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Discovery of OT4003, a Novel, Potent, and Orally Active cys-LT_1 Receptor Antagonist

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Abstract—The present paper describes the structural modifications leading to the discovery of a new series of quinoline-containing cys-LT_1 receptor (LTD_4 receptor) antagonists. A structural optimization with respect to the in vitro receptor binding, the in vivo brochoconstriction, and the toxicological effect in the form of peroxisomal proliferation was performed in order to achieve the target compound OT4003. OT4003 ((*S*)-(+)-*E*-2-[2-(3-(2-(7-chloroquinolin-2-yl)ethenyl)phenylaminomethyl)-phenoxy]-hexanoic acid) was found to be a potent and selective inhibitor of [^{3}H]LTD_4 specific binding to guinea pig lung membranes (IC_{50} 2.4±1.0 nM), and also a potent, orally active, antagonist of LTD_4 induced bronchoconstriction in guinea pigs [ED_{50} 0.14 (ED_{16} 0.1–ED_{84} 0.4) mg/kg; 4 h pretreatment]. The enantiomerically pure OT4003 was prepared using a short convergent synthesis, including an enzymatic resolution step.

Introduction

The importance of eicosanoids, and in particular of leukotrienes, as mediators of the inflammatory process in asthma has been recently demonstrated clinically.[1] An increased production of leukotrienes has been found in antigen challenged allergic patients,[2] and therefore a series of new high affinity cys-LT_1 receptor [leukotriene D_4 (LTD_4) receptor] antagonists have been clinically tested. These include SKF-104353,[3] ICI 204,219,[4] MK-571/MK-679,[5] MK-476,[6] and ONO-1078,[7] which all block LTD_4 induced/antigen-induced bronchoconstriction in asthmatic patients. Furthermore, ICI 204,219, MK-679, MK-476, and ONO-1078 have shown a beneficial clinical effect in chronic asthma.[8]

The cys-LT_1 receptor antagonists mentioned above represent different structural classes of inhibitors: the agonist-based analogues, like SKF-104353,[9] the indole-based analogues, like ICI 204,219,[10] the quinoline-based analogues, like MK-476,[11] and the miscellaneous analogues, like ONO-1078.[12] These different compound series have been developed in parallel, and several of them, such as ONO-1078, (Pranlukast), ICI 204,219 (Zafirlukast), and MK-476 (Montelukast sodium), are today in an advanced stage of development as therapeutic agents for the treatment of asthma.

In this paper we report our discovery of a new series of quinoline containing cys-LT_1 receptor antagonists and in particular the optimization of these structures leading to the potent orally active cys-LT_1 receptor antagonist OT4003.

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Results and Discussion

Structure–activity

One of the initial leads in our search for leukotriene antagonists was the quinoline containing compound **1** (Fig. 1). This early candidate identified in our screening assays showed a weak inhibitory effect on the binding of [^{3}H]LTD_4 (IC_{50} 5 mM) to guinea pig lung membranes. We found the preferred position for amino substitution to be at carbon 3 in the phenyl ring. This positional preference has also been reported for other substituents, such as REV 5901,[13] and MK-571.[14] A simple benzylation of the 3-amino group in **1**, led to the more active compound **2**, (OT3473).[15] Compound **2** was found to inhibit both the [^{3}H]LTD_4 binding (IC_{50} 200 nM) and the 5-lipoxygenase (5-LO) activity (IC_{50} 100 nM), thus displaying a dual inhibitory effect on both the formation and the action of leukotrienes.

A further modification of this lead compound (**1**) was made by a systematic change of substituent and substitution pattern in ring B and C (Fig. 1). Several compound groups with altered biological in vitro profile emerged from this work. Examples of these are SR2640,[16] **3** ([^{3}H]LTD_4 IC_{50} 23 nM;[15] 5-LO IC_{50} 10 mM), and ETH615,[16] **4** ([^{3}H]LTD_4 IC_{50} 300 nM; 5-LO IC_{50} 10 nM).[17] These compounds were subsequently characterized in additional in vitro and in vivo studies.[18]

In parallel with this work, a substitution in the C ring to yield 2-phenoxy acetic acids gave the next improvement in the in vitro activity for the inhibition of binding of [^{3}H]LTD$_4$. The most active compounds in this series were found to be the 2-substituted analogues, among which the most potent was compound **5**, whose IC_{50} was 7.2 nM (Fig. 2).

This interesting compound (**5**) was further tested in vivo in guinea pigs challenged with LTD$_4$. However, its efficacy in this test was not as high as expected. One explanation for this result might be the negative effect of human serum albumin (HSA) on the binding affinity for the LTD$_4$ receptor in vitro, as observed with several compounds of this class. To overcome the influence of HSA, modifications of the quinoline ring substitution were made. Guided by the literature,[19] we performed a halogen substitution at position 7 and a simultaneous replacement of the methyloxy function with a double bond, which led to compound **8**, for which virtually no loss of binding affinity was found in the presence of HSA. Moreover, compound **8** was shown to have the desired high in vivo activity (Fig. 2).

This high in vivo activity could not be obtained either by a single modification replacing the methyloxy group with a double bond, compound **6**, or by the addition of a 7-Cl in the quinoline of compound **5** as in compound **7** (Fig. 2).

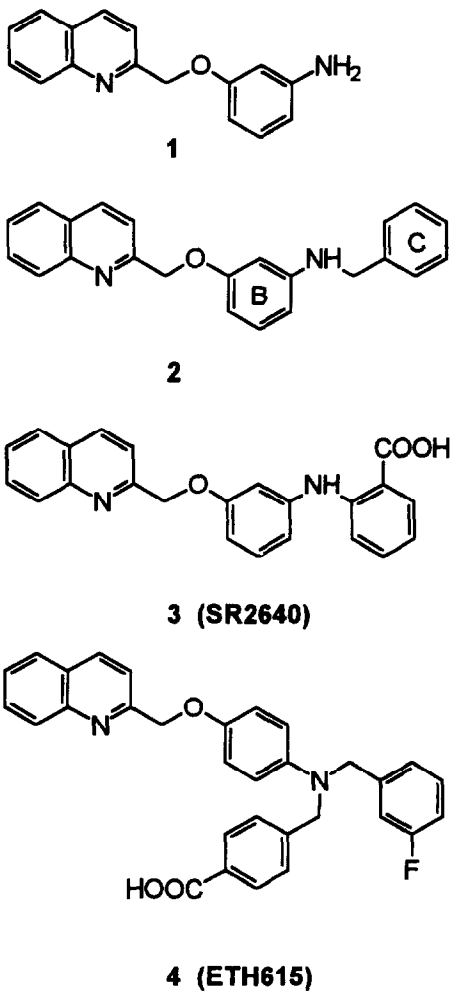
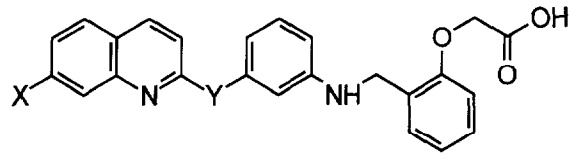


Figure 1. Lead compounds and analogues.



5-8

Figure 2.

	5	6	7	8
X	H	H	Cl	Cl
Y	CH_2O	CH=CH[c]	CH_2O	CH=CH[c]
In vitro[a] IC_{50} (nm)				
+HSA	54±7(3)	31±16(3)	24±14(3)	3.4±0.7(4)
-HSA	7.2±2.0(7)	3.2±1.0(2)	2.0±0.0(2)	2.2±0.3(3)
In vivo[b] ED_{50} (mg/kg)	11.6	5.9	>30	0.8

[a]Inhibition of the [^{3}H]LTD$_4$ specific binding to guinea pig lung membranes, in the presence or absence of 0.1% HSA.
[b]LTD$_4$-induced bronchoconstriction in guinea pig. Drugs were administered p.o. 4 h prior to LTD$_4$ challenge.
[c]*E* isomer.

In vitro SAR of alkyl analogues of compound 8

Alkyl substitution in the a position of the phenoxy acetic acid **8** led to a further improvement of the in vitro activity (Table 1). In this test, the mono ethyl substituted compound **10** was found to be the most potent, having an IC_{50} of 1.0±0.2 nM (−HSA). The in vitro binding to guinea pig lung membranes was performed in the presence or absence of 0.1% HSA.[15] For this set of compounds, **8–14** (Scheme 1), no significant difference in the binding affinity was found in the presence or absence of HSA. For the short chain homologues, **8–12**, we also found a good correlation between the in vitro and in vivo data. To begin with, compounds **9–15** were all tested as racemates, and their IC_{50} values were in the range of 0.5–2.8 nM in the presence of HSA (Table 1). Only the long chain compound **16** was found less potent (IC_{50} 10 nM), which indicates a structural preference for the short and medium chain homologues.

To investigate whether there was an enantioselective preference in the interaction between the cys-LT$_1$ receptor and these structures, the pure enantiomers of compound **12** were prepared. OT4003, the (*S*)-(+) enantiomer (ee >99%) of compound **12**, was found to be about 10 times more potent than OT4002, the (*R*)-(−) enantiomer (ee >99%). OT4003 was not only potent, but also selective for the cys-LT$_1$ receptor. Accordingly, OT4003 was not found to show any significant activity on arachidonic acid release and metabolism in rat peritoneal leukocytes[15] [arachidonic acid release IC_{20} ~4 mM, LTB$_4$ and 5-HETE biosynthesis

IC_{50} ~2 mM, thromboxane biosynthesis IC_{50} ~10 mM, prostacyclin biosynthesis IC_{50} >10 mM].

Both structural and stereochemical modifications of LTD_4 had previously been made in order to study the structural requirements for activation of the cys-LT_1 receptor. These studies demonstrated that the stereochemical arrangement played an important role in retaining a high activity.[20] In the recent discovery of the leukotriene antagonists MK-679 and MK-476, the importance of enantioselective interactions has also been investigated both in respect to activity and to toxicology.[11,13,21]

Table 1. Inhibition of the [^{3}H]LTD_4 specific binding (IC_{50} (nM)) to guinea pig lung membrane in the presence or absence of 0.1% HSA

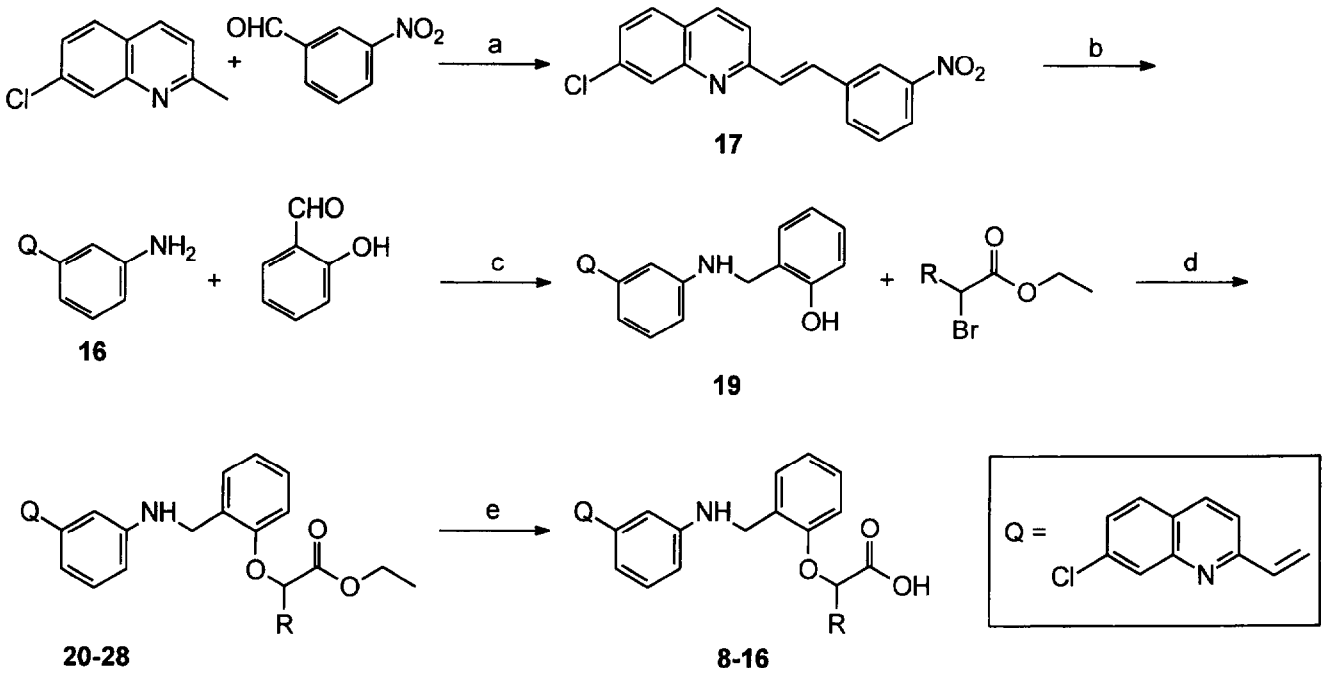
Compound	IC_{50} (nM) (mean ± SD (n))	
	+HSA	−HSA
8	3.4 ± 0.7 (4)	2.2 ± 0.3 (3)
9	1.0 ± 0.3 (3)	1.6 ± 0.4 (3)
10	0.5 ± 0.0 (3)	1.0 ± 0.2 (3)
11	1.3 ± 1.0 (3)	1.0 ± 0.2 (3)
12	2.1 ± 0.8 (5)	3.2 ± 0.8 (5)
13	1.0 ± 0.2 (3)	2.0 ± 1.2 (3)
14	1.0 ± 0.2 (3)	2.7 ± 2.1
15	2.8 ± 1.0 (3)	18 ± 19 (3)
16	10 ± 2 (3)	22 ± 12 (3)
(*S*)(+)-(**12**), OT4003	1.9 ± 1.6 (12)	2.4 ± 1.0 (12)
(*R*)(−)-(**12**), OT4002	31 ± 19 (5)	17 ± 5 (5)
ICI 204,219	3.9 ± 1.6 (5)	2.1 ± 0.8 (5)
MK-476	7.8 ± 3.1 (6)	1.7 ± 1.2 (5)

A structural comparison of OT 4003 with the MK-compounds showed that the chiral center of the compound did not directly overlap with the chiral center of MK-476. This indicates that there is more than one enantioselective interaction between the substrate and the cys-LT_1 receptor, which can be of use in the design of new cys-LT_1 receptor antagonists.

However, a structural based design of a cys-LT_1 receptor antagonist is hampered by the lack of structural information on the receptor itself, therefore already identified cys-LT_1 receptor antagonists may serve as a source of inspiration for the design of new receptor antagonists.

Toxicology; peroxisomal proliferation

In the process of structural optimization of our early lead compounds, the key substances were screened for their ability to induce peroxisome proliferation in rodents. Peroxisome proliferators induce a variety of liver enzyme systems in rodents, amongst others the peroxisomal fatty acid β-oxidation system and the cytochrome P450–4A.[22–25] Morphologically, an increase in the number and size of peroxisomes in the hepatocytes, leading to an increased size of the rodent liver, is observed. Peroxisome proliferators can also induce liver tumors after long-term administration to



Scheme 1. General synthesis of the OT4003 analogues. (a) acetic anhydride, Δ; (b) $SnCl_2$/HCl; (c) $NaBH_4$; (d) K_2CO_3/acetone; (e) LiOH.

R =	H	CH_3	C_2H_5	C_3H_7	C_4H_9	C_5H_{11}	C_6H_{13}	C_8H_{17}	$C_{10}H_{21}$
Compound	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
Compound	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**

rodents.[23,25,26] The peroxisome proliferator activated receptor (PPAR) has been suggested to be involved in both the activation of peroxisomal enzymes and in the induction of liver tumors.[27,28] However, these data are only indicative of such an involvement, and no specific structural requirements for this interaction have yet been determined. The relevance of these effects of peroxisome proliferators in rodents for the safety evaluation of the compounds in humans still remains to be established.[29]

These undesired toxic events have been noted in some of the earlier studies on leukotriene antagonists. Fortunately, specific structural and stereochemical changes have in some cases led to compounds with retained pharmacological activity, but without peroxisome proliferation activity.[21,30,31]

In the toxicological tests with our series of alkyl homologues, compounds **8**–**13**, we found an increased liver weight in mice for compound **8** and for the short chain methyl **9** and ethyl **10** homologues. Fortunately, the propyl **11** butyl **12**, and pentyl **13** homologues did not induce any significant liver weight increase in mice (Fig. 3). Moreover, OT 4003, the (*S*)-(+) enantiomer of **12**, did not induce either an increased liver weight in mice, or an increased β-oxidation in rats (Fig. 3), indicating that this compound is not a peroxisomal proliferator. Thus, seemingly minor structural changes with limited influence on the leukotriene antagonistic potency gave compounds virtually free of peroxisome proliferation activity.

In vivo

In parallel with the in vitro screening and the toxicology, the primary in vivo selection was made using a model of LTD_4-induced bronchoconstriction in guinea pigs. The ED_{50} values were determined in anesthetized guinea pigs treated orally with the drug 4 h before challenge with LTD_4. In our homologous series, an improved ED_{50} was found for the compounds with a side chain containing up to four carbons (Fig. 4). In the guinea pig, OT4003 was found to have an ED_{50} of 0.14 (ED_{16} 0.1–ED_{84} 0.4) mg/kg , as compared with the opposite enantiomer compound, (*R*)-(−)-OT4002, which had an ED_{50} of 13.3 (ED_{16} 6.5–ED_{84} 27) mg/kg . Similarly, high ED_{50} values were found for the compounds with longer side chains **13**–**15**. In this model, the ED_{50} of OT4003 is compar-

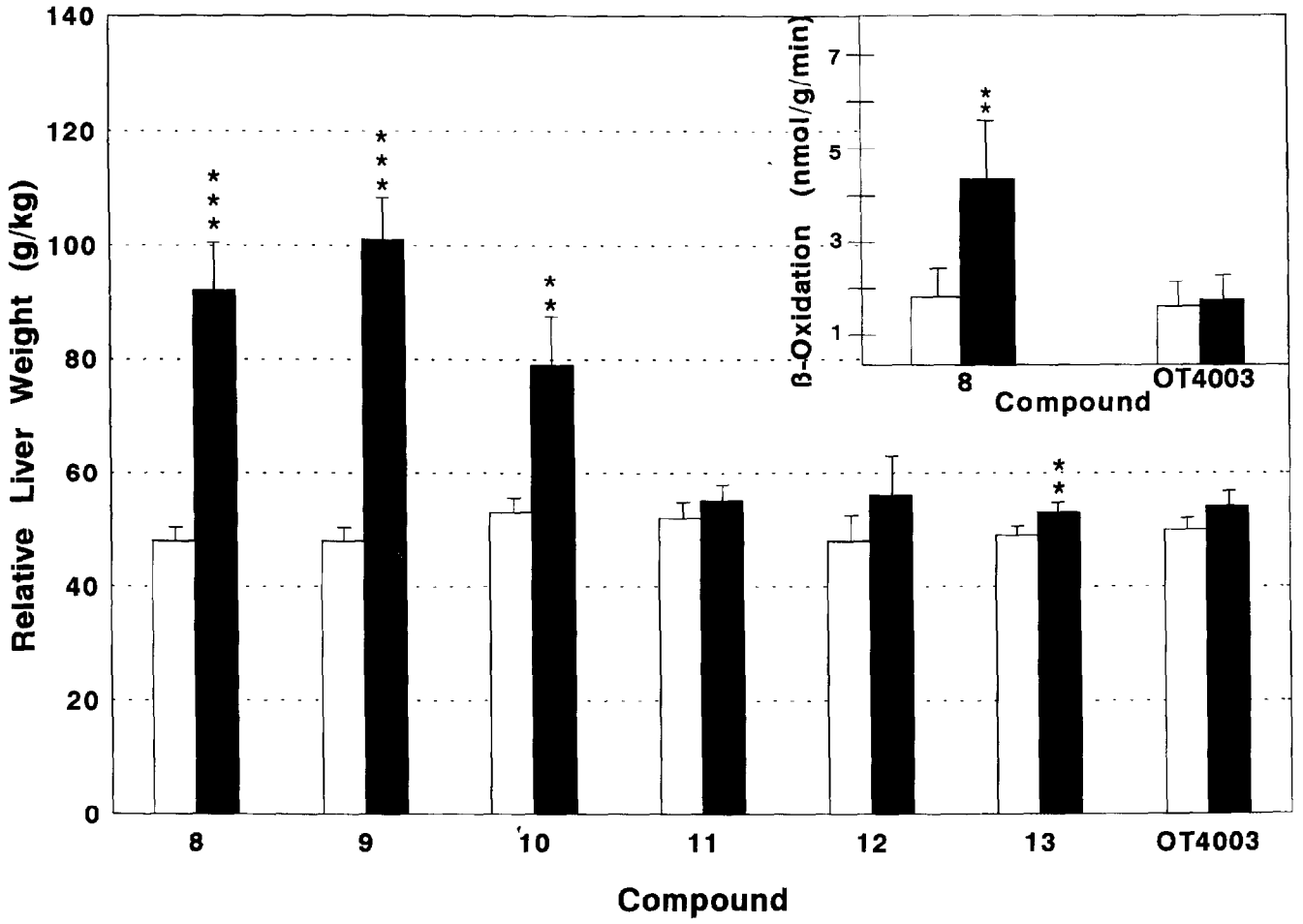


Figure 3. Peroxisome proliferation as determined by the relative liver weight in mice. The animals were treated once daily during 9 days at dose levels of 0 (vehicle control) and 50 mg/kg/day using ip administration. The inserted diagram shows the peroxisomal β-oxidation for compound **8** and OT4003 in the rat. Drugs were administered p.o. for 10 days (300 mg/kg/day).

able with the ED_{50} values of our selected reference compounds ICI 204,219 and MK-476 (Fig. 4).

In the further characterization of OT4003, the duration of the effect and the shift of the LTD_4 dose response curve in a guinea pig model were determined. The ED_{50} values were determined for the compound administered orally up to 24 h before LTD_4-challenge. In this model, OT4003 was found to potently inhibit bronchoconstriction up to 24 h, having an ED_{50} of 8.1 (ED_{16} 3.3–ED_{84} 20.3) mg/kg (Table 2). For comparison, data for the reference compounds ICI 204,219, and MK-476 were included. In an additional study, the dose response curves of OT4003 and of the reference compounds were compared with regard to the effect on bronchoconstriction after i.v. administration of LTD_4 to guinea pigs. Intravenous administration of OT4003 at the high dose level of 10 mg/kg (10 min pretreatment) produced a greater than 200-fold shift of the LTD_4 dose response curve, which was comparable with the effect found for the two reference compounds. In this in vivo model, OT4003 as well as the two reference compounds were found to be competitive inhibitors of the LTD_4 induced bronchoconstriction. Thus, the maximal response returned to control values when increased concentrations of LTD_4 vs inhibitors were used (data not shown).

Chemistry

Preparation of compounds 1, 2, 5, and 7. Compound **1** was synthesized by coupling 2-chloromethylquinoline with 3-acetaminophenol, followed by acidic hydrolysis. Benzylation of **1** via Schiff base formation and reduction using benzaldehyde or 2-formylphenoxyacetic acid gave compound **2** or **5**, respectively. Similarly, compound **7** was obtained by coupling 2-chloromethyl-7-chloroquinoline with 3-acetaminophenol, followed by hydrolysis. This intermediate was then benzylated using 2-formylphenoxyacetic acid to yield compound **7**.

Preparation of the OT4003 homologues. The general synthesis of the OT4003 analogues is outlined in Scheme 1. 7-Chloroquinaldine was coupled with the 3-nitrobenzaldehyde, followed by reduction to the corresponding amine **18**. Formation of the Schiff base with 2-hydroxybenzaldehyde, followed by reduction, gave the key intermediate **19**. Coupling of **19** with an

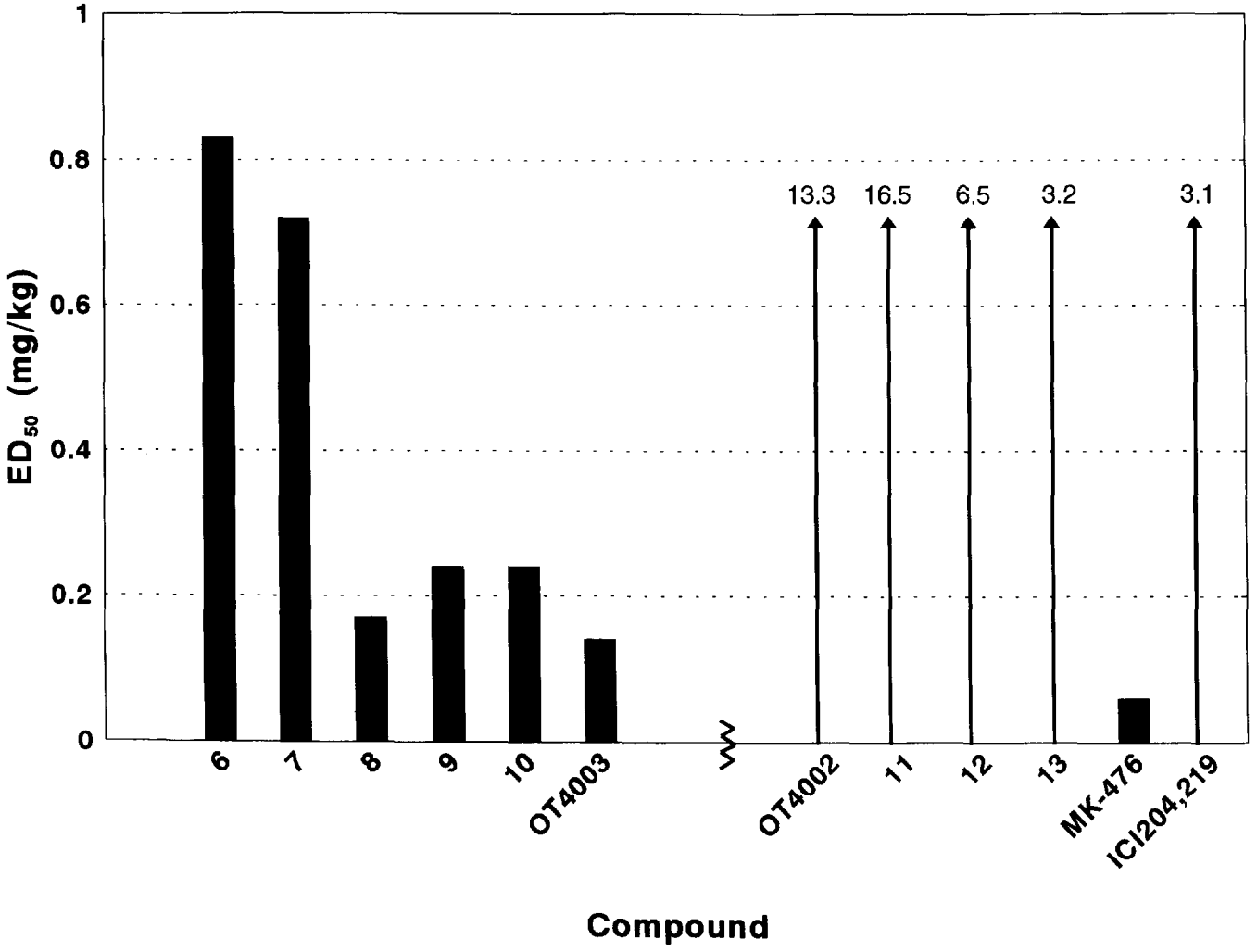


Figure 4. LTD_4-induced bronchoconstriction (ED_{50}) in guinea pigs. Drugs were administered p.o. 4 h prior to LTD_4 challenge.

appropriate bromoester gave compounds **20–28**, which after base catalyzed hydrolysis gave the target acids **8–16** in excellent overall yields.

Preparation of OT4003. To obtain the optically pure OT4003, the intermediate ester **24** was subjected to chiral resolution by enzymatic hydrolysis (Scheme 2). Lipozyme®, an immobilized lipase from the fungus *Mucor miehei*, was found to stereospecifically hydrolyse the (*R*)-(−) ethyl ester (*R*)-(−)-**24** in an excellent yield (47%), producing (*R*)-(−)-OT4002 (ee >99%). The remaining (*S*)-(+) ester was chemically hydrolysed to the target (*S*)-(+)-OT4003 (ee >99%). The optical purity of both the intermediate ester and of the final products was determined by chiral HPLC.

The absolute configuration of OT4003 was determined by alkylation of the phenolic intermediate **19** with the enantiomerically pure ethyl (*R*)-2-bromo hexanoate, obtained from the corresponding (*S*)-(L)-norleucine.[32] Thus, the optical rotation of this ester was found to be identical with that of the intermediate ester (*S*)-(+)-**24** from the OT4003 synthesis (Scheme 2). This synthesis could also serve as an alternative preparation of OT4003.

Yet another synthesis of OT4003, perhaps the most convergent and efficient, was used for the labeling of the compound. In this preparation, salicylaldehyde was alkylated with ethyl 2-bromohexanoate, followed by lipase catalysed resolution to obtain the (*S*)-(+) ester, (*S*)-(+)-**29**. After hydrolysis of (*S*)-(+)-**29**, the chiral acid (*S*)-(+)-**30** was used to form a Schiff base with the intermediate aniline **18**. This was further in situ reduced with, respectively, sodium borohydride or sodium borotritide, to obtain unlabeled or labeled (*S*)-(+)-OT4003 (Scheme 3). The labeled compound is being used for pharmacokinetic studies to be reported elsewhere.

Table 2. LTD_4-induced bronchoconstriction in guinea pigs. ED_{50} (mg/kg) was determined for drugs administered i.v. or p.o. at 10 min or 4–24 h, respectively, prior to challenge with LTD_4

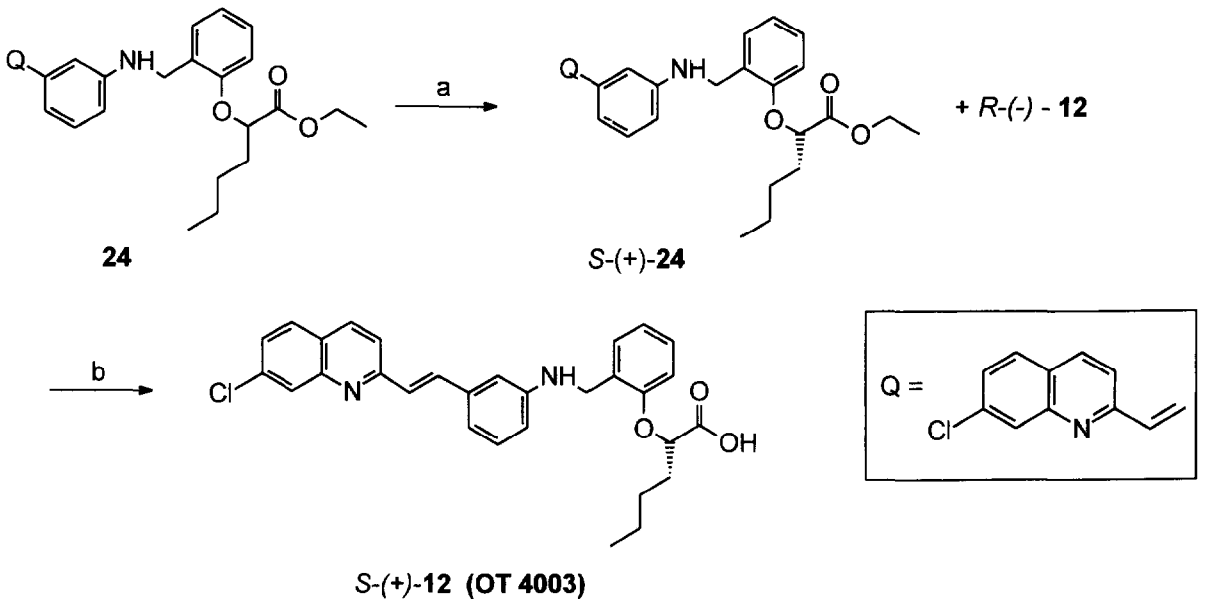
Compound ED_{50} (mg/kg)	OT4003	MK-476	ICI 204219
10 min i.v.	0.0057	0.0043	0.020
4 h p.o.	0.14	0.034	3.1
8 h p.o.	0.19	0.047	16
16 h p.o.	1.7	0.042	14
24 h p.o.	8.1	0.14	>100.0

Conclusion

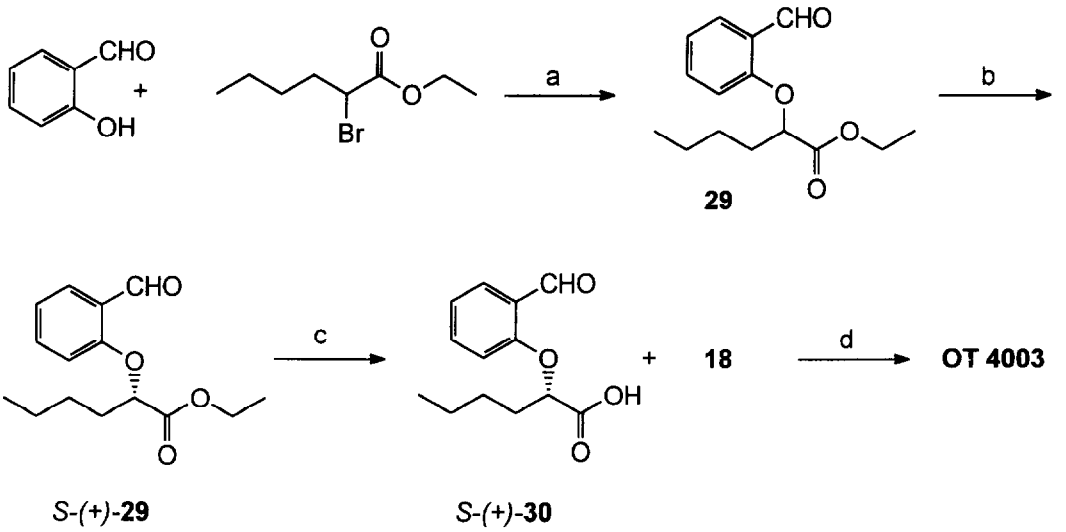
A novel series of quinoline containing cys-LT_1 receptor antagonists was optimized with respect to their in vitro and in vivo potency. Undesired side effects such as peroxisomal proliferation, seen in rodents, were removed, while retaining the potent cys-LT_1 receptor antagonistic activity.

The optimal compound, OT4003, was found to be potent both in vitro inhibiting the binding of [^{3}H]LTD_4 to guinea pig lung membranes, and in vivo inhibiting LTD_4 induced bronchoconstriction in guinea pigs. In these assays OT4003 compared well with state of the art cys-LT_1 receptor antagonists, which have shown beneficial effects in the clinic.

OT4003 was prepared in a high yield, using a convergent synthesis including a stereospecific enzyme catalysed resolution.



Scheme 2. Synthesis of (*S*)-(+)-10, OT4003. (a) Lipozyme®/sodium phosphate buffer, *t*-butyl-methylether; (b) LiOH.



Scheme 3. Synthesis of ^{3}H labeled (*S*)-(+)-10, OT4003. (a) K_2CO_3/acetone; (b) Lipozyme®/sodium phosphate buffer, *t*-butyl-methylether; (c) LiOH; (d) $NaBH_4$ (NaB^3H_4).

Experimental

In vitro: binding of [^{3}H]LTD$_4$ to guinea pig lung membranes

The cys-LT$_1$ receptor binding assays were performed in Millipore MultiScreen-GV Filtration Plates, essentially as described previously.[15] Briefly, the antagonist was incubated with [^{3}H]LTD$_4$ (0.1 nM) and membrane protein (100 µg) in a final volume of 0.2 mL for 45 min at ambient temperature. The membrane protein was freshly prepared from the lungs of young female guinea pigs using a standard dilution factor from the initial lung weight. The assays were performed in the presence or absence of 0.1% human serum albumin (HSA). [14,15,19,20-^{3}H(n)]LTD$_4$, specific activity 3.70–8.88 TBq/mmol (DuPont NEN, Belgium). LTD$_4$ (Ultrafine Chemicals, Manchester, U.K.).

In vivo: LTD$_4$-induced bronchoconstriction in guinea pigs

Guinea pigs were anesthetized by intraperitoneal administration of urethane (1.2 g/kg). One of the jugular veins was cannulated with a polyethylene catheter to allow administration of the drugs by the intravenous route. To control anesthesia, blood pressure was measured with a Statham pressure transducer (P23 ID) connected to a polyethylene catheter placed in the carotid artery. Through a third cannula inserted into the trachea, the animals were ventilated with a fixed volume of air by means of a rodent respirator (rate: 60 strokes/min) connected to a bronchoconstriction transducer (Ugo Basile, Comerio-Varese, Italy, pressure: 9 cm H_2O) measuring the air-overflow a.m. Konzett and Rössler.

LTD$_4$ was injected intravenously at a dose of 0.75 mg/kg 4 min after administration of suxamethonium (1.2 mg/kg i.v.).

Responses were quantified as the percentage of maximum bronchoconstriction, as defined by clamping of the trachea distal to the cannula. This manoeuvre was carried out at the end of each experiment.

The test compounds were administered intravenously or orally at, respectively, 10 min or 4–24 h before the challenge with LTD$_4$.

The results were reported as ED_{50} (ED_{16}–ED_{84}) values calculated by regression analysis of 2–3 doses of the antagonist.

Peroxisome proliferation

Groups of 6 female NMRI mice (BomMice, Ry, Denmark) were treated intraperitoneally with the test compound dissolved in a vehicle of hydroxypropyl-β-cyclodextrin (30% w/v) in phosphate buffer (0.1 M, pH 7). The animals were treated once daily during 9 days at dose levels of 0 (vehicle control), 12.5, 50, and 200 mg/kg/day. Clinical signs were recorded daily. At the end of the treatment period the mice were killed by exsanguination under CO_2/O_2 anesthesia. The body and liver weights were recorded, and the liver to body weight ratio was calculated for each mouse as an expression of the peroxisome proliferating activity of the compound. The group mean values were analyzed using a parametric one-way analysis of variance (ANOVA). For the comparative screening of the OT4003 analogues, the liver to body weight ratio for each compound after treatment with 50 mg/kg/day was used, since no signs of general toxicity which might have influenced the result were observed at this dose level with any of the compounds.

Assay for peroxisomal β-oxidation

The peroxisomal β-oxidation was measured in fresh liver homogenate from Sprague–Dawley rats treated

orally with OT4003 or compound **8** at a dose level of 300 mg/kg/day for 10 days. Briefly, liver samples from the treated rats were placed in a measured volume of ice-cold 0.25 M sucrose, homogenized and centrifuged at 600 *g*. The supernatant was analysed for the cyanide-insensitive reduction of NAD using palmiotyl-CoA as substrate. The enzymatic activity was measured spectroscopically by recording the appearance of NADH from the oxidation.[33]

Chemistry

^1H and ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer at 300 and 75 MHz, respectively, using TMS as internal standard. Determination of the enantiomeric purity was performed by chiral HPLC. A Daicel Chiracel OD (250×4.6 mm^2) column using heptane:ethanol, 1:1, 1 mL/min elution, and UV detection (λ 270 nm) was used for esters, and a Phenomenex Chirex (S) Val & DNAn (250×4 mm^2) column using NH_4OAc (4 mM) in MeOH, 1.5 mL/min elution, and UV detection (λ 272 nm) was used for acids. Optical rotations were measured using a Perkin–Elmer 241 polarimeter. Microanalysis was performed in the Microanalytical Department, Leo Pharmaceutical Products. The reference compounds ICI 204,219[10] and MK-476[12] were prepared according to the literature.

Preparation of compounds 1 and 2

3-(2′-Quinolylmethoxy)acetanilide·HCl·H_2O. A mixture of 3-acetaminophenol (31 g, 205 mmol), 2-chloromethylquinoline hydrochloride (43 g, 200 mmol), K_2CO_3 (85 g, 615 mmol) and dimethylformamide (400 mL) was stirred at ambient temperature for about 20 h and then stirred at 60–80 °C for a further 3–4 h. The resulting mixture was diluted with water to precipitate the product, which was filtered off, washed with water and dried in air: yield 60 g (86%); mp 160–161.5 °C; ^1H NMR (DMSO) δ 2.05 (s, 3H), 5.61 (s, 2H), 6.79 (m, 1H), 7.15–7.30 (m, 2H), 7.54 (m, 1H), 8.00 (d, 1H), 8.05 (m, 1H), 8.25 (d, 1H), 8.37 (d, 1H), 8.93 (d, 1H), 10.19 (s, 1H). Anal. calcd for $C_{18}H_{16}N_2O_2 \cdot HCl \cdot H_2O$: C, 62.33; H, 5.52; N, 8.08; found: C, 62.37; H, 5.63; N, 8.12%.

3-(2′-Quinolylmethoxy)aniline·$2H_2O$ (1). A mixture of 3-(2′-quinolylmethoxy)acetanilide, HCl, H_2O, (60 g, 173 mmol), HCl (170 mL, 4 N) and EtOH (330 mL) was refluxed for 3 h. After cooling, the resulting solution was neutralized to pH 8 with a soln of NaOH to precipitate the product, which was filtered off, washed with water and dried in air: yield 46.5 g (94%); mp 98 °C; ^1H NMR ($CDCl_3$): δ 3.67 (bs, 2H), 5.34 (s, 2H), 6.29 (m, 1H), 6.36 (t, 1H), 6.42 (m, 1H), 7.04 (t, 1H), 7.53 (m, 1H), 7.65 (d, 1H), 7.72 (m, 1H), 7.81 (d, 1H), 8.07 (d, 1H), 8.16 (d, 1H) Anal. calcd for $C_{16}H_{14}N_2O \cdot 2H_2O$: C, 67.11; H, 6.33; N, 9.79; found: C, 67.22; H, 6.43; N, 9.81%.

3-(2′-Quinolylmethoxy)-*N*-benzylaniline·HCl (2), (OT-3473). A mixture of 3-(2′-quinolylmethoxy)aniline (2.5 g, 8.7 mmol), benzaldehyde (157 g, 14.8 mmol) and MeOH (30 mL) was stirred at ambient temperature for 20 h and was then refluxed for a further hour. After cooling, $NaBH_4$ (0.8 g, 21.2 mmol) was added in portions during about 1 h, keeping the temperature of the reaction mixture between 15 and 20 °C by external cooling. The mixture was stirred at ambient temperature for a further 5 h, whereafter an excess of HCl (4 M) was added carefully, and the acidified mixture was stirred at ambient temperature for a further 16–20 h. The resulting mixture was extracted twice with EtOAc, and the combined extracts were dried and evapd. The resulting product was recrystallized (ethanol:water) to yield the title compound: yield 2 g (55%); mp 210–212 °C; ^1H NMR (DMSO): δ 4.44 (s, 2H), 5.65 (s, 2H), 6.82 (bd, 2H), 6.96 (bs, 1H), 7.27 (m, 4H), 7.45 (m, 2H), 7.86 (m, 1H), 7.97 (d, 1H), 8.06 (m, 1H), 8.27 (d, 1H), 8.41 (d, 1H), 8.95 (d, 1H), 10.5 (bs, 3H). Anal. calcd for $C_{23}H_{20}N_2O \cdot 2HCl \cdot 1.25H_2O$: C, 66.11; H, 5.43; N, 6.71; Cl, 16.97; found: C, 66.08; H, 5.50; N, 6.71; Cl, 16.72%.

Preparation of compounds 5 and 7

3-(2′-Quinolylmethoxy)-*N*-(2-carboxymethoxybenzylidene)aniline. To a soln of the aniline **1** (7.5 g, 30 mmol) in Et_2O (400 mL), 2-formylphenoxyacetic acid (5.4 g, 30 mmol) in Et_2O (500 mL) was added. The mixture was stirred at ambient temperature for 2.5 h. The precipitate that was formed was then filtered off, washed with Et_2O, and dried. This mataerial was used directly in the next step: yield 10 g (78%).

3-(2′-Quinolylmethoxy)phenylaminomethylphenoxyacetic acid (5). To a suspension of the Schiff base (3.3 g, 8 mmol) in EtOH (100 mL), sodium borohydride (1.2 g, 32 mmol) was added in portions during 1.5 h, while stirring at ambient temperature. The reaction mixture was treated with water and was acidified by vigorous stirring to pH 5–6 by the addition of acetic acid (10 mL, 3 M). The mixture was stirred at ambient temperature for 12 h, filtered off, and washed with water. The precipitate was dried and then recrystallized: yield 1.45 g (35%): mp 151–152 °C; ^1H NMR (DMSO): δ 4.26 (s, 2H), 4.76 (s, 2H), 5.25 (s, 2H), 6.23 (m, 3H), 6.88 (m, 2H), 6.93 (t, 1H), 7.17 (m, 1H), 7.22 (m, 1H), 7.61 (m, 2H), 7.78 (m, 1H), 8.00 (m, 2H), 8.38 (d, 1H). Anal. calcd for $C_{25}H_{22}N_2O_4$: C, 72.45; H, 5.35; N, 6.76; found: C, 72.36; H, 5.41; N, 6.73%.

3-[2-(7-Chloroquinoline-2-yl)methoxy]phenylaminomethylphenoxyacetic acid (7). By following the synthesis procedure described above for compound **5**, but by replacing 3-(2′-quinolylmethoxy)-*N*-(2-carboxymethoxybenzylidene)aniline with 3-(2-(7-chloroquinoline-2-yl)methoxy)-*N*-(2-carboxymethoxybenzylidene)aniline, compound **7** was obtained: mp 167–169 °C; ^1H NMR (DMSO): δ 4.16 (d, 2H), 4.29 (s, 2H), 5.27 (s, 2H), 6.17 (dd, 1H), 6.28 (d, 1H), 6.33 (m, 1H), 6.74 (t,

1H), 6.90 (m, 2H), 7.00–7.20 (m, 3H), 7.66 (m, 2H), 8.06 (m, 2H), 8.44 (d, 1H). Anal. calcd for $C_{25}H_{20}ClN_2O_4 \cdot 2H_2O$: C, 59.17; H, 4.73; N, 5.52; Cl, 7.00; found: C, 59.55; H, 4.78; N, 5.53; Cl, 7.07%.

General method for the synthesis of compounds 8–16, (Scheme 1)

***E*-2-[2-(3-(2-(7-Chloroquinolin-2-yl)ethenyl)phenylaminomethyl)phenoxy]hexanoic acid (12)**

Step 1

***E*-2-[3-(2-(7-Chloroquinolin-2-yl)ethenyl)]nitrobenzene (17)**. A soln of 7-chloroquinaldine (3.6 g, 20 mmol) and 3-nitrobenzaldehyde (3.0 g, 20 mmol) in acetic anhydride (20 mL) was stirred for 4.5 h at 130 °C. The mixture was cooled to ambient temperature and the precipitate was filtered off, washed with water, and Et_2O. The title compound obtained was used directly in the next step: yield 4.8 g (78%); mp 178–180 °C; ^{1}H NMR ($CDCl_3$): δ 7.46 (d, J=16.3 Hz, 1H), 7.48 (m, 1H), 7.58 (t, 1H), 7.62 (d, 1H), 774 (d, 1H), 7.79 (d, J=16.3 Hz, 1H), 7.92 (d, 1H), 8.08 (m, 1H), 8.14 (m, 1H), 8.17 (m, 1H), 8.49 (t, 1H). Anal. calcd for $C_{17}H_{11}ClN_2O_2$: C, 65.71; H, 3.57; N, 9.02; Cl, 11.41; found: C, 65.65; H, 3.67; N, 8.88; Cl, 11.42%.

Step 2

***E*-2-[3-(2-(7-Chloroquinolin-2-yl)ethenyl)]aniline (18)**. To a soln of $SnCl_2$ (15.0 g) in concd HCl (40 mL), the nitrobenzene **17**, (5.1 g, 16 mmol) from step 1 in acetic acid (75 mL) was added, and the mixture stirred at 80 °C for 1 h. The reaction mixture was cooled to ambient temperature and evapd to dryness. The residue was treated with H_2O (160 mL) and NaOH (aq 10 M) added to an alkaline reaction (pH 9). Extraction with ethyl acetate (2 × 150 mL), followed by drying and evapn, gave the title compound: yield 13.1 g (94%); mp 127–128 °C; ^{1}H NMR (DMSO): δ 5.19 (s, 2H), 6.60 (dd, 1H), 6.90 (m, 2H), 7.10 (t, 1H), 7.30 (d, J=16.3 Hz, 1H), 7.58 (dd, 1H), 7.72 (d, J=16.3 Hz), 1H), 7.90 (d, 1H), 7.99 (d, 1H), 8.02 (d, 1H), 8.38 (d, 1H). Anal. calcd for $C_{17}H_{13}ClN_2$: C, 72.72; H, 4.67; N, 9.98; found: C, 72.71; H, 4.81; N, 9.95%.

Step 3

***E*-2-[3-(2-(7-Chloroquinolin-2-yl)ethenyl)phenylaminomethyl]phenol (19)**. By following the synthesis procedure for compound **5**, steps 1 and 2 described above, but by replacing 2-formylphenoxyacetic acid with salicylaldehyde, *E*-3-[2-(7-chloroquinolin-2-yl)-ethenyl]-*N*-(2-hydroxybenzylidene)aniline was obtained as an intermediate, which was then reduced to the title compound: yield 90%; mp 151–152 °C; ^{1}H NMR ($CDCl_3$): δ 4.05 (bs, 1H), 4.44 (bs, 2H), 6.80 (m, 1H), 6.90 (m, 2H), 7.07 (m, 1H), 7.12–7.30 (m, 3H), 7.26 (t, 1H), 7.30 (d, J=16.3 Hz, 1H), 7.44 (dd, 1H), 7.62 (d, 1H), 7.62 (d, J=16.3 Hz, 1H), 7.71 (d, 1H), 7.71 (d, 1H), 8.07 (s, 1H). Anal. calcd for $C_{24}H_{19}ClN_2O$: C, 74.51; H, 4.95; N, 7.24; found: C, 74.40; H, 4.88; N, 7.23%.

Step 4

***E*-Ethyl-2-[2-(3-(2-(7-chloroquinolin-2-yl)ethenyl)-phenylaminomethyl)phenoxy]hexanoate (24)**. A mixture of *E*-2-[3-(2-(7-chloroquinolin-2-yl)ethenyl)-phenylaminomethyl]phenol (7.0 g, 18 mmol), ethyl 2-bromohexanoate (4.9 g, 22 mmol), K_2CO_3 (10 g, 72 mmol), and Me_2CO (350 mL) was refluxed for 40 h, whereafter volatile material was removed by evapn. The residue was dissolved in Et_2O and the hydrochloride of the title compound was precipitated by acidification with a slight excess of HCl (aq 1 N). The obtained hydrochloride was filtered and treated with $NaHCO_3$ (200 mL, 1 N) and Et_2O. The organic layer was separated, dried ($MgSO_4$) and evapd to give the title compound. For data, see Table 3.

The compounds included in Table 3 were obtained by following the synthesis procedure for compound **24**, see step 4 above, but by replacing ethyl 2-bromohexanoate with the appropriate bromoesters.

Step 5

***E*-2-[2-(3-(2-(7-Chloroquinolin-2-yl)ethenyl)phenylaminomethyl)phenoxy]hexanoic acid (12)**. A mixture of the ethyl ester **24** (6.3 g, 12 mmol), lithium hydroxide hydrate (10 g, 250 mmol), water (100 mL), MeOH (200 mL), and THF (140 mL) was stirred at ambient temperature for 4 h. After filtration, the mixture was evapd to give the crude product. Recrystallization from ethanol gave the title compound. For data, see Table 4.

The compounds in Table 4 were obtained by following the synthesis procedure for compound **12**, see step 5 above, but by replacing *E*-ethyl-2-[2-(3(2-(7-chloroquinolin-2-yl)ethenyl)-phenylaminomethyl)phenoxy]-hexanoate with the esters in Table 3.

***E*-2-[2-(3-(2-quinolyl)ethenyl)phenylaminomethyl]-phenoxyacetic acid (6)**. By following the general synthesis procedure, steps 1–5, for compounds **8–16**, but by replacing 7-chloroquinaldine with quinaldine, compound **6** was obtained: mp 108–111 °C; ^{1}H NMR (DMSO): δ 4.37 (bs, 2H), 4.83 (s, 2H), 6.61 (m, 1H), 6.93 (m, 4H), 7.11 (t, 1H), 7.20 (m, 1H), 7.31 (d, 1H, J=16.3 Hz), 7.32 (m, 1H), 7.55 (m, 1H), 7.69 (d, 1H, J=16.3 Hz), 7.74 (m, 1H), 7.87 (d, 1H), 7.93 (d, 1H), 7.99 (d, 1H), 8.33 (d, 1H). Anal. calcd for $C_{26}H_{22}N_2O_3 \cdot H_2O$: C, 72.82; H, 5.60; N, 6.54; found: C, 73.69; H, 5.60; N, 6.56%.

Synthesis of OT4003, (*S*)-(+)-*E*-2-[2-(3-(2-(7-chloroquinolin-2-yl)ethenyl)phenylaminomethyl)phenoxy]-hexanoic acid ((*S*)-(+)-12) (Scheme 2)

Step 1

(*S*)-(+)-*E*-Ethyl-2-[2-(3-(2-(7-chloroquinolin-2-yl)-ethenyl)phenylaminomethyl)phenoxy]hexanoate ((*S*)-(+)-24). A mixture of *E*-ethyl-2-[2-(3-(2-(7-chloroquinolin-2-yl)ethenyl)phenylaminomethyl)phenoxy]-hexanoate (5.3 g, 10 mmol) and Lipozyme® (immobilized *Mucor miehei* lipase) (530 mg, 6.1 Baun/g) in *t*-butyl ether (100 mL) and phosphate buffer (100 mL, pH 8) was stirred at ambient temperature for 120 h. Filtration through Celite followed by washing with EtOAc gave two phases that were separated. The organic phase was dried ($MgSO_4$) and evaporated. The product was then purified by silica gel chromatography (EtOAc:Et_2O), and crystallized from EtOH (96%): yield: 2.5 g (50%); mp 100–101 °C; $[\alpha]_D^{20}$ 30.3° (*c* 1.0, Me_2CO); ee >99% (HPLC). Anal. calcd for $C_{32}H_{33}ClN_2O_3$: C, 72.64; H, 6.29; N, 5.30; Cl, 6.70; found: C, 72.65; H, 6.23; N, 5.30; Cl, 6.71%.

Table 3.

Compound	Microanalysis calculated (%)		^{1}H NMR (δ)	Melting point (°C)	Yield (%)
	Calcd	Found			
20[a]	$C_{28}H_{25}ClN_2O_3$		—	—	—
21[a]	$C_{29}H_{27}ClN_2O_3$		—	—	—
22	$C_{30}H_{29}ClN_2O_3$ C, 71.91 H, 5.83 N, 5.59 Cl, 7.08	 C, 71.80 H, 5.86 N, 5.56 Cl, 7.15	(DMSO) δ 1.06 (t, 3H), 1.19 (t, 3H), 2.00 (m, 2H), 4.2 (m, 2H), 4.40 (m, 2H), 4.98 (t, 1H), 6.24 (t, 1H), 6.62 (d, 1H), 7.31 (m, 1H), 7.57 (dd, 1H), 7.72 (d, J=16.4 Hz, 1H), 7.88 (d, 1H), 7.99 (d, 1H), 8.00 (s, 1H), 8.37 (d, 1H)	85–87	50
23	$C_{31}H_{31}ClN_2O_3$ C, 72.29 H, 6.07 N, 5.44 Cl, 6.88	 C, 72.24 H, 6.15 N, 5.47 Cl, 6.90	(DMSO) δ 0.95 (t, 3H), 1.19 (t, 3H), 1.54 (m, 2H), 1.94 (m, 2H), 4.19 (m, 2H), 4.39 (m, 2H), 4.99 (t, 1H), 6.22 (t, 1H), 6.61 (d, 1H), 6.90 (m, 4H), 7.14 (m, 2H), 7.29 (d, J=16.4 Hz, 1H), 7.31 (d, 1H), 7.57 (dd, 1H), 7.72 (d, J=16.4 Hz, 1H), 7.88 (d, 1H), 7.98 (d, 1H), 8.00 (s, 1H), 8.36 (d, 1H)	90–92	58
24	$C_{32}H_{33}ClN_2O_3$ C, 72.64 H, 6.29 N, 5.30 Cl, 6.70	 C, 72.70 H, 6.41 N, 5.29 Cl, 6.79	($CDCl_3$) δ 0.88 (t, 3H), 1.24 (t, 3H), 1.37 (m, 2H), 1.50 (m, 2H), 1.98 (m, 2H), 4.23 (m, 2H), 4.39 (dd, 1H), 4.50 (bd, 1H), 4.58 (bs, 1H), 4.78 (t, 1H), 6.67 (dd, 1H), 6.76 (d, 1H), 6.96 (m, 3H), 7.19 (m, 2H), 7.31 (d, J=16.3 Hz, 1H), 7.35 (m, 1H), 7.41 (dd, 1H), 7.62 (d, 1H), 7.63 (d, J=16.3 Hz, 1H), 7.68 (d, 1H), 8.05 (m, 2H)	84–86	72
25[a]	$C_{33}H_{35}ClN_2O_3$			—	—
26	$C_{34}H_{37}ClN_2O_3$ C, 73.29 H, 6.70 N, 5.03 Cl, 6.36	 C, 73.47 H, 6.78 N, 5.17 Cl, 6.40	(DMSO) δ 0.78 (t, 3H), 1.19 (t, 3H), 1.21 (m, 4H), 1.31 (m, 2H), 1.50 (m, 2H), 1.94 (m, 2H), 4.19 (m, 2H), 4.37 (m, 2H), 4.98 (t, 1H), 6.22 (t, 1H), 6.61 (d, 1H), 6.90 (m, 4H), 7.15 (m, 2H), 7.29 (d, J=16.3 Hz, 1H), 7.31 (m, 1H), 7.57 (dd, 1H), 7.72 (d, J=16.3 Hz, 1H), 7.88 (d, 1H), 7.98 (d, 1H), 8.00 (s, 1H), 8.37 (d, 1H)	76–77.5	50
27	$C_{36}H_{41}ClN_2O_3$ C, 73.89 H, 7.06 N, 4.79 Cl, 6.06	 C, 73.92 H, 7.17 N, 4.77 Cl, 6.21	(DMSO) δ 0.75 (t, 3H), 1.15 (m, 8H), 1.19 (t, 3H), 1.30 (m, 2H), 1.50 (m, 2H), 1.94 (m, 2H), 4.19 (m, 2H), 4.37 (m, 2H), 4.98 (t, 1H), 6.21 (t, 1H), 6.61 (m, 1H), 6.90 (m, 4H), 7.11 (t, 1H), 7.18 (m, 1H), 7.28 (d, J=16.3 Hz, 1H), 7.32 (m, 1H), 7.57 (dd, 1H), 7.71 (d, J=16.3 Hz, 1H), 7.87 (d, 1H), 7.98 (d, 1H), 8.00 (s, 1H), 8.36 (d, 1H)	87–88	68
28	$C_{38}H_{45}ClN_2O_3$ C, 74.42 H, 7.40 N, 4.57 Cl, 5.78	 C, 74.23 H, 7.30 N, 4.59 Cl, 5.93	(DMSO) δ 0.77 (t, 3H), 1.19 (t, 3H), 1.05–1.25 (m, 12H), 1.29 (m, 2H), 1.50 (m, 2H), 1.93 (m, 2H), 4.19 (m, 2H), 4.37 (m, 2H), 4.98 (t, 1H), 6.20 (t, 1H), 6.62 (m, 1H), 6.90 (m, 4H), 7.11 (t, 1H), 7.18 (m, 1H), 7.28 (d, J=16.3 Hz, 1H), 7.32 (m, 1H), 7.57 (dd, 1H), 7.71 (d, J=16.3 Hz, 1H), 7.87 (d, 1H), 7.98 (d, 1H), 8.00 (s, 1H), 8.36 (d, 1H)	64–65	

[a]The compound was not isolated, but was used as crude material in the next step.

Step 2

(*S*)-(+)-*E*-2-[2-(3-(2-(7-Chloroquinolin-2-yl)ethenyl)phenylaminomethyl)phenoxy] hexanoic acid ((*S*)-(+)-12). To (*S*)-(+)-*E*-ethyl-2-[2-(3-(2-(7-chloroquinolin-2-yl)ethenyl)phenylaminomethyl)phenoxy]-hexanoate (10.6 g, 20 mmol) (from step 1) dissolved in MeOH (300 mL) and THF (150 mL), lithium hydroxide monohydrate (4.3 g, 102 mmol) in water (45 mL) was added, and the mixture was stirred for 4 h.

Table 4.

Compound	Microanalysis calculated (%)		^{1}H NMR (δ)	Melting point (°C)	Yield (%)
	Calcd	Found			
8	$C_{26}H_{21}ClN_2O_3$ C, 70.19 H, 4.76 N, 6.30	 C, 69.90 H, 4.79 N, 6.31	(DMSO) δ 4.37 (s, 2H), 4.83 (s, 2H), 6.30 (bs, 1H), 6.62 (m, 1H), 6.92 (m, 4H), 7.12 (t, 1H), 7.20 (m, 1H), 7.30 (d, J = 16.4 Hz, 1H), 7.32 (m, 1H), 7.57 (dd, 1H), 7.72 (d, J = 16.4 Hz, 1H), 7.90 (d, 1H), 7.99 (d, 1H), 8.03 (s, 1H), 8.37 (d, 1H)	187–189	92
9	$C_{27}H_{25}ClN_2O_3$ C, 70.65 H, 5.05 N, 6.10 Cl, 7.73	 C, 70.68 H, 5.09 N, 6.06 Cl, 7.80	(DMSO) δ 1.60 (d, 3H), 4.37 (m, 2H), 4.98 (q, 1H), 6.25 (bs. 1H), 6.62 (d, 1H), 6.90 (m, 4H), 7.12 (t, 1H), 7.19 (m, 1H), 7.30 (d, J = 16.3 Hz, 1H), 7.31 (m, 1H), 7.57 (dd, 1H), 7.72 (d, J = 16.3 Hz, 1H), 7.90 (d, 1H), 7.99 (d, 1H), 8.02 (d, 1H), 8.37 (d, 1H)	183–184	76
10	$C_{32}H_{33}ClN_2O_3$ C, 71.10 H, 5.33 N, 5.92 Cl, 7.50	 C, 71.13 H, 5.37 N, 5.97 Cl, 7.60	(DMSO) δ 1.07 (t, 3H), 1.98 (m, 2H), 4.38 (m, 2H), 4.85 (t, 1H), 6.24 (bs, 1H), 6.61 (d, 1H), 6.90 (m, 4H), 7.12 (t, 1H), 7.18 (m, 1H), 7.30 (d, J = 16.3 Hz, 1H), 7.31 (m, 1H), 7.57 (dd, 1H), 7.72 (d, J = 16.3 Hz, 1H), 7.90 (d, 1H), 7.99 (d, 1H), 8.02 (d, 1H), 8.37 (d, 1H)	201–202	78
11	$C_{29}H_{27}ClN_2O_3$ C, 70.22 H, 5.69 N, 5.65 Cl, 7.15	 C, 70.58 H, 5.70 N, 5.79 Cl, 7.28	(DMSO) δ 0.95 (t, 3H), 1.55 (m, 2H), 1.94 (m, 2H), 4.37 (m, 2H), 4.87 (t, 1H), 6.23 (bs, 1H), 6.61 (d, 1H), 6.90 (m, 4H), 7.12 (t, 1H), 7.18 (m, 1H), 7.30 (d, J = 16.3 Hz, 1H), 7.31 (m, 1H), 7.57 (dd, 1H), 7.72 (d, J = 16.3 Hz, 1H), 7.89 (d, 1H), 7.98 (d, 1H), 8.02 (d, 1H), 8.37 (d, 1H)	218–219	66
12	$C_{30}H_{29}ClN_2O_3$ C, 71.91 H, 5.83 N, 5.59	 C, 72.11 H, 5.84 N, 5.60	(DMSO) δ 0.86 (t, 3H), 1.35 (m, 2H), 1.50 (m, 2H), 1.95 (m, 2H), 4.37 (s, 2H), 4.86 (t, 1H), 6.22 (bs, 1H), 6.61 (d, 1H), 6.90 (m, 4H), 7.07–7.23 (m, 2H), 7.30 (d, J = 16.3 Hz, 1H), 7.31 (m, 1H), 7.58 (dd, 1H), 7.72 (d, J = 16.3 Hz, 1H), 7.89 (d, 1H), 7.99 (d, 1H), 8.02 (m, 1H), 8.37 (d, 1H)	181–182	56
13	$C_{31}H_{31}ClN_2O_3$ C, 72.29 H, 6.07 N, 5.44 Cl, 6.88	 C, 72.23 H, 6.16 N, 5.39 Cl, 6.94	(DMSO) δ 0.82 (t, 3H), 1.28 (m, 4H), 1.53 (m, 2H), 1.94 (m, 2H), 4.37 (s, 2H), 4.87 (t, 1H), 6.21 (bs, 1H), 6.61 (m, 1H), 6.90 (m, 4H), 7.12 (t, 1H), 7.19 (m, 1H), 7.30 (d, J = 16.3 Hz, 1H), 7.31 (m, 1H), 7.57 (dd, 1H), 7.72 (d, J = 16.3 Hz, 1H), 7.89 (d, 1H), 7.99 (d, 1H), 8.02 (d, 1H), 8.37 (d, 1H)	196–197	80
14	$C_{32}H_{33}ClN_2O_3$ C, 72.64 H, 6.29 N, 5.30 Cl, 6.70	 C, 72.28 H, 6.46 N, 5.73 Cl, 6.75	(DMSO) δ 0.78 (t, 3H), 1.21 (m, 4H), 1.31 (m, 2H), 1.51 (m, 2H), 1.94 (m, 2H), 4.36 (s, 2H), 4.86 (t, 1H), 6.20 (bs, 1H), 6.61 (m, 1H), 6.90 (m, 4H), 7.12 (t, 1H), 7.19 (m, 1H), 7.30 (d, J = 16.4 Hz, 1H), 7.31 (m, 1H), 7.57 (dd, 1H), 7.72 (d, J = 16.4 Hz, 1H), 7.89 (d, 1H), 7.99 (d, 1H), 8.02 (d, 1H), 8.37 (d, 1H)	188–189	85
15	$C_{34}H_{37}ClN_2O_3$ C, 73.29 H, 6.70 N, 5.03 Cl, 6.36	 C, 73.38 H, 6.72 N, 5.04 Cl, 6.49	(DMSO) δ 0.75 (t, 3H), 1.15 (m, 8H), 1.28 (m, 2H), 1.49 (m, 2H), 1.93 (m, 2H), 4.35 (m, 2H), 4.86 (t, 1H),, 6.20 (bs, 1H), 6.61 (m, 1H), 6.89 (m, 4H), 7.11 (t, 1H), 7.18 (m, 1H), 7.29 (d, J = 16.3 Hz, 1H), 7.31 (m, 1H), 7.57 (dd, 1H), 7.71 (d, J = 16.3 Hz, 1H), 7.88 (d, 1H), 7.99 (d, 1H), 8.01 (d, 1H), 8.37 (d, 1H)	130–132	48
16	$C_{36}H_{41}ClN_2O_3$ C, 73.89 H, 7.06 N, 4.79 Cl, 6.06	 C, 73.75 H, 7.03 N, 4.80 Cl, 6.20	(DMSO) δ 0.77 (t, 3H), 1.13 (m, 12H), 1.29 (m, 2H), 1.51 (m, 2H), 1.93 (m, 2H), 4.36 (m, 2H), 4.85 (t, 1H), 6.20 (bs, 1H), 6.62 (m, 1H), 6.89 (m, 4H), 7.11 (t, 1H), 7.19 (m, 1H), 7.29 (d, J = 16.3 Hz, 1H), 7.32 (m, 1H), 7.57 (dd, 1H), 7.71 (d, J = 16.3 Hz, 1H), 7.88 (d, 1H), 7.99 (d, 1H), 8.01 (d, 1H), 8.37 (d, 1H)	115–117	54

The mixture was then evapd and redissolved in H_2O:MeOH (3:1), followed by acidification with acetic acid (50% aq) to pH 3–4. The precipitate was filtered off, dried, and recrystallized from EtOH (abs) yielding a crude product (10.3 g) containing 0.9 equiv EtOH: mp 150 °C (dec); $[\alpha]_D^{20}$ 23.4° (*c* 1.0, DMSO). Further careful recrystallization from acetonitrile gave the pure product in a yield of 95%: mp 187–188 °C, $[\alpha]_D^{20}$ 24.5° (*c* 1.1, DMSO); ee >99% (HPLC). Anal. calcd for $C_{30}H_{29}ClN_2O_3$: C, 71.91; H, 5.83; N, 5.59; Cl, 7.08; found: C, 72.16; H, 5.90; N, 5.64; Cl, 7.08%.

Alternative synthesis of OT4003 ((*S*)-(+)-12), (Scheme 3)

Ethyl 2-(2-formylphenoxy)hexanoate (29). A mixture of 2-formylphenol (6.1 g, 50 mmol), ethyl 2-bromohexanoate (11.2 g, 50 mmol), and K_2CO_3 (21 g) in DMF (100 mL) was stirred for 20 h, poured into water and extracted with Et_2O, and then washed with NaCl (satd aq) and water. The organic phase was dried ($MgSO_4$) and evaporated: yield 12.7 g (96%); ^{1}H NMR ($CDCl_3$): δ 0.94 (t, 3H), 1.24 (t, 3H), 1.42 (m, 2H), 1.54 (m, 2H), 2.03 (m, 2H), 4.21 (q, 2H), 4.75 (m, 1H), 6.82 (d, 1H), 7.06 (m, 1H), 7.50 (m, 1H), 7.86 (dd, 1H), 10.60 (s, 1H). Anal. calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.62; found: C, 68.03; H, 7.64%.

***S*-(+)-Ethyl 2-(2-formylphenoxy)hexanoate (*S*-(+)-29).** To ethyl 2-(2-formylphenoxy)hexanoate (**28**) (13.2 g, 50 mmol) in *t*-butyl-methyl ether (300 mL), and phosphate buffer (300 mL, pH 8), Lipozyme® (4 g) was added. The mixture was stirred for 96 h at ambient temperature and then filtered through Celite, taken up in EtOAc, washed with $NaHCO_3$, and water. The organic phase was dried ($MgSO_4$) and evapd: yield 6.2 g (47%); ^{1}H NMR (DMSO): δ 0.90 (t, 3H), 1.16 (t, 3H), 1.37 (m, 2H), 1.48 (m, 2H), 1.97 (m, 2H), 4.17 (m, 2H), 5.10 (t, 1H), 7.10 (d. 1H), 7.12 (t, 1H), 7.62 (m, 1H), 7.72 (dd, 1H), 10.48 (s, 1H). Anal. calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.62; found: C, 67.30; H, 7.53%.

***S*-(+)-2-(2-Formylphenoxy)hexanoic acid (*S*-(+)-30).** To *S*-(+)-ethyl 2-(2-formylphenoxy)hexanoate (*S*-(+)-**29**) (2.7 g, 10 mmol) in MeOH:THF (60 mL, 2:1) LiOH (20 mL, 10% aq) was added, and the mixture stirred for 3 h, then evapd to one-third, and taken up in water and EtOAc. The mixture was acidified (HCl, 4 M) and extracted with EtOAc, and was then washed with NaCl (aq) and water. The organic phase was dried ($MgSO_4$) and evapd: yield 2.4 g (100%); mp 84–87 °C; $[\alpha]_D^{20}$ 26.5° (*c* 1.0, DMSO); ^{1}H NMR (DMSO): δ 0.90 (t, 3H), 1.37 (m, 2H), 1.48 (m, 2H), 1.96 (m, 2H), 4.97 (t, 1H), 7.08 (d, 1H), 7.10 (t, 1H), 7.62 (m, 1H), 7.71 (dd, 1H), 10.47 (s, 1H). Anal. calcd for $C_{13}H_{16}O_4$: C, 66.08, H, 6.83; found: C, 66.06; H, 6.92%.

OT4003 ((*S*)-(+)-12). Using the synthesis procedure for compound **5**, steps 1 and 2 described above, but by replacing 2-formylphenoxyacetic acid with *S*-(+)-2-(2-formylphenoxy)hexanoic acid (*S*-(+)-**29**), and then reducing the intermediate Schiff base, the title compound was obtained in a yield of 42%.

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