Rational Design and Synthesis of Selective PRMT4 Inhibitors: A New Chemotype for Development of Cancer Therapeutics**

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Protein arginine *N*-methyl transferase 4 (PRMT4) asymmetrically dimethylates the arginine residues of histone H3 and nonhistone proteins. The overexpression of PRMT4 in several cancers has stimulated interest in the discovery of inhibitors as biological tools and, potentially, therapeutics. Although several PRMT4 inhibitors have been reported, most display poor selectivity against other members of the PRMT family of methyl transferases. Herein, we report the structure-based design of a new class of alanine-containing 3-arylindoles as potent and selective PRMT4 inhibitors, and describe key structure–activity relationships for this class of compounds.

Introduction

Arginine methyl transferases catalyze both symmetric and asymmetric methylation of arginine residues in the histone H3 proteins using the methyl group from *S*-adenosyl-L-methionine (SAM).[1] These methyl transferases regulate a variety of biological processes including transcriptional activation,[2] RNA splicing,[3] cell-cycle regulation,[4] DNA damage response,[5] and cell differentiation,[6] while also catalyzing the methylation of a variety of nonhistone proteins.[7] Type I arginine methyl transferases (PRMTs), PRMT1, -2, -3, -4, -6, and -8, catalyze mono and asymmetric dimethylation. The type II PRMTs PRMT5 and -9 catalyze mono and symmetric demethylation, whereas PRMT7 catalyzes mono methylation of arginines.[8]

PRMT4 has been implicated in several malignancies and is highly overexpressed in ~75% of colorectal cancers[9] as well as in prostate carcinoma and androgen-independent prostate carcinoma.[10] PRMT4 is a critical factor in the pathway of estrogen-stimulated breast cancer growth[11] and its overexpression is associated with poor prognosis in this disease.[12] Knockout studies in breast cancer cell lines show that PRMT4 regulates breast cancer cell migration and metastasis.[13] Moreover, pharmacological inhibition of PRMT4 with selective inhibitors is effective in reducing the growth of multiple myeloma cell lines[14] as well as in vivo mouse models of multiple myeloma[15]

The majority of reported PRMT4 inhibitors shows moderate to poor selectivity against other type I PRMTs[16,17] and/or lack of cellular activity,[18–20] with the notable exceptions of PRMT4 selective chemical probes 1 and 2 reported by Structural Genomics Consortium[14,21] and Epizyme,[15] respectively. Here, we report the development of indole based, potent and PRMT4 selective inhibitors starting from a dual PRMT4/6 inhibitor scaffold. Notably, we relied on the co-crystal structure of a hit compound **3a** (Figure 1) with PRMT6 and molecular modeling with reported PRMT4 structures to design PRMT4 selective inhibitors and identify key structural features relevant to PRMT4 selectivity.

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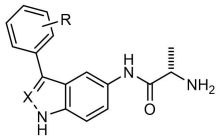
Table 1. PRMT4 and PRMT6 activity of indazoles and indoles **3** and **4**.



3a: X= N, R = 3-SO_2NMe_2
3b: X = N, R = 4-$CONH_2$
4a: X = CH, R = 4-$CONH_2$
4b: X = CH, R = 3-SO_2Me

	Compound	IC_{50} [μM][a] PRMT4	PRMT6	Selectivity PRMT4
1	**3a**	0.06 ± 0.02	0.5 ± 0.1	8.3
2	**3b**	> 10	0.2 ± 0.1	< 0.02
3	**4a**	2.30 ± 0.20	0.3 ± 0.1	0.13
4	**4b**	0.04 ± 0.02	7.9 ± 1.0	197

[a] Average ± SD of three IC_{50} values from three experiments.

Results and Discussion

Based on the similarity with the reported PRMT inhibitors, we selected a library of 5000 compounds from our own collection and screened for PRMT4 and PRMT6 activity using the reported methods[14] and identified novel indazoles as inhibitors of PRMT4 and PRMT6. Owing to its potent PRMT4 inhibitory activity (IC_{50} 0.06 μM) and moderate selectivity over PRMT6 (eightfold), the indazole **3a** (Table 1, entry 1) was selected as a starting point for the development of a selective PRMT4 inhibitor.

Unfortunately, attempts to co-crystallize **3a** or the structurally related indazole **3b** (Figure 1) with PRMT4 were unsuccessful. However, we were able to obtain the co-crystal structure of **3a** with PRMT6 (PDB entry: 7NR4, Figure 2A). Based on this structural insight, superimposition of the PRMT6-bound structure of indazole **3a** in the reported PRMT4 structure[19] (Figure 2B) suggested that the corresponding indole might improve selectivity for PRMT4. Specifically, in the PRMT6-bound structure of **3a**, H-bonding between Glu59 and the indazole NH was identified as a key binding interaction. Thus, it was proposed that decreasing the acidity of the N–H from indazole (pK_a ~ 14) to indole (pK_a ~ 21) would attenuate interactions with PRMT6. Additionally, the reduced polar surface area (PSA) of the corresponding indole would expectedly result in improved hydrophobic interactions with PRMT4 (Figure 2B).

To test this hypothesis, indole **4a** was synthesized following the synthetic sequence described in Scheme 1 and we were pleased to find that this compound showed a moderate loss in PRMT6 activity and coincident gain in PRMT4 activity compared to **3b** (Table 1, entries 2 and 3). Furthermore, replacement of the amide with a *meta*-sulfonamide (entries 1 and 2) resulted in improved PRMT4 activity. Inspired by these observations, the corresponding *meta*-methyl sulfone analogue of indole **4a** was prepared (entry 4), resulting in further improved PRMT4 activity and selectivity. Notably, the *meta*-methyl sulfone **4b** (entry 4) proved to be 197-fold selective against PRMT6 (PRMT4 IC_{50} = 40 nM) and furthermore > 50-fold selective against other arginine methyltransferases PRMT1, -3, -5 to -9. Docking studies of the sulfonamide **3a** in both PRMT4 and PRMT6 suggested that the improved PRMT4 activity is likely due to interactions

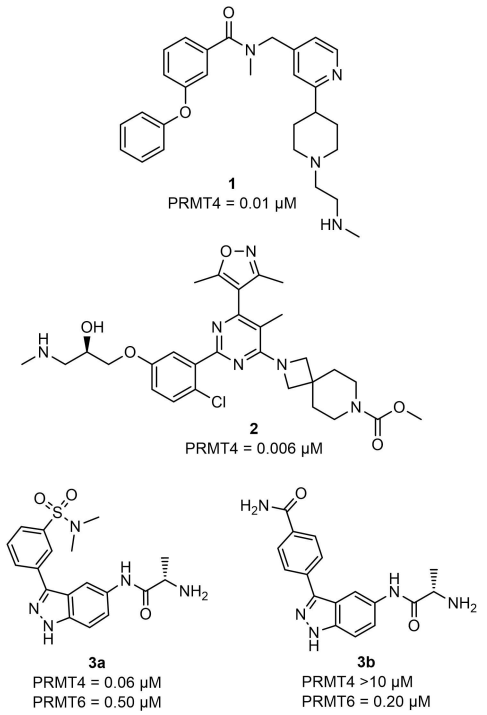


Figure 1. PRMT4 inhibitors **1** and **2** reported by the Structural Genomics Consortium and Epizyme, respectively, and dual PRMT4/6 inhibitors identified by screening a focused library.

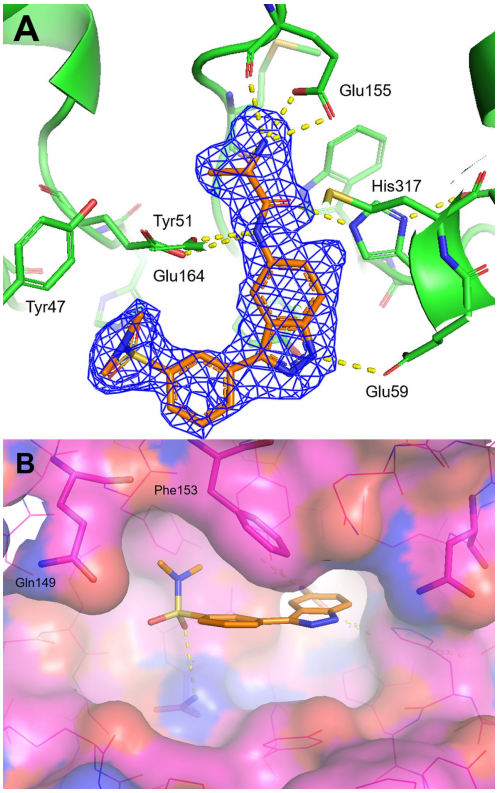
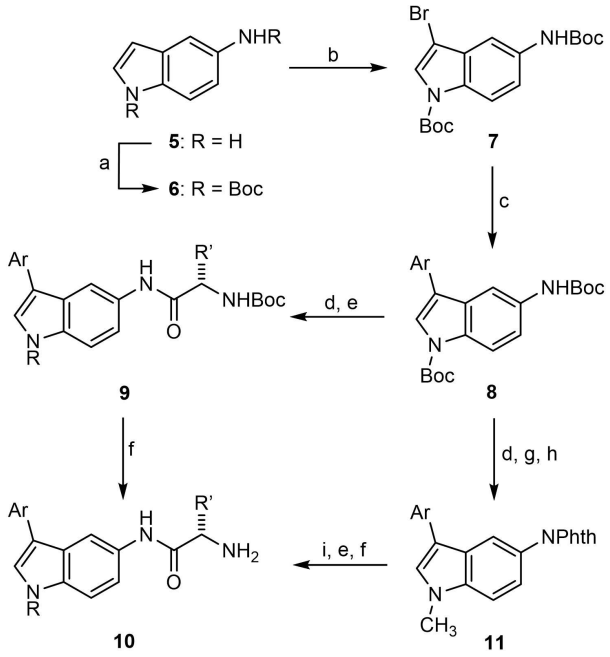


Figure 2. A) The PRMT6-bound structure of **3a**. B) The PRMT6-bound conformation of **3a** superimposed in the binding site of PRMT4 highlights a hydrophobic pocket (occupied by sulfonamide group) not present in PRMT6.

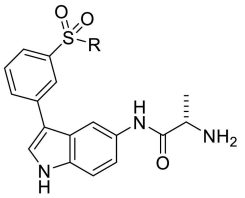


Scheme 1. General synthetic route for preparing indole-based PRMT4 inhibitors. a) Boc_2O, THF, 52%; b) NBS, THF, 77%; c) $ArB(OR)_2$, K_2CO_3, $Pd(PPh_3)_4$ or $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, THF/H_2O (3:1), 80 °C; d) TFA; e) *N*-Boc-amino acid, DIPEA, PyBOP, DMF; f) TFA; g) phthalic anhydride, toluene, reflux; h) K_2CO_3, CH_3I, DMF, RT; i) H_2NNH_2, MeOH, RT.

within the unique hydrophobic binding pocket in PRMT4 (Figure 2B). This additional subpocket in PRMT4 is mainly created by Gln149 and Phe153, while the corresponding less-space-demanding residues Leu46 and Cys50 do not generate a similar pocket in PRMT6.

To further probe the effect of modifications at the meta-position in indole **4b**, a series of sulfones and sulfonamides was prepared in a straightforward manner as summarized in Scheme 1 and Table 2. Here, we found that exchanging the methyl sulfone for a dimethyl sulfonamide (e.g., compound **12**, entries 1 and 2) resulted in a 2.5-fold gain in PRMT4 activity ($IC_{50} = 0.01$ μM) and a threefold improvement in selectivity against PRMT6 ($IC_{50} = 9.1$ μM, 650-fold). This result indicated that a more lipophilic dimethyl sulfonamide better exploits the hydrophobic binding pocket in the PRMT4 active site.

Table 2. Biological evaluation of sulfone and sulfonamide series.



	Compound	R	[IC_{50} μM][a] PRMT4
1	**4b**	Me	0.04 ± 0.02
2	**12**	NMe_2	0.01 ± 0.00
3	**13**	NEt_2	3.50 ± 0.30
4	**14**	$N(CH_2)_5$	0.89 ± 0.18
5	**15**	$N(CH_2)_4$	0.18 ± 0.04
6	**16**	*i*Pr	0.04 ± 0.01
7	**17**	$C(CH_2)_4$	3.30 ± 0.10

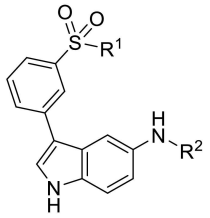
[a] Average ± SD of three IC_{50} values from three experiments.

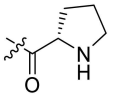
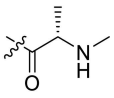
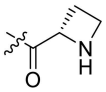
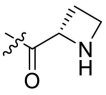
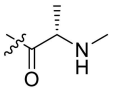
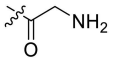
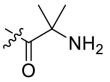
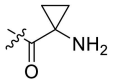
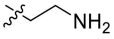
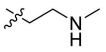
However, this modification was accompanied by a loss in potency when more sterically hindered sulfonamides were examined. For example, the diethyl sulfonamide **13** (entry 3, $IC_{50} = 3.5$ μM) and cyclic sulfonamide **14** (entry 4, $IC_{50} = 0.89$ μM) proved to be less active against PRMT4. While the smaller five membered ring sulfonamide **15** (entry 5) was tolerated ($IC_{50} = 0.18$ μM), this compound was still less potent and selective than original methyl sulfone **4b**. Several analogues of the methyl sulfone **4b** were also synthesized and it was found that the isopropyl sulfone **16** (entry 6) was a potent PRMT4 inhibitor ($IC_{50} = 0.04$ μM) and selective against PRMT6 ($IC_{50} = 8$ μM). Here again, a similar trend to that seen with sulfonamides was observed. Specifically, increasing the size of the alkyl sulfone led to a significant loss in potency (e.g., **17**; $IC_{50} = 3.3$ μM). This data suggested that the hydrophobic binding pocket in PRMT4 could not accommodate groups larger than the dimethyl sulfonamide or isopropyl sulfone. As a result, dimethyl sulfonamide **12** (entry 2) and isopropyl sulfone **16** (entry 6) were selected as the lead molecules for further optimization.

Having identified that both the indole sulfone and sulfonamide confer excellent PRMT4 activity and selectivity against PRMT6, we then turned our attention towards the amino acid side of the molecule. Here, we aimed to increase lipophilicity to improve cellular permeability and perhaps potency and selectivity. With this in mind, we probed the size of the amino acid with the L-proline methyl sulfone analogue **18** (Table 3, entry 1) and found this compound was not active (PRMT4 $IC_{50} >$ 10 μM). A similar amino acid SAR study on the isopropyl sulfone and dimethyl sulfonamide scaffolds was undertaken through the synthesis of compounds **19**–**20** and **21**–**25**, respectively (Table 3, entries 2–8). Here, we examined methylation and incorporation of an azetidine for the isopropyl sulfone and in the case of the sulfonamide, we investigated incorporation of an azetidine, methylation, glycine incorporation, geminal dimethylation and cyclopropanation. In the case of the isopropyl sulfone, each modification resulted in a decrease in PRMT4 activity (PRMT4 $IC_{50} = 0.60$ to > 10 μM). In general, the dimethyl sulfonamide analogues **21**–**25** were more potent. In particular, the glycine analogue **23** proved to be a low-nanomolar inhibitor of PRMT4 ($IC_{50} = 0.01$ μM) and maintained selectivity against PRMT6. The ethyl amine and *N*-methyl ethyl amine **26** and **27**, respectively, were also synthesized based on the common use of ethyl amine in PRMT inhibitors.[16,17,22] Unfortunately, in both cases we observed a significant loss in potency (PRMT4 $IC_{50} > 10$ μM in both cases).

At this point, we examined the cell permeability of the most promising compounds **4b**, **12**, **16** and **21**. The results of the Caco-2 assay are summarized in Figure 3. From the series methyl sulfone **4b**, dimethyl sulfonamide **12** and isopropyl sulfone **16**, the dimethyl sulfonamide proved to be the most permeable (2.13 nm/s for **12** vs 0.23 nm/s for **4b**). Disappointingly, replacement of alanine for azetidine in an effort to reduce

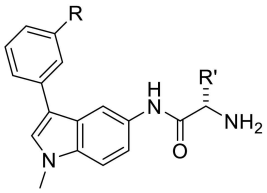
Table 3. SAR of the amino acid moiety.



	Compound	R^1	R^2	$[IC_{50}\ \mu M]^{[a]}$ PRMT4
1	**18**	Me		>10
2	**19**	iPr		>10
3	**20**	*i*Pr		0.60 ± 0.10
4	**21**	NMe_2		0.11 ± 0.00
5	**22**	NMe_2		4.20 ± 0.20
6	**23**	NMe_2		0.01 ± 0.00
7	**24**	NMe_2		0.33 ± 0.00
8	**25**	NMe_2		>10
9	**26**	Me		>10
10	**27**	Me		>10

[a] Average ± SD of three IC_{50} values from three experiments.

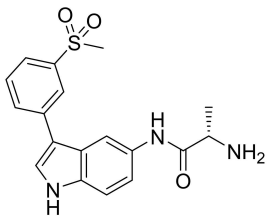
Table 4. Evaluation of methylated indole derivatives.



	Compound	R	R′	$[IC_{50}\ \mu M]^{[a]}$ PRMT4
1	**28**	SO_2NMe_2	Me	<0.002
2	**29**	SO_2NMe_2	CH_2F	0.181 ± 0.019
3	**30**	$CONMe_2$	Me	0.225 ± 0.049

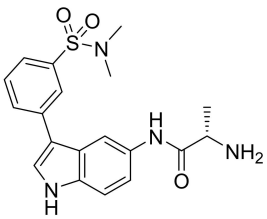
[a] average ± SD of three IC_{50} values from three experiments.

H-bond donors, increased lipophilicity and maintained cellular permeability, but increased the efflux ratio compared with compound **11** by approximately threefold (Figure 3). Based on this data we further explored a series of analogues that incorporated the dimethyl sulfonamide core.



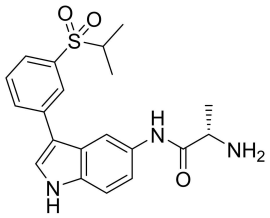
4b

Caco-2: 0.23 nm/s (34)



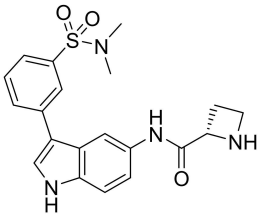
12

Caco-2: 2.13 nm/s (12)



16

Caco-2: 1 nm/s (9)



21

Caco-2: 2 nm/s (30)

Figure 3. Caco-2 data for key compounds (efflux ratio in parenthesis).

As the co-crystal structure of indazole **3a** bound to PRMT6 indicated that the aryl ring was largely solvent exposed, we next focused on modifications aimed to increase lipophilicity using the most potent dimethylsulfonamide core. Thus, analogues **28**–**30** were synthesized as summarized in Scheme 1. Of this series, the *N*-methylindole **28** proved to be the most potent and selective compound (PRMT4 $IC_{50} = < 2$ nM, >500-fold selectivity against PRMT6, Table 4, entry 1). In order to assess the effect of attenuating the basicity of the free amine on membrane permeability, the fluoroalanine **29** was synthesized[23] (entry 2, Table 4), which unfortunately proved to be approximately ten times less potent and selective. To further reduce the total polar surface area of the molecule, we examined the dimethyl amide **30**, however, this analogue proved also to be less active and selective. In summary, the N-methylated derivative **28** is the most potent compound of our series against PRMT4, indicating that H-bonding of the indole-NH to Asn 162 is by far less relevant for PRMT4 binding, while it is important for the charge-reinforced interaction to Glu 59 in PRMT6. Hence, methylation of the indole-N is a key driver to achieve selectivity against PRMT6.

Chemistry

The general preparation of compounds described herein was performed as shown in Scheme 1, starting from commercially available 5-aminoindole **5**. Boc protection of the amine and indole nitrogen atoms[24] gave the bis-Boc-protected compound **6**, which was exposed to NBS to furnish the corresponding 3-brominated product **7**.[25] This later material then engaged in a

Suzuki reaction[26] with a suitable *meta*-substituted sulfone or sulfonamide derivative. Following the Suzuki reaction, global deprotection was accomplished by treatment with trifluoroacetic acid. The 5-amino group was then coupled to a Boc-protected amino acid using PyBOP coupling conditions.[27,28] Finally, deprotection of the amino acid using trifluoroacetic acid yielded the desired indole analogues as their corresponding trifluoroacetate salts. In the case of analogues **28**–**30**, prior to amino acid coupling, the 5-amino group was protected as a phthalyl group and the indole nitrogen was methylated using methyl iodide and potassium carbonate (**11**, Scheme 1). Deprotection of the phthalimide was effected by treatment with hydrazine and the free amine was then carried through a similar sequence of steps as described above (i.e., peptide coupling and Boc-deprotection).

Evaluation of cellular activity of PRMT4 inhibitors

PRMT4 has been shown to methylate BAF155 at R1064.[13] We have evaluated key compounds **4b**, **28** and **30** following the reported methods;[14] however, none of the compounds showed significant reduction in BAF155 methylation when tested up to 30 to 100 μM (48 h of exposure in HEK293 cells), while methylation of BAF155 was abrogated by 2–3 μM of the PRMT4 selective inhibitor TP-064 (Figure 4).[14] The absence of any significant cellular activity of compound **4b** can be attributed to its poor permeability. Even though compounds **28** and **30** were expected to show enhanced permeability because of reduced H-bond donors (**28** and **30**) and reduced polar surface area (**30**), the absence of cellular activity indicates these changes were not sufficient to increase the cellular permeability of these series of compounds.

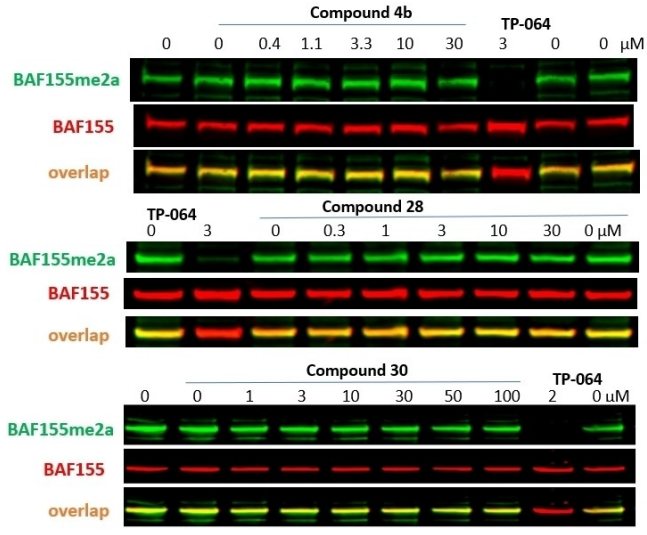


Figure 4. Effect of PRMT4-dependent BAF155 asymmetric dimethylation in HEK293T cells upon treatment with up to 100 μM compounds **4b**, **28** and **30** over 2 days.

Conclusion

In conclusion, we report the design, synthesis, and evaluation of a new series of 3-arylindole alanine-based PRMT4 inhibitors that are both potent and selective over the closely related PRMT6. Based on the cocrystal structure of our initial hit compound **3a** with PRMT6 and comparing it with the reported PRMT4 structures, we demonstrated that selectivity for PRMT4 can be achieved. Furthermore, methylation of the indole nitrogen resulted in a potent and selective *in-vitro* PRMT4 inhibitor. However, despite these efforts, none of the compounds described herein achieved on-target effects in cells. Nonetheless, these indoles represent a new chemotype for further development of cell active PRMT4 inhibitors, which will deepen our understanding of the intricate biology of PRMT4 and might engender the design of new anti-tumor PRMT4-selective inhibitors. These compounds could possibly be used as handles to develop PROTACs to degrade PRMT4 selectively. Linker attachment and E3 antagonist components used for developing PROTACs will likely influence the cellular permeability of the PROTAC compounds and therefore the poor cellular permeability of our PRMT4 inhibitors is not likely to be a hinderance for cell active PROTAC development.

Experimental Section

General chemistry: All reagents and starting materials were purchased from Sigma Aldrich, TCI, Alfa Aesar, CarboSynth, and AK Sci and were used without further purification. Dichloromethane was distilled from CaH_2 and stored under nitrogen, THF was distilled from sodium wire/benzophenone ketyl radical and stored under nitrogen. Column chromatography was carried out with 230–400 mesh silica gel (Merck, Silica Gel 60). Concentration and removal of trace solvents was done in a Buchi rotary evaporator using acetone-dry-ice condenser and a Welch vacuum pump. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$, CD_3OD, CD_3CN or [D_6]DMSO as the solvent. Signal positions (δ) are given in parts per million from tetramethylsilane (δ 0) and were measured relative to the signal of the solvent (^{1}H NMR: $CDCl_3$: δ 7.26; CD_3OD: δ3.31; CD_3CN: δ1.96; [D_6]DMSO: δ 2.50; ^{13}C NMR: $CDCl_3$: δ 77.16; CD_3OD: δ 49.00; CD_3CN: δ1.32; [D_6]DMSO: 39.5). Coupling constants (*J* values) are given in Hertz and are reported to the nearest 0.1 Hz. ^{1}H NMR spectral data are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; sept, septet; m, multiplet; br, broad), coupling constants, number of protons. NMR spectra were recorded on a Bruker Avance 600 equipped with a QNP or TCI cryoprobe (600 MHz), Bruker 400 (400 MHz) or Bruker 500 (500 MHz). High performance liquid chromatography (HPLC) analysis was performed on an Agilent 1100 HPLC, equipped with a variable wavelength UV/Vis detector. High-resolution mass spectrometry was performed on an Agilent 6210 TOF LC/MS.

General procedures

Complete synthetic procedures including intermediates synthesis are provided in the Supporting Information.

General procedure A: Suzuki-Miyaura coupling. A pressure vial charged with a stir bar, bromoindole **7** (1.0 equiv.), boronic acid or ester (1.0–1.6 equiv.), K_2CO_3 (3.0 equiv.), and $Pd(PPh_3)_4$ or Pd(dppf) $Cl_2CH_2Cl_2$ (0.10 equiv.) was placed under vacuum and then filled

with nitrogen. A mixture of degassed THF and H_2O (0.09 M THF/H_2O 3:1 unless otherwise indicated) was then added and the resulting mixture was stirred under an atmosphere of nitrogen at 80 °C for 18 h or until the reaction was complete as monitored by TLC analysis. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The residue was then dissolved in EtOAc and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated to afford the crude aryl-indole product. Purification of the crude product by flash chromatography (silica gel, Et_2O or EtOAc and hexanes) afforded the pure coupled product.

General procedure B: Amide coupling and deprotection. To a stirred solution of the aryl-indole intermediate (1.0 equiv.) in dry dimethylformamide (DMF; 0.1 M) at room temperature was added *N,N*-diisopropylethylamine (DIPEA; 5 equiv.), followed by benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP; 1–2 equiv.) and the protected amino acid (1–2 equiv.). The resulting solution was stirred at room temperature until completion of the reaction as monitored by TLC. The reaction mixture was then diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc (3×). The combined organic layers were washed with saturated aqueous LiCl (3×), dried over $MgSO_4$, and concentrated to afford the crude product (brown gum), which was used directly in the next step without further purification. The crude coupled product was dissolved in TFA (neat, 0.1 M) and stirred at room temperature until the reaction was complete as monitored by TLC analysis. Purification of the crude indole by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semipreparative column (5 μm, 100 Å, 10× 250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in H_2O B: 0.1% TFA in ACN) on gradients of 2→30%, or 2→100% solvent B over 15 minutes as indicated afforded the final compounds.

General procedure C: Synthesis of sulfonamides. To a stirred solution of amine (1.05 equiv.) in dry pyridine (0.2 M) at 0 °C was added dropwise (for liquids) or in small portions (for solids) the sulfonyl chloride (1.0 equiv.). The reaction mixture was warmed to room temperature and stirred until the reaction was complete as monitored by TLC analysis. The reaction mixture was then concentrated under reduced pressure and the residue was dissolved in EtOAc and washed with 0.5 M HCl (2×). The organic layer was then dried over $MgSO_4$, filtered and concentrated to afford the sulfonamide. The sulfonamide was used in subsequent reactions without further purification.

General procedure D: Boronic ester synthesis. A flask was charged with a stir bar, aryl bromide (1.0 equiv.), B_2pin_2 (1.0 equiv.), $NaHCO_3$ (2.50 equiv.), and $Pd(ddpf)Cl_2$ (0.05 equiv.). The flask was then placed under vacuum and filled with nitrogen. Degassed DMSO (0.2 M) was added to the reaction vessel, and the reaction mixture was stirred under an atmosphere of nitrogen at 80 °C for 18 h or until the reaction was complete as monitored by TLC analysis. The reaction mixture was then cooled to room temperature and diluted with equal parts H_2O and EtOAc, then filtered through Celite and the Celite was rinsed with EtOAc. The filtrate was then washed with H_2O and brine, dried over $MgSO_4$ and concentrated to afford the crude product. Purification of the crude product by flash chromatography (silica gel, Et_2O or EtOAc and hexanes) afforded the arylboronic ester.

General procedure E: Sulfone synthesis from thiophenol precursors. A stirred solution of substituted thiophenol (1 equiv.), K_2CO_3 (1.4 equiv.), and secondary bromoalkane (1.2 equiv.) in dry acetone (0.3 M), was stirred under nitrogen at reflux until completion of the reaction was observed by TLC (ca. 18 h). The reaction mixture was cooled to room temperature, diluted with H_2O, and extracted with Et_2O (3×). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated to afford the crude aryl thioether intermediate. To a stirred solution of the crude aryl thioether intermediate (1.0 equiv.) in MeOH (0.16 M) at 0 °C was added oxone (potassium peroxymonosulfate; 3.0 equiv.) in H_2O (0.5 M). The resulting white suspension was warmed to room temperature over 2 h and stirred at room temperature until completion was observed by TLC. The reaction mixture was then diluted with H_2O and extracted with EtOAc (2×). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated to afford the aryl sulfone. The aryl sulfone was used in subsequent reactions without further purification.

General procedure F: Phthalimide protection. To a stirred solution of aminoindole intermediate (1.0 equiv.) in toluene (0.2 M) was added phthalic anhydride (1.3 equiv.). The resultant solution was heated to reflux until completion of the reaction was observed by TLC. The reaction mixture was then cooled down to room temperature and concentrated under reduced pressure to afford a crude product which was used without further purification unless otherwise indicated.

General procedure G: N-Methylation of indole scaffolds. To a stirred solution of protected indole intermediate (1.0 equiv.) in dry dimethylformamide (DMF; 0.2 M) was added potassium carbonate (5 equiv.) followed by methyl iodide (3 equiv.). The resultant solution was stirred at 60 °C until completion of the reaction was observed by TLC. The reaction mixture was then diluted with water and extracted with EtOAc (3×). The combined organic layers were washed with aqueous saturated LiCl (3×), dried over $MgSO_4$, and concentrated to afford the crude methylated product, which was used directly in the next step without further purification unless otherwise indicated.

General procedure H: Phthalimide deprotection. To a stirred solution of *N*-methylindole intermediate (1.0 equiv.) in methanol (0.06 M) was added hydrazine hydrate (1.3 equiv.). The resultant solution was stirred at room temperature until completion of the reaction was observed by TLC. The reaction mixture was then concentrated, diluted in dichloromethane and filtered. The resulting filtrate was concentrated under reduced pressure to afford the amino indole product, which was used directly in the next step without further purification.

General procedure I: Boc-deprotection. A solution of Boc-protected intermediate (1 equiv.) in TFA (0.1 M) was stirred at room temperature until completion of the reaction was observed by TLC. Concentration of the reaction mixture under reduced pressure afforded the deprotected aryl-indole product which was used without further purification unless otherwise indicated.

(*S*)-2-Amino-*N*-(3-(3-(*N*,*N*-dimethylsulfamoyl)phenyl)-1*H*-indazol-5-yl)propenamide (3 a). To a solution of **31** (57.5 mg, 0.15 mmol) in 1-methyl-2-pyrrolidinone (NMP) (1 mL), were added subsequently [3-(dimethylsulfamoyl)phenyl]boronic acid (68.7 mg, 0.30 mmol, dissolved in 0.53 mL 1-methyl-2-pyrrolidinone), [1,1′-bis (diphenylphosphino)ferrocene] dichloropalladium(II), complex with dichloromethane (24.5 mg, 0.03 mmol, dissolved in 1 mL NMP) and potassium carbonate (62.2 mg, 0.45 mmol, dissolved in water 0.5 mL). The reaction mixture was heated to 100 °C and shaken for 24 h. The crude mixture was filtered through a pad of activated MP Alumina N (EcoChrom TM) and washed with NMP and concentrated under vacuum. The residue was dissolved in a TFA/CH_2Cl_2 mixture (1:1, 2 mL) and shaken for 24 h and finally dried using a Christ-centrifuge to give 4.62 mg of the title compound (7% yield). LC–MS method: Instrument MS: Waters ZQ; Instrument HPLC: Waters UPLC Acquity; column: Acquity BEH C_{18} (Waters), 50 mm × 2.1 mm, 1.7 μm; Solvent: A: 0.1% formic acid in water, solvent B: MeCN; gradient: 0.0 min 99% A–1.6 min 1% A–1.8 min 1%A–1.81 min 99%

A–2.0 min 99% A; oven: 60 °C; flow: 0.800 mL/min; UV-Detection PDA 210–400 nm. t_R = 0.83 min; LRMS (ESI): *m/z* 388 $[M+H]^+$

(*S*)-4-(5-(2-Aminopropanamido)-1*H*-indazol-3-yl)benzamide (3 b). Carbamate **31** (57.5 mg, 0.15 mmol, dissolved in 1 mL NMP), (4-carbamoylphenyl)boronic acid (49.5 mg, 0.30 mmol, dissolved in 0.53 mL NMP) and [1,1′-Bis(diphenylphosphino)ferrocene] dichloropalladium(II), complex with dichloromethane (24.5 mg, 0.03 mmol, dissolved in 1 mL NMP) along with potassium carbonate (62.2 mg, 0.45 mmol, dissolved in water 0.5 mL) were heated to 100 °C and shaken for 24 h. The microtitre plates (MTPs) were dried by Zirbus-centrifuge and then were dissolved again in 2 mL of a TFA/acetonitrile (1 : 1) mixture. The reaction mixture was further shaken for 1 d at room temperature. The MTPs were dried again and 2 mL NMP were added. The precipitated material was filtered off and purified by preparative HPLC to give 4.01 mg of the title compound (8% yield). LC-MS method: Instrument MS: Waters ZQ; Instrument HPLC: Waters UPLC Acquity; column: Acquity BEH C_{18} (Waters), 50 mm × 2.1 mm, 1.7 µm; Solvent A: 0.1% formic acid in water, solvent B: MeCN; Gradient: 0.0 min 99% A–1.6 min 1% A–1.8 min 1% A–1.81 min 99% A–2.0 min 99% A; oven: 60 °C; flow: 0.800 mL/min; UV-Detection PDA 210–400 nm. t_R = 0.50 min; LRMS (ESI): *m/z* 324 $[M+H]^+$

(*S*)-4-(5-(2-Aminopropanamido)-1*H*-indol-3-yl)benzamide (4 a). The title compound was prepared according to general procedure B using the aryl-indole **32** (25 mg, 0.068 mmol), (*tert*-butoxycarbonyl)-L-alanine (17 mg, 0.072 mmol), PyBOP (43 mg, 0.082 mmol), DIPEA (0.06 mL, 0.34 mmol), and dry DMF (0.68 mL). Purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semipreparative column (5 µm, 100 Å, 10 × 250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→100) % solvent B over 15 min, t_R = 4.52 min) of the crude deprotected product afforded the TFA salt of 4a as a colorless solid (9 mg, 32%). ^{1}H NMR: (500 MHz, CD_3OD) δ (ppm) = 8.23 (d, *J* = 1.7 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 2H), 7.77 (d, *J* = 8.5 Hz, 2H), 7.64 (s, 1H), 7.43 (d, *J* = 8.7 Hz, 1H), 7.32 (dd, *J* = 8.7, 1.7 Hz, 1H), 4.09 (q, *J* = 7.1 Hz, 1H), 1.64 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR: (125 MHz, CD_3OD) δ (ppm) = 172.4, 169.0, 141.4, 136.3, 131.8, 131.4, 129.3, 127.5, 126.6, 126.0, 117.3, 117.2, 113.1, 112.5, 50.9, 17.7. HRMS: (ESI) *m/z* calcd for $C_{18}H_{18}N_4O_2$: 323.1503 $[M+H]^+$; found: 323.1477.

(*S*)-2-Amino-*N*-(3-(3-(methylsulfonyl)phenyl)-1*H*-indol-5-yl) propanamide (4 b). The title compound was prepared according to general procedure B using the aryl-indole **33** (63.2 mg, 0.158 mmol), (*tert*-butoxycarbonyl)-L-alanine (60 mg, 0.32 mmol), PyBOP (165 mg, 0.32 mmol), DIPEA (0.21 mL, 0.79 mmol), and dry DMF (1.6 mL). Purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semipreparative column (5 µm, 100 Å, 10 × 250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→100) % solvent B over 15 min, t_R = 5.68 min) of the crude deprotected product afforded the TFA salt of **3 b** as a colorless solid (11 mg, 18%). ^{1}H NMR: (600 MHz, CD_3OD) δ (ppm) = 8.24 (d, *J* = 1.9 Hz, 1H), 8.22 (s, 1H), 7.99 (d, *J* = 7.7 Hz, 1H), 7.80 (d, *J* = 7.6 Hz, 1H), 7.70–7.64 (m, 2H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.33 (dd, *J* = 8.7, 2.0 Hz, 1H), 4.10 (q, *J* = 7.0 Hz, 1H), 3.20 (s, 3H), 1.63 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR: (150 MHz, CD_3OD) δ (ppm) = 169.1, 142.5, 139.1, 136.2, 132.9, 132.1, 131.0, 126.4, 126.0, 126.0, 124.9, 117.2, 116.4, 113.2, 111.8, 50.9, 44.5, 17.8. HRMS: (ESI) *m/z* calcd for $C_{18}H_{19}N_3O_3S$: 358.1220 $[M+H]^+$; found: 358.1230.

(*S*)-2-Amino-*N*-(3-(3-(*N*,*N*-dimethylsulfamoyl)phenyl)-1*H*-indol-5-yl)propenamide (12). The title compound was prepared according to the general procedures I and B using the Boc-protected-aryl-indole **36** (55 mg, 0.106 mmol) and TFA (1.0 mL) followed by treatment with (*tert*-butoxycarbonyl)-L-alanine (24 mg, 0.13 mmol), PyBOP (66 mg, 0.53 mmol), DIPEA (0.13 mL, 0.13 mmol), and dry DMF (1.0 mL). Final Boc-deprotection of the crude mixture using TFA (1.0 mL) and subsequent purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semipreparative column (5 µm, 100 Å, 10 × 250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→100) %) solvent B over 15 min, t_R = 6.2 min) afforded the TFA salt of **12** as a colorless solid (8 mg, 19%). ^{1}H NMR: (400 MHz, CD_3OD) δ (ppm) = 8.32 (d, *J* = 1.9 Hz, 1H), 8.06 (br s, 1H), 7.95 (dt, *J* = 6.7, 2.1 Hz, 1H), 7.68-7.61 (m, 3H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.26 (dd, *J* = 8.8, 2.1 Hz, 1H), 4.08 (q, *J* = 7.1 Hz, 1H), 2.78 (s, 6H), 1.63 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR: (150 MHz, CD_3OD) δ (ppm) = 168.9, 138.8, 136.9, 136.2, 132.2, 132.1, 130.7, 126.6, 126.4, 125.9, 125.5, 117.2, 116.6, 113.2, 111.8, 50.89, 38.56, 17.75 HRMS: (ESI) *m/z* calcd for $C_{19}H_{22}N_4O_3S$: 387.1491 $[M+H]^+$; found: 387.1452.

(*S*)-2-Amino-*N*-(3-(3-(*N*,*N*-diethylsulfamoyl)phenyl)-1*H*-indol-5-yl) propanamide (13). The title compound was prepared according to the general procedures I and B using the Boc-protected-aryl-indole **39** (50 mg, 0.091 mmol) and TFA (0.9 mL) followed by treatment with (*tert*-butoxycarbonyl)-L-alanine (20 mg, 0.11 mmol), PyBOP (56 mg, 0.45 mmol), DIPEA (0.08 mL, 0.11 mmol), and dry DMF (0.9 mL). Final Boc-deprotection of the crude mixture using TFA (0.9 mL) and subsequent purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semipreparative column (5 µm, 100 Å, 10 × 250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→100) %) solvent B over 15 min, t_R = 6.6 min) afforded the TFA salt of **13** as a colorless solid (6 mg, 16%). ^{1}H NMR: (400 MHz, [D_6]DMSO) δ (ppm) = 11.61 (s, 1H), 10.37 (s, 1H), 8.27 (s, 1H), 8.20 (br s, 2H), 7.98 (s, 1H), 7.90 (dt, *J* = 1.6, 7.1 Hz, 1H), 7.88 (d, *J* = 2.5 Hz, 1H), 7.68–7.59 (m, 2H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.31 (dd, *J* = 8.8, 1.6 Hz, 1H), 4.01 (q, *J* = 7.0 Hz, 1H), 3.23 (q, *J* = 7.0 Hz, 4H), 1.47 (d, *J* = 7.0 Hz, 3H), 1.06 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR: (150 MHz, [D_6]DMSO) δ (ppm) = 167.5, 140.4, 136.8, 134.0, 131.1, 130.0, 129.7, 125.5, 124.4, 123.6, 123.1, 115.5, 114.1, 112.3, 109.4, 48.92, 41.83, 17.14, 14.03. HRMS: (ESI) *m/z* calcd for $C_{21}H_{27}N_4O_3S$: 415.1804 $[M+H]^+$; found: 415.

(*S*)-2-Amino-*N*-(3-(3-(piperidin-1-ylsulfonyl)phenyl)-1*H*-indol-5-yl) propenamide (14). The title compound was prepared according to the general procedures I and B using the aryl-indole **42** (49 mg, 0.088 mmol) and TFA (0.9 mL) followed by treatment with (*tert*-butoxycarbonyl)-L-alanine (20 mg, 0.11 mmol), PyBOP (55 mg, 0.44 mmol), DIPEA (0.08 mL, 0.11 mmol), and dry DMF (0.9 mL). Final Boc-deprotection of the crude mixture using TFA (0.9 mL) and subsequent purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semipreparative column (5 µm, 100 Å, 10 × 250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→100) %) solvent B over 15 min, t_R = 6.7 min) afforded the TFA salt of **14** as a colorless solid (7 mg, 18%). ^{1}H NMR: (400 MHz, CD_3CN) δ 9.69 (s, 1H), 9.06 (s, 1H), 8.28 (s, 1H), 8.00 (d, *J* = 2.0 Hz, 1H), 7.92 (d, *J* = 7.6 Hz, 1H), 7.70–7.58 (m, 3H), 7.48 (d, *J* = 8.7 Hz, 1H), 7.27 (dd, *J* = 8.6, 2.0 Hz, 1H), 4.19 (q, *J* = 7.0 Hz, 1H), 3.03 (t, *J* = 5.7 Hz, 4H), 1.64 (quint, *J* = 5.7 Hz, 4H), 1.59 (d, *J* = 7.0 Hz, 3H), 1.45-1.37 (m, 2H). ^{13}C NMR: (150 MHz, [D_6] DMSO) δ (ppm) = 167.7, 137.0, 136.0, 134.1, 131.4, 130.6, 130.0, 125.9, 124.5, 124.4, 124.0, 115.5, 114.1, 112.5, 109.3, 49.11, 46.90, 24.82, 22.98, 17.39. HRMS: (ESI) *m/z* calcd for $C_{22}H_{27}N_4O_3S$: 427.1804 $[M+H]^+$; found: 427.1753.

(*S*)-2-Amino-*N*-(3-(3-(pyrrolidin-1-ylsulfonyl)phenyl)-1*H*-indol-5-yl) propanamide (15). The title compound was prepared according to general procedure B using the aryl-indole **45** (27 mg, 0.06 mmol), (*tert*-butoxycarbonyl)-L-alanine (11 mg, 0.06 mmol), PyBOP (31 mg, 0.06 mmol), DIPEA (0.08 mL, 0.30 mmol), and dry DMF (0.6 mL). RP-HPLC (gradient: 2–50 shortprep, t_R = 8.99 min) of the crude deprotected product afforded the TFA salt of **15** as a colorless solid (11 mg, 34%). ^{1}H NMR: (500 MHz, CD_3OD) δ (ppm) = 8.29 (s, 1H), 8.10 (s, 1H), 7.94 (d, *J* = 7.6 Hz, 1H), 7.68 (d, *J* = 7.7 Hz, 1H), 7.644 (s,

1H), 7.636 (dd, J=7.7, 7.6 Hz, 1H), 7.45 (d, J=8.7 Hz, 1H), 7.27 (dd, J=8.7 Hz, 1H), 4.09 (q, J=7.0 Hz, 1H), 3.34–3.30 (m, 4H), 1.79–1.75 (m, 4H), 1.63 (d, J=7.0 Hz, 3H). ^{13}C NMR: (125 MHz, CD_3OD) δ (ppm)=169.0, 138.8, 138.3, 136.2, 132.1, 132.0, 130.7, 126.4, 126.3, 125.9, 125.3, 117.3, 116.6, 113.2, 111.9, 50.9, 49.4, 26.2, 17.8. HRMS: (ESI) m/z calcd for $C_{21}H_{24}N_4O_3S$: 413.1642 $[M+H]^+$; found: 413.1643.

(*S*)-2-Amino-*N*-(3-(3-(isopropylsulfonyl)phenyl)-1*H*-indol-5-yl) propanamide (16). The title compound was prepared according to general procedure B using the aryl-indole **48** (56 mg, 0.14 mmol), (*tert*-butoxycarbonyl)-L-alanine (30 mg, 0.16 mmol), PyBOP (85 mg, 0.16 mmol), DIPEA (0.18 mL, 0.68 mmol), and dry DMF (1.4 mL). Purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semi-preparative column (5 μm, 100 Å, 10×250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→100) % solvent B over 15 min, t_R= 6.11 min) of the crude deprotected product afforded the TFA salt of **16** as a colorless solid (10 mg, 15%). ^{1}H NMR: (600 MHz, CD_3CN) δ (ppm)=9.71 (s, 1H), 9.31 (s, 1H), 8.19 (d, J=2.0 Hz, 1H), 8.07 (t, J= 1.8 Hz, 1H), 7.95 (ddd, J=7.7, 1.5, 1.4 Hz, 1H), 7.71 (ddd, J=7.9, 1.5, 1.4 Hz, 1H), 7.65 (dd, J=7.9, 7.7 Hz, 1H), 7.63 (d, J=2.7 Hz, 1H), 7.44 (d, J=8.7 Hz, 1H), 7.33 (dd, J=8.7, 2.0 Hz, 1H), 4.24 (q, J=7.1 Hz, 1H), 3.35 (septet, J=6.8 Hz, 1H), 1.60 (d, J=7.1 Hz, 3H), 1.26 (d, J= 6.8 Hz, 6H). ^{13}C NMR: (150 MHz, CD_3CN) δ (ppm)=168.5, 138.7, 137.9, 135.2, 132.6, 132.3, 130.6, 127.4, 126.6, 125.9, 125.8, 116.9, 116.0, 113.2, 110.9, 55.9, 50.9, 17.6, 15.88, 15.87. HRMS: (ESI) m/z calcd for $C_{20}H_{23}N_3O_3S$: 386.1533 $[M+H]^+$; found: 386.1538.

Synthesis of sulfone (17). The title compound was prepared according to general procedure B using the aryl-indole **51** (45 mg, 0.10 mmol), (*tert*-butoxycarbonyl)-L-alanine (19 mg, 0.10 mmol), PyBOP (52 mg, 0.10 mmol), DIPEA (0.14 mL, 0.50 mmol), and dry DMF (1.0 mL). RP-HPLC (gradient: 2–50 shortprep, t_R=7.52 min) of the crude deprotected product afforded the TFA salt of **17** as a colorless solid (27 mg, 50%). ^{1}H NMR: (500 MHz, CD_3CN) δ (ppm)= 9.74 (s, 1H), 9.18 (s, 1H), 8.17 (d, J=1.9 Hz, 1H), 8.09 (dd, J=1.8, 1.4 Hz, 1H), 7.93 (dd, J=7.8, 1.4, 1.4 Hz, 1H), 7.72 (dd, J=7.9, 1.4, 1.4 Hz, 1H), 7.64 (dd, J=7.9, 7.8 Hz, 1H), 7.62 (d, J=2.6 Hz, 2H), 7.45 (d, J=8.7 Hz, 1H), 7.30 (dd, J=8.8, 2.4 Hz, 1H), 4.23 (q, J=7.0 Hz, 1H), 3.68 (tt, J=8.9, 7.0 Hz, 1H), 2.04–1.96 (m, 2H), 1.91–1.82 (m, 2H), 1.73–1.66 (m, 2H), 1.63–1.55 (m, 5H). ^{13}C NMR: (125 MHz, CD_3CN) δ (ppm)=168.6, 140.7, 138.1, 135.3, 132.5, 132.2, 130.7, 126.9, 126.1, 126.0, 125.9, 117.1, 116.1, 113.2, 111.2, 64.6, 51.0, 28.0, 27.9, 26.6, 17.6. HRMS: (ESI) m/z calcd for $C_{22}H_{25}N_3O_3S$: 412.1689 $[M+H]^+$; found: 412.1706.

(S)-2-Amino-*N*-(3-(3-(cyclopentylsulfonyl)phenyl)-1*H*-indol-5-yl) propanamide (18). The title compound was prepared according to general procedure B using the aryl-indole **33** (100 mg, 0.25 mmol), (*tert*-butoxycarbonyl)-L-proline (65 mg, 0.30 mmol), PyBOP (156 mg, 0.30 mmol), DIPEA (0.33 mL, 1.25 mmol), and dry DMF (2.5 mL). Purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semi-preparative column (5 μm, 100 Å, 10×250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→100) % solvent B over 15 min, t_R= 5.88 min) of the crude deprotected product afforded the TFA salt of **18a** as a pale yellow solid (21 mg, 17%). ^{1}H NMR: (600 MHz, CD_3OD) δ (ppm)=8.25 (d, J=1.9 Hz, 1H), 8.22 (dd, J=1.9, 1.9 Hz, 1H), 7.99 (ddd, J=7.7, 1.9, 1.8 Hz, 1H), 7.80 (ddd, J=7.6, 1.9 1.8 Hz, 1H), 7.67 (s, 1H), 7.65 (dd, J=7.7, 7.6 Hz, 1H), 7.45 (d, J=8.7 Hz, 1H), 7.34 (dd, J=8.7, 1.9 Hz, 1H), 4.43 (dd, J=7.7, 7.7 Hz, 1H), 3.48 (ddd, J=11.4, 7.1, 7.0 Hz, 1H), 3.39 (ddd, J=11.4, 71, 7.0 Hz, 1H), 3.19 (s, 3H), 2.55 (dddd, J=13.6, 7.1, 7.0, 7.0 Hz, 1H), 2.26–2.04 (m, 3H). ^{13}C NMR: (150 MHz, CD_3OD) δ (ppm)=167.7, 142.5, 139.1, 136.2, 132.9, 132.1, 131.0, 126.3, 126.0, 126.0, 124.9, 117.2, 116.4, 113.2, 111.8, 61.7, 47.5, 44.5, 31.2, 25.2. HRMS: (ESI) m/z calcd for $C_{20}H_{21}N_3O_3S$: 384.1376 $[M+H]^+$; found: 384.1386.

(S)-*N*-(3-(3-(Isopropylsulfonyl)phenyl)-1*H*-indol-5-yl)-2-(meth-ylamino)propanamide (19). The title compound was prepared according to general procedure B using the aryl-indole **48** (50 mg, 0.12 mmol), *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanine (25 mg, 0.12 mmol), PyBOP (63 mg, 0.12 mmol), DIPEA (0.16 mL, 0.61 mmol), and dry DMF (1.22 mL). RP-HPLC (gradient: 2–30 shortprep, t_R=11.84 min) of the crude deprotected product afforded the TFA salt of **18b** as a colorless solid (19 mg, 30%). ^{1}H NMR: (600 MHz, CD_3CN) δ (ppm)=9.86 (s, 1H), 9.39 (s, 1H), 8.19 (s, 1H), 8.07 (d, J=1.8 Hz, 1H), 7.95 (dd, J=7.6, 1.7 Hz, 1H), 7.70 (dd, J=7.8, 1.7 Hz, 1H), 7.65 (dd, J=7.8, 7.6 Hz, 1H), 7.62 (d, J=2.5 Hz, 1H), 7.46 (d, J=8.6 Hz, 1H), 7.32 (d, J=8.7 Hz, 1H), 4.06 (q, J= 7.0 Hz, 1H), 3.33 (*septet*, J=6.7 Hz, 1H), 2.68 (s, 3H), 1.59 (d, J= 6.8 Hz, 3H), 1.25 (d, J=6.8 Hz, 6H). ^{13}C NMR: (150 MHz, CD_3CN) δ (ppm)=167.8, 138.7, 137.9, 135.3, 132.7, 132.0, 130.6, 127.4, 126.6, 126.0, 125.8, 117.1, 116.0, 113.2, 111.3, 58.8, 56.0, 32.1, 16.3, 15.91, 15.90. HRMS: (ESI) m/z calcd for $C_{21}H_{25}N_3O_3S$: 400.1689 $[M+H]^+$; found: 400.1703.

(S)-*N*-(3-(3-(Isopropylsulfonyl)phenyl)-1*H*-indol-5-yl)azetidine-2-carboxamide (20). The title compound was prepared according to general procedure B using the aryl-indole **48** (50 mg, 0.122 mmol), *N*-Boc-L-azetidine-2-carboxylic acid (25 mg, 0.122 mmol), PyBOP (63 mg, 0.122 mmol), DIPEA (0.16 mL, 0.61 mmol), and dry DMF (1.22 mL). Purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semipreparative column (5 μm, 100 Å, 10×250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→30) % solvent B over 15 min, t_R=11.69 min) of the crude deprotected product afforded the TFA salt of **18c** as a colorless solid (14 mg, 23%). ^{1}H NMR: (600 MHz, CD_3CN) δ (ppm)=9.81 (s, 1H), 9.36 (s, 1H), 8.21 (s, 1H), 8.08 (s, 1H), 7.96 (d, J=7.5 Hz, 1H), 7.72 (d, J=7.7 Hz, 1H), 7.66 (dd, J=7.7, 7.5 Hz, 1H), 7.63 (s, 1H), 7.47 (d, J=8.6 Hz, 1H), 7.32 (d, J= 8.7 Hz, 1H), 5.23 (dd, J=9.4, 7.7 Hz, 1H), 4.16 (q, J=9.3 Hz, 1H), 3.96 (td, J=10.1, 6.4 Hz, 1H), 3.35 (*septet*, J=6.4 Hz, 1H), 2.82 (qd, J= 10.1, 6.5 Hz, 1H), 2.64 (dt, J=18.6, 8.4 Hz, 1H), 1.27 (d, J=6.5 Hz, 6H). ^{13}C NMR: (150 MHz, CD_3CN) δ (ppm)=166.4, 138.8, 138.0, 135.3, 132.7, 132.2, 130.6, 127.4, 126.6, 126.0, 125.9, 116.9, 116.1, 113.3, 111.0, 59.6, 56.0, 44.7, 24.0, 15.9. HRMS: (ESI) m/z calcd for $C_{21}H_{23}N_3O_3S$: 398.1533 $[M+H]^+$; found: 398.1564.

(S)-*N*-(3-(3-(*N*,*N*-Dimethylsulfamoyl)phenyl)-1*H*-indol-5-yl) azetidine-2-carboxamide (21). The title compound was prepared according to general procedure B using the aryl indole **52** (58 mg, 0.14 mmol), *N*-Boc-L-azetidine-2-carboxylic acid (27 mg, 0.14 mmol), PyBOP (70 mg, 0.14 mmol), DIPEA (0.179 mL, 0.68 mmol) and dry DMF (1.45 mL). Purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semipreparative column (5 μm, 100 Å, 10×250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→50) % solvent B over 15 min, t_R=8.60 min) of the crude deprotected product afforded the TFA salt of **18d** as a colorless solid (14 mg, 23%) (14 mg, 20%). ^{1}H NMR: (400 MHz, CD_3CN) δ (ppm)=δ 9.77 (s, 1H), 9.06 (s, 1H), 8.27 (d, J=1.8 Hz, 1H), 8.01 (t, J=1.7, 1H), 7.96–7.86 (m, 1H), 7.70–7.59 (m, 3H), 7.44 (d, J=8.7 Hz, 1H), 7.26 (dd, J=8.7, 2.0 Hz, 1H), 5.16 (t, J=8.6 Hz, 1H), 4.31–3.82 (m, 2H), 2.91–2.76 (m, 1H), 2.74 (s, 6H), 2.72–2.55 (m, 1H). ^{13}C NMR: (150 MHz, CD_3CN) δ (ppm)=137.9, 136.6, 135.2, 132.3, 132.0, 130.7, 126.4, 126.0, 125.7, 116.7, 113.3, 110.7, 59.8, 44.7, 41.3, 38.7, 24.6. *^{13}C=O not observed. HRMS: (ESI) m/z calcd for $C_{20}H_{23}N_4O_3S$: 399.1485 $[M+H]^+$; found: 399.1490.

(S)-*N*-(3-(3-(*N*,*N*-Dimethylsulfamoyl)phenyl)-1*H*-indol-5-yl)-2-(methylamino)propanamide (22). The title compound was prepared according to general procedure B using the aryl-indole **52** (50 mg, 0.116 mmol), *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-alanine (24 mg, 0.116 mmol), PyBOP (61 mg, 0.116 mmol), DIPEA (0.15 mL, 0.58 mmol), and dry DMF (1.2 mL). Purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semipreparative column (5 μm, 100 Å,

10×250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→30) % solvent B over 15 min, t_R = 11.84 min) of the crude deprotected product afforded the TFA salt of **18e** as a colorless solid (20 mg, 34%). ^{1}H NMR: (500 MHz, CD_3OD) δ (ppm) = 8.31 (d, J = 2.0 Hz, 1H), 8.06 (dd, J = 1.8 Hz, 1H), 7.96 (ddd, J = 7.2, 1.8, 1.7 Hz, 1H), 7.70–7.61 (m, 3H), 7.45 (d, J = 8.7 Hz, 1H), 7.27 (dd, J = 8.7, 2.0 Hz, 1H), 3.87 (q, J = 7.0 Hz, 1H), 2.77 (s, 6H), 2.70 (s, 3H), 1.60 (d, J = 7.0 Hz, 3H). ^{13}C NMR: (150 MHz, CD_3OD) δ (ppm) = 169.14, 138.8, 136.8, 136.2, 132.2, 132.0, 130.7, 126.6, 126.4, 125.9, 125.6, 117.1, 116.5, 113.2, 111.8, 59.3, 38.6, 32.2, 16.9. HRMS: (ESI) m/z calcd for $C_{20}H_{24}N_4O_3S$: 401.1642 $[M+H]^+$; found: 401.1655.

2-Amino-*N*-(3-(3-(*N*,*N*-dimethylsulfamoyl)phenyl)-1*H*-indol-5-yl) acetamide (23). The title compound was prepared according to general procedure B using the aryl-indole **52** (45 mg, 0.10 mmol), (*tert*-butoxycarbonyl)glycine (18 mg, 0.10 mmol), PyBOP (54 mg, 0.10 mmol), DIPEA (0.14 mL, 0.52 mmol), and dry DMF (1.0 mL). RP-HPLC (gradient: 2–50 shortprep, t_R = 10.38 min) of the crude deprotected product afforded the TFA salt of **18f** as a colorless solid (13 mg, 25%). ^{1}H NMR: (500 MHz, CD_3OD:CD_3CN 1:1, calibrated to CD_3OD) δ (ppm) = 8.32 (d, J = 2.0 Hz, 1H), 8.07 (s, 1H), 7.98 (d, J = 7.2 Hz, 1H), 7.72–7.65 (m, 3H), 7.50 (d, J = 8.7 Hz, 1H), 7.29 (dd, J = 8.7, 2.0 Hz, 1H), 3.85 (s, 2H), 2.78 (s, 6H). ^{13}C NMR: (125 MHz, CD_3OD:CD_3CN 1:1, calibrated to CD_3OD) δ (ppm) = 164.9, 138.3, 136.7, 135.6, 132.13, 132.07, 130.7, 126.4, 126.12, 126.08, 125.6, 116.9, 116.2, 113.3, 111.2, 42.0, 38.6. HRMS: (ESI) m/z calcd for $C_{18}H_{20}N_4O_3S$: 373.1329 $[M+H]^+$; found: 373.1343.

2-Amino-*N*-(3-(3-(*N*,*N*-dimethylsulfamoyl)phenyl)-1*H*-indol-5-yl)-2-methylpropanamide (24). The title compound was prepared according to general procedure B using the aryl-indole **52** (50 mg, 0.116 mmol), N-Boc-α-methyl alanine (24 mg, 0.116 mmol), PyBOP (601 mg, 0.116 mmol), DIPEA (0.15 mL, 0.58 mmol), and dry DMF (1.2 mL). RP-HPLC (gradient: 2–50 shortprep, t_R = 8.50 min) of the crude deprotected product afforded the TFA salt of **18g** as a colorless solid (17 mg, 28%). ^{1}H NMR: (500 MHz, CD_3CN) δ (ppm) = 9.75 (s, 1H), 8.75 (s, 1H), 8.20 (d, J = 1.9 Hz, 1H), 8.01 (dd, J = 1.7, 1.6 Hz, 1H), 7.95 (ddd, J = 7.3, 1.7, 1.6 Hz, 1H), 7.71–7.60 (m, 3H), 7.50 (d, J = 8.7 Hz, 1H), 7.34 (dd, J = 8.7, 1.9 Hz, 1H), 2.73 (s, 6H), 1.72 (s, 6H). ^{13}C NMR: (125 MHz, CD_3CN) δ (ppm) = 170.6, 137.8, 136.5, 135.4, 131.9, 131.8, 130.6, 126.3, 125.9, 125.8, 125.6, 117.9, 116.2, 113.1, 112.2, 59.2, 38.6, 24.1. HRMS: (ESI) m/z calcd for $C_{20}H_{24}N_4O_3S$: 401.1642 $[M+H]^+$; found: 401.1652.

1-Amino-*N*-(3-(3-(*N*,*N*-dimethylsulfamoyl)phenyl)-1*H*-indol-5-yl) cyclopropane-1-carboxamide (25). The title compound was prepared according to general procedure B using the aryl-indole **52** (50 mg, 0.116 mmol), 1-((*tert*-butoxycarbonyl)amino)cyclopropane-1-carboxylic acid (23 mg, 0.116 mmol), PyBOP (61 mg, 0.116 mmol), DIPEA (0.15 mL, 0.58 mmol), and dry DMF (1.16 mL). RP-HPLC (gradient: 2–50 shortprep, t_R = 8.43 min) of the crude deprotected product afforded the TFA salt of **18h** as a colorless solid (16 mg, 27%). ^{1}H NMR: (500 MHz, CD_3CN) δ (ppm) = 9.68 (s, 1H), 8.12 (d, J = 1.9 Hz, 1H), 8.01 (dd, J = 1.7, 1.7 Hz, 1H), 7.93 (ddd, J = 7.2, 1.8, 1.7 Hz, 1H), 7.89 (s, 1H), 7.70–7.62 (m, 3H), 7.49 (d, J = 8.7 Hz, 1H), 7.26 (dd, J = 8.8, 2.0 Hz, 1H), 2.74 (s, 6H), 1.74–1.68 (m, 2H), 1.60–1.54 (m, 2H). ^{13}C NMR: (125 MHz, CD_3CN) δ (ppm) = 167.1, 136.9, 135.8, 134.6, 131.0, 130.3, 129.7, 125.4, 125.0, 124.9, 124.7, 118.2, 115.3, 112.2, 111.7, 37.6, 36.6, 12.4. HRMS: (ESI) m/z calcd for $C_{20}H_{22}N_4O_3S$: 399.1485 $[M+H]^+$; found: 399.1495.

***N*1-(3-(3-(Methylsulfonyl)phenyl)-1*H*-indol-5-yl)ethane-1,2-diamine (26)**. To a stirred solution of the aryl-indole **33** (35 mg, 0.12 mmol, 2.0 equiv.) in water (*ca.* 0.15 mL, 2.0 M) was added 2-bromoethan-1-amine hydrochloride (13 mg, 0.061 mmol, 1.0 equiv.) at room temperature. The reaction mixture was then stirred at 95 °C for 22 h and cooled to room temperature. The reaction mixture was then diluted with water and extracted with EtOAc (3x). The remaining aqueous layer was purified by RP-HPLC (gradient: 2–30 shortprep, t_R = 8.33 min) to afford the TFA salt of **19a** as a brown gum (4 mg, 13%). ^{1}H NMR: (500 MHz, CD_3CN) δ (ppm) = 9.47 (s, 1H), 8.17 (dd, J = 1.9, 1.4 Hz, 1H), 8.01 (ddd, J = 7.8, 1.4, 1.1 Hz, 1H), 7.76 (ddd, J = 7.8, 1.9, 1.1 Hz, 1H), 7.67 (dd, J = 7.8, 7.8 Hz, 1H), 7.57 (d, J = 2.6 Hz, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.16 (s, 1H), 6.75 (d, J = 8.5 Hz, 1H), 3.49 (t, J = 5.9 Hz, 2H), 3.22 (t, J = 5.9 Hz, 2H), 3.12 (s, 3H).

***N*1-Methyl-*N*2-(3-(3-(methylsulfonyl)phenyl)-1*H*-indol-5-yl)ethane-1,2-diamine (27)**. To a stirred solution of the aryl-indole **33** (22 mg, 0.077 mmol, 1.0 equiv.) in 1,2-dichloroethane (0.77 mL, 0.1 M) was added *tert*-butyl methyl(2- oxoethyl)carbamate (13 mg, 0.077 mmol, 1.0 equiv.) at room temperature. The reaction mixture was then stirred at room temperature for 30 minutes, after which $NaBH(OAc)_3$ (24 mg, 0.12 mmol, 1.5 equiv.) was added. The solution was then stirred for 18 h, diluted with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3x). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude protected indole (brown gum) which was used in the next step without further purification. The crude protected product was dissolved in TFA (neat, 0.77 mL, 0.1 M) and stirred at room temperature for 30 min. The reaction mixture was then concentrated under reduced pressure to afford the crude product, which was purified by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semipreparative column (5 μm, 100 Å, 10×250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→30) % solvent B over 15 min, t_R = 8.95 min) to afford the TFA salt of **19b** as a brown gum (3 mg, 8%). ^{1}H NMR: (600 MHz, CD_3OD) δ (ppm) = 8.26 (s, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.68 (dd, J = 7.8, 7.8 Hz, 1H), 7.58 (s, 1H), 7.34 (d, J = 8.7 Hz, 1H), 7.23 (d, J = 2.1 Hz, 0H), 6.80 (dd, J = 8.7, 2.1 Hz, 1H), 3.53 (t, J = 6.0 Hz, 2H), 3.31 (t, J = 6.1 Hz, 2H), 3.19 (s, 3H), 2.78 (s, 3H). ^{13}C NMR: (150 MHz, CD_3OD) δ (ppm) = 143.0, 142.4, 139.7, 133.6, 132.6, 131.0, 127.1, 125.8, 125.1, 124.5, 115.4, 113.9, 113.8, 102.5, 49.6, 44.4, 42.9, 33.7. HRMS: (ESI) m/z calcd for $C_{18}H_{21}N_3O_2S$: 344.1427 $[M+H]^+$; found: 344.1427.

(*S*)-2-Amino-*N*-(3-(3-(*N*,*N*-dimethylsulfamoyl)phenyl)-1-methyl-1*H*-indol-5-yl)propanamide (28). The title compound was prepared according to general procedure B using the aryl-indole **55** (28 mg, 0.080 mmol), (*tert*-butoxycarbonyl)-L-alanine (18 mg, 0.095 mmol), PyBOP (50 mg, 0.38 mmol), DIPEA (0.070 mL, 0.095 mmol), and dry DMF (0.8 mL) followed by Boc-deprotection using TFA (0.7 mL). Purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semi-preparative column (5 μm, 100 Å, 10×250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→100) %) solvent B over 15 min, t_R = 6.5 min) of the crude deprotected product afforded the TFA salt of **20** as a colorless solid (10 mg, 15%). ^{1}H NMR: (400 MHz, CD_3CN) δ (ppm) = 9.23 (s, 1 H), 8.23 (d, J = 1.6 Hz, 1H), 7.96 (br s, 1H), 7.87 (dt, J = 7.4, 1.6 Hz, 1H), 7.63 (t, J = 7.4 Hz, 1H), 7.60 (t, J = 1.6 Hz, 1H), 7.55 (s, 1H), 7.35 (dq, J = 8.9, 0.8 Hz, 1H), 7.29 (dd, J = 8.9, 1.9 Hz, 1H), 4.24 (q, J = 7.0 Hz, 1H), 3.79 (s, 3H), 2.71 (s, 6H), 1.58 (d, J = 7.0 Hz, 3H). ^{13}C NMR: (150 MHz, CD_3CN) δ (ppm) = 168.6, 137.7, 136.6, 136.0, 132.2, 131.6, 130.6, 130.2, 126.2, 126.1, 125.4, 116.8, 114.9, 111.4, 111.3, 50.96, 38.60, 33.47, 17.62. HRMS: (ESI) m/z calcd for $C_{21}H_{24}N_4O_3S$: 401.1647 $[M+H]^+$; found: 401.1602.

(*R*)-2-Amino-*N*-(3-(3-(*N*,*N*-dimethylsulfamoyl)phenyl)-1-methyl-1*H*-indol-5-yl)-3-fluoropropanamide (29). To a solution of **61** (12 mg, 0.02 mmol, 1 equiv.) in dry CH_2Cl_2 (200 μL), TFA (200 μL, 2.6 mmol, 131 equiv.) was added. After 1 h at room temperature, the solvents were evaporated. Purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semipreparative column (5 μm, 100 Å, 10× 250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→100) %) solvent B over 15 min, t_R = 6.60 min) afforded the TFA salt of **29** as a

colourless solid (3 mg, 24%). ^{1}H NMR: (400 MHz, CD_3CN) δ (ppm) = 9.44 (s, 1H), 8.24 (s, 1H), 7.98 (s, 1H), 7.91–7.89 (m, 1H), 7.66–7.58 (m, 3H), 7.40 (d, J = 8.7 Hz, 1H), 7.32 (d, J = 8.8 Hz, 1H), 4.96 (d, J = 46.5 Hz, 2H), 4.46 (s, 1H), 3.82 (s, 3H), 2.72 (s, 6H). ^{19}F NMR: (376 MHz, CD_3CN) δ (ppm) = −231.3 (s, 1F). HRMS: (ESI) *m/z* calcd for $C_{20}H_{23}FN_4O_3S$: 419.1548 $[M+H]^+$; found: 419.1565.

(S)-3-(5-(2-Aminopropanamido)-1-methyl-1*H*-indol-3-yl)-*N*,*N*-dimethylbenzamide (30). The title compound was prepared according to general procedure B using the aryl-indole **64** (14 mg, 0.048 mmol), (*tert*-butoxycarbonyl)-L-alanine (11 mg, 0.057 mmol), PyBOP (30 mg, 0.24 mmol), DIPEA (0.041 mL, 0.057 mmol), and dry DMF (0.5 mL) followed by Boc-deprotection using TFA (0.5 mL). Purification by RP-HPLC (using a SiliCycle SiliaChrom dtC18 semi-preparative column (5 μm, 100 Å, 10×250 mm) with a flow rate of 5 mL/min eluting with solvent (A: 0.1% TFA in water B: 0.1% TFA in MeCN) on a gradient of (2→100) %) solvent B over 15 min, t_R = 5.95 min) of the crude deprotected product afforded the TFA salt of **22** as a colorless solid (7 mg, 40%). ^{1}H NMR: (400 MHz, CD_3CN) δ (ppm) = 9.10 (s, 1H), 8.22 (s, 1H), 7.67 (d, J = 7.3, 2H), 7.49 (s, 1H), 7.46 (t, J = 7.9 Hz, 1H), 7.38 (d, J = 8.8 Hz, 1H), 7.29 (br d, J = 8.8 Hz, 1H), 7.25 (br d, J = 7.6 Hz, 1H), 4.19 (q, J = 6.4 Hz, 1H), 3.80 (s, 3H), 3.05 (br s, 3H), 2.99 (br s, 3H), 1.58 (d, J = 6.4 Hz, 3H). ^{13}C NMR: (150 MHz, CD_3CN) δ (ppm) = 172.1, 168.6, 138.4, 136.7, 135.9, 131.9, 129.8, 129.6, 128.5, 126.4, 126.0, 125.0, 116.6, 116.0, 111.6, 111.3, 51.02, 39.89, 35.39, 33.50, 30.20, 17.57. HRMS: (ESI) *m/z* calcd for $C_{21}H_{24}N_4O_2$ 365.4565 $[M+H]^+$; found: 365.1915.

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Conflict of Interest

The authors declare no conflict of interest.

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[1] B. T. Schurter, S. S. Koh, D. Chen, G. J. Bunick, J. M. Harp, B. L. Hanson, A. Henschen-Edman, D. R. Mackay, M. R. Stallcup, D. W. Aswad, *Biochemistry* **2001**, *40*, 5747–5756.
[2] W. An, J. Kim, R. G. Roeder, *Cell* **2004**, *117*, 735–748.
[3] D. Cheng, J. Cote, S. Shaaban, M. T. Bedford, *Mol. Cell* **2007**, *25*, 71–83.
[4] S. El Messaoudi, E. Fabbrizio, C. Rodriguez, P. Chuchana, L. Fauquier, D. Cheng, C. Theillet, L. Vandel, M. T. Bedford, C. Sardet, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 13351–13356.
[5] Y. H. Lee, M. R. Stallcup, *Cell Cycle* **2011**, *10*, 1343–1344.
[6] S. L. Chen, K. A. Loffler, D. Chen, M. R. Stallcup, G. E. Muscat, *J. Biol. Chem.* **2002**, *277*, 4324–4333.
[7] H. Wei, R. Mundade, K. C. Lange, T. Lu, *Cell Cycle* **2014**, *13*, 32–41.
[8] J. Fuhrmann, K. W. Clancy, P. R. Thompson, *Chem. Rev.* **2015**, *115*, 5413–5461.
[9] Y. R. Kim, B. K. Lee, R. Y. Park, N. T. Nguyen, J. A. Bae, D. D. Kwon, C. Jung, *BMC Cancer* **2010**, *10*, 197.
[10] H. Hong, C. Kao, M. H. Jeng, J. N. Eble, M. O. Koch, T. A. Gardner, S. Zhang, L. Li, C. X. Pan, Z. Hu, G. T. MacLennan, L. Cheng, *Cancer* **2004**, *101*, 83–89.
[11] S. Frietze, M. Lupien, P. A. Silver, M. Brown, *Cancer Res.* **2008**, *68*, 301–306.
[12] H. O. Habashy, E. A. Rakha, I. O. Ellis, D. G. Powe, *Breast Cancer Res. Treat.* **2013**, *140*, 307–316.
[13] L. Wang, Z. Zhao, M. B. Meyer, S. Saha, M. Yu, A. Guo, K. B. Wisinski, W. Huang, W. Cai, J. W. Pike, M. Yuan, P. Ahlquist, W. Xu, *Cancer Cell* **2014**, *25*, 21–36.
[14] K. Nakayama, M. M. Szewczyk, C. Dela Sena, H. Wu, A. Dong, H. Zeng, F. Li, R. F. de Freitas, M. S. Eram, M. Schapira, Y. Baba, M. Kunitomo, D. R. Cary, M. Tawada, A. Ohashi, Y. Imaeda, K. S. Saikatendu, C. E. Grimshaw, M. Vedadi, C. H. Arrowsmith, D. Barsyte-Lovejoy, A. Kiba, D. Tomita, P. J. Brown, *Oncotarget* **2018**, *9*, 18480–18493.
[15] A. E. Drew, O. Moradei, S. L. Jacques, N. Rioux, A. P. Boriack-Sjodin, C. Allain, M. P. Scott, L. Jin, A. Raimondi, J. L. Handler, H. M. Ott, R. G. Kruger, M. T. McCabe, C. Sneeringer, T. Riera, G. Shapiro, N. J. Waters, L. H. Mitchell, K. W. Duncan, M. P. Moyer, R. A. Copeland, J. Smith, R. Chesworth, S. A. Ribich, *Sci. Rep.* **2017**, *7*, 17993.
[16] Y. Shen, M. M. Szewczyk, M. S. Eram, D. Smil, H. U. Kaniskan, R. F. de Freitas, G. Senisterra, F. Li, M. Schapira, P. J. Brown, C. H. Arrowsmith, D. Barsyte-Lovejoy, J. Liu, M. Vedadi, J. Jin, *J. Med. Chem.* **2016**, *59*, 9124–9139.
[17] M. S. Eram, Y. Shen, M. Szewczyk, H. Wu, G. Senisterra, F. Li, K. V. Butler, H. U. Kaniskan, B. A. Speed, C. Dela Sena, A. Dong, H. Zeng, M. Schapira, P. J. Brown, C. H. Arrowsmith, D. Barsyte-Lovejoy, J. Liu, M. Vedadi, J. Jin, *ACS Chem. Biol.* **2016**, *11*, 772–781.
[18] H. Hu, K. Qian, M. C. Ho, Y. G. Zheng, *Expert Opin. Invest. Drugs* **2016**, *25*, 335–358.
[19] J. S. Sack, S. Thieffine, T. Bandiera, M. Fasolini, G. J. Duke, L. Jayaraman, K. F. Kish, H. E. Klei, A. V. Purandare, P. Rosettani, S. Troiani, D. Xie, J. A. Bertrand, *Biochem. J.* **2011**, *436*, 331–339.
[20] R. Ferreira de Freitas, M. S. Eram, D. Smil, M. M. Szewczyk, S. Kennedy, P. J. Brown, V. Santhakumar, D. Barsyte-Lovejoy, C. H. Arrowsmith, M. Vedadi, M. Schapira, *J. Med. Chem.* **2016**, *59*, 6838–6847.
[21] X. C. Cai, T. Zhang, E. J. Kim, M. Jiang, K. Wang, J. Wang, S. Chen, N. Zhang, H. Wu, F. Li, C. C. Dela Sena, H. Zeng, V. Vivcharuk, X. Niu, W. Zheng, J. P. Lee, Y. Chen, D. Barsyte, M. Szewczyk, T. Hajian, G. Ibanez, A. Dong, L. Dombrovski, Z. Zhang, H. Deng, J. Min, C. H. Arrowsmith, L. Mazutis, L. Shi, M. Vedadi, P. J. Brown, J. Xiang, L. X. Qin, W. Xu, M. Luo, *eLife* **2019**, *8*, e47110.
[22] L. H. Mitchell, A. E. Drew, S. A. Ribich, N. Rioux, K. K. Swinger, S. L. Jacques, T. Lingaraj, P. A. Boriack-Sjodin, N. J. Waters, T. J. Wigle, O. Moradei, L. Jin, T. Riera, M. Porter-Scott, M. P. Moyer, J. J. Smith, R. Chesworth, R. A. Copeland, *ACS Med. Chem. Lett.* **2015**, *6*, 655–659.
[23] C. Carpentier, R. Godbout, F. Otis, N. Voyer, *Tetrahedron Lett.* **2015**, *56*, 244–1246.
[24] C. B. De Koning, J. P. Michael, A. L. J. Rousseau, *Chem. Soc. Perkin Trans. 1* **2000**, 1705–1713.
[25] B. E. Blough, A. Landavazo, J. S. Partilla, A. M. Decker, K. M. Page, M. H. Baumann, R. B. Rothman, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4754–4758.
[26] M. Livendahl, J. Jamroskovic, S. Ivanova, P. Demirel, N. Sabouri, E. Chorell, *Chem. Eur. J.* **2016**, *22*, 13004–13009.
[27] M. Taddei, E. Cini, L. Giannotti, G. Giannini, G. Battistuzzi, D. Vignola, L. Vesci, W. Cabri, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 61–64.
[28] E. Valeur, M. Bradley, *Chem. Soc. Rev.* **2009**, *38*, 606–631.

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