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Benzenesulfonamide indole inhibitors of cytosolic phospholipase $A_2\alpha$: Optimization of in vitro potency and rat pharmacokinetics for oral efficacy

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Abstract—The synthesis and structure–activity relationship of a series of benzenesulfonamide indole inhibitors of $cPLA_2\alpha$ are described. Substitution of the benzenesulfonamide led to analogues with 50-fold improvement in potency versus the unsubstituted benzenesulfonamide lead compound. Rat pharmacokinetics in a minimal formulation was used to prioritize compounds, leading to the discovery of a potent inhibitor of $cPLA_2\alpha$ with oral efficacy in models of rat carrageenan paw edema and *Ascaris suum* airway challenge in naturally sensitized sheep.

1. Introduction

Cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$, type IVA phospholipase) catalyzes the selective release of arachidonic acid from the *sn*-2 position of glycerophospholipids to initiate the production of leukotrienes, prostaglandins and thromboxanes, all of which are inflammatory mediators. The 1-*O*-alkyl-2-OH-glycerophosphocholine fragment of the glycerophospholipid can undergo acetylation to form platelet-activating factor (PAF), another inflammatory mediator. $cPLA_2\alpha$ deficient mice are generally healthy with a defect in induction of labor that is also seen with the cyclooxygenase-1 (COX-1) deficient mice[1,2] and have slightly reduced litter size, which was more pronounced in the COX-2 deficient mice.[3,4] $cPLA_2\alpha$ deficient mice are resistant to disease in multiple models including an ova-induced anaphylaxis model of asthma,[5] a bleomycin-induced model of idiopathic pulmonary fibrosis,[1] and lipopolysaccharide (LPS) induced adult respiratory distress syndrome,[6] a collagen-induced arthritis model of rheumatoid arthritis,[7] a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced Parkinson's model,[8] a model of colon cancer in the APC mouse,[9] an ischemia reperfusion model of stroke[10] and a model of multiple sclerosis.[11]

$cPLA_2\alpha$ inhibitors have been recently reviewed by Lehr[12] and Kokotos.[13] In a recent publication,[14] Dennis and Kokotos described their work on 2-oxoamide $cPLA_2\alpha$ inhibitors. At Wyeth we have disclosed $cPLA_2\alpha$ inhibitors bearing an indole core and three pharmacophores:

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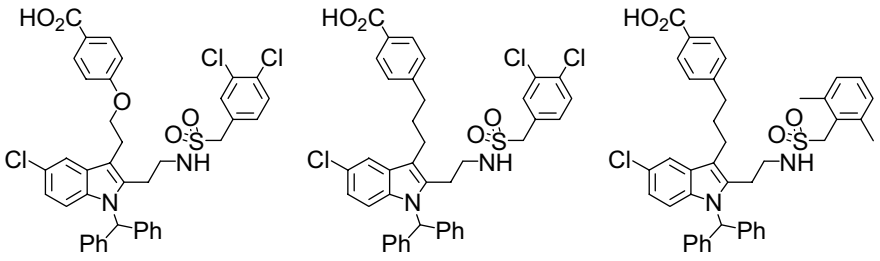
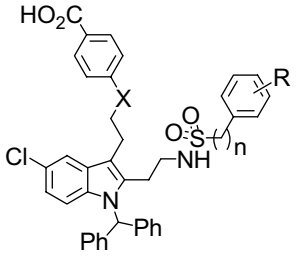


Figure 1. Examples of Wyeth indole phenylmethane sulfonamide cPLA$_2\alpha$ inhibitors.

a benzoic acid, a benzhydryl group and a sulfonamide.[15,16] Phenylmethane sulfonamide indoles **1**,[15] **2**[16] and **3**[16] (Fig. 1) all are potent inhibitors of cPLA$_2\alpha$ in our primary in vitro assays and demonstrated oral efficacy in animal models of inflammation.

The behavior of the three lead compounds and unsubstituted phenylmethane sulfonamide analogues **4** and **5** in our two primary assays and rat pharmacokinetic studies is summarized in Table 1. In the GLU micelle assay, purified human cPLA$_2\alpha$ cleaves an artificial substrate, 7-hydroxycoumarinyl-γ-linolenate (GLU), in a detergent- and lipid-rich medium, while the rat whole blood (RWB) assay utilizes pooled rat blood stimulated with A23187, a calcium ionophore.[15] These compounds demonstrate structure–activity relationship trends that we have observed earlier[16] for this class of indole cPLA$_2\alpha$ inhibitors, for example, that at C3, the propyl linker is preferred to the ethoxy linker, and that at C2, *ortho* substitution of the sulfonamide phenylmethane moiety leads to increased potency. The pharmacokinetic data of these compounds are also representative: the rat iv clearance of compounds with the C3 propyl linker is typically lower than that of the corresponding ethoxy analogue (**2** vs **1**, **5** vs **4**). These compounds are highly lipophilic and have poor aqueous solubility, and the oral bioavailability (*F*) of these inhibitors is poor, particularly in a minimal formulation such as 0.5% methyl cellulose (MC)/2% Tween (TW) 80. Use of a lipid-based formulation (55.5% Phosal 53 medium chain triglyceride (MCT), 5.6% Tween 80, 16.7% Labrasol and 22.2% propylene carbonate, in which the compound was dissolved at 37.5 mg compound per mL vehicle, diluted with water where necessary, and dosed at 4 mL/kg) led to improved oral bioavailability for the three lead compounds (**1**, **2**, and **3**). This Phosal formulation was employed for in vivo studies in which these phenylmethanesulfonamides displayed oral efficacy. However, because the Phosal formulation is a non-conventional, lipid-based formulation, there are some potential disadvantages. Such a vehicle requires a gel capsule, increasing the complexity and cost of production. Chronic dosing of lipid-

Table 1. In vitro potency and rat pharmacokinetic data of phenylmethane- and benzenesulfonamides



Compound	*n*	X	R	GLU IC_{50}[a] (μM)	RWB IC_{50}[a] (μM)	iv Cl[b] (mL/min/kg)	*F*[c] (%) MC/TW formulation	*F*[c] (%) Phosal formulation
1	1	O	3,4-diCl	0.15	0.16	14	6.0	12
2	1	CH_2	3,4-diCl	0.04	0.07	5.0	1.0	6.0
3	1	CH_2	2,6-diMe	0.014	0.03	4.0	1.0	3.5
4	1	O	H	0.11	0.12	69	3.9	—
5	1	CH_2	H	0.04	0.08	13	3.8[d]	7.2[e]
6	0	O	H	3.0	1.0	73	—	—

[a] Data are the average of two or more independent measurements.
[b] iv clearance (Cl) was determined at a dose of 2 mg/kg in 50% PEG-400, 50% DMSO formulation unless otherwise specified.
[c] Oral bioavailability (*F*) was determined by the dose normalized AUC ratio between oral and iv administration. A po dose of 25 mg/kg was used unless otherwise specified.
[d] po dose 5 mg/kg.
[e] po dose 10 mg/kg.

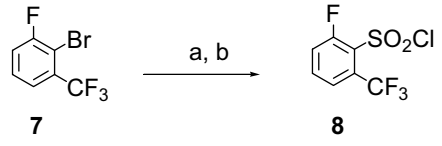
based formulations may change the plasma lipoprotein profile.[17] Because of these concerns, we sought to avoid the use of a lipid-based formulation such as Phosal, and instead to use a minimal formulation in selection of a new lead.

The focus of the work reported here is on benzenesulfonamide cPLA$_2\alpha$ inhibitors. The starting point was compound **6**, which as a low μM inhibitor in both primary assays was significantly less potent than the corresponding phenylmethane analogue (**4**). The goals of this effort were twofold: to improve the potency of the benzenesulfonamide class by exploring substitution of the benzene moiety and demonstrate oral efficacy in a minimal formulation. Compounds were triaged based on rat pharmacokinetics, that is, iv clearance (Cl) and oral exposure in a conventional MC/TW formulation; for all of these studies, the benchmark compounds were the phenylmethane lead compounds exemplified by **1**, **2** and **3**.

2. Chemistry

There are hundreds of commercially available benzenesulfonyl chlorides, and for this structure–activity study, we employed mono- or disubstituted benzenesulfonyl chlorides, mainly with *ortho* substitution. One sulfonyl chloride (**8**) was prepared by lithium–halogen exchange of bromide **7**, reaction with sulfur dioxide, and subsequent reaction with sulfuryl chloride (Scheme 1).

Scheme 2 shows the synthesis of our benzenesulfonamide cPLA$_2\alpha$ inhibitors via a two-step procedure. Sulfonylation of amino esters **9**[15] and **10**[16] with a sulfonyl chloride under Schotten–Baumann conditions and subsequent hydrolysis of the methyl ester of intermediates **11** and **12** afforded the desired products (**13** and **14**).



Scheme 1. Reagents and conditions: (a) *n*-BuLi/hexanes, SO_2, Et_2O–THF, −78 °C–rt; (b) SO_2Cl_2/CH_2Cl_2, hexanes, 0 °C–rt, 48% (two steps).

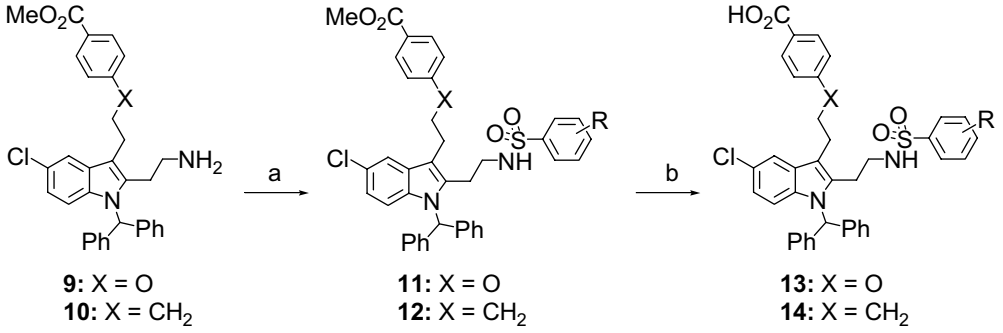
3. Results

3.1. In vitro and pharmacokinetic data

The in vitro data of the benzenesulfonamides **13a**–**k** and **14a**–**k** in the GLU micelle and RWB assays (Table 2) indicate that as with the phenylmethane sulfonamides, the propyl linker (X = CH_2) generally is preferred over the ethoxy linker (X = O). Substitution of the benzenesulfonamide led to increased potency compared to the unsubstituted parent compound **6**. The IC_{50} values of these compounds in the RWB assay ranged from 0.06 to 0.60 μM, with the more potent compounds comparing favorably to the lead compounds in the phenylmethane series (**1**, **2**, and **3**).

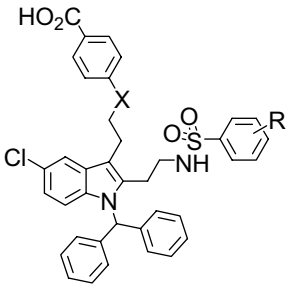
Rat pharmacokinetics of these compounds were then determined. Again, in agreement with the phenylmethane sulfonamide series, compounds bearing the propyl linker (X = CH_2) displayed lower iv clearance than their ethoxy linked (X = O) analogues. The majority of the compounds had reasonable clearance, of less than 20 mL/min/kg. Oral bioavailability (*F*) was calculated upon dosing the compounds orally using a minimal formulation of MC/TW. Most compounds showed better *F* than the more potent phenylmethane derivatives did in the same formulation (**2** and **3** displayed 1% *F* in rats). Only compounds **14d**, **13d**, **13h**, and **14k** had >10% *F*, and of these, **14k** offered the best combination of in vitro potency and pharmacokinetic parameters in rats: **13d** displayed both a higher clearance and was fivefold less potent, and **13d** and **13h** were 2- to 3-fold less potent in the RWB assay than **14k**. Thus, **14k** was further studied by pharmacokinetic determination in two formulations in rat and dog, isothermal calorimetry, selectivity assays, a human whole blood assay, and animal models of acute inflammation and asthma.

Rat and dog pharmacokinetics of **14k** and phenylmethane sulfonamide lead compound **3** were determined in both the lipid-based Phosal formulation which had been developed to increase the oral exposure of the phenylmethane sulfonamides and the minimal MC/TW



Scheme 2. Reagents and conditions: (a) $ArSO_2Cl$, satd $NaHCO_3$, CH_2Cl_2, 61–93%; (b) 1 N NaOH, MeOH, THF, 50 °C, 36–100%.

Table 2. In vitro and rat pharmacokinetic data of benzene sulfonamides



Compound	R	X	GLU IC_{50}[a] (μM)	RWB IC_{50}[a] (μM)	iv Cl[b] (mL/min/kg)	F[c] (%) MC/TW formulation
6	H	O	3.0	1.0	73	—
13a	2-Ph	O	0.11	0.15	4.5	<0.1
14a	2-Ph	CH_2	0.04	0.06	2.3	0.4
13b	3-Ph	O	0.57	0.60	—	—
14b	3-Ph	CH_2	0.18	0.22	—	—
13c	2-F	O	0.60	0.15	28.0	6.0
14c	2-F	CH_2	0.19	0.22	8.8	4.0
13d	2-Cl	O	0.40	0.55	31.0	39.0
14d	2-Cl	CH_2	0.10	0.20	9.4	13.7
13e	2-Br	O	0.52	0.32	3.1	<0.2
14e	2-Br	CH_2	0.10	0.12	6.5	1.9
13f	2-Me	O	0.34	0.16	61.0	—
14f	2-Me	CH_2	0.09	0.095	10.3	8.6
13g	2-OMe	O	0.18	0.06	18.6	<0.1
14g	2-OMe	CH_2	0.10	0.20	10.7	7.8
13h	2-OCF_3	O	0.35	0.36	12.4	15.4
14h	2-OCF_3	CH_2	0.10	0.20	5.2	4.6
13i	2,6-diCl	O	0.26	0.21	16.3	2.9
14i	2,6-diCl	CH_2	0.06	0.10	8.5	3.8
13j	2,6-diMe	O	0.10	0.095	6.1	6.0
14j	2,6-diMe	CH_2	0.047	0.075	10.5	1.3
13k	2-F, 6-CF_3	O	0.18	0.15	15.3	3.0
14k	2-F, 6-CF_3	CH_2	0.065	0.10	14.0	13.0

[a] Data are the average of two or more independent measurements.
[b] iv clearance (Cl) was determined at a dose of 2 mg/kg in 50% PEG-400, 50% DMSO formulation.
[c] Oral bioavailability (F) was determined by the dose normalized AUC ratio between oral and iv administration. A po dose of 25 mg/kg was used.

formulation which had been used to filter the benzenesulfonamides (Table 3). In both rat and dog, the phenylmethane derivative **3** had higher %F in the lipid-based formulation than in the conventional MC/TW formulation. Benzenesulfonamide **14k**, however, had a slightly higher %F in the MC/TW formulation than in the Phosal formulation. Although 13–16%F is generally considered low, this represented an improvement compared to all $cPLA_2\alpha$ inhibitors of suitable activity in whole blood assays, which are highly lipophilic and have high molecular weight, and typically have <5% oral bioavailability. Benzenesulfonamide **14k** demonstrated >10-fold higher %F than **3** in the MC/TW formulation in both rat and dog, thus fulfilling one of the goals of this effort.

3.2. Isothermal calorimetry data

The affinity and stoichiometry of compound **14k** for human $cPLA_2\alpha$ were determined using isothermal calorimetry at 30 °C using buffer conditions similar to that of the GLU micelle assay. An exothermic reaction was observed. The binding isotherm displayed a good fit ($CHI^2 = 92793.8$) with a single site model ($N = 1.03$), indicating that one molecule of compound **14k** bound

Table 3. Rat and dog pharmacokinetic parameters of **3** and **14k** in two formulations

Compound	Species	iv Cl[a] (mL/min/kg)	F[b] (%) Phosal formulation	F[b] (%) MC/TW formulation
3	Rat	3.5	3.5	1.0
14k	Rat	14	5.0	13
3	Dog	0.6	1.5	0.08
14k	Dog	1.4	13	16

[a] For rats, iv dose 2 mg/kg in 50% PEG-400, 50% DMSO formulation. For dogs, iv dose 2 mg/kg in 20% DMSO, 80% PEG-200 formulation.
[b] For rats, po dose 25 mg/kg. For dogs, a po dose of 50 mg/kg was used for compound **3** and 5 mg/kg was used for compound **14k**.

to one molecule of cPLA$_2\alpha$. The K_d was 80 nM, in agreement with that IC_{50} seen in the GLU micelle assay (65 nM).

3.3. Selectivity data

Compound **14k** was also evaluated in assays for COX inhibition. At a compound concentration of 100 μM, **14k** led to 45.0% inhibition of COX-1 and 9.9% inhibition of COX-2, while its IC_{50} in the GLU micelle assay measuring inhibition of cPLA$_2\alpha$ is 0.065 μM. Thus, **14k** was shown to be greater than 1000-fold selective for cPLA$_2\alpha$ versus COX-1 and -2.

3.4. Human whole blood data

The human whole blood assay employed at Wyeth has been previously described.[15] Compound **14k** was tested using blood from 3 different donors. At 0.3 μM, **14k** blocked the production of TXB_2, PGE_2, $PGF_2\alpha$, and LTB_4, as indicated in Table 4. The activity of **14k** in these assays is consistent with its behavior as a cPLA$_2\alpha$ inhibitor in the GLU micelle and RWB assays and demonstrates that **14k** effectively inhibited the production all of the arachidonate metabolites tested.

3.5. Rat carrageenan paw edema (CPE) model

The CPE model is an acute inflammation model that is primarily driven by prostaglandins.[18,19] It has been highly predictive of NSAID and COX-2 utility. In this study, all of the inhibitors were dosed orally (Fig. 2). The positive controls in this study were the NSAID Naproxen, dosed in the Phosal formulation, and the COX-2 selective inhibitor Celecoxib, for which the vehicle was MC/TW. Inhibitor **14k** was dosed orally at two concentrations in both vehicles. While **14k** when dosed at 25 mg/kg in the Phosal vehicle led to a 16% decrease in paw volume, **14k** dosed at 25 mg/kg in the minimal formulation of MC/TW was more efficacious, with a 44% decrease in paw volume. At 10 mg/kg orally in MC/TW, **14k** led to a 34% decrease in paw volume. **14k** was more efficacious at the 25 mg/kg dose (44% decrease in paw volume) than the earlier lead compound, phenylmethane sulfonamide **3**, at the same dose (36% decrease in paw volume) when both compounds were administered in their preferred formulations (*p*-value from Student's *t*-test: 0.03). The ED_{50} (defined as the dose giving 50% of the maximum response) of compound **14k** in MC/TW formulation is <10 mg/kg.[20]

Table 4. Inhibition of inflammatory mediators in human whole blood by **14k**

Product	IC_{50}[a] (μM)	Std. dev.
TXB_2	0.12	0.048
LTB_4	0.082	0.009
PGE_2	0.060	0.017
$PGF_2\alpha$	0.11	0.03

[a] Compound **14k** was tested using blood from three different donors.

3.6. *Ascaris suum* airway challenge in naturally sensitized sheep

This large animal model[21] can be utilized to measure the effect of inhibitors in decreasing allergen-induced bronchoconstriction and associated airway hyperresponsiveness (AHR), both of which are cardinal features of asthma. Sheep that are naturally sensitized to the parasitic nematode worm *A. suum* when challenged via the airways with the *A. suum* antigen develop both an early phase (EAR) and late phase (LAR) bronchoconstriction and AHR to aerosolized carbachol. This model has proved useful in helping to elucidate the pathogenesis of asthma as well as in predicting corticosteroid utility in the disease. Efficacy of **14k** was demonstrated in this model by iv dosing (10 mg/kg BID) and oral dosing (3 mg/kg BID) the day prior to antigen challenge and BID the day of challenge administration (Table 5). Additionally, **14k** was orally efficacious at a lower dose (1 mg/kg) when dosed BID for 4 days prior to challenge and BID on the day of challenge. With all three dosing regimens, **14k** had minimal impact on the early asthmatic response (EAR) while demonstrating a significant impact versus historical control on the late asthmatic response (LAR); for example, when **14k** was dosed 5 days at 1 mg/kg, the mean maximum LAR was reduced from

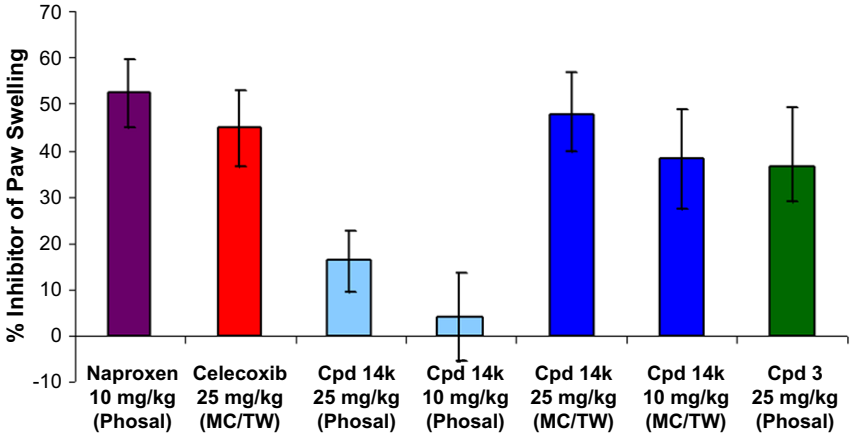


Figure 2. Percent inhibition of paw swelling in the rat carrageenan paw edema model by Naproxen, Celecoxib, **14k** and **3**.

Table 5. Efficacy of compound **14k** in *Ascaris suum* airway challenge in naturally sensitized sheep[a]

Route and frequency of administration	Duration of dosing (days)	Dose (mg/kg)	Mean Max. EAR[b] (%)	Mean Max. LAR[b] (%)	Mean Max. AHR[c]
iv BID	1	Control[a]	405	136	14.5
		10	346	29	23.5
po BID	2	Control[a]	526	136	13.0
		3	379	29	24.0
po BID	5	Control[a]	396	124	11.7
		1	431	68	26.0

[a] Control values are from historical data obtained from the same sheep.
[b] Mean maximum percentage increase in specific lung resistance.
[c] Mean cumulative carbachol concentration that increased specific lung resistance by 400% over the post saline value.

124% to 68%. Also, in all three dosing regimens, **14k** had a significant impact on AHR, that is, the mean cumulative carbachol concentration required to increase the specific lung resistance by 400% versus saline in sheep treated with **14k** was comparable to the amount needed for untreated animals which had not been exposed to antigen.

4. Conclusion

Starting from a benzenesulfonamide indole cPLA$_2\alpha$ inhibitor with in vitro potency in the low μM range, we synthesized substituted benzenesulfonamide analogues with at least 10-fold improved potency in isolated enzyme and rat whole blood assays. The SAR of these inhibitors indicates that at C3, the propyl linker is preferred to the ethoxy linker, and that *ortho* substitution of the benzene moiety of the sulfonamide is favorable. Triage of these inhibitors by rat pharmacokinetics, by first determining iv clearance, then measuring oral exposure in a minimal formulation, led to the selection of **14k**, which showed superior oral exposure in the MC/TW formulation in rat and dog compared to the phenylmethane sulfonamide lead compound **3**. In the rat CPE model, benzenesulfonamide **14k** demonstrated oral efficacy at 10 mg/kg when administered in the MC/TW formulation. Furthermore, in the same in vivo model, **14k**, when administered in MC/TW, the preferred, minimal formulation, showed superior oral efficacy to the lead phenylmethane sulfonamide WAY-196025 (**3**) administered in a lipid-based formulation. Indole **14k** also was orally efficacious at 1 mg/kg in the attenuation of both the LAR and the associated AHR to aerosolized carbachol in naturally sensitized sheep that had been challenged via the airways with *A. suum* antigen.

5. Experimental

5.1. General methods: chemistry

All solvents and reagents were used as obtained. Proton NMR spectra were recorded at 300 MHz on a Varian Gemini 2000 or 400 MHz Bruker AV-400 spectrometer using TMS (δ 0.0) as a reference. Combustion analyses were obtained using a Perkin Elmer Series II 2400 CHNS/O analyzer. CHN analyses were carried out by Robertson-Microlit. Low resolution mass spectra were obtained using a Micromass Platform Electrospray Ionization Quadrupole mass spectrometer. High resolution mass spectra were obtained using a Bruker (Billerica, MA) APEXIII Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an actively shielded 7 Tesla superconducting magnet (Magnex Scientific Ltd, UK) and an external Bruker APOLLO electrospray ionization (ESI) source. Flash chromatography was performed using EM Science 230–400 mesh silica gel or Biotage flash columns packed with KP-SIL 60 Angstrom silica gel. Thin-layer chromatography (TLC) was performed using EMD 250 μm prescored silica gel 60 F_{254} plates. Purity in two solvent systems (H_2O–CH_3CN and H_2O–MeOH) was determined using an Agilent 1100HPLC instrument.

5.1.1. 2-Fluoro-6-trifluoromethylbenzenesulfonyl chloride (8). To a solution of 2-bromo-3-fluoro-benzotrifluoride **7** (15 g, 62 mmol) in THF (150 mL) and Et_2O (150 mL) cooled to −78 °C was added *n*-BuLi (2.5 M in hexanes, 25 mL, 62 mmol) dropwise. The mixture was stirred at −78 °C for 30 min. Sulfur dioxide (200 mL, 3.9 mol) was condensed into a separate flask at −78 °C and Et_2O (200 mL), precooled to −78 °C, was added via cannula. The *n*-BuLi mixture was added via cannula to the sulfur dioxide mixture, and the resulting suspension was allowed to warm to room temperature overnight. The mixture was concentrated, diluted with Et_2O (200 mL), and filtered. The off-white solid was washed with Et_2O (200 mL), transferred to a flask, and suspended in hexanes (500 mL), and cooled to 0 °C. Sulfuryl chloride (1.0 M in CH_2Cl_2, 65 mL, 65 mmol) was added dropwise. The mixture was allowed to warm to room temperature overnight and concentrated. The residue was triturated with hexanes (500 mL) at 40 °C for 30 min, filtered, and dried to afford sulfonyl chloride **8** (7.8 g, 48% yield), an off-white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39–7.50 (m, 1H), 7.50–7.57 (m, 2H).

5.2. General procedure 1: sulfonylation of amines via Schotten–Baumann reaction

To a solution of the amine **9**[15] or **10**[16] (1.0 mmol) in CH_2Cl_2 (10 mL) were added sulfonyl chloride

(1.2 mmol) and sat. $NaHCO_3$ (10 mL). The resulting suspension was stirred until the amine was consumed (TLC analysis in 10% MeOH–CH_2Cl_2). The mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (20 mL) and brine, dried ($MgSO_4$), and concentrated. Purification of the crude product by flash chromatography (EtOAc–hexanes) afforded the sulfonamide.

5.2.1. Methyl 4-[2-(1-benzhydryl-2-{2-[(1,1′-biphenyl-2-ylsulfonyl)amino]ethyl}-5-chloro-1*H*-indol-3-yl)ethoxy]-benzoate (11a). Sulfonylation of **9** with 2-phenylbenzenesulfonyl chloride, white foam, 93% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.47–2.66 (m, 2H), 2.7–2.96 (m, 2H), 3.09 (t, J = 6.7 Hz, 2H), 3.59 (t, J = 6.4 Hz, 1H), 3.89 (s, 3H), 4.08 (d, 2H), 6.47 (d, J = 8.8 Hz, 1H), 6.66–6.85 (m, 4H), 6.92–7.04 (m, 4H), 7.20–7.42 (m, 13H), 7.46–7.57 (m, 2H), 7.85–7.98 (m, 2H), 8.02 (dd, J = 8.0, 1.1 Hz, 1H).

5.2.2. Methyl 4-[2-(1-benzhydryl-2-{2-[(1,1′-biphenyl-3-ylsulfonyl)amino]ethyl}-5-chloro-1*H*-indol-3-yl)ethoxy]-benzoate (11b). Sulfonylation of **9** with 3-phenylbenzenesulfonyl chloride, white foam, 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.98–3.10 (m, 2H), 3.14–3.34 (m, 4H), 3.96 (s, 3H), 4.24 (t, J = 6.6 Hz, 2H), 4.57 (t, J = 6.3 Hz, 1H), 6.59 (d, J = 8.8 Hz, 1H), 6.75–6.94 (m, 3H), 6.96 (s, 1H), 7.02–7.17 (m, 4H), 7.25–7.40 (m, 6H), 7.43–7.55 (m, 4H), 7.56–7.64 (m, 3H), 7.64–7.73 (m, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.96–8.08 (m, 3H).

5.2.3. Methyl 4-{2-[1-benzhydryl-5-chloro-2-(2-{[(2-fluorophenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}-benzoate (11c). Sulfonylation of **9** with 2-fluorobenzenesulfonyl chloride, white foam, 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.82–2.94 (m, 2H), 2.98–3.06 (m, 2H), 3.10 (t, J = 6.7 Hz, 2H), 3.82 (s, 3H), 4.12 (t, J = 6.6 Hz, 2H), 4.68 (t, J = 6.3 Hz, 1H), 6.46 (d, J = 8.6 Hz, 1H), 6.69–6.78 (m, 3H), 6.83 (s, 1H), 6.93–7.01 (m, 4H), 7.01–7.09 (m, 2H), 7.16–7.30 (m, 6H), 7.37–7.48 (m, 2H), 7.60–7.70 (m, 1H), 7.87 (d, J = 9.1 Hz, 2H).

5.2.4. Methyl 4-{2-[1-benzhydryl-5-chloro-2-(2-{[(2-chlorophenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}-benzoate (11d). Sulfonylation of **9** with 2-chlorobenzenesulfonyl chloride, white foam, 86% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.86–2.98 (m, 2H), 2.98–3.07 (m, 2H), 3.07–3.21 (m, 2H), 3.81 (s, 3H), 4.19 (t, J = 6.7 Hz, 2H), 6.49 (d, J = 8.8 Hz, 1H), 6.80 (dd, J = 8.8, 2.0 Hz, 1H), 6.96 (d, J = 8.8 Hz, 2H), 6.99–7.14 (m, 5H), 7.28–7.36 (m, 6H), 7.37–7.44 (m, 1H), 7.53–7.61 (m, 1H), 7.60–7.69 (m, 2H), 7.80 (dd, J = 7.8, 1.5 Hz, 1H), 7.86 (d, J = 8.8 Hz, 2H), 8.11 (t, J = 6.1 Hz, 1H).

5.2.5. 4-{2-[1-Benzhydryl-5-chloro-2-(2-{[(2-bromophenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}benzoic acid (11e). Sulfonylation of **9** with 2-bromobenzenesulfonyl chloride, white foam, 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.72–2.96 (m, 2H), 3.04–3.13 (m, 2H), 3.16 (t, J = 6.6 Hz, 2H), 3.89 (s, 3H), 4.17 (t, J = 6.6 Hz, 2H), 5.20 (t, J = 6.4 Hz, 1H), 6.52 (d, J = 8.8 Hz, 1H), 6.73–6.85 (m, 3H), 6.89 (s, 1H), 6.99–7.10 (m, 4H), 7.21–7.39 (m, 9H), 7.51 (d, J = 2.0 Hz, 1H), 7.65 (dd, J = 7.8, 1.3 Hz, 1H), 7.88–8.01 (m, 3H).

5.2.6. Methyl 4-{2-[1-benzhydryl-5-chloro-2-(2-{[(2-methylphenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}benzoate (11f). Sulfonylation of **9** with 2-methylbenzenesulfonyl chloride, white foam, 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.87–2.00 (m, 2H), 2.48 (s, 3H), 2.71 (appar q, J = 7.8 Hz, 4H), 2.78–2.88 (m, 2H), 2.91–3.07 (m, 2H), 3.91 (s, 3H), 4.36 (t, J = 6.3 Hz, 1H), 6.48 (d, J = 8.8 Hz, 1H), 6.79 (dd, J = 8.8, 2.0 Hz, 1H), 6.84 (s, 1H), 6.97–7.08 (m, 4H), 7.12–7.20 (m, 1H), 7.20–7.34 (m, 9H), 7.36–7.45 (m, 3H), 7.74 (dd, J = 7.8, 1.3 Hz, 1H), 7.96 (d, J = 8.3 Hz, 2H).

5.2.7. Methyl 4-{2-[1-benzhydryl-5-chloro-2-(2-{[(2-methoxyphenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}benzoate (11g). Sulfonylation of **9** with 2-methoxybenzenesulfonyl chloride, white foam, 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.01–3.15 (m, 2H), 3.25 (t, J = 7.6 Hz, 2H), 3.34 (t, J = 6.7 Hz, 2H), 3.87 (s, 3H), 4.05 (s, 3H), 4.33 (t, J = 6.3 Hz, 2H), 5.18 (t, J = 6.1 Hz, 1H), 6.63 (d, J = 8.8 Hz, 1H), 6.97 (appar d, J = 9.1 Hz, 3H), 7.03–7.15 (m, 3H), 7.18 (appar dd, J = 7.3, 1.8 Hz, 4H), 7.34–7.53 (m, 6H), 7.61–7.69 (m, 1H), 7.70 (d, J = 2.0 Hz, 1H), 7.94 (dd, J = 7.7, 1.6 Hz, 1H), 8.11 (d, J = 8.8 Hz, 2H).

5.2.8. Methyl 4-(2-{5-chloro-1-(diphenylmethyl)-2-[2-({[2-(trifluoromethoxy)phenyl]sulfonyl}amino)ethyl]-1*H*-indol-3- yl}ethoxy)benzoate (11h). Sulfonylation of **9** with 2-trifluoromethoxybenzenesulfonyl chloride, white foam, 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.81–2.95 (m, 2H), 3.03–3.13 (m, 2H), 3.16 (t, J = 6.6 Hz, 2H), 3.88 (s, 3H), 4.17 (t, J = 6.6 Hz, 2H), 4.71 (t, J = 6.2 Hz, 1H), 6.53 (d, J = 8.8 Hz, 1H), 6.78 (d, J = 8.8 Hz, 2H), 6.81 (dd, J = 8.8, 2.3 Hz, 1H), 6.90 (s, 1H), 6.99–7.09 (m, 4H), 7.15–7.39 (m, 8H), 7.45–7.62 (m, 2H), 7.82 (dd, J = 7.8, 1.8 Hz, 1H), 7.92 (d, J = 8.8 Hz, 2H).

5.2.9. Methyl 4-{2-[1-benzhydryl-5-chloro-2-(2-{[(2,6-dichlorophenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}-benzoate (11i). Sulfonylation of **9** with 2,6-dichlorobenzenesulfonyl chloride, white foam, 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.83–3.03 (m, 2H), 3.07–3.17 (m, 2H), 3.16–3.31 (m, 2H), 3.89 (s, 3H), 4.20 (t, J = 6.6 Hz, 2H), 5.33 (t, J = 5.3 Hz, 1H), 6.54 (d, J = 8.8 Hz, 1H), 6.72–6.86 (m, 3H), 6.90 (s, 1H), 6.98–7.15 (m, 4H), 7.18–7.32 (m, 7H), 7.37 (d, J = 8.3 Hz, 2H), 7.53 (s, 1H), 7.93 (d, J = 8.8 Hz, 2H).

5.2.10. Methyl 4-{2-[1-benzhydryl-5-chloro-2-(2-{[(2,6-dimethylphenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}benzoate (11j). Sulfonylation of **9** with 2,6-dimethylbenzenesulfonyl chloride, white foam, 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) 2.41–2.55 (m, 6H), 2.81–2.98 (m, 2H), 2.99–3.10 (m, 2H), 3.16 (t, J = 6.7 Hz, 2H), 3.89 (s, 3H), 4.17 (t, J = 6.7 Hz, 2H), 4.50 (t, J = 5.9 Hz, 1H), 6.53 (d, J = 8.8 Hz, 1H), 6.74–6.86

(m, 4H), 6.97–7.05 (m, 4H), 7.07 (s, 1H), 7.19–7.25 (m, 1H), 7.25–7.32 (m, 7H), 7.53 (d, J = 2.0 Hz, 1H), 7.93 (d, J = 8.8 Hz, 2H).

5.2.11. Methyl 4-(2-{1-benzhydryl-5-chloro-2-[2-({[2-fluoro-6-(trifluoromethyl)phenyl]sulfonyl}amino)ethyl]-1*H*-indol-3-yl}ethoxy)benzoate (11k). Sulfonylation of **9** with 2-fluoro-6-trifluoromethylbenzenesulfonyl chloride (**8**), pale yellow foam, 89% yield. ^{1}H NMR (400 MHz, $CDCl_3$) 3.00 (q, J = 6.8 Hz, 2H), 3.08–3.16 (m, 2H), 3.20 (t, J = 6.4 Hz, 2H), 3.88 (s, 3H), 4.20 (t, J = 6.4 Hz, 2H), 4.99 (t, J = 6.2 Hz, 1H), 6.54 (d, J = 8.8 Hz, 1H), 6.79 (d, J = 9.1 Hz, 2H), 6.84 (dd, J = 9.0, 2.1 Hz, 1H), 6.88 (s, 1H), 7.01–7.07 (m, 4H), 7.22–7.37 (m, 7H), 7.54 (d, J = 2.0 Hz, 1H), 7.57–7.67 (m, 2H), 7.93 (d, J = 9.1 Hz, 2H).

5.2.12. Methyl 4-[3-(1-benzhydryl-2-{2-[(1,1′-biphenyl-2-ylsulfonyl)amino]ethyl}-5-chloro-1*H*-indol-3-yl)propyl]-benzoate (12a). Sulfonylation of **10** with 2-phenylbenzenesulfonyl chloride, white foam, 83% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.78–1.93 (m, 2H), 2.44–2.56 (m, 2H), 2.56–2.72 (m, 4H), 2.73–2.83 (m, 2H), 3.55 (t, J = 6.1 Hz, 1H), 3.91 (s, 3H), 6.44 (d, J = 8.8 Hz, 1H,) 6.73 (s, 1H), 6.79 (dd, J = 8.8, 2.0 Hz, 1H), 6.90–7.04 (m, 4H), 7.10–7.33 (m, 14H), 7.35–7.46 (m, 2H), 7.48–7.63 (m, 1H), 7.90–7.99 (m, 2H), 8.02 (dd, J = 8.0, 1.1 Hz, 1H).

5.2.13. Methyl 4-[3-(1-benzhydryl-2-{2-[(1,1′-biphenyl-3-ylsulfonyl)amino]ethyl}-5-chloro-1*H*-indol-3-yl)propyl]-benzoate (12b). Sulfonylation of **10** with 3-phenylbenzenesulfonyl chloride, white foam, 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.82–2.02 (m, 2H), 2.56–2.84 (m, 4H), 2.84–2.97 (m, 2H), 2.97–3.13 (m, 2H), 3.92 (s, 3H), 4.64 (t, J = 6.3 Hz, 1H), 6.49 (d, J = 8.8 Hz, 1H), 6.81 (dd, J = 8.8, 2.3 Hz, 1H), 6.86 (s, 1H), 6.96–7.10 (m, 4H), 7.15–7.35 (m, 8H), 7.37–7.51 (m, 5H), 7.51–7.58 (m, 2H), 7.63 (dd, J = 8.1, 1.5 Hz, 1H), 7.76 (dd, J = 6.4, 1.6 Hz, 1H), 7.87–8.05 (m, 3H).

5.2.14. Methyl 4-{3-[1-benzhydryl-5-chloro-2-(2-{[(2-fluorophenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}benzoate (12c). Sulfonylation of **10** with 2-fluorobenzenesulfonyl chloride, white foam, 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.82–2.00 (m, 2H), 2.72 (q, J = 8.1 Hz, 4H), 2.82–2.91 (m, 2H), 2.92–3.02 (m, 2H), 3.91 (s, 3H), 4.63 (t, J = 6.1 Hz, 1H), 6.49 (d, J = 9.1 Hz, 1H), 6.80 (dd, J = 8.8, 2.3 Hz, 1H), 6.85 (s, 1H), 7.00–7.07 (m, 3H), 7.08–7.19 (m, 2H), 7.21–7.35 (m, 9H), 7.40 (d, J = 1.8 Hz, 1H), 7.46–7.58 (m, 1H), 7.71 (dt, J = 7.6, 1.8 Hz, 1H), 7.96 (d, J = 8.6 Hz, 2H).

5.2.15. Methyl 4-{3-[1-benzhydryl-5-chloro-2-(2-{[(2-chlorophenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}-benzoate (12d). Sulfonylation of **10** with 2-chlorobenzenesulfonyl chloride, white foam, 66% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.72–1.91 (m, 2H), 2.59–2.74 (m, 4H), 2.79–3.04 (m, 4H), 3.84 (s, 3H), 6.45 (d, J = 8.8 Hz, 1H), 6.77 (dd, J = 9.0, 2.1 Hz, 1H), 6.99 (s, 1H), 7.04 (dd, J = 7.5, 1.9 Hz, 4H), 7.26–7.38 (m, 8H), 7.39–7.47 (m, 2H), 7.54–7.70 (m, 2H), 7.82 (dd, J = 7.7, 1.4 Hz, 1H), 7.87 (d, J = 8.3 Hz, 2H), 8.06 (t, J = 5.7Hz, 1H).

5.2.16. Methyl 4-{3-[1-benzhydryl-2-(2-{[(2-bromophenyl)sulfonyl]amino}ethyl)-5-chloro-1*H*-indol-3-yl]propyl}-benzoate (12e). Sulfonylation of **10** with 2-bromobenzenesulfonyl chloride, white foam, 76% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.85–2.02 (m, 2H), 2.49–2.86 (m, 6H), 2.88–3.06 (m, 2H), 3.91 (s, 3H), 5.10 (t, J = 6.3 Hz, 1H), 6.48 (d, J = 8.8 Hz, 1H), 6.79 (dd, J = 9.0, 2.1 Hz, 1H), 6.83 (s, 1H), 6.99–7.06 (m, 4H), 7.24 (d, J = 8.3 Hz, 2H), 7.25–7.43 (m, 10H), 7.62–7.69 (m, 1H), 7.89–8.01 (m, 3H).

5.2.17. Methyl 4-{3-[1-benzhydryl-5-chloro-2-(2-{[(2-methylphenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}-benzoate (12f). Sulfonylation of **10** with 2-methylbenzenesulfonyl chloride, white foam, 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.42 (s, 3H), 2.76–2.94 (m, 2H), 2.95–3.05 (m, 2H), 3.08 (t, J = 6.7 Hz, 2H), 3.80 (s, 3H), 4.10 (t, J = 6.6 Hz, 2H), 4.41 (t, J = 6.3 Hz, 1H), 6.44 (d, J = 8.8 Hz, 1H), 6.66–6.78 (m, 3H), 6.82 (s, 1H), 6.92–7.00 (m, 4H), 7.05 (t, J = 8.3 Hz, 1H), 7.12–7.26 (m, 8H), 7.25–7.36 (m, 1H), 7.44 (d, J = 2.0 Hz, 1H), 7.66 (dd, J = 8.0, 1.4 Hz, 1H), 7.85 (d, J = 8.8 Hz, 2H).

5.2.18. Methyl 4-{3-[1-benzhydryl-5-chloro-2-(2-{[(2-methoxyphenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}benzoate (12g). Sulfonylation of **10** with 2-methoxybenzenesulfonyl chloride, pale yellow foam, 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.84–2.00 (m, 2H), 2.60–2.78 (m, 4H), 2.80–2.91 (m, 2H), 2.96 (t, J = 7.2 Hz, 2H), 3.57 (s, 3H), 3.91 (s, 3H), 4.97 (t, J = 6.1 Hz, 1H), 6.41 (d, J = 9.3 Hz, 1H), 6.77 (dd, J = 8.8, 2.3 Hz, 1H), 6.80 (s, 1H), 6.89 (d, J = 8.3 Hz, 1H), 6.94–7.03 (m, 5H), 7.12–7.34 (m, 9H), 7.40 (d, J = 2.0 Hz, 1H), 7.45–7.57 (m, 1H), 7.78 (dd, J = 7.8, 1.8 Hz, 1H), 7.95 (d, J = 8.3 Hz, 2H).

5.2.19. Methyl 4-(3-{5-chloro-1-(diphenylmethyl)-2-[2-({[2-(trifluoromethoxy)phenyl]sulfonyl}amino)ethyl]-1*H*-indol-3-yl}propyl)benzoate (12h). Sulfonylation of **10** with 2-trifluoromethoxybenzenesulfonyl chloride, white foam, 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.85–2.00 (m, 2H), 2.65–2.76 (m, 4H), 2.76–2.86 (m, 2H), 2.91–3.15 (m, 2H), 3.91 (s, 3H), 4.63 (t, J = 6.2 Hz, 1H), 6.50 (d, J = 8.8 Hz, 1H), 6.80 (dd, J = 8.8, 2.3 Hz, 1H), 6.85 (s, 1H), 6.98–7.11 (m, 4H), 7.17–7.35 (m, 10H), 7.39 (d, J = 2.0 Hz, 1H), 7.48–7.66 (m, 1H), 7.82 (dd, J = 7.8, 1.5 Hz, 1H), 7.95 (d, J = 8.6 Hz, 2H).

5.2.20. Methyl 4-{3-[1-benzhydryl-5-chloro-2-(2-{[(2,6-dichlorophenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}-benzoate (12i). Sulfonylation of **10** with 2,6-dichlorobenzenesulfonyl chloride, pale orange foam, 93% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.84–2.01 (m, 2H), 2.66–2.78 (m, 4H), 2.82–2.91 (m, 2H), 2.94–3.06 (m, 2H), 3.91 (s, 3H), 5.25 (t, J = 6.2 Hz, 1H), 6.49 (d, J = 8.8 Hz, 1H), 6.80 (dd, J = 8.8, 2.0 Hz, 1H), 6.84 (s, 1H), 7.00–7.08 (m, 4H), 7.20–7.32 (m, 9H), 7.35–7.43 (m, 3H), 7.95 (d, J = 8.3 Hz, 2H).

5.2.21. Methyl 4-{3-[1-benzhydryl-5-chloro-2-(2-{[(2,6-dimethylphenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}benzoate (12j). Sulfonylation of **10** with 2,6-dimethylbenzenesulfonyl chloride, white foam, 66% yield. ^{1}H NMR (400 MHz, $CDCl_3$) 1.87–1.99 (m, 2H), 2.50 (s, 6H), 2.64–2.74 (m, 4H), 2.78–2.87 (m, 2H), 2.88–2.97 (m, 2H), 3.91 (s, 3H), 4.40 (t, J = 6.4 Hz, 1H), 6.47 (d, J = 8.8 Hz, 1H), 6.77 (s, 1H), 6.80 (dd, J = 8.8, 2.3 Hz, 1H), 6.95–7.03 (m, 4H), 7.07 (d, J = 7.6 Hz, 2H), 7.22 (d, J = 8.1 Hz, 2H), 7.24–7.32 (m, 7H), 7.40 (d, J = 2.0 Hz, 1H), 7.95 (d, J = 8.3 Hz, 2H).

5.2.22. Methyl 4-(3-{1-benzhydryl-5-chloro-2-[2-({[2-fluoro-6-(trifluoromethyl)phenyl]sulfonyl}amino)ethyl]-1*H*-indol-3-yl}propyl)benzoate (12k). Sulfonylation of **10** with 2-fluoro-6-trifluoromethylbenzenesulfonyl chloride (**8**), pale yellow foam, 62% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.84–2.02 (m, 2H), 2.56–2.82 (m, 4H), 2.84–2.95 (m, 2H), 2.95–3.11 (m, 2H), 3.91 (s, 3H), 4.88 (t, J = 6.3 Hz, 1H), 6.50 (d, J = 8.8 Hz, 1H), 6.72–6.92 (m, 2H), 7.02 (dd, J = 5.7, 3.4 Hz, 4H), 7.15–7.35 (m, 9H), 7.41 (d, J = 1.8 Hz, 1H), 7.52–7.73 (m, 2H), 7.95 (d, J = 8.1 Hz, 2H).

5.3. General procedure 2: ester hydrolysis

To a solution of the ester (1.0 mmol) in inhibitor-free THF (20 mL) were added 1 N aq NaOH (3.0 mL, 3.0 mmol) and MeOH (10 mL). The mixture was heated at 50 °C until the ester starting material was consumed (TLC analysis in 50% EtOAc–hexanes). The reaction mixture was concentrated and the residue was diluted with H_2O (10 mL) and acidified to pH 1 using 1 N HCl. The resulting mixture was extracted with EtOAc (2× 20 mL). The organic extracts were washed with H_2O (20 mL) and brine (20 mL), dried, and concentrated. The residue was lyophilized to afford the carboxylic acid.

5.3.1. 4-[2-(1-Benzhydryl-2-{2-[(1,1′-biphenyl-2-ylsulfonyl)amino]ethyl}-5-chloro-1*H*-indol-3-yl)ethoxy]benzoic acid (13a). Ester hydrolysis of **11a**, white solid, 94% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.75–2.87 (m, 2H), 2.92–3.00 (m, 2H), 3.11 (t, J = 6.7 Hz, 2H), 4.17 (t, J = 6.7 Hz, 2H), 6.47 (d, J = 8.8 Hz, 1H), 6.80 (dd, J = 8.8, 2.3 Hz, 1H), 6.88–6.94 (m, 2H), 6.95 (s, 1H), 6.96–7.03 (m, 4H), 7.21–7.36 (m, 12H), 7.40–7.53 (m, 2H), 7.56–7.63 (m, 1H), 7.64 (d, J = 2.3 Hz, 1H), 7.77–7.90 (m, 3H); HRMS calcd for [$C_{44}H_{37}ClN_2O_5S$+H]: 741.21845: found 741.21709. Anal. Calcd for [$C_{44}H_{37}ClN_2O_5S$]: C, H, N.

5.3.2. 4-[2-(1-Benzhydryl-2-{2-[(1,1′-biphenyl-3-ylsulfonyl)amino]ethyl}-5-chloro-1*H*-indol-3-yl)ethoxy]benzoic acid (13b). Ester hydrolysis of **11b**, white solid, 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.81–2.96 (m, 2H), 2.96–3.06 (m, 2H), 3.11 (t, J = 5.8 Hz, 2H), 4.14 (t, J = 6.6 Hz, 2H), 6.49 (d, J = 8.6 Hz, 1H), 6.79 (dd, J = 8.8, 2.3 Hz, 1H), 6.85 (d, J = 8.6 Hz, 2H), 6.91–7.10 (m, 5H), 7.21–7.37 (m, 6H), 7.38–7.53 (m, 3H), 7.55–7.71 (m, 5H), 7.80 (d, J = 8.6 Hz, 2H), 7.88 (d, J = 7.6 Hz, 1H), 7.94–8.07 (m, 2H); HRMS calcd for [$C_{44}H_{.37}ClN_2O_5S$+H] 741.21845: found 741.21879. HPLC purity: CH_3CN–H_2O, 99.0%, MeOH–H_2O, 100%.

5.3.3. 4-{2-[1-Benzhydryl-5-chloro-2-(2-{[(2-fluorophenyl)-sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}benzoic acid (13c). Ester hydrolysis of **11c**, white solid, 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.88–2.99 (m, 2H), 2.99–3.07 (m, J = 5.8 Hz, 2H), 3.10 (t, J = 6.6 Hz, 2H), 4.13 (t, J = 6.6 Hz, 2H), 6.49 (d, J = 9.1 Hz, 1H), 6.79 (appar dd, J = 8.8, 2.0 Hz, 3H), 7.04 (appar dd, J = 7.7, 1.6 Hz, 5H), 7.20–7.42 (m, 8H), 7.55–7.68 (m, 3H), 7.80 (s, 2H), 8.19 (s, 1H); HRMS calcd for [$C_{38}H_{33}ClFN_2O_5S$+H] 683.17773: found 683.17694. Anal. Calcd for [$C_{38}H_{33}ClFN_2O_5S$]: C, H, N.

5.3.4. 4-{2-[1-Benzhydryl-5-chloro-2-(2-{[(2-chlorophenyl)-sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}benzoic acid (13d). Ester hydrolysis of **11d**, white solid, 74% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.83–2.98 (m, 2H), 3.05–3.15 (m, 2H), 3.18 (t, J = 6.6 Hz, 2H), 4.20 (t, J = 6.6 Hz, 2H), 5.09 (t, J = 6.3 Hz, 1H), 6.53 (d, J = 8.8 Hz, 1H), 6.78–6.86 (m, 3H), 6.90 (s, 1H), 7.01–7.12 (m, 4H), 7.22–7.34 (m, 7H), 7.42–7.47 (m, 2H), 7.52 (d, J = 2.0 Hz, 1H), 7.85–7.94 (m, 1H), 8.00 (d, J = 8.8 Hz, 2H); HRMS calcd for [$C_{38}H_{32}Cl_2N_2O_5S$+H]: 699.14818: found 699.14786. Anal. Calcd for [$C_{38}H_{32}Cl_2N_2O_5S$]: C, H, N.

5.3.5. 4-{2-[1-Benzhydryl-2-(2-{[(2-bromophenyl)sulfonyl]amino}ethyl)-5-chloro-1*H*-indol-3-yl]ethoxy}benzoic acid (13e). Ester hydrolysis of **11e**, off-white powder, 91% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.87–2.98 (m, J = 7.2, 3.9 Hz, 2H), 3.00–3.07 (m, 2H), 3.11 (t, J = 6.7 Hz, 2H), 4.18 (t, J = 6.6Hz, 2H), 6.49 (d, J = 8.8 Hz, 1H), 6.80 (dd, J = 8.8, 2.3 Hz, 1H), 6.93 (d, J = 9.1 Hz, 2H), 7.00–7.08 (m, 5H), 7.27–7.39 (m, 7H), 7.39–7.54 (m, 2H), 7.65 (d, J = 2.0 Hz, 1H), 7.76–7.89 (m, 4H), 8.08 (t, J = 5.8 z, 1H); HRMS calcd for [$C_{38}H_{32}BrClN_2O_5S$+H] 743.09766: found 743.09697. Anal. Calcd for [$C_{38}H_{32}BrClN_2O_5S$]: C, H, N.

5.3.6. 4-{2-[1-Benzhydryl-5-chloro-2-(2-{[(2-methylphenyl)-sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}benzoic acid (13f). Ester hydrolysis of **11f**, white solid, 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.66–1.91 (m, 2H), 2.52 (s, 3H), 2.65 (t, J = 6.7 Hz, 4H), 2.73–2.84 (m, 2H), 2.86–2.96 (m, 2H), 6.45 (d, J = 8.8 Hz, 1H), 6.77 (dd, J = 9.0, 2.1 Hz, 1H), 6.99 (s, 1H), 7.01–7.10 (m, 4H), 7.14–7.27 (m, 3H), 7.26–7.40 (m, 8H), 7.41–7.51 (m, 2H), 7.65 (dd, J = 7.8, 1.3 Hz, 1H), 7.83 (d, J = 7.3 Hz, 2H), 7.94 (t, J = 5.8 Hz, 1H); HRMS calcd for [$C_{39}H_{35}ClN_2O_5S$+H] 679.20280: found 679.20197. HPLC purity: CH_3CN–H_2O, 100%, MeOH–H_2O, 98.2%.

5.3.7. 4-{2-[1-Benzhydryl-5-chloro-2-(2-{[(2-methoxyphenyl)-sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}benzoic acid (13g). Ester hydrolysis of **11g**, white solid, 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.83–3.03 (m, 4H), 3.02–3.14 (m, 2H), 3.81 (s, 3H), 4.11 (t, J = 6.6 Hz, 2H), 6.46 (d, J = 8.8 Hz, 1H), 6.72–6.86 (m, 3H), 6.94 (t, J = 7.8 Hz, 1H), 6.98–7.09 (m, 5H), 7.14 (d, J = 8.3 Hz, 1H), 7.25–7.43 (m, 6H), 7.45–7.56 (m, 2H),

7.58–7.68 (m, 2H), 7.84 (s, 2H); HRMS calcd for $[C_{39}H_{35}ClN_2O_6S+H]$ 695.19722: found 695.19701. HPLC purity: CH_3CN–H_2O, 100%, MeOH–H_2O, 100%.

5.3.8. 4-(2-{5-Chloro-1-(diphenylmethyl)-2-[2-({[2-(trifluoromethoxy)phenyl]sulfonyl}amino)ethyl]-1*H*-indol-3-yl}ethoxy)benzoic acid (13h). Ester hydrolysis of **11h**, white solid, 90% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.01 (dd, J = 19.6, 6.2 Hz, 4H), 3.11 (t, J = 6.4 Hz, 2H), 4.13 (t, J = 6.7 Hz, 2H), 6.49 (d, J = 8.8 Hz, 1H), 6.66–6.85 (m, 3H), 6.94–7.12 (m, 5H), 7.23–7.37 (m, 6H), 7.42 (t, J = 7.5 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.65 (d, J = 2.3 Hz, 1H), 7.67–7.73 (m, 1H), 7.74–7.90 (m, 3H), 8.22 (br s, 1H); HRMS calcd for $[C_{39}H_{35}ClF_3N_2O_6S+H]$ 749.16945: found 749.16813. HPLC purity: CH_3CN–H_2O, 98.4%, MeOH–H_2O, 97.9%.

5.3.9. 4-{2-[1-Benzhydryl-5-chloro-2-(2-{[(2,6-dichlorophenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}benzoic acid (13i). Ester hydrolysis of **11i**, white solid, 82% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.04 (br s, 4H), 3.14 (t, J = 6.6 Hz, 2H), 4.20 (t, J = 6.7 Hz, 2H), 6.49 (d, J = 8.8 Hz, 1H), 6.81 (dd, J = 9.0, 2.1 Hz, 1H), 6.92 (d, J = 8.8 Hz, 2H), 6.99–7.08 (m, 5H), 7.25–7.38 (m, 6H), 7.44–7.54 (m, 1H), 7.55–7.60 (m, 2H), 7.66 (d, J = 2.3Hz, 1H), 7.83 (d, J = 8.8 Hz, 2H), 8.30–8.41 (m, 1H), 12.59 (br s, 1H); HRMS calcd for $[C_{38}H_{31}Cl_3N_2O_5S+H]$ 733.10921: found 733.10836. Anal. Calcd for $[C_{38}H_{31}Cl_3N_2O_5S]$: found C, H, N.

5.3.10. 4-{2-[1-Benzhydryl-5-chloro-2-(2-{[(2,6-dimethylphenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]ethoxy}benzoic acid (13j). Ester hydrolysis of **11j**, white solid, 79% yield. ^{1}H NMR (400 MHz, DMF) δ 3.07 (s, 6H), 3.37–3.50 (m, 2H), 3.49–3.60 (m, 2H), 3.67 (t, J = 6.7 Hz, 2H), 4.74 (t, J = 6.8 Hz, 2H), 7.07 (d, J = 8.8 Hz, 1H), 7.38 (dd, J = 8.8, 2.0 Hz, 1H), 7.49 (d, J = 8.8 Hz, 2H), 7.54 (s, 1H), 7.60 (dd, J = 7.2, 2.1 Hz, 4H), 7.73 (d, J = 7.6 Hz, 2H), 7.81–7.97 (m, 7H), 8.22 (d, J = 2.0 Hz, 1H), 8.32 (t, J = 5.2 Hz, 1H), 8.41 (d, J = 8.8 Hz, 2H), 13.16 (br s, 1 H); HRMS calcd for $[C_{40}H_{37}ClN_2O_5S+H]$ 693.21845: found 693.21791. HPLC purity: CH_3CN–H_2O, 98.8%, MeOH–H_2O, 97.8%.

5.3.11. 4-(2-{1-Benzhydryl-5-chloro-2-[2-({[2-fluoro-6-(trifluoromethyl)phenyl]sulfonyl}amino)ethyl]-1*H*-indol-3-yl}ethoxy)benzoic acid (13k). Ester hydrolysis of **11k**, white solid, 36% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.96–3.06 (m, 2H), 3.08–3.17 (m, 2H), 3.21 (t, J = 6.4 Hz, 2H), 4.22 (t, J = 6.4 Hz, 2H), 5.06 (t, J = 6.2 Hz, 1H), 6.55 (d, J = 9.1 Hz, 1H), 6.79–6.86 (m, 3H), 6.88 (s, 1H), 7.01–7.07 (m, 4H), 7.24–7.37 (m, 7H), 7.55 (d, J = 2.3 Hz, 1H), 7.56–7.69 (m, 2H), 7.98 (d, J = 8.8 Hz, 2H); HRMS calcd for $[C_{39}H_{31}ClF_4N_2O_5S+H]$ 751.16511: found 751.16431. HPLC purity: CH_3CN–H_2O, 98.8%, MeOH–H_2O, 98.5%.

5.3.12. 4-[3-(1-Benzhydryl-2-{2-[(1,1′-biphenyl-2-ylsulfonyl)amino]ethyl}-5-chloro-1*H*-indol-3-yl)propyl]benzoic acid (14a). Ester hydrolysis of **12a**, white powder, 98% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.69–1.88 (m, 2H), 2.59–2.78 (m, 6H), 2.80–2.94 (m, 2H), 6.44 (d, J = 8.8 Hz, 1H), 6.77 (dd, J = 8.8, 2.3 Hz, 1H), 6.93 (s, 1H), 6.99 (dd, J = 6.8, 2.5 Hz, 4H), 7.19–7.41 (m, 15H), 7.41–7.50 (m, 2H), 7.58–7.68 (m, 1H), 7.81 (dd, J = 8.0, 1.1 Hz, 1H), 7.83–7.89 (m, 2H); HRMS calcd for $[C_{45}H_{39}ClN_2O_4S+H]$ 739.23919: found 739.23825. HPLC purity: CH_3CN–H_2O, 97.5%, MeOH–H_2O, 97.0%.

5.3.13. 4-[3-(1-Benzhydryl-2-{2-[(1,1′-biphenyl-3-ylsulfonyl)amino]ethyl}-5-chloro-1*H*-indol-3-yl)propyl]benzoic acid (14b). Ester hydrolysis of **12b**, white solid, 100% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.67–1.89 (m, 2H), 2.53–2.74 (m, 4H), 2.75–2.87 (m, 2H), 2.87–2.98 (m, 2H), 6.44 (d, J = 8.8 Hz, 1H), 6.75 (dd, J = 9.0, 2.1 Hz, 1H), 6.93–7.17 (m, 7H), 7.20–7.37 (m, 6H), 7.37–7.54 (m, 4H), 7.54–7.71 (m, 4H), 7.78 (d, J = 7.6 Hz, 2H), 7.88 (d, J = 8.1 Hz, 1H), 7.98 (s, 1H), 8.05 (s, 1H); HRMS calcd for $[C_{45}H_{39}ClN_2O_4S+H]$ 739.23919: found 739.23896. HPLC purity: CH_3CN–H_2O, 97.6%, MeOH–H_2O, 98.7%.

5.3.14. 4-{3-[1-Benzhydryl-5-chloro-2-(2-{[(2-fluorophenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}benzoic acid (14c). Ester hydrolysis of **12c**, white solid, 100% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.72–1.90 (m, 2H), 2.57–2.73 (m, 4H), 2.92 (s, 4H), 6.45 (d, J = 8.8 Hz, 1H), 6.77 (dd, J = 8.8, 2.3 Hz, 1H), 7.01 (s, 1H), 7.02–7.09 (m, 4H), 7.15 (d, J = 8.3 Hz, 2H), 7.23–7.43 (m, 8H), 7.45 (d, J = 2.3 Hz, 1H), 7.59–7.71 (m, 2H), 7.81 (d, J = 8.8 Hz, 2H), 8.16 (s, 1H); HRMS calcd for $[C_{39}H_{34}ClFN_2O_4S+H]$ 681.19846: found 681.19854. HPLC purity: CH_3CN–H_2O, 99.1%, MeOH–H_2O, 97.9%.

5.3.15. 4-{3-[1-Benzhydryl-5-chloro-2-(2-{[(2-chlorophenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}benzoic acid (14d). Ester hydrolysis of **12d**, white foam, 84% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89–2.01 (m, 2H), 2.63–2.84 (m, 6H), 2.94–3.07 (m, 2H), 5.01 (t, J = 5.6 Hz, 1H), 6.48 (d, J = 8.8 Hz, 1H), 6.79 (dd, J = 8.8, 2.0 Hz, 1H), 6.84 (s, 1H), 7.04 (dd, J = 6.6, 2.8 Hz, 4H), 7.22–7.33 (m, 9H), 7.40 (d, J = 1.8 Hz, 1H), 7.42–7.50 (m, 2H), 7.90 (dd, J = 7.7, 1.1 Hz, 1H), 8.02 (d, J = 8.3 Hz, 2H); HRMS calcd for $[C_{39}H_{34}Cl_2N_2O_4S–H]$ 695.15435: found 695.15363. HPLC purity: CH_3CN–H_2O, 97.0%, MeOH–H_2O, 96.2%.

5.3.16. 4-{3-[1-Benzhydryl-2-(2-{[(2-bromophenyl)sulfonyl]amino}ethyl)-5-chloro-1*H*-indol-3-yl]propyl}benzoic acid (14e). Ester hydrolysis of **12e**, off-white powder, 95% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.72–1.90 (m, 2H), 2.67 (q, J = 7.6 Hz, 4H), 2.78–2.99 (m, 4H), 6.45 (d, J = 8.8 Hz, 1H), 6.77 (dd, J = 8.8, 2.0 Hz, 1H), 6.99 (s, 1H), 7.05 (dd, J = 7.6, 1.8 Hz, 4H), 7.24–7.40 (m, 9H), 7.42–7.55 (m, 3H), 7.78–7.91 (m, 4H), 8.03 (t, J = 5.8 Hz, 1H), 12.74 (br s, 1H); HRMS calcd for $[C_{39}H_{34}BrClN_2O_4S+H]$ 741.1184: found 741.11696. Anal. Calcd for $[C_{39}H_{34}BrClN_2O_4S]$: C, H, N.

5.3.17. 4-{3-[1-Benzhydryl-5-chloro-2-(2-{[(2-methylphenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}benzoic acid (14f). Ester hydrolysis of **12f**, white solid, 100% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.52 (s, 3H), 2.87 (br s, 2H), 2.93–3.03 (m, 2H), 3.09 (t, J = 7.3 Hz, 2H), 4.12 (t, J = 6.7 Hz, 2H), 6.49 (d, J = 8.8 Hz, 1H),

6.72–6.87 (m, 3H), 7.04 (d, J = 7.8 Hz, 5H), 7.20 (t, J = 7.6 Hz, 1H), 7.26–7.38 (m, 7H), 7.40–7.50 (m, 1H), 7.58–7.69 (m, 2H), 7.74–7.87 (m, 2H), 7.95 (t, J = 5.6 Hz, 2H); HRMS calcd for [$C_{40}H_{37}ClN_2O_4S$+H] 677.22354: found 677.22244. HPLC purity: CH_3CN–H_2O, 98.6%, MeOH–H_2O, 98.4%.

5.3.18. 4-{3-[1-Benzhydryl-5-chloro-2-(2-{[(2-methoxyphenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}benzoic acid (14g). Ester hydrolysis of **12g**, off-white solid, 100% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.72–1.91 (m, 2H), 2.59–2.75 (m, 4H), 2.87 (br s, 4H), 3.81 (s, 3H), 6.43 (d, J = 8.8 Hz, 1H), 6.76 (dd, J = 8.8, 2.3 Hz, 1H), 6.93–7.07 (m, 6H), 7.18 (d, J = 7.8 Hz, 1H), 7.27–7.39 (m, 9H), 7.38–7.48 (m, 2H), 7.53–7.60 (m, 1H), 7.64 (dd, J = 7.8, 1.8 Hz, 1H), 7.85 (d, J = 8.3 Hz, 2H), 12.80 (br s, 1H); HRMS calcd for [$C_{40}H_{37}ClN_2O_5S$+H] 693.2185: found 693.21852. Anal. Calcd for [$C_{40}H_{37}ClN_2O_5S$]: C, H, N.

5.3.19. 4-(3-{5-Chloro-1-(diphenylmethyl)-2-[2-({[2-(trifluoromethoxy)phenyl]sulfonyl}amino)ethyl]-1*H*-indol-3-yl}propyl)benzoic acid (14h). Ester hydrolysis of **12h**, white solid, 100% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.69–1.90 (m, 2H), 2.54–2.77 (m, 4H), 2.93 (br s, 4H), 6.45 (d, J = 8.8 Hz, 1H), 6.77 (dd, J = 8.8, 2.0 Hz, 1H), 6.96–7.08 (m, 5H), 7.14 (d, J = 7.8 Hz, 2H), 7.23–7.37 (m, 6H), 7.39–7.48 (m, 2H), 7.53 (d, J = 8.3 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.75–7.90 (m, 3H), 8.16 (br s, 1H); HRMS calcd for [$C_{40}H_{37}ClF_3N_2O_5S$+H] 747.19019: found 747.18848. HPLC purity: CH_3CN–H_2O, 98.5%, MeOH–H_2O, 100%.

5.3.20. 4-{3-[1-Benzhydryl-5-chloro-2-(2-{[(2,6-dichlorophenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}benzoic acid (14i). Ester hydrolysis of **12i**, pale yellow solid, 71% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.69–1.91 (m, 2H), 2.61–2.78 (m, 4H), 2.94 (br s, 4H), 6.44 (d, J = 8.8 Hz, 1H), 6.78 (dd, J = 8.8, 2.0 Hz, 1H), 6.99 (s, 1H), 7.02–7.13 (m, 4H), 7.23–7.39 (m, 9H), 7.48–7.56 (m, 1H), 7.57–7.68 (m, 2H), 7.84 (d, J = 8.3 Hz, 2H), 8.29 (t, J = 5.1 Hz, 1H), 12.74 (br s, 1H); HRMS calcd for [$C_{39}H_{33}Cl_3N_2O_4S$+H] 731.12994: found 731.13005. Anal. Calcd for [$C_{39}H_{33}Cl_3N_2O_4S$]: C, H, N.

5.3.21. 4-{3-[1-Benzhydryl-5-chloro-2-(2-{[(2,6-dimethylphenyl)sulfonyl]amino}ethyl)-1*H*-indol-3-yl]propyl}benzoic acid (14j). Ester hydrolysis of **12j**, white solid, 96% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.70–1.89 (m, 2H), 2.50 (s, 6H), 2.60–2.70 (m, 4H), 2.74–2.84 (m, 2H), 2.84–2.92 (m, 2H), 6.45 (d, J = 8.8 Hz, 1H), 6.77 (dd, J = 8.8, 2.3 Hz, 1H), 6.94 (s, 1H), 7.03 (d, J = 2.3 Hz, 4H), 7.17 (d, J = 7.3 Hz, 2H), 7.24–7.38 (m, 9H), 7.43 (d, J = 2.3 Hz, 1H), 7.69 (t, J = 6.3 Hz, 1H), 7.85 (d, J = 8.3 Hz, 2H), 12.74 (br s, 1H); HRMS calcd for [$C_{41}H_{39}ClN_2O_4S$+H] 691.23919: found 691.23872. HPLC purity: CH_3CN–H_2O, 97.1%, MeOH–H_2O, 96.0%.

5.3.22. 4-(3-{1-Benzhydryl-5-chloro-2-[2-({[2-fluoro-6-(trifluoromethyl)phenyl]sulfonyl}amino)ethyl]-1*H*-indol-3-yl}propyl)benzoic acid (14k). Ester hydrolysis of **12k**, pale yellow powder, 86% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.73–1.89 (m, 2H), 2.67 (t, J = 7.5 Hz, 4H), 2.94 (s, 4H), 6.44 (d, J = 8.8 Hz, 1H), 6.77 (dd, J = 8.8, 2.0 Hz, 1H), 6.98 (s, 1H), 7.03 (d, J = 7.1 Hz, 4H), 7.20–7.40 (m, 8H), 7.45 (d, J = 2.0 Hz, 1H), 7.68–7.99 (m, 5H), 8.40 (t, J = 5.1 Hz, 1H), 12.74 (br s, 1H); HRMS calcd for [$C_{40}H_{33}ClF_4N_2O_4S$+H] 749.18585: found 749.18578. Anal. Calcd for [$C_{40}H_{33}ClF_4N_2O_4S$]: C, H, N.

5.4. General procedure 3: GLU micelle assay conditions

The assay was carried out in a 96-well format using a fluorescent plate reader with a 355 nm excitation filter and a 460 nm emission filter (Lab Systems Fluoroscan II, Helsinki, Finland). The assay buffer contained 940 μM Triton X-100, 50 mM Hepes, pH 7.4, 0.3 mM EDTA, 1 mM $CaCl_2$, and 300 mM KCl. DTPC (1, 2-*O*-tetradecyl-*sn*-glycero-3-phosphocholine, Avanti) at a final concentration of 120 μM was added the day of the experiment and GLU (7-hydroxycoumarinyl-γ-linolenate, Biomol Research Lab, Inc.) at a final concentration of 90 μM was added immediately prior to each assay.

Compounds (10 μL) dissolved in DMSO were placed in duplicate wells of a black 96-well plate. Wells corresponding to the positive and negative controls contained DMSO without inhibitors. Just prior to the experiment, 200 μL assay buffer containing 90 μM GLU and 120 μM DTPC was added to all wells in the assay plate. Assay buffer (50 μL) was added to the negative, and 50 μL cPLA$_2$α solution (5 mg/mL in assay buffer) was added to all other wells to initiate the reaction. The final concentration of enzyme was 1 μg/mL. The content of each well was mixed gently during the addition of the enzyme, and the plate was rapidly transferred to the fluorescent plate reader. The increase in fluorescence was read every 4 min for 84 min. The slope of the resulting line was determined and the inhibition was calculated.

5.5. General procedure 4: rat whole blood assay conditions

Fresh blood was collected in heparinized tubes by cardiac puncture of male Sprague–Dawley rats. Aliquots of blood (0.6 mL) were incubated with 6 μL vehicle (DMSO) containing various concentrations of the test compounds. After 15 min pre-incubation at 37 °C, blood was stimulated with 6 μL calcium ionophore, A23187 (Sigma C-7522), in DMSO for 10 min at 37 °C. The final concentration of A23187 was 5 μM. DMSO (6 μL) was added in the unstimulated controls. The reactions were stopped by mixing 60 μL cold EDTA to give a final concentration of 20 mM. The blood was centrifuged at 6500 rpm for 10 min on a microcentrifuge to obtain plasma. A 70 μL aliquot of plasma was mixed with 400 μL cold methanol for protein precipitation. After incubation at −80 °C for 30 min, the supernatant was obtained by centrifuging at 6500 rpm for 10 min and was assayed for TXB_2 according to the manufacturer's procedure (Assay Designs, Inc.).

5.6. General procedure 5: human whole blood assay conditions

Fresh blood was collected in heparinized tubes by venipuncture of healthy male volunteers. Aliquots of blood (0.9 mL) were incubated with 9 μL DMSO vehicle containing various concentrations of test compound. After 15 min pre-incubation at 37 °C, the blood was stimulated with 9 μL of calcium ionophore A23187 (Sigma C-7522) in DMSO for 15 min at 37 °C. The final concentration of A23187 was 30 μM. The unstimulated controls received 9 μL DMSO. The reactions were stopped by mixing 100 μL cold EDTA to give a final concentration of 20 mM and processed as described for the rat whole blood assay. Supernatant was assayed for TXB_2, LTB_4, PGE_2, and $PGF_2\alpha$ according to the manufacturer's procedures (Assay Designs, Inc.).

5.7. Isothermal calorimetry assay conditions

Purified human cPLA$_2\alpha$ was dialyzed in 50 mM Hepes, pH 7.4, 0.3 mM EDTA, 1 mM $CaCl_2$, and 300 mM KCl. DMSO and Triton X-100 were added to make a 2 mL protein solution consisting of 2.5 μM cPLA$_2\alpha$, 1% DMSO, and 940 μM Triton X-100. A stock solution of compound (7.85 mM in 100% DMSO) was diluted with the dialysis buffer in which cPLA$_2\alpha$ was dialyzed previously to make 2 mL working solution that contained 78.5 μM compound **14k**, 940 μM Triton X-100, and 1% DMSO. A control solution containing the dialysis buffer, 940 μM Triton X-100 and 1% DMSO was made, and both solutions were degassed. The cPLA$_2\alpha$ protein solution was loaded carefully into the sample cell of the calorimeter (VP-Isothermal Titration Calorimeter, Microcal Inc., Northampton, MA), while the syringe was filled with compound **14k** working solution (250 μL). The titration was carried out at 30 °C. A total of 20 injections (10 μL each) of the compound were made and settings were as recommended by Microcal. A separate control experiment was done in which compound **14k** was titrated against the buffer solution without any protein to determine the background. The data were analyzed using the Origin software supplied with the VP-isothermal titration calorimeter and the binding parameters were determined.

5.8. Cyclooxygenase assay conditions

A colorimetric COX assay (Cayman, cat. # 760111) was used to measure inhibition of both COX-1 and COX-2. SC-560-7 (Cayman, cat. # 70340) was used as the reference compound for selective COX-1 inhibition and Celecoxib was used as the reference for COX-2 inhibition. The assay was performed according to the manufacturer's directions.

5.9. Pharmacokinetics in rats and dogs

Male Sprague–Dawley rats (200–300 g, Taconic, Germantown, NY) were used for PK assessment. For iv administration, animals ($n = 3$) received a single bolus dose of 2 mg/kg in vehicle (50% PEG-400, 50% DSMO) via tail vein injection. For oral administration, overnight fasted rats ($n = 3$) were dosed via gavage at 25 mg/kg in MC/TW vehicle (0.5% methylcellulose, Sigma and 2% Tween 80, Spectrum) or Phosal formulation (55.5% Phosal 53 MCT, American Lecithin Co.; 5.6% Tween 80, Spectrum; 16.7% Labrasol, Gattefosse; and 22.2% propylene carbonate, Spectrum) with test compound dissolved at 37.5 mg/mL vehicle, diluted with water prior to use, and dosed at 4 mL/kg. Blood samples were collected into EDTA/NaF tubes via the jugular vein cannula at pre-selected time points over a 24-h period. After a centrifugation at 4 °C for 10 min, plasma samples (200 μL each) were harvested into a tube containing 10 μL of 2 N HCl (final pH of plasma samples approximately 4). Plasma samples were kept at −70 °C until assay.

Male Beagle dogs (8.5–13.0 kg) were used in the study. Dogs ($n = 3$ per treatment group) were fasted overnight and received either iv administration at 2 mg/kg in 20% DMSO/80% PEG-200 in a dosing volume of 0.2 mL/kg or po administration at 5 mg/kg for compound **3** or 50 mg/kg for compound **14k** in MC/TW or Phosal formulation (for details of the formulations, see the rat PK section). For compound **3**, the po dosing volume was 4 mL/kg and for compound **14k**, the po dosing volume was 6 mL/kg. Blood samples were collected into EDTA/NaF tubes via the saphenous vein at pre-selected time points over a 24-h period. After a centrifugation at 4 °C for 10 min, plasma samples (200 μL each) were harvested into tubes containing 10 μL of 2 N HCl (final pH of plasma samples approximately 4). Plasma samples were kept at −70 °C until assay.

To determine plasma concentrations of cPLA$_2\alpha$ inhibitors, aliquots (50 μL) of plasma samples were used and the plasma protein was precipitated with 100 μL of ACN containing a structural analogue of the test article as the internal standard. The supernatants (10 μL) were injected directly onto the LC/MS/MS system for analysis. The test article and its internal standard were separated with a Waters XTerra MS C18 column (20×2.1 mm ID, 2.5 μm). The mobile phase consisted of Solvent A with 0.1% TFA in H_2O and Solvent B with 0.1% TFA in CH_3CN. At a constant flow rate of 200 μL/min, a linear mobile phase gradient from 100% Solvent A to 100% Solvent B over a 4-min period was applied. An API-3000-1 (Applied Biosystems, Concord, Ontario, Canada) mass spectrometer fitted with an electrospray ionization interface (ESI) was used for the mass detection. The positive MRM acquisition conditions were optimized by infusing the standard solutions of the test article and the internal standard. Under these experimental conditions, the low quantitation limit was 5 ng/mL for most cPLA$_2\alpha$ inhibitors. The pharmacokinetic parameters (AUC and clearance) were calculated with non-compartment methods (WinNonlin, Version 4.0, Pharsight Corp., Mountain View, CA).

5.10. Rat carrageenan paw edema (CPE) model

Male Sprague–Dawley rats ($n = 10$ per treatment group) weighing 190–250 g from Taconic Farms were housed for 1 week prior to experimentation and fed food and

water ad libitum. The protocol for the CPE model was adapted from procedures previously described.[18,22] The volume of the left hind footpad of the rat was measured using an Ugo Basile plethysmometer prior to dosing. The animal was then dosed orally with the test compound in Phosal vehicle (55.5% Phosal 53 MCT, American Lecithin Co.; 5.6% Tween 80, Spectrum; 16.7% Labrasol, Gattefosse; and 22.2% propylene carbonate, Spectrum) or MC/TW vehicle (0.5% methylcellulose, Sigma and 2% Tween 80, Spectrum), orally with 10 mg/kg of Naproxen in Phosal vehicle, orally with 25 mg/kg Celecoxib in MC/TW vehicle, or with vehicle alone as described above. Two hours after dosing, 60 μL of 1% carrageenan in saline was injected subplantar into the left hind footpad. The paw volume measurement was repeated 3 h after the carrageenan injection. Inhibition was calculated using the following formula:

$$\begin{aligned}\text{Percent inhibition} = \{1 - [&3\text{ h paw volume} \\ &- 0\text{ h paw volume(test group)}] \\ &/[3\text{ h paw volume} \\ &- 0\text{ h paw volume(vehicle group)}]\} \\ &\times 100\end{aligned}$$

5.11. *Ascaris suum* airway challenge in naturally sensitized sheep

Allergic sheep ($n = 2$–3 per test group) weighing 27–50 kg were used to assess efficacy in the *A. suum* asthma model.[21] All sheep had previously been shown to develop both early and late bronchial responses to inhaled *A. suum* antigen. The sheep were conscious and were restrained in a modified shopping cart in the prone position with their heads immobilized. After topical anesthesia of the nasal passages with 2% lidocaine, a balloon catheter was advanced through one nostril into the lower esophagus. The animals were intubated with a cuffed endotracheal tube through the other nostril with a flexible fiberoptic bronchoscope as a guide. Animals were challenged via the airways with *A. suum* antigen (Greer Diagnostics, Lenoir, NC) and breath-by breath determination of mean pulmonary resistance (R_L) was measured with the esophageal balloon technique as described previously.[21] To assess bronchial responsiveness (AHR), the day following allergen challenge, cumulative concentration–response curves to carbachol were generated by measuring specific lung resistance immediately after inhalation of buffer and after each consecutive administration of 10 breaths of increasing concentrations of carbachol. The provocation test was discontinued when specific lung resistance increased by more than 400% from the post saline value, or after the highest carbachol concentration had been administered.

To assess efficacy after intravenous administration, compound **14k** was dissolved in 100% DMSO and administered 30 min prior to *A. suum* antigen challenge and 8 h post challenge. To determine efficacy by oral administration, **14k** was administered at 3 mg/kg BID on the day prior to antigen challenge and again 2 h prior to challenge and 8 h post challenge. To evaluate efficacy after long term oral administration, **14k** was dosed BID for 4 d prior to challenge and on the day of challenge (day 5) 2 h prior to challenge and 8 h post challenge. For oral administration at 3 mg/kg, **14k** was dissolved in Phosal vehicle (150 mg/mL stock solution in 55.5% Phosal 53 MCT, American Lecithin Co.; 5.6% Tween 80, Spectrum; 16.7% Labrasol, Gattefosse; and 22.2% propylene carbonate, Spectrum). On the day of the experiment the stock solution was diluted to 12 mg/mL with water and dosed at 0.25 mL/kg. For evaluation at 1 mg/kg po the 150 mg/mL stock solution was diluted to 4 mg/mL with water and dosed at 0.25 mL/kg. Changes in airway resistance after administration of **14k** were compared to historical control values for each individual sheep.

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