5-Oxo-ETE Receptor Antagonists

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Abstract

5-Oxo-ETE is the most powerful eosinophil chemoattractant among lipid mediators. Eosinophil infiltration into the lungs of asthmatics may be responsible for the late phase of inflammatory asthma. We have designed and synthesized a 5-oxo-ETE receptor antagonist, the purpose of which is to prevent eosinophil migration to the lung during an asthma attack and thereby reduce asthma symptoms.

Keywords

5-oxo-ETE; HETE; eosinophil; neutrophil; antagonist; asthma; SAR; intracellular calcium; oxovaleryl indole; design; synthesis

INTRODUCTION

5-Oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-oxo-ETE **5**) is a metabolite of arachidonic acid (AA) that is formed as a result of the oxidation of the 5-lipoxygenase product 5*S*-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-HETE **3**) by 5-hydroxyeicosanoid dehydrogenase (5-HEDH). We have performed the synthesis of 5-oxo-ETE[1] and a tetra deutero derivative[2] and developed a mass spectrometric assay for its quantitation.[3] This enzyme oxidizes eicosanoids containing a 5*S*-hydroxyl group followed by a 6,7-*trans* double bond and is highly selective for 5-HETE (Figure 1).[4] It is present in neutrophils, monocytes, lymphocytes, eosinophils, platelets, and various structural cells.[5,6] 5-Oxo-ETE **5** acts through a specific G_i-coupled receptor[7–10] that is expressed by eosinophils > neutrophils > monocytes[10] as well as by certain tumor cell lines[11] and has been designated as the OXE receptor (**OXE-R**).[12] Its biological activity is limited by its metabolism to biologically inactive products, including 5-oxo-12-HETE and 5-oxo-20-HETE (Figure 1).

Although 5-oxo-ETE **5** is a chemoattractant for both neutrophils[13] and monocytes,[14] it is less potent than LTB$_4$. In contrast, among lipid mediators, it is the strongest chemoattractant known for human eosinophils[15] and also induces a variety of other responses in these cells,[16–18] some of which are markedly enhanced by the proinflammatory cytokines GM-CSF and TNF .[19] It is active *in vivo*, eliciting infiltration of eosinophils and, to a lesser extent, neutrophils, into human skin.[20] **OXE-R** is highly selective for 5-oxo-ETE **5** over a variety of its metabolites, leukotrienes (LTs) and other eicosanoids.[21]

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Because of the above effects, 5-oxo-ETE **5** may be an important regulator of the tissue infiltration and activation of eosinophils and neutrophils in diseases such as asthma, allergic rhinitis, arthritis, and psoriasis. For this reason we set out to design a selective OXE receptor antagonist and report here the progress made.

RESULTS AND DISCUSSION

In a previous study we identified 5-oxo-12*S*-HETE **6** (Figure 1) as a major metabolite of 5-HETE by human platelets.[22] We have performed the total synthesis of **6**,[23] and we found that this compound had virtually no effect on intracellular Ca^{++} levels in neutrophils, but blocked 5-oxo-ETE-induced Ca^{++} mobilization in the low μmolar range, suggesting that it has antagonist properties.[22] Consistent with this, 5-oxo-12*S*-HETE inhibited 5-oxo-ETE-induced neutrophil migration. However, this compound was not a suitable candidate for drug development because of its instability and susceptibility to metabolism.

It has previously been shown that the conjugated triene systems of LTB_4[24] and LTD_4[25] are essentially flat in solution. With that in mind, we decided to synthesize a series of conformationally restricted analogs in which the carboxyl end of 5-oxo-ETE, containing the 5-oxo group, was coupled to a flat aromatic system such as benzene, naphthalene, quinoline, benzofuran, or indole. Figure 2 represents a small sample of the 45 such compounds we prepared.

To screen for antagonist activity we used a calcium mobilization assay[21] in which human neutrophils, loaded with the calcium-sensitive fluorescent dye indo-1, were treated first with the potential antagonist and then two min later with 5-oxo-ETE. A major advantage of this assay is that it enables real-time testing simultaneously for both agonist and antagonist activity, in contrast to a binding assay, for example, which measures only receptor affinity. Using this assay, we showed that none of these monosubstituted aromatic compounds had any agonist activity. However, they had little or no effect on 5-oxo-ETE-induced calcium mobilization and therefore showed little promise as **OXE-R** antagonists. A typical example is shown in Figure 3. A high concentration (30 μM) of compound **10** neither mobilized intracellular calcium when added to neutrophils, nor altered the response to 5-oxo-ETE (10 nM), which was added 2 min later. Similar results were obtained with the other three compounds shown in Figure 2.

At this juncture, we embarked on a detailed structure-activity study to identify the major features of 5-oxo-ETE required for interaction with and activation of its receptor.[21] The resulting data highlighted the importance of a free carboxyl group and a 5-oxo group conjugated with a *trans-cis* diene system (Figure 4A). In addition, and of particular relevance to the present study, was the requirement for the terminal hydrophobic portion of 5-oxo-ETE, as analogs with carbon chain lengths of less than 18 showed very little activity.

Because of the importance of the terminal hydrophobic region of 5-oxo-ETE, we decided to investigate the antagonist effects of a series of aromatic derivatives containing two substituents: one mimicking the 5-oxo-valerate portion of 5-oxo-ETE (i.e. C_1-C_5) and the other mimicking the -end of the molecule in the form of a hexyl group. These were joined together by an aromatic group that provides the required unsaturated portion of the molecule (Figure 4B). A variety of conformations are available for 5-oxo-ETE, one of which will be preferentially bound by the OXE receptor. These different conformations can be mimicked by placing the two side chains in different positions on the aromatic scaffold.

We synthesized a series of 1-(5-oxovalerate)indoles containing a hexyl group at C-2 **26**, C-3 **16**, C-5 **17**, C-6 **18**, or C-7 **19**, and examined their effects on 5-oxo-ETE-induced calcium

mobilization in human neutrophils. None of these compounds affected intracellular calcium levels in the absence of 5-oxo-ETE. Compounds **16–19** had only modest inhibitory effects on the response to 5-oxo-ETE, and all had IC_{50} values above 10 μM. In contrast, the 2-hexylindole **26**, the only compound in which the two side chains are *ortho* to one another, strongly inhibited 5-oxo-ETE-induced calcium mobilization with an IC_{50} value of 1.55 μM. To ensure that the effect of **26** was selective for the OXE receptor, we determined whether it could inhibit the response to the closely related 5-lipoxygenase product leukotriene LTB_4. As shown in Figure 5A, **26** (10 μM) had virtually no effect on the response of neutrophils to LTB_4 while completely inhibiting their response to 5-oxo-ETE (Figure 5B). A lower concentration (1 μM) of **26** also substantially inhibited the effect of 5-oxo-ETE. The syntheses of the above molecules are shown in Schemes 1 and 2; Table 1 summarizes their IC_{50} values.

We next investigated the effects of adding a chloro substituent to the benzene ring (Figure 6). The 5-chloro derivative **33** inhibited 5-oxo-ETE-induced calcium mobilization with a potency (IC_{50}, 1.2 μM) similar to the non-chlorinated compound **26**. In contrast, the addition of chlorine in the C-6 position of compound **39** (Scheme 4), increased potency by about 4-fold (IC_{50}, 400 nM; Figure 5C). The syntheses of the two chloro analogs are shown in Schemes 3 and 4.

For the synthesis of indole-based antagonists, we started from the indole carboxaldehydes **11**. Carboxaldehydes in positions 2, 6, 7 were prepared from their corresponding carboxylic acids, as illustrated for compound **20** (Scheme 2). Carboxaldehydes at C-3 and C-5 were obtained commercially. Reduction of **20** with $LiAlH_4$ gave the alcohol which was further oxidized to aldehyde **11** by MnO_2. The reaction crude was filtered through celite; however, some MnO_2 filtered through. A better alternative was centrifugation followed by decanting. Next, a Wittig reaction was carried out using alkyl phosphonium salt to give the olefin which was hydrogenated to afford the alkyl indole. Glutaric anhydride was used under KOH/DMSO conditions to substitute the proton on the nitrogen of the indole ring. Schemes 1 and 2 show the synthetic approach. The synthesis of the 5-chloro analog **33** was achieved similarly as illustrated in Scheme 3.

For the synthesis of 5-(6-Chloro-2-hexyl-1*H*-indol-1-yl)-5-oxo-valeric acid **39** (Scheme 4), a known literature procedure was used to obtain ethyl ester **37**.[26] From this point onwards we used the same strategy described for the synthesis of **33** (Scheme 3). It is noteworthy that the hydrogenation of **31** and **38** with Pd/C in some instances led to a partial hydrogenolysis of the chlorine substituent.

The progression of the SAR leading to compound **39** is summarized in Figure 7, with the corresponding IC_{50} values. Moreover, **39** is numbered so as to show its structural similarities to 5-oxo-ETE.

CONCLUSION

We have described herein the design and synthesis of series of potent and selective 5-oxo-ETE antagonists with the lead compound having low micromolar activity. The initial monosubstituted aromatic oxovalerate compounds showed little to no activity. The addition of the hexyl chain proved crucial for antagonist properties to 5-oxo-ETE receptors. Moreover, optimal activity was observed with the hexyl at the C-2 position. Finally, submicromolar activity was obtained by the addition of a chlorine at C-6 of the indole ring. Figure 7 shows the progression of the structure-activity from the inactive **10** to the lead compound **39**.

EXPERIMENTAL SECTION

Measurement of intracellular calcium levels

Neutrophils suspended in phosphate-buffered saline were prepared from whole blood obtained from healthy human subjects and loaded with the calcium-sensitive dye indo-1 as previously described.[21] Five min before commencement of the experiment, the cells were transferred to a cuvette and $CaCl_2$ (1.8 mM) and $MgCl_2$ (1 mM) were added. Intracellular calcium was then measured at 37 °C in a spectrofluorimeter.[21] After a stable baseline was obtained either vehicle or a synthetic indole was added, followed 2 min later by 5-oxo-ETE (10 nM). One min later digitonin was added to determine the maximal response.

Reagents and Methods

Unless stated otherwise, all reagents and chemicals were obtained from commercial sources and were used without further purification. All reactions were carried out with dry, freshly distilled solvents under anhydrous conditions. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. HRMS of positive ions were obtained on an AccuTOF mass spectrometer with a DART ion source. ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER AMX 360 MHz and BRUKER AMX 400 MHz spectrometers using $CDCl_3$ as solvent at 25 °C. The chemical shifts ( ) for ^{1}H NMR are expressed in ppm, followed by the multiplicity (br, broad; s, singlet; d, doublet; t, triplet; q, quadruplet; qt, quintet and m, multiplet), coupling constants (J in Hertz, Hz), and integration. All compounds were analyzed by TLC, NMR, and HRMS. All final compounds were further quantified and purified to be > 95% pure by HPLC prior to each biological assay. HPLC conditions: Waters 2695 Alliance System with Waters Novapak C18 (150 × 3.9 mm) column and photodiode array detector (Waters Model 2996), gradient mobile phase between H_2O/MeCN/MeOH (56:22:22) to H_2O/MeCN/MeOH (16:42:42) over 30 min, both solvents contained 0.02% acetic acid, and a flow rate of 1 mL/min.

Synthesis of monosubstituted aromatic compounds 7–10

Compounds **7** and **8** were synthesized as follows for **7**:

(E)-7-(naphthalen-1-yl)-5-oxohept-6-enoic acid (7)—To a solution of methyl 5-chloro-5-oxopentanoate, **40** (3.00 g, 18.2 mmol) in dry Et_2O was added 105 mL of freshly prepared CH_2N_2 at 0 °C over 30 min. The reaction was quenched with water; the organic layer dried with Na_2SO_4, and then bubbled with dry HCl. Next, it was washed with brine and the organic layer dried with Na_2SO_4. The crude **41** was further added to excess NaI in dry MeCN and stirred overnight. The solvent was evaporated, the crude re-suspended in CH_2Cl_2, washed with water and dried with Na_2SO_4. To this crude iodoketone (3.46 g, 12.8 mmol) in MeCN was added trimethoxyphosphine (3.23 g; 1.5 equiv). The mixture was stirred at rt for 12 h, the solvent was evaporated, and the crude was washed with brine. The organic layer was separated and dried to afford **methyl 6-(dimethoxyphosphoryl)-5-oxohexanoate, 42** (3.02 g, 93.5%). ^{1}H NMR ($CDCl_3$): 3.79 (s, 3H), 3.76 (s, 3H), 3.66 (s, 3H), 3.11 (s, 1H), 3.05 (s, 1H), 2.69 (t, $J = 7.0$ Hz, 2H), 2.34 (t, $J = 7.2$ Hz, 2H), 1.90 (qt, $J = 7.1$ Hz, 2H). To a stirred suspension of this intermediate (242 mg, 0.960 mmol) in anhydrous THF (1 mL) was added NaHMDS (1.0M in THF, 0.800 mL, 0.800 mmol) at rt under argon. The mixture was stirred for 15 min, cooled to −78°C, and a solution of 1-naphthaldehyde, **43** (50 mg, 0.320 mmol) in 1 mL dry THF was added. The reaction mixture was stirred at −78°C for 1 h, warmed to 25°C, stirred for 3 h, quenched with saturated NH_4Cl solution, and extracted with EtOAc. The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the crude residue was purified by silica gel chromatography using EtOAc/Hex as eluent to afford **(E)-methyl 7-(naphthalen-1-yl)-5-oxohept-6-enoate** (83.8 mg, 92.8%). ^{1}H NMR ($CDCl_3$): 8.44 (d, $J =$

15.8 Hz, 1H), 8.22 (d, $J = 7.4$ Hz, 1H), 7.94 (t, $J = 7.6$ Hz, 2H), 7.80 (d, $J = 6.6$ Hz, 1H), 7.48–7.67 (m, 3H), 6.85 (d, $J = 15.9$ Hz, 1H), 3.71 (s, 3H), 2.84 (t, $J = 7.0$ Hz, 2H), 2.48 (t, $J = 7.0$ Hz, 2H), 2.05–2.14 (m, 2H). To 10 mg of this ester was added a solution of 5% KOH in dioxane. Stirring was allowed under N_2 for 2 h, then the reaction was quenched with dilute HCl, extracted with EtOAc, and dried with Na_2SO_4. The solvent was evaporated to afford **(E)-7-(naphthalen-1-yl)-5-oxohept-6-enoic acid, 7** (9.4 mg, 99%). ^{1}H NMR (CDCl$_3$): 8.45 (d, $J = 15.8$ Hz, 1H), 8.22 (d, $J = 8.1$ Hz, 1H), 7.90 (t, $J = 8.5$ Hz, 2H), 7.80 (d, $J = 7.1$ Hz, 1H), 7.44–7.65 (m, 3H), 6.85 (d, $J = 15.9$ Hz, 1H), 2.87 (t, $J = 6.9$ Hz, 2H), 2.55 (t, $J = 7.0$ Hz, 2H), 2.19 (t, $J = 7.0$ Hz, 2H).

(E)-5-oxo-7-phenylhept-6-enoic acid (8)—^{1}H NMR (CDCl$_3$): 7.52–7.66 (m, 3H), 7.40 (br s, 3H), 6.73 (d, $J = 16.0$ Hz, 1H), 2.82 (t, $J = 6.9$ Hz, 2H), 2.50 (t, $J = 7.0$ Hz, 2H), 1.95–2.15 (m, 2H).

4-(benzofuran-2-yl)butanoic acid (9)—To a stirred suspension of 2-((triphenylphosphonium bromide)methyl)phenol, **45** (895 mg, 1.99 mmol) and Et$_3$N (605 mg, 3 equiv) in toluene (8 mL) was added glutaric anhydride, **46** (250 mg, 2.19 mmol). The mixture was refluxed for 8 h, then quenched with saturated NH$_4$Cl, extracted with EtOAc, dried and purified by silica gel chromatography to afford **9** (197 mg, 48%). ^{1}H NMR (CDCl$_3$): 7.52 (d, $J = 7.4$ Hz, 1H), 7.43 (d, $J = 7.4$ Hz, 1H), 7.18–7.28 (m, 2H), 6.42 (s, 1H), 2.82 (t, $J = 6.9$ Hz, 2H), 2.40 (t, $J = 7.0$ Hz, 2H), 1.77–1.88 (m, 2H).

5-(1H-indol-1-yl)-5-oxopentanoic acid (10)—To a stirred suspension of indole, **47** (100 mg, 0.854 mmol) in anhydrous THF (4 mL) was added EtMgBr (3M, 0.600 mL) at rt under argon. Glutaric anhydride, **46** (194 mg, 1.70 mmol) was added and stirred for 3 h. The reaction was quenched with saturated NH$_4$Cl, extracted with EtOAc and dried to afford **10** (172 mg, 87.6%). ^{1}H NMR (CDCl$_3$): 8.46 (d, J = 8.2 Hz, 1H), 7.57 (d, J = 7.7 Hz, 1H), 7.47 (d, J = 3.5 Hz, 1H), 7.36 (t, J = 7.7 Hz, 1H), 7.31 – 7.26 (m, 1H), 6.65 (d, J = 3.8 Hz, 1H), 3.04 (td, J = 7.0, 1.6 Hz, 2H), 2.60 (t, J = 7.0 Hz, 2H), 2.18 (qt, J = 7.1 Hz, 2H). ^{13}C NMR (CDCl$_3$): 178.62 (s), 170.60 (s), 135.61 (s), 130.34 (s), 125.21 (s), 124.46 (s), 123.74 (s), 120.88 (s), 116.59 (s), 109.39 (s), 34.56 (s), 32.70 (s), 19.41 (s).

Synthesis of indole carboxaldehydes

1H-indole carboxaldehydes (at C-2, C-6 and C-7), **11**, and the 5-chloro-analog were synthesized from their commercially available carboxylic acid precursors (**20** and **30**) as described below for 1H-indole-2-carboxaldehyde (**11**).

1H-Indole-2-carboxaldehyde (11)—To a stirred solution of 1H-indole-2-carboxylic acid (**20**) (2.0 g, 12 mmol) in THF (20 mL) at 0 °C was added LiAlH$_4$ (1M in THF, 24.8 mmol, 25 mL) under argon. The reaction mixture was stirred at 0 °C for 30 min, then was gradually warmed to 25 °C over a period of 3 h. The reaction mixture was quenched with aqueous NH$_4$Cl solution and then was extracted with EtOAc. The organic layers were combined and dried over Na$_2$SO$_4$. The solvent was evaporated under reduced pressure and the crude mixture was dried under vacuum pump. To this crude mixture in MeCN (50 mL) was added MnO$_2$ (5.6 g, 65 mmol) and the reaction mixture was stirred overnight. Next day, the reaction mixture was diluted with ether and filtered through celite and florisil layers. The solvent was evaporated under reduced pressure and the crude residue purified by silica gel chromatography using (3:7) EtOAc/Hex solvent as eluent to afford **11** as pale brown solid (1.53 g, 81.0%). HRMS (ESI) m/z calcd for [C$_9$H$_7$NO+H]$^+$: 146.0606, found 146.0613. ^{1}H NMR (CDCl$_3$): 9.85 (s, 1H), 9.01 (s, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.40 (t, J = 7.5 Hz, 1H), 7.28 (s, 1H), 7.19 (t, J = 7.4 Hz, 1H). ^{13}C NMR (CDCl$_3$):

182.00 (s), 137.87 (s), 135.95 (s), 127.36 (s), 123.48 (s), 121.30 (s), 114.71 (s), 112.37 (s), 102.68 (s).

Synthesis of alk-1-en-1-yl-1*H*-indoles

Compounds **13, 22** ([alk-1-en-1-yl] derivatives at C-2), **31** (5-Cl analog) and **38** (6-Cl analog) were synthesized as shown for **22**:

2-Hex-1-enyl-1*H*-indole (22)—To a stirred suspension of phosphonium salt **21** (973 mg, 2.36 mmol) in anhydrous THF (4 mL) was added LiHMDS (1.0M in THF, 2.12 mL, 2.12 mmol) at −40°C under argon. The mixture was stirred for 20 min, cooled to −78°C, and a solution of aldehyde **11** (114 mg, 0.785 mmol) in 0.5 mL dry THF was added. The reaction mixture was stirred at −78°C for 1 h, warmed to 25°C, stirred for 3 h, quenched with saturated NH$_4$Cl solution, and extracted with EtOAc. The organic layers were combined and dried over Na$_2$SO$_4$. The solvent was evaporated under reduced pressure and the crude residue was purified by silica gel chromatography using (15:85) EtOAc/Hex as eluent to afford **22**, as pale yellow solid (142 mg, 85.0%). HRMS (ESI) *m/z* calcd for [C$_{14}$H$_{17}$N+H]$^+$: 200.1439, found 200.1443. ^{1}H NMR (CDCl$_3$): 7.98 (s, 1H), 7.45–7.47 (d, 1H), 7.21–7.23 (d, 1H), 7.06 (t, 1H), 6.98 (t, 1H), 6.32–6.44 (m, 2H), 5.95–6.02 (m, *J* = 16.04 Hz, 1H), 2.15–2.20 (q, 2H), 1.29–1.44 (m, 4H), 0.86 (t, 3H).

Synthesis of alkyl-1H-indoles

Compounds **14, 23, 32** (5-Cl analog) and **38** (6-Cl analog) were synthesized as shown for 2-hex-1-enyl-1*H*-indole (**23**):

2-Hexyl-1*H*-indole (23)—To a stirred solution of **22** (35 mg, 0.16 mmol) in anhydrous EtOH (1 mL) was added 10% PD/C (10 % wt) at rt under H$_2$ atmosphere and stirred for 6 h. The reaction mixture was filtered through celite/florisil. The filtrate was concentrated under reduced pressure and the crude residue purified by silica gel chromatography using (1:9) Et$_2$O/Hex solvent as eluent to afford **23** as white solid (32 mg, 90%). HRMS (ESI) *m/z* calcd for [C$_{14}$H$_{19}$N+H]$^+$: 202.1596, found 202.1596. ^{1}H NMR (CDCl$_3$): 7.8 (s, 1H), 7.45 (d, 1H), 7.22 (d, 1H), 6.93–7.08 (m, 2H), 6.14 (s, 1H), 2.68 (t, 2H), 1.59–1.60 (m, 2H), 1.15–1.38 (m, 6H), 0.82 (t, 3H).

Synthesis of alkyl-(5-oxo-valeric acid)indoles

Compounds **16–19** (hexyl at C-3, 5, 6, and 7), **24–29** (alkyl at C-2 = butyl **24**, pentyl **25**, hexyl **26**, heptyl **27**, octyl **28**, and undecyl **29**), **33** (5-Cl analog), **39** (6-Cl analog) were synthesized using the general procedure for **24** as follows:

5-(2-Hexyl-1*H*-indol-1-yl)-5-oxo-valeric acid (26)—To a solution of **23** (32 mg, 0.16 mmol) in anhydrous DMSO was added crushed KOH (44.5 mg, 0.795 mmol) at rt under nitrogen. The reaction mixture was stirred for 30 min followed by addition of glutaric anhydride **15** (90.6 mg, 0.795 mmol). The stirring was continued for 3 h. The reaction mixture was quenched with saturated NH$_4$Cl solution, extracted with EtOAc and the organic layers combined and dried over Na$_2$SO$_4$. The solvent was evaporated under reduced pressure and the crude residue was purified by silica gel chromatography using 50% EtOAc/Hex as eluent to afford **26** as a white solid (30.1 mg, 60%). HRMS (ESI) *m/z* calcd for [C$_{19}$H$_{25}$NO$_3$+H]$^+$: 316.1913, found 316.1913. ^{1}H NMR (CDCl$_3$): 7.82 (1H, d), 7.49–7.51 (1H, m), 7.21 –7.28 (2H, m), 6.43 (1H, s), 3.15 (2H, t), 3.02 (2H, t), 2.6 (2H, t), 2.18–2.25 (2H, m), 1.68–1.76 (2H, m), 1.28–1.47 (6H, m), 0.93 (3H, t). ^{13}C NMR (CDCl$_3$): 179.1, 172.7, 143.1, 136.0, 130.1, 123.4, 123.0, 120.2, 114.8, 108.3, 37.9, 32.9, 31.7, 30.7, 29.2, 28.9, 22.6, 19.8, 14.1.

5-(3-Hexyl-1*H*-indol-1-yl)-5-oxo-valeric acid (16)—Yield: 72%. HRMS (ESI) *m/z* calcd for $[C_{19}H_{25}NO_3+H]^+$: 316.1913, found 316.1904. ^1H NMR (CDCl$_3$): 8.46 (1H, s), 7.55 (1H, d), 7.37 (1H, t), 7.28–7.32 (2H, m), 7.24 (1H, s), 3.03 (2H, t), 2.67–2.72 (2H, m), 2.62 (2H, t), 2.16–2.23 (2H, m), 1.69–1.77 (2H, m), 1.28–1.45 (6H, m), 0.92 (3H, t). ^{13}C NMR (CDCl$_3$): 178.1, 170.3, 136.1, 130.8, 125.2, 123.8, 123.4, 120.7, 119.0, 116.7, 34.6, 32.7, 31.7, 29.3, 29.1, 25.0, 22.6, 19.4, 14.1.

5-(5-Hexyl-1*H*-indol-1-yl)-5-oxo-valeric acid (17)—Yield: 54%. HRMS (ESI) *m/z* calcd for $[C_{19}H_{25}NO_3+H]^+$: 316.1913, found 316.1921. ^1H NMR (CDCl$_3$): 8.24 (s, 1H), 7.38 (d, *J* = 8.08 Hz, 1H), 7.32 (d, *J* = 3.54 Hz, 1H), 7.04 (d, *J* = 8.08 Hz, 1H), 6.52 (d, *J* = 3.54 Hz, 1H), 2.95 (t, *J* = 6.95 Hz, 2H), 2.65 (t, *J* = 7.71 Hz, 2H), 2.52 (t, *J* = 6.82 Hz, 2H), 2.05 – 2.16 (m, 2H), 1.59 (qt, *J* = 7.33 Hz, 2H), 1.16 – 1.33 (m, 6H), 0.80 (t, *J* = 6.44 Hz, 3H). ^{13}C NMR (CDCl$_3$): 177.9, 170.6, 140.6, 136.0, 128.2, 124.6, 123.9, 120.4, 116.3, 109.3, 36.5, 34.5, 32.6, 32.1, 31.7, 29.0, 22.6, 19.5, 14.1.

5-(6-Hexyl-1*H*-indol-1-yl)-5-oxo-valeric acid (18)—Yield: 66%. HRMS (ESI) *m/z* calcd for $[C_{19}H_{25}NO_3+H]^+$: 316.1913, found 316.1909. ^1H NMR (CDCl$_3$): 8.24 (s, 1H), 7.38 (d, *J* = 8.08 Hz, 1H), 7.32 (d, *J* = 3.54 Hz, 1H), 7.04 (d, *J* = 8.08 Hz, 1H), 6.52 (d, *J* = 3.54 Hz, 1H), 2.95 (t, *J* = 6.95 Hz, 2H), 2.65 (t, *J* = 7.71 Hz, 2H), 2.52 (t, *J* = 6.82 Hz, 2H), 2.05 – 2.17 (m, 2H), 1.59 (qt, *J* = 7.33 Hz, 2H), 1.15 – 1.33 (m, 6H), 0.80 (t, *J* = 6.44 Hz, 3H). ^{13}C NMR (CDCl$_3$): 177.9, 170.6 140.7, 136.0, 128.2, 124.6, 123.9, 116.3, 109.3, 36.5, 34.5, 32.6, 32.1, 31.7, 29.0, 22.6, 19.4, 14.1.

5-(7-Hexyl-1*H*-indol-1-yl)-5-oxo-valeric acid (19)—Yield: 74%. HRMS (ESI) *m/z* calcd for $[C_{19}H_{25}NO_3+H]^+$: 316.1913, found 316.1917. ^1H NMR (CDCl$_3$): 7.47 (d, *J* = 3.79 Hz, 1H), 7.42 (d, *J* = 7.33 Hz, 1H), 7.19 – 7.27 (m, 2H), 6.66 (d, *J* = 3.79 Hz, 1H), 3.01 – 3.12 (m, 4H), 2.61 (t, *J* = 6.95 Hz, 2H), 2.18 – 2.27 (m, 2H), 2.01 – 2.09 (m, 2H), 1.46 – 1.55 (m, 2H), 1.24 – 1.37 (m, 4H), 0.88 (t, 3H). ^{13}C NMR (CDCl$_3$): 177.6, 169.9, 134.3, 132.3, 131.7, 127.3, 126.0, 124.1, 118.7, 109.3, 35.7, 35.4, 31.8, 30.2, 29.7, 29.3, 22.6, 20.2, 14.1.

5-(2-Butyl-1*H*-indol-1-yl)-5-oxo-valeric acid (24)—Yield: 68.0%. HRMS (ESI) *m/z* calcd for $[C_{17}H_{21}NO_3+H]^+$: 288.1600, found 288.1596. ^1H NMR (CDCl$_3$): 7.72 (d, *J* = 7.83 Hz, 1H), 7.37 – 7.42 (m, 1H), 7.09 – 7.19 (m, 2H), 6.33 (s, 1H), 3.05 (t, *J* = 6.95 Hz, 2H), 2.92 (t, *J* = 7.58 Hz, 2H), 2.50 (t, *J* = 7.07 Hz, 2H), 2.12 (qt, *J* = 7.01 Hz, 2H), 1.61 (qt, *J* = 7.52 Hz, 2H), 1.37 (m, 2H), 0.89 (t, *J* = 7.20 Hz, 3H). ^{13}C NMR (CDCl$_3$): 178.5, 172.7, 143.1, 136.0, 130.1, 123.4, 123.0, 120.2, 114.7, 108.3, 37.9, 32.8, 31.1, 30.4, 22.5, 19.9, 13.9.

5-(2-Pentyl-1*H*-indol-1-yl)-5-oxo-valeric acid (25)—Yield: 40%. HRMS (ESI) *m/z* calcd for $[C_{18}H_{23}NO_3+H]^+$: 302.1756, found 302.1756. ^1H NMR (CDCl$_3$): 7.69 (d, *J* = 6.93 Hz, 1H), 7.32 – 7.39 (m, 1H), 6.98 – 7.12 (m, 2H), 6.27 (s, 1H), 3.18 (t, *J* = 7.23 Hz, 2H), 2.98 (t, *J* = 7.68 Hz, 2H), 2.50 (t, *J* = 7.16 Hz, 2H), 2.21 (m 2H), 1.58 (qt, *J* = 7.52 Hz, 2H), 1.04 (m, 4H), 0.92 (t, *J* = 6.97 Hz, 3H). ^{13}C NMR (CDCl$_3$): 179.1, 173.2, 144.7, 135.6, 129.8, 122.7, 121.9, 119.5, 113.8, 109.1, 38.2, 32.7, 31.5, 30.8, 23.1, 22.5, 19.7, 13.9.

5-(2-Heptyl-1*H*-indol-1-yl)-5-oxo-valeric acid (27)—Yield: 46%. HRMS (ESI) *m/z* calcd for $[C_{20}H_{27}NO_3+H]^+$: 330.2069, found 330.2058. ^1H NMR (CDCl$_3$): 7.72 (d, J = 7.83 Hz, 1H), 7.37 – 7.43 (m, 1H), 7.10 – 7.20 (m, 2H), 6.34 (s, 1H), 3.06 (t, J = 7.07 Hz, 2H), 2.92 (t, J = 7.58 Hz, 2H), 2.51 (t, J = 7.07 Hz, 2H), 2.12 (qt, J = 7.07 Hz, 2H), 1.62 (qt, J = 7.45 Hz, 2H), 1.15 – 1.39 (m, 8H), 0.77 – 0.85 (t, 3H). ^{13}C NMR (CDCl$_3$): 172.7,

143.1, 136.0, 130.0, 123.4, 122.9, 120.0, 114.7, 108.3, 37.9, 32.8, 31.8, 30.7, 29.4, 29.2, 29.0, 22.6, 20.0, 14.1.

5-(2-Octyl-1*H*-indol-1-yl)-5-oxo-valeric acid (28)—Yield: 65.5%. HRMS (ESI) *m/z* calcd for [C$_{21}$H$_{29}$NO$_3$+H]$^+$: 344.2226, found 344.2222. ^{1}H NMR (CDCl$_3$):   7.71 (d, *J* = 7.58 Hz, 1H), 7.36 – 7.43 (m, 1H), 7.10 – 7.20 (m, 2H), 6.33 (s, 1H), 3.03 – 3.12 (m, 1H), 2.87 – 2.96 (m, 3H), 2.71 (qd, *J* = 6.68, 13.29 Hz, 1H), 2.52 (dd, *J* = 6.32, 15.66 Hz, 1H), 2.34 (dd, *J* = 7.20, 15.54 Hz, 1H), 1.62 (qt, *J* = 7.52 Hz, 2H), 1.28 –1.39 (m, 3H), 1.14 – 1.28 (m, 8H), 1.08 (d, *J* = 6.82 Hz, 3H), 0.76 – 0.85 (t, 3H). ^{13}C NMR (CDCl$_3$):   177.6, 172.3, 143.0, 136.0, 130.0, 123.3, 122.9, 120.2, 114.7, 108.2, 45.2, 40.4, 31.8, 30.6, 29.5, 29.2, 29.0, 27.2, 22.6, 19.9, 14.1.

5-(2-Undecyl-1*H*-indol-1-yl)-5-oxo-valeric acid (29)—Yield: 46%. HRMS (ESI) *m/z* calcd for [C$_{24}$H$_{35}$NO$_3$+H]$^+$: 386.2695, found 386.2674. ^{1}H NMR (CDCl$_3$):   7.80 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 6.8 Hz, 1H), 7.22 (dd, J = 12.1, 7.1 Hz, 2H), 6.41 (s, 1H), 3.13 (t, J = 6.4 Hz, 2H), 3.00 (t, J = 7.2 Hz, 2H), 2.58 (t, J = 6.5 Hz, 2H), 2.26 – 2.14 (m, 2H), 1.77 – 1.62 (m, 2H), 1.49 – 1.18 (m, 18H), 0.89 (t, J = 5.8 Hz, 3H). ^{13}C NMR (CDCl$_3$):   178.72 (s), 172.70 (s), 143.16 (s), 135.99 (s), 130.06 (s), 123.42 (s), 122.96 (s), 120.25 (s), 114.78 (s), 108.27 (s), 37.91 (s), 32.84 (s), 31.93 (s), 30.72 (s), 29.77 – 29.59 (m), 29.52 (d, J = 5.4 Hz), 29.36 (s), 28.95 (s), 22.70 (s), 19.85 (s), 14.14 (s).

5-(5-Chloro-2-hexyl-1*H*-indol-1-yl)-5-oxo-valeric acid (33)—Yield: 64%. HRMS (ESI) *m/z* calcd for [C$_{19}$H$_{24}$ClNO$_3$+H]$^+$: 350.1523 and 352.1493, found 350.1523 and 352.1567. ^{1}H NMR (CDCl$_3$):   7.72 (d, *J* = 7.58 Hz, 1H), 7.38 – 7.42 (m, 1H), 7.12 – 7.18 (m, 1H), 6.34 (s, 1H), 3.06 (t, *J* = 7.07 Hz, 2H), 2.92 (t, *J* = 7.71 Hz, 2H), 2.51 (t, *J* = 7.20 Hz, 2H), 2.13 (qt, *J* = 6.76 Hz, 2H), 1.62 (qt, *J* = 7.52 Hz, 2H), 1.14 – 1.40 (m, 6H), 0.83 (t, *J* = 6.95 Hz, 3H). ^{13}C NMR (CDCl$_3$):   177.63, 172.70, 143.12, 136.03, 130.07, 123.40, 122.94, 120.23, 114.74, 108.27, 37.90, 32.68, 31.70, 30.67, 29.68, 29.13, 28.93, 22.60, 19.91, 14.04.

5-(6-Chloro-2-hexyl-1*H*-indol-1-yl)-5-oxo-valeric acid (39)—Yield: 38.1%. HRMS (ESI) *m/z* calcd for [C$_{19}$H$_{24}$ClNO$_3$+H]$^+$: 350.1523 and 352.1493, found 350.1519 and 352.1568. ^{1}H NMR (CDCl$_3$):   7.83 (1H, s), 7.27–7.29 (1H, d), 7.09–7.12 (1H, dd), 6.29 (1H, s), 3.00–3.06 (1H, dd), 2.82–2.89 (2H, m), 2.64–2.73 (1H, m), 2.49–2.54 (1H, dd), 2.32–2.38 (1H, dd), 1.58– 1.65 (2H, m), 1.18–1.38 (6H, m), 0.82 (3H, t). ^{13}C NMR (CDCl$_3$):   178.0, 172.1, 143.1, 136.6, 129.3, 128.3, 123.4, 120.7, 115.3, 107.9, 44.9, 40.3, 31.7, 30.6, 29.1, 28.9, 22.6, 20.0, 14.0.

Synthesis of 3-(4-chloro-2-nitro-phenyl)-2-oxo-propionic acid ethyl ester (36)

An ethereal solution of anhydrous EtOH (15 mL EtOH + 36 mL ether) was added dropwise to a stirred mixture of potassium metal (2.26 g, 57.9 mmol) in anhydrous ether (22 mL) at rt. A large amount of effervescence was observed. After the potassium metal was completely dissolved, an ethereal solution of diethyl oxalate, **35** (9.43 mL, 69.3 mmol, 22 mL ether) was added dropwise to the reaction mixture. Then an ethereal solution of 4-chloro-1-methyl-2-nitrobenzene, **34** (10 g, 58.28 mmol) in anhydrous ether (15 mL) was added to the reaction mixture. The reaction was stirred for 15 h and then sonicated for another 7 h. The reaction mixture was neutralized by adding 1N HCl at 0°C. It was extracted with EtOAc and the organic layers washed with brine and dried over Na$_2$SO$_4$. The solvent was evaporated under reduced pressure to afford **36** in crude form which was used without any further purification. ^{1}H NMR (CDCl$_3$):   8.15 (d, J = 2.2 Hz, 1H), 7.58 (dd, J = 8.2, 2.2 Hz, 1H), 7.23 (d, J = 3.4 Hz, 1H), 4.49 (s, 2H), 4.36 (q, J = 7.1 Hz, 2H), 1.37 (t, J = 7.2 Hz, 3H).

Synthesis of 6-chloro-1*H*-indole-2-carboxylic acid ethyl ester (37)

To a solution of **36** (1 g, 3.68 mmol) in 1:1 mixture of anhydrous ethanol and glacial acetic acid (7 mL each) was added Fe powder (1.83 g, 32.76 mmol) and the reaction mixture was refluxed for 4 h. It was then cooled to 5 °C and filtered through celite and florisil layers. The filtrate was partitioned between CH_2Cl_2. The organic layer was washed with 1N HCl followed by saturated $NaHCO_3$ solution and finally with brine. The crude residue was purified by crystallization to yield **37** as a pale brown solid (421 mg, 51%). HRMS (ESI) *m/z* calcd for $[C_{11}H_{10}ClNO_2+H]^+$: 224.0478 and 226.0449, found 224.0477 and 226.0451. ^{1}H NMR ($CDCl_3$): 8.84 (br s, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.42 (s, 1H), 7.19 (s, 1H), 7.13 (dd, J = 8.6, 1.1 Hz, 1H), 4.41 (q, J = 7.2 Hz, 2H), 1.42 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$): 161.7, 137.0, 131.3, 128.2, 126.0, 123.5, 121.8, 111.6, 108.6, 61.2, 14.3.

Synthesis of 6-chloro-2-(hex-1-en-1-yl)-1*H*-indole (38)

To a stirred solution of **37** (200 mg, 0.894 mmol) in THF (5 mL) at 0 °C was added a $LiAlH_4$ (1M in THF, 1.34 mL, 1.34 mmol) under argon. The reaction mixture was stirred at 0 °C for 30 min, then was gradually warmed to rt over a period of 3 h. The reaction mixture was quenched with aqueous NH_4Cl solution and then was extracted with EtOAc. The organic layers were combined and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the crude mixture was dried under vacuum pump. To this crude mixture in acetonitrile (5 mL) was added MnO_2 (405 mg, 4.68 mmol) and the reaction mixture was stirred for 18 h. The reaction mixture was diluted with ether and filtered through celite and florisil layers. The solvent was evaporated under reduced pressure and the crude residue purified by silica gel chromatography using 30% EtOAc/Hex solvent as eluent to afford **6-chloro-1*H*-indole-2-carbaldehyde** as a brown solid (156 mg, 92%). HRMS (ESI) *m/z* calcd for $[C_9H_6ClNO+H]^+$: 180.0216 and 182.0187, found 180.0215 and 182.0189. ^{1}H NMR ($CDCl_3$): 9.77 (1H, s), 9.11 (1H, s), 7.58–7.60 (1H, d), 7.39 (1H, s), 7.18–7.19 (1H, m), 7.07–7.10 (1H, dd). ^{13}C NMR ($CDCl_3$): 181.96 (s), 124.41 (s), 122.48 (s), 121.43 (s), 120.66 (s), 114.68 (s), 112.23 (s), 110.88 (s), 100.13 (s). This aldehyde was reacted with the bromopentyl triphenyl phosphonium salt as described for the synthesis of **22** to afford **38**: HRMS (ESI) *m/z* calcd for $[C_{14}H_{16}ClN+H]^+$: 234.1050 and 236.1020, found 234.1053 and 236.1027. ^{1}H NMR ($CDCl_3$): 8.05 (s, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.37 – 7.26 (m, 2H), 7.02 (d, J = 8.4 Hz, 1H), 6.42 – 6.31 (m, 2H), 6.11 – 6.00 (m, 1H), 2.24 (q, J = 7.1 Hz, 2H), 1.42 (ddd, J = 31.6, 14.9, 7.4 Hz, 4H), 0.93 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$): 137.31 (s), 136.76 (s), 130.92 (s), 127.78 (s), 127.56 (s), 121.10 (s), 120.63 (s), 120.41 (s), 110.34 (s), 101.26 (s), 32.67 (s), 31.37 (s), 22.24 (s), 13.94 (s).

Acknowledgments

ABBREVIATIONS USED

5-HEDH	5-hydroxyeicosanoid dehydrogenase
5-oxo-ETE	5-Oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid
$CaCl_2$	calcium chloride

CDCl₃	deuterated chloroform
MeCN	acetonitrile
CH₂N₂	diazomethane
DMSO	dimethylsulfoxide
Et₂O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
GM-CSF	granulocyte/macrophage colony stimulating factor
HETE	hydroxy-eicosatetraenoic acid
Hex	hexanes
KHMDS	potassium hexamethyldisilazide
KOH	potassium hydroxide
LiAlH₄	lithium aluminum hydride
LiHMDS	lithium hexamethyldisilazide
LO	lipoxygenase
LT	leukotriene
MgCl₂	magnesium chloride
MnO₂	manganese dioxide
NaHMDS	sodium hexamethyldisilazide
Na₂SO₄	sodium sulfate
NaI	sodium iodide
NH₄Cl	ammonium chloride
OXE-R	oxoeicosanoid receptor
SE	standard error
THF	tetrahydrofuran

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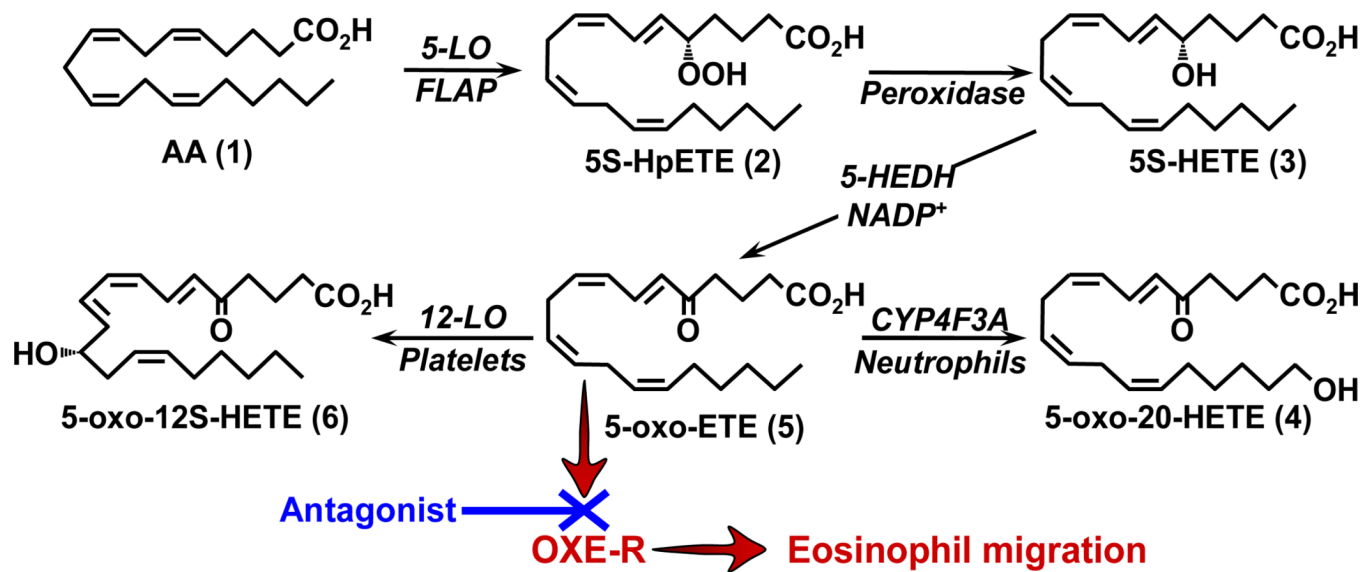
Figure 1.
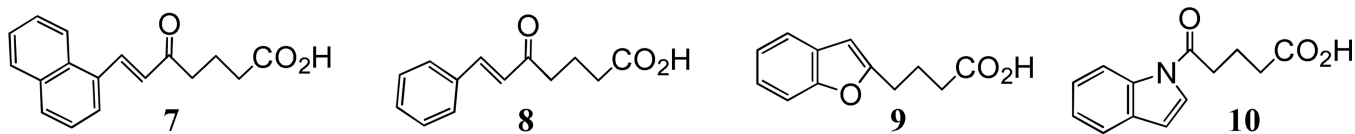Biosynthesis and metabolism of 5-oxo-ETE.

Figure 2.
Examples of some monosubstituted aromatic compounds that have been synthesized and found to be without antagonist activity.
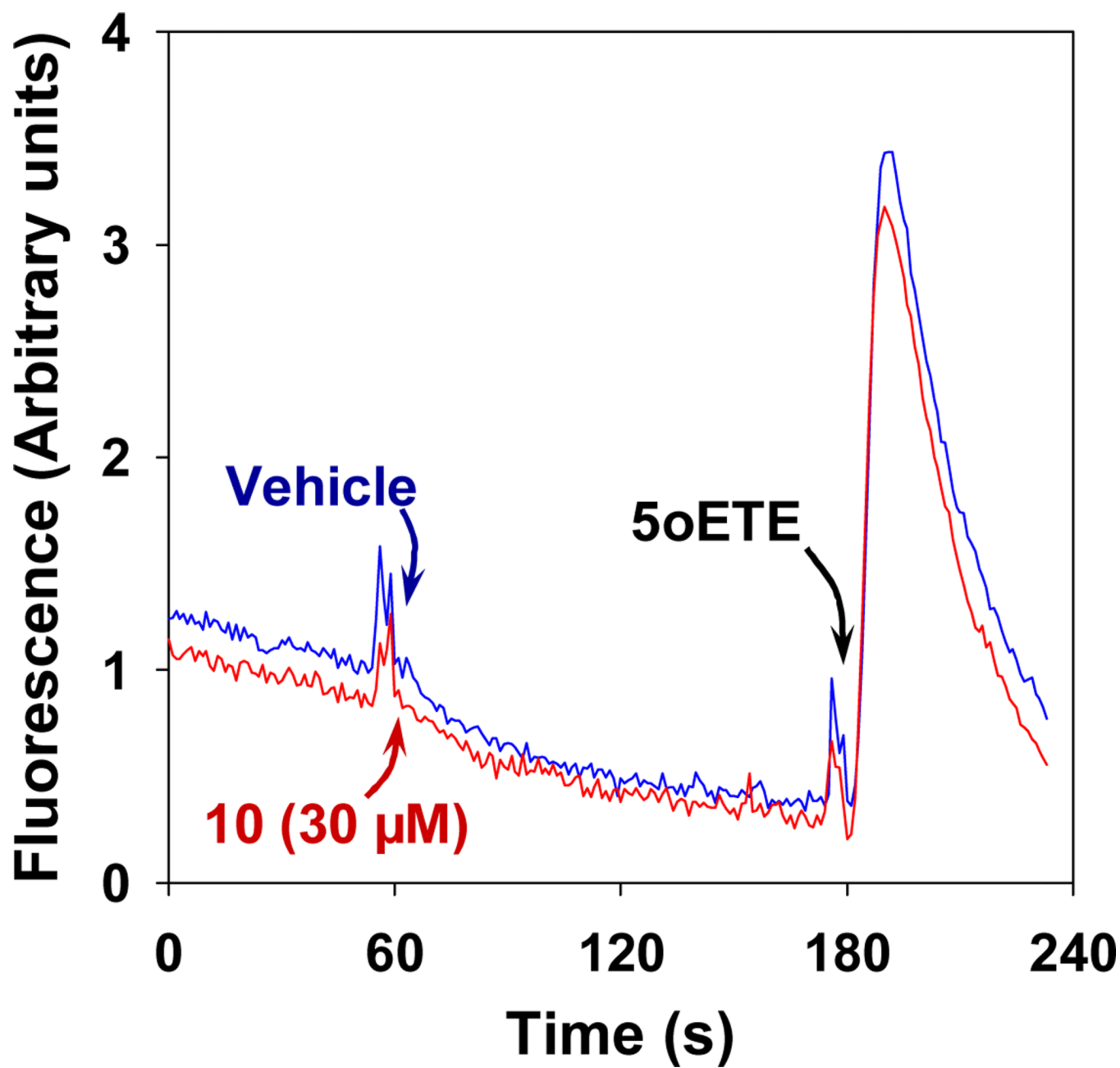
Figure 3.
The monosubstituted indole **10** does not affect calcium mobilization in human neutrophils induced by 5-oxo-ETE. Neutrophils were loaded with the calcium-sensitive dye indo-1 and changes in fluorescence were monitored after addition of **10** (30 μM) and, 2 min later, 5-oxo-ETE (10 nM).
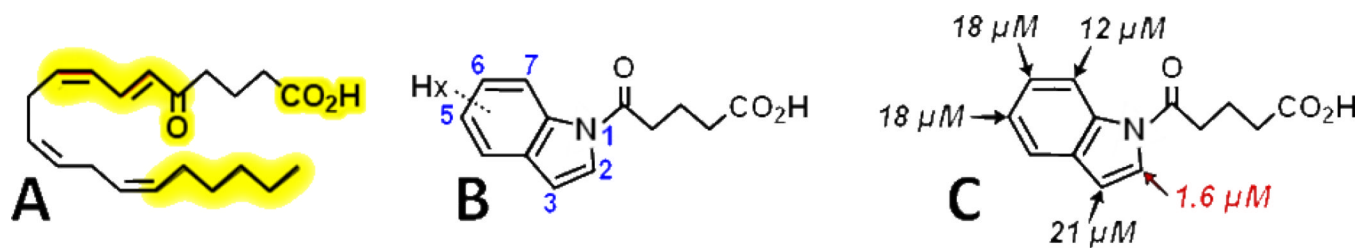
Figure 4.
Structures of 5-oxo-ETE and indole-based conformationally restricted analogs. A: 5-Oxo-ETE with the regions required for activation of the OXE receptor in neutrophils highlighted in yellow. B: Monohexyl-substituted 1-(5-oxovalerate)indoles. C: IC_{50} values for 1-(5-oxovalerate)indoles containing a hexyl group in different positions.
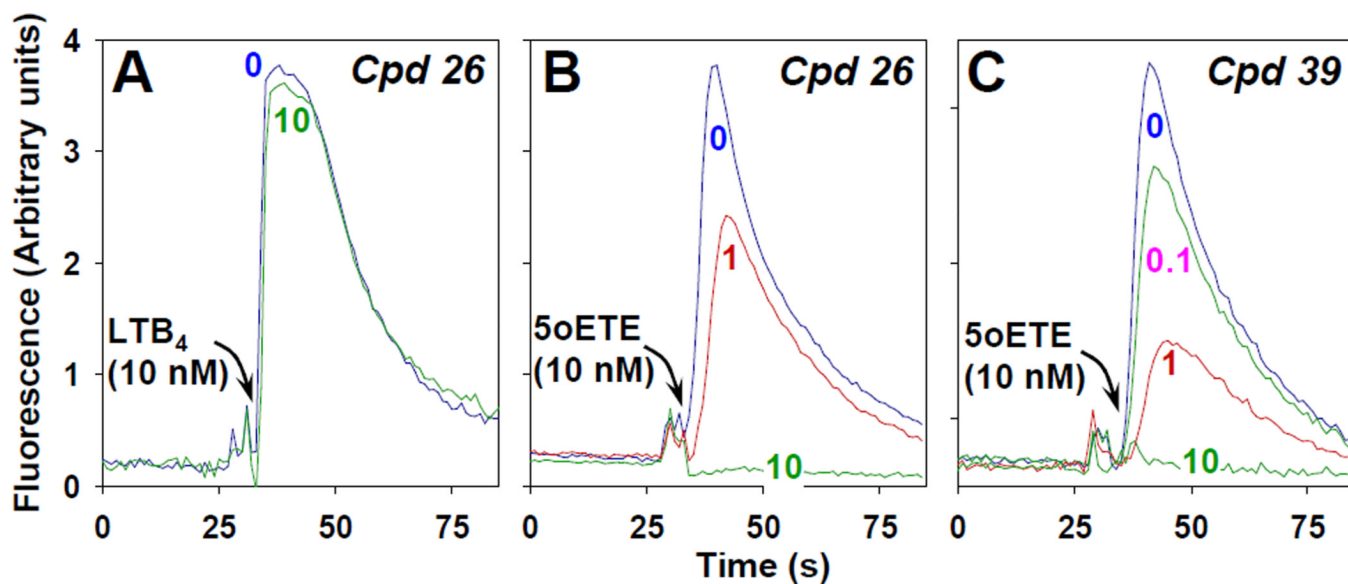
Figure 5.
Effects of OXE receptor antagonists on calcium mobilization in human neutrophils. Various concentrations of either **26** (**A** and **B**) or **39** (**C**) were added to indo-1-loaded neutrophils followed 2 min later by the addition of either LTB_4 (10 nM; **A**) or 5-oxo-ETE (10 nM; **C**).
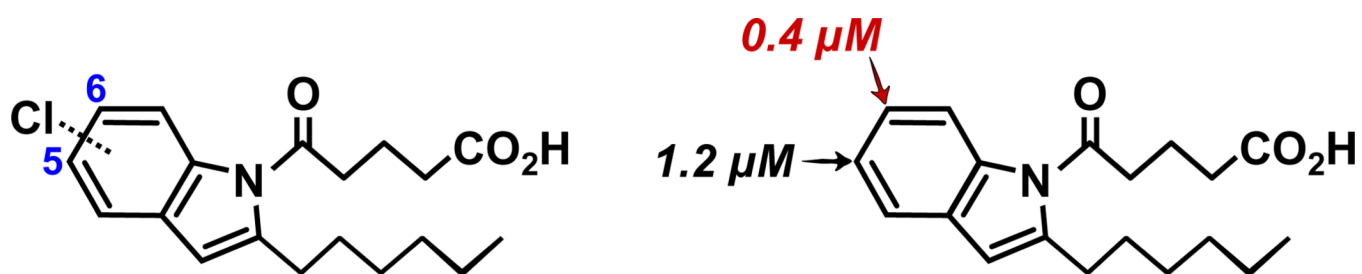
Figure 6.
Structures of chloro-substituted indole antagonists. The IC_{50} values of the 5- and 6-chloro compounds are shown on the right.
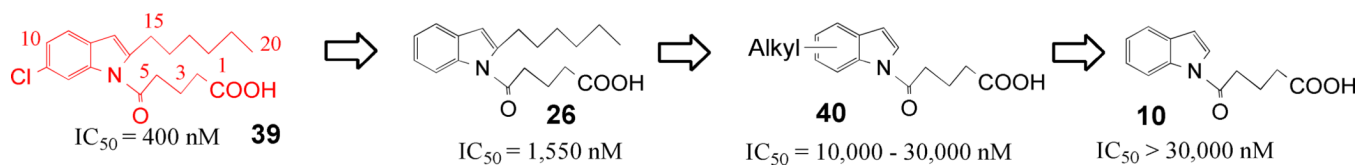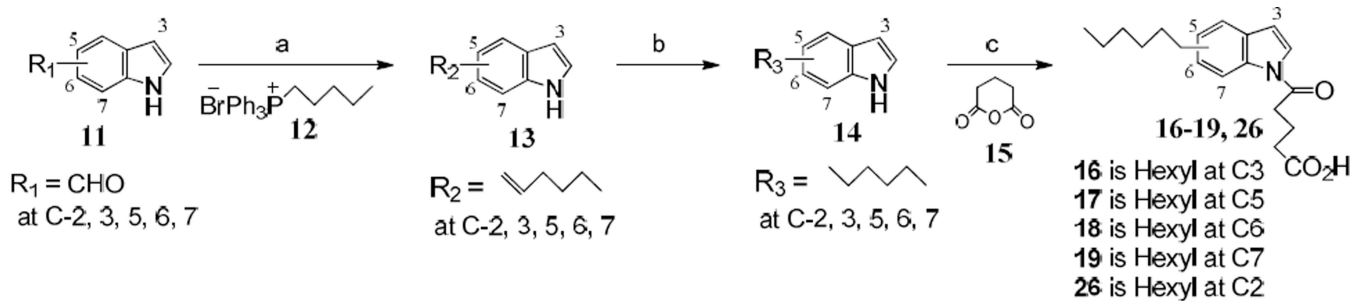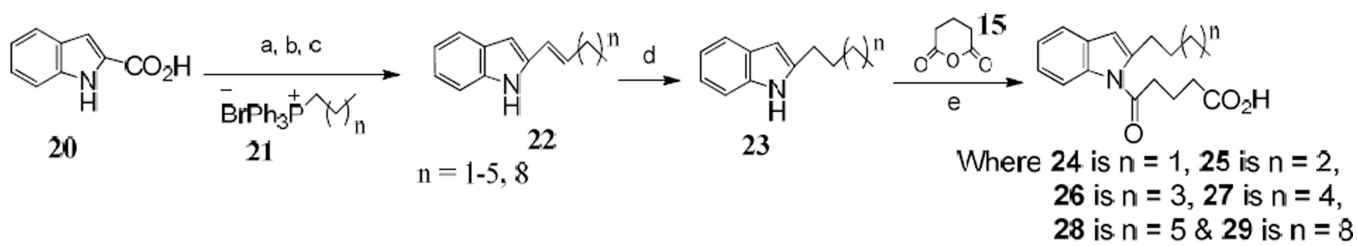
Figure 7.
Summary of structure activity leading to **39**. The numbering on **39** refers to the comparable carbons on 5-oxo-ETE.
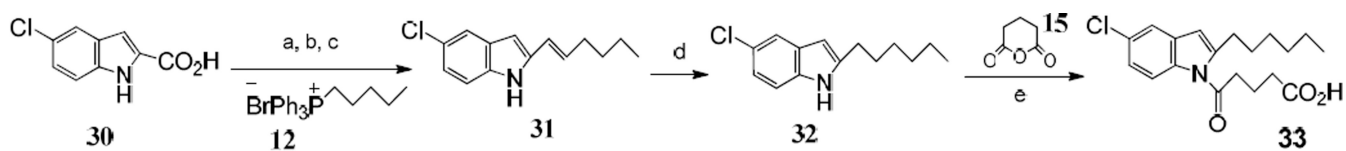
Reagents & Conditions : a) **12**, LiHMDS, THF, -78 ^{0}C; b) 10% Pd/C, H_2, EtOH, rt; c) **15**, KOH, DMSO, rt.

Scheme 1.
Synthesis of indole derivatives.

Reagents & Conditions : a) LiAlH$_4$, THF, rt; b) MnO$_2$, dry CH$_3$CN, rt; c) **21**, LiHMDS, THF, −78 ^{0}C; d) 10% Pd/C, H$_2$, EtOH, rt; e) **15**, KOH, DMSO, rt.
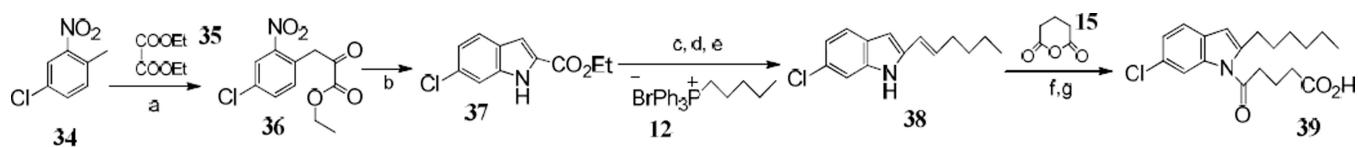
Scheme 2.
Synthetic approach for indole derivatives.

Reagents & Conditions : a) LiAlH$_4$, THF, rt; b) MnO$_2$, dry CH$_3$CN, rt; c) **12**, LiHMDS, THF, -78 ^{0}C; d) 10% Pd/C, H$_2$, EtOH, rt; e) **15**, KOH, DMSO, rt.
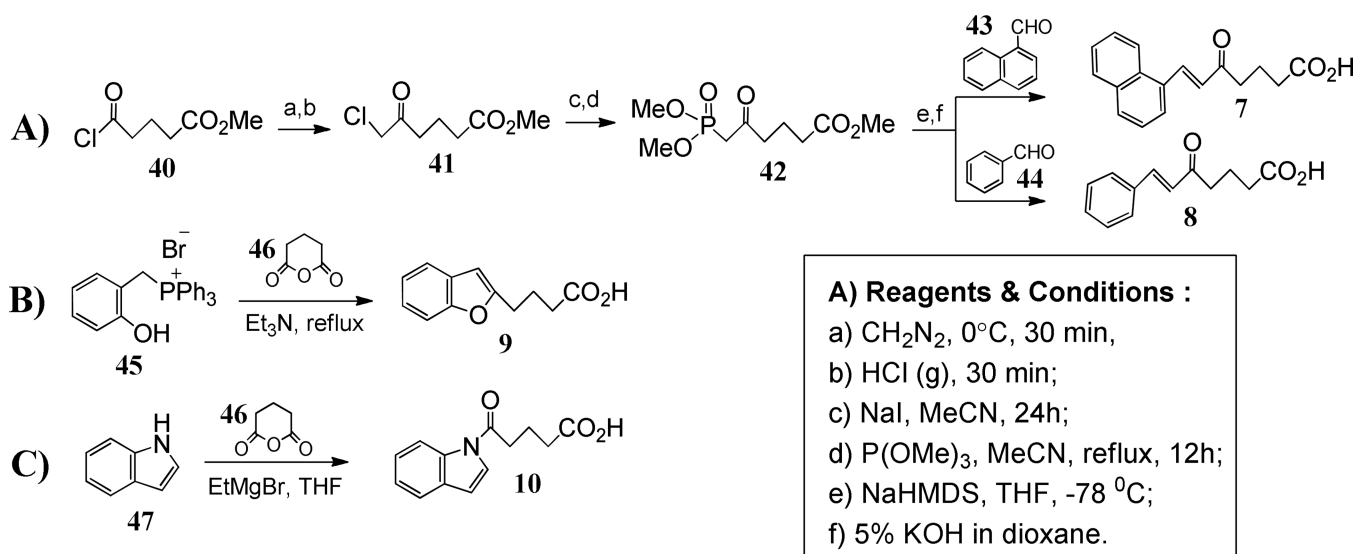
Scheme 3.
Synthesis of 5-(5-Chloro-2-hexyl-1*H*-indol-1-yl)-5-oxo-valeric acid **33**.

Reagents & Conditions : a) **35**, EtOK, EtOH, Et₂O, rt; b) Fe, AcOH, EtOH, reflux; c) LiAlH₄, THF, rt; d) MnO₂, dry CH₃CN, rt; e) **12**, LiHMDS, THF, -78 °C; f) 10% Pd/C, H₂, EtOH, rt; g) **15**, KOH, DMSO, rt.

Scheme 4.
Synthesis of 5-(6-Chloro-2-hexyl-1*H*-indol-1-yl)-5-oxo-valeric acid **39**.

Scheme 5.
Synthesis of monosubstituted aromatic compounds **7–10**.

A) Reagents & Conditions :
a) CH$_2$N$_2$, 0°C, 30 min,
b) HCl (g), 30 min;
c) NaI, MeCN, 24h;
d) P(OMe)$_3$, MeCN, reflux, 12h;
e) NaHMDS, THF, -78 ^{0}C;
f) 5% KOH in dioxane.

Table 1

IC$_{50}$ values of hexyl-substituted 1-(5-oxovalerate)indoles. A series of 1-(5-oxovalerate)indoles containing a hexyl substituent at various positions as well as 2-hexyl-1-(5-oxovalerate)indoles containing a chloro substituent at positions 5 or 6 were tested in the calcium mobilization assay as shown in Figure 5. IC$_{50}$ values were determined from concentration- response curves for each individual experiment.

Indole	IC$_{50}$ (µM)
2-Hx (26)	**1.55 ± 0.25 (11)**
3-Hx (**16**)	20.68 ± 5.60 (4) [***]
5-Hx (**17**)	17.67 ± 3.59 (3) [**]
6-Hx (**18**)	18.00 ± 2.65 (3) [**]
7-Hx (**19**)	11.77 ± 2.51 (3) [*]
5-Cl (**33**)	1.17 ± 0.32 (4)
6-Cl (**39**)	0.40 ± 0.03 (7) [**]

The values are means ± SE with the numbers of independent determinations shown in brackets. Statistically significant differences compared to **26** are shown as follows:

[*] $p<0.05$;

[**] $p<0.005$;

[***] $p<0.001$

(one-way ANOVA with a Holm-Sidak post-hoc test).