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Article

Synthesis and Structure−Activity Relationships of Arylsulfonamides as AIMP2-DX2 Inhibitors for the Development of a Novel Anticancer Therapy

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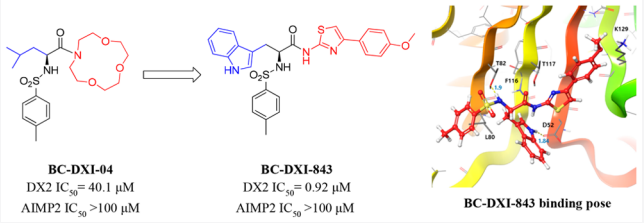
ABSTRACT: AIMP2-DX2, a splicing variant of AIMP2, is up-regulated in lung cancer, possesses oncogenic activity, and results in tumorigenesis. Specifically inhibiting the interaction between AIMP2-DX2 and HSP70 to suppress AIMP2-DX2-dependent cancers with small molecules is considered a promising avenue for cancer therapeutics. Optimization of hit **BC-DXI-04** (IC_{50} = 40.1 μM) provided new potent sulfonamide based AIMP2-DX2 inhibitors. Among these, **BC-DXI-843** showed improved inhibition against AIMP2-DX2 (IC_{50} = 0.92 μM) with more than 100-fold selectivity over AIMP2 in a luciferase assay. Several binding assays indicated that this compound effectively induces cancer cell apoptosis by specifically interrupting the interaction between DX2 and HSP70, which leads to the degradation of DX2 via Siah1-mediated ubiquitination. More importantly, **BC-DXI-843** demonstrated *in vivo* efficacy in a tumor xenograft mouse model (H460 cells) at a dosage of 50 mg/kg, suggesting it as a promising lead for development of novel therapeutics targeting AIMP2-DX2 in lung cancer.



INTRODUCTION

Aminoacyl-tRNA synthetases (ARSs) are a family of 20 essential enzymes (one for each amino acid) that play an important role in conjugating amino acids to their cognate tRNAs during protein synthesis. In higher eukaryotic systems, nine different ARSs form a multisynthetase complex (MSC) with three auxiliary factor ARS-interacting multifunctional proteins (AIMPs). Although AIMP1−3 provide scaffolding for the assembly and integrity of the MSC, they also dissociate from the MSC to perform diverse regulatory functions that are not directly related to protein synthesis.[1−4] Among them, AIMP2/p38 helps control the fate of a cell and performs various functions in response to stress signals. It displays antiproliferative activity by enhancing the growth-arresting signal of TGF-β, and it also promotes cell death by activating p53 and the apoptotic signal of TNF-α. The antiproliferative and proapoptotic roles of AIMP2 have been supported by *in vivo* mouse models. *Aimp2*-heterozygous mice, when compared with wild-type mice, are more vulnerable to tumorigenesis in lung, colon, and skin carcinogenesis models, suggesting that AIMP2 is a haploinsufficient tumor suppressor with a unique mechanism.[5−9]

The gene encoding AIMP2 consists of four exons and is subject to alternative splicing. A variant of AIMP2, known as AIMP2-DX2 (DX2 from here onward), is created by deleting exon 2 via alternative splicing and has been detected in various cancer cell lines and tissues. Although this variant has lost the ability to associate with the MSC, it can still inhibit the tumor-suppressive function of the native AIMP2 through competitive interactions with p53, FBP, and TRAF2. Furthermore, the specific interaction of DX2 with p14 and the subsequent inactivation of p14 are critical for tumor growth in KRAS-driven lung cancers. An increased ratio of DX2 versus AIMP2 shows a positive correlation with poor clinical outcome of lung cancer as well as chemotherapy resistance in ovarian cancer. siRNA or chemical inhibition of DX2 expression reduces tumor growth, indicating its potential as a therapeutic target.[10−14]

More recently, we reported that the 70 kDa heat shock proteins (HSP70s) are critical determinants of DX2 levels in cells. A positive correlation between HSP70 and DX2 levels has been shown in various lung cancer cell lines and patient tissues. The binding of the substrate-binding domain (SBD) of HSP70 to the flexible N-terminal region (NFR) and the GST-N-terminal domain (GST-N) of DX2 protect it from Siah1-

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mediated ubiquitination. Moreover, preventing HSP70 from binding to DX2 with mutational and chemical inhibition destabilizes DX2, suggesting HSP70 is functionally significant in maintaining DX2 levels in cells. Thus, inhibiting the protein−protein interaction (PPI) between AIMP2-DX2 and HSP70 may lead to a therapeutic strategy against cancer (Figure 1).[15,16]

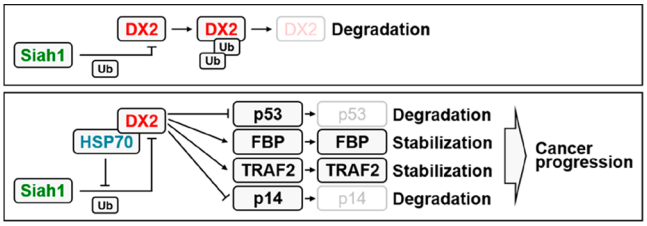

Figure 1. Schematic model for DX2-dependent cancer progression. DX2 is degraded by Siah1-mediated ubiquitination (upper). But in the presence of HSP70, DX2 binds with HSP70 and is protected from Siah1, stabilizing DX2 protein levels. Stabilized DX2 reduces p53 and p14 protein levels upon UV exposure and oncogenic signals, respectively. Stabilized DX2 also increases FBP and TRAF2 upon TGFβ and TNF-α transduction, respectively, resulting in enhanced cancer progression (bottom). Ub = ubiquitin.

Among the previously identified small molecules targeting DX2,[15−20] **BC-DXI-01** suppresses DX2 cellular levels through selective degradation of DX2 mRNA transcripts, leading to inhibition of DX2 activity and tumor suppression.[17] **SLCB050** specifically inhibits the interaction between DX2 and p14ARF, suppressing proliferation of p14ARF-positive lung cancer cells.[18,19] The sulfonamide **BC-DXI-04** (**1a**) was identified as a screening hit of DX2 that decreases the proliferation of lung cancer cells in a concentration-dependent manner.[20] Using one of its synthetic derivatives (**BC-DXI-495**, **2**) as a chemical probe, the mode of action was elucidated. These compounds destabilize DX2 by inhibiting its interaction with HSP70.[15,16] Herein, we report the synthesis and structure−activity relationships of a series of arylsulfonamides (Figure 2), which led to the novel compound **BC-DXI-843** (**20d**). It modulates the HSP70-DX2 interaction with greater efficacy than the starting compound. We also demonstrate the tumor-suppressive function of **BC-DXI-843** in a DX2-dependent manner via the inhibition of two protein interactions.

RESULTS AND DISCUSSION

Chemistry. The synthetic approaches used to generate the arylsulfonamides are described in Schemes 1−6. The hit compound and its morpholine derivative were synthesized following the procedure shown in Scheme 1. L-Leucine was coupled with *p*-toluenesulfonyl chloride in basic medium to generate the sulfonamide **4a**, which yielded derivatives **1a** (**BC-DXI-04**) and **5a** under standard amide coupling conditions. Using similar conditions, the enantiomers, **1b** and **5b**, were synthesized from D-leucine (Scheme 1).

Anilines **8a−e** were prepared from 4-fluoronitrobenzene (**6**) by using nucleophilic aromatic substitution followed by reduction with Pd/C and H_2 as previously described.[21,22] Subsequent condensation with intermediate **4a** under the standard conditions yielded amide derivatives **9a−e** (Scheme 2). L-Leucine benzyl ester was initially coupled with *p*-toluic acid to generate intermediate **11**, which was subsequently deprotected under hydrogenation and further coupled with 4-morpholinoaniline using the aforementioned conditions to yield the diamide derivative **12** (Scheme 3).

The arylsulfonamide derivatives **14a−f** were prepared following the procedures shown in Scheme 4. Various arylsulfonyl chlorides were coupled with L-leucine under basic conditions yielding the intermediates **13a−f**. Under the standard amide coupling conditions, these acids yielded compounds **14a−f**. *p*-Toluene sulfonamides, **16a−k**, of various L-amino acids were prepared as shown in Scheme 5. Compounds **17a−f**, **17i**, and **2** (**BC-DXI-495**) were obtained from their respective acids under the aforementioned conditions. The tyrosine derivative **17j** was prepared by the deprotection of the O-sulfonated compound under basic conditions, and derivatives **17g** and **17h** were prepared through the deprotection of the benzyl groups from their respective benzyl ester by hydrogenation.

As shown in Scheme 6, various L-tryptophan sulfonamides (**16k**, **18a−c**) were prepared using the established conditions. Subsequent condensation with various amines under the standard conditions generated derivatives **19a,b**, **20a−d**, and **21a−f**. The thiazole amines (**d**, **e**, and **f**) were prepared in one-step with the corresponding phenacyl bromides and thiourea using previously described conditions.[23,24]

Structure−Activity Relationship Exploration. High-throughput screening (HTS) involving our in-house chemical library identified a substituted sulfonamide, **BC-DXI-04** (**1a**), that suppresses intracellular levels of DX2 and inhibits the proliferation of cancer cells with moderate activity and good selectivity. It demonstrated 55.80% inhibition at 40 μM in a DX2-luciferase assay, which is an assay for determining the level of luciferase-tagged DX2 via detection of luminescence signal. The IC_{50} and EC_{50} values measured by DX2-luciferase and cell

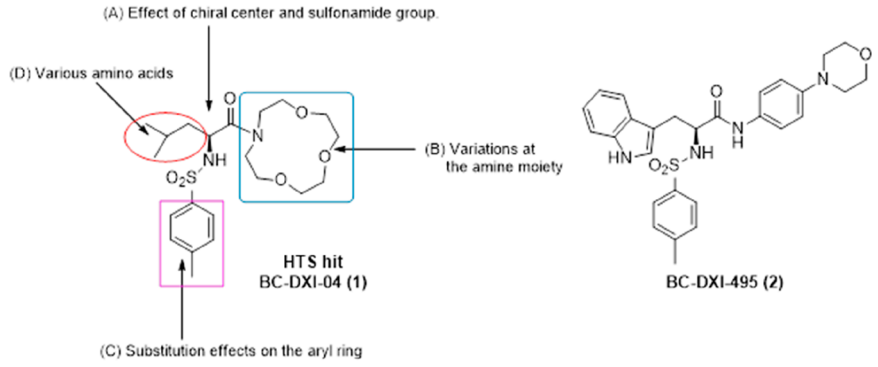

Figure 2. Chemical structures of known sulfonamide based small molecule inhibitors and strategy for SAR exploration.

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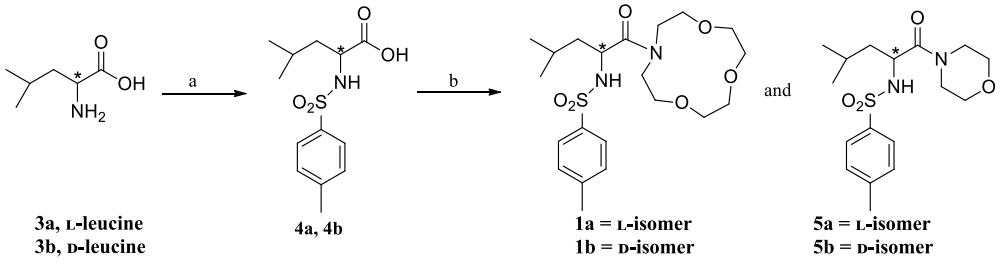
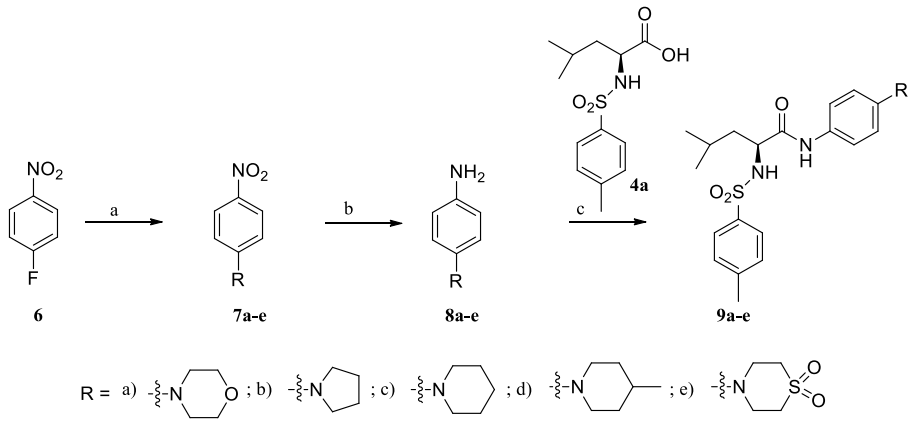
Scheme 1[a]



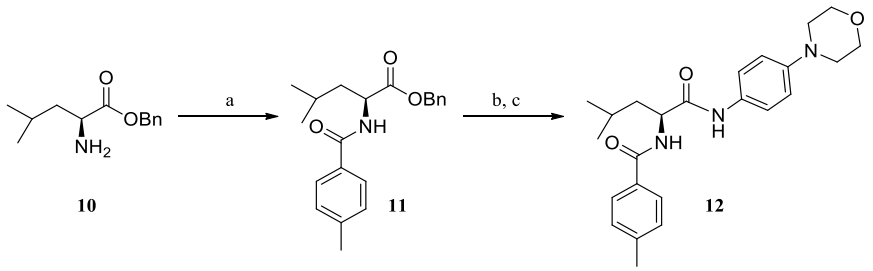
[a]Reagents and condition: (a) *p*-toluenesulfonyl chloride, NaOH, H_2O, rt, 12 h, 52% and 58%; (b) amine, EDCI·HCl, HOBt, TEA, DCM, rt, 18 h, 21−91%.

Scheme 2[a]



[a]Reagents and condition: (a) amine, K_2CO_3, DMF, 70°C, 5−6 h, 83−93%; (b) H_2, Pd/C, MeOH, rt, 2−3 h, 94−99%; (c) amine, EDCI·HCl, HOBt, TEA, DCM, rt, 18 h, 21−91%.

Scheme 3[a]



[a]Reagents and condition: (a) *p*-toluic acid, EDCI·HCl, HOBt, TEA, DCM, rt, 12 h, 18−94%; (b) H_2, Pd/C, MeOH, 2 h; (c) 4-morpholinoaniline, EDCI·HCl, HOBt, TEA, DCM, rt, 18 h, 24%.

viability assay were 40.1 μM and 40.8 μM, respectively. On the basis of this hit, a series of arylsulfonamides were screened using a nanoluciferase assay at 40 μM. To define the key structural requirements for the biological activity of **BC-DXI-04**, our optimization strategy was focused on four discrete areas: (A) the effect of the chiral center and the requirement of a sulfonamide linker, (B) the 1,4,7-trioxa-10-azacyclododecane moiety, which could be replaced by an arylamine containing various substituents, (C) substitution effects on the phenyl ring of the sulfonamide part, and (D) variations in the amino acid chain (Figure 2).

Initial replacement of the 1,4,7-trioxa-10-azacyclododecane moiety with morpholine yielded a simplified derivative, **5a**, with a comparable inhibition of 57.30% in the DX2-luciferase assay (part B). In parallel, we also synthesized enantiomers (D-isomer) of these compounds, **1b** and **5b**, to understand the effect of the chiral center on their activity (part A). As shown in Table 1, the inhibition values of these isomers dramatically decreased, suggesting that the presence of an L-configuration is essential for their activity. Furthermore, we replaced the morpholine group of **5a** with 4-morpholinoaniline, resulting in an equipotent molecule **9a**, (Table 2) with 56.90% inhibition. Considering that the aryl linker could be a useful handle for optimizing the properties of the molecule, a detailed SAR study of this molecule (**9a**) was designed.

We explored the effects of various substitutions on the phenyl ring of the amide chain and the sulfonamide moiety on inhibitory activity as shown in Table 2. Continuing the SAR study in Table 2, replacing morpholine with pyrrolidine and piperidine moieties resulted in the less potent derivatives **9b−d**. Substituting with thiomorpholine 1,1-dioxide also decreased the inhibitory activity as in **9e**. Further modifications to the amide

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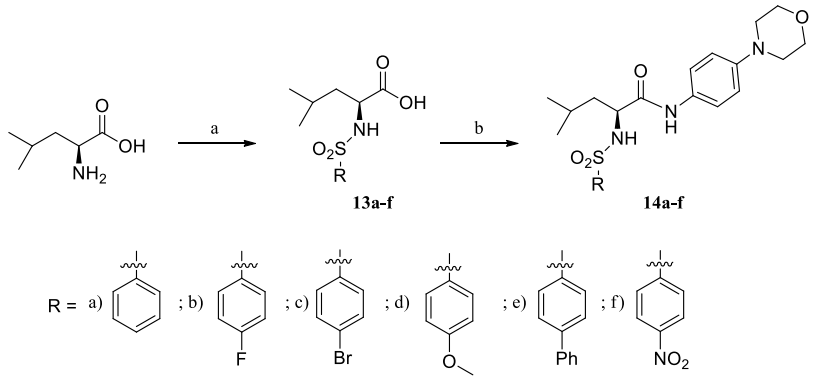
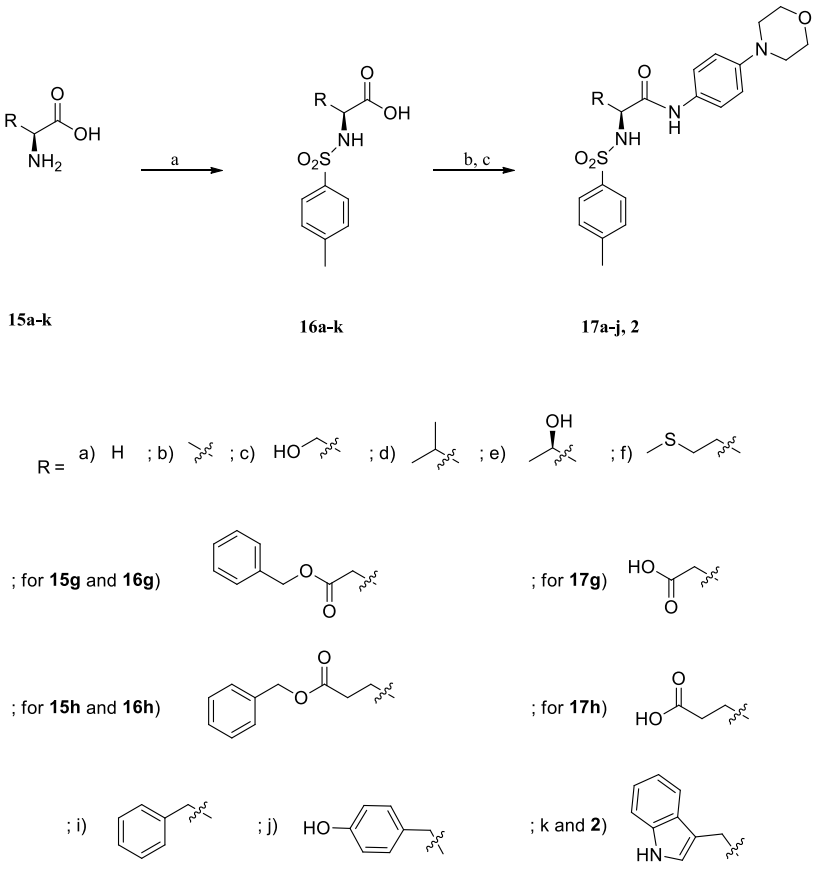
Scheme 4[a]



[a]Reagents and condition: (a) arylsulfonyl chloride, NaOH, H_2O, rt, 12 h, 14−59%; (b) 4-morpholinoaniline, EDCI·HCl, HOBt, TEA, DCM, rt, 18 h, 31−80%.

Scheme 5[a]



[a]Reagents and condition: (a) *p*-toluenesulfonyl chloride, NaOH, H_2O, rt, 12 h, 13−92%; (b) 4-morpholinoaniline, EDCI·HCl, HOBt, TEA, DCM, rt, 18 h, and then (c) 5 M NaOH, EtOH, reflux, 4 h for **17j** and H_2, Pd/C, MeOH, rt, 3 h for **17g** and **17h**, 31−68%.

chain were planned in the later stages. The diamide analog, **12**, of compound **9a** exhibited significantly diminished inhibitory effects (30.20% inhibition), suggesting that the sulfonamide moiety is essential for the activity. We further explored the effects of substituents on the phenyl ring of the sulfonamide part on the inhibitory activity. When the methyl group was removed, the resulting compound **14a** exhibited a loss of potency (14.46% inhibition). The effects of electron withdrawing and donating groups were also examined (**14b−f**). However, except for 4-bromo, all other substitutions resulted in a significant loss in activity and the 4-bromobenzenesulfonamide derivative **14c** demonstrated a potency similar to **9a** with 54.55% inhibition in the DX2-luciferase assay.

The impact of the amino acid chain on the inhibitory effects was then evaluated as shown in Table 3. A series of L-amino acid derivatives (**17a−j, 2**) were synthesized and tested. Compounds **17a−j** showed significantly diminished inhibitory effects when compared with **9a**. However, the tryptophan derivative **2** (**BC-DXI-495**) showed significantly increased activity (67.10% inhibition) and is the most potent DX2 inhibitor synthesized in this series so far. **BC-DXI-495**, a representative compound in this series, was also used to understand their mode of action. It

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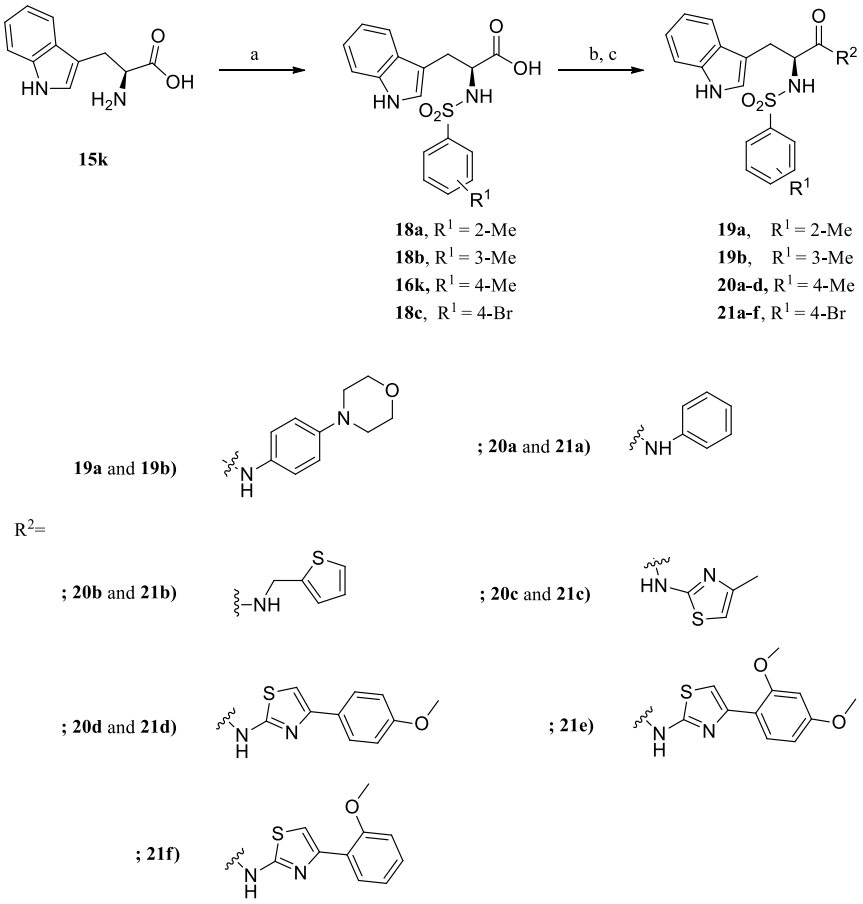
Scheme 6[a]



[a]Reagents and condition: (a) arylsulfonyl chloride, NaOH, H_2O, rt, 12 h, 40−54%; (b) amine, EDCI·HCl, HOBt, TEA, DCM, rt, 3−4 h, 31−92%; (c) amine, EDCI·HCl, HOBt, DIPEA, DMF, 60 °C, 16 h, for **20d−21f**, 31−75%.

exerts tumor-suppressive activity by specifically inhibiting the tumor-promoting interaction between DX2 and HSP70.[16]

Further structural modifications of **BC-DXI-495** were explored (Table 4). The orientation effect of the methyl group on the phenyl ring was initially investigated. The *ortho* and *meta* derivatives **19a** and **19b** resulted in reduced potency with 59.23% and 40.78% inhibition, respectively, which suggests the importance of a *para*-methyl group. Further optimization focused on amide derivatives (part B) with 4-methyl (from **2**) and 4-bromo (from **14c**) sulfonamide moieties. Decreased potency was observed for the **20a** and **21a** derivatives without morpholine substitution on the phenyl ring. The introduction of thiophen-2-ylmethanamine resulted in decreased potency for the 4-methylbenzenesulfonamide derivative **20b**, while a moderate effect (42.99% inhibition) was observed with the 4-bromobenzenesulfonamide derivative **21b**. Similar results were obtained for the five-membered heteroarylamine derivatives **20c** and **21c**. Better potencies observed with the heterocycles compared to phenyl ring suggested an opportunity to introduce heteroarylamines. In addition, loss of potency with removal of the morpholine group (**20a** and **21a**) indicated the requirement of bulky amino groups. Introduction of 4-(4-methoxyphenyl)-thiazol-2-amine provided the 4-methylbenzenesulfonamide derivative **20d**, which exhibited the best inhibitory results (72.56% inhibition), and the 4-bromobenzenesulfonamide analog **21d**, showing moderately good results (45.43% inhibition). We also examined the orientation effects of methoxy substitution on the benzene ring for the 4-bromobenzene-sulfonamide derivative. The inhibitory effects were abolished for the synthesized molecules **21e** and **21f**, which suggests that the *p*-toluenesulfonamide and 4-(4-methoxyphenyl)thiazol-2-amine moieties are essential for inhibiting DX2 as exemplified by **20d** (**BC-DXI-843**).

BC-DXI-843 Specifically Inhibits AIMP2-DX2. Driven by the improved inhibitory effect of **BC-DXI-843**, we assessed the selectivity profile of candidates to identify lead compounds. Six novel derivatives (**BC-DXI-04**, **5a**, **9a**, **14c**, **BC-DXI-495**, and **BC-DXI-843**) showing over 50% inhibition were subjected to secondary screening using nanoluciferase-DX2 and -AIMP2. Among the screened compounds, **BC-DXI-843** demonstrated an improved DX2 inhibition with better AIMP2 vs DX2 selectivity (Figure 3A). Therefore, this compound was selected for further evaluation. To generate IC_{50} values, A549 cells expressing nanoluciferase-DX2 or nanoluciferase-AIMP2 were treated with **BC-DXI-843** in a dose-dependent manner for 4 h, and the luminescence was measured. The IC_{50} for inhibition of DX2 and AIMP2 shows more than 100-fold selectivity (DX2 IC_{50} = 0.92 μM and AIMP2 IC_{50} > 100 μM, Figure 3B). **BC-DXI-495** was previously reported to be a DX2 inhibitor, and its IC_{50} for DX2 degradation was 4.20 μM.[15,16] To observe any improvement of **BC-DXI-843** over the previously reported compounds **BC-DXI-04** and **BC-DXI-495**, we compared their IC_{50} values regarding inhibition of AIMP2 and DX2 expression and their EC_{50} values in lung cancer and normal cells (Table 5).

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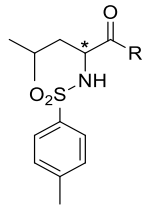
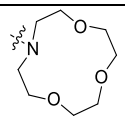
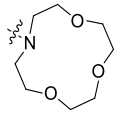
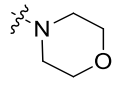
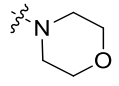
Table 1. DX2-Inhibitory Activity of the Sulfonamide Derivatives 1a,b and 5a,b Using a DX2-Nanoluciferase Assay



No.	R	% inhibition of DX2-luciferase (at 40 μM)[a]
1a (BC-DXI-495) (L-isomer)		55.80 ± 14.80
1b (D-isomer)		10.69 ± 12.75
5a (L-isomer)		57.30 ± 6.80
5b (D-isomer)		-14.04 ± 15.60

[a]The change of the nanoluciferase-tagged DX2 level upon treating chemicals was determined by detecting the luminescence signal.

The IC_{50} of the compounds with respect to DX2 gradually decreased according to the modification of the compound but did not affect AIMP2 levels. The EC_{50} measurements trended similarly to the IC_{50} measurements; they gradually decreased. But the EC_{50} values generated by normal lung cells were all 100-fold higher than those generated by cancer cells (Table 5, right). To determine the mode of action of **BC-DXI-843**, we first examined the interaction between **BC-DXI-843** and DX2 using a fluorescence-based equilibrium binding assay. The binding affinity (K_d) was approximately 3.07 μM (Figure 4A). Previously it was found that the analog **BC-DXI-495** was bound to the hydrophobic pocket of DX2, which comprises L80, T82, F116, T117, and K129 residues. This hydrophobic pocket is proximal to the binding surface of HSP70, suggesting that when **BC-DXI-495** binds to DX2, it may interfere with DX2/HSP70 binding.[16] To characterize this relationship further, a docking study was performed with **BC-DXI-843** that focused on the hydrophobic pocket identified in the previous study. This study used our in-house homology model of DX2 (Figure 4B). In addition, **BC-DXI-843** docking was analyzed through the binding configuration of five important residues in the hydrophobic pocket. According to the results, **BC-DXI-843** was positioned very close to L80, T82, F116, T117, and K129. Interestingly, **BC-DXI-843** forms two hydrogen bonds with the hydrophobic pocket via T82 and D52 (Figure 4C). To validate the significance of T82 and D52 to the interaction between **BC-DXI-843** and DX2, we substituted several residues surrounding the hydrophobic pocket, including these two residues, to alanine and conducted the functional assay. When either D52 or T82 was mutated, **BC-DXI-843** could not interfere with the binding of DX2 to HSP70 (Figure S1A) and induce the degradation of DX2 protein (Figure S1B). This confirmed the significance of the hydrogen bonds formed between **BC-DXI-843** and the D52 and T82 amino acid residues of DX2. Therefore, the improved biological activity of **BC-DXI-843** may be explained by the formation of these hydrogen bonds.

BC-DXI-843 Specifically Induces the Degradation of DX2 via Ubiquitination. To confirm that **BC-DXI-843** is a DX2-specific inhibitor, we compared the inhibition of endogenous DX2 protein expression in H460 cells using immunoblotting after treating the cells with **BC-DXI-843** or **BC-DXI-495**. Both compounds exhibited dose-dependent inhibition of DX2 protein levels, and **BC-DXI-843** efficiently induced degradation of DX2 at a much lower concentration (1.20 μM) when compared to **BC-DXI-495** (Figure S1C). This implies that **BC-DXI-843** is a more potent regulator of DX2, and it regulates DX2 levels by specifically inducing its degradation. However, AIMP2 was not affected in any of the test conditions by **BC-DXI-843** or **BC-DXI-495** (Figure S1C). We also confirmed that BC-DXI-843 has no effect on the level of DX2 mRNA (Figure S1C), suggesting the specific inhibitory effect on protein level of DX2. Since the degradation of DX2 is mediated by ubiquitination,[15,16] we evaluated the amount of ubiquitinated DX2 after treatment with **BC-DXI-843** in a dose-dependent manner. **BC-DXI-843** induced the ubiquitination of endogenous DX2, and the amount of ubiquitinated DX2 was saturated at 1.20 μM (Figure S1D). These results are similar to the upper results in Figure S1C, suggesting that **BC-DXI-843** leads to the degradation of DX2 via ubiquitination.

BC-DXI-843 Inhibits DX2 from Binding to HSP70. It has been reported that DX2 is stabilized when bound to HSP70.[16] Therefore, we tested whether **BC-DXI-843** interferes with the

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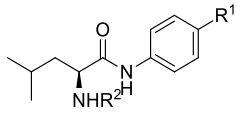
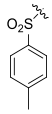
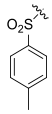
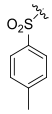
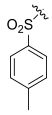
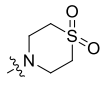
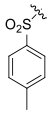
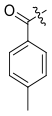
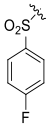
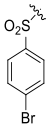
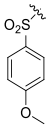
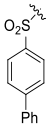
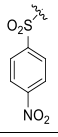
Table 2. DX2-Inhibitory Activity of the Sulfonamide Derivatives 9a−e, 12, and 14a−f Using a Nanoluciferase Assay



No.	R^1	R^2	% inhibition of DX2-luciferase (at 40 μM)[a]
9a			56.90 ± 7.20
9b			33.44 ± 6.60
9c			31.70 ± 6.06
9d			35.45 ± 2.45
9e			16.10 ± 20.61
12			30.20 ± 8.46
14a			14.46 ± 9.45
14b			22.83 ± 7.33
14c			54.55 ± 4.84
14d			29.61 ± 11.71
14e			-6.12 ± 10.90
14f			11.05 ± 23.55

[a]The change of the nanoluciferase-tagged DX2 level upon treating chemicals was determined by detecting the luminescence signal.

interaction between DX2 and HSP70. First, we confirmed that **BC-DXI-843** interferes with DX2 and HSP70 binding by performing an *in vitro* pull-down assay using purified DX2 and HSP70 proteins in the presence of **BC-DXI-843**. **BC-DXI-843** interfered with DX2 and HSP70 binding at a dose similar to that which caused degradation of DX2 (Figure S2A). Next, we

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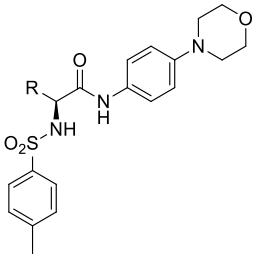
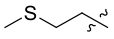
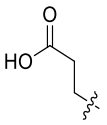
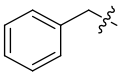
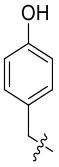
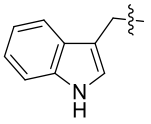
Table 3. DX2-Inhibitory Activity of the Sulfonamide Derivatives 17a−j and 2 Using a Nanoluciferase Assay



No.	R	% inhibition of DX2-luciferase (at 40 μM)[a]
17a	H	12.93 ± 12.66
17b	Me	2.17 ± 20.23
17c		-0.39 ± 15.72
17d		20.45 ± 5.02
17e		-12.81 ± 10.58
17f		4.17 ± 22.57
17g		-11.05 ± 19.25
17h		21.57 ± 4.53
17i		10.24 ± 10.30
17j		-8.00 ± 16.11
2 (BC-DXI-495)		67.10 ± 10.80

[a]The change of the nanoluciferase-tagged DX2 level upon treating chemicals was determined by detecting the luminescence signal.

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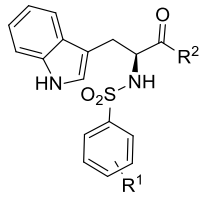
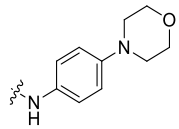
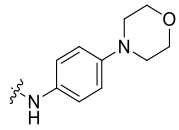
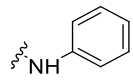
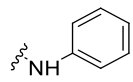
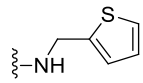
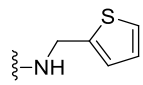
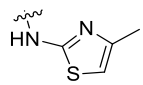
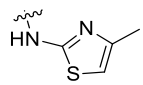
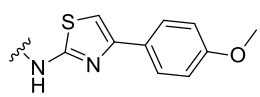
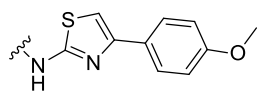
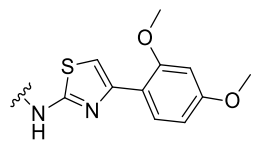
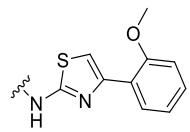
Table 4. DX2-Inhibitory Activity of the Sulfonamide Derivatives 19a,b, 20a−d, and 21a−f Using a Nanoluciferase Assay



No.	R^1	R^2	% inhibition of DX2-luciferase (at 40 μM)[a]
19a	2-Me		59.23 ± 8.02
19b	3-Me		40.78 ± 5.82
20a	4-Me		-1.85 ± 20.00
21a	4-Br		1.08 ± 10.46
20b	4-Me		-17.70 ± 16.6
21b	4-Br		42.99 ± 22.28
20c	4-Me		-15.84 ± 6.29
21c	4-Br		25.61 ± 22.32
20d (BC-DXI-843)	4-Me		72.56 ± 6.91
21d	4-Br		45.43 ± 9.78
21e	4-Br		-4.70 ± 10.8
21f	4-Br		11.38 ± 11.84

[a]The change of the nanoluciferase-tagged DX2 level upon treating chemicals was determined by detecting the luminescence signal.

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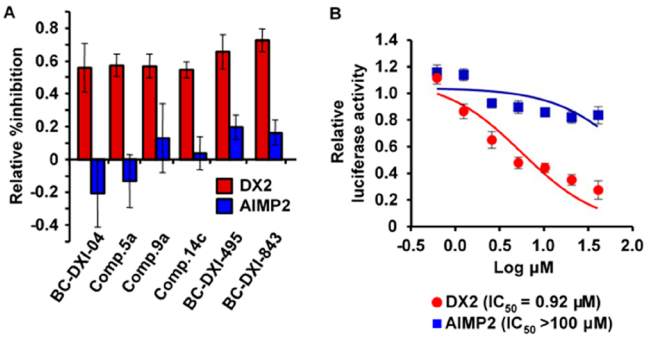

Figure 3. BC-DXI-843 is a specific inhibitor of DX2. (A) DX2 and AIMP2 levels were measured using nanoluciferase-DX2 and AIMP2, respectively, after treatment with each of the six compounds (40 μM, 4 h). The experiment was independently repeated three times with error bars denoting SD. The presented value was the inhibited luciferase activity upon treatment with each chemicals compared to DMSO as a control. (B) A549 cells expressing nanoluciferase-DX2 or AIMP2 were treated with **BC-DXI-843** in a dose-dependent manner for 4 h, and the resulting luminescence was measured. IC_{50} values for the inhibition of DX2 and AIMP2 were calculated as shown. The experiment was independently repeated three times with error bars denoting SD.

Table 5. Comparison of the Inhibition Efficacy on DX2 and AIMP2 Protein Levels and Cell Proliferation with BC-DXI-04, -495, and -843[a]

	IC_{50} (μM)[b]		EC_{50} (μM)[b]	
compd	DX2	AIMP2	A549	WI-26
BC-DXI-04	40.1	>100	40.8	>100
BC-DXI-495	4.20	>100	14.2	>100
BC-DXI-843	0.92	>100	1.20	>100

[a]IC_{50} and EC_{50} indicate 50% inhibition concentration for protein expression and cell viability, respectively. [b]IC_{50} and EC_{50} were determined by DX2- or AIMP2-nanoluciferase and cell viability assay, respectively

determined whether **BC-DXI-843**-mediated inhibition is specific to DX2 and HSP70 binding using a nanoluciferase-based complementation assay. Luminescence generated via DX2 and HSP70 binding was detected in the absence of **BC-DXI-843**, but the signal decreased in a dose-dependent manner in the presence of **BC-DXI-843** (Figure S2B, red). However, a dose-dependent decline was not observed in another binding pair, PRKACA and PRKAR2A (Figure S2B, gray), suggesting that **BC-DXI-843** specifically inhibits DX2 and HSP70 binding. HSP70 is reported to block Siah1 from interacting with DX2, resulting in the inhibition of Siah1-mediated ubiquitination.[15,16] Therefore, we evaluated whether **BC-DXI-843** could affect the relationship between HSP70, Siah1, and DX2. Immunoprecipitation of DX2 in the presence of **BC-DXI-843** revealed diminished and enhanced binding of HSP70 and Siah1 to DX2, respectively (Figure S2C). These results demonstrate that **BC-DXI-843** specifically inhibits HSP70 and DX2 binding, which causes ubiquitin-dependent degradation of DX2 by Siah1.

BC-DXI-843 Inhibits DX2-Dependent Cancer Cell Proliferation. Since DX2 is a significant oncogenic protein,[11,12,17] we examined whether **BC-DXI-843**-mediated degradation of DX2 could inhibit cancer cell progression. A549 lung cancer cells and WI-26 normal cell lines were treated with **BC-DXI-843** in a dose-dependent manner, and EC_{50} values for cell viability were determined. The EC_{50} in A549 cells was 1.20 μM (Figure 5A, red), which was similar to the IC_{50} for inhibition of DX2 (Figure 3B). However, no inhibition of WI-26 cells was observed (Figure 5A, blue), suggesting that **BC-DXI-843** specifically reduces the viability of cancer cells. Since DX2 levels in A549 cells were higher than in WI-26 cells, the above results suggest a relationship between **BC-DXI-843** and DX2 levels. Next, we demonstrated that the mechanism of action for **BC-DXI-843** is dependent on DX2 by using a DX2-inducible cell line. The DX2-induced cell line showed lower cell viability than control cells after treatment with **BC-DXI-843** (Figure 5B), demonstrating that the level of DX2 is critical to the activity of **BC-DXI-843**. We further characterized the effect of **BC-DXI-843** on cell viability using a 3D cell culture system. Spheroids formed with H460 cells decreased in size after treatment with **BC-DXI-843** in a dose-dependent manner, although the functional concentration was higher than in the 2D cell culture condition (Figure 5C).[25] To further test the *in vivo* efficacy of **BC-DXI-843**, we xenografted H460 cells into the backs of mice, and **BC-DXI-843** (50 mg/kg) was intraperitoneally injected every other day for 15 days. The embedded tumor volume gradually declined (Figure 6A,B) after **BC-DXI-843** administration, but no changes in body weight were observed (Figure 6D). The weight of the excised tumors after sacrifice had decreased in mice treated with **BC-DXI-843** (Figure 6C). These data suggest that **BC-DXI-843** decreases DX2-dependent cancer cell viability.

CONCLUSIONS

DX2, a splicing variant of AIMP2, is highly expressed in various cancers, including histologically small cell lung cancer. Therefore, inhibiting DX2 with small molecules is a promising therapeutic treatment for cancers expressing DX2. In this study, a series of arylsulfonamides was synthesized and evaluated as potent PPI inhibitors that specifically interrupt the interaction between DX2 and HSP70. Structure−activity relationship studies revealed that the L-configuration at the chiral center and the arylsulfonamide moiety are essential for anticancer activity. The replacement of L-leucine by L-tryptophan and the incorporation of a substituted arylamine group are critical in improving antiproliferation efficacy in cancer cells.

Thus, we identified a series of potent anticancer compounds. The most potent compound, **BC-DXI-843**, exhibited an IC_{50} of 0.92 μM on DX2 with more than 100-fold selectivity over AIMP2. **BC-DXI-843** also showed an EC_{50} value of 1.20 μM on cancer cell viability but had no effect on normal cells. A docking study revealed that **BC-DXI-843** exhibits favorable binding characteristics with key residues in a hydrophobic pocket of AIMP2-DX2, which may interfere with the interaction between DX2 and HSP70.

The biological activity and mechanism of action of **BC-DXI-843** were analyzed. **BC-DXI-843** specifically decreased endogenous DX2 protein levels by inducing DX2 degradation via Siah1, an E3 ligase specific to DX2. In comparison with **BC-DXI-04** and **BC-DXI-495**, small molecules previously reported to decrease DX2 levels, **BC-DXI-843** showed approximately 40- and 5-fold improvements in potency, respectively. **BC-DXI-843** specifically interrupts the interaction between DX2 and HSP70 according to several binding assays. This proves that the binding of the two proteins is significant to DX2 stabilization. Furthermore, **BC-DXI-843** significantly suppressed the progression of cancer cells *in vitro* and *in vivo* in a DX2-dependent manner, which emphasizes the tumor-promoting function of DX2. The proliferation of normal lung cells and the bodyweight of mice were not affected after treatment with **BC-DXI-843**,

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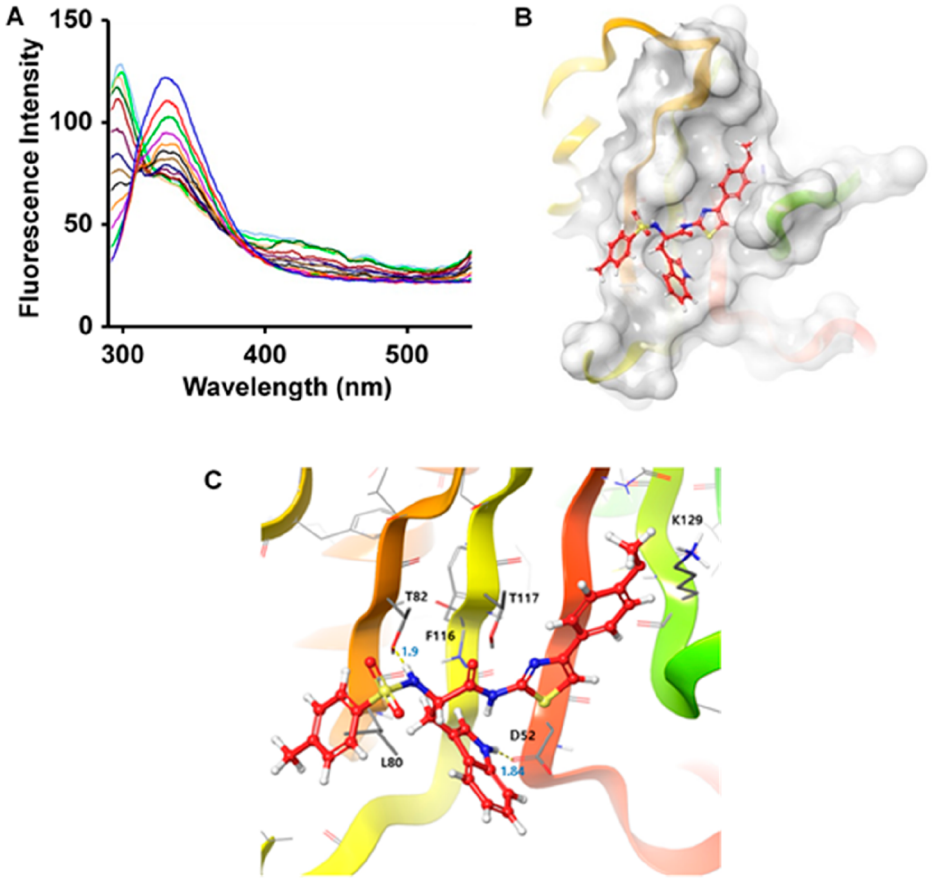

Figure 4. Determining the binding mode of **BC-DXI-843** to DX2. (A) Fluorescence-based binding titration measurement of **BC-DXI-843** to DX2. Protein samples were excited at 280 nm, and the decrease in fluorescence emission upon binding was measured at 335 nm. (B) Docking pose with the binding site of our in-house homology model of DX2 and **BC-DXI-843** (red). (C) Close-up view of **BC-DXI-843** with the relevant interaction residues (D52, T82) and distances between them and **BC-DXI-843**'s closest atoms. **BC-DXI-843** was also found to be docked close to the residues in the hydrophobic pocket. The structure of DX2-GST was obtained from PDB code 5A34.

implying that there are no significant side effects associated with **BC-DXI-843**. Thus, this study identified **BC-DXI-843** as a potential lead for the development of novel therapeutics that could target DX2 and combat lung cancer, especially SCLC.

EXPERIMENTAL SECTION

Chemistry. General Procedures. All commercial chemicals were reagent-grade and were used without further purification. Solvents were dried with standard procedures. All reactions were carried out under an atmosphere of dried argon in flame-dried glassware. The proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Varian 400 MHz spectrometer (Varian Medical Systems, Inc., Palo Alto, CA, USA). ^{13}C NMR spectra were recorded on a Varian 100 MHz spectrometer. The chemical shifts are provided in parts per million (ppm) downfield with coupling constants in hertz (Hz). The mass spectra were recorded using high-resolution mass spectrometry (HRMS) (electron ionization MS) on a JMS-700 mass spectrometer (Jeol, Japan) or by HRMS (electrospray ionization MS) on a G2 QTOF mass spectrometer. The products from all reactions were purified by flash column chromatography using silica gel 60 (230−400 mesh Kieselgel 60). Additionally, thin-layer chromatography on 0.25 mm silica plates (E. Merck; silica gel 60 F254) was used to monitor reactions. Final product purity was determined by reversed phase high-pressure liquid chromatography (RP-HPLC) using a Waters Corp. HPLC system equipped with an ultraviolet (UV) detector set at 254 nm. The mobile phases used were (A) H_2O containing 0.05% TFA and (B) CH_3CN. HPLC employed a YMC Hydrosphere C18 (HS-302) column (5 μm particle size, 12 nm pore size) that was 4.6 mm in diameter ×150 mm in size with a flow rate of 1.0 mL/min. The compound purity was assessed using either a gradient of 25% B to 100% B in 30 min (method A) or a gradient of 5% B to 100% B in 30 min (method B). The purity of all biologically evaluated compounds was ≥95% in method A or in method B. Melting points were measured on a Fisherbrand digital melting point apparatus.

General Procedure for the Preparation of Sulfonamide. Arylsulfonyl chloride (1 equiv) was added to a stirred solution of amino acid (1 equiv) in 1 M aqueous sodium hydroxide solution (20 volumes), and the mixture was stirred at rt for 8 h. The reaction mass was extracted with *n*-hexane, and the aqueous layer was acidified with 1 N HCl up to pH 4 at 0 °C. The resulting white precipitate was filtered, washed with cold water, and collected.

(*S*)-4-Methyl-2-(4-methylphenylsulfonamido)pentanoic Acid (4a). The title compound was prepared from **3a** and *p*-toluenesulfonyl chloride according to the general procedure as a colorless solid (1.12 g, 52%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (brs, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.64 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 3.64−3.40 (m, 1H), 2.36 (s, 3H), 1.55−1.45 (m, 1H), 1.40−1.25 (m, 2H), 0.79 (d, J = 6.4 Hz, 3H), 0.68 (d, J = 6.4 Hz, 3H). HRMS (ESI): $[M + H]^+$ $C_{13}H_{20}NO_4S$ calcd 286.1113, found 286.1113.

(*R*)-4-Methyl-2-(4-methylphenylsulfonamido)pentanoic Acid (4b). The title compound was prepared from **3b** and *p*-toluenesulfonyl chloride according to the general procedure as a colorless solid (1.26 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (brs, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.64 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 3.64−3.40 (m, 1H), 2.36 (s, 3H), 1.55−1.45 (m, 1H), 1.40−1.25 (m, 2H), 0.79 (d, J = 6.4 Hz, 3H), 0.68 (d, J = 6.4 Hz, 3H). HRMS (ESI): $[M + H]^+$ $C_{13}H_{20}NO_4S$ calcd 286.1113, found 286.1107.

(*S*)-4-Methyl-2-(phenylsulfonamido)pentanoic Acid (13a). The title compound was prepared from **3a** and benzenesulfonyl

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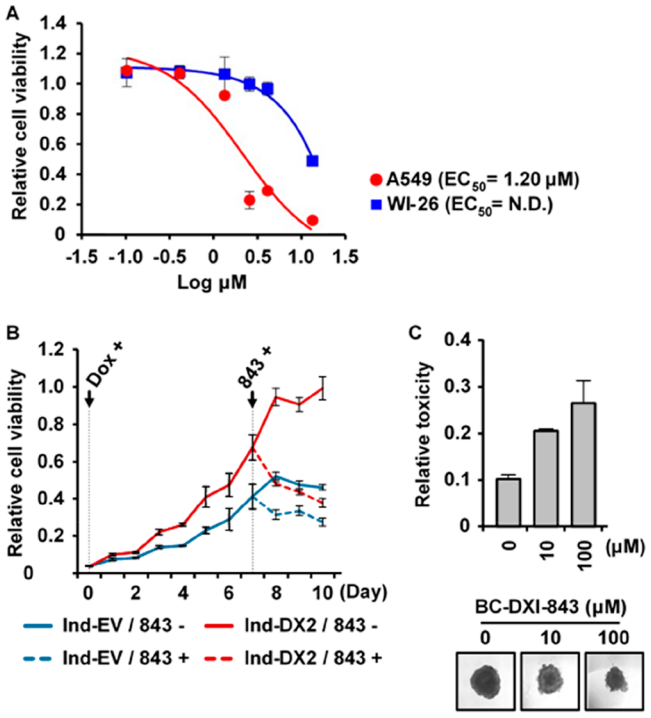

Figure 5. BC-DXI-843 suppresses cancer cell proliferation in a DX2-dependent manner. (A) **BC-DXI-843**-specific inhibition of cancer cell proliferation. A549 cancer cells and WI-26 normal cells were treated with **BC-DXI-843** as indicated for 72 h, and cell viability was determined with an MTT assay. The EC_{50}, 50% inhibiting concentration of cell viability, is shown on the right. N.D. = not determined. (B) Suppression of DX2-mediated cancer cell proliferation by **BC-DXI-843**. Empty vector (EV)- or DX2-inducible A549 cells (Ind-EV and Ind-DX2, respectively) were treated with doxycycline (Dox) and **BC-DXI-843** (**843**) as indicated, and cell viability was determined in the same manner as described above. (C) Inhibitory effect of **BC-DXI-843** on cancer cell spheroids formed in a 3D culture system. Several doses of **BC-DXI-843** were added to H460 spheroids over the course of 72 h. Cell toxicity was determined by a CellTiter-Glo 3D assay, and representative photos of the spheroids are shown. (A–C) All experiments were independently repeated three times with error bars denoting SD.

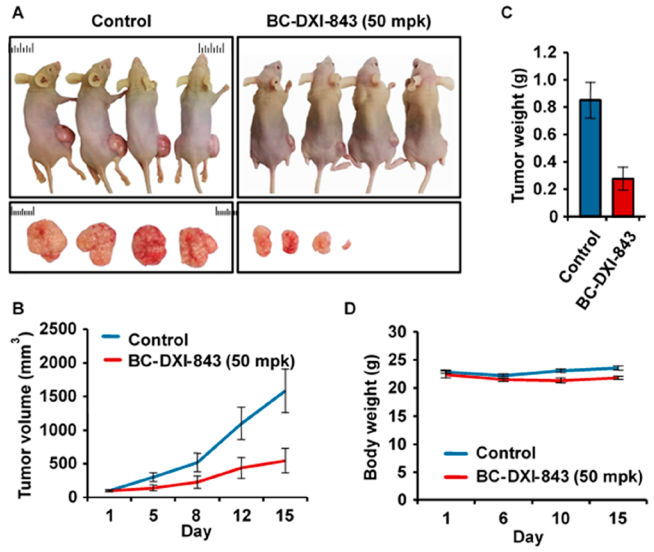

Figure 6. *In vivo* antitumor effect of **BC-DXI-843**. (A–D) Mice ($n = 4$) subcutaneously injected with H460 cells were intraperitoneally administered **BC-DXI-843** (50 mg/kg) every other day for 15 days. The sizes of the embedded tumors (B) and body weights (D) were measured throughout the experiment. After sacrifice, the weights of the harvested tumors were determined (C) and photos of whole-body bearing the tumor and the excised tumors were taken (A).

chloride according to the general procedure as a colorless solid (0.68 g, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 8.0 Hz, 2H), 7.58 (t, J = 7.2 Hz, 1H), 7.50 (t, J = 7.6 Hz, 2H), 5.10 (d, J = 1.2 Hz, 1H), 3.98–3.92 (m, 1H), 1.76–1.74 (m, 1H), 1.54–1.51 (m, 2H), 0.90 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 6.8 Hz, 3H). HRMS (ESI): $[M + H]^+$ $C_{12}H_{18}NO_4S$ calcd 272.0957, found 272.0944.

(*S*)-2-(4-Fluorophenylsulfonamido)-4-methylpentanoic Acid (13b). The title compound was prepared from **3a** and 4-fluorobenzenesulfonyl chloride according to the general procedure as an off white solid (0.95 g, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89–7.82 (m, 2H), 7.16–7.09 (m, 2H), 5.05 (d, J = 1.2 Hz, 1H), 3.98–3.92 (m, 1H), 1.82–1.75 (m, 1H), 1.59–1.47 (m, 2H), 0.92 (d, J = 5.2 Hz, 3H), 0.87 (d, J = 5.2 Hz, 3H). HRMS (ESI): $[M + H]^+$ $C_{12}H_{17}FNO_4S$ calcd 290.0862, found 290.0851.

(*S*)-2-(4-Bromophenylsulfonamido)-4-methylpentanoic Acid (13c). The title compound was prepared from **3a** and 4-bromobenzenesulfonyl chloride according to the general procedure as a light yellow solid (1.57 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 8.8 Hz, 2H), 5.10 (d, J = 1.2 Hz, 1H), 4.01–3.95 (m, 1H), 1.83–1.76 (m, 1H), 1.61–1.53 (m, 2H), 0.91 (d, J = 6.8 Hz, 3H), 0.88 (d, J = 6.4 Hz, 3H). HRMS (ESI): $[M + H]^+$ $C_{12}H_{17}BrNO_4S$ calcd 350.0062, found 350.0055.

(*S*)-2-(4-Methoxyphenylsulfonamido)-4-methylpentanoic Acid (13d). The title compound was prepared from **3a** and 4-methoxybenzenesulfonyl chloride according to the general procedure as a colorless solid (1.15 g, 48%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (brs, 1H), 7.97 (d, J = 5.6 Hz, 1H), 7.74 (d, J = 8.0 Hz, 2H), 6.96 (d, J = 8.0 Hz, 2H), 3.82 (s, 3H), 3.63–3.59 (m, 1H), 1.58–1.53 (m, 1H), 1.39–1.34 (m, 2H), 0.80 (d, J = 6.4 Hz, 3H), 0.69 (d, J = 6.4 Hz, 3H). HRMS (ESI): $[M + H]^+$ $C_{13}H_{20}NO_5S$ calcd 302.1062, found 302.1055.

(*S*)-2-(Biphenyl-4-ylsulfonamido)-4-methylpentanoic Acid (13e). The title compound was prepared from **3a** and 4-phenylbenzenesulfonyl chloride according to the general procedure as a pink solid (0.37 g, 14%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 8.2 Hz, 2H), 7.71–7.65 (m, 2H), 7.63–7.57 (m, 2H), 7.53–7.40 (m, 3H), 4.97 (d, J = 9.3 Hz, 1H), 4.02–3.96 (m, 1H), 1.85–1.73 (m, 1H), 1.56–1.40 (m, 2H), 0.89 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H). HRMS (ESI): $[M + H]^+$ $C_{18}H_{22}NO_4S$ calcd 348.1270, found 348.1270.

(*S*)-4-Methyl-2-(4-nitrophenylsulfonamido)pentanoic Acid (13f). The title compound was prepared from **3a** and 4-nitrobenzenesulfonyl chloride according to the general procedure as a light yellow solid (0.43 g, 18%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.67 (brs, 1H), 8.5 (s, 1H), 8.40 (d, J = 8.0 Hz, 2H), 8.01 (d, J = 8.0 Hz, 2H), 3.78–3.34 (m, 1H), 1.61–1.58 (m, 1H), 1.45–1.41 (m, 2H), 0.84 (d, J = 6.4 Hz, 3H), 0.75 (d, J = 6.4 Hz, 3H). HRMS (ESI): $[M + H]^+$ $C_{12}H_{17}N_2O_6S$ calcd 317.0807, found 317.0808.

2-(4-Methylphenylsulfonamido)acetic Acid (16a). The title compound was prepared from glycine (**15a**) and *p*-toluenesulfonyl chloride according to the general procedure as a colorless solid (1.03 g, 34%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (brs, 1H), 7.94 (t, J = 6.0 Hz, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 3.54 (d, J = 6.0 Hz, 2H), 2.35 (s, 3H). HRMS (ESI): $[M + H]^+$ $C_9H_{12}NO_4S$ calcd 230.0487, found 230.0486.

(*S*)-2-(4-Methylphenylsulfonamido)propanoic Acid (16b). The title compound was prepared from L-alanine (**15b**) and *p*-toluenesulfonyl chloride according to the general procedure as a colorless solid (1.50 g, 55%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.62 (brs, 1H), 8.04 (d, J = 8.4 Hz, 1H) (s, 1H), 7.66 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 3.76–3.68 (m, 1H), 2.37 (s, 3H), 1.12 (d, J = 7.2 Hz, 3H). HRMS (ESI): $[M + H]^+$ $C_{10}H_{14}NO_4S$ calcd 244.0644, found 244.0638.

(*S*)-3-Hydroxy-2-(4-methylphenylsulfonamido)propanoic Acid (16c). The title compound was prepared from L-serine (**15c**) and

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p-toluenesulfonyl chloride according to the general procedure as a colorless solid (0.57 g, 23%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60 (s, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 4.95 (brs, 1H), 3.73−3.68 (m, 1H), 3.50−3.40 (m, 2H), 2.37 (s, 3H). HRMS (ESI): $[M + H]^+$ $C_{10}H_{14}NO_5S$ calcd 260.0593, found 260.0585.

(*S*)-3-Methyl-2-(4-methylphenylsulfonamido)butanoic Acid (16d). The title compound was prepared from L-valine (**15d**) and *p*-toluenesulfonyl chloride according to the general procedure as a colorless solid (0.58 g, 25%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.55 (s, 1H), 7.91 (d, J = 9.6 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 3.50−3.44 (m, 1H), 2.34 (s, 3H), 1.92−1.85 (m, 1H), 0.81 (d, J = 6.8 Hz, 3H), 077 (d, J = 6.8 Hz, 3H). HRMS (ESI): $[M + H]^+$ $C_{12}H_{18}NO_4S$ calcd 272.0957, found 272.0950.

(2*S*,3*S*)-3-Hydroxy-2-(4-methylphenylsulfonamido)butanoic Acid (16e). The title compound was prepared from L-(−)-threonine (**15e**) and *p*-toluenesulfonyl chloride according to the general procedure as a colorless solid (0.94 g, 41%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 (d, J = 8.0 Hz, 2H), 7.51 (d, J = 9.2 Hz, 1H), 7.33 (d, J = 8.0 Hz, 2H), 3.96−3.93 (m, 1H), 3.63−3.61 (m, 1H), 2.28 (s, 3H), 1.00 (d, J = 6.4 Hz, 3H). HRMS (ESI); $[M + H]^+$ $C_{11}H_{16}NO_5S$ calcd 274.0749, found 274.0749.

(*S*)-2-(4-Methylphenylsulfonamido)-4-(methylthio)butanoic Acid (16f). The title compound was prepared from L-methionine (**15f**) and *p*-toluenesulfonyl chloride according to the general procedure as a colorless solid (0.26 g, 13%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 3.81−3.79 (m, 1H), 2.44−2.24 (m, 5H), 1.91 (s, 3H), 1.86−1.64 (m, 2H). HRMS (ESI): $[M + H]^+$ $C_{12}H_{18}NO_4S_2$ calcd 304.0677, found 304.0677.

(*S*)-4-(Benzyloxy)-2-(4-methylphenylsulfonamido)-4-oxobutanoic Acid (16g). The title compound was prepared from 5-benzyl L-aspartate (**15g**) and *p*-toluenesulfonyl chloride according to the general procedure as a colorless solid (1.10 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 8.4 Hz, 2H), 7.39−7.25 (m, 7H), 5.64 (d, J = 8.0 Hz, 1H), 5.04 (s, 2H), 4.21−4.17 (m, 1H), 3.04 (dd, J = 17.4, 4.2 Hz, 1H), 2.87 (dd, J = 17.6, 5.2 Hz, 1H), 2.39 (s, 3H). HRMS (ESI): $[M + H]^+$ $C_{18}H_{20}NO_6S$ calcd 378.1011, found378.0995.

(*S*)-5-(Benzyloxy)-2-(4-methylphenylsulfonamido)-5-oxopentanoic Acid (16h). The title compound was prepared from 5-benzyl L-glutamate (**15h**) and *p*-toluenesulfonyl chloride according to the general procedure as a colorless solid (1.15 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 8.4 Hz, 2H), 7.36−7.30 (m, 5H), 7.22 (d, J = 8.4 Hz, 2H), 5.67 (d, J = 9.2 Hz, 1H), 5.07 (s, 2H), 4.01−3.96 (m, 1H), 2.51−2.38 (m, 2H), 2.34 (s, 3H), 2.17−2.12 (m, 1H), 1.93−1.88 (m, 1H). HRMS (ESI): $[M + H]^+$ $C_{19}H_{22}NO_6S$ calcd 392.1168, found 392.1168.

(*S*)-2-(4-Methylphenylsulfonamido)-3-phenylpropanoic Acid (16i). The title compound was prepared from L-phenylalanine (**15i**) and *p*-toluenesulfonyl chloride according to the general procedure as a colorless solid (0.60 g, 31%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (s, 1H), 8.18 (d, J = 9.2 Hz, 1H), 7.44 (d, J = 8.4 Hz, 2H), 7.21−7.16 (m, 5H), 7.10−7.08 (m, 2H), 3.85−3.76 (m, 1H), 2.94−2.89 (m, 1H), 2.72−2.67 (m, 1H), 2.34 (s, 3H). HRMS (ESI): $[M + H]^+$ $C_{16}H_{18}NO_4S$ calcd 320.0957, found 320.0949.

(*S*)-2-(4-Methylphenylsulfonamido)-3-(4-(tosyloxy)phenyl)propanoic Acid (16j). The title compound was prepared from L-tyrosine (**15j**) and *p*-toluenesulfonyl chloride according to the general procedure as a colorless solid (0.81 g, 30%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, J = 9.2 Hz, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.48−7.41 (m, 4H), 7.21 (d, J = 8.0 Hz, 2H), 7.09 (d, J = 8.8 Hz, 1H), 6.81 (d, J = 8.8 Hz, 2H), 3.83−3.77 (m, 1H), 3.07−3.03 (m, 1H), 2.69−2.63 (m, 1H), 2.33 (s, 3H), 2.28 (s, 3H). HRMS (ESI): $[M + H]^+$ $C_{23}H_{24}NO_7S_2$ calcd 490.0994, found 490.0992.

(*S*)-3-(1*H*-Indol-3-yl)-2-(4-methylphenylsulfonamido)-propanoic Acid (16k). See ref 16.

(*S*)-3-(1*H*-Indol-3-yl)-2-(2-methylphenylsulfonamido)-propanoic Acid (18a). The title compound was prepared from L-tryptophan (**15k**) and *o*-toluenesulfonyl chloride according to the general procedure as a light brown solid (0.70 g, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.61 (brs, 1 H), 10.78 (s, 1H), 8.26 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 7.6 Hz, 2H), 7.35 (t, J = 8.0 Hz, 1H), 7.29 (t, J = 8.0 Hz, 1H), 7.29 (d, J = 8.0 Hz, 2H), 7.03 (d, J = 8.0 Hz, 2H), 6.89 (t, J = 7.6 Hz, 1H), 3.83 (q, J = 7.6 Hz, 1H), 3.05 (dd, J = 14.5, 6.7 Hz, 1H), 2.89 (dd, J = 14.5, 7.8 Hz, 1H), 2.43 (s, 3H). HRMS (ESI): $[M + H]^+$ $C_{18}H_{19}N_2O_4S$ calcd 359.1066, found 359.1066.

(*S*)-3-(1*H*-Indol-3-yl)-2-(3-methylphenylsulfonamido)-propanoic Acid (18b). The title compound was prepared from L-tryptophan (**15k**) and *m*-toluenesulfonyl chloride according to the general procedure as a light brown solid (0.750 g, 43%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.61 (brs, 1 H), 10.79 (s, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.39 (s, 2H), 7.30−7.25 (m, 4H), 7.05−7.01 (m, 2H), 6.92 (t, J = 8.0 Hz, 1H), 3.90 (q, J = 7.6 Hz, 1H), 3.03 (dd, J = 14.5, 6.7 Hz, 1H), 2.82 (dd, J = 14.5, 7.8 Hz, 1H), 2.26 (s, 3H). HRMS (ESI): $[M + H]^+$ $C_{18}H_{19}N_2O_4S$ calcd 359.1066, found 359.1066.

(*S*)-2-(4-Bromophenylsulfonamido)-3-(1*H*-indol-3-yl)-propanoic Acid (18c). The title compound was prepared from L-tryptophan (**15k**) and 4-bromobenzenesulfonyl chloride according to the general procedure as a colorless solid (0.82 g, 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.61 (brs, 1 H), 10.78 (s, 1H), 8.38 (d, J = 8.4 Hz, 1H), 7.51 (d, J = 8.0 Hz, 2H), 7.40 (t, J = 8.0 Hz, 2H), 7.31 (d, J = 7.2 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.07 (s, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.92 (t, J = 7.6 Hz, 1H), 3.92 (q, J = 7.6 Hz, 1H), 3.05 (dd, J = 14.5, 6.7 Hz, 1H), 2.89 (dd, J = 14.5, 7.8 Hz, 1H). HRMS (ESI): $[M + H]^+$ $C_{17}H_{16}BrN_2O_4S$ calcd 423.0014, found 424.0009.

(*S*)-Benzyl 4-Methyl-2-(4-methylbenzamido)pentanoate (11). The title compound was prepared from L-leucine benzylester (**10**) and *p*-toluenebenzoic acid following general procedure for amide coupling reaction as a colorless solid (0.76 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 8.0 Hz, 2H), 7.39−7.32 (m, 5H), 7.22 (d, J = 8.4 Hz, 2H), 6.51 (d, J = 7.6 Hz, 1H), 5.22−5.16(m, 2H), 4.93−4.87 (m, 1H), 2.39 (s, 3H), 1.80−1.62 (m, 3H), 0.97 (d, J = 6.0 Hz, 3H), 0.95 (d, J = 6.0 Hz, 3H). HRMS (ESI): $[M + H]^+$ $C_{21}H_{26}NO_3S$ calcd 340.1913, found 340.1913.

General Procedure for Amide Coupling. EDCI·HCl (1.1 equiv) and HOBt (1.1 equiv) were added to a stirred solution of carboxylic acid (1 equiv), amine (1 equiv), and TEA (2.5 equiv) in DCM at room temperature, and the reaction mixture was stirred at the same temperature for 18 h. The solvent was evaporated, and the residue was treated with saturated sodium bicarbonate and extracted with DCM. The combined organic layer was then washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified through flash column chromatography (hexane/ethyl acetate).

(*S*)-4-Methyl-*N*-(4-methyl-1-oxo-1-(1,4,7-trioxa-10-azacyclododecan-10-yl)pentan-2-yl)benzenesulfonamide (1a). The title compound was prepared from **4a** and 1,4,7-trioxa-10-azacyclododecane according to the general procedure as a colorless solid (0.14 g, 31%). Mp: 78−80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 8.0 Hz, 2H), 5.59 (d, J = 10.0 Hz, 1H), 4.25−4.19 (m, 1H), 3.78−3.71 (m, 2H), 3.69−3.37 (m, 11H), 3.22−3.12 (m, 3H), 2.40 (s, 3H), 1.98−1.91 (m, 1H), 1.49−1.33 (m, 2H), 0.91 (d, J = 4.4 Hz, 3H) 0.89 (d, J = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.37, 143.28, 137.20, 129.43, 127.30, 71.44, 70.49, 69.80, 69.68, 69.22, 68.93, 51.64, 49.99, 48.96, 42.69, 24.22, 23.52, 21.51, 21.05, 20.93. HRMS (ESI): $[M + H]^+$ $C_{21}H_{35}N_2O_6S$ calcd 443.2216, found 443.2216; purity ≥99.29% (as determined by RP-HPLC, method A, t_R = 13.35 min).

(*R*)-4-Methyl-*N*-(4-methyl-1-oxo-1-(1,4,7-trioxa-10-azacyclododecan-10-yl)pentan-2-yl)benzenesulfonamide (1b). The title compound was prepared from **4b** and 1,4,7-trioxa-10-azacyclododecane according to the general procedure as a colorless solid (0.09 g, 21%). Mp: 69−71 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 7.6 Hz, 2H), 7.27 (d, J = 7.6 Hz, 2H), 5.59 (d, J = 9.6 Hz, 1H), 4.25−4.19 (m, 1H), 3.78−3.7 (m, 2H), 3.61−3.39 (m, 11H), 3.23−3.12 (m, 3H), 2.40 (s, 3H), 1.97−1.90 (m, 1H), 1.49−1.33 (m, 2H), 0.92 (d, J = 4.2 Hz, 3H), 0.89 (d, J = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.33, 143.25, 137.19, 129.39, 127.26, 71.41, 70.46, 69.77, 69.66, 69.18, 68.89, 51.60, 49.96, 48.93, 42.64, 24.18, 23.49, 21.49, 20.90. HRMS (ESI): $[M + H]^+$ $C_{21}H_{35}N_2O_6S$ calcd 443.2216, found

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443.2208; purity ≥98.18% (as determined by RP-HPLC, method A, t_R = 13.33 min).

(*S*)-4-Methyl-*N*-(4-methyl-1-morpholino-1-oxopentan-2-yl)-benzenesulfonamide (5a). The title compound was prepared from **4a** and morpholine according to the general procedure as a colorless solid (0.09 g, 74%). Mp: 162−164 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 7.6 Hz, 2H), 7.29 (d, J = 7.6 Hz, 2H), 5.60 (d, J = 9.6 Hz, 1H), 4.04−4.01 (m, 1H), 3.61−3.51 (m, 2H), 3.41−3.36 (m, 1H), 3.31−3.14 (m, 5H), 2.42 (s, 3H), 2.00−1.93 (m, 1H), 1.50−1.43 (m, 1H), 1.17−1.11 (m, 1H), 0.95 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.91, 143.56, 136.56, 129.46, 127.53, 66.36, 66.04, 51.04, 45.41, 42.55, 42.25, 24.07, 23.27, 21.50, 21.11. HRMS (ESI): $[M + H]^+$ $C_{17}H_{27}N_2O_4S$ calcd 355.1692, found 355.1692; purity ≥98.57% (as determined by RP-HPLC, method A, t_R = 12.34 min).

(*R*)-4-Methyl-*N*-(4-methyl-1-morpholino-1-oxopentan-2-yl)-benzenesulfonamide (5b). The title compound was prepared from **4b** and morpholine according to the general procedure as a colorless solid (0.10 g, 91%). Mp: 163−165 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 7.6 Hz, 2H), 7.29 (d, J = 7.6 Hz, 2H), 5.61 (d, J = 9.6 Hz, 1H), 4.06−4.01 (m, 1H), 3.60−3.50 (m, 2H), 3.41−3.36 (m, 1H), 3.31−3.14 (m, 5H), 2.42 (s, 3H), 2.00−1.93 (m, 1H), 1.50−1.43 (m, 1H), 1.17−1.11 (m, 1H), 0.95 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.90, 143.55, 136.57, 129.45, 127.52, 66.39, 66.04, 51.02, 45.41, 42.53, 42.24, 24.06, 23.26, 21.50, 21.11. HRMS (ESI): $[M + H]^+$ $C_{17}H_{27}N_2O_4S$ calcd 355.1692, found 355.1692; purity ≥97.81% (as determined by RP-HPLC, method A, t_R = 12.35 min).

(*S*)-4-Methyl-2-(4-methylphenylsulfonamido)-N-(4-morpholinophenyl)pentanamide (9a). The title compound was prepared from **4a** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.04 g, 25%). Mp: 176−178 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 8.0 Hz, 2H), 7.69 (s, 1H), 7.28−7.16 (m, 4H), 6.83 (d, J = 8.8 Hz, 2H), 5.03 (d, J = 7.6 Hz, 1H), 3.85 (t, J = 4.8 Hz, 4H), 3.78−3.73 (m, 1H), 3.08 (t, J = 4.8 Hz, 4H), 2.33 (s, 3H), 1.70−1.60 (m, 2H), 1.52−1.46 (m, 1H), 0.86 (d, J = 6.0 Hz, 3H), 0.70 (d, J = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.30, 148.49, 144.06, 136.20, 129.80, 129.75, 127.32, 121.52, 116.03, 66.84, 56.16, 49.64, 42.08, 24.39, 22.89, 21.51, 21.50, 21.31. HRMS (ESI): $[M + H]^+$ $C_{23}H_{32}N_3O_4S$ calcd 446.2114, found 446.2115; purity ≥99.99% (as determined by RP-HPLC, method A, t_R = 11.48 min).

(*S*)-4-Methyl-2-(4-methylphenylsulfonamido)-*N*-(4-(pyrrolidin-1-yl)phenyl)pentanamide (9b). The title compound was prepared from **4a** and 4-(pyrrolidin-1-yl)aniline (**8b**) according to the general procedure as a colorless solid (0.04 g, 24%). Mp: 183−185 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 7.6 Hz, 2H), 7.42 (s, 1H), 7.27 (d, J = 8.0 Hz, 2H), 7.08 (d, J = 8.8 Hz, 2H), 6.45 (d, J = 8.8 Hz, 2H), 5.09 (d, J = 7.6 Hz, 1H), 3.78−3.73 (m, 1H), 3.24 (t, J = 6.4 Hz, 4H), 2.43 (s, 3H), 2.03−1.96 (m, 4H), 1.64−1.47 (m, 3H), 0.88 (d, J = 5.6 Hz, 3H), 0.75 (d, J = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.11, 145.65, 143.87, 136.43, 129.76, 127.32, 125.44, 122.34, 111.46, 56.14, 47.75, 42.32, 25.41, 24.38, 22.94, 21.51, 21.39. HRMS (ESI): $[M + H]^+$ $C_{23}H_{32}N_3O_3S$ calcd 430.2164, found 430.2164; purity ≥99.99% (as determined by RP-HPLC, method A, t_R = 11.83 min).

(*S*)-4-Methyl-2-(4-methylphenylsulfonamido)-*N*-(4-(piperidin-1-yl)phenyl)pentanamide (9c). The title compound was prepared from **4a** and 4-(piperidin-1-yl)aniline (**8c**) according to the general procedure as a colorless solid (0.06 g, 40%). Mp: 178−180 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 8.0 Hz, 2H), 7.59 (s, 1H), 7.25 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.8 Hz, 2H), 5.10 (d, J = 7.2 Hz, 1H), 3.77−3.74 (m, 1H), 3.09 (t, J = 5.6 Hz, 4H), 2.32 (s, 3H), 1.72−1.68 (m, 4H), 1.67−1.46 (m, 5H), 0.86 (d, J = 6.0 Hz, 3H), 0.73 (d, J = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.24, 149.57, 144.01, 136.30, 129.80, 128.91, 127.32, 121.48, 116.81, 56.19, 50.97, 42.18, 25.79, 24.40, 24.20, 22.93, 21.52, 21.50, 21.37. HRMS (ESI): $[M + H]^+$ $C_{24}H_{34}N_3O_3S$ calcd 444.2321, found 444.2321; purity ≥99.99% (as determined by RP-HPLC, method B, t_R = 18.20 min).

(*S*)-4-Methyl-2-(4-methylphenylsulfonamido)-*N*-(4-(4-methylpiperidin-1-yl)phenyl)pentanamide (9d). The title compound was prepared from **4a** and 4-(4-methylpiperidin-1-yl)aniline (**8d**) according to the general procedure as a colorless solid (0.11 g, 72%). Mp: 184−186 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 8.0 Hz, 2H), 7.51 (s, 1H), 7.27 (d, J = 8.0 Hz, 2H), 7.17 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 4.98 (d, J = 8.0 Hz, 1H), 3.77−3.72 (m, 1H), 3.59−3.56 (m, 2H), 2.65−2.58 (m, 2H), 2.35 (s, 3H), 1.73−1.70 (m, 2H), 1.62−1.56 (m, 2H), 1.50−1.45 (m, 2H), 1.37−1.25 (m, 2H), 0.97 (d, J = 6.4 Hz, 3H), 0.84 (d, J = 6.0 Hz, 3H), 0.71 (d, J = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.10, 149.23, 144.04, 136.22, 129.80, 128.83, 127.32, 121.44, 116.76, 56.15, 50.32, 42.18, 34.02, 30.61, 24.39, 22.91, 21.87, 21.51, 21.35. HRMS (ESI): $[M + H]^+$ $C_{25}H_{36}N_3O_3S$ calcd 458.2477, found 458.2477; purity ≥99.32% (as determined by RP-HPLC, method B, t_R = 19.59 min).

(*S*)-4-Methyl-2-(4-methylphenylsulfonamido)-*N*-(4-thiomorpholine-1,1-dioxophenyl)pentanamide (9e). The title compound was prepared from **4a** and 4-(4-aminophenyl)thiomorpholine 1,1-dioxide (**8e**) according to the general procedure as a colorless solid (0.12 g, 70%). Mp: 195−197 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.77 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 8.8 Hz, 2H), 7.28 (d, J = 8.0 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 4.97 (d, J = 6.8 Hz, 1H), 3.82−3.76 (m, 5H), 3.10 (t, J = 5.2 Hz, 4H), 2.37 (s, 3H), 1.67−1.64(m, 1H), 1.55−1.45 (m, 2H), 0.84 (d, J = 6.4 Hz, 3H), 0.65 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.51, 144.80, 144.19, 136.02, 130.78, 129.85, 127.36, 121.86, 117.00, 56.12, 50.53, 48.10, 41.93, 24.40, 22.89, 21.54, 21.21. HRMS (ESI): $[M + H]^+$ $C_{23}H_{32}N_3O_5S_2$ calcd 494.1783, found 494.1783; purity ≥99.99% (as determined by RP-HPLC, method A, t_R = 13.21 min).

(*S*)-4-Methyl-*N*-(4-methyl-1-(4-morpholinophenylamino)-1-oxopentan-2-yl)benzamide (12). Palladium charcoal was added to the solution of **11** in methanol, and the reaction solution was stirred at room temperature under H_2 pressure for 3 h. After deprotection of the benzyl group from the starting material indicated by TLC, the reaction mass was filtered through Celite and concentrated. The residue was used without further purification. The title compound was prepared from the above acid and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.06 g, 24% over 2 steps). Mp: 208−210 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H), 7.69 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 8.8 Hz, 2H), 6.84−6.80 (m, 3H) 4.90−4.86 (m, 1H), 3.84 (t, J = 4.8 Hz, 4H), 3.08 (t, J = 4.8 Hz, 4H), 2.39 (s, 3H), 1.90−1.88 (m, 1H), 1.86−1.71 (m, 2H), 0.97 (d, J = 6.4 Hz, 3H), 0.90 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.73, 167.78, 140.09, 142.19, 131.08, 130.95, 129.19, 127.23, 116.18, 66.87, 53.05, 49.79, 41.41, 24.98, 22.93, 22.27, 21.44. HRMS (ESI): $[M + H]^+$ $C_{24}H_{32}N_3O_3$ calcd 410.2444, found 410.2439; purity ≥99.74% (as determined by RP-HPLC, method A, t_R = 11.43 min).

(*S*)-4-Methyl-*N*-(4-morpholinophenyl)-2-(phenylsulfonamido)pentanamide (14a). The title compound was prepared from **13a** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.11 g, 69%). Mp: 217−219 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 7.6 Hz, 2H), 7.75 (s, 1H), 7.56 (t, J = 7.6 Hz, 1H), 7.49 (t, J = 7.6 Hz, 2H), 7.24 (d, J = 8.8 Hz, 2H), 6.83 (d, J = 8.8 Hz, 2H), 5.07 (d, J = 7.2 Hz, 1H), 3.85 (t, J = 4.8 Hz, 4H), 3.81−3.77 (m, 1H), 3.10 (t, J = 4.8 Hz, 4H), 1.66−1.46 (m, 3H), 0.85 (d, J = 6.0 Hz, 3H), 0.69 (d, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.09, 147.56, 138.16, 132.15, 128.58, 128.25, 126.26, 120.56, 11.05, 65.83, 55.11, 48.60, 41.12, 28.68, 23.39, 21.89, 20.26. HRMS (ESI): $[M + H]^+$ $C_{22}H_{30}N_3O_4S$ calcd 432.1957, found 432.1956; purity ≥98.65% (as determined by RP-HPLC, method A, t_R = 10.31 min).

(*S*)-2-(4-Fluorophenylsulfonamido)-4-methyl-*N*-(4-morpholinophenyl)pentanamide (14b). The title compound was prepared from **13b** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.05 g, 31%). Mp: 168−170 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91−7.87 (m, 2H), 7.65 (s, 1H), 7.22 (d, J = 8.8 Hz, 2H), 7.13 (t, J = 8.4 Hz, 2H), 6.82 (d J = 8.8 Hz, 2H), 5.26 (d, J = 8.0 Hz, 1H), 3.82 (t, J = 4.8 Hz, 4H), 3.80−3.77 (m, 1H), 3.10 (t, J = 4.8 Hz, 4H), 1.63−1.57 (m, 2H), 1.53−1.48 (m, 1H), 0.87 (d, J = 6.4 Hz, 3H), 0.74 (d, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.28, 166.50, 163.96, 148.64, 135.48, 135.45, 130.08,

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129.99, 129.43, 121.61, 116.52, 116.29, 116.06, 66.81, 56.20, 49.55, 42.12, 24.41, 22.87, 21.35. HRMS (ESI): $[M + H]^+$ $C_{22}H_{29}FN_3O_4S$ calcd 450.1863, found 450.1863; purity ≥99.15% (as determined by RP-HPLC, method A, t_R = 11.15 min).

(*S*)-2-(4-Bromophenylsulfonamido)-4-methyl-*N*-(4-morpholinophenyl)pentanamide (14c). The title compound was prepared from **13c** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.10 g, 70%). Mp: 185−187 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 8.4 Hz, 2H), 7.56 (s, 1H), 7.17 (d, J = 8.4 Hz, 2H), 6.82 (d, J = 8.4 Hz, 2H), 5.38 (d, J = 7.6 Hz, 1H), 3.86 (t, J = 4.6 Hz, 4H), 3.82−3.77 (m, 1H), 3.12 (t, J = 5.0 Hz, 4H), 1.67−1.50 (m, 3H), 0.88 (d, J = 6.4 Hz, 3H), 0.78 (d, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.18, 148.70, 138.50, 132.41, 129.25, 128.74, 128.12, 121.70, 116.07, 66.82, 56.26, 49.53, 42.12, 24.41, 22.87, 21.43. HRMS (ESI): $[M + H]^+$ $C_{22}H_{29}BrN_3O_4S$ calcd 510.1062, found 510.1064; purity ≥99.99% (as determined by RP-HPLC, method A, t_R = 12.75 min).

(*S*)-2-(4-Methoxyphenylsulfonamido)-4-methyl-*N*-(4-morpholinophenyl)pentanamide (14d). The title compound was prepared from **13d** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.12 g, 76%). Mp: 192−194 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.8 Hz, 2H), 7.74 (s, 1H), 7.25 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.8 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 5.01 (d, J = 6.8 Hz, 1H), 3.85 (t, J = 4.8 Hz, 4H), 3.79 (, 3H), 3.77−3.71 (m, 1H), 3.10 (t, J = 4.8 Hz, 4H), 1.66−1.60 (m, 2H), 1.51−1.47 (m, 1H), 0.87 (d, J = 6.4 Hz, 3H), 0.71 (d, J = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.37, 163.23, 148.48, 130.60, 129.80, 129.50, 121.47, 116.04, 114.36, 66.84, 56.24, 55.62, 49.63, 42.08, 24.41, 22.91, 21.32. HRMS (ESI): $[M + H]^+$ $C_{23}H_{32}N_3O_5S$ calcd 462.2063, found 462.2071; purity ≥99.16% (as determined by RP-HPLC, method A, t_R = 10.57 min).

(*S*)-2-(Biphenyl-4-ylsulfonamido)-4-methyl-*N*-(4-morpholinophenyl)pentanamide (14e). The title compound was prepared from **13e** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.12 g, 80%). Mp: 213-215 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 8.0 Hz, 2H), 7.67 (s, 1H), 7.63 (d, J = 8.4 Hz, 2H), 7.46−7.39 (m, 5H), 7.18 (d, J = 8.8 Hz, 2H), 6.74 (d, J = 8.4 Hz, 2H), 5.27 (d, J = 8.0 Hz, 1H), 3.87−382 (m, 5H), 3.03 (t, J = 4.8 Hz, 4H), 1.66−1.60 (m, 2H), 1.56−1.51 (m, 1H), 0.88 (d, J = 5.6 Hz, 3H), 0.76 (d, J = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.19, 148.44, 146.01, 139.10, 137.78, 129.54, 128.95, 128.50, 127.80, 127.78, 127.33, 121.45, 115.97, 66.82, 56.31, 49.48, 42.14, 24.44, 22.91, 21.40. HRMS (ESI): $[M + H]^+$ $C_{28}H_{34}N_3O_4S$ calcd 508.2270, found 508.2271; purity ≥98.94% (as determined by RP-HPLC, method A, t_R = 15.08 min).

(*S*)-4-Methyl-*N*-(4-morpholinophenyl)-2-(4-nitrophenylsulfonamido)pentanamide (14f). The title compound was prepared from **13g** and 4-morpholinoaniline (**8a**) according to the general procedure as yellowish solid (0.03g, 21%). Mp: 215−217 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 8.8 Hz, 2H), 8.02 (d, J = 8.8 Hz, 2H), 7.32 (s, 1H), 7.122 (d, J = 8.8 Hz, 2H), 6.78 (d, J = 8.8 Hz, 2H), 5.55 (d, J = 7.2 Hz, 1H), 3.91−3.84 (m, 5H), 3.09 (t, J = 4.8 Hz, 4H), 1.76−1.70 (m, 1H), 1.61−1.51 (m, 2H), 0.93 (d, J = 6.8 Hz, 3H), 0.87 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$,) δ 167.51, 148.93, 147.89, 144.35, 127.77, 127.44, 123.26, 120.29, 114.95, 65.78, 55.45, 48.33, 41.19, 28.69, 23.40, 21.81, 20.56. HRMS (ESI): $[M + H]^+$ $C_{22}H_{29}N_4O_6S$ calcd 477.1808, found 477.1808; purity ≥99.99% (as determined by RP-HPLC, method A, t_R = 11.86 min).

2-(4-Methylphenylsulfonamido)-*N*-(4-morpholinophenyl)-acetamide (17a). The title compound was prepared from **16a** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.09 g, 55%). Mp: 182−184 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.76 (d, J = 8.4 Hz, 2H), 7.35−7.32 (m, 4H), 6.85 (d, J = 8.8 Hz, 2H), 5.31 (t, J = 6.8 Hz, 1H), 3.85 (t, J = 4.8 Hz, 4H), 3.69 (d, J = 6.4 Hz, 2H), 3.11 (t, J = 4.8 Hz, 4H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.61, 148.65, 144.40, 135.40, 130.05, 129.43, 127.27, 121.54, 116.10, 66.84, 49.53, 46.49, 21.56. HRMS (ESI): $[M + H]^+$ $C_{19}H_{24}N_3O_4S$ calcd 390.1488, found 390.1485; purity ≥99.8% (as determined by RP-HPLC, method B, t_R = 17.55 min).

(*S*)-2-(4-Methylphenylsulfonamido)-*N*-(4-morpholinophenyl)propanamide (17b). The title compound was prepared from **16b** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.10 g, 60%). Mp: 215−217 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.77 (d, J = 8.4 Hz, 2H), 7.32−7.30 (m, 4H), 6.85 (d, J = 8.4 Hz, 2H), 5.04 (d, J = 7.6 Hz, 1H), 3.89−3.85 (m, 5H), 3.11 (t, J = 5.2 Hz, 4H), 2.40(s, 3H), 1.31 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.09, 148.50, 144.22, 136.09, 129.96, 129.76, 127.23, 121.44, 116.07, 66.83, 53.15, 49.61, 21.54, 18.86. HRMS (ESI): $[M + H]^+$ $C_{20}H_{26}N_3O_4S$ calcd 404.1644, found 404.1643; purity ≥99.99% (as determined by RP-HPLC, method B, t_R = 17.87 min).

(*S*)-3-Hydroxy-2-(4-methylphenylsulfonamido)-*N*-(4-morpholinophenyl)propanamide (17c). The title compound was prepared from **16c** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.10 g, 61%). Mp: 208−210 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.78 (d, J = 8.0 Hz, 2H), 7.37−7.32 (m, 4H), 6.86 (d, J = 8.4 Hz, 2H), 5.70 (d, J = 6.8 Hz, 1H), 4.10 (d, J = 10.4 Hz, 1H), 3.85−3.87 (m, 5H), 3.39−3.36 (m, 1H), 3.12 (t, J = 4.8 Hz, 4H), 2.43 (s, 3H), 2.30 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.28, 148.67, 144.43, 135.87, 130.89, 129.50, 127.24, 121.49, 116.06, 66.83, 62.12, 57.57, 49.54. HRMS (ESI): $[M + H]^+$ $C_{20}H_{26}N_3O_5S$ calcd 420.1593, found 420.1593; purity ≥95.59% (as determined by RP-HPLC, method B, t_R = 15.24 min).

(*S*)-3-Methyl-2-(4-methylphenylsulfonamido)-*N*-(4-morpholinophenyl)butanamide (17d). The title compound was prepared from **16d** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.10 g, 63%). Mp: 240−242 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 7.2 Hz, 2H), 7.51 (s, 1H), 7.25 (d, J = 8.0 Hz, 2H), 7.19 (d, J = 8.0 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 5.15 (d, J = 6.8 Hz, 1H), 3.85 (t, J = 4.4 Hz, 4H), 3.55(t, J = 5.6 Hz, 1H), 3.10 (t, J = 4.8 Hz, 4H), 2.35 (s, 3H), 2.21−2.18 (m, 1H), 0.90 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.29, 148.64, 144.05, 136.08, 129.76, 129.45, 127.38, 121.62, 116.06, 66.83, 62.81, 49.63, 31.24, 21.53, 19.12, 17.36. HRMS (ESI): $[M + H]^+$ $C_{22}H_{30}N_3O_4S$ calcd 432.1957, found 432.1946; purity ≥99.99% (as determined by RP-HPLC, method B, t_R = 21.16 min).

(2*S*,3*S*)-3-Hydroxy-2-(4-methylphenylsulfonamido)-*N*-(4-morpholinophenyl)butanamide (17e). The title compound was prepared from **16e** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.07 g, 42%). Mp: 202−203 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.78 (d, J = 7.2 Hz, 2H), 7.32−7.26 (m, 4H), 6.84 (d, J = 7.2 Hz, 2H), 5.80 (d, J = 5.6 Hz, 1H), 4.43 (brs, 1H), 3.85 (t, J = 4.4 Hz, 4H), 3.75 (d, J = 5.6 Hz, 1H), 3.11 (t, J = 4.4 Hz, 4H), 2.79 (s, 1H), 2.41 (s, 3H), 0.94 (d, J = 5.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.37, 148.59, 144.18, 136.06, 129.93, 129.59, 127.22, 121.47, 116.06, 67.64, 66.82, 60.80, 49.56, 21.55, 18.09. HRMS (ESI): $[M + H]^+$ $C_{21}H_{28}N_3O_5S$ calcd 434.1750, found 434.1745; purity ≥98.74% (as determined by RP-HPLC, method B, t_R = 16.15 min).

(*S*)-2-(4-Methylphenylsulfonamido)-4-(methylthio)-*N*-(4-morpholinophenyl)butanamide (17f). The title compound was prepared from **16f** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.07 g, 45%). Mp: 200−202 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.77 (d, J = 8.0 Hz, 2H), 7.30−7.26 (m, 4H), 6.84 (d, J = 8.4 Hz, 2H), 5.84 (d, J = 8.4 Hz, 1H), 4.03−3.98 (m, 1H), 3.85 (t, J = 4.8 Hz, 4H), 3.11 (t, J = 4.4 Hz, 4H), 2.53−2.47 (m, 1H), 2.44−2.41 (m, 1H), 2.39 (s, 3H), 2.03 (s, 3H), 1.99−1.95 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.19, 148.61, 144.17, 136.25, 129.90, 129.55, 127.30, 121.48, 116.06, 66.83, 56.59, 49.58, 31.17, 30.04, 21.56, 15.16. HRMS (ESI): $[M + H]^+$ $C_{22}H_{30}N_3O_4S_2$ calcd 464.1678, found 464.1669; purity ≥99.06% (as determined by RP-HPLC, method B, t_R = 21.91 min).

(*S*)-3-(4-Methylphenylsulfonamido)-4-(4-morpholinophenylamino)-4-oxobutanoic Acid (17g). A general amide coupling reaction was used to prepare intermediate (*S*)-benzyl 3-(4-methylphenylsulfonamido)-4-(4-morpholinophenylamino)-4-oxobutanoate from **16g** and 4-morpholinoaniline (**8a**) as a colorless solid. To the solution of the above prepared intermediate (1 equiv) in MeOH/EA palladium/charcoal (0.1 equiv) was added, and the reaction mixture

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was stirred at room temperature under H_2 pressure for 3 h. The reaction solution was filtered through Celite and the solvent was evaporated to yield the title compound as a colorless solid (0.13 g, 55% over 2 steps). Mp: 166−168 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 1H), 7.65−7.20 (m, 4H), 7.21 (d, J = 4.0 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 4.17 (t, J = 7.0 Hz, 1H), 3.72 (t, J = 4.4 Hz, 4H), 3.02 (t, J = 4.6 Hz, 4H), 2.57 (dd, J = 16.2, 6.6 Hz, 1H), 2.35 (dd, J = 16.0, 7.6 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.53, 167.93, 147.78, 142.88, 138.51, 131.19, 129.66, 127.05, 120.99, 115.59, 66.53, 54.29, 49.40, 38.09, 21.38. HRMS (ESI): $[M + H]^+$ $C_{21}H_{26}N_3O_6S$ calcd 448.1542, found 448.1541; purity ≥99.99% (as determined by RP-HPLC, method B, t_R = 16.29 min).

(*S*)-4-(4-Methylphenylsulfonamido)-5-(4-morpholinophenylamino)-5-oxopentanoic Acid (17h). A general amide coupling reaction was used to prepare intermediate (*S*)-benzyl 4-(4-methylphenylsulfonamido)-5-(4-morpholinophenylamino)-5-oxopentanoate from **16h** and 4-morpholinoaniline (**8a**) as a colorless solid. To the solution of above prepared intermediate (1 equiv) in MeOH/EA palladium/charcoal (0.1 equiv) was added, and the reaction mixture was stirred at room temperature under H_2 pressure for 3 h. The reaction solution was filtered through Celite, and the solvent was evaporated to yield the title compound as white solid (0.12 g, 52%). Mp: 139−141 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67 (s, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 7.6 Hz, 2H), 7.19 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 3.83−3.79 (m, 1H), 3.72 (t, J = 4.6 Hz, 4H), 3.02 (t, J = 4.8 Hz, 4H), 2.26 (s, 3H), 2.20−2.11 (m, 2H), 1.80−1.70 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.06, 168.74, 147.88, 142.92, 138.48, 130.98, 129.71, 126.97, 121.09, 115.68, 66.53, 56.73, 49.37, 30.53, 28.58, 21.37. HRMS (ESI): $[M + H]^+$ $C_{22}H_{28}N_3O_6S$ calcd 462.1699, found 462.1697; purity ≥99.14% (as determined by RP-HPLC, method B, t_R = 16.43 min).

(*S*)-2-(4-Methylphenylsulfonamido)-*N*-(4-morpholinophenyl)-3-phenylpropanamide (17i). The title compound was prepared from **16i** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.10 g, 65%). Mp: 223−225 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.57 (d, J = 7.6 Hz, 2H), 7.26−7.17 (m, 7H), 6.97 (d, J = 6.8 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 5.03 (d, J = 6.8 Hz, 1H), 3.98−3.93 (m, 1H), 3.85 (t, J = 4.4 Hz, 4H), 3.10 (t, J = 4.4 Hz, 4H), 3.09−3.04 (m, 1H), 2.969−2.91 (m, 1H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.00, 148.59, 144.04, 135.53, 135.22, 129.88, 129.49, 129.21, 128.99, 127.31, 127.19, 121.69, 116.05, 66.84, 58.56, 49.59, 38.41, 21.57. HRMS (ESI): $[M + H]^+$ $C_{26}H_{30}N_3O_4S$ calcd 480.1957, found 480.1956; purity ≥99.99% (as determined by RP-HPLC, method A, t_R = 12.01 min).

(*S*)-3-(4-Hydroxyphenyl)-2-(4-methylphenylsulfonamido)-*N*-(4-morpholinophenyl)propanamide (17j). A general amide coupling reaction was used to prepare intermediate (*S*)-4-(2-(4-methylphenylsulfonamido)-3-(4-morpholinophenylamino)-3-oxopropyl)phenyl 4-methylbenzenesulfonate from **16j** and 4-morpholinoaniline (**8a**) as a colorless solid. To the solution of the above prepared intermediate (1 equiv) in ethanol was added sodium hydroxide (5 equiv), and the solution was refluxed for 4 h. The reaction mixture was cooled down to room temperature, and the ethanol was evaporated under reduced pressure. The residue was acidified with 1.5 N HCl and extracted with ethyl acetate. The combined organic fraction was dried over magnesium sulfate, filtered, and concentrated. The residue was purified through flash column chromatography eluting with 0−5% MeOH in DCM to yield the title compound as a white solid (0.03 g, 40%). Mp: 224−226 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (s, 1H), 9.18 (s, 1H), 8.05 (s, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.15 (d, J = 8.4 Hz, 4H), 6.93 (d, J = 7.6 Hz, 2H), 6.82 (d, J = 9.2 Hz, 2H), 6.57 (d, J = 8.8 Hz, 2H), 3.98−3.85 (m, 1H), 3.72 (t, J = 4.6 Hz, 4H), 3.01 (t, J = 4.6 Hz, 4H), 2.78−2.73 (m, 1H), 2.67−2.59 (m, 1H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.85, 156.33, 147.79, 142.60, 138.63, 131.05, 130.62, 129.51, 127.39, 126.84, 121.06, 115.65, 115.25, 66.53, 59.01, 49.37, 21.37. HRMS (ESI): $[M + H]^+$ $C_{26}H_{30}N_3O_5S$ calcd 496.1906, found 496.1898; purity ≥99.99% (as determined by RP-HPLC, method B, t_R = 19.93 min).

(*S*)-3-(1*H*-Indol-2-yl)-2-(4-methylphenylsulfonamido)-*N*-(4-morpholinophenyl)propanamide (BC-DXI-495, 2). See ref 16.

(*S*)-3-(1*H*-Indol-3-yl)-2-(2-methylphenylsulfonamido)-*N*-(4-morpholinophenyl)propanamide (19a). The title compound was prepared from **18a** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.08 g, 60%). Mp: 125−127 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.71 (s, 1H), 7.34 (d, J = 7.2 Hz, 3H), 7.24−7.18 (m, 4H), 7.05−6.96 (m, 3H), 6.82 (d, J = 8.8 Hz, 2H), 5.23 (d, J = 5.2 Hz, 1H), 3.98−3.95 (m, 1H), 3.85 (t, J = 4.8 Hz, 4H), 3.23 (d, J = 6.8 Hz, 2H), 3.10 (t, J = 4.8 Hz, 4H), 2.24 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.65, 148.55, 136.99, 136.34, 135.89, 133.19, 132.64, 129.72, 129.53, 126.72, 126.09, 123.49, 122.62, 121.72, 120.15, 118.41, 116.06, 111.32, 109.32, 66.84, 57.47, 49.49, 29.03, 19.97. HRMS (ESI): $[M + H]^+$ $C_{28}H_{31}N_4O_4S$ calcd 519.2066, found 519.2066; purity ≥99.99% (as determined by RP-HPLC, method A, t_R = 11.94 min).

(*S*)-3-(1*H*-Indol-3-yl)-2-(3-methylphenylsulfonamido)-*N*-(4-morpholinophenyl)propanamide (19b). The title compound was prepared from **18b** and 4-morpholinoaniline (**8a**) according to the general procedure as a colorless solid (0.07 g, 55%). Mp: 210−212 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.77 (s, 1H), 9.73 (s, 1H), 8.14 (s, 1H), 7.46−7.40 (m, 3H), 7.29−7.21 (m, 5H), 7.07−7.01 (m, 2H), 6.94 (d, J = 8.0 Hz, 1H), 6.82 (d, J = 8.8 Hz, 2H), 4.10−4.05 (m,1H), 3.72 (t, J = 4.4 Hz, 4H), 3.07−3.05 (m, 1H), 3.02 (t, J = 4.4 Hz, 4H), 2.87−2.83 (m, 1H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.23, 147.81, 141.40, 138.67, 136.36, 132.98, 131.16, 128.91, 127.58, 127.04, 124.30, 123.94, 121.26, 121.00, 118.71, 118.63, 115.62, 111.69, 109.59, 66.53, 58.08, 49.36, 29.08, 21.15. HRMS (ESI): $[M + H]^+$ $C_{28}H_{31}N_4O_4S$ calcd 519.2066, found 519.2066; purity ≥99.99% (as determined by RP-HPLC, method A, t_R = 11.98 min).

(*S*)-3-(1*H*-Indol-3-yl)-2-(4-methylphenylsulfonamido)-*N*-phenylpropanamide (20a). The title compound was prepared from **16k** and aniline according to the general procedure as a colorless solid (0.04 g, 35%). Mp: 186−188 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.10 (s, 1H), 7.45 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 7.6 Hz, 2H), 7.30−7.24 (m, 4H), 7.17−7.11 (m, 2H), 7.09−6.92 (m, 3H), 6.91 (s, 1H), 5.19 (d, J = 5.6 Hz, 1H), 4.05−4.01 (m, 1H), 3.20−3.17 (m, 2H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.86, 144.03, 137.00, 136.33, 134.91, 129.63, 128.89, 126.95, 126.69, 124.71, 123.49, 120.18, 119.99, 118.41, 111.35, 109.13, 57.43, 28.45, 21.51. HRMS (ESI): $[M + H]^+$ $C_{24}H_{24}N_3O_3S$ calcd 434.1538, found 434.1538; purity ≥99.99% (as determined by RP-HPLC, method A, t_R = 16.45 min).

(*S*)-2-(4-Bromophenylsulfonamido)-3-(1*H*-indol-3-yl)-*N*-phenylpropanamide (21a). The title compound was prepared from **18c** and aniline according to the general procedure as a colorless solid (0.08 g, 40%). Mp: 215−217 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78 (s, 1H), 9.98 (s, 1H), 8.40 (s, 1H), 7.40−7.39 (m, 5H), 7.36 (d, J = 8.4 Hz, 2H), 7.30−7.23 (m, 3H), 7.10 (s, 1H), 7.06−7.01 (m, 2H), 6.93 (t, J = 7.2 Hz, 1H), 4.16−4.13 (m, 1H), 3.05 (dd, J = 14.2, 6 Hz, 1H), 2.87 (dd,, J = 14.7, 8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.75, 140.44, 138.78, 136.38, 132.12, 131.86, 128.97, 128.55, 127.44, 126.22, 124.49,123.95, 121.27, 119.92, 118.73, 118.62, 111.76, 109.38, 58.08, 28.95. HRMS (ESI): $[M + H]^+$ $C_{23}H_{21}BrN_3O_3S$ calcd 498.0487, found 498.0487; purity ≥96.56% (as determined by RP-HPLC, method A, t_R = 19.59 min).

(*S*)-3-(1*H*-Indol-3-yl)-2-(4-methylphenylsulfonamido)-*N*-(thiophen-2-ylmethyl)propanamide (20b). The title compound was prepared from **16k** and thiophen-2-ylmethanamine according to the general procedure as a colorless solid (0.09 g, 66%). Mp: 158−160 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.41 (d, J = 8.4 Hz, 2H), 7.28−7.24 (m, 2H), 7.16−7.11 (m, 2H), 7.01−6.88 (m, 3H), 6.87 (s, 1H), 6.81 (s, 1H), 6.74 (s, 1H), 5.13 (d, J = 6.0 Hz, 1H), 4.48−4.45 (m, 2H), 3.94−3.91 (m, 1H), 3.11−3.09 (m, 2H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.55, 143.79, 140.17, 136.31, 135.18, 129.55, 126.85, 126.76, 126.09, 125.12, 123.52, 122.35, 119.86, 118.41, 111.30, 109.03, 56.85, 38.35, 28.44, 21.55. HRMS (ESI): $[M + H]^+$ $C_{23}H_{24}N_3O_3S_2$ calcd 454.1259, found 454.1270; purity ≥99.41% (as determined by RP-HPLC, method A, t_R = 15.40 min).

(*S*)-2-(4-Bromophenylsulfonamido)-3-(1*H*-indol-3-yl)-*N*-(thiophen-2-ylmethyl)propanamide (21b). The title compound was prepared from **18c** and thiophen-2-ylmethanamine according to the general procedure as a colorless solid (0.08 g, 60%). Mp: 170−170 °C.

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^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (s, 1H), 8.62 (t, J = 6.0 Hz, 1H), 8.22 (s, 1H), 7.42−7.33 (m, 6H), 7.27 (d, J = 8.4 Hz, 1H), 7.05−7.01 (m, 2H), 6.94−6.88 (m, 2H), 6.86 (d, J = 2.4 Hz, 1H), 4.30−4.26 (m, 2H), 3.99−3.95 (m, 1H), 2.99 (dd, J = 14.2, 6.0 Hz, 1H), 2.80 (dd,, J = 14.7, 8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.92, 142.19, 140.45, 136.42, 131.74, 128.36, 127.33, 127.03, 126.07, 125.71, 125.41, 124.60, 121.16, 118.54, 111.17, 109.41, 51.31, 37.66, 29.12. HRMS (ESI): [M + H]$^+$ $C_{22}H_{21}BrN_3O_3S_2$ calcd 518.0208, found 518.0223; purity ≥98.48% (as determined by RP-HPLC, method A, t_R = 16.60 min).

(*S*)-3-(1*H*-Indol-3-yl)-2-(4-methylphenylsulfonamido)-*N*-(4-methylthiazol-2-yl)propanamide (20c). The title compound was prepared from **16k** and 4-methylthiazol-2-amine according to the general procedure as a colorless solid (0.08 g, 60%). Mp: 299−301 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (s, 1H), 10.84 (s, 1H), 8.24 (s, 1H), 7.44 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 7.6 Hz, 1H), 7.27 (d, J = 8.0 Hz, 1H), 7.06−7.00 (m, 4H), 6.89 (t, J = 7.6 Hz, 1H), 6.71 (s, 1H), 4.24−4.19 (m, 1H), 3.08−3.02 (m, 1H), 2.87−2.81 (m, 1H), 2.23 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.12, 157.14, 147.01, 142.66, 137.91, 136.39, 129.39, 127.41, 126.75, 124.41, 121.21, 118.74, 118.57, 111.67, 108.93, 108.29, 57.02, 28.79, 21.33, 17.28. HRMS (ESI): [M + H]$^+$ $C_{22}H_{23}N_4O_3S_2$ calcd 455.1212, found 455.1234; purity ≥99.16% (as determined by RP-HPLC, method A, t_R = 14.99 min).

(*S*)-2-(4-Bromophenylsulfonamido)-3-(1*H*-indol-3-yl)-*N*-(4-methylthiazol-2-yl)propanamide (21c). The title compound was prepared from **18c** and 4-methylthiazol-2-amine according to the general procedure as a colorless solid (0.10 g, 92%). Mp: 303−305 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 1H), 10.85 (s, 1H), 8.53 (d, J = 8.5 Hz, 1H), 7.44 (d, J = 8 Hz, 1H), 7.44−7.36 (m, 4H), 7.27 (d, J = 8 Hz, 1H), 7.07−7.02 (m, 2H), 6.90 (t, J = 8.4 Hz, 1H), 6.73 (s, 1H), 4.29−4.25 (m, 1H), 3.07 (dd, J = 14.2, 6 Hz, 1H), 2.87 (dd,, J = 14.7, 8 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.89, 157.01, 147.10, 139.96, 136.39, 131.85, 128.49, 127.30, 126.29, 124.64, 121.24, 118.77, 118.58, 111.76, 108.84, 104.45, 56.98, 28.76, 17.31. HRMS (ESI): [M + H]$^+$ $C_{21}H_{20}BrN_4O_3S_2$ calcd 519.0160, found 519.0176; purity ≥98.96% (as determined by RP-HPLC, method A, t_R = 16.20 min).

Synthesis of Compounds 20d, 21d, 21e, and 21f. EDCI·HCl (1.1 equiv) and HOBt (1.1 equiv) were added to a stirred solution of carboxylic acid (1 equiv), amine (1 equiv) and DIPEA (2.5 equiv) in DMF at room temperature, and the reaction mixture was stirred at 60 °C for 16 h. The reaction mass was cooled to room temperature, acidified with 1.5 N HCl and extracted with ethyl acetate. The combined organic layer was then washed with saturated sodium bicarbonate, brine and concentrated under reduced pressure. The residue was purified through flash column chromatography (hexane/ethyl acetate).

(*S*)-3-(1*H*-Indol-3-yl)-*N*-(4-(4-methoxyphenyl)thiazol-2-yl)-2-(4-methylphenylsulfonamido)propanamide (BC-DXI-843, 20d). The title compound was prepared from **16k** and 4-(4-methoxyphenyl)thiazol-2-amine as a colorless solid (0.13 g, 65%). Mp: 249−251 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (s, 1H), 10.77 (s, 1H), 8.21 (d, J = 8.4 Hz, 1H), 7.79 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 7.41 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 8.0 Hz, 1H), 7.06−6.97 (m, 6H), 6.90 (t, J = 7.6 Hz, 1H), 4.33−4.27 (m, 1H), 3.79 (s, 3H), 3.11−3.05 (m, 1H), 2.88−2.83 (m, 1H), 2.18 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.39, 159.41, 157.63, 149.14, 142.68, 137.90, 136.42, 129.41, 127.56, 127.43, 127.41, 126.79, 124.48, 121.26, 118.82, 118.61, 114.55, 111.66, 108.95, 106.69, 56.88, 55.56, 28.80, 21.33. HRMS (ESI): [M + H]$^+$ $C_{28}H_{27}N_4O_4S_2$ calcd 547.1474, found 547.1474; purity ≥99.99% (as determined by RP-HPLC, method A, t_R = 19.53 min).

(*S*)-2-(4-Bromophenylsulfonamido)-3-(1*H*-indol-3-yl)-*N*-(4-(4-methoxyphenyl)thiazol-2-yl)propanamide (21d). The title compound was prepared from **18c** and 4-(4-methoxyphenyl)thiazol-2-amine as a colorless solid (0.12 g, 62%). Mp: 260−262 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40 (s, 1H), 10.77 (s, 1H), 8.48 (s, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.48−7.37 (m, 5H), 7.27 (d, J = 8.0 Hz, 1H), 7.08−6.97 (m, 5H), 6.92 (t, J = 8.0 Hz, 1H), 4.34−4.31 (m, 1H), 3.79 (s, 3H), 3.12−3.07 (m, 1H), 2.91−2.85 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.34, 159.41, 157.59, 149.21, 139.95, 136.41, 131.85, 128.51, 127.56, 127.44, 127.31, 126.32, 124.69, 121.28, 118.84, 118.60, 114.53, 111.75, 108.86, 106.83, 56.84, 54.41, 28.77. HRMS (ESI): [M + H]$^+$ $C_{27}H_{24}BrN_4O_4S_2$ calcd 611.0422, found 611.0422; purity ≥99.88% (as determined by RP-HPLC, method A, t_R = 20.42 min).

(*S*)-2-(4-Bromophenylsulfonamido)-*N*-(4-(2,4-dimethoxyphenyl)thiazol-2-yl)-3-(1*H*-indol-3-yl)propanamide (21e). The title compound was prepared from **18c** and 4-(2,4-dimethoxyphenyl)thiazol-2-amine as a colorless solid (0.05 g, 31%). Mp: 165−168 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (s, 1H), 10.76 (s, 1H), 8.48 (s, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.96 (d, J = 8.8 Hz, 1H), 7.47 (s, 2H), 7.46−7.40 (m, 4H), 7.37 (d, J = 9.2 Hz, 1H), 7.07−7.02 (m, 2H), 6.92 (d, J = 8.8 Hz, 1H), 6.89−6.60 (m, 2H), 4.34−4.30 (m, 1H), 3.90 (s, 3H), 3.80 (s, 3H), 3.12−3.07 (m, 1H), 2.91−2.85 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.29, 160.41, 158.19, 155.95, 145.30, 139.98, 136.41, 131.82, 130.11, 128.416, 127.31, 126.32, 124.73, 121.28, 118.85, 118.60, 116.26, 111.76, 110.21, 108.88, 105.49, 99.16, 56.86, 55.97, 55.72, 28.77. HRMS (ESI): [M + H]$^+$ $C_{28}H_{26}BrN_4O_5S_2$ calcd 641.0528, found 641.0524; purity ≥99.99% (as determined by RP-HPLC, method A, t_R = 21.01 min).

(*S*)-2-(4-Bromophenylsulfonamido)-3-(1*H*-indol-3-yl)-*N*-(4-(2-methoxyphenyl)thiazol-2-yl)propanamide (21f). The title compound was prepared from **18c** and 4-(2-methoxyphenyl)thiazol-2-amine as a colorless solid (0.09 g, 75%). Mp: 196−198 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (s, 1H), 10.76 (s, 1H), 8.48 (s, 1H), 7.64 (s, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.41−7.11 (m, 6H), 7.08−7.01 (m, 5H), 6.91(t, J = 7.6 Hz, 1H), 4.34−4.30 (m, 1H), 3.91 (s, 3H), 3.12−3.07 (m, 1H), 2.91−2.85 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.38, 157.06, 156.11, 145.24, 139.98, 136.43, 131.83, 129.27, 128.48, 127.32, 126.34, 124.74, 122.99, 121.29, 120.88, 118.84, 118.62, 112.52, 112.12, 111.77, 108.88, 56.88, 55.91, 28.78. HRMS (ESI): [M + H]$^+$ $C_{27}H_{24}BrN_4O_4S_2$ calcd 611.0422, found 611.0404; purity ≥99.15% (as determined by RP-HPLC, method A, t_R = 21.07 min).

Biology. Cell Culture and Materials. A549, H460, and CHO cells were cultured in RPMI media supplemented with 10% FBS and 1% penicillin/streptomycin in 5% CO_2 at 37 °C. WI-26 cells were cultivated in DMEM media using the same conditions as above. All cell lines, antibodies specific for DX2 and AIMP2, and purified DX2 proteins were kindly provided by Biobank of Biocon (Medicinal Bioconvergence Research Center, Seoul National University). Antibodies specific for Siah1 (no. ab2237) and actin (no. A1978) were purchased from Abcam and Sigma, respectively. Anti-ubiquitin (no. sc-8017) and anti-HSP70 (no. sc-29352) antibodies were purchased from Santa Cruz Biotechnology. MG-132 (no. 474790) and doxycycline (no. D3447) were purchased from Millipore and Sigma, respectively.

Screening. A549 cells (4×10^4 cells/mL) expressing nanoluciferase-tagged DX2 were seeded in 96-well, flat-bottom white plates (no. 3903 Corning) and incubated for 12 h. The cells were treated with the synthetic compounds (40 μM), individually, for 4 h, and luminescence was measured according to the manufacturer's protocol (Promega). A549 cells were treated with six compounds (40 μM), individually, and each showed over 50% inhibition of DX2 using the same conditions as above; nanoluciferase-tagged AIMP2 was introduced ectopically for negative screening. The compound showing the highest and lowest inhibition of DX2 and AIMP2, respectively (**BC-DXI-843**), was identified as a lead. All experiments were independently repeated three times.

***In Vitro* Pull-down Assay.** DX2-His 6 (0.1 mg) and HSP70 (0.25 mg) were mixed at an equal molar ratio (1:1) in 50 mM Tris-HCl (pH 7.4) lysis buffer containing 100 mM NaCl, 0.5% Triton X-100, 10% glycerol, 1 mM EDTA, and protease inhibitors (Calbiochem). **BC-DXI-843** was then added in a dose-dependent manner and incubated for 4 h. After incubation, DX2-His 6 proteins were precipitated with Ni-IDA beads (ELPIS-BIOTECH) and washed with cold lysis buffer three times. HSP70 that co-precipitated with DX2-His 6 was separated and detected by SDS−PAGE and coomassie staining, respectively.

Complementation Assay Using Nanoluciferase Binary Technology. DX2 and HSP70 were cloned into pBiT1.1-N[TK/LgBiT] and pBiT2.1-N[TK/SmBiT] to express LgBiT-tagged DX2 and

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SmBiT-tagged HSP70, respectively. A549 cells were ectopically induced with LgBiT-DX2 and SmBiT-HSP70 and then treated with **BC-DXI-843** in a dose-dependent manner for 4 h. After incubation, luciferase activity was measured with a nanoluciferase assay system following the manufacturer's protocol (Promega). For a negative binding control, PRKACA (protein kinase A catalytic subunit) and PKRAR2A (protein kinase A type 2A regulatory subunit) were cloned into pBiT1.1-N[TK/LgBiT] and pBiT2.1-N[TK/SmBiT], respectively, following the same experimental procedures as above. The experiments were independently repeated three times.

Immunoprecipitation. The cells were lysed with 50 mM Tris-HCl (pH 7.4) lysis buffer containing 100 mM NaCl, 0.5% Triton X-100, 0.1% SDS, 10% glycerol, 1 mM EDTA, and protease inhibitors (Calbiochem). Total cell extracts were mixed with a DX2-specific antibody and incubated with agarose A for 12 h. After incubation, co-precipitates with agarose A were washed with lysis buffer without SDS and subjected to SDS−PAGE and immunoblotting using specific antibodies against the proteins of interest. To monitor the ubiquitination of DX2, the cells were cultured in media supplemented with MG-132 (50 μM) following the same experimental procedure described above.

Cell Viability Assay. A549 (1.5×10^4 cells/mL) or WI-26 (4×10^4 cells/mL) cells were cultured in 96-well, flat-bottom plates for 24 h. Diluted compounds in serum-free media were added to the cells and incubated for 72 h. To test each compound's DX2 dependency, stable cells expressing DX2 upon induction with DMSO (Sigma) were cultured in 96-well plates and treated with each compound. An amount of 10 μL of MTT solution (5 mg/mL, Sigma) was added to the cells and incubated for 1.5 h at 37 °C. After discarding the culture media with the MTT solution, the precipitated formazan crystals in each well were dissolved with 100 μL of DMSO (Duchefa). Absorbance was measured at 560 nm using a microplate reader (Sunrise, TECAN). All experiments were independently repeated three times.

3D Cell Culture Assay. H460 cells cultivated in 2D culture conditions were detached using Accumax (EMD Millipore) for 3D cell culture. Cells were counted with a disposable hemocytometer, C-Chip (INCYTO), and diluted in media supplemented with 1% penicillin/streptomycin and 10% FBS to a concentration of 5000 cells/100 μL. The cells were seeded onto ultralow attachment, 96-well, 3D-culture plates (Corning). After incubation for 48 h, spheroid, 3D-cultured cancer cells were treated with each compound for 72 h. Cell viability was determined using the CellTiter-Glo 3D cell viability assay (Promega) according to the manufacturer's instructions.

Xenograft. H460 cells (1×10^7) were subcutaneously inoculated into the back of 7-week-old female BALB/cSLC-nu/nu mice (Central Lab. Animal Inc., Korea) ($n = 4$/group). **BC-DXI-843** (50 mg/kg) was intraperitoneally injected every other day for 15 days. The embedded tumor volume and body weight were measured five and four times, respectively, over the experimental period. After 15 days, all mice were sacrificed, and the size and weight of the excised tumors were measured after photos of the mice were taken. Animal experiments complied with the University Animal Care and Use Committee guidelines at Seoul National University.

Docking and System Preparation for Computational Modeling. Modeling and initial docking calculations were performed using the Schrödinger Suite molecular modeling package (version 2017-3). The default parameters were used unless otherwise noted. The homology structure that was built in our previous study was prepared using the Protein Preparation Wizard.[26] First, force field atom types and bond orders were assigned, missing atoms were added, and tautomer/ionization states were assigned so that their tautomers were adjusted to optimize the hydrogen bond. Constrained energy minimization was then performed. Typically, docking sites were explored and characterized using the Site Map[27] tool. On the basis of previous binding studies using NMR, X-ray, and site-directed mutagenesis, we prepared Grid box and docked using Glide.[28,29]

Fluorescence-Based Equilibrium Binding Assay. All titration experiments were conducted at 20 °C using a Jasco FP 6500 spectrofluorometer (Easton, MD, USA). Purified DX2 protein was equilibrated with various concentrations of ligands before measuring fluorescence emission. Ligand stock solutions were titrated into a protein sample dissolved in phosphate buffer (pH 7.4) containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4. Protein samples were excited at 280 nm, and the decrease in fluorescence emission upon ligand binding was measured at 335 nm as a function of ligand concentration. All titration data were fit to a hyperbolic binding equation to obtain K_d values.

RT-PCR. Total RNA extracted by using an RNeasy Mini Kit (Qiagen) was subjected to RT-PCR with dNTP, random hexamer, and Moloney murine leukemia virus (MMLV). The same volume of cDNA was used in a PCR assay to detect the mRNA expression of DX2, AIMP2, and actin using the following specific primers: DX2, CTGGCCACGTGCAGGATTACGGGG and AAGTGAATCCCAG-CTGATAG; AIMP2, ATGCCGATGTACCAGGTAAAG and CTTAAGGAGCTTGAGGGCCGT; actin, CCTTCCTGGGCATG-GAGTCCT and GGAGCAATGATCTTGATCTT.

Statistics. Statistical tests were performed with Prism (GraphPad). All error bars represent standard deviation (SD). For calculation of IC_{50}, EC_{50}, and K_d values, we used Prism (GraphPad).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.9b01961.

Determining the significant residues for binding of **BC-DXI-843** to DX2 (Figure S1A,B); DX2 degradation caused by **BC-DXI-843** (Figure S1C,D); **BC-DXI-843**-dependent inhibition of DX2 and HSP70 binding (Figure S2); ^{1}H NMR and ^{13}C NMR spectra of the final compounds (PDF)

Coordinates information for structure representation (PDB)

Molecular formula strings and some data (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AIMP2, aminoacyl-tRNA synthetase-interacting multifunctional protein 2; HSP70s, 70 kDa heat shock proteins; p53, phosphoprotein p53; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; FBP, fuse-binding protein; TRAF2, TNF receptor-associated factor 2; GST, Glutathione *S*-transferase; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; TEA, triethylamine; DIPEA, *N*,*N*-diisopropylethylamine; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimidehydrochloride; HOBt, hydroxybenzotriazole; EtOH, ethanol; MeOH, methanol; EtOAc, ethyl acetate; HRMS, high-resolution mass spectrometry; HPLC, high-performance liquid chromatography; mp, melting point.

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