcd for $C_{14}H_{11}N_2O_3F$ [M–H]$^-$ 273.0823, found 273.0968; Anal. calcd. for $C_{14}H_{12}N_2O_3F$: C, 61.31; H, 4.04; N, 6.93. Found: C, 61.03; H, 4.43; N, 6.97.

5-Amino-2-(3-hydroxybenzamido)benzoic acid (32).

Brown crystals (prisms); Yield: 78%; R_f 0.69 (MeOH); Mp: 241.0–245.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.78 (dd, *J* = 8.9, 3.0 Hz, 1H, Ar-*H*), 6.92–7.01 (m, 1H, Ar-*H*), 7.24–7.38 (m, 4H, Ar-*H*), 8.34 (d, *J* = 8.9 Hz, 1H, Ar-*H*), 9.78 (br s, 1H, O*H*), 12.23 (br s, 1H, N*H*CO), resonances for NH_2 and COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 113.85, 115.71, 117.10, 118.23, 119.72, 119.86, 129.48, 129.60, 130.65, 136.80, 143.77, 157.58, 163.34, 167.26; HRMS (ESI) m/z calcd for $C_{14}H_{12}N_2O_4Na$ [M+Na]$^+$ 295.0695, found 295.0692; HPLC purity: 96.02%, retention time: 15.64 min.

2-(4-Amino-3-butoxybenzamido)benzoic acid (33).

Brown crystals (prisms); Yield: 97%; R_f 0.60 (CH_2Cl_2/MeOH/AcOH = 9/1/0.1); Mp: 189.0–191.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.96 (t, *J* = 7.5 Hz, 3H, $OCH_2CH_2CH_2CH_3$), 1.39–1.51 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.70–1.80 (m, 2H, $OCH_2CH_2CH_2CH_3$), 4.03 (t, *J* = 6.3 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 6.68 (d, *J* = 8.7 Hz, 1H, Ar-*H*), 6.90 (td, *J* = 7.5, 1.2 Hz, 1H, Ar-*H*), 7.25 (td, *J* = 7.2, 1.5 Hz, 1H, Ar-*H*), 7.42–7.49 (m, 2H, Ar-*H*), 8.00 (dd, *J* = 7.5, 1.5 Hz, 1H, Ar-*H*), 8.63 (dd, *J* = 8.1, 1.2 Hz, 1H, Ar-*H*), 12.25 (br s, 1H, N*H*CO), resonances for NH_2 and COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.74, 18.76, 30.83, 67.36, 110.46, 112.23, 117.98, 120.59, 120.85, 122.65, 124.61, 129.81, 131.18, 141.24, 141.45, 144.74, 164.38, 170.34; HRMS (ESI) m/z calcd for $C_{18}H_{20}N_2O_4Na$ [M+Na]$^+$ 351.1321, found 351.1320; HPLC purity: 94.31%, retention time: 20.28 min.

3. 6. General Procedure for the Preparation of Compounds 34–48.

The corresponding sulfonyl chloride (1.0 mmol) was dissolved in CH_2Cl_2 (5 mL) and slowly added to a solution of the appropriate amine (1.2 mmol) in CH_2Cl_2 (5 mL), followed by addition of Py (237 mg, 3.0 mmol) after 10 min. The reaction mixture was stirred for 24 h at room temperature. After the reaction was complete (monitored by TLC), 2 M HCl (10 mL) was added and the product extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with brine (2 × 30 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting crude products were purified by crystallization or column chromatography, to yield compounds **34**–**48**.

Methyl 2-(naphthalene-2-sulfonamido)benzoate (34).

Column chromatography (EtOAc/hexane = 1/5); White crystals (needles); Yield: 49%; R_f 0.29 (EtOAc/hexane = 1/3); Mp: 126.5–128.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 3H, CH_3), 7.11–7.17 (m, 1H, Ar-*H*), 7.49–7.55 (m, 2H, Ar-*H*), 7.62–7.83 (m, 4H, Ar-*H*), 8.01 (dd, *J* = 7.4, 1.4 Hz, 1H, Ar-*H*), 8.08 (d, *J* = 8.9 Hz, 1H, Ar-*H*), 8.13–8.18 (m, 1H, Ar-*H*), 8.53 (d, *J* = 1.7 Hz, 1H, Ar-*H*), 10.52 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.55, 118.03, 119.99, 121.84, 123.94, 127.77, 127.78, 128.46, 129.24, 129.29, 129.59, 130.92, 131.45, 134.34, 134.36, 135.55, 138.38, 167.45; HRMS (ESI) m/z calcd for $C_{18}H_{16}NO_4S$ [M+H]$^+$ 342.0800, found 342.0785; HPLC purity: 100.00%, retention time: 19.95 min.

Methyl 3-(naphthalene-2-sulfonamido)benzoate (35).

Column chromatography (EtOAc/hexane = 1/2); White crystals (prisms); Yield: 42%; R_f 0.15 (EtOAc/hexane = 1/3); Mp: 192.5–193.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 3H, CH_3), 7.36 (ddd, *J* = 8.0, 7.5, 0.4 Hz, 1H, Ar-*H*), 7.39–7.43 (m, 1H, Ar-*H*), 7.57 (dt, *J* = 7.5, 1.5 Hz, 1H, Ar-*H*), .7.62–7.72 (m, 2H, Ar-*H*), 7.73 (m, 1H, Ar-*H*), 7.76 (dd, *J* = 8.7, 1.8 Hz, 1H, Ar-*H*), 7.99 (d, *J* = 8.0 Hz, 1H, Ar-*H*), 8.09 (d, *J* = 8.7 Hz, 1H, Ar-*H*), 8.11–8.15 (m, 1H, Ar-*H*), 8.45 (d, *J* = 1.8 Hz, 1H, Ar-*H*), 10.68 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.21, 120.18, 121.76, 124.25, 124.59, 127.72, 127.78, 128.00, 129.04, 129.19, 129.56, 129.70, 130.45, 131.43, 134.22, 136.05, 138.06, 165.53; HRMS (ESI) m/z calcd for $C_{18}H_{16}NO_4S$ [M+H]$^+$ 342.0800, found 342.0797; HPLC purity: 100.00%, retention time: 16.94 min.

Dimethyl 5-(naphthalene-2-sulfonamido)isophthalate (36).

Column chromatography (EtOAc/hexane = 1/2); White amorphous solid; Yield: 72%; R_f 0.46 (EtOAc/hexane = 1/1); Mp: 211.5–213.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 6H, 2 × CH_3), 7.62–7.74 (m, 2H, Ar-*H*), 7.77 (dd, *J* = 8.7, 1.9 Hz, 1H, Ar-*H*), 7.96–8.03 (m, 3H, Ar-*H*), 8.08 (t, *J* = 1.5 Hz, 1H, Ar-*H*), 8.09–8.17 (m, 2H, Ar-*H*), 8.47 (d, *J* = 1.5 Hz, 1H, Ar-*H*), 10.94 (br s, 1H,

N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.11, 52.57, 116.55, 118.01, 121.61, 124.67, 127.80, 127.82, 128.08, 129.18, 129.24, 129.77, 130.57, 131.11 (2C), 131.44, 134.30, 138.77, 164.72, 165.92; HRMS (ESI) m/z calcd for $C_{20}H_{18}NO_6S$ $[M+H]^+$ 400.0855, found 400.0858; HPLC purity: 96.08%, retention time: 18.39 min.

Methyl 5-bromo-2-(naphthalene-2-sulfonamido)benzoate (37).

Column chromatography (Et_2O/petroleum ether = 1/3); White crystals (prisms); Yield: 71%; R_f 0.19 (Et_2O/petroleum ether = 1/3); Mp: 128.0–130.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.76 (s, 3H, CH_3), 7.43 (dd, *J* = 8.8, 0.5 Hz, 1H, Ar-*H*), 7.65–7.75 (m, 3H, Ar-*H*), 7.76 (dd, *J* = 8.8, 2.2 Hz, Ar-*H*), 7.88 (dd, *J* = 2.2, 0.5 Hz, 1H, Ar-*H*), 8.00–8.04 (m, 1H, Ar-*H*), 8.10 (dd, *J* = 8.7, 0.5 Hz, 1H, Ar-*H*), 8.15–8.18 (m, 1H, Ar-*H*), 8.52–8.54 (m, 1H, Ar-*H*), 10.45 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.79, 115.93, 120.98, 121.84, 122.71, 127.81, 128.42, 129.30, 129.33, 129.66, 131.47, 133.04, 134.42, 135.48, 136.69, 137.26, 166.02; HRMS (ESI) m/z calcd for $C_{18}H_{15}NO_4SBr$ $[M+H]^+$ 419.9905, found 419.9913; HPLC purity: 99.75%, retention time: 23.92 min.

N-(3-Hydroxyphenyl)naphthalene-2-sulfonamide (38).

Column chromatography (EtOAc/hexane = 1/1); Brown solid; Yield: 96%; R_f 0.13 (EtOAc/hexane = 1/2); Mp:121.0–125.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.36 (d, *J* = 7,9 Hz, 1H, Ar-*H*), 6.52–6.62 (m, 2H, Ar-*H*), 6.95 (t, *J* = 8.1 Hz, 1H, Ar-*H*), 7.60–7.73 (m, 2H, Ar-*H*), 7.77 (d, *J* = 8.8 Hz, 1H, Ar-*H*), 8.00 (d, *J* = 7.5 Hz, 1H, Ar-*H*), 8.05–8.17 (m, 2H, Ar-*H*), 8.40–8.44 (m, 1H, Ar-*H*), 9.39 (br s, 1H, O*H*), 10.25 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 107.99, 112.14, 112.50, 122.92, 128.86, 129.06, 129.09, 130.03,130.36, 130.58, 131.28, 132.39, 135.31, 136.79, 139.31, 158.28; HRMS (ESI) m/z calcd for $C_{16}H_{14}NO_3S$ $[M+H]^+$ 300.0694, found 300.0696; HPLC purity: 97.50%, retention time: 12.48 min.

Methyl 2-(naphthalene-1-sulfonamido)benzoate (39).

Column chromatography (EtOAc/hexane = 1/2); White crystals (prisms); Yield: 61%; R_f 0.30 (EtOAc/hexane = 1/2); Mp: 191.5–192.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.74 (s, 3H, CH_3), 7.08 (ddd, *J* = 7.9, 7.3, 1.2 Hz, 1H, Ar-*H*), 7.39–7.42 (m, 1H, Ar-*H*), 7.46–7.52 (m, 1H, Ar-H), 7.62–7.69 (m, 2H, Ar-*H*), 7.73–7.78 (m, 2H, Ar-*H*), 8.07–8.10 (m, 1H, Ar-*H*), 8.24–8.27 (m, 1H, Ar-*H)*, 8.28 (dd, *J* = 7.4, 1.2 Hz, 1H, Ar-*H*), 8.50–8.55 (m, 1H, Ar-*H*), 10.87 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.61, 117.12, 119.03, 123.49, 123.59. 124.44, 126.94, 127.11, 128.44, 129.29, 130.39, 130.87, 133.12, 133.70, 134.40, 135.06, 138.46, 167.58; HRMS (ESI) m/z calcd for $C_{18}H_{16}NO_4S$ $[M+H]^+$ 342.0800, found 342.080; HPLC purity: 99.13%, retention time: 19.35 min.

Methyl 3-(naphthalene-1-sulfonamido)benzoate (40).

Crystallization from EtOAc/hexane mixture (1/2); White crystals (needles); Yield: 64%; R_f 0.49 (EtOAc/hexane = 1/1); Mp: 173.0–174.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.78 (s, 3H, CH_3), 7.27–7.34 (m, 2H, Ar-*H*), 7.46–7.54 (m, 1H, Ar-*H*), 7.59–7.70 (m, 3H, Ar-*H*), 7.75 (ddd, *J* = 8.6, 6.9, 1.4 Hz, 1H, Ar-*H*), 8.07 (dd, *J* = 7.9, 1.0 Hz, 1H, Ar-*H*), 8.19–8.25 (m, 2H, Ar-*H*), 8.71 (d, *J* = 8.6 Hz, 1H, Ar-*H*), 10.94 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.20, 118.96, 122.98, 123.98, 124.02, 124.41, 127.02, 127.24, 128.25, 129.10, 129.65, 129.96, 130.37, 133.69, 133.84, 134.62, 137.97, 165.50; HRMS (ESI) m/z calcd for $C_{18}H_{16}NO_4S$ $[M+H]^+$ 342.0800, found 342.0807; HPLC purity: 100.00%, retention time: 16.15 min.

Dimethyl 5-(naphthalene-1-sulfonamido)isophthalate (41).

Crystallization from EtOAc/hexane mixture (1/2); Off-white crystals (needles); Yield: 91%; R_f 0.37 (EtOAc/hexane = 1/1); Mp: 208.5–209.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.82 (s, 6H, 2 × CH_3), 7.62–7.71 (m, 2H, Ar-*H*), 7.77 (ddd, *J* = 7.7, 6.8, 1.3 Hz, 1H, Ar-*H*), 7.88 (d, *J* = 1.5 Hz, 2H, Ar-*H*), 8.01 (t, *J* = 1.5 Hz, 1H, Ar-*H*), 8.08 (dd, *J* = 8.1, 1.3 Hz, 1H, Ar-*H*), 8.22–8.27 (m, 2H, Ar-*H*), 8.70 (d, *J* = 8.3 Hz, 1H, Ar-*H*), 11.25 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.11, 52.55, 116.54, 118.01, 122.52, 124.02, 124.43, 127.13, 127.15, 128.43, 129.19, 130.00, 130.57, 131.03, 133.48, 133.73, 134.91, 138.64, 164.69, 165.92; HRMS (ESI) m/z calcd for $C_{20}H_{18}NO_6S$ $[M+H]^+$ 400.0855, found 400.0859; HPLC purity: 96.41%, retention time: 17.72 min.

Methyl 5-bromo-2-(naphthalene-1-sulfonamido)benzoate (42).

Column chromatography (EtOAc/hexane = 1/5); Brown crystals (prisms); Yield: 44%; R_f 0.21 (EtOAc/hexane = 1/3); Mp: 151.0–153.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3,71 (s, 3H, CH_3), 7.34 (d, *J* = 8.8 Hz, 1H, Ar-*H*), 7.62–7.79 (m, 4H, Ar-*H*), 7.83 (d, *J* = 2.4 Hz, 1H, Ar-*H*), 8.08–8.13 (m, 1H, Ar-*H*), 8.22–8.31 (m, 2H, Ar-*H*), 8.52 (d, *J* = 8.5 Hz, 1H, Ar-*H*), 10.72 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.84, 115.58, 120.08, 121.90, 123.54, 124.47, 126.92, 127.16, 128.48, 129.30, 130.23, 132.97, 133.07, 133.72, 135.15, 136.75, 137.34, 166.15; HRMS (ESI) m/z calcd for $C_{18}H_{15}NO_4SBr$ $[M+H]^+$ 419.9905, found 419.9892; HPLC purity: 89.86%, retention time: 23.37 min.

N-(3-Hydroxyphenyl)naphthalene-1-sulfonamide (43).

Column chromatography (EtOAc/hexane = 2/1); Yellow crystals (needles); Yield: 71%; R_f 0.53 (EtOAc/hexane = 2/1); Mp: 137.0–139.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.83 (ddd, *J* = 8.1, 2.1, 0.9 Hz, 1H, Ar-H), 6.20 (t, *J* = 2.1 Hz, 1H, Ar-H), 6.38 (ddd, *J* = 8.1, 2.1, 0.9 Hz, 1H, Ar-*H*), 6.82 (t, *J* = 8.1 Hz, 1H, Ar-*H*), 7.66 (dd, *J*

= 8.1, 7.4 Hz, 1H, Ar-*H*), 7.74–7.81 (m, 1H, Ar-*H*), 7.89 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, Ar-*H*), 8.12 (dd, *J* = 7.4, 1.1 Hz, 1H, Ar-*H*), 8.20 (d, *J* = 8.1, 1.1 Hz, 1H, Ar-*H*), 8.39 (d, *J* = 8.1 Hz, 1H, Ar-*H*), 8.60–8.65 (m, 1H, Ar-*H*), resonances for O*H* and N*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 106.19, 107.45, 112.54, 124.11, 124.54, 127.45, 127.57, 129.09, 129.34, 129.75, 130.08, 131.00, 133.67, 136.05, 150.05, 150.36; HRMS (ESI) m/z calcd for $C_{16}H_{14}NO_3S$ $[M+H]^+$ 300.0694, found 300.0684; HPLC purity: 100.00%, retention time: 12.79 min.

Methyl 2-(5-(dimethylamino)naphthalene-1-sulfonamido)benzoate (44).

Column chromatography (EtOAc/hexane = 1/3); Yellow crystals (needles); Yield: 56%; R_f 0.24 (EtOAc/hexane = 1/3); Mp: 146.0–148.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.80 (s, 6H, N(CH_3)$_2$), 3.77 (s, 3H, CH_3), 7.05–7.12 (m, 1H, Ar-*H*), 7.22–7.27 (m, 1H, Ar-*H*), 7.41 (dd, *J* = 8.3, 1.0 Hz, 1H, Ar-*H*), 7.50 (ddd, *J* = 8.3, 7.2, 1.5 Hz, 1H, Ar-*H*), 7.59–7.67 (m, 2H, Ar-*H*), 7.78 (dd, *J* = 8.1, 1.5 Hz, 1H, Ar-*H*), 8.17 (dd, *J* = 8.6, 1.2 Hz, 1H, Ar-*H*), 8.28 (dd, *J* = 7.3, 1.2 Hz, 1H, Ar-*H*), 8.48 (dd, *J* = 8.6, 1.5 Hz, 1H, Ar-*H)*, 10.85 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.93 (2C), 52.65, 115.36, 116.64, 117.69, 118.59, 123.36, 123.49, 128.52, 128.53, 128.87, 130.27, 130.79, 130.90, 133.52, 134.48, 138.69, 151.61, 167.66; HRMS (ESI) m/z calcd for $C_{20}H_{21}N_2O_4S$ $[M+H]^+$ 385.1222, found 385.1217; HPLC purity: 99.62%, retention time: 20.21 min.

Methyl 3-(5-(dimethylamino)naphthalene-1-sulfonamido)benzoate (45).

Column chromatography (EtOAc/hexane = 1/3); Fluorescent yellow crystals (prisms); Yield: 72%; R_f 0.15 (EtOAc/hexane = 1/3); Mp: 140.0–141.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.80 (s, 6H, N(CH_3)$_2$), 3.78 (s, 3H, CH_3), 7.25 (d, *J* = 7.3 Hz, 1H, Ar-*H*), 7.29–7.33 (m, 2H, Ar-*H*), 7.48–7.53 (m, 1H, Ar-*H*), 7.57–7.66 (m, 3H, Ar-*H*), 8.22 (dd, *J* = 7.3, 1.1 Hz, 1H, Ar-*H*), 8.35 (d, *J* = 8.7 Hz, 1H, Ar-*H*), 8.45 (d, *J* = 8.5 Hz, 1H, Ar-*H*), 10.91 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.82 (2C), 53.39, 116.42, 116.95, 119.02, 119.83, 124.31, 124.40, 125.35, 129.71, 129.74, 130.81, 131.14, 131.30, 131.61, 134.66, 138.66, 152.46, 167.16; HRMS (ESI) m/z calcd for $C_{20}H_{21}N_2O_4S$ $[M+H]^+$ 385.1222, found 385.1225; HPLC purity: 100.00%, retention time: 15.23 min.

Dimethyl 5-(5-(dimethylamino)naphthalene-1-sulfonamido)isophthalate (46).

Column chromatography (EtOAc/hexane = 1/3); Amorphous white solid; Yield: 43%; R_f 0.44 (EtOAc/hexane = 1/1); Mp: 207.5–209.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.80 (s, 6H, N(CH_3)$_2$), 3.83 (s, 6H, CH_3), 7.27 (d, *J* = 7.5 Hz, 1H, Ar-*H*), 7.59–7.69 (m, 2H, Ar-*H*), 7.89 (d, *J* = 1.5 Hz, 2H, Ar-*H*), 8.02 (t, *J* = 1.5 Hz, 1H, Ar-*H*), 8.23 (dd, *J* = 7.5, 1.0 Hz, 1H, Ar-*H*), 8.34 (d, *J* = 8.5 Hz, 1H, Ar-*H*), 8.46 (d, *J* = 8.5 Hz, 1H, Ar-*H*), 11.21 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.99 (2C), 52.14, 52.56, 115.50, 116.92, 118.33, 118.36, 122.47, 123.55, 128.50, 128.74, 128.86, 129.84, 130.54, 130.61, 131.04, 133.95, 138.75, 151.25, 164.73, 165.89; HRMS (ESI) m/z calcd for $C_{22}H_{23}N_2O_6S$ $[M+H]^+$ 443.1277, found 443.1263; HPLC purity: 95.04%, retention time: 11.71 min.

Methyl 5-bromo-2-(5-(dimethylamino)naphthalene-1-sulfonamido)benzoate (47).

Column chromatography (Et$_2$O/petroleum ether = 1/2); Fluorescent yellow crystals (needles); Yield: 41%; R_f 0.25 (Et$_2$O/petroleum ether = 1/2); Mp: 148.0–150.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.81 (s, 6H, N(CH_3)$_2$), 3.74 (s, 3H, CH_3), 7.26 (d, *J* = 7.4 Hz, 1H, Ar-*H*), 7.35 (d, *J* = 8.9 Hz, 1H, Ar-*H*), 7.59–7.66 (m, 2H, Ar-*H*), 7.69 (dd, *J* = 8.9, 2.1 Hz, 1H, Ar-*H*), 7.85 (d, *J* = 2.1 Hz, 1H, Ar-*H*), 8.15 (d, *J* = 8.6 Hz, 1H, Ar-*H*), 8.24 (dd, *J* = 7.4, 1.0 Hz, 1H, Ar-*H*), 8.49 (d, *J* = 8.6 Hz, 1H, Ar-*H)*, 10.72 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.95 (2C), 52.90, 115.30, 115.40, 117.71, 119.51, 121.41, 123.52, 128.51, 128.60, 128.90, 130.15, 130.91, 132.99, 133.44, 136.84, 137.61, 151.63, 166.27; HRMS (ESI) m/z calcd for $C_{20}H_{20}N_2O_4SBr$ $[M+H]^+$ 463.0327, found 463.0319; HPLC purity: 95.41%, retention time: 20.17 min.

5-(Dimethylamino)-N-(3-hydroxyphenyl)naphthalene-1-sulfonamide (48).

Column chromatography (EtOAc/hexane = 1/1); Fluorescent yellow crystals (prisms); Yield: 97%; R_f 0.30 (EtOAc/hexane = 1/1); Mp: 82.0–86.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.80 (s, 6H, N(CH_3)$_2$), 6.31 (dd, *J* = 8.1, 2.1 Hz, 1H, Ar-*H*), 6.44–6.53 (m, 2H, Ar-*H*), 6.91 (t, *J* = 8.1 Hz, 1H, Ar-*H*), 7.25 (d, *J* = 7.5 Hz, 1H, Ar-*H*), 7.57–7.64 (m, 2H, Ar-*H*), 8.19 (d, *J* = 7.5 Hz, 1H, Ar-*H*), 8.37 (d, *J* = 8.6 Hz, 1H, Ar-*H*), 8.44 (d, *J* = 8.6 Hz, 1H, Ar-*H*), 9.35 (br s, 1H, O*H*), 10.50 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.98 (2C), 105.57, 109.13, 110.37, 115.20, 118.61, 123.45, 128.07, 128.90, 128.94, 129.64, 129.70, 129.96, 134.83, 138.65, 151.37, 157.72; HRMS (ESI) m/z calcd for $C_{18}H_{19}N_2O_3S$ $[M+H]^+$ 343.1116, found 343.1121; HPLC purity: 98.61%, retention time: 8.22 min.

3. 7. General procedure for the Preparation of Compounds 49–60.

Alkaline hydrolysis.

To a stirred solution of the corresponding protected sulfonamide derivative (1.0 mmol) in dioxane (2 mL), 1 M NaOH (1.5 mL) was added, and the reaction mixture stirred at room temperature until the starting material had completely reacted (monitored by TLC). The solvent was then evaporated under reduced pressure, and the residue

was diluted with H_2O (10 mL) and washed with EtOAc (2 × 10 mL). The aqueous phase was acidified to pH 2 using 1 M HCl, and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine (3 × 10 mL), then dried over Na_2SO_4, filtered, and evaporated to dryness, to provide compounds **49**–**60**. If necessary, these were additionally purified by column chromatography.

2-(Naphthalene-2-sulfonamido)benzoic acid (49).

Off-white solid; Yield: 67%; R_f 0.40 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 223.0–225.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.04–7.12 (m, 1H, Ar-*H)*, 7.49–7.60 (m, 2H, Ar-*H*), 7.63–7.74 (m, 2H, Ar-*H*), 7.76 (dd, *J* = 8.7, 2.1 Hz, 1H, Ar-*H)*, 7.86 (dd, *J* = 8.0, 1.3 Hz, 1H, Ar-*H*), 7.97–8.03 (m, 1H, Ar-*H*), 8.08 (d, *J* = 8.7 Hz, 1H, Ar-*H*), 8.13–8.19 (m, 1H, Ar-*H*), 8.59 (d, *J* = 1.2 Hz, 1H, Ar-*H*), 11.26 (br s, 1H, N*H*), resonance for COO*H* missing; ^{13}C NMR (100 MHz, CD_3COCD_3-d_6): δ 117.62, 120.32, 123.92, 124.89, 129.63, 129.79, 130.75, 131.03, 131.18, 131.51, 133.54, 133.91, 136.55, 136.85, 138.16, 142.67, 171.43; HRMS (ESI) m/z calcd for $C_{17}H_{12}NO_4S$ $[M–H]^-$ 326.0487, found 326.0479; HPLC purity: 97.65%, retention time: 18.02 min.

3-(Naphthalene-2-sulfonamido)benzoic acid (50).

Brown amorphous solid; Yield: 57%; R_f 0.43 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 226.0–228.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.33 (ddd, *J* = 8.1, 7.5, 0.5 Hz, 1H, Ar-*H*), 7.38 (ddd, *J* = 8.1, 2.2, 1.4 Hz, 1H, Ar-*H*), 7.55 (dt, *J* = 7.5, 1.4 Hz, 1H, Ar-*H*), 7.62–7.72 (m, 3H, Ar-*H*), 7.77 (dd, *J* = 8.7, 1.8 Hz, 1H, Ar-*H*), 7.98–8.01 (m, 1H, Ar-*H*), 8.09 (d, *J* = 8.7 Hz, 1H, Ar-*H*), 8.11–8.14 (m, 1H, Ar-*H*); 8.44 (d, *J* = 1.8 Hz, 1H, Ar-*H*), 10.64 (br s, 1H, N*H*), 13.00 (br s, 1H, COO*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 120.51, 121.80, 123.93, 124.80, 127.71, 127.79, 127.96, 129.02, 129.17, 129.47, 129.54, 131.44, 131.65, 134.21, 136.14, 137.92, 166.60; HRMS (ESI) m/z calcd for $C_{17}H_{12}NO_4S$ $[M–H]^-$ 326.0487, found 326.0499; HPLC purity: 100.00%, retention time: 14.12 min.

5-(Naphthalene-2-sulfonamido)isophthalic acid (51).

Off-white solid; Yield: 81%; R_f 0.18 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 273.0–277.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.61–7.73 (m, 2H, Ar-*H*), 7.74–7.80 (m, 1H, Ar-*H*), 7.92–7.96 (m, 2H, Ar-*H*), 8.00 (d, *J* = 8.1 Hz, 1H, Ar-*H*), 8.05–8.16 (m, 3H, Ar-*H*), 8.45 (m, 1H, Ar-*H*), 10.85 (br s, 1H, N*H*), 13.27 (br s, 2H, COO*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.32, 118.07, 121.66, 123.98, 125.23, 127.82 (2C), 127.99, 129.14, 129.21, 129.71, 131.45, 132.17, 134.27, 135.92, 138.45, 165.90, 167.14; HRMS (ESI) m/z calcd for $C_{18}H_{12}NO_6S$ $[M–H]^-$ 370.0385, found 370.0374; HPLC purity: 99.30%, retention time: 12.45 min.

5-Bromo-2-(naphthalene-2-sulfonamido)benzoic acid (52).

Brown solid; Yield: 61%; R_f 0.36 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 200.0–202.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.51 (dd, *J* = 8.8, 0.5 Hz, 1H, Ar-*H*), 7.65–7.74 (m, 3H, Ar-*H*), 7.76 (dd, *J* = 8.9, 2.0 Hz, 1H, Ar-*H*), 7.93 (dd, *J* = 2.3, 0.5 Hz, 1H, Ar-*H*), 8.00–8.04 (m, 1H, Ar-*H*), 8.10 (d, *J* = 8.9 Hz, 1H, Ar-*H*), 8.15–8.19 (m, 1H, Ar-*H*), 8.60 (d, *J* = 2.0 Hz, 1H, Ar-*H*), 11.24 (br s, 1H, N*H*), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 114.92, 118.99, 120.56, 121.62, 127.82, 127.84, 128.59, 129.35 (2C), 129.81, 131.46, 133.47, 134.44, 135.27, 136.87, 138.82, 168.28; HRMS (ESI) m/z calcd for $C_{17}H_{11}NO_4SBr$ $[M–H]^-$ 403.9592, found 403.9609; HPLC purity: 99.74%, retention time: 22.10 min.

2-(Naphthalene-1-sulfonamido)benzoic acid (53).

Yellow solid; Yield: 52%; R_f 0.40 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 232.0–235.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.98–7.05 (m, 1H, Ar-*H*), 7.43–7.49 (m, 2H, Ar-*H*), 7.64–7.69 (m, 2H, Ar-*H*), 7.73 (ddd, *J* = 8.6, 6.9, 1.5 Hz, 1H, Ar-*H*), 7.79–7.83 (m, 1H, Ar-*H*), 8.07–8.10 (m, 1H, Ar-*H*), 8.24–8.28 (m, 1H, Ar-*H*), 8.35 (dd, J_1 = 7.4, 1.2 Hz, 1H, Ar-*H*), 8.53 (dd, *J* = 8.6, 0.8 Hz, 1H, Ar-*H*), 11.68 (br s, 1H, N*H*), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 115.59, 117.01, 122.78, 123.30, 124.45, 126.89, 127.13, 128.49, 129.35, 130.60, 131.44, 132.93, 133.73, 134.51, 135.12, 139.73, 169.89; HRMS (ESI) m/z calcd for $C_{17}H_{12}NO_4S$ $[M–H]^-$ 326.0487, found 326.0497; HPLC purity: 100.00%, retention time: 17.07 min.

3-(Naphthalene-1-sulfonamido)benzoic acid (54).

White amorphous solid; Yield: 97%; R_f 0.41 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 195.0–196.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.25–7.32 (m, 2H, Ar-*H*), 7.47–7.52 (m, 1H, Ar-*H*), 7.59–7.71 (m, 3H, Ar-*H*), 7.75 (ddd, *J* = 8.5, 6.9, 1.4 Hz, 1H, Ar-*H*), 8.05–8.10 (m, 1H, Ar-*H*), 8.20–8.25 (m, 2H, Ar-*H*), 8.72 (d, *J* = 8.5 Hz, 1H, Ar-*H*), 10.90 (br s, 1H, N*H*), 12.91 (br s, 1H, COO*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 119.30, 122.70, 124.07, 124.25, 124.42, 127.03, 127.29, 128.24, 129.11, 129.43, 129.93, 131.61, 133.71, 133.94, 134.59, 137.82, 166.60; HRMS (ESI) m/z calcd for $C_{17}H_{12}NO_4S$ $[M–H]^-$ 326.0487, found 326.0502; HPLC purity: 100.00%, retention time: 13.28 min.

5-(Naphthalene-1-sulfonamido)isophthalic acid (55).

Off-white amorphous solid; Yield: 72%; R_f 0.16 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 298.0–300.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.60–7.88 (m, 5H, Ar-*H*), 7.99–8.03 (m, 1H, Ar-*H*), 8.05–8.12 (m, 1H, Ar-*H*), 8.20–8.27 (m, 2H, Ar-*H*), 8.67–8.74 (m, 1H, Ar-*H*), 11.16 (br s, 1H, N*H*), 13.25 (br s, 2H, COO*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 122.66 (2C), 123.93, 124.43, 124.62, 127.12, 127.19, 128.38, 129.18, 129.89, 132.09 (2C), 133.64, 133.73, 134.81, 138.33, 165.87 (2C); HRMS (ESI) m/z calcd for $C_{18}H_{14}NO_6S$ $[M+H]^+$

372.0542, found 372.0541; HPLC purity: 99.64%, retention time: 11.89 min.

5-Bromo-2-(naphthalene-1-sulfonamido)benzoic acid (56).

Brown amorphous solid; Yield: 86%; R_f 0.35 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 187.0–189.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.40 (dd, J =8.9, 0.5 Hz, 1H, Ar-*H*), 7.63–7.70 (m, 3H, Ar-*H*), 7.74 (ddd, J = 8.6, 6.9, 1.6 Hz, 1H, Ar-*H*), 7.88 (dd, J = 2.5, 0.5 Hz, 1H, Ar-*H*), 8.08–8.12 (m, 1H, Ar-*H*), 8.26–8.29 (m, 1H, Ar-*H*), 8.32 (dd, J = 7.4, 1.2 Hz, 1H, Ar-*H*), 8.51 (dd, J = 8.6, 1.0 Hz, 1H, Ar-*H*), 11.72 (br s, 1H, N*H*), resonance for COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 114.42, 118.08, 119.46, 123.30, 124.51, 126.86, 127.23, 128.61, 129.43, 130.56, 132.79, 133.47, 133.78, 135.28, 136.94, 139.00, 168.54; HRMS (ESI) m/z calcd for $C_{17}H_{13}NO_4SBr$ $[M+H]^+$ 405.9749, found 405.9744; HPLC purity: 98.01%, retention time: 21.33 min.

2-(5-(Dimethylamino)naphthalene-1-sulfonamido)benzoic acid (57).

Green amorphous solid; Yield: 87%; R_f 0.51 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 190.5–192.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.78 (s, 6H, N(CH_3)$_2$), 6.77–6.87 (m, 1H, Ar-*H*), 7.16–7.36 (m, 3H, Ar-*H*), 7.49–7.64 (m, 2H, Ar-*H*), 7.76–7.80 (m, 1H, Ar-*H*), 8.24–8.35 (m, 2H, Ar-*H*), 8.37–8.43 (m, 1H, Ar-*H*), resonances for N*H* and COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.96 (2C), 115.09, 116.52, 117.80, 118.73, 120.50, 123.45, 127.82, 128.93, 128.95, 129.21, 129.50, 131.06, 133.06, 135.90, 142.84, 151.31, 169.59; HRMS (ESI) m/z calcd for $C_{19}H_{17}N_2O_4S$ $[M–H]^–$ 369.0909, found 369.0904; HPLC purity: 99.26%, retention time: 17.01 min.

3-(5-(Dimethylamino)naphthalene-1-sulfonamido)benzoic acid (58).

Yellow crystals (needles); Yield: 87%; R_f 0.40 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 226.5–228.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.80 (s, 6H, N(CH_3)$_2$), 7.23–7.33 (m, 3H, Ar-*H*), 7.45–7.52 (m, 1H, Ar-*H*), 7.56–7.66 (m, 3H, Ar-*H*), 8.21 (dd, J = 7.4, 1.1 Hz, 1H, Ar-*H*), 8.34–8.38 (m, 1H, Ar-*H*), 8.42–8.47 (m, 1H, Ar-*H*), 10.86 (br s, 1H, N*H*), 12.93 (br s, 1H, COO*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.97 (2C), 115.29, 118.40, 119.13, 122.50, 123.46, 124.09, 128.27, 128.84, 128.90, 129.41, 129.74, 130.26, 131.60, 134.38, 137.91, 151.44, 166.61; HRMS (ESI) m/z calcd for $C_{19}H_{17}N_2O_4S$ $[M–H]^–$ 369.0909, found 369.0900; HPLC purity: 99.26%, retention time: 17.01 min.

5-(5-(Dimethylamino)naphthalene-1-sulfonamido)isophthalic acid (59).

Yellow crystals (needles); Yield: 89%; R_f 0.18 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 283.5–286.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.80 (s, 6H, N(CH_3)$_2$), 7.26 (dd, J = 7.6, 0.5 Hz, 1H, Ar-*H*), 7.58–7.67 (m, 2H, Ar-*H*), 7.85 (d, J = 1.4 Hz, 2H, Ar-*H*), 8.01 (t, J = 1.4 Hz, 1H, Ar-*H*), 8.21 (dd, J = 7.3, 1.1 Hz, 1H, Ar-*H*), 8.32–8.37 (m, 1H, Ar-*H*), 8.43–8.48 (m, 1H, Ar-*H*), 11.11 (br s, 1H, N*H*), 13.25 (br s, 1H, COO*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.96 (2C), 115.37, 118.25, 122.57 (2C), 123.46, 124.52, 128.43, 128.77, 128.92, 129.70, 130.48, 132.13 (2C), 134.07, 138.41, 151.50, 165.91 (2C); HRMS (ESI) m/z calcd for $C_{20}H_{17}N_2O_6S$ $[M–H]^–$ 413.0807, found 413.0800; HPLC purity: 99.75%, retention time: 9.57 min.

5-Bromo-2-(5-(dimethylamino)naphthalene-1-sulfonamido)benzoic acid (60).

Yellow crystals (needles); Yield: 97%; R_f 0.43 (CH_2Cl_2/MeOH/AcOH = 5/1/0.1); Mp: 186.5–189.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.78 (s, 6H, N(CH_3)$_2$), 7.15–7.21 (m, 2H, Ar-*H*), 7.26 (dd, J = 8.8, 2.6 Hz, 1H, Ar-*H)*, 7.47–7.59 (m, 2H, Ar-*H*), 7.82 (d, J = 2.6 Hz, 1H, Ar-*H*), 8.18 (dd, J = 7.3, 1.1 Hz, 1H, Ar-*H*), 8.33–8.42 (m, 2H, Ar-*H*), resonances for N*H* and COO*H* missing; ^{13}C NMR (100 MHz, DMSO-d_6): δ 45.06 (2C), 110.52, 115.00, 118.42, 119.55, 122.65, 123.47, 127.47, 128.37, 128.78, 129.05, 129.23, 132.89, 134.02, 137.37, 144.20, 151.18, 167.89; HRMS (ESI) m/z calcd for $C_{19}H_{18}N_2O_4SBr$ $[M+H]^+$ 449.0171, found 449.0160; HPLC purity: 99.86%, retention time: 22.20 min.

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