

Discovery of Gemilukast (ONO-6950), a Dual CysLT₁ and CysLT₂ Antagonist As a Therapeutic Agent for Asthma

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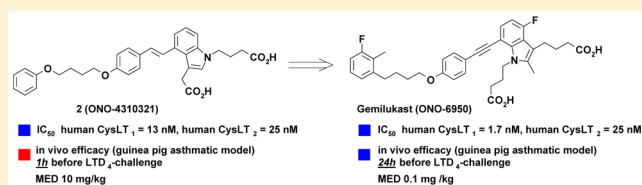
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S Supporting Information

ABSTRACT: An orally active dual CysLT₁ and CysLT₂ antagonist possessing a distinctive structure which consists of triple bond and dicarboxylic acid moieties is described. Gemilukast (ONO-6950) was generated via isomerization of the core indole and the incorporation of a triple bond into a lead compound. Gemilukast exhibited antagonist activities with IC₅₀ values of 1.7 and 25 nM against human CysLT₁ and human CysLT₂, respectively, and potent efficacy at an oral dose of 0.1 mg/kg given 24 h before LTD₄ challenge in a CysLT₁-dependent guinea pig asthmatic model. In addition, gemilukast dose-dependently reduced LTC₄-induced bronchoconstriction in both CysLT₁- and CysLT₂-dependent guinea pig asthmatic models, and it reduced antigen-induced constriction of isolated human bronchi. Gemilukast is currently being evaluated in phase II trials for the treatment of asthma.



■ INTRODUCTION

The cysteinyl leukotrienes (CysLTs), LTC₄, LTD₄, and LTE₄ are lipid mediators derived from arachidonic acid via the 5-lipoxygenase pathway,^{1–4} and they are involved in various inflammatory diseases, including asthma and allergic rhinitis. CysLTs are known to cause constriction of smooth muscle and the migration of inflammatory cells via certain receptors. Pharmacological studies have revealed the existence of two classes of receptors to which CysLTs can bind. They are the CysLT₁ and CysLT₂ receptors, which were identified and cloned in 1999 and 2000, respectively.^{5,6} Figure 1 shows several CysLT₁ selective antagonists. Pranlukast, montelukast, and zafirlukast, which are in clinical use, have demonstrated clinical benefit for the treatment of bronchial asthma and allergic rhinitis. Nonetheless, approximately half of the patients treated with these agents are nonresponders.^{7–9} Thus, more effective drugs than CysLT₁-selective antagonists are needed.

CysLT₂ receptors were reported to be expressed on airway smooth muscle cells,⁶ inflammatory cells,^{10–13} and vascular endothelial cells¹⁴ similar to CysLT₁ receptors. In addition, the urine concentration of LTE₄, which is a metabolite of LTD₄, is increased in aspirin-sensitive asthmatics or severe asthmatic patients.^{15–20} LTD₄ likely binds to both receptors in severe asthma but more tightly to CysLT₁ than CysLT₂ because the

severity is linked to the amount of LTD₄ production. The involvement of CysLT₂ in severe asthma is anticipated. Therefore, dual CysLT₁ and CysLT₂ antagonists may be more attractive as therapeutics for asthma.

As shown in Figure 2, two potent CysLT₂ selective antagonists have been reported.^{21,22} However, there are no reports of effective dual antagonists.^{23–27}

In our previous report,²⁸ (2S)-4-(3-carboxypropyl)-8-[[4-(4-phenylbutoxy)benzoyl]amino]-3,4-dihydro-2H-1,4-benzoxazine-2-carboxylic acid (ONO-2050297, the S-isomer of 1) was identified as the first potent dual antagonist, with IC₅₀ values of 17 and 0.87 nM for human CysLT₁ (hCysLT₁) and human CysLT₂ (hCysLT₂), respectively. As shown in Table 1, the PK profile of 1 was very poor, and its bioavailability was only 1.5% in rats.²⁸ However, further modification resulted in 4-(3-(carboxymethyl)-4-[(E)-2-[4-(4-phenoxybutoxy)phenyl]-vinyl]-1H-indol-1-yl)butanoic acid (2, ONO-4310321), which had a significantly improved PK profile in rats.²⁹ Compound 2 demonstrated a reduction in bronchoconstriction in a dose-dependent manner and complete inhibition at an oral dose of 10 mg/kg in a CysLT₁-dependent guinea pig bronchoconstric-

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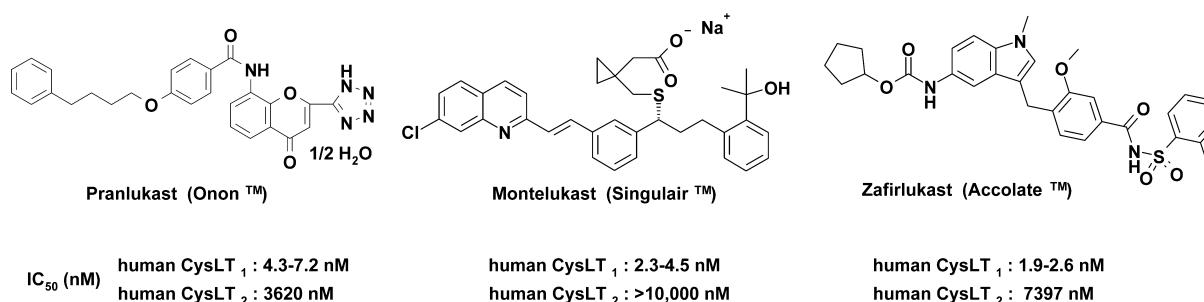


Figure 1. CysLT₁-selective antagonists. IC₅₀ values were quoted from refs 5 (CysLT₁ antagonist activities) and 6 (CysLT₂ antagonist activities).

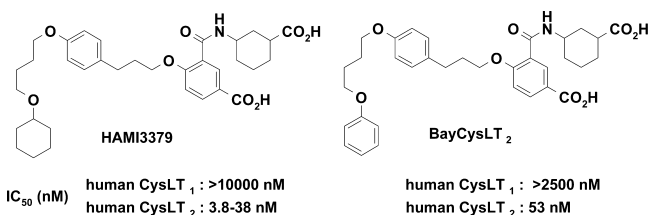


Figure 2. CysLT₂-selective antagonists. IC₅₀ values were quoted from refs 21 (HAMI3378) and 22 (BayCysLT₂).

tion model. However, compound 2 was unfit as a clinical candidate because the strength and duration of its in vivo efficacy were inadequate.

In this article, further modification to create an orally active dual CysLT₁ and CysLT₂ antagonist compatible with once daily administration will be described. After structural optimizations based on the duration of efficacy and penetration into lung tissue, 4,4'-[4-fluoro-7-(2-{4-[4-(3-fluoro-2-methylphenyl)-butoxy]phenyl}ethynyl)-2-methyl-1*H*-indole-1,3-diyl]-dibutanoic acid (35, ONO-6950, gemilukast) was identified as a clinical candidate.

RESULTS AND DISCUSSION

Lead Optimization. The isomer of the core indole ring was initially investigated. The indole-3-acetic acid derivative 2 was

previously reported.²⁹ Table 2 shows that indole-1-acetic acid derivative 3, an isomer, is a more potent dual antagonist, with IC₅₀ values of 1.5 and 6.0 nM against hCysLT₁ and hCysLT₂, respectively. In addition, both compounds 2 and 3 were orally administered 1 h before LTD₄ challenge at 1 mg/kg, and compound 3 showed potent in vivo efficacy (97% inhibition of bronchoconstriction) compared to compound 2 (48% inhibition). Indole-1-acetic acid derivatives appeared more amenable to further modification, although their bioavailability in guinea pigs was moderate.

As shown in Table 2, both compounds 2 and 3 demonstrated much lower bioavailability in guinea pigs than in rats. Generally, the more basic pH of the small intestine reduces the permeability of acidic compounds. Indeed, compounds 2 and 3 showed lower permeability at pH 7.4 than at pH 6.2 (Table 2). The pH in the small intestine of guinea pigs is greater than that for other species (pH 7.6–8.2 for guinea pigs, pH 6.5–7.1 for rats, pH 6.6–7.5 for dogs, pH 5.5–6.0 for monkeys, and pH 5.0–7.0 for humans),^{30,31} and this basic pH explains why these compounds are less bioavailable in guinea pigs. This species difference should be considered when evaluating compounds in guinea pigs.

Table 3 shows the effects of introducing a methyl group at the 2-position of the indole and other substitutions on the terminal phenyl group. As indicated by a comparison of compounds 9–16 and 4–8, the incorporation of the methyl

Table 1. Pharmacokinetic Profiles and In Vivo Efficacy of 1 and 2

hCysLT ₁ /CysLT ₂		
IC ₅₀ (nM) ^c	8.5 (5.0–14) / 2.3 (1.6–3.5)	12 (8.4–17) / 27 (21–35)
Pharmacokinetics profile		
<i>F</i> (%) : rat / guinea pig	1.5 / 2.8	100 / 26
In vivo efficacy ^d 1h p.o.		
% inhibition at 10mg/kg	0%	99%

^aCompound 1: Rat bioavailability was calculated based on an intravenous (iv) dose of 1 mg/kg and an oral administration (po) dose of 30 mg/kg. Guinea pig bioavailability was calculated based on an iv dose of 0.1 mg/kg and a po dose of 30 mg/kg. Mean values (*n* = 3). ^bCompound 2: Rat and guinea pig bioavailability was calculated based on an iv dose of 1 mg/kg and a po dose of 10 mg/kg. Mean values (*n* = 3). ^cIC₅₀ values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad) (*n* ≥ 2). ^dLTD₄-induced bronchoconstriction model dependent on CysLT₁. Mean values (*n* = 3).

Table 2. Indole-3-acetic Acid (2) and Indole-1-acetic Acid (3)

$R =$

cmpd	structure	IC ₅₀ (nM) ^a		in vivo efficacy ^b		Permeability		Carboxylic Acid pK _a ^c	Bioavailability ^d	
				% inhibition					F (%)	
		hCysLT ₁	hCysLT ₂	mg/kg	% inhibition	PAMPA x 10 ⁻⁶ cm/s			Guinea pig	Rat
						pH 6.2	pH 7.4			
2		12 (8.4-17)	27 (21-35)	1	48	6.9	1.8	4.9 /4.1	26	100
3		1.5 (0.87-2.7)	6.0 (3.9-9.4)	1	97	1.6	<0.05	4.8 /3.4	12	66

^a Assay protocols are provided in the [Experimental Section](#). IC₅₀ values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad) ($n \geq 2$). ^b Effects on a LTD₄-induced bronchoconstriction model dependent on CysLT₁. The values represent the mean for $n = 3$. The compounds were orally administered 1 h before LTD₄ challenge. ^c pK_a: Predicted by ADMET Predictor version 7 (Simulations Plus, Inc.). ^d F (%) was calculated using the mean of AUC (po) and the mean of AUC (iv) ($n = 3$).

group into the indole improved permeability at pH 7.4. This improvement appears to result from the diminished acidity of the 1-acetic acid moiety due to the electron donation of the methyl group.

In addition, the hCysLT₂ antagonist activity of 2H-indole-1-acetic acid derivatives tended to be much weaker in guinea pigs (IC₅₀ value of >100 nM, data not shown) compared with humans. Ultimately, 2-methyl-indole was considered a more suitable core structure.

Upon the addition of substitutions to the terminal phenyl group, no significant differences in hCysLT₁ or hCysLT₂ antagonist activities were achieved. However, the compounds possessing certain substituents on the terminal phenyl group had a tendency toward improved antagonist activities and permeability. Thus, these compounds were evaluated in a CysLT₁-dependent guinea pig asthmatic model at doses of 1, 0.3, or 0.1 mg/kg. The compounds were orally administered 1 h before LTD₄ challenge.

As shown in [Table 3](#), some of the compounds demonstrated high inhibitory activity at 0.3 mg/kg. In particular, compounds **8** and **16**, both possessing a 3-chloro-2-methylphenyl group at the terminus, exhibited approximately 50% inhibition at 0.1 mg/kg. Both compounds appeared equivalent. However, compound **16** was selected for further profiling because 2-methyl-indole-1-acetic acid derivatives are more promising in terms of the species differences in antagonist activities and permeability, as discussed above.

[Table 4](#) shows the pharmacokinetic profile of **16** in rats, guinea pigs, and dogs. Compound **16** demonstrated a low oral bioavailability of 11% in guinea pigs, whereas it demonstrated good oral bioavailability of 56% and a long half-life of 8.9 h in rats. As shown above, compound **16** showed in vivo efficacy even at the lowest dose. Thus, the concentration of **16** in the plasma and lung tissue of guinea pigs was measured ([Figure 3](#)). The concentration of **16** remained high (K_p value of 15) for over 24 h in lung tissue.

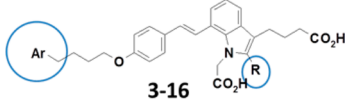
Lakshminarayana et al. reported that tuberculosis drugs whose K_p values are at least 2 can be efficient.³² In addition, the K_p value of montelukast was reported to be greater than 5 at 24 h according to its FDA pharmacology review.³³ Thus, the 2-methyl-indole derivative represented by **16** appears to possess a profile appropriate for the treatment agent of asthma.

Further Optimization of Advanced Lead toward a Once-Daily Drug. From [Figure 3](#), it appeared that there was room to improve the pharmacokinetic properties of compound **16** as a clinical candidate, although it was active in the in vivo model. In particular, the permeability of **16** was still low. Two options for improving the low permeability were deduced: reducing (1) flexibility and (2) acidity. In a situation where target proteins are related to lipid mediators, in our experience there is a trend toward an elongated and flapping compound as a ligand. Thus, it is difficult to make dual antagonists rigid. For example, shortening or fixing the side chain to reduce flexibility decreases antagonist activity. Therefore, we focused on the trans-double bond in the core structure and attempted its replacement with a triple bond. Because a triple bond is more linear than a double bond, the potential spatial area occupied by the side chain was expected to decrease.

Compounds possessing a triple bond instead of the double bond were synthesized and evaluated as shown in [Table 5](#). Interestingly, compound **19**, which corresponds to compound **16**, retained dual antagonist activities; if anything, hCysLT₂ antagonist activity increased. Thus, the compatibility of the triple bond was confirmed. In addition, the permeability of **19** was improved by P_{app} (pH 7.4) = 3.2×10^{-6} cm/s as expected.

The acidity of carboxylic acids should correlate with distance from the indole ring. As previously reported for the benzoxazine analogues,²⁸ the length of the carboxylic acid side chain is an important factor that affects antagonist activities. Thus, several analogues with various carboxylic acid side chains lengths were synthesized to better balance

Table 3. Introduction of a Methyl Group into the 2-Position of the Indole and Substitutions on the Terminal Phenyl Group



3-16

cmpd	Ar	R	IC ₅₀ (nM) ^a		Permeability PAMPA x 10 ⁻⁶ cm/s		in vivo efficacy ^b %Inhibition, po, 1h		
			hCysLT ₁	hCysLT ₂	pH 6.2	pH 7.4	0.1 mg/kg	0.3 mg/kg	1 mg/kg
Montelukast			2.3-4.5 ^c	>10,000 ^c	-	-	-	-	-
HAMI3379			>10,000 ^d	3.8-38 ^d	-	-	-	-	-
3		H	1.5 (0.87-2.7)	6.0 (3.9-9.4)	1.6	<0.05	-	37%	97%
4		H	3.4 (2.7-4.2)	25 (12-50)	1.3	<0.05	-	-	75% at 3mg/kg
5		H	3.1 (1.9-4.9)	23 (11-47)	2.5	<0.05	-	-	97%
6		H	1.0 (0.60-1.6)	2.1 (1.5-2.7)	1.4	<0.05	0%	45%	94%
7		H	2.9 (2.9-3.4)	14 (9.1-23)	3.5	<0.05	37%	73%	97%
8		H	0.62 (0.41-0.95)	4.6 (2.7-7.6)	1.9	1.4	67%	-	-
9		Me	4.1 (3.1-5.4)	4.7 (3.5-6.4)	1.3	<0.05	-	-	33%
10		Me	46 (13-170)	15 (9.7-24)	-	-	-	-	24%
11		Me	2.4 (1.6-3.6)	1.0 (0.71-1.5)	4.4	2.6	-	-	25%
12		Me	2.2 (1.2-4.2)	3.7 (2.6-5.2)	9.4	0.5	-	9%	-
13		Me	0.80 (0.55-1.2)	1.5 (1.2-1.8)	1.8	0.3	-	55%	-
14		Me	1.6 (0.81-3.1)	2.2 (1.7-2.8)	2.9	0.3	-	87%	-
15		Me	1.1 (0.82-1.5)	7.2 (5.2-10)	4.0	1.5	-	83%	94%
16		Me	0.56 (0.45-0.70)	5.1 (3.9-6.5)	4.6	1.5	46%	89%	99%

^aAssay protocols are provided in the [Experimental Section](#). IC₅₀ values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad) ($n \geq 2$). ^bEffects on LTD₄-induced bronchoconstriction model dependent on CysLT₁ ($n = 3$). ^cIC₅₀ values were quoted from refs 5 (CysLT₁ antagonist activity) and 6 (CysLT₂ antagonist activity). ^dIC₅₀ values were quoted from ref 21.

Table 4. Pharmacokinetic Parameters of 16

species	dose (mg/kg)		iv $t_{1/2}$ ^a (h)	iv CL ^a (mL/min/kg)	V_{dss} ^a (L/kg)	po AUC ^a (μg·h/mL)	F^b (%)
	iv	po					
rat	1	3	8.9 ± 8.5	1.1 ± 0.7	0.30 ± 0.14	39 ± 12	56
guinea pig	1	10	5.3 ± 0.7	1.8 ± 0.1	0.23 ± 0.01	10 ± 4.1	11
dog	1	1	4.7 ± 5.0	5.5 ± 1.1	0.38 ± 0.33	0.65 ± 0.16	21

^aThe value are expressed as the mean ± standard deviation ($n = 3$). ^b F (%) was calculated using the mean of AUC (po) and the mean of AUC (iv).

permeability with dual antagonist activities. Compound 25 was the best among these analogues in [Table 5](#).

A triple bond is generally considered unstable under a certain condition. Thus, the stability of 25 was assessed under various severe conditions. Surprisingly, 25 was stable under acidic

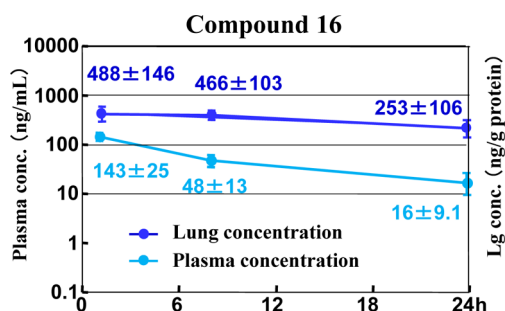


Figure 3. Concentration of **16** in plasma or lung tissue of guinea pigs. The value are expressed as the mean $\pm$ SD ($n = 4$).

conditions (1 mol/L H_2SO_4 in DME/ H_2O at 50 °C for 24 h) or basic conditions (1 mol/L NaOH in DME/ $\text{EtOH}/\text{H}_2\text{O}$ at 50 °C for 24 h). Furthermore, degradation of **25** in ambient light was not observed even under the conditions where the compounds with a double bond such as **16** easily decomposed. In the case of **16**, the isomerization of the trans-double bond by ambient light caused oxidation and decomposition.

Next, the effects of substitution at the benzene ring of the core indole were investigated. Table 6 shows 4-, 5-, and 6-fluoro derivatives of **25**. Only compound **29** exhibited dual antagonist activities comparable to **25**. Table 6 also shows the in vivo efficacies and PK profiles of **25** and **29**. Both compounds were evaluated in a LTD₄-induced bronchoconstriction model. They demonstrated sufficient efficacies at an oral dose even 24 h before LTD₄ challenge. Compound **29** demonstrated similar in vivo efficacy despite its poor PK profile, likely attributable to better penetration into lung tissue.

Table 7 shows the optimization of substitutions to the phenyl group in the 2-methyl-indole-1-butanoic acid derivatives **25** and **29**. All compounds exhibited potent antagonist activities against not only hCysLT₁ and hCysLT₂ but also guinea pig CysLT₁ (gCysLT₁) and guinea pig CysLT₂ (gCysLT₂).

These compounds were evaluated in a LTD₄-induced bronchoconstriction model at an oral dose of 0.3 or 0.1 mg/kg 24 h prior to LTD₄ challenge. As shown in Table 7, 2,3,4,6-tetrafluorophenyl derivatives **32** and **33** and 3-fluoro-2-methylphenyl derivatives **34** and **35** demonstrated more than 70% inhibition of bronchoconstriction at 0.3 mg/kg. Furthermore, compounds **34** and **35** demonstrated potent in vivo efficacy (approximately 70% inhibition) and a long duration of action even at 0.1 mg/kg. Compound **35** (ONO-6950, gemilukast) was ultimately chosen as a clinical candidate based upon further evaluation, including a safety study.

Further Biological Profiling of 35. Table 8 shows that **35** demonstrated acceptable bioavailability (35% in rats, 51% in dogs, 56% in guinea pigs) and low clearance values (0.68 mL/min/kg in rats, 1.10 mL/min/kg in guinea pigs, 0.64 mL/min/kg in dogs). In particular, the clearance of **35** in dogs was much lower compared to **16**, and this resulted in an improved pharmacokinetic profile in dogs. For example, **35** had a long half-life (7.6 h) and abundant blood exposure (28.2 $\mu\text{g}\cdot\text{h}/\text{mL}$). An excellent pharmacokinetic profile for **35** in humans was expected because much lower clearance (0.2 mL/min/kg) in humans was estimated based upon allometric scaling of the animal results.^{34,35} As shown in Figure 4, 2-methyl-indole derivatives such as **35** tended to penetrate lung tissue well. Thus, a ratio (K_p value) of the concentration of **35** in lung tissue to the concentration in plasma was calculated from the observed data in guinea pigs. As expected, the K_p value was very high: approximately 8 at an oral dose of 3 mg/kg or 30 at an oral dose of 0.3 mg/kg.

Regarding the risk assessment of **35**, the result (11% inhibition at 10 μM) of an in vitro hERG assay indicated low risk for QT interval prolongation, and the Ames test was negative. Further evaluation of **35** as a clinical candidate was then conducted.

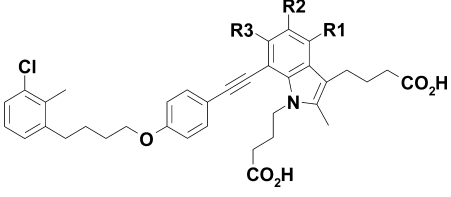
The value are expressed as the mean $\pm$ SE ($n = 8$). The compound was administered once daily.

Table 5. Length of Two Carboxylic Acid Side Chains of 2-Methyl-indole Derivatives

compd	m	n	IC ₅₀ ^a (nM)		permeability PAMPA $\times 10^{-6}$ cm/s pH 7.4
			hCysLT ₁	hCysLT ₂	
17	1	1	0.47 (0.29–0.76)	0.89 (0.81–0.97)	<0.05
18	1	2	0.32 (0.20–0.50)	5.7 (4.9–6.8)	0.7
19	1	3	0.22 (0.17–0.27)	0.88 (0.71–1.1)	3.2
20	2	1	12 (8.3–16)	96 (84–110)	7.2
21	2	2	10 (6.2–17)	78 (48–127)	3.6
22	2	3	17 (13–23)	22 (14–35)	3.0
23	3	1	5.5 (3.6–8.5)	55 (46–65)	4.4
24	3	2	0.79 (0.54–1.2)	15 (11–20)	7.2
25	3	3	1.5 (1.2–2.0)	5.5 (4.3–7.1)	7.9
26	4	1	65 (56–75)	248 (73–847)	1.0
27	4	2	25 (17–36)	60 (26–138)	4.6
28	4	3	56 (46–69)	30 (23–41)	5.0

^a Assay protocols are provided in the Experimental Section. IC₅₀ values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad) ($n \geq 2$).

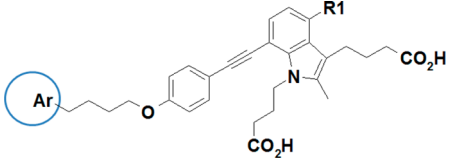
Table 6. Incorporation of a Fluorine Atom into the Core Indole Ring

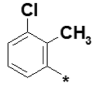
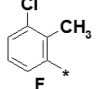
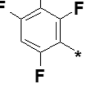
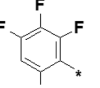
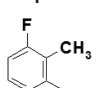
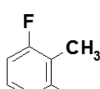


compd	R1	R2	R3	IC ₅₀ ^a (nM)		In vivo efficacy ^b , po % Inhibition		pharmacokinetics guinea pig ^c	
				hCysLT ₁	hCysLT ₂	0.3 mg/kg, 1 h	1 mg/kg, 24 h	AUC ^c (μg·h/mL)	F ^d (%)
25	H	H	H	1.5 (1.2–2.0)	5.5 (4.3–7.1)	96	97	10.5 ± 2.7	66
29	F	H	H	3.4 (2.1–5.3)	12 (9.9–15)	99	79	3.4 ± 0.9	52
30	H	F	H	16 (6.6–38)	31 (15–63)				
31	H	H	F	11 (8.2–15)	48 (33–70)				

^aAssay protocols are provided in the [Experimental Section](#). IC₅₀ values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad) ($n \geq 2$). ^bEffects on LTD₄-induced bronchoconstriction model dependent on CysLT₁. The values represent the means for $n = 6$. ^cGuinea pig cassette dosing at 0.2 mg/kg, iv and 1 mg/kg, po (nonfasted). The value are expressed as the mean ± SD ($n = 3$). ^dF (%) was calculated using the mean of AUC (po) and the mean of AUC (iv).

Table 7. Substitution on the Terminal Phenyl Group of 2-Methyl-indole-1-butanoic Acid Derivatives



cmpd	Ar	R ¹	IC ₅₀ (nM) ^a				Permeability PAMPA × 10 ⁻⁶ cm/s pH 7.4	in vivo Efficacy ^b % inhibition, po, 24 h	
								0.3 mg/kg	0.1 mg/kg
			hCysLT ₁	hCysLT ₂	gCysLT ₁	gCysLT ₂			
25		H	1.5 (1.2–2.0)	5.5 (4.3–7.1)	2.2 (2.0–2.5)	24 (21–28)	7.9	57	25
29		F	3.4 (2.1–5.3)	12 (9.9–15)	3.0 (2.1–4.5)	33 (26–44)	-	12	-
32		H	2.8 (2.2–3.5)	3.6 (3.1–4.2)	2.6 (2.3–3.0)	9.9 (8.3–12)	6.8	81	36
33		F	7.4 (6.0–9.2)	13 (10–16)	4.3 (3.7–5.0)	11 (9.6–12)	7.9	76	40
34		H	1.1 (8.7–14)	8.8 (6.9–11)	2.0 (1.7–2.3)	12 (9.6–15)	12.4	88	67
35 Gemilukast		F	1.7 (1.2–2.4)	25 (19–32)	6.3 (5.3–7.5)	8.2 (6.8–9.9)	5.1	86	74

^aAssay protocols are provided in the [Experimental Section](#). IC₅₀ values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad) ($n \geq 6$). ^bEffects on LTD₄-induced bronchoconstriction model dependent on CysLT₁. The values represent the means for $n = 6$.

Asthmatic Model Mediated via CysLT₂ in Guinea Pigs.

LTC₄ is reported to be a guinea pig CysLT₂ selective ligand, unlike the case in humans.^{36,37} It is difficult to evaluate pharmacological effects involving CysLT₂ in guinea pigs

because LTC₄ is immediately metabolized by γ -glutamyltranspeptidase. Yonetomi et al. reported a novel asthmatic model mediated via the CysLT₂ receptor in guinea pigs.³⁸ In the reported CysLT₂ dependent model, the metabolism of LTC₄

Table 8. Pharmacokinetic Parameters of 35 in Rats and Dogs

species	dose (mg/kg)		iv $t_{1/2}^c$ (h)	CL ^c (mL/min/kg)	V_{dss}^c (L/kg)	po C_{max}^c (μg/mL)	po AUC ^c (μg·h/mL)	F^{d1} (%)
	iv	po						
rat ^a	1	1	9.6 ± 3.1	0.68 ± 0.26	0.24 ± 0.06	1.19 ± 0.59	9.33 ± 1.45	35
guinea pig ^b	0.2	1	7.1 ± 3.8	1.10 ± 0.10	0.31 ± 0.19	1.01 ± 0.29	8.51 ± 1.49	56
dog ^a	1	1	7.6 ± 1.0	0.64 ± 0.24	0.14 ± 0.05	3.69 ± 0.74	28.2 ± 8.70	51

^aSingle intravenous and oral administration. ^bCassette dosing. ^cThe value are expressed as the mean ± standard deviation ($n = 3$). ^d F (%) was calculated using the mean of AUC (po) and the mean of AUC (iv).

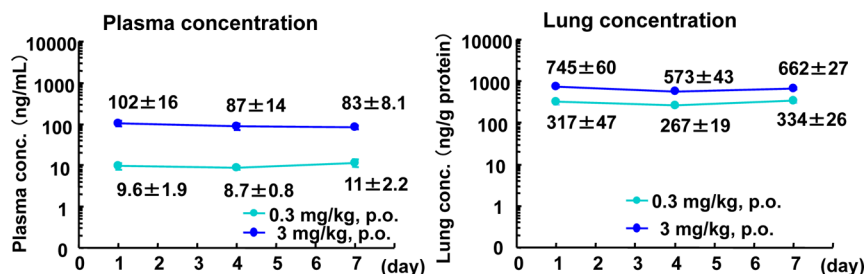


Figure 4. Plasma and lung tissue concentrations of 35 in guinea pigs.

was inhibited, and the effect of montelukast (CysLT₁ selective antagonist) on LTC₄-induced bronchoconstriction was only partial. In contrast, as shown in Figure 5, 35 (dual CysLT₁ and

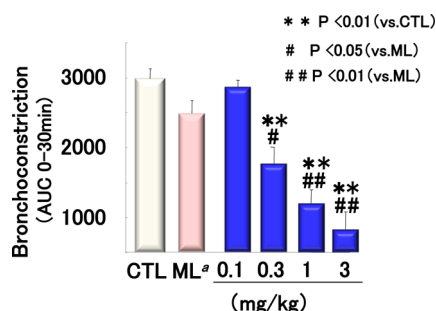


Figure 5. Efficacy of 35 in an asthmatic model mediated via CysLT₂ in guinea pigs. (a) ML (montelukast) was orally administered at 0.1 mg/kg. Each column represents the means ± SE of 5–10 animals.

CysLT₂ antagonist) significantly reduced the bronchoconstriction in a dose-dependent manner. Thus, 35 is expected to be a more useful treatment agent for asthma because of the potential involvement of CysLT₂ in severe symptoms.

Antigen-Induced Constriction in Isolated Human Bronchi. A study using human tissue increases the probability of success in clinical trials. An evaluation of 35 using human bronchi was performed. Isolated human bronchi were passively sensitized by incubation with human atopic serum, and the resulting bronchial strip was suspended in a Magnus bath. Test compound or vehicle was applied 30 min before mite antigen challenge. In addition, a cyclooxygenase inhibitor (indomethacin) and an antihistamine drug (pyrilamine) were applied 10 and 5 min, respectively, before antigen challenge such that the antigen-induced contractile response was dependent on CysLTs. The detailed procedure is described in the Experimental Section.

Figure 6 shows that 100 nmol/L of 35 reduced the constriction of isolated human bronchi. The *in vitro* CysLT₁ or CysLT₂ antagonist activity of 35 was several times weaker than that of montelukast (CysLT₁) or BayCysLT₂RA³⁹ (CysLT₂), so compound 35 was used at a higher concentration.

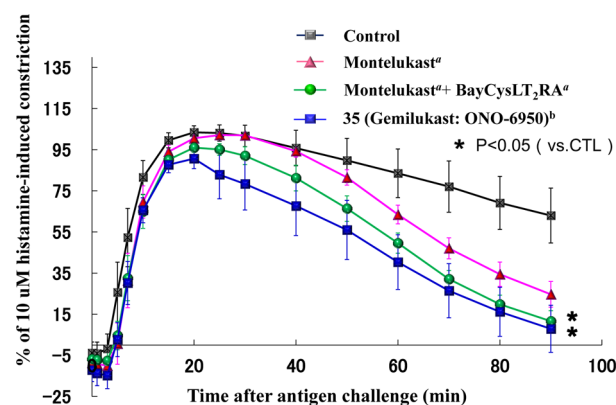


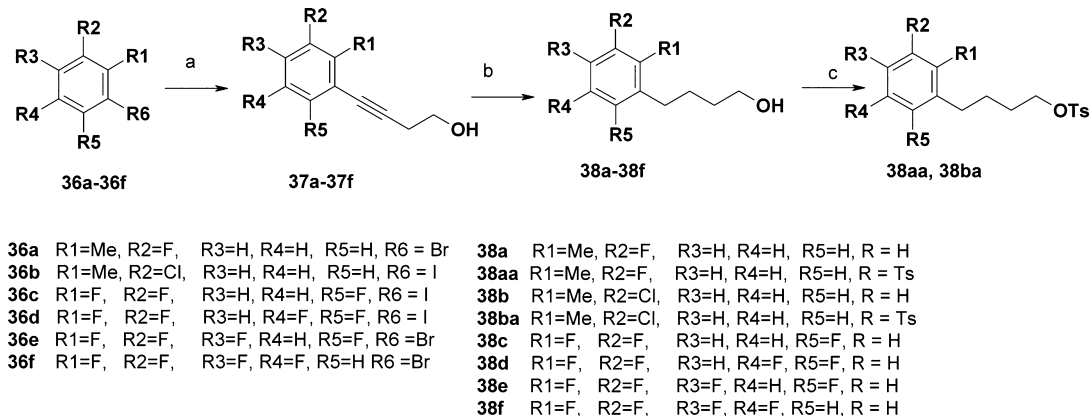
Figure 6. Effect of compound 35 on antigen-induced constriction of isolated human bronchi. (a) Concentration of both montelukast and BayCysLT₂RA was 10 nmol/L ($n = 4$). (b) Concentration of 35 was 100 nmol/L ($n = 4$).

As a result, the inhibitory activity of 35 (blue line) was comparable to that of a combination treatment of montelukast and BayCysLT₂RA (green line). Recently, the expression of CysLT₂ in airways of asthma subjects was reported to be higher than for nonasthma subjects.⁴⁰ This result suggests that dual CysLT₁ and CysLT₂ antagonists could be more effective and useful.

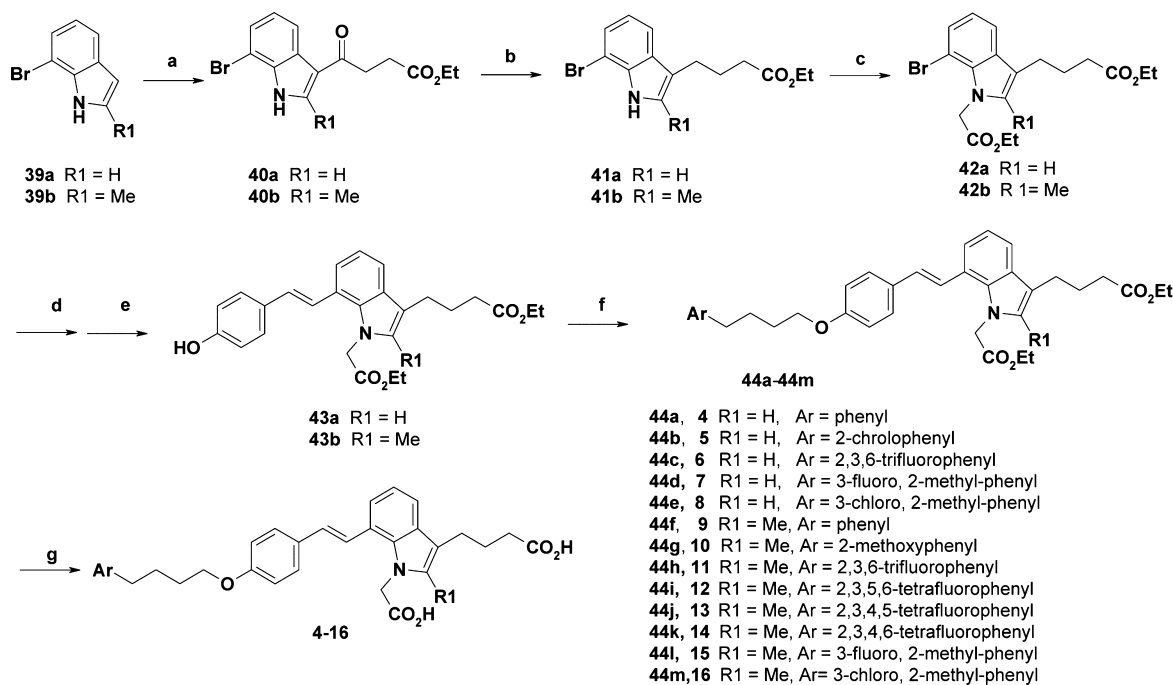
CHEMISTRY

Syntheses of the compounds herein are outlined in Schemes 1–6. Syntheses of 38a–f are described in Scheme 1. Compounds 36a–f were subjected to Sonogashira coupling with 3-buten-1-ol. The resulting acetylene derivatives 37a–f were converted to alcohol derivatives 38a–f with Pd/C under a hydrogen atmosphere. Tosyl derivatives 38aa and 38ba were afforded by tosylation of 38a and 38b.

Compounds 4–16 were prepared as shown in Scheme 2. Commercially available indole derivatives 39a or 39b were converted to ketoesters 40a or 40b by Friedel–Crafts acylation followed by reduction with sodium borohydride to prepare monoester 41a or 41b. *N*-Alkylation of 41a and 41b with ethyl bromoacetate using K₂CO₃ as the base afforded diesters 42a

Scheme 1. Syntheses of Side Chains 38a–f^a

^aReagents and conditions: (a) 3-butyn-1-ol, PdCl₂(PPh₃)₂, Ph₃P, CuI, Et₃N, rt–80 °C, 33–99%; (b) H₂ gas, 10% Pd–C(wet), EtOH, rt, 52–95%; (c) *p*-toluenesulfonyl chloride, Et₃N, Me₃N·HCl, toluene, rt, 80–95%.

Scheme 2. Syntheses of Compounds 4–16^a

^aReagents and conditions: (a) AlCl₃, ethyl succinyl chloride, CH₂Cl₂, 0 °C, 83–34%; (b) NaBH₄, BF₃·OEt₂, THF, 0 °C, 54–76%; (c) ethyl bromoacetate, Cs₂CO₃, DMF, 50 °C, 99–86%; (d) 4-acetoxystyrene, palladium(II)acetate, tri-*o*-tolylphosphine, Et₃N, dioxane, 100 °C; (e) K₂CO₃, THF, EtOH, 50 °C, 89–96% (2 steps); (f) 4-phenyl-1-butanol, 4-(2-methoxyphenyl)-1-butanol, 4-(3-chlorophenyl)-1-butanol, or 38a–g, ADDP, PPh₃, CH₂Cl₂, rt; (g) NaOHaq, EtOH–DME, rt–50 °C, 51–97% (2 steps).

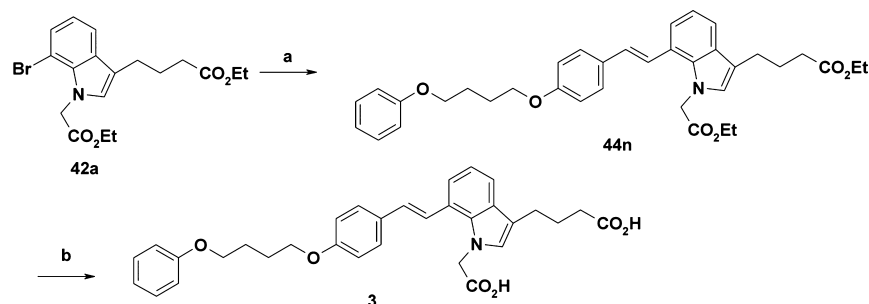
and 42b. Heck reaction of 42a and 42b and 4-acetoxystyrene, deprotection of the acetoxy group, and Mitsunobu reaction with the corresponding alcohols gave diesters 44a–m. Carboxylic acid derivatives 4–16 were afforded by alkaline hydrolysis of 44a–m.

Compound 3 was prepared as shown in Scheme 3. Heck reaction of the indole derivative 42a with 1-(4-phenoxybutoxy)-4-vinylbenzene followed by alkaline hydrolysis gave carboxylic acid derivative 3.

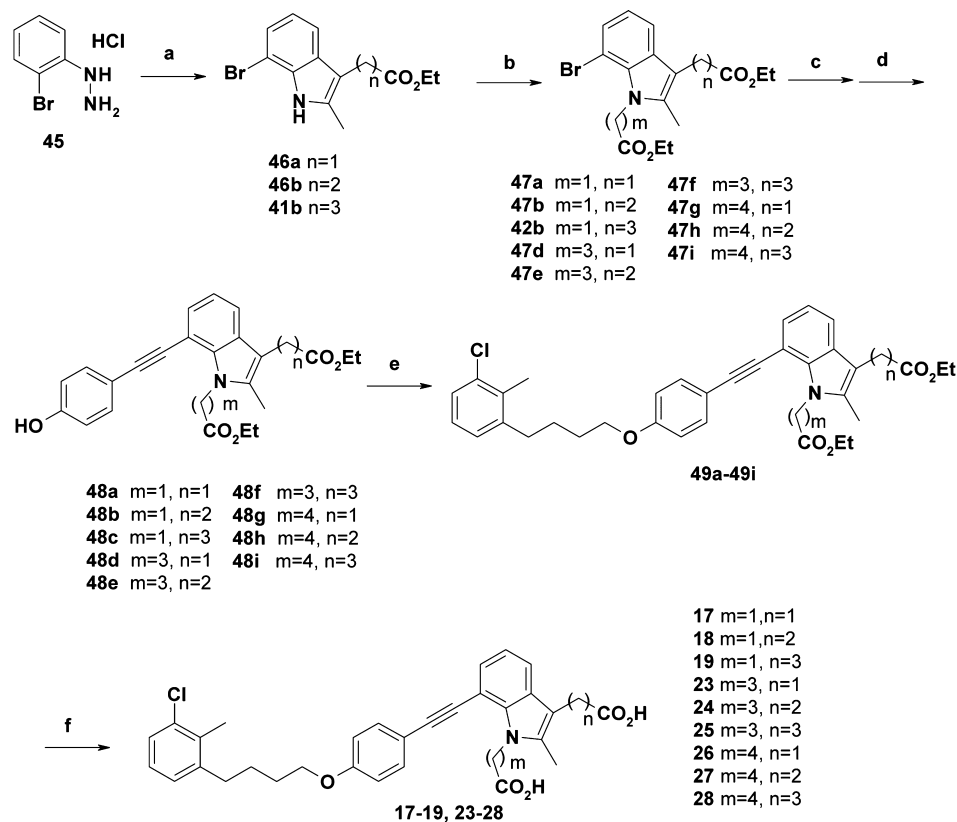
Syntheses of 17–19 and 23–28 are described in Scheme 4. *N*-alkylation of indole derivatives 46a, 46b, and 41b prepared via Fischer indole synthesis was performed with the corresponding bromide to afford diesters 47a and 47b, 42b, and 47d–i. These compounds were subjected to Sonogashira

coupling with 4-ethynylphenyl acetate, and then the acetoxy group was deprotected. The resulting phenols 48a–i were converted to dicarboxylic acid derivatives 17–19 and 23–28 by *O*-alkylation with tosylate 38ba, which was followed by alkaline hydrolysis.

Syntheses of 20–22 are described in Scheme 5. *N*-Alkylation and selective deprotection of indole derivatives 46a, 46b, and 41b resulted in monocarboxylic acids 51a–c. For one-carbon extension of 51a–c, diazoketone intermediates 52a–c were prepared with the use of trimethylsilyldiazomethane and treated under Wolff rearrangement conditions. The resulting C1 extended diesters 53a–c were converted to dicarboxylic acid compounds 20–22 as in Scheme 4.

Scheme 3. Synthesis of Compound 3^a

^aReagents and conditions: (a) 1-(4-phenoxybutoxy)-4-vinylbenzene, Pd(dba)₃, *tert*-Bu₃P, Cy₂NMe, 1,4-dioxane, 100 °C; (b) NaOHaq, EtOH–DME, rt–50 °C, 92% (2 steps).

Scheme 4. Syntheses of Compounds 17–19 and 23–28^a

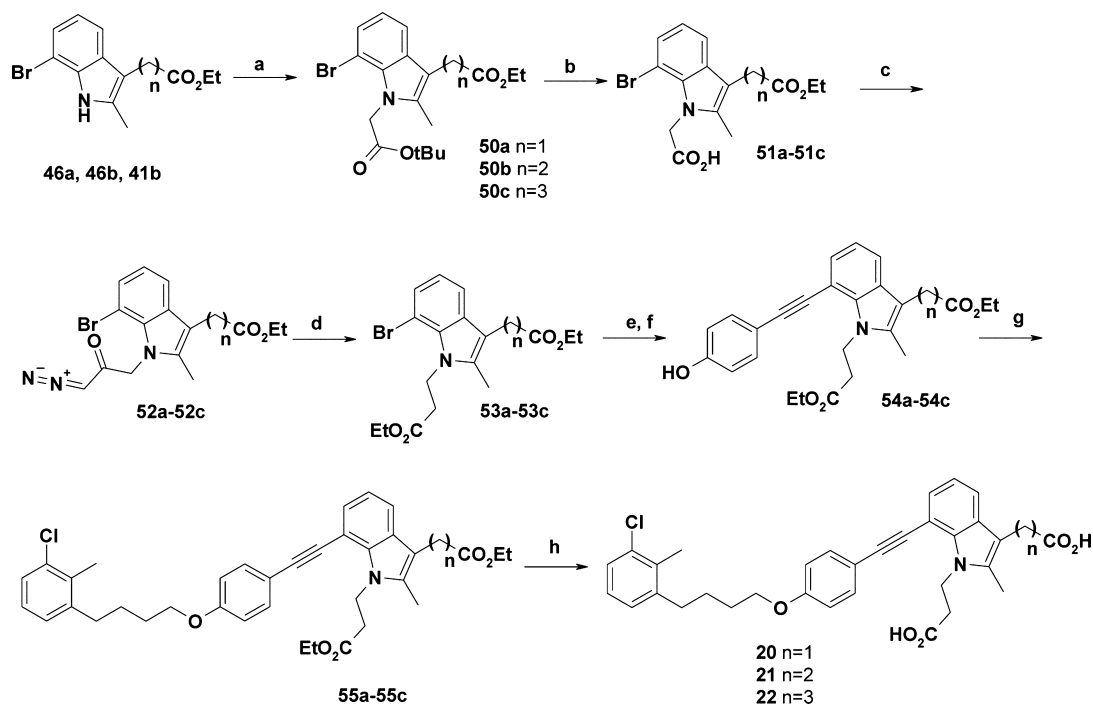
^aReagents and conditions: (a) H₂SO₄, EtOH, 4-oxopentanoic acid ($n=1$), 5-oxohexanoic acid ($n=2$), or 6-oxoheptanoic acid ($n=3$), 100 °C, 58–60%; (b) Br-(CH₂)_m-CO₂Et ($m=1–4$), Cs₂CO₃, rt–50 °C, 58–99%; (c) 4-ethynylphenyl acetate, Pd(*tert*-Bu₃P)₂, Pr₃NH, CH₃CN, rt; (d) K₂CO₃, DME–EtOH, rt, 62–78% (2 steps); (e) 38ba, Cs₂CO₃, DMF, rt; (f) NaOHaq, EtOH–DME, rt–50 °C, 67–97% (2 steps).

Scheme 6 shows the preparation of the fluorine-substituted derivatives 29–35. These compounds were also synthesized with the use of the corresponding synthetic materials according to the method described in Scheme 4.

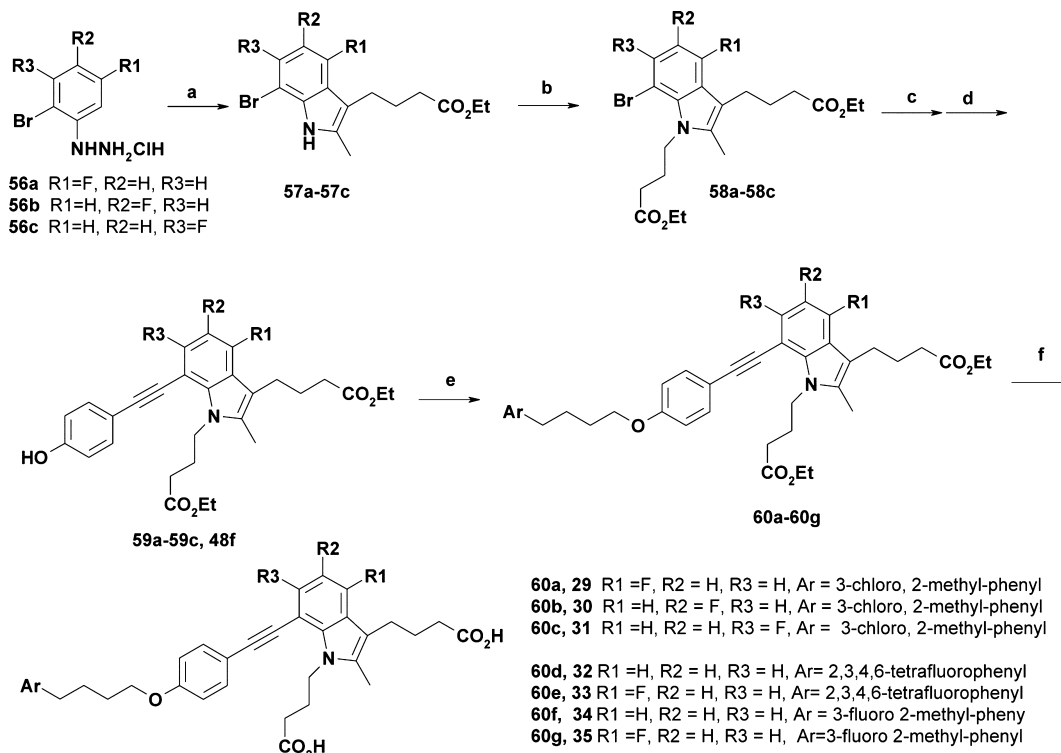
As shown in Figure 7, the structure of 35 (gemilukast) was confirmed with an X-ray crystallographic analysis of a single crystal (see Supporting Information). Interestingly, the terminal alkyloxyphenyl ring is orthogonal to the indole ring. In general, two aromatic rings at either end of a triple bond tend to be in the same plane. The fluorine atom at the 4-position of the indole is thought to be causally related to this twisted formation based on the tendencies of its analogues. This conformation is considered reasonable for gemilukast and its analogues.

CONCLUSION

In summary, the isomerization of the core indole and the incorporation of a triple bond significantly improved in vivo efficacy, and 35 (ONO-6950, gemilukast) was determined to be a novel, highly potent, and orally available dual CysLT₁ and CysLT₂ antagonist. Gemilukast showed good PK properties in rats, guinea pigs, and dogs without species differences, promising excellent PK in humans. Gemilukast also showed excellent efficacy in two guinea pig asthmatic models: 74% inhibition of bronchoconstriction in a CysLT₁-dependent model at an oral dose of 0.1 mg/kg 24 h before LTD₄ challenge, and 87% inhibition of bronchoconstriction in both CysLT₁- and CysLT₂-dependent models at an oral dose of 3

Scheme 5. Syntheses of Compounds 20–22^a

^aReagents and conditions: (a) 2-methyl-2-propenyl bromoacetate, Cs₂CO₃, 45 °C, 100%; (b) HCO₂H, CH₂Cl₂, rt, 91–100%; (c) isobutyl chloroformate, triethylamine, THF then CH₂N₂/diethyl ether, –10 °C to 0 °C; (d) silver(I) benzoate, triethylamine, EtOH, rt–50 °C, 44–52% (2 steps); (e) 4-ethynylphenyl acetate, Pd(Pt-Bu₃)₂, ¹Pr₂NH, CH₃CN, rt; (f) K₂CO₃, DME–EtOH, rt–50 °C, 35–84% for 2 steps; (g) 38ba, Cs₂CO₃, DMF, rt; (h) NaOHaq, DME–EtOH, rt–50 °C, 86–95%.

Scheme 6. Syntheses of Compounds 29–35^a

^aReagents and conditions: (a) H₂SO₄, EtOH, 6-oxoheptanoic acid 100 °C, 35–75%; (b) ethyl 4-bromobutanoate, Cs₂CO₃, 55 °C, 95–97%; (c) 4-ethynylphenyl acetate, Pd(*tert*-Bu₃P)₂, ¹Pr₂NH, CH₃CN, rt; (d) K₂CO₃, DME–EtOH, rt, 77–95% (2 steps); (e) 38a or 38e, ADPP, PPh₃, CH₂Cl₂, rt; (f) NaOHaq, EtOH–DME, rt–50 °C, 72–98% (2 steps).

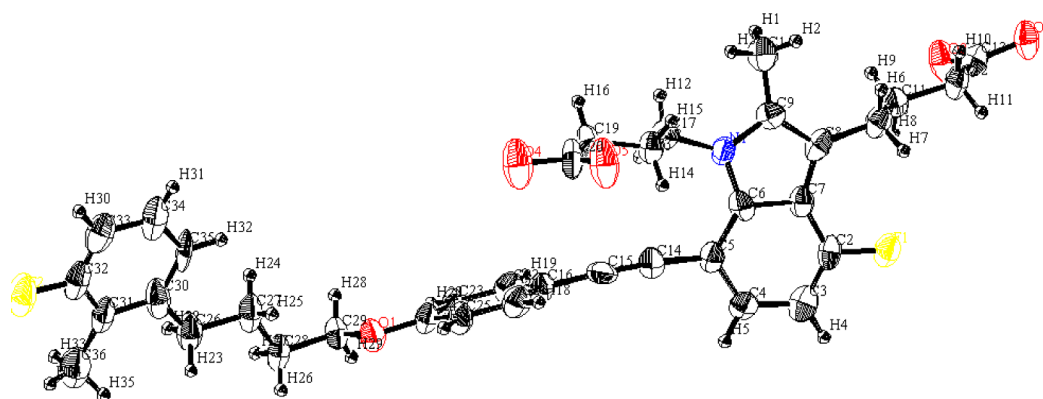


Figure 7. ORTEP drawing of compound **35** (ONO-6950, gemilukast). Data and refinement statistics are summarized in [Supporting Information](#).

mg/kg 24 h before LTC₄ challenge. Furthermore, gemilukast reduced the antigen-induced constriction of isolated human bronchi, and its effect was comparable to that of combination treatment with antagonists against CysLT₁ and CysLT₂. On the basis of the strength of the data presented herein, gemilukast was selected as a clinical candidate. Gemilukast is currently being evaluated in phase II clinical trials. The results will be reported in due course.

EXPERIMENTAL SECTION

General Remarks. Analytical samples were homogeneous as confirmed by TLC, and spectroscopic results were consistent with the assigned structures. NMR spectra were recorded as designated on either a Varian Mercury 300 spectrometer or INOVA-500 spectrometer using deuterated chloroform (CDCl₃), deuterated methanol (CD₃OD), or deuterated dimethyl sulfoxide (DMSO-*d*₆) as the solvent. Fast atom bombardment (FABMS, HRMS) and electron ionization (EI) were performed on a JEOL JMS-DX303HF spectrometer. Atmospheric pressure chemical ionization (APCI) mass spectra were determined with a Hitachi M-1200H spectrometer. IR spectra were measured on a JASCO FTIR-430 spectrometer. Elemental analyses were performed with a Perkin–Elmer PE2400 series II CHNS/O analyzer and were only indicated as the elements within ±0.4% of the theoretical values unless otherwise noted. Purity analyses were performed on the following HPLC systems: HPLC, Agilent Technologies 1200 series with Unison UK-C18 (4.6 mm × 150 mm). Method A: Unison UK-C18 (4.6 mm × 150 mm), eluting over 60 min with 30–80% acetonitrile in 20 mM KH₂PO₄ aq (pH = 2.0), flow rate of 1 mL/min, column temperature of 35 °C, detection with UV (210 nm). Method B: Unison UK-C18 (4.6 mm × 150 mm), eluting over 60 min with 30–80% acetonitrile in 20 mM K₂HPO₄ aq (pH = 7.0), flow rate of 1 mL/min, column temperature of 35 °C, detection with UV (210 nm). Column chromatography was performed with silica gel [Merck Silica Gel 60 (0.063–0.200 μm), Wako gel C-200, or Fuji Silysia FL60D]. Thin layer chromatography was performed with silica gel (Merck TLC or HPTLC plates, Silica Gel 60 F254) or with a medium-pressure preparative liquid chromatograph W-prep 2XY (manufactured by Yamazen Corporation; column: main column size S-5L, inject column size SS-2L). The following abbreviations for solvents and reagents are used: DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; EtOH, ethanol; EtOAc, ethyl acetate; MeOH, methanol; THF, tetrahydrofuran; CH₂Cl₂, dichloromethane; *tert*-BuOMe, *tert*-butyl methyl ether; ¹Pr₂O, diisopropyl ether; CH₃CN, acetonitrile; Et₃N, triethylamine; TFA, trifluoroacetic acid; ADDP, 1,1'-(azodicarbonyl)-dipiperidine. All animal experiments were approved by the Animal Ethical Committee of Ono Pharmaceutical Co., Ltd. and were performed in accordance with institutional animal care guidelines.

4-(3-Fluoro-2-methylphenyl)-1-butanol (38a). 3-Butyn-1-ol (0.38 mL, 5.0 mmol), PdCl₂(PPh₃)₂ (175 mg, 0.26 mmol), Ph₃P (131 mg, 0.53 mmol), and CuI (95 mg, 0.53 mmol) were added to a solution of

36a (1.0 g 5.29 mmol) in Et₃N (5.0 mL) at room temperature. The reaction mixture was stirred for 1 h at 80 °C. ¹Pr₂O was added to the reaction mixture; this mixture was filtered and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give **37a** (357 mg, 40%). **37a** (313 mg, 1.76 mmol) and platinum dioxide (30 mg) in EtOH (2.0 mL) were stirred for 3 h under a hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated to give **38a** (304 mg, 95%). ¹H NMR (300 MHz, CDCl₃) δ 1.14–1.44 (m, 1H), 1.57–1.73 (m, 4H), 2.16–2.26 (m, 3H), 2.62 (t, *J* = 7.2 Hz, 2H), 3.61–3.76 (m, 2H), 6.82–6.90 (m, 1H), 6.90–6.95 (m, 1H), 7.02–7.12 (m, 1H).

4-(3-Chloro-2-methylphenyl)-1-butanol (38b). Compound **38b** was prepared from compound **36b** in a manner similar to that described for compound **38a**. ¹H NMR (300 MHz, CDCl₃) δ 1.27 (t, *J* = 5.1 Hz, 1H), 1.61–1.68 (m, 4H), 2.35 (s, 3H), 2.62–2.72 (m, 2H), 3.63–3.75 (m, 2H), 7.00–7.09 (m, 2H), 7.17–7.26 (m, 1H).

4-(3-Chloro-2-methylphenyl)butyl 4-methylbenzenesulfonate (38ba). Triethylamine (7.9 mL, 56.9 mmol), trimethylamine hydrochloride (272 mg, 2.84 mmol), and *p*-toluenesulfonyl chloride (7.05 g, 37.0 mmol) were added to a solution of compound **38b** (5.65 g, 28.4 mmol) in toluene (57 mL) at 0 °C, and the mixture was stirred for 2.5 h at 0 °C. The reaction mixture was poured into H₂O, and the resulting mixture was extracted with EtOAc. The organic layer was washed with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give **38ba** (9.49 g, 95%). ¹H NMR (300 MHz, CDCl₃) δ 1.51–1.64 (m, 2H), 1.64–1.78 (m, 2H), 2.29 (s, 3H), 2.44 (s, 3H), 2.52–2.65 (m, 2H), 4.05 (t, *J* = 6.2 Hz, 2H), 6.93–7.05 (m, 2H), 7.21 (dd, *J* = 7.7 Hz, 1.5 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.78 (d, *J* = 8.2 Hz, 2H).

4-(3-Fluoro-2-methylphenyl)butyl 4-Methylbenzenesulfonate (38aa). Compound **38aa** was prepared from compound **38a** in a manner similar to that described for compound **38ba**. ¹H NMR (300 MHz, CDCl₃) δ 1.51–1.64 (m, 2H), 1.65–1.78 (m, 2H), 2.15 (d, *J* = 2.2 Hz, 3H), 2.44 (s, 3H), 2.57 (t, *J* = 7.7 Hz, 2H), 4.05 (t, *J* = 6.2 Hz, 2H), 6.77–6.91 (m, 2H), 6.97–7.13 (m, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H).

4-(4,5-Difluoro-2-methylphenyl)-1-butanol (38c). Compound **38c** was prepared from compound **36c** in a manner similar to that described for compound **38a**. ¹H NMR (300 MHz, CDCl₃) δ 1.59–1.69 (m, 5H), 2.24 (s, 3H), 2.53–2.58 (m, 2H), 3.67–3.71 (m, 2H), 6.89–6.95 (m, 2H).

4-(2,3,6-Trifluorophenyl)-1-butanol (38d). Compound **38d** was prepared from compound **36d** in a manner similar to that described for compound **38a**. ¹H NMR (300 MHz, CDCl₃) δ 1.14–1.15 (m, 1H), 1.52–1.82 (m, 4H), 2.63–2.86 (m, 2H), 3.58–3.77 (m, 2H), 6.71–6.81 (m, 1H), 6.88–7.05 (m, 1H).

4-(2,3,5,6-Tetrafluorophenyl)-1-butanol (38e). Compound **38e** was prepared from compound **36e** in a manner similar to that described for compound **38a**. ¹H NMR (300 MHz, CDCl₃) δ 1.20–

1.35 (m, 1H), 1.52–1.80 (m, 4H), 2.69–2.87 (m, 2H), 3.56–3.77 (m, 2H), 6.82–7.01 (m, 1H).

4-(2,3,4,6-Tetrafluorophenyl)-1-butanol (38f). Compound 38f was prepared from compound 36f in a manner similar to that described for compound 38a. ¹H NMR (300 MHz, CDCl₃) δ 1.15–1.39 (m, 1H), 1.51–1.79 (m, 4H), 2.68 (t, *J* = 6.0 Hz, 2H), 3.67 (t, *J* = 5.9 Hz, 2H), 6.71 (m, 1H).

4-(2,3,4,5-Tetrafluorophenyl)-1-butanol (38g). Compound 38g was prepared from compound 36g in a manner similar to that described for compound 38a. ¹H NMR (300 MHz, CDCl₃) δ 1.39 (brs, 1H), 1.60–1.74 (m, 4H), 2.63–2.68 (m, 2H), 3.65–3.69 (m, 2H), 6.75–6.84 (m, 1H).

Ethyl 4-(7-Bromo-1H-indol-3-yl)-4-oxobutanoate (40a). AlCl₃ (1.36 g, 10.2 mmol) and 39a (1.0 g, 5.10 mmol) were added to ethyl 4-chloro-4-oxobutanoate (1.68 g, 10.2 mmol) in CH₂Cl₂ at 0 °C, which was followed by stirring for 5 h at room temperature. H₂O was added to the reaction mixture, and the resulting mixture was extracted with EtOAc. The organic layer was washed sequentially with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was stirred for 30 min in EtOAc and filtered to obtain a solid. The resulting solid was dried to give 40a (1.37 g, 83%). ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.27 (t, *J* = 7.2 Hz, 3H), 2.79 (t, *J* = 6.9 Hz, 2H), 3.23 (t, *J* = 6.9 Hz, 2H), 4.17 (q, *J* = 7.2 Hz, 2H), 7.16 (t, *J* = 7.8 Hz, 1H), 7.43 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.96 (t, *J* = 0.9 Hz, 1H), 8.30 (dd, *J* = 7.8, 0.9 Hz, 1H), 8.76 (brs, 1H).

Ethyl 4-(7-Bromo-2-methyl-1H-indol-3-yl)-4-oxobutanoate (40b). Compound 40b was prepared from compound 39b in a manner similar to that described for compound 40a. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.28 (t, *J* = 7.2 Hz, 3H), 2.79 (s, 3H), 2.79 (t, *J* = 6.6 Hz, 2H), 3.28 (t, *J* = 6.6 Hz, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 7.09 (t, *J* = 8.1 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 8.75 (brs, 1H).

Ethyl 4-(7-Bromo-1H-indol-3-yl)butanoate (41a). NaBH₄ (118 mg, 3.11 mmol) and boron trifluoride–ethyl ether complex (0.66 mL, 5.19 mmol) were added to compound 40a (560 mg, 1.73 mmol) in THF at –20 °C, which was followed by stirring for 2.5 h at –10 °C. Saturated ammonium chloride solution was added to the reaction mixture, and the resulting mixture was extracted with EtOAc. The organic layer was washed sequentially with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 41a (292 mg, 54%). ¹H NMR (300 MHz, CDCl₃) δ 1.24 (t, *J* = 7.2 Hz, 3H), 1.98–2.08 (m, 2H), 2.36 (t, *J* = 7.5 Hz, 2H), 2.79 (t, *J* = 7.2 Hz, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 6.99 (t, *J* = 7.5 Hz, 1H), 7.10–7.08 (m, 1H), 7.34 (dd, *J* = 7.5, 0.6 Hz, 1H), 7.55 (dt, *J* = 7.5, 0.6 Hz, 1H), 8.12 (brs, 1H).

Ethyl 4-(7-Bromo-2-methyl-1H-indol-3-yl)butanoate (41b). Procedure 1. Starting with 40b, following the procedures above resulted in compound 41a.

Procedure 2. Fisher indole synthesis: Concentrated H₂SO₄ (12 mL) was added to a solution of 45 (25 g, 112 mmol) and 6-oxoheptanoic acid (16.1 g, 112 mmol) in EtOH (120 mL) at room temperature. The reaction mixture was stirred for 1.5 h at reflux. The resulting mixture was extracted with EtOAc. The organic layer was washed sequentially with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 41b (27.5 g, 76%). ¹H NMR (300 MHz, CDCl₃) δ 1.23 (t, *J* = 7.2 Hz, 3H), 1.89–2.00 (m, 2H), 2.30 (t, *J* = 7.2 Hz, 2H), 2.39 (s, 3H), 2.71 (t, *J* = 7.2 Hz, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 6.94 (t, *J* = 8.1 Hz, 1H), 7.24 (d, *J* = 8.1 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 7.91 (brs, 1H).

Ethyl 4-[7-Bromo-1-(2-ethoxy-2-oxoethyl)-1H-indol-3-yl]butanoate (42a). Cs₂CO₃ (8.4 g, 25.8 mmol) and ethyl bromoacetate (1.51 mL, 13.5 mmol) were added to compound 41a (4.0 g, 12.9 mmol) in DMF (40 mL) at 0 °C, which was followed by stirring for 1 h at 0 °C. H₂O was added to the reaction mixture, and the resulting mixture was extracted with EtOAc. The organic layer was washed sequentially with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 42a (4.98 g,

97%). ¹H NMR (300 MHz, CDCl₃) δ 1.25 (t, *J* = 7.2 Hz, 3H), 1.26 (t, *J* = 7.2 Hz, 3H), 1.93–2.09 (m, 2H), 2.36 (t, *J* = 7.4 Hz, 2H), 2.69–2.82 (m, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 4.23 (q, *J* = 7.2 Hz, 2H), 5.22 (s, 2H), 6.80 (s, 1H), 6.94 (dd, *J* = 7.9, 7.6 Hz, 1H), 7.33 (dd, *J* = 7.6, 1.0 Hz, 1H), 7.52 (dd, *J* = 7.9, 1.1 Hz, 1H).

Ethyl 4-[7-Bromo-1-(2-ethoxy-2-oxoethyl)-2-methyl-1H-indol-3-yl]butanoate (42b). Compound 42b was prepared from compound 41b in a manner similar to that described for compound 42a. ¹H NMR (300 MHz, CDCl₃) δ 1.23 (t, *J* = 7.2 Hz, 3H), 1.26 (t, *J* = 7.2 Hz, 3H), 1.86–1.97 (m, 2H), 2.27 (s, 3H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.74 (t, *J* = 7.2 Hz, 2H), 4.11 (q, *J* = 7.2 Hz, 2H), 4.23 (q, *J* = 7.2 Hz, 2H), 5.27 (s, 2H), 6.90 (t, *J* = 7.8 Hz, 1H), 7.25 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.44 (dd, *J* = 7.5, 0.9 Hz, 1H).

Ethyl 4-[1-(2-Ethoxy-2-oxoethyl)-7-[(E)-2-(4-hydroxyphenyl)-vinyl]-1H-indol-3-yl]butanoate (43a). Dicyclohexylmethylamine (3.24 mL, 15.1 mmol) and a solution of ^tBu₃P (7.08 mL, 2.52 mmol, 10 w/w % hexane solution) were added to a solution of 42a (5.00 g, 12.6 mmol), 4-acetoxystyrene (2.02 mL, 13.2 mmol), and tris(dibenzylideneacetone)dipalladium(0) (1.15 g, 1.26 mmol) in dioxane (60 mL) at room temperature under an argon atmosphere. The reaction mixture was stirred for 3 h at 100 °C. The reaction mixture was filtered, the filtrate was poured into water, and the resulting mixture was extracted with EtOAc. The organic layer was washed sequentially with aqueous saturated ammonium chloride solution, water, and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 43a (4.61 g, 87%). ¹H NMR (300 MHz, CDCl₃) δ 1.19 (t, *J* = 7.2 Hz, 3H), 1.25 (t, *J* = 7.2 Hz, 3H), 2.78 (t, *J* = 7.5 Hz, 2H), 2.04 (t, *J* = 7.5 Hz, 2H), 2.39 (t, *J* = 7.5 Hz, 2H), 4.13 (q, *J* = 7.2 Hz, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 4.98 (s, 2H), 5.19 (s, 1H), 6.80 (s, 1H), 6.82 (d, *J* = 15.0 Hz, 1H), 6.83 (d, *J* = 8.7 Hz, 2H), 7.10 (t, *J* = 7.5 Hz, 1H), 7.24 (d, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 8.7 Hz, 2H), 7.42 (d, *J* = 15.0 Hz, 1H), 7.51 (d, *J* = 7.5 Hz, 1H).

Ethyl 4-[1-(2-Ethoxy-2-oxoethyl)-7-[(E)-2-(4-hydroxyphenyl)-vinyl]-2-methyl-1H-indol-3-yl]butanoate (43b). Compound 43b was prepared from compound 42b in a manner similar to that described for compound 43a. ¹H NMR (300 MHz, CDCl₃) δ 1.17–1.29 (m, 6H), 1.90–1.98 (m, 2H), 2.29 (s, 3H), 2.32 (t, *J* = 7.3 Hz, 2H), 2.76 (t, *J* = 7.3 Hz, 2H), 4.11 (q, *J* = 7.0 Hz, 2H), 4.20 (q, *J* = 7.2 Hz, 2H), 4.89 (s, 1H), 4.97 (s, 2H), 6.77–6.86 (m, 3H), 7.06 (t, *J* = 7.3 Hz, 1H), 7.15 (d, *J* = 7.3 Hz, 1H), 7.35–7.46 (m, 4H).

4-(1-(Carboxymethyl)-7-[(E)-2-[4-(4-phenoxybutoxy)phenyl]-vinyl]-1H-indol-3-yl)butanoic Acid (3). 1-(4-Phenoxybutoxy)-4-vinylbenzene²⁸ (0.78 g, 2.91 mmol), tris(dibenzylideneacetone)dipalladium(0) (243 mg, 0.27 mmol), dicyclohexylmethylamine (0.62 g, 3.2 mmol), and a solution of *tert*-Bu₃P (1.08 g, 0.53 mmol, 10 w/w % hexane solution) were added to a solution of 42a (1.05 g, 2.65 mmol) in dioxane (14 mL) at room temperature under an argon atmosphere. The reaction mixture was stirred for 3 h at 105 °C. The reaction mixture was then filtered, the filtrate was poured into water, and the resulting mixture was extracted with EtOAc. The organic layer was washed sequentially with aqueous saturated ammonium chloride solution, water, and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 44n (1.29 g, 83%). A 2 mol/L aqueous solution of sodium hydroxide (2.8 mL) was added to a solution of 44n (1.1 g, 1.9 mmol) in DME (18 mL), and the mixture was stirred for 5 h at room temperature. Hydrochloric acid (1 mol/L) and water were added to the reaction mixture, and the resulting mixture was extracted with EtOAc. The organic layer was

washed with water and brine sequentially, dried over anhydrous sodium sulfate, and concentrated. The residue was washed with Pr_2O to give **3** (890 mg, 90%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.78–1.93 (m, 6H), 2.28 (t, J = 7.2 Hz, 2H), 2.67 (t, J = 7.4 Hz, 2H), 3.97–4.12 (m, 4H), 5.13 (s, 2H), 6.86–6.97 (m, 6H), 7.02 (dd, J = 7.6 Hz, 1H), 7.08 (s, 1H), 7.22–7.31 (m, 3H), 7.40–7.58 (m, 4H), 12.29 (s, 2H). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 23.67, 25.07, 25.39, 33.30, 50.40, 66.92, 67.11, 113.62, 114.39, 114.64, 118.02, 119.02, 120.14, 120.36, 122.41, 123.07, 127.65, 128.84, 129.24, 129.40, 129.89, 130.14, 133.62, 158.23, 158.56, 171.15, 174.37. MS (FAB, neg) m/z 526 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{32}\text{H}_{33}\text{NO}_6$ ($\text{M} - \text{H}$) $^-$ calcd mass 526.2230, found 526.2233. LCMS (ELSD) RT = 1.17 min (>98%). HPLC purity 96% (method A).

4-[(1-(Carboxymethyl)-7-[(E)-2-[4-(4-phenylbutoxy)phenyl]vinyl]-1H-indol-3-yl)]butanoic Acid (4). Compound **4** was prepared from compound **43a** in a manner similar to that described for compound **16**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.66–1.78 (m, 4H), 1.78–1.93 (m, 2H), 2.28 (t, J = 7.5 Hz, 2H), 2.58–2.76 (m, 4H), 3.91–4.10 (m, 2H), 5.11 (s, 2H), 6.87–6.95 (m, 4H), 7.01 (t, J = 7.8 Hz, 1H), 7.07 (s, 1H), 7.14–7.32 (m, 6H), 7.41–7.57 (m, 3H), 12.05 (brs, 2H). MS (FAB, neg) m/z 510 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{32}\text{H}_{33}\text{NO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 510.2280, found 510.2269. HPLC purity 95% (method B).

4-[(1-(Carboxymethyl)-7-[(E)-2-[4-(4-(3-chlorophenyl)butoxy]phenyl]vinyl]-1H-indol-3-yl)]butanoic Acid (5). Compound **5** was prepared from compound **43a** in a manner similar to that described for compound **16**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.67–1.77 (m, 4H), 1.78–1.91 (m, 2H), 2.28 (t, J = 7.5 Hz, 2H), 2.61–2.72 (m, 4H), 3.96–4.04 (m, 2H), 5.11 (s, 2H), 6.91 (d, J = 15.9 Hz, 1H), 6.92 (d, J = 9.0 Hz, 2H), 7.01 (t, J = 7.5 Hz, 1H), 7.07 (s, 1H), 7.16–7.57 (m, 9H), 12.15 (brs, 2H). MS (FAB, neg) m/z 544 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{32}\text{H}_{32}\text{ClNO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 544.1891, found 544.1895. HPLC purity 93% (method B).

4-[(1-(Carboxymethyl)-7-[(E)-2-[4-(4-(2,3,6-trifluorophenyl)butoxy]phenyl]vinyl]-1H-indol-3-yl)]butanoic Acid (6). Compound **6** was prepared from compound **43a** in a manner similar to that described for compound **16**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.62–1.92 (m, 6H), 2.28 (t, J = 7.2 Hz, 2H), 2.66 (t, J = 7.2 Hz, 2H), 2.70–2.79 (m, 2H), 4.00 (t, J = 6.0 Hz, 2H), 5.13 (s, 2H), 6.87–6.96 (m, 3H), 7.01 (t, J = 7.5 Hz, 1H), 7.08 (s, 1H), 7.10–7.17 (m, 1H), 7.25 (d, J = 7.5 Hz, 1H), 7.30–7.57 (m, 5H), 12.31 (brs, 1H), 12.85 (brs, 1H). MS (FAB, neg) m/z 564 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{33}\text{H}_{30}\text{F}_3\text{NO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 564.1998, found 564.1996. HPLC purity 99% (method B).

4-[(1-(Carboxymethyl)-7-[(E)-2-[4-(4-(3-fluoro-2-methylphenyl)butoxy]phenyl]vinyl]-1H-indol-3-yl)]butanoic Acid (7). Compound **7** was prepared from compound **43a** in a manner similar to that described for compound **16**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.59–1.92 (m, 6H), 2.17 (d, J = 2.0 Hz, 3H), 2.22–2.34 (m, 2H), 2.61–2.73 (m, 4H), 4.02 (t, J = 6.2 Hz, 2H), 5.13 (s, 2H), 6.86–7.05 (m, 6H), 7.06–7.18 (m, 2H), 7.26 (d, J = 7.3 Hz, 1H), 7.40–7.58 (m, 4H), 12.15 (brs, 1H), 12.95 (brs, 1H). MS (FAB, neg) m/z 542 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{33}\text{H}_{34}\text{FNO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 542.5343, found 542.2343. HPLC purity 95% (method B).

4-[(1-(Carboxymethyl)-7-[(E)-2-[4-(4-(3-chloro-2-methylphenyl)butoxy]phenyl]vinyl]-1H-indol-3-yl)]butanoic Acid (8). Compound **8** was prepared from compound **43a** in a manner similar to that described for compound **16**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.53–1.94 (m, 6H), 2.23–2.35 (m, 5H), 2.60–2.77 (m, 4H), 4.02 (t, J = 5.9 Hz, 2H), 5.13 (s, 2H), 6.86–6.97 (m, 3H), 7.02 (t, J = 7.7 Hz, 1H), 7.06–7.18 (m, 3H), 7.22–7.29 (m, 2H), 7.40–7.57 (m, 4H), 12.21 (brs, 1H), 12.81 (brs, 1H). MS (FAB, neg) m/z 558 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{33}\text{H}_{34}\text{ClNO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 558.2047, found 558.2058. HPLC purity 96% (method B).

4-[(1-(Carboxymethyl)-2-methyl-7-[(E)-2-[4-(4-phenylbutoxy)phenyl]vinyl]-1H-indol-3-yl)]butanoic Acid (9). Compound **9** was prepared from compound **43b** in a manner similar to that described for compound **16**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.61–1.84 (m, 6H), 2.13–2.30 (m, 5H), 2.58–2.76 (m, 4H), 3.93–4.07 (m, 2H), 5.01 (s, 2H), 6.81–7.03 (m, 4H), 7.09–7.32 (m, 6H), 7.38 (d, J = 7.3

Hz, 1 H), 7.43–7.55 (m, 3H), 12.20 (brs, 1H), 13.10 (brs, 1H). MS (FAB, neg) m/z 524 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{33}\text{H}_{35}\text{NO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 524.2437, found 524.2446. HPLC purity 96% (method B).

4-[(1-(Carboxymethyl)-7-[(E)-2-[4-(4-(2-methoxyphenyl)butoxy]phenyl]vinyl]-2-methyl-1H-indol-3-yl)]butanoic Acid (10). Compound **10** was prepared from compound **43b** in a manner similar to that described for compound **16**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.62–1.80 (m, 6H), 2.21 (t, J = 7.3 Hz, 2H), 2.25 (s, 3H), 2.61 (t, J = 7.3 Hz, 2H), 2.66 (t, J = 7.1 Hz, 2H), 3.77 (s, 3H), 3.95–4.04 (m, 2H), 5.01 (s, 2H), 6.82–7.02 (m, 6H), 7.11–7.21 (m, 3H), 7.39 (d, J = 7.7 Hz, 1H), 7.48 (d, J = 8.6 Hz, 2H), 7.50 (d, J = 15.7 Hz, 1H), 12.05 (brs, 1H), 13.40 (brs, 1H). MS (FAB, neg) m/z 554 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{34}\text{H}_{37}\text{NO}_6$ ($\text{M} - \text{H}$) $^-$ calcd mass 554.2543, found 554.2532. HPLC purity 97% (method B).

4-[(1-(Carboxymethyl)-2-methyl-7-[(E)-2-[4-(4-(2,3,6-trifluorophenyl)butoxy]phenyl]vinyl]-1H-indol-3-yl)]butanoic Acid (11). Compound **11** was prepared from compound **43b** in a manner similar to that described for compound **16**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.64–1.84 (m, 6H), 2.22 (t, J = 7.2 Hz, 2H), 2.24–2.30 (s, 3H), 2.67 (t, J = 7.2 Hz, 2H), 2.70–2.80 (m, 2H), 3.97–4.08 (m, 2H), 5.02 (s, 2H), 6.81–7.06 (m, 4H), 7.05–7.21 (m, 2H), 7.28–7.44 (m, 2H), 7.44–7.60 (m, 3H), 12.06 (brs, 1H), 13.10 (brs, 1H). MS (FAB, neg) m/z 578 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{33}\text{H}_{32}\text{F}_3\text{NO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 578.2154, found 578.2151. HPLC purity 95% (method B).

4-[(1-(Carboxymethyl)-2-methyl-7-[(E)-2-[4-(4-(2,3,5,6-tetrafluorophenyl)butoxy]phenyl]vinyl]-1H-indol-3-yl)]butanoic Acid (12). Compound **12** was prepared from compound **43b** in a manner similar to that described for compound **16**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.65–1.84 (m, 6H), 2.21 (t, J = 7.2 Hz, 2H), 2.25 (s, 3H), 2.62–2.71 (m, 2H), 2.75–2.85 (m, 2H), 3.97–4.08 (m, 2H), 4.97–5.09 (m, 2H), 6.83–7.04 (m, 4H), 7.14 (d, J = 7.1 Hz, 1H), 7.36–7.41 (m, 1H), 7.44–7.56 (m, 3H), 7.64–7.83 (m, 1H), 12.11 (brs, 1H), 13.07 (brs, 1H). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 9.85, 22.12, 22.97, 25.16, 25.72, 28.08, 33.00, 47.13, 66.98, 104.33 (m), 110.58, 114.65, 117.13, 118.95, 120.13, 120.64 (m), 122.31, 123.03, 127.59, 128.75, 129.89, 130.24, 133.50, 134.11, 144.18 (m), 145.22 (m), 158.17, 171.19, 174.42. MS (FAB, neg) m/z 596 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{33}\text{H}_{31}\text{F}_4\text{NO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 596.2060, found 596.2060. HPLC purity 95% (method B).

4-[(1-(Carboxymethyl)-2-methyl-7-[(E)-2-[4-(4-(2,3,4,5-tetrafluorophenyl)butoxy]phenyl]vinyl]-1H-indol-3-yl)]butanoic Acid (13). Compound **13** was prepared from compound **43b** in a manner similar to that described for compound **16**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.65–1.85 (m, 6H), 2.21 (t, J = 7.3 Hz, 2H), 2.25 (s, 3H), 2.62–2.76 (m, 4H), 3.90–4.09 (m, 2H), 5.02 (s, 2H), 6.87 (d, J = 15.9 Hz, 1H), 6.89–7.04 (m, 3H), 7.14 (d, J = 7.3 Hz, 1H), 7.33–7.42 (m, 2H), 7.43–7.58 (m, 3H), 12.02 (brs, 1H), 13.01 (brs, 1H). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 27.23, 27.95, 33.00, 47.12, 67.09, 110.59, 112.09 (m), 114.65, 117.13, 118.95, 120.13, 122.31, 123.02, 125.87 (m), 127.58, 128.76, 129.87, 130.25, 133.49, 134.11, 137.66 (m), 139.74 (m), 145.04 (m), 146.05 (m), 158.19, 171.19, 174.42. MS (FAB, neg) m/z 596 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{33}\text{H}_{31}\text{F}_4\text{NO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 596.2060, found 596.2060. HPLC purity 95% (method B).

4-[(1-(Carboxymethyl)-2-methyl-7-[(E)-2-[4-(4-(2,3,4,6-tetrafluorophenyl)butoxy]phenyl]vinyl]-1H-indol-3-yl)]butanoic Acid (14). Compound **14** was prepared from compound **43b** in a manner similar to that described for compound **16**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.60–1.84 (m, 6H), 2.21 (t, J = 7.1 Hz, 2H), 2.25 (s, 3H), 2.61–2.77 (m, 4H), 3.99 (t, J = 5.9 Hz, 2H), 4.99 (s, 2H), 6.82–6.94 (m, 3H), 6.98 (t, J = 7.6 Hz, 1H), 7.14 (d, J = 7.3 Hz, 1H), 7.38 (d, J = 7.3 Hz, 1H), 7.41–7.58 (m, 4H), 12.20 (brs, 1H), 13.10 (brs, 1H). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 9.85, 21.45, 22.98, 25.28, 25.73, 28.06, 33.01, 47.24, 66.99, 101.31 (m), 110.52, 114.63, 114.95 (m), 117.11, 118.90, 120.07, 122.31, 123.08, 127.58, 128.73, 129.89, 130.17, 133.50, 134.11, 136.38 (m), 148.17 (m), 148.93 (m), 154.94 (m), 158.15, 171.22, 174.42. MS (FAB, neg) m/z 596 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{33}\text{H}_{31}\text{F}_4\text{NO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 596.2060, found 596.2060. HPLC purity 98% (method B).

4-[1-(Carboxymethyl)-7-[(E)-2-{4-[4-(3-fluoro-2-methylphenyl)-butoxy]phenyl]vinyl]-2-methyl-1H-indol-3-yl]butanoic Acid (**15**). Compound **15** was prepared from compound **43b** in a manner similar to that described for compound **16**. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.59–1.85 (m, 6H), 2.17 (d, *J* = 2.2 Hz, 3H), 2.21 (t, *J* = 7.4 Hz, 2H), 2.25 (s, 3H), 2.60–2.74 (m, 4H), 4.02 (t, *J* = 6.1 Hz, 2H), 5.01 (s, 2H), 6.81–7.05 (m, 6H), 7.08–7.18 (m, 2H), 7.38 (d, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.49 (d, *J* = 16.5 Hz, 1H), 12.10 (brs, 1H), 13.20 (brs, 1H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ 9.85, 10.01, 22.98, 25.72, 26.16, 28.36, 31.97, 33.00, 47.13, 67.25, 110.590, 112.31 (*J*_{C–F} = 23.0 Hz), 114.660, 117.130, 118.950, 120.130, 122.200, 122.320, 122.990, 124.61 (*J*_{C–F} = 2.8 Hz), 126.67 (*J*_{C–F} = 9.1 Hz), 127.590, 128.760, 129.830, 130.260, 133.490, 134.100, 143.08 (*J*_{C–F} = 4.1 Hz), 158.260, 160.70 (*J*_{C–F} = 239.9 Hz), 171.190, 174.420. MS (FAB, neg) *m/z* 556 (M – H)[–]. HRMS (FAB, neg) C₃₄H₃₆FNO₅ (M – H)[–] calcd mass 556.2499, found 556.2502. HPLC purity 98% (method A).

4-[1-(Carboxymethyl)-7-[(E)-2-{4-[4-(3-chloro-2-methylphenyl)-butoxy]phenyl]vinyl]-2-methyl-1H-indol-3-yl]butanoic Acid (**16**). **38b** (37 mg, 0.19 mmol), ADDP (98 mg, 0.37 mmol), and PPh₃ (94 mg, 0.37 mmol) were added to a solution of **43b** (70 mg, 0.16 mmol) in CH₂Cl₂ (1.0 mL), and the mixture was stirred for 15 h at room temperature. Triphenylphosphine oxide was removed by filtration, and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give **44m** (109 mg, quant). A 2 mol/L aqueous solution of sodium hydroxide (1.0 mL) was added to a mixture of **44m** (98 mg, 0.16 mmol), DME (2.0 mL), and EtOH (2.0 mL), and the mixture was stirred for 2.5 h at 50 °C. The reaction mixture was acidified with 2 mol/L hydrochloric acid and extracted with EtOAc. The organic layer was washed sequentially with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was stirred for 30 min in ³Pr₂O and filtered to obtain a solid. The resulting solid was dried to give **16** (82 mg, 92%). ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.52–1.86 (m, 6H), 2.21 (t, *J* = 7.3 Hz, 2H), 2.25 (s, 3H), 2.31 (s, 3H), 2.61–2.79 (m, 4H), 4.02 (t, *J* = 6.2 Hz, 2H), 5.01 (s, 2H), 6.81–7.05 (m, 4H), 7.07–7.19 (m, 3H), 7.22–7.31 (m, 1H), 7.34–7.43 (m, 1H), 7.43–7.59 (m, 3H), 12.20 (brs, 1H), 13.31 (brs, 1H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ 9.85, 15.50, 22.98, 25.72, 26.21, 28.35, 33.00, 33.06, 47.13, 67.24, 110.58, 114.66, 117.13, 118.95, 120.13, 122.32, 123.00, 126.63, 126.86, 127.59, 127.86, 128.76, 129.84, 130.26, 133.27, 133.50, 133.83, 134.11, 142.74, 158.25, 171.20, 174.42. MS (FAB, neg) *m/z* 572 (M – H)[–]. HRMS (FAB, neg) C₃₄H₃₆ClNO₅ (M – H)[–] calcd mass 572.2204, found 572.2201. HPLC purity 95% (method A).

Ethyl (7-Bromo-2-methyl-1H-indol-3-yl)acetate (**46a**). Compound **46a** was prepared from compound **45** in a manner similar to that described for compound **41b** (Procedure 2). ¹H NMR (300 MHz, CDCl₃) δ 1.23 (t, *J* = 7.2 Hz, 3H), 2.44 (s, 3H), 3.65 (s, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 6.96 (t, *J* = 7.8 Hz, 1H), 7.25 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.41–7.50 (m, 1H), 8.01 (s, 1H).

Ethyl 3-(7-Bromo-2-methyl-1H-indol-3-yl)propanoate (**46b**). Compound **46b** was prepared from compound **45** in a manner similar to that described for compound **41b** (Procedure 2). ¹H NMR (300 MHz, CDCl₃) δ 1.21 (t, *J* = 7.2 Hz, 3H), 2.42 (s, 3H), 2.59 (t, *J* = 6.6 Hz, 2H), 3.01 (t, *J* = 7.2 Hz, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 6.95 (t, *J* = 7.8 Hz, 1H), 7.25 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.92 (brs, 1H).

Diethyl 2,2'-[7-Bromo-2-methyl-1H-indole-1,3-diyl]diacetate (**47a**). Compound **47a** was prepared from compound **46a** in a manner similar to that described for compound **42a**. ¹H NMR (300 MHz, CDCl₃) δ 1.22 (t, *J* = 7.2 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 3H), 2.33 (s, 3H), 3.68 (s, 2H), 4.11 (q, *J* = 7.2 Hz, 2H), 4.24 (q, *J* = 7.2 Hz, 2H), 5.29 (s, 2H), 6.92 (t, *J* = 7.8 Hz, 1H), 7.23–7.31 (m, 1H), 7.47 (dd, *J* = 7.8, 1.1 Hz, 1H).

Ethyl 3-[7-Bromo-1-(2-ethoxy-2-oxoethyl)-2-methyl-1H-indol-3-yl]propanoate (**47b**). Compound **47b** was prepared from compound **41b** in a manner similar to that described for compound **42a**. ¹H NMR (300 MHz, CDCl₃) δ 1.20–1.32 (m, 6H), 2.31 (s, 3H), 2.57 (t, *J* = 7.2 Hz, 2H), 3.03 (t, *J* = 7.5 Hz, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 4.26 (q, *J* =

7.4 Hz, 2H), 5.27 (s, 2H), 6.92 (dd, *J* = 7.5, 7.8 Hz, 1H), 7.27 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.44 (dd, *J* = 7.8, 1.0 Hz, 1H).

Ethyl 4-[7-Bromo-3-(2-ethoxy-2-oxoethyl)-2-methyl-1H-indol-1-yl]butanoate (**47d**). Compound **47d** was prepared from compound **46a** in a manner similar to that described for compound **42a**. ¹H NMR (300 MHz, CDCl₃) δ 1.22 (t, *J* = 7.2 Hz, 3H), 1.26 (t, *J* = 7.2 Hz, 3H), 1.99–2.14 (m, 2H), 2.40 (t, *J* = 7.2 Hz, 2H), 2.42 (s, 3H), 3.67 (s, 2H), 4.11 (q, *J* = 7.2 Hz, 2H), 4.14 (q, *J* = 7.2 Hz, 2H), 4.47–4.58 (m, 2H), 6.91 (t, *J* = 7.8, 1.0 Hz, 1H), 7.29 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.47 (dd, *J* = 7.8, 1.0 Hz, 1H).

Ethyl 4-[7-Bromo-3-(3-ethoxy-3-oxopropyl)-2-methyl-1H-indol-1-yl]butanoate (**47e**). Compound **47e** was prepared from compound **46b** in a manner similar to that described for compound **42a**. ¹H NMR (300 MHz, CDCl₃) δ 1.22 (t, *J* = 6.9 Hz, 3H), 1.26 (t, *J* = 6.8 Hz, 3H), 1.99–2.09 (m, 2H), 2.36–2.41 (m, 2H), 2.39 (s, 3H), 2.55 (t, *J* = 7.9 Hz, 2H), 3.02 (t, *J* = 7.9 Hz, 2H), 4.07–4.17 (m, 4H), 4.48–4.53 (m, 2H), 6.90 (dd, *J* = 7.5, 7.8 Hz, 1H), 7.28 (d, *J* = 7.5 Hz, 1H), 7.44 (d, *J* = 7.8 Hz, 1H).

Diethyl 4,4'-[7-Bromo-2-methyl-1H-indole-1,3-diyl]dibutanoate (**47f**). Compound **47f** was prepared from compound **41b** in a manner similar to that described for compound **42a**. ¹H NMR (300 MHz, CDCl₃) δ 1.23 (t, *J* = 7.2 Hz, 3H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.82–2.12 (m, 4H), 2.29 (t, *J* = 7.8 Hz, 2H), 2.36 (s, 3H), 2.39 (t, *J* = 7.5 Hz, 2H), 2.73 (t, *J* = 7.5 Hz, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 4.13 (q, *J* = 7.2 Hz, 2H), 4.46–4.55 (m, 2H), 6.88 (t, *J* = 7.5 Hz, 1H), 7.27 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.43 (dd, *J* = 7.5, 0.9 Hz, 1H).

Ethyl 5-[7-Bromo-3-(2-ethoxy-2-oxoethyl)-2-methyl-1H-indol-1-yl]pentanoate (**47g**). Compound **47g** was prepared from compound **46a** in a manner similar to that described for compound **42a**. ¹H NMR (300 MHz, CDCl₃) δ 1.17–1.29 (m, 6H), 1.65–1.87 (m, 4H), 2.35 (t, *J* = 7.0 Hz, 2H), 2.39 (s, 3H), 3.66 (s, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 4.39–4.52 (m, 2H), 6.89 (t, *J* = 7.8, 7.5 Hz, 1H), 7.27 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.45 (dd, *J* = 7.8, 0.9 Hz, 1H).

Ethyl 5-[7-Bromo-3-(3-ethoxy-3-oxopropyl)-2-methyl-1H-indol-1-yl]pentanoate (**47h**). Compound **47h** was prepared from compound **46b** in a manner similar to that described for compound **42a**. ¹H NMR (300 MHz, CDCl₃) δ 1.19–1.28 (m, 6H), 1.69–1.95 (m, 4H), 2.33–2.36 (m, 2H), 2.36 (s, 3H), 2.54 (t, *J* = 7.8 Hz, 2H), 3.01 (t, *J* = 7.8 Hz, 2H), 4.05–4.15 (m, 4H), 4.41–4.46 (m, 2H), 6.87 (dd, *J* = 7.8, 7.5 Hz, 1H), 7.27 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.41 (dd, *J* = 7.8, 0.9 Hz, 1H).

Ethyl 5-[7-Bromo-3-(4-ethoxy-4-oxobutyl)-2-methyl-1H-indol-1-yl]pentanoate (**47i**). Compound **47i** was prepared from compound **41b** in a manner similar to that described for compound **42a**. ¹H NMR (300 MHz, CDCl₃) δ 1.24 (t, *J* = 7.1 Hz, 3H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.70–1.81 (m, 4H), 1.85–1.94 (m, 2H), 2.27–2.40 (m, 4H), 2.34 (s, 3H), 2.73 (t, *J* = 7.4 Hz, 2H), 4.07–4.16 (m, 4H), 4.42–4.47 (m, 2H), 6.88 (dd, *J* = 7.8, 7.5 Hz, 1H), 7.27 (dd, *J* = 7.5, 1.1 Hz, 1H), 7.43 (dd, *J* = 7.8, 1.1 Hz, 1H).

Diethyl 2,2'-[7-[(4-Hydroxyphenyl)ethynyl]-2-methyl-1H-indole-1,3-diyl]diacetate (**48a**). Compound **48a** was prepared from compound **47a** in a manner similar to that described for compound **43a**. ¹H NMR (300 MHz, CDCl₃) δ 1.11 (t, *J* = 7.2 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 3H), 2.32 (s, 3H), 3.73 (s, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 4.16 (q, *J* = 7.2 Hz, 2H), 5.35 (s, 2H), 5.72 (s, 1H), 6.62 (d, *J* = 8.7 Hz, 2H), 7.06 (t, *J* = 7.6 Hz, 1H), 7.31 (dd, *J* = 7.4, 1.2 Hz, 1H), 7.35 (d, *J* = 8.7 Hz, 2H), 7.50 (dd, *J* = 7.6, 1.2 Hz, 1H).

Ethyl 3-[1-(2-Ethoxy-2-oxoethyl)-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indol-3-yl]propanoate (**48b**). Compound **48b** was prepared from compound **47b** in a manner similar to that described for compound **43a**. ¹H NMR (300 MHz, CDCl₃) δ 1.12 (t, *J* = 7.1 Hz, 3H), 1.22 (t, *J* = 7.2 Hz, 3H), 2.32 (s, 3H), 2.59 (t, *J* = 7.5 Hz, 2H), 3.05 (t, *J* = 7.5 Hz, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 4.13 (q, *J* = 7.2 Hz, 2H), 5.12 (s, 1H), 5.43 (s, 2H), 6.81–6.84 (m, 2H), 7.04 (dd, *J* = 7.8, 7.5 Hz, 1H), 7.31 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.43–7.46 (m, 2H), 7.49 (dd, *J* = 7.8, 1.0 Hz, 1H).

Ethyl 4-[1-(2-Ethoxy-2-oxoethyl)-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indol-3-yl]butanoate (**48c**). Compound **48c** was prepared from compound **42b** in a manner similar to that described for compound **43a**. ¹H NMR (300 MHz, CDCl₃) δ 1.12 (t, *J* = 7.1 Hz, 3H), 1.22 (t, *J* = 7.2 Hz, 3H), 1.86–1.98 (m, 2H), 2.29 (s, 3H), 2.28–

2.36 (m, 2H), 2.76 (t, $J = 7.4$ Hz, 2H), 4.11 (q, $J = 7.2$ Hz, 2H), 4.13 (q, $J = 7.2$ Hz, 2H), 5.09 (s, 1H), 5.44 (s, 2H), 6.82 (d, $J = 8.8$ Hz, 2H), 7.03 (dd, $J = 7.8, 7.5$ Hz, 2H), 7.30 (dd, $J = 7.5, 1.1$ Hz, 1H), 7.44 (d, $J = 8.8$ Hz, 2H), 7.49 (dd, $J = 7.8, 1.1$ Hz, 1H).

Ethyl 4-{3-(2-Ethoxy-2-oxoethyl)-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indol-1-yl}butanoate (48d). Compound 48d was prepared from compound 47d in a manner similar to that described for compound 43a. ^1H NMR (300 MHz, CDCl_3) δ 1.12 (t, $J = 7.2$ Hz, 3H), 1.25 (t, $J = 7.2$ Hz, 3H), 2.07–2.20 (m, 2H), 2.30 (t, $J = 7.4$ Hz, 2H), 2.40 (s, 3H), 3.70 (s, 2H), 4.08 (q, $J = 7.2$ Hz, 2H), 4.14 (q, $J = 7.2$ Hz, 2H), 4.53–4.64 (m, 2H), 5.50 (s, 1H), 6.71 (d, $J = 8.8$ Hz, 2H), 7.05 (t, $J = 7.8$ Hz, 1H), 7.33 (dd, $J = 7.8, 1.1$ Hz, 1H), 7.38 (d, $J = 8.8$ Hz, 2H), 7.51 (dd, $J = 7.8, 1.1$ Hz, 1H).

Ethyl 4-{3-(3-Ethoxy-3-oxopropyl)-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indol-1-yl}butanoate (48e). Compound 48e was prepared from compound 47e in a manner similar to that described for compound 43a. ^1H NMR (300 MHz, CDCl_3) δ 1.19–1.25 (m, 6H), 2.12–2.19 (m, 2H), 2.28–2.33 (m, 2H), 2.39 (s, 3H), 2.58 (t, $J = 7.7$ Hz, 2H), 3.04 (t, $J = 7.7$ Hz, 2H), 4.05–4.14 (m, 4H), 4.62 (t, $J = 7.5$ Hz, 2H), 5.35 (s, 1H), 6.81–6.84 (m, 2H), 7.03 (dd, $J = 7.8, 7.2$ Hz, 1H), 7.32 (d, $J = 7.2$ Hz, 1H), 7.41–7.44 (m, 2H), 7.49 (d, $J = 7.8$ Hz, 1H).

Diethyl 4,4'-[7-[(4-Hydroxyphenyl)ethynyl]-2-methyl-1H-indole-1,3-diyl]dibutanoate (48f). Compound 48f was prepared from compound 47f in a manner similar to that described for compound 43a. ^1H NMR (300 MHz, CDCl_3) δ 1.22 (t, $J = 7.2$ Hz, 3H), 1.26 (t, $J = 7.2$ Hz, 3H), 1.82–1.99 (m, 2H), 2.05–2.21 (m, 2H), 2.29–2.35 (m, 7H), 2.75 (t, $J = 7.5$ Hz, 2H), 4.04–4.14 (m, 4H), 4.62 (t, $J = 7.2$ Hz, 2H), 5.39 (s, 1H), 6.83 (d, $J = 8.7$ Hz, 2H), 7.01 (t, $J = 7.8$ Hz, 1H), 7.30 (dd, $J = 7.8, 1.2$ Hz, 1H), 7.42 (d, $J = 8.7$ Hz, 2H), 7.48 (dd, $J = 7.2, 1.2$ Hz, 1H).

Ethyl 5-{3-(2-Ethoxy-2-oxoethyl)-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indol-1-yl}pentanoate (48g). Compound 48g was prepared from compound 47g in a manner similar to that described for compound 43a. ^1H NMR (300 MHz, CDCl_3) δ 1.19 (t, $J = 7.2$ Hz, 3H), 1.26 (t, $J = 7.2$ Hz, 3H), 1.56–1.70 (m, 2H), 1.74–1.90 (m, 2H), 2.24 (t, $J = 7.4$ Hz, 2H), 2.37 (s, 3H), 3.71 (s, 2H), 4.06 (q, $J = 7.2$ Hz, 2H), 4.15 (q, $J = 7.2$ Hz, 2H), 4.45–4.59 (m, 2H), 5.69 (s, 1H), 6.65 (d, $J = 8.8$ Hz, 2H), 7.04 (t, $J = 7.8$ Hz, 1H), 7.29–7.40 (m, 3H), 7.50 (dd, $J = 8.7, 1.1$ Hz, 1H).

Ethyl 5-{3-(3-Ethoxy-3-oxopropyl)-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indol-1-yl}pentanoate (48h). Compound 48h was prepared from compound 47h in a manner similar to that described for compound 43a. ^1H NMR (300 MHz, CDCl_3) δ 1.17–1.24 (m, 6H), 1.64–1.86 (m, 4H), 2.25 (t, $J = 7.4$ Hz, 2H), 2.37 (s, 3H), 2.57 (t, $J = 7.8$ Hz, 2H), 3.04 (t, $J = 7.8$ Hz, 2H), 4.03–4.14 (m, 4H), 4.54 (t, $J = 7.7$ Hz, 2H), 5.36 (s, 1H), 6.81–6.84 (m, 2H), 7.02 (dd, $J = 7.8, 7.7$ Hz, 1H), 7.31 (dd, $J = 7.7, 0.9$ Hz, 1H), 7.41–7.44 (m, 2H), 7.48 (dd, $J = 7.8, 0.9$ Hz, 1H).

Ethyl 5-{3-(4-Ethoxy-4-oxobutyl)-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indol-1-yl}pentanoate (48i). Compound 48i was prepared from compound 47i in a manner similar to that described for compound 43a. ^1H NMR (300 MHz, CDCl_3) δ 1.20 (t, $J = 7.1$ Hz, 3H), 1.24 (t, $J = 7.2$ Hz, 3H), 1.65–1.97 (m, 6H), 2.26 (t, $J = 7.5$ Hz, 2H), 2.31 (t, $J = 8.0$ Hz, 2H), 2.34 (s, 3H), 2.75 (t, $J = 7.5$ Hz, 2H), 4.07 (q, $J = 7.1$ Hz, 2H), 4.10 (q, $J = 7.2$ Hz, 2H), 4.58 (t, $J = 7.7$ Hz, 2H), 5.36 (s, 1H), 6.82–6.85 (m, 2H), 7.01 (dd, $J = 8.0, 7.4$ Hz, 1H), 7.31 (dd, $J = 7.4, 1.1$ Hz, 1H), 7.41–7.44 (m, 2H), 7.48 (dd, $J = 8.0, 1.1$ Hz, 1H).

2,2'-[7-[(4-{3-Chloro-2-methylphenyl}butoxy)phenyl]ethynyl]-2-methyl-1H-indole-1,3-diyl]diacetic Acid (17). 38ba (65 mg, 0.18 mmol) and Cs_2CO_3 (109 mg, 0.33 mmol) were added to a solution of 48a (70 mg) in DMF (0.5 mL), and the reaction mixture was stirred for 14 h at room temperature. The reaction mixture was filtered and concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give the ester derivative 49a. A 2 mol/L aqueous solution of sodium hydroxide (0.6 mL) was added to a mixture of 49a, DME (1.5 mL), and EtOH (1.5 mL), and the mixture was stirred for 7 h at 50 °C. The reaction mixture was acidified with 2 mol/L hydrochloric acid and extracted with EtOAc. The organic layer

was washed sequentially with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was stirred for 30 min in $^1\text{Pr}_2\text{O}$ and filtered to obtain a solid. The resulting solid was dried to give 17 (88 mg, 89%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.57–1.85 (m, 4H), 2.28 (s, 3H), 2.31 (s, 3H), 2.65–2.76 (m, 2H), 3.62 (s, 2H), 4.00–4.10 (m, 2H), 5.40 (s, 2H), 6.94–7.04 (m, 3H), 7.08–7.17 (m, 2H), 7.18–7.28 (m, 2H), 7.42–7.54 (m, 3H), 12.15 (brs, 1H), 13.02 (brs, 1H). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 9.83, 15.50, 26.16, 28.26, 29.87, 33.04, 45.45, 67.36, 85.83, 92.69, 104.52, 105.18, 114.39, 114.78, 118.85, 126.23, 126.64, 126.86, 127.86, 128.64, 132.52, 133.27, 133.83, 134.09, 135.82, 142.71, 158.81, 170.56, 172.73. MS (FAB, neg) m/z 542 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{32}\text{H}_{30}\text{ClNO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 542.1734, found 542.1725. HPLC purity 97% (method A).

3-[1-(Carboxymethyl)-7-[(4-{3-chloro-2-methylphenyl}butoxy)phenyl]ethynyl]-2-methyl-1H-indol-3-yl]propanoic Acid (18). Compound 18 was prepared from compound 48b and 38ba in a manner similar to that described for compound 17. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.59–1.72 (m, 2H), 1.72–1.85 (m, 2H), 2.27 (s, 3H), 2.31 (s, 3H), 2.40–2.47 (m, 2H), 2.67–2.73 (m, 2H), 2.91 (t, $J = 7.5$ Hz, 2H), 4.05 (t, $J = 6.3$ Hz, 2H), 5.38 (s, 2H), 7.00 (t, $J = 8.0$ Hz, 3H), 7.08–7.17 (m, 2H), 7.18–7.23 (m, 1H), 7.23–7.29 (m, 1H), 7.45–7.54 (m, 3H), 12.06 (brs, 1H), 12.91 (brs, 1H). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 9.68, 15.50, 19.33, 26.16, 28.26, 33.04, 34.90, 45.36, 67.36, 85.92, 92.65, 104.51, 110.11, 114.42, 114.79, 118.59, 118.73, 126.12, 126.64, 126.87, 127.86, 128.25, 132.50, 133.27, 133.83, 134.23, 134.63, 142.71, 158.80, 170.63, 173.89. MS (FAB, neg) m/z 556 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{33}\text{H}_{32}\text{ClNO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 556.1891, found 556.1890. HPLC purity 95% (method B).

4-[1-(Carboxymethyl)-7-[(4-{3-chloro-2-methylphenyl}butoxy)phenyl]ethynyl]-2-methyl-1H-indol-3-yl]butanoic Acid (19). Compound 19 was prepared from compound 48c and 38ba in a manner similar to that described for compound 17. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.58–1.86 (m, 6H), 2.21 (t, $J = 7.2$ Hz, 2H), 2.25 (s, 3H), 2.31 (s, 3H), 2.62–2.75 (m, 4H), 3.99–4.09 (m, 2H), 5.31–5.46 (m, 2H), 6.94–7.04 (m, 3H), 7.08–7.16 (m, 2H), 7.20 (d, $J = 7.1$ Hz, 1H), 7.22–7.29 (m, 1H), 7.44–7.54 (m, 3H), 12.09 (brs, 1H), 12.88 (brs, 1H). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 26.16, 28.25, 32.96, 33.03, 45.34, 67.36, 85.94, 92.62, 104.47, 110.73, 114.43, 114.79, 118.59, 118.65, 126.06, 126.64, 126.86, 127.85, 128.57, 132.48, 133.27, 133.83, 134.25, 134.48, 142.71, 158.79, 170.67, 174.38. MS (FAB, neg) m/z 570 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{34}\text{H}_{34}\text{ClNO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 570.2047, found 570.2048. HPLC purity 95% (method B).

4-[3-(Carboxymethyl)-7-[(4-{3-chloro-2-methylphenyl}butoxy)phenyl]ethynyl]-2-methyl-1H-indol-1-yl]butanoic Acid (23). Compound 23 was prepared from compound 48d and 38ba in a manner similar to that described for compound 17. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.57–1.86 (m, 4H), 1.87–2.05 (m, 2H), 2.20 (t, $J = 7.4$ Hz, 2H), 2.31 (s, 3H), 2.33–2.38 (m, 3H), 2.64–2.79 (m, 2H), 3.61 (s, 2H), 4.05 (t, $J = 6.2$ Hz, 2H), 4.49–4.62 (m, 2H), 6.93–7.04 (m, 3H), 7.06–7.18 (m, 2H), 7.19–7.28 (m, 2H), 7.41–7.54 (m, 3H), 12.25 (brs, 2H). MS (FAB, neg) m/z 570 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{34}\text{H}_{34}\text{ClNO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 570.2047, found 570.2026. HPLC purity 95% (method B).

4-[3-(2-Carboxyethyl)-7-[(4-{3-chloro-2-methylphenyl}butoxy)phenyl]ethynyl]-2-methyl-1H-indol-1-yl]butanoic Acid (24). Compound 24 was prepared from compound 48e and 38ba in a manner similar to that described for compound 17. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.59–1.72 (m, 2H), 1.72–1.83 (m, 2H), 1.86–2.02 (m, 2H), 2.13–2.22 (m, 2H), 2.31 (s, 3H), 2.35 (s, 3H), 2.39–2.46 (m, 2H), 2.65–2.77 (m, 2H), 2.84–2.99 (m, 2H), 4.05 (t, $J = 6.3$ Hz, 2H), 4.47–4.60 (m, 2H), 6.94–7.04 (m, 3H), 7.09–7.18 (m, 2H), 7.19–7.30 (m, 2H), 7.45–7.55 (m, 3H), 12.10 (s, 2H). MS (FAB, neg) m/z 584 ($\text{M} - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{35}\text{H}_{36}\text{ClNO}_5$ ($\text{M} - \text{H}$) $^-$ calcd mass 584.2204, found 584.2206. HPLC purity 99% (method A).

4,4'-[7-[(4-{3-Chloro-2-methylphenyl}butoxy)phenyl]ethynyl]-2-methyl-1H-indole-1,3-diyl]dibutanoic Acid (25). Compound 25 was prepared from compound 48f and 38ba in a manner similar to

that described for compound 17. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.59–1.87 (m, 6H), 1.88–2.03 (m, 2H), 2.12–2.23 (m, 4H), 2.31 (s, 3H), 2.33 (s, 3H), 2.61–2.77 (m, 4H), 4.05 (t, *J* = 6.2 Hz, 2H), 4.54 (t, *J* = 7.5 Hz, 2H), 6.93–7.03 (m, 3H), 7.07–7.18 (m, 2H), 7.19–7.29 (m, 2H), 7.42–7.58 (m, 3H), 12.05 (s, 2H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ 9.74, 15.49, 22.82, 25.74, 26.14, 26.61, 28.25, 30.48, 33.01, 33.03, 42.57, 67.36, 86.39, 91.64, 104.35, 110.76, 114.44, 114.94, 118.40, 118.70, 126.21, 126.63, 126.85, 127.85, 128.66, 132.42, 133.27, 133.47, 133.83, 134.09, 142.71, 158.82, 173.70, 174.38. MS (FAB, neg) *m/z* 598 (M – H)[–]. HRMS (FAB, neg) C₃₆H₃₈ClNO₅ (M – H)[–] calcd mass 598.2360, found 598.2371. HPLC purity 98% (method B).

5-[3-(Carboxymethyl)-7-({4-[4-(3-chloro-2-methylphenyl)-butoxy]phenyl}ethynyl)-2-methyl-1H-indol-1-yl]pentanoic Acid (26). Compound 26 was prepared from compound 48g and 38ba in a manner similar to that described for compound 17. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.43–1.58 (m, 2H), 1.58–1.85 (m, 6H), 2.19 (t, *J* = 7.3 Hz, 2H), 2.31 (s, 3H), 2.35 (s, 3H), 2.64–2.76 (m, 2H), 3.61 (s, 2H), 4.04 (t, *J* = 6.3 Hz, 2H), 4.45–4.64 (m, 2H), 6.91–7.03 (m, 3H), 7.07–7.18 (m, 2H), 7.19–7.29 (m, 2H), 7.40–7.55 (m, 3H), 12.15 (brs, 2H). MS (FAB, neg) *m/z* 584 (M – H)[–]. HRMS (FAB, neg) C₃₅H₃₆ClNO₅ (M – H)[–] calcd mass 584.2204, found 584.2200. HPLC purity 96% (method B).

5-[3-(2-Carboxyethyl)-7-({4-[4-(3-chloro-2-methylphenyl)-butoxy]phenyl}ethynyl)-2-methyl-1H-indol-1-yl]pentanoic Acid (27). Compound 27 was prepared from compound 48h and 38ba in a manner similar to that described for compound 17. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.41–1.57 (m, 2H), 1.59–1.84 (m, 6H), 2.18 (t, *J* = 7.2 Hz, 2H), 2.31 (s, 3H), 2.34 (s, 3H), 2.38–2.46 (m, 2H), 2.65–2.76 (m, 2H), 2.90 (t, *J* = 7.4 Hz, 2H), 4.04 (t, *J* = 6.2 Hz, 2H), 4.47–4.61 (m, 2H), 6.92–7.02 (m, 3H), 7.07–7.17 (m, 2H), 7.20 (dd, *J* = 7.3, 0.9 Hz, 1H), 7.23–7.28 (m, 1H), 7.43–7.53 (m, 3H), 11.99 (brs, 2H). MS (FAB, neg) *m/z* 598 (M – H)[–]. HRMS (FAB, neg) C₃₆H₃₈ClNO₅ (M – H)[–] calcd mass 598.2360, found 598.2352. HPLC purity 99% (method B).

5-[3-(3-Carboxypropyl)-7-({4-[4-(3-chloro-2-methylphenyl)-butoxy]phenyl}ethynyl)-2-methyl-1H-indol-1-yl]pentanoic Acid (28). Compound 28 was prepared from compound 48i and 38ba in a manner similar to that described for compound 17. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.43–1.57 (m, 2H), 1.59–1.85 (m, 8H), 2.13–2.24 (m, 4H), 2.31 (s, 3H), 2.33 (s, 3H), 2.62–2.75 (m, 4H), 3.99–4.09 (m, 2H), 4.50–4.60 (m, 2H), 6.93–7.01 (m, 3H), 7.07–7.17 (m, 2H), 7.20 (dd, *J* = 7.4, 0.8 Hz, 1H), 7.23–7.28 (m, 1H), 7.43–7.52 (m, 3H), 11.96 (brs, 2H). MS (FAB, neg) *m/z* 612 (M – H)[–]. HRMS (FAB, neg) C₃₇H₄₀ClNO₅ (M – H)[–] calcd mass 612.2517, found 612.2516. HPLC purity 99% (method B).

Ethyl 2-Methyl-2-propanyl 2,2'-(7-bromo-2-methyl-1H-indole-1,3-diyl)diacetate (50a). Compound 50a was prepared from compound 46a in a manner similar to that described for compound 42a. ¹H NMR (300 MHz, CDCl₃) δ 1.22 (t, *J* = 7.2 Hz, 3H), 1.46 (s, 9H), 2.33 (s, 3H), 3.68 (s, 2H), 4.11 (q, *J* = 7.2 Hz, 2H), 5.20 (s, 2H), 6.92 (t, *J* = 7.8 Hz, 1H), 7.24–7.31 (m, 1H), 7.47 (dd, *J* = 7.8, 1.0 Hz, 1H).

Ethyl 3-(7-Bromo-2-methyl-1-[(2-methyl-2-propanyl)oxy]-2-oxoethyl)-1H-indol-3-yl]propanoate (50b). Compound 50b was prepared from compound 46b in a manner similar to that described for compound 42a. ¹H NMR (300 MHz, CDCl₃) δ 1.22 (t, *J* = 7.2 Hz, 3H), 1.45 (s, 9H), 2.30 (s, 3H), 2.51–2.62 (m, 2H), 2.98–3.08 (m, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 5.17 (s, 2H), 6.90 (dd, *J* = 7.8, 7.6 Hz, 1H), 7.26 (dd, *J* = 7.6, 1.0 Hz, 1H), 7.43 (dd, *J* = 7.8, 1.1 Hz, 1H).

Ethyl 4-(7-Bromo-2-methyl-1-[(2-methyl-2-propanyl)oxy]-2-oxoethyl)-1H-indol-3-yl]butanoate (50c). Compound 50c was prepared from compound 41b in a manner similar to that described for compound 42a. ¹H NMR (300 MHz, CDCl₃) δ 1.24 (t, *J* = 7.2 Hz, 3H), 1.44 (s, 9H), 1.84–1.98 (m, 2H), 2.24–2.34 (m, 5H), 2.74 (t, *J* = 7.4 Hz, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 5.17 (s, 2H), 6.88 (t, *J* = 7.6 Hz, 1H), 7.24 (dd, *J* = 7.6, 1.0 Hz, 1H), 7.42 (dd, *J* = 7.6, 1.0 Hz, 1H).

[7-Bromo-3-(2-ethoxy-2-oxoethyl)-2-methyl-1H-indol-1-yl]acetic Acid (51a). HCO₂H (15 mL) was added to a solution of 50a (890 mg, 2.17 mmol) in CH₂Cl₂ (10 mL), and the reaction mixture was stirred for 14 h at room temperature. The residue was stirred for 30 min in

¹Pr₂O and filtered to obtain a solid. The resulting solid was dried to give 51a (768 mg, 93%). ¹H NMR (300 MHz, CDCl₃) δ 1.22 (t, *J* = 7.2 Hz, 3H), 2.34 (s, 3H), 3.68 (s, 2H), 4.11 (q, *J* = 7.2 Hz, 2H), 5.37 (s, 2H), 6.94 (t, *J* = 7.8 Hz, 1H), 7.29 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.48 (dd, *J* = 7.8, 0.9 Hz, 1H).

[7-Bromo-3-(3-ethoxy-3-oxopropyl)-2-methyl-1H-indol-1-yl]acetic Acid (51b). Compound 51b was prepared from compound 50b in a manner similar to that described for compound 51a. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.12 (t, *J* = 7.2 Hz, 3H), 2.25 (s, 3H), 2.47–2.55 (m, 2H), 2.93 (t, *J* = 7.2 Hz, 2H), 3.99 (q, *J* = 7.2 Hz, 2H), 5.23 (s, 2H), 6.90 (t, *J* = 7.7 Hz, 1H), 7.22 (dd, *J* = 7.7, 0.9 Hz, 1H), 7.47 (dd, *J* = 7.7, 0.9 Hz, 1H), 13.01 (s, 1H).

[7-Bromo-3-(4-ethoxy-4-oxobutyl)-2-methyl-1H-indol-1-yl]acetic Acid (51c). Compound 51c was prepared from compound 50c in a manner similar to that described for compound 51a. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.15 (t, *J* = 7.2 Hz, 3H), 1.65–1.83 (m, 2H), 2.20–2.32 (m, 5H), 2.67 (t, *J* = 7.5 Hz, 2H), 4.03 (q, *J* = 7.2 Hz, 2H), 5.24 (s, 2H), 6.90 (t, *J* = 7.8 Hz, 1H), 7.21 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.47 (dd, *J* = 7.8, 0.9 Hz, 1H), 13.01 (s, 1H).

Ethyl 3-[7-Bromo-3-(2-ethoxy-2-oxoethyl)-2-methyl-1H-indol-1-yl]propanoate (53a). Triethylamine (0.42 mL, 3.0 mmol) and isobutyl chloroformate (0.25 mL, 2.6 mmol) were added to a solution of 51a (708 mg, 2.0 mmol) in THF (10.0 mL) at –10 °C. The reaction mixture was stirred for 3 h at –10 °C. Then CH₂N₂/diethyl ether (10 mL) was added to the reaction mixture at –10 °C. The reaction mixture was stirred for 3 h at 0 °C. H₂O was added to the reaction mixture, and the resulting mixture was extracted with EtOAc. The organic layer was washed sequentially with water and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 52a. Triethylamine (0.16 mL, 1.15 mmol) and silver(I) benzoate (30 mg, 0.13 mmol) were added to a solution of 52a in EtOH (4.0 mL), and the reaction mixture was stirred for 3 h at 50 °C. The reaction mixture was filtered, the filtrate was poured into water, and the resulting mixture was extracted with EtOAc. The organic layer was washed sequentially with aqueous saturated ammonium chloride solution, water, and saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc) to give 53a (408 mg, 51%, 2 steps). ¹H NMR (300 MHz, CDCl₃) δ 1.23 (t, *J* = 7.2 Hz, 3H), 1.25 (t, *J* = 7.2, 3H), 1.82–1.98 (m, 2H), 2.29 (t, *J* = 7.2 Hz, 2H), 2.36 (s, 3H), 2.68–2.81 (m, 4H), 4.10 (q, *J* = 7.2 Hz, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 4.72–4.84 (m, 2H), 6.90 (t, *J* = 7.8 Hz, 1H), 7.28 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.43 (dd, *J* = 7.8, 0.9 Hz, 1H).

Diethyl 3,3'-(7-Bromo-2-methyl-1H-indole-1,3-diyl)dipropoanoate (53b). Compound 53b was prepared from compound 51b in a manner similar to that described for compound 53a. ¹H NMR (300 MHz, CDCl₃) δ 1.22 (t, *J* = 7.2 Hz, 3H), 1.25 (t, *J* = 7.2 Hz, 3H), 2.38 (s, 3H), 2.49–2.60 (m, 2H), 2.67–2.81 (m, 2H), 2.95–3.06 (m, 2H), 4.09 (q, *J* = 7.2 Hz, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 4.71–4.83 (m, 2H), 6.90 (t, *J* = 7.8 Hz, 1H), 7.28 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.42 (dd, *J* = 7.8, 1.0 Hz, 1H).

Ethyl 4-[7-Bromo-1-(3-ethoxy-3-oxopropyl)-2-methyl-1H-indol-3-yl]butanoate (53c). Compound 53c was prepared from compound 51c in a manner similar to that described for compound 53a. ¹H NMR (300 MHz, CDCl₃) δ 1.23 (t, *J* = 7.2 Hz, 3H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.82–1.98 (m, 2H), 2.29 (t, *J* = 7.2 Hz, 2H), 2.36 (s, 3H), 2.68–2.81 (m, 4H), 4.10 (q, *J* = 7.2 Hz, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 4.72–4.84 (m, 2H), 6.90 (t, *J* = 7.8 Hz, 1H), 7.28 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.43 (dd, *J* = 7.8, 0.9 Hz, 1H).

Ethyl 3-[3-(2-Ethoxy-2-oxoethyl)-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indol-1-yl]propanoate (54a). Compound 54a was prepared from compound 53a in a manner similar to that described for compound 43a. ¹H NMR (300 MHz, CDCl₃) δ 1.11 (t, *J* = 7.2 Hz, 3H), 1.26 (t, *J* = 7.2 Hz, 3H), 2.39 (s, 3H), 2.77–2.89 (m, 2H), 3.70 (s, 2H), 3.99 (q, *J* = 7.2 Hz, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 4.74–4.89 (m, 2H), 5.65–5.77 (m, 1H), 6.60 (d, *J* = 8.6 Hz, 2H), 7.04 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.28–7.39 (m, 3H), 7.48 (dd, *J* = 7.8, 1.0 Hz, 1H).

Diethyl 3,3'-(7-[(4-Hydroxyphenyl)ethynyl]-2-methyl-1H-indole-1,3-diyl)dipropoanoate (54b). Compound 54b was prepared from

compound **53b** in a manner similar to that described for compound **43a**. ^1H NMR (300 MHz, CDCl_3) δ 1.12 (t, J = 7.2 Hz, 3H), 1.22 (t, J = 7.2 Hz, 3H), 2.39 (s, 3H), 2.52–2.62 (m, 2H), 2.80–2.89 (m, 2H), 2.98–3.08 (m, 2H), 4.01 (q, J = 7.2 Hz, 2H), 4.11 (q, J = 7.2 Hz, 2H), 4.83–4.94 (m, 2H), 5.16 (s, 1H), 6.81 (d, J = 8.7 Hz, 2H), 7.04 (dd, J = 7.8, 7.4 Hz, 1H), 7.32 (dd, J = 7.4, 1.1 Hz, 1H), 7.42 (d, J = 8.7 Hz, 2H), 7.48 (dd, J = 7.8, 1.1 Hz, 1H).

Ethyl 4-[(3-Ethoxy-3-oxopropyl)-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indol-3-yl]butanoate (54c). Compound **54c** was prepared from compound **53c** in a manner similar to that described for compound **43a**. ^1H NMR (300 MHz, CDCl_3) δ 1.12 (t, J = 7.2 Hz, 3H), 1.24 (t, J = 7.2 Hz, 3H), 1.85–1.99 (m, 2H), 2.30 (t, J = 7.4 Hz, 2H), 2.36 (s, 3H), 2.74 (t, J = 7.4 Hz, 2H), 2.79–2.91 (m, 2H), 4.01 (q, J = 7.2 Hz, 2H), 4.10 (q, J = 7.2 Hz, 2H), 4.82–4.94 (m, 2H), 5.08 (s, 1H), 6.80 (d, J = 8.8 Hz, 2H), 7.02 (t, J = 7.8 Hz, 1H), 7.31 (dd, J = 7.4, 1.1 Hz, 1H), 7.41 (d, J = 8.8 Hz, 2H), 7.47 (dd, J = 7.8, 1.1 Hz, 1H).

3-[3-(Carboxymethyl)-7-[(4-[4-(3-chloro-2-methylphenyl)butoxy]phenyl)ethynyl]-2-methyl-1H-indol-1-yl]propanoic Acid (20). Compound **20** was prepared from compound **54a** in a manner similar to that described for compound **17**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.56–1.85 (m, 4H), 2.31 (s, 3H), 2.37 (s, 3H), 2.63–2.80 (m, 4H), 3.60 (s, 2H), 4.04 (t, J = 6.1 Hz, 2H), 4.69–4.86 (m, 2H), 6.95 (d, J = 8.6 Hz, 2H), 7.00 (t, J = 7.7 Hz, 1H), 7.08–7.18 (m, 2H), 7.21–7.30 (m, 2H), 7.41–7.56 (m, 3H), 12.30 (brs, 2H). MS (FAB, neg) m/z 556 ($M - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{33}\text{H}_{32}\text{ClNO}_5$ ($M - \text{H}$) $^-$ calcd mass 556.1891, found 556.1903. HPLC purity 95% (method B).

3,3'-[7-[(4-[4-(3-Chloro-2-methylphenyl)butoxy]phenyl)ethynyl]-2-methyl-1H-indole-1,3-diyl]dipropanoic Acid (21). Compound **21** was prepared from compound **54b** in a manner similar to that described for compound **17**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.56–1.86 (m, 4H), 2.30 (s, 3H), 2.36 (s, 3H), 2.41 (t, J = 7.5 Hz, 2H), 2.62–2.75 (m, 4H), 2.89 (t, J = 7.5 Hz, 2H), 4.03 (t, J = 6.1 Hz, 2H), 4.66–4.85 (m, 2H), 6.89–7.04 (m, 3H), 7.07–7.17 (m, 2H), 7.20–7.29 (m, 2H), 7.44–7.54 (m, 3H), 12.41 (brs, 2H). MS (FAB, neg) m/z 570 ($M - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{34}\text{H}_{34}\text{ClNO}_5$ ($M - \text{H}$) $^-$ calcd mass 570.2047, found 570.2039. HPLC purity 98% (method B).

4-[1-(2-Carboxyethyl)-7-[(4-[4-(3-chloro-2-methylphenyl)butoxy]phenyl)ethynyl]-2-methyl-1H-indol-3-yl]butanoic Acid (22). Compound **22** was prepared from compound **54c** in a manner similar to that described for compound **17**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.57–1.86 (m, 6H), 2.19 (t, J = 7.3 Hz, 2H), 2.30 (s, 3H), 2.35 (s, 3H), 2.59–2.77 (m, 6H), 4.04 (t, J = 6.4 Hz, 2H), 4.69–4.83 (m, 2H), 6.90–7.04 (m, 3H), 7.07–7.18 (m, 2H), 7.19–7.29 (m, 2H), 7.44–7.54 (m, 3H), 12.30 (brs, 2H). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 9.63, 15.49, 22.83, 25.78, 26.15, 28.26, 33.02, 33.10, 35.95, 67.33, 86.12, 91.73, 104.33, 110.85, 114.44, 114.73, 118.50, 118.61, 126.26, 126.63, 126.85, 127.85, 128.76, 132.55, 133.21, 133.26, 133.82, 134.04, 142.70, 158.73, 172.43, 174.49. MS (FAB, neg) m/z 584 ($M - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{35}\text{H}_{36}\text{ClNO}_5$ ($M - \text{H}$) $^-$ calcd mass 584.2204, found 584.2200. HPLC purity 99% (method A).

Ethyl 4-(7-Bromo-4-fluoro-2-methyl-1H-indol-3-yl)butanoate (57a). Compound **57a** was prepared from compound **56a** in a manner similar to that described for compound **41b** (procedure 2). ^1H NMR (300 MHz, CDCl_3) δ 1.22 (t, J = 7.2 Hz, 3H), 1.86–2.03 (m, 2H), 2.32 (t, J = 7.4 Hz, 2H), 2.38 (s, 3H), 2.78 (t, J = 7.4 Hz, 2H), 4.08 (q, J = 7.2 Hz, 2H), 6.63 (dd, J = 10.7, 8.4 Hz, 1H), 7.11 (dd, J = 8.4, 4.2 Hz, 1H), 7.96 (s, 1H).

Ethyl 4-(7-Bromo-5-fluoro-2-methyl-1H-indol-3-yl)butanoate (57b). Compound **57b** was prepared from compound **56b** in a manner similar to that described for compound **41b** (procedure 2). ^1H NMR (300 MHz, CDCl_3) δ 1.24 (t, J = 7.2 Hz, 3H), 1.84–1.99 (m, 2H), 2.30 (t, J = 7.3 Hz, 2H), 2.39 (s, 3H), 2.67 (t, J = 7.4 Hz, 2H), 4.11 (q, J = 7.2 Hz, 2H), 7.05 (dd, J = 8.9, 2.2 Hz, 1H), 7.11 (dd, J = 9.3, 2.2 Hz, 1H), 7.87 (s, 1H).

Ethyl 4-(7-Bromo-6-fluoro-2-methyl-1H-indol-3-yl)butanoate (57c). Compound **57c** was prepared from compound **56c** in a manner similar to that described for compound **41b** (procedure 2). ^1H NMR (300 MHz, CDCl_3) δ 1.23 (t, J = 7.2 Hz, 3H), 1.85–2.01 (m, 2H), 2.30 (t, J = 7.4 Hz, 2H), 2.38 (s, 3H), 2.70 (t, J = 7.4 Hz, 2H), 4.10 (q,

J = 7.2 Hz, 2H), 6.89 (dd, J = 9.4, 8.5 Hz, 1H), 7.33 (dd, J = 8.5, 5.5 Hz, 1H), 7.90 (s, 1H).

Diethyl 4,4'-(7-Bromo-4-fluoro-2-methyl-1H-indole-1,3-diyl)-dibutanoate (58a). Compound **58a** was prepared from compound **57a** in a manner similar to that described for compound **42a**. ^1H NMR (300 MHz, CDCl_3) δ 1.21–1.29 (m, 6H), 1.81–2.11 (m, 4H), 2.31 (t, J = 7.2 Hz, 2H), 2.34 (s, 3H), 2.39 (t, J = 7.2 Hz, 2H), 2.81 (t, J = 7.2 Hz, 2H), 4.05–4.17 (m, 4H), 4.49 (t, J = 7.8 Hz, 2H), 6.56 (dd, J = 10.2 Hz, 8.4 Hz, 1H), 7.14 (dd, J = 8.4, 4.8 Hz, 1H).

Diethyl 4,4'-(7-Bromo-5-fluoro-2-methyl-1H-indole-1,3-diyl)-dibutanoate (58b). Compound **58b** was prepared from compound **57b** in a manner similar to that described for compound **42a**. ^1H NMR (300 MHz, CDCl_3) δ 1.25 (t, J = 7.2 Hz, 3H), 1.26 (t, J = 7.2 Hz, 3H), 1.79–1.95 (m, 2H), 1.96–2.10 (m, 2H), 2.29 (t, J = 7.4 Hz, 2H), 2.35 (s, 3H), 2.38 (t, J = 7.4 Hz, 2H), 2.68 (t, J = 7.4 Hz, 2H), 4.05–4.20 (m, 4H), 4.40–4.53 (m, 2H), 7.01–7.14 (m, 2H).

Diethyl 4,4'-(7-Bromo-6-fluoro-2-methyl-1H-indole-1,3-diyl)-dibutanoate (58c). Compound **58c** was prepared from compound **57c** in a manner similar to that described for compound **42a**. ^1H NMR (300 MHz, CDCl_3) δ 1.18–1.33 (m, 6H), 1.81–1.95 (m, 2H), 1.97–2.11 (m, 2H), 2.29 (t, J = 7.3 Hz, 2H), 2.34 (s, 3H), 2.40 (t, J = 7.2 Hz, 2H), 2.70 (t, J = 7.5 Hz, 2H), 4.10 (q, J = 7.2 Hz, 2H), 4.13 (q, J = 7.2 Hz, 2H), 4.42–4.54 (m, 2H), 6.88 (dd, J = 9.2, 8.5 Hz, 1H), 7.32 (dd, J = 8.5, 5.1 Hz, 1H).

Diethyl 4,4'-(4-Fluoro-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indole-1,3-diyl)dibutanoate (59a). Compound **59a** was prepared from compound **58a** in a manner similar to that described for compound **43a**. ^1H NMR (300 MHz, CDCl_3) δ 1.21 (t, J = 7.2 Hz, 3H), 1.23 (t, J = 7.2 Hz, 3H), 1.82–1.99 (m, 2H), 2.05–2.21 (m, 2H), 2.21–2.38 (m, 7H), 2.81 (t, J = 7.2 Hz, 2H), 4.04–4.13 (m, 4H), 4.61 (t, J = 7.5 Hz, 2H), 5.43 (s, 1H), 6.66 (dd, J = 10.8, 8.4 Hz, 1H), 6.82 (d, J = 8.4 Hz, 2H), 7.21 (dd, J = 8.1, 5.1 Hz, 1H), 7.40 (d, J = 8.4 Hz, 2H).

Diethyl 4,4'-(5-Fluoro-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indole-1,3-diyl)dibutanoate (59b). Compound **59b** was prepared from compound **58b** in a manner similar to that described for compound **43a**. ^1H NMR (300 MHz, CDCl_3) δ 1.23 (t, J = 7.2 Hz, 3H), 1.25 (t, J = 7.2 Hz, 3H), 1.82–1.97 (m, 2H), 2.07–2.20 (m, 2H), 2.26–2.34 (m, 4H), 2.35 (s, 3H), 2.70 (t, J = 7.4 Hz, 2H), 4.08 (q, J = 7.2 Hz, 2H), 4.12 (q, J = 7.2 Hz, 2H), 4.52–4.64 (m, 2H), 5.22–5.32 (m, 1H), 6.84 (d, J = 8.8 Hz, 2H), 7.04 (dd, J = 9.7, 2.6 Hz, 1H), 7.13 (dd, J = 9.2, 2.6 Hz, 1H), 7.43 (d, J = 8.8 Hz, 2H).

Diethyl 4,4'-(6-Fluoro-7-[(4-hydroxyphenyl)ethynyl]-2-methyl-1H-indole-1,3-diyl)dibutanoate (59c). Compound **59c** was prepared from compound **58c** in a manner similar to that described for compound **43a**. ^1H NMR (300 MHz, CDCl_3) δ 1.15–1.33 (m, 6H), 1.82–1.99 (m, 2H), 2.06–2.22 (m, 2H), 2.23–2.40 (m, 7H), 2.72 (t, J = 7.4 Hz, 2H), 4.09 (q, J = 7.2 Hz, 2H), 4.11 (q, J = 7.2 Hz, 2H), 4.50–4.66 (m, 2H), 5.40 (s, 1H), 6.77–6.93 (m, 3H), 7.37 (dd, J = 8.6, 5.3 Hz, 1H), 7.45 (d, J = 8.8 Hz, 2H).

4,4'-[7-[(4-[4-(3-Chloro-2-methylphenyl)butoxy]phenyl)ethynyl]-4-fluoro-2-methyl-1H-indole-1,3-diyl]dibutanoic Acid (29). Compound **29** was prepared from compound **59a** in a manner similar to that described for compound **17**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 1.57–1.84 (m, 6H), 1.86–2.03 (m, 2H), 2.12–2.23 (m, 4H), 2.30 (s, 3H), 2.32 (s, 3H), 2.65–2.79 (m, 4H), 4.05 (t, J = 6.3 Hz, 2H), 4.55 (t, J = 7.7 Hz, 2H), 6.76 (dd, J = 10.8, 8.2 Hz, 1H), 6.99 (d, J = 9.0 Hz, 2H), 7.07–7.30 (m, 4H), 7.48 (d, J = 9.0 Hz, 2H), 12.08 (brs, 2H). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ 9.54, 15.49, 23.95, 26.14, 26.49, 28.25, 30.40, 32.83, 33.03, 42.70, 67.36, 85.49, 91.32, 101.31 ($J_{\text{C-F}}$ = 3.3 Hz), 104.52 ($J_{\text{C-F}}$ = 20.6 Hz), 109.2 ($J_{\text{C-F}}$ = 3.3 Hz), 114.31, 114.93, 116.46 ($J_{\text{C-F}}$ = 19.1 Hz), 126.63, 126.85, 127.05 ($J_{\text{C-F}}$ = 8.3 Hz), 127.85, 132.42, 133.27, 133.83, 134.87, 136.13 ($J_{\text{C-F}}$ = 12.9 Hz), 142.70, 155.98 ($J_{\text{C-F}}$ = 246.8 Hz), 158.85, 173.62, 174.30. MS (FAB, neg) m/z 616 ($M - \text{H}$) $^-$. HRMS (FAB, neg) $\text{C}_{36}\text{H}_{37}\text{ClFNO}_5$ ($M - \text{H}$) $^-$ calcd mass 616.2266, found 616.2272. HPLC purity 99% (method A).

4,4'-[7-[(4-[4-(3-Chloro-2-methylphenyl)butoxy]phenyl)ethynyl]-5-fluoro-2-methyl-1H-indole-1,3-diyl]dibutanoic Acid (30). Compound **30** was prepared from compound **59b** in a manner similar to that described for compound **17**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ

1.58–1.85 (m, 6H), 1.86–2.01 (m, 2H), 2.11–2.24 (m, 4H), 2.30 (s, 3H), 2.33 (s, 3H), 2.59–2.76 (m, 4H), 4.05 (t, $J = 6.2$ Hz, 2H), 4.51 (t, $J = 7.6$ Hz, 2H), 6.99 (d, $J = 8.8$ Hz, 2H), 7.04 (dd, $J = 9.9$, 2.6 Hz, 1H), 7.08–7.18 (m, 2H), 7.25 (dd, $J = 7.0$, 2.4 Hz, 1H), 7.29 (dd, $J = 9.3$, 2.6 Hz, 1H), 7.50 (d, $J = 8.8$ Hz, 2H), 12.22 (s, 2 H). MS (FAB, neg) m/z 616 ($M - H$)[−]. HRMS (FAB, neg) $C_{36}H_{37}ClFNO_5$ ($M - H$)[−] calcd mass 616.2266, found 616.2272. HPLC purity 98% (method A).

4,4'-[7-((4-[4-(3-Chloro-2-methylphenyl)butoxy]phenyl)ethynyl)-6-fluoro-2-methyl-1H-indole-1,3-diyl]dibutanoic Acid (31). Compound 31 was prepared from compound 59c in a manner similar to that described for compound 17. ¹H NMR (300 MHz, DMSO- d_6) δ 1.57–1.85 (m, 6H), 1.88–2.01 (m, 2H), 2.14–2.25 (m, 4H), 2.30 (s, 3H), 2.31 (s, 3H), 2.59–2.76 (m, 4H), 4.05 (t, $J = 6.2$ Hz, 2H), 4.50 (t, $J = 7.1$ Hz, 2H), 6.94 (dd, $J = 10.0$, 8.7 Hz, 1H), 7.00 (d, $J = 8.8$ Hz, 2H), 7.07–7.19 (m, 2H), 7.26 (dd, $J = 6.8$, 2.2 Hz, 1H), 7.42–7.55 (m, 3H), 12.09 (s, 2H). MS (FAB, neg) m/z 616 ($M - H$)[−]. HRMS (FAB, neg) $C_{36}H_{37}ClFNO_5$ ($M - H$)[−] calcd mass 616.2266, found 616.2272. HPLC purity 98% (method A).

4,4'-[2-Methyl-7-((4-[4-(2,3,4,6-tetrafluorophenyl)butoxy]phenyl)ethynyl)-1H-indole-1,3-diyl]dibutanoic Acid (32). Compound 32 was prepared from compound 48f in a manner similar to that described for compound 16. ¹H NMR (300 MHz, DMSO- d_6) δ 1.60–1.82 (m, 6H), 1.86–2.03 (m, 2H), 2.13–2.25 (m, 4H), 2.34 (s, 3H), 2.60–2.78 (m, 4H), 4.02 (t, $J = 5.8$ Hz, 2H), 4.54 (t, $J = 7.1$ Hz, 2H), 6.91–7.04 (m, 3H), 7.21 (d, $J = 7.3$ Hz, 1H), 7.38–7.54 (m, 4H), 12.07 (brs, 2H). ¹³C NMR (125 MHz, DMSO- d_6) δ 9.74, 21.43, 22.82, 25.23, 25.74, 26.60, 27.98, 30.47, 33.00, 42.56, 67.12, 86.39, 91.61, 101.33 (m), 104.34, 110.76, 114.49, 114.77, 114.91 (m), 118.40, 118.69, 126.21, 128.66, 132.41, 133.47, 134.08, 136.38 (m), 146.67 (m), 148.93 (m), 154.98, 158.74, 173.69, 174.37. MS (FAB, neg) m/z 622 ($M - H$)[−]. HRMS (FAB, neg) $C_{35}H_{33}F_4NO_5$ ($M - H$)[−] calcd mass 622.2217, found 622.2215. HPLC purity 98% (method A).

4,4'-[4-Fluoro-2-methyl-7-((4-[4-(2,3,4,6-tetrafluorophenyl)butoxy]phenyl)ethynyl)-1H-indole-1,3-diyl]dibutanoic Acid (33). Compound 33 was prepared from compound 59a in a manner similar to that described for compound 16. ¹H NMR (300 MHz, DMSO- d_6) δ 1.57–1.83 (m, 6H), 1.86–2.03 (m, 2H), 2.12–2.24 (m, 4H), 2.32 (s, 3H), 2.60–2.78 (m, 4H), 3.99 (t, $J = 5.8$ Hz, 2H), 4.53 (t, $J = 7.3$ Hz, 2H), 6.73 (dd, $J = 10.7$, 8.3 Hz, 1H), 6.94 (d, $J = 8.8$ Hz, 2H), 7.18 (dd, $J = 8.3$, 5.1 Hz, 1H), 7.34–7.49 (m, 1H), 7.45 (d, $J = 8.8$ Hz, 2H), 12.07 (s, 2H). ¹³C NMR (75 MHz, DMSO- d_6) δ 9.68, 21.54, 24.08, 25.35, 26.59, 28.08, 30.48, 32.98, 42.74, 67.04, 85.38, 91.11, 101.09 (m), 101.13, 104.42 (d, $J_{C-F} = 20.3$ Hz), 108.80 (d, $J_{C-F} = 3.0$ Hz), 114.13, 114.61, 114.50 (m), 116.21 (d, $J_{C-F} = 19.5$ Hz), 126.75 (d, $J_{C-F} = 8.3$ Hz), 132.09, 134.53, 135.88 (d, $J_{C-F} = 8.3$ Hz), 136.12 (m), 147.81 (m), 148.59 (m), 154.59 (m), 155.60 (m), 158.35, 173.20, 173.88. MS (FAB, neg) m/z 640 ($M - H$)[−]. HRMS (FAB, neg) $C_{35}H_{32}F_5NO_5$ ($M - H$)[−] calcd mass 640.2122, found 640.2101. HPLC purity 99% (method A). Elemental analysis calculated (%) for $C_{35}H_{32}F_5NO_5$: C 65.52, H 5.03, N 2.18. Found: C 65.42, H 5.19, N 2.03.

4,4'-[7-((4-[4-(3-Fluoro-2-methylphenyl)butoxy]phenyl)ethynyl)-2-methyl-1H-indole-1,3-diyl]dibutanoic Acid (34). Compound 34 was prepared from compound 48f in a manner similar to that described for compound 17. ¹H NMR (300 MHz, DMSO- d_6) δ 1.59–1.85 (m, 6H), 1.88–2.02 (m, 2H), 2.13–2.24 (m, 7H), 2.33 (s, 3H), 2.67 (t, $J = 7.4$ Hz, 4H), 4.05 (t, $J = 6.3$ Hz, 2H), 4.54 (t, $J = 7.4$ Hz, 2H), 6.91–7.03 (m, 5H), 7.08–7.17 (m, 1H), 7.21 (d, $J = 6.6$ Hz, 1H), 7.43–7.52 (m, 3H), 12.07 (brs, 2H). ¹³C NMR (75 MHz, DMSO- d_6) δ 10.82, 11.08, 23.85, 26.78, 27.11, 27.64, 29.29, 31.47, 32.96, 33.98, 43.52, 68.19, 87.19, 92.39, 105.07, 111.44, 113.00 (d, $J_{C-F} = 22.5$ Hz), 115.12, 115.56, 119.23 (d, $J_{C-F} = 21.8$ Hz), 122.92 (d, $J_{C-F} = 14.4$ Hz), 125.22 (d, $J_{C-F} = 3.0$ Hz), 126.85, 127.30 (d, $J_{C-F} = 9.0$ Hz), 129.29, 133.03, 134.06, 134.68, 143.60 (d, $J_{C-F} = 3.8$ Hz), 159.33, 159.61, 162.79, 174.21, 174.89. MS (FAB, neg) m/z 582 ($M - H$)[−]. HRMS (FAB, neg) $C_{36}H_{38}FNO_5$ ($M - H$)[−] calcd mass 582.2656, found 582.2661. HPLC purity >99% (method A).

4,4'-[4-Fluoro-7-(2-[4-[4-(3-fluoro-2-methylphenyl)butoxy]phenyl)ethynyl)-2-methyl-1H-indole-1,3-diyl]dibutanoic Acid (35):

Gemilukast, ONO-6950). Compound 35 was prepared from compound 59a in a manner similar to that described for compound 17. ¹H NMR (300 MHz, DMSO- d_6) δ 1.64–1.71 (m, 2H), 1.74–1.83 (m, 4H), 1.93–2.00 (m, 2H), 2.17–2.23 (m, 7H), 2.35 (s, 3H), 2.69 (t, $J = 7.5$ Hz, 2H), 2.75 (t, $J = 7.5$ Hz, 2H), 4.07 (t, $J = 6.0$ Hz, 2H), 4.57 (t, $J = 7.5$ Hz, 2H), 6.78 (dd, $J = 10.5$, 8.5 Hz, 1H), 6.96–7.04 (m, 4H), 7.12–7.18 (m, 1H), 7.22 (dd, $J = 8.5$, 5.0 Hz, 1H), 7.50 (d, $J = 9.0$ Hz, 2H), 12.09 (brs, 2H). ¹³C NMR (DMSO- d_6) δ 9.58, 10.02 ($J_{C-F} = 5.5$ Hz), 24.98, 26.13, 26.52, 28.30, 30.42, 31.98 ($J_{C-F} = 2.8$ Hz), 32.84, 42.74, 67.39, 85.52, 91.34, 101.32 ($J_{C-F} = 3.8$ Hz), 104.56 ($J_{C-F} = 21$ Hz), 109.02, 112.37 ($J_{C-F} = 23$ Hz), 114.31, 114.95, 116.56 ($J_{C-F} = 20$ Hz), 122.30 ($J_{C-F} = 16$ Hz), 124.65 ($J_{C-F} = 2.8$ Hz), 126.72 ($J_{C-F} = 9.1$ Hz), 127.08 ($J_{C-F} = 7.8$ Hz), 132.47, 134.96 ($J_{C-F} = 0.9$ Hz), 136.14 ($J_{C-F} = 13$ Hz), 143.08 ($J_{C-F} = 4.1$ Hz), 156.00 ($J_{C-F} = 246$ Hz), 158.88, 160.72 ($J_{C-F} = 240$ Hz), 173.69, 174.37. MS (FAB, neg) m/z 600 ($M - H$)[−]. HRMS (FAB, neg) $C_{36}H_{37}F_2NO_5$ ($M - H$)[−] calcd mass 600.2562, found 600.2562. HPLC purity >99% (method A). Elemental analysis calculated (%) for $C_{36}H_{37}F_2NO_5$: C 71.86, H 6.20, N 2.33. Found: C 71.88, H 6.19, N 2.25.

In Vitro Assay. Method 1. Calcium mobilization assay with compounds 1–32: The intracellular calcium mobilization response was measured using the fluorescent Ca^{2+} indicator dye Fura 2-AM (Dojindo). CHO-K1 cells stably expressing human CysLT₁, guinea pig CysLT₁, or guinea pig CysLT₂ receptors, and the HEK293 cells stably expressing human CysLT₂ receptor were used. Before the assay was performed, these cells were incubated at 37 °C in 5% CO₂ for at least 24 h with the following culture conditions: cells expressing human CysLT₁ receptor (4×10^4 cells/well) were cultured with Ham's F-12 supplemented with 10% vol fetal bovine serum, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 600 μ g/mL Geneticin. Cells expressing human CysLT₂ receptor (10×10^4 cells/well) were cultured with Dulbecco's Modified Eagle's Medium supplemented with 10% vol fetal bovine serum, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 400 μ g/mL geneticin. Cells expressing guinea pig CysLT₁ or CysLT₂ receptors (3×10^4 cells/well) were cultured with Ham's F-12K supplemented with 10% vol fetal bovine serum, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 500 μ g/mL geneticin. After removing the culture medium, loading medium containing 7.5 μ M Fura 2-AM, 20 mmol/L HEPES, and 2.5 mmol/L probenecid was added to each well. After a 1 h incubation, loading medium was removed and the cells were incubated at room temperature for 1 h in the assay buffer (Hank's Balanced Salt Solution containing 20 mM HEPES). The cells were alternately irradiated at two excitation wavelengths (340 and 380 nm) using FDSS-2000 (Hamamatsu Photonics Co., Ltd., Shizuoka, Japan) to measure the ratio of fluorescence intensities (f_{340}/f_{380}) at 500 nm. To evaluate the antagonism of the compounds, they were added 30 min before the addition of the ligand: 100, 100, 10, or 100 nM LTD₄ for human CysLT₁, human CysLT₂, guinea pig CysLT₁ or guinea pig CysLT₂ receptors, respectively.

Method 2. Calcium mobilization assay of compound 35 (gemilukast): The intracellular calcium response was measured using Fura 2-AM (Dojindo). Briefly, CHO-K1 cells stably expressing human or guinea pig CysLT₁ or CysLT₂ receptors were cultured for at least 24 h (3×10^4 cells/well). After removing the culture medium (Ham's F-12 supplemented with 10% fetal bovine serum and 500 μ g/mL geneticin), loading medium containing 5 μ M Fura 2-AM was added to each well. After a 1 h incubation, the loading medium was removed and the cells were incubated at room temperature for 1 h in the assay buffer (Hank's Balanced Salt Solution containing 20 mM HEPES). The cells were alternately irradiated at two excitation wavelengths (340 and 380 nm) using FDSS-3000 (Hamamatsu Photonics Co., Ltd., Shizuoka, Japan) to measure the ratio of fluorescence intensities (f_{340}/f_{380}) at 500 nm. To evaluate the antagonism of the compounds, they were added 30 min before the addition of the ligand: 100 nM LTD₄ for human CysLT₁ receptor, 0.3 nM LTD₄ for human CysLT₂ receptor, 10 nM LTD₄ for guinea pig CysLT₁ receptor, or 3 nM LTC₄ for guinea pig CysLT₂ receptor.

Parallel Artificial Membrane Permeability Assay (PAMPA). The PAMPA System (Pion) was used. The System Solution

Concentrate (Pion) was diluted with water, and DMSO was added to prepare a system solution (in 5% DMSO). NaOH at 0.5 mol/L was added using an automated buffer preparation system to make three different buffers with pH values of 5.0, 6.2, and 7.4. A volume of 150 μL of each buffer was added per well in a UV plate to serve as blanks for UV absorption (190–498 nm). Next, 5 μL of DMSO solution containing the test compounds at 10 mM was added to 1000 μL of the system solution, and this was filtered by suction through a 96-well filter plate (0.2 μm PVDF hydrophilic membrane, Corning) (final compound concentration of 50 μM). A volume of 150 μL of the filtrate was added per well in the UV plate to serve as the reference for UV absorption (190–498 nm). Then, 200 μL of the remaining filtrate was added per well in the donor plate and used as the donor solution. A membrane was prepared by dropping 4 μL of oil (GIT-0 Liquid, Pion) onto the filter of the acceptor plate. A 200 μL aliquot of the acceptor solution (Acceptor Sink Buffer, pH 7.4, Pion) was added to each well in the acceptor plate. The acceptor plate was placed on the donor plate. After a 4 h incubation, 150 μL aliquots were transferred from the donor and acceptor solutions to the UV plate to measure UV absorption (190–498 nm). The membrane permeability coefficient was calculated from the observed values obtained using the PAMPA Evolution Command software.

Pharmacokinetic Analysis in Rat, Guinea Pig, and Dog. The pharmacokinetics of compounds were studied following single intravenous and oral administration. The iv and po solution formulations contained wellsolve/ H_2O = 1/9 (Celeste) and sodium hydroxide (2 equiv). All iv and po formulations were given as solutions. Animals were fasted prior to oral dosing in single-dose studies. Oral bioavailability was estimated using noncrossover study designs for the rat, guinea pig, and dog ($n = 3$). Plasma samples were assayed for compounds using protein precipitation by acetonitrile/ethanol (70/30, v/v), followed by HPLC/MS/MS analysis employing negative-ion Turbo IonSpray ionization. Plasma concentration–time data were analyzed by noncompartmental methods.

Pharmacokinetic Analysis in Guinea Pig Cassette Dosing. The pharmacokinetics of compounds were studied following single intravenous and oral administration. The iv and po solution formulations contained wellsolve/ H_2O = 5/95 (Celeste) and sodium hydroxide (1.2 equiv). All iv and po formulations were given as cassette dosing solutions containing five compounds. Animals were fasted prior to oral dosing in single-dose studies. Oral bioavailability was estimated using noncrossover study designs for the guinea pig ($n = 3$). Plasma samples were assayed for compounds using protein precipitation by acetonitrile/ethanol (70/30, v/v), followed by HPLC/MS/MS analysis employing negative-ion Turbo IonSpray ionization. Plasma concentration–time data were analyzed by noncompartmental methods.

Measurement of the Concentration in Lung. The isolated lungs were homogenized in saline. The resulting lung homogenate (0.5 mL) was incubated in 2 mol/L NaOH (0.5 mL) at 60 °C for 3 h, and then diluted 50-fold with PBS. The amount of protein in the sample was spectrophotometrically determined using the BCA Protein Assay Kit (Thermo Scientific). Meanwhile, the amount of compound was measured by HPLC/MS/MS analysis employing negative-ion Turbo IonSpray ionization after protein precipitation with ethanol. The concentrations of compounds in lung tissues were expressed in ng/g protein.

In Vitro Hepatocyte Metabolic Stability. The metabolic stability of test compounds (1 μM test concentration) was determined in human cryopreserved hepatocytes (Celsis In Vitro Technologies, Baltimore, USA, pooled donors). Incubations (final incubation volume = 800 μL) were performed at 37 °C under an atmosphere of 95%/5% CO_2/O_2 using 1 million cells mL^{-1} in hepatocyte incubation media (In Vitro Technologies, Baltimore, MD, USA). Then, 150 μL aliquots (cells and media) were taken at zero, 0.5, 1, and 2 h after the start of the incubation and quenched using 300 μL of acetonitrile/ethanol (7/3). The resulting samples were centrifuged at 3000g for 10 min. An aliquot (10 μL) of the supernatant was directly injected onto the LC–MS/MS for analysis. All incubations were performed in duplicate.

Effects of Compounds on LTD₄ and LTC₄-Induced Bronchoconstriction in Guinea Pigs. Ventilation pressure was measured using the method of Konzett and Rössler. Briefly, guinea pigs were anesthetized with intraperitoneal pentobarbital sodium (75 mg/kg). A polyethylene cannula was inserted into the trachea. The other end of the cannula was connected to a volume-limited ventilator (model SN-480-7, Shinano Manufacturing Co., Ltd., Tokyo, Japan), and artificial ventilation was provided at a supply rate of 5 mL/stroke and 70 strokes/min. Another catheter was inserted into the jugular vein, securing the route for administration. The ventilation pressure was measured via a pneumotachometer (M-I-P-S Co., Ltd., Osaka, Japan) connected to a lateral port of the tracheal cannula, using a WinPULMOS-III system (version 3.6, M-I-P-S Co., Ltd., Osaka, Japan). After the basal ventilation pressure was confirmed to be stabilized, LTD₄ (0.25 $\mu\text{g}/\text{kg}$) or LTC₄ (15 $\mu\text{g}/\text{kg}$) was intravenously administered via the catheter secured in the jugular vein. In the experiment in which LTC₄-induced bronchoconstriction was examined, S-hexyl GSH (30 mg/kg) was intravenously administered 10 s prior to LTC₄ (15 $\mu\text{g}/\text{kg}$) injection. The ventilation pressure was measured for at least 10 min after LTD₄ or 30 min after LTC₄ injection, and then the trachea was completely blocked to obtain the maximum ventilation pressure. After measurement of the maximum ventilation pressure, artificial ventilation was stopped and the animal was euthanized. The area under the percentage bronchoconstriction curve (AUC) from 0 to 10 min after LTD₄ injection or from 0 to 30 min after LTC₄ injection was calculated.

Antigen-Induced Contraction in Isolated Human Bronchial Strips. As described in the previous report,⁴¹ bronchi (outer diameter 2–6 mm) were isolated from macroscopic normal portions of the lung tissue and cut into 1.5 mm wide spirals. In the experiments to evaluate the effects of CysLT antagonists on antigen-induced contraction, the bronchi were passively sensitized by incubation in human atopic serum for 2 h at 37 °C. Bronchial strips (2 cm long, outer diameter 2–4 mm) were then cut and suspended under an isotonic resting tension of 300 mg at 37 °C in a Magnus bath containing Tyrode's solution gassed with 95% O_2 –5% CO_2 . Before starting the experiments, acetylcholine (5 μM) and then histamine (10 μM) were repeatedly applied to the preparations until almost equal contractions were obtained. When the resting tonus of the histamine-treated smooth muscle stabilized, guinea pig serum albumin at a final concentration of 0.1 mg/mL was added to prevent adsorption of ONO-6950 and montelukast to the inner wall of the Magnus bath. Immediately after the addition of guinea pig serum albumin, ONO-6950 (100 nM), BayCysLT₂RA (10 nM), montelukast (10 nM), or DMSO (final concentration: 0.1%) were applied, and 30–35 min after the addition, antigen challenge (mite extract from *Dermatophagoides farinae*, 3 $\mu\text{g}/\text{mL}$) was performed. As previously reported,^{42,43} a cyclooxygenase inhibitor, indomethacin (3 μM), and an antihistamine drug, pyrilamine (1 μM), were applied 10 and 5 min, respectively, before antigen challenge. Data were expressed as the % of 10 μM histamine-induced contraction. Each compound was added at 30 min before the antigen challenge.

■ ASSOCIATED CONTENT

§ Supporting Information

This information includes the experimental details for data collection, data reduction, and refinement statistics of the X-ray crystallography of 35 (PDF). Molecular formula strings (XLSX). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00741.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CysLTs, cysteinyl leukotrienes; hCysLT₁, human CysLT₁; hCysLT₂, human CysLT₂; gCysLT₁, guinea pig CysLT₁; gCysLT₂, guinea pig CysLT₂; LTC₄, leukotriene C₄; LTD₄, leukotriene D₄; LTE₄, leukotriene E₄

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Discovery of Gemilukast (ONO-6950), a Dual CysLT₁ and CysLT₂ Antagonist as a Therapeutic Agent for Asthma

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X-ray crystallography of compound 35

Data Collection

The crystals of compound **35** $C_{36}H_{37}F_2NO_5$ was mounted on a glass fiber. All measurements were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated Cu-K α radiation.

Indexing was performed from 3 oscillations that were exposed for 90 seconds. The crystal-to-detector distance was 127.40 mm.

Cell constants and an orientation matrix for data collection corresponded to a primitive triclinic cell with dimensions:

$$\begin{array}{ll} a = 9.0230(4) \text{ \AA} & \alpha = 105.550(2)^\circ \\ b = 12.5570(5) \text{ \AA} & \beta = 107.943(2)^\circ \\ c = 15.0447(7) \text{ \AA} & \gamma = 91.581(2)^\circ \\ V = 1550.98(12) \text{ \AA}^3 & \end{array}$$

For $Z = 2$ and F.W. = 601.69, the calculated density is 1.288 g/cm^3 . Based on a statistical analysis of intensity distribution, and the successful solution and refinement of the structure, the space group was determined to be:

$$P-1 (\#2)$$

The data were collected at a temperature of $20 \pm 1^\circ\text{C}$ to a maximum 2θ value of 136.5° . A total of 30 oscillation images were collected. A sweep of data was done using ω scans from 80.0 to 260.0° in 30.0° step, at $\chi=54.0^\circ$ and $\phi = 0.0^\circ$. The exposure rate was $2.0 \text{ [sec./}^\circ]$. A second sweep was performed using ω scans from 80.0 to 260.0° in 30.0° step, at $\chi=54.0^\circ$ and $\phi = 90.0^\circ$. The exposure rate was $2.0 \text{ [sec./}^\circ]$. Another sweep was performed using ω scans from 80.0 to 260.0° in 30.0° step, at $\chi=54.0^\circ$ and $\phi = 180.0^\circ$. The exposure rate was $2.0 \text{ [sec./}^\circ]$. Another sweep was performed using ω scans from 80.0 to 260.0° in 30.0° step, at $\chi=54.0^\circ$ and $\phi = 270.0^\circ$. The exposure rate was $2.0 \text{ [sec./}^\circ]$. Another sweep was performed using ω scans from 80.0 to 260.0° in 30.0° step, at $\chi=0.0^\circ$ and $\phi = 0.0^\circ$. The exposure rate was $2.0 \text{ [sec./}^\circ]$. The crystal-to-detector distance was 127.40 mm. Readout was performed in the 0.100 mm pixel mode.

Data Reduction

Of the 5479 reflections that were collected, 5479 were unique ($R_{\text{int}} = 0.111$).

The linear absorption coefficient, μ , for Cu-K α radiation is 7.685 cm^{-1} . The data were corrected for Lorentz and polarization effects.

Structure Solution and Refinement

The structure was solved by direct methods¹ and expanded using Fourier techniques². The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement³ on F was based on 1709 observed reflections ($I > 2.00\sigma(I)$) and 432 variable parameters and converged (largest parameter shift was 0.04 times its esd) with unweighted and weighted

agreement factors of:

$$R = \Sigma ||F_o| - |F_c|| / \Sigma |F_o| = 0.0893$$

$$R_w = [\Sigma w (|F_o| - |F_c|)^2 / \Sigma w F_o^2]^{1/2} = 0.0876$$

The standard deviation of an observation of unit weight⁴ was 5.29. Unit weights were used. Plots of $\Sigma w (|F_o| - |F_c|)^2$ versus $|F_o|$, reflection order in data collection, $\sin \theta/\lambda$ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.17 and 0.17 e-/ Å³, respectively.

Neutral atom scattering factors were taken from Cromer and Waber⁵. Anomalous dispersion effects were included in Fcalc⁶; the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley⁷. The values for the mass attenuation coefficients are those of Creagh and Hubbell⁸. All calculations were performed using the CrystalStructure^{9,10} crystallographic software package.

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- (3) Least Squares function minimized:

$$\Sigma w(|F_o| - |F_c|)^2 \quad \text{where } w = \text{Least Squares weights.}$$
- (4) Standard deviation of an observation of unit weight:

$$[\Sigma w(|F_o| - |F_c|)^2 / (N_o - N_v)]^{1/2}$$

where: N_o = number of observations
 N_v = number of variables
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