Published in final edited form as:

Org Biomol Chem. 2011 April 21; 9(8): 2999–3010. doi:10.1039/c0ob00714e.

Synthesis and Evaluation of 5-Lipoxygenase Translocation Inhibitors from Acylnitroso Hetero-Diels-Alder Cycloadducts†

Joshua K. Bolger^a, Wen Tian^b, William R. Wolter^c, Wonhwa Cho^b, Mark A. Suckow^c, and Marvin J. Miller^a

Wonhwa Cho: wcho@uic.edu; Mark A. Suckow: suckow.1@nd.edu; Marvin J. Miller: mmiller1@nd.edu

^aDepartment of Chemistry and Biochemistry, 251 Nieuwland Science Hall, University of Notre Dame, Notre Dame, IN 46556. Tel: 574-631-7571

^bDepartment of Chemistry, University of Illinois at Chicago, 845 West Taylor Street, Chicago, Illinois 60607-7061 Tel: (312)-996-4883

^cFreimann Animal Care Facility, 400 Galvin Life Science, University of Notre Dame, Notre Dame, IN 46556 Tel: (574) 631-6085

Abstract

Acylnitroso cycloadducts have proven to be valuable intermediates in the syntheses of a plethora of biologically active molecules. Recently, organometallic reagents were shown to open bicyclic acylnitroso cycloadducts and, more interestingly, the prospect of highly regioselective openings was raised. This transformation was employed in the synthesis of a compound with excellent inhibitory activity against 5-lipoxygenase ((±)-**4a**, IC₅₀ 51 nM), an important mediator of inflammation intimately involved in a number of disease states including asthma and cancer. Optimization of the copper-mediated organometallic ring opening reaction was accomplished allowing the further exploration of the biological activity. Synthesis of a number of derivatives with varying affinity for metal binding as well as pendant groups in a range of sizes was accomplished. Analogues were tested in a whole cell assay which revealed a subset of the compounds to be inhibitors of enzyme translocation, a mode of action not previously known and, potentially, extremely important for better understanding of the enzyme and inhibitor development. Additionally, the lead compound was tested *in vivo* in an established colon cancer model and showed very encouraging anti-tumorigenic properties.

Introduction

Arachidonic acid (AA), a C20 polyunsaturated fatty acid, possesses a fundamental role in the regulation of many cell functions as a precursor to potent mediators with far-ranging effects. These mediators arise when arachidonic acid is metabolized by one of two possible pathways. The most well known and widely studied pathway involves metabolism by cyclooxygenase (COX) to give rise to a variety of compounds known as prostaglandins and thromboxanes. There exists two isoforms of cyclooxygenase, COX-1 and COX-2. The second, less studied pathway concerns metabolism of AA by lipoxygenase of which there are at least 5 isoforms, the most common and interesting being 5-lipoxygenase (5-LOX).

†Electronic Supplementary Information (ESI) available: [Spectral data for all compounds and additional images from cellular study]. See DOI: 10.1039/b000000x/

This journal is © The Royal Society of Chemistry [year]

Correspondence to: Marvin J. Miller, mmiller1@nd.edu.

The action of 5-LOX on arachidonic acid gives rise to leukotrienes which, along with the prostanoids, are potent pro-inflammatory mediators known as eicosanoids.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most prescribed drugs worldwide.¹ Non-selective COX inhibitors, such as acetylsalicylic acid, have been used for the treatment of pain, inflammation and fever for over a century. The COX-1 isoform is ubiquitously expressed and produces prostaglandins which control normal physiological functions, particularly with respect to the gastrointestinal tract and renal function.^{2, 3} COX-2, on the other hand, is an inducible enzyme and is produced in response to proinflammatory stimuli, growth factors and other means. The beneficial effects of COX-1 coupled with the transient nature of COX-2 led to an extensive search for selective COX-2 inhibitors.⁴ Several selective COX-2 inhibitors have been approved by the FDA in the last 10 years including Vioxx (1999), Celebrex (1998) and Bextra (2001). Many of the selective COX-2 inhibitors displayed reduced gastrointestinal side effects caused by inhibiting the production of chemoprotective prostaglandins by COX-1.⁵ In 2004 Vioxx was removed from the market after it was found to increase the risk of myocardial infarction, other COX-2 inhibitors were pulled shortly after for the same reason. In light of the obstacles associated with COX-2 inhibitors and the limited efficacy in other inflammation driven disease states, such as asthma, the lipoxygenase pathway has seen increased effort to develop effective inhibitors and leukotriene receptor antagonists. To date no essential protective roles have been identified for leukotrienes, as in the case of prostaglandins, making it an attractive target for drug development.

5-Lipoxygenase, downstream enzymes and leukotriene receptors have been the subject of intense study in light of the potential therapeutic value.⁶ Leukotrienes, being potent mediators of inflammation, are intimately involved in a number of disease states.^{7, 8} In the arachidonic acid metabolic pathway both COX^{9, 10} and LOX^{11, 12} play an important role in asthma. In fact the study of anaphylaxis and bronchoconstriction is directly responsible for the discovery of leukotrienes and the 5-LOX pathway. Cyclooxygenase inhibitors have been shown to delay tumor growth in animals and humans.^{13, 14} Yet the high doses and resulting increase in detrimental side effects described earlier have limited their development as chemopreventative agents. LOX inhibition, considering its lack of mechanistic side effects, is a considerably more attractive target for the development of anticancer drugs.^{15, 16} As a result of the intense biological activity of leukotrienes and apparent lack of an essential protective role as in cyclooxygenase, the lipoxygenase pathway is an attractive target for drug discovery (Figure 1).

We previously reported initial studies on the opening of acylnitroso hetero-Diels-Alder (HDA) cycloadducts¹⁷ with Grignard reagents.¹⁸ In the same work the synthesis and *in vitro* activity of an extremely potent inhibitor of 5-LOX was described (Scheme 1). Building upon those preliminary studies, here we describe the facile synthesis of a small library of iron-binding 5-lipoxygenase inhibitors. Cellular experiments are described along with the unexpected, yet very encouraging, emergence of an unprecedented mode of inhibition. Finally, the tumor suppression results from an oncogenic mouse model are reported.

Results

Chemical Synthesis

In order to effectively utilize the discovery of Grignard-mediated ring opening of acylnitroso-derived cycloadducts in the synthesis of biologically relevant molecules, optimization of the reaction conditions was necessary (Table 1). The study utilized Grignard reagents derived from benzyl bromide and 3-phenoxybenzyl bromide, as well as, commercial solutions of benzylmagnesium chloride and ethylmagnesium bromide. The

reactions were performed with both the methyl and *t*-butyl *N*-hydroxycarbamate-derived cycloadducts. The catalyst (CuCN) loading, addition time of the Grignard solution, and reaction temperature were varied in order to determine the most efficient reaction with respect to yield of the *anti*-1,2-product as well as production of byproducts. The 1,4-products, when formed, were not quantified.

The catalytic reactions were initially performed in diethyl ether with 10% copper(I) cyanide in accordance with the studies of Bäckvall *et al.*^{19, 20, 21, 22} Thus, benzylmagnesium bromide, was added to a solution of the copper catalyst and substrate at room temperature over 45 minutes (Table 1, entry 1). Consistent with our previous study, the reaction gave a 31% yield of the *anti*-1,2-product as determined by 2D NMR and X-ray crystallography. Slower (3 hour) addition of the Grignard increased the yield to 50% of *anti*-1,2-product (Table 1, entry 2). Increasing the catalyst load to 20% and then 50% further increased the yield to 58% and 73%, respectively (entries 3 and 4). Repetition of the reaction with the Grignard reagent generated from 3-phenoxybenzyl bromide displayed similar, although more variable, results. Catalyst loading of 20% resulted in the product being formed in 27–74% yield with 1.2 equivalents of the Grignard reagent, while 50% catalyst loading gave 67–79% with 2 equivalents of Grignard.

When conditions that were expected to provide exclusive α -addition were employed in order to produce the *anti*-1,4-product, the result was unexpected. Thus, use of tetrahydrofuran as the solvent, 10 mol% copper(I) cyanide, and a 5 minute addition of the Grignard with a reaction temperature of -78°C was anticipated to produce the *anti*-1,4-product, yet gave the *anti*-1,2-product in 62% yield with no isolated 1,4-products (Table 1, entry 9). The reaction also did not produce polymeric byproducts which had complicated purification in previous reactions.

Utilizing the previous finding with γ -selective conditions (diethyl ether, 40–50 mol% CuCN) resulted in formation of 72–97% yield of the *anti*-1,2-product with significantly less formation of byproducts (Table 1, entry 10). In fact, unreacted cycloadduct was isolated in some cases. Use of the Grignard derived from 3-phenoxybenzyl bromide gave the desired product in 78% yield (Table 1, entry 11). These examples also utilized a more rapid addition (1.5 hours) of the Grignard solution. The identification of reaction conditions which consistently produced the desired product in >70% yield allowed efforts to be shifted towards the synthesis of 5-LOX inhibitors.

Examination of the putative catalytic pocket of 5-LOX described by Charlier *et al.* (Figure 2) allowed development of a synthetic strategy for elaboration of a structure-activity relationship (SAR).²³ Thus, analysis of the 5-LOX active site led to identification of areas accessible to four major features of an inhibitor, two hydrophobic groups, a hydrogen bond acceptor, and an aromatic ring. By placing our lead inhibitor (**4a**) in the most logical orientation within the binding pocket, three sites, or zones, were identified for exploration (Figure 3). Preparation of analogues of the first zone focused on variation of the essential iron-binding group (IBG), including pendant groups and substituents thereof, are described below. Synthetic efforts towards the production of analogues focusing on the second zone, substituents and derivatives of the biaryl ether, will be described in a separate manuscript. Variation of the third zone by incorporating substituents on the cyclopentene ring, was explored only briefly.

In order to vary iron binding groups for SAR of zone 1, deprotection of the hydroxylamine carbamate was necessary. Two carbamates, methoxycarbonyl and *t*-butoxycarbonyl, were used in the optimization of the Cu-catalyzed ring opening of the acylnitroso Diels-Alder cycloadduct. Deprotection of the methoxycarbonyl group was unsuccessful under either

acidic or basic conditions²⁴ (Table 2, entries 1–5) although similar conditions have been successful in the case of *O*-alkyl *N*-hydroxy carbamates.^{25, 26, 27, 28} This pitfall, coupled with the increased yield and stability of the *t*-butyl carbamate (Boc) version, prompted us to use the Boc hydroxylamine derived acylnitroso cycloadduct for all subsequent synthetic efforts.

Cleavage of Boc protecting groups is generally carried out under acidic conditions (Table 2, entries 6–11). When either trifluoroacetic acid or hydrochloric acid was used the reaction led to a complex mixture of products presumably due to the generated *t*-butyl cation undergoing an electrophilic aromatic substitution with the diphenyl ether. Inclusion of a cation scavenger, either anisole or triethylsilane, improved the yields significantly (Table 2, entry 10). However, Using *in situ* generated trimethylsilyl iodide²⁹ led to a complex mixture. The hydroxylamine product was characterized both as the free base and after formation of the *p*-toluenesulfonic acid salt. The free hydroxylamine required refrigeration and fairly rapid use (< 1 week) to avoid significant decomposition that occurred upon prolonged storage.

With the hydroxylamines available, representative examples of hydroxamates, *N*-hydroxy carbamates, and *N*-hydroxy ureas were synthesized in order to explore the scope and limitations of Zone 1. Thus, treatment of the free hydroxylamine with acid anhydrides and triethylamine allowed access to several examples, namely the acetyl and succinyl hydroxamates (Scheme 2).³⁰ The formyl hydroxamate was formed upon heating the hydroxylamine in the presence of 2,2,2-trifluoroethyl formate.^{31, 32} Benzoyl chloride and Hunig's base were used to give the benzoyl hydroxamate. In the cases above, with exception of installation of the formyl group, extensive *N,O*-diacylation was observed, although hydrolysis of the crude reaction mixture with sodium hydroxide readily provided the desired compounds.

N-Hydroxy ureas were formed either by reaction with the corresponding isocyanate³³ or carbamoyl chloride, while chloroformates were employed for generation of *N*-hydroxy carbamates (Scheme 3). The free hydroxylamine was treated with trimethylsilyl isocyanate in hot 1,4-dioxane and methyl isocyanate in acetonitrile at room temperature to give the unsubstituted *N*-hydroxy urea and *N'*-methyl-*N*-hydroxy urea, respectively. Dimethylcarbamoyl chloride and Hunig's base provided *N',N'*-dimethyl-*N*-hydroxy urea, (±)-**7c**. Carbamate (±)-**7d** was synthesized by reaction with the corresponding chloroformate and subsequent hydrolytic removal of the simultaneously formed *O*-carboalkoxy group.

In order to determine if the primary cause of the potency of (±)-**4a** was the ability to chelate the catalytic iron, an analogue (**8**) devoid of an IBG was synthesized. Reduction of the N–O bond of (±)-**4a** was accomplished in good yield by reaction with Ti(III)Cl₃^{34, 35} (Scheme 4). It was anticipated that (±)-**8** would lose most or all 5-LOX inhibitory activity. Retention of activity would indicate competitive inhibition which could be exploited.

Biological Evaluation

As indicated earlier, the lead compound, (±)-**4a**, was found to have potent inhibitory activity against 5-LOX. The initial biochemical testing of (±)-**4a** was performed by MDS-Pan Labs, an outside contract laboratory that gave only single point data with no ability to potentially determine mode of action.¹⁸ Thus, derivatives described here were first tested utilizing a commercially available kit and then in cells transfected with GFP tagged 5-LOX. This section will describe the efforts at biochemical evaluation, the results of the cellular assay, and the results of an *in vivo* tumorigenesis experiment.

Several methods for determining 5-LOX activity and inhibition have been described in the literature. The direct spectrophotometric measurement of the leukotriene product at 234 nm

is the simplest method, but is prone to interference since many organic molecules, including inhibitors, absorb light at this wavelength.^{36, 37} Because of this shortcoming, colorimetric assays that take advantage of the lipid peroxide oxidation state have been developed. The assays are conducted in two steps with the variation coming in the reagents used in the second step. After the hydroperoxide product is formed, an oxidation susceptible, color-forming reagent or mixture of reagents is added and the developed color is measured on a spectrophotometer at a wavelength not normally absorbed by inhibitors. While the primary developing reagent employed is an iron(II)/xylene orange (FOX) complex,³⁸ several alternatives exist including hemoglobin/*N*-benzoyl leucomethylene³⁹ and Amplex UltraRed/MicroPeroxidase,⁴⁰ among others.^{41, 42, 43}

A commercially available 96-well plate colorimetric assay (Cayman Chemical) was used to test several of the synthesized compounds, including our initial lead ($\pm$)-**4a**. The developing reagent was not specified by the manufacturer, but believed to be the FOX reagent or a variation thereof. All wells showed no enzymatic activity. An explanation for this may lie in a report from the research laboratories of Pfizer.⁴¹ In the development of a new high-throughput assay they found that a 2.5% final concentration of DMSO resulted in a 10–15% loss in activity, although the cause was not specified. A 10% final DMSO concentration was required to obtain a final concentration of 1 μ M for the compounds in this study resulting in a potential loss of 40–60% of activity. Also, while Zileuton has been successfully tested using the FOX reagent,⁴⁴ potential underestimation of lipid peroxide levels may result from interference of the iron(III)/xylene orange complex by iron chelators.⁴⁵

Assays employing whole cells or cell lysates appear to be more tolerant of endogenous factors. A number of methods exist for the evaluation of lipoxygenase inhibitors in this manner. Procedures employing human whole blood, human neutrophils, rat peritoneal leukocytes,⁴⁶ and rat basophil leukemia cell lysates have been in use for years.^{47, 48} The procedures involve incubation of the cells or partially purified lysates with substrate and an inhibitor followed by addition of a calcium ionophore and a second incubation. The reaction is quenched by freezing or addition of alcohol. The samples are extracted with organic solvent and analyzed for 5-lipoxygenase metabolites, i.e. 5-HETE or LTB₄, as well as metabolites of cyclooxygenase, 12-, and 15-lipoxygenase.⁴⁹ Analysis can be performed by HPLC, enzyme immunoassay, or radioimmunoassay; kits for the latter two methods are commercially available.

Several of the zone 1 compounds were tested in a whole cell assay.⁵⁰ COS-7 cells were transfected with N-terminal green fluorescent protein tagged 5-lipoxygenase and, prior to the experiment, treated diphenyl-1-pyrenyl phosphine (DPPP). GFP-tagged lipoxygenase allows the protein to be “tracked” within the cells, while DPPP reacts with lipid peroxides produced by lipoxygenase allowing the enzymatic activity to be quantified. After addition of calcium ionophore and arachidonic acid or ionophore, AA, and inhibitor the fluorescence was measured for both the GFP and DPPP at regular time intervals.

In total nine of the zone 1 analogues were tested in this assay with interesting results (Table 3). Two analogues (($\pm$)-**6d** and, unsurprisingly, ($\pm$)-**8**, that lacks the iron binding group) were inactive, while three (($\pm$)-**6a**, ($\pm$)-**6b**, and ($\pm$)-**7b**) inhibited lipid peroxide formation to a lesser degree than Zileuton, and one compound (($\pm$)-**7c**) showed greater inhibition than Zileuton. The two remaining compounds (($\pm$)-**4a** and ($\pm$)-**7a**) were quite interesting in that they showed little to no peroxide formation indicating extensive lipoxygenase inhibition. In fact, the compounds shut down the translocation of 5-LOX from both the nucleoplasm and the cytoplasm to the nuclear membrane (Figure 4) that is essential for its enzymatic activity. Inhibitors which interfere with membrane translocation have not been reported for 5-LOX. Although, the FLAP inhibitor MK-886 was originally believed to inhibit translocation it has

since been shown otherwise.^{51, 52} This was a very exciting result that opens new avenue for 5-LOX inhibitor development and warrants further research in order to determine the exact nature of the enzyme-inhibitor interaction.

Grossly visible tumors

The *In vivo* activity of ($\pm$)-**4a** was explored using a colorectal tumorigenesis study employing the APC^(Min/+) mouse model. This mouse line is genetically predisposed to the spontaneous development of multiple intestinal adenomas mimicking the hereditary human condition familial adenomatous polyposis (FAP).^{53, 54} This model has been utilized in numerous studies concerning adenoma formation^{55, 56, 57} and the anti-tumorigenesis effects of nonsteroidal anti-inflammatory drugs.^{58, 59} Sulindac, a selective COX-2 inhibitor, has been shown to effectively reduce tumor progression in FAP studies.^{60, 61}

The study with ($\pm$)-**4a** contained five groups of six mice each. The groups consisted of two dosed with ($\pm$)-**4a** in olive oil at 1.0 mg and 0.1 mg twice daily by oral gavage, two dosed with sulindac under the same regiment, and one vehicle group given only olive oil. All mice remained clinically normal for the duration of the study. The mice were dosed for 40 days, sacrificed, and the numbers of tumors in the digestive tract were determined (Figure 5). Sulindac greatly inhibited tumor development at 1.0 mg b.i.d with tumor numbers fewer than 10% versus the control and when dosed at 0.1 mg b.i.d a 40% reduction was observed. Compound ($\pm$)-**4a** inhibited tumor formation to a lesser, but still very promising, degree with tumor numbers reduced by 62% and 73% relative to the control at 0.1 mg and 1.0 mg, respectively.

Histological analysis

Because GIN lesions are only observable microscopically, rolled intestinal samples were evaluated to assess and characterize lesions histologically. All samples were free of carcinoma, except for a single carcinoma present in a sample from a mouse treated with 0.10 mg ($\pm$)-**4a**. As a means to evaluate progression of neoplasia from GIN to adenoma, the mean ratio of GIN:adenoma was calculated for each group and compared between groups. Because two mice in the 1.0 mg sulindac group had no microscopic lesions observed in the cut sections and one mouse had only one GIN lesion and no adenoma lesions observed, their data was excluded from this analysis. A higher GIN:adenoma ratio indicates that a greater proportion of the lesions are GINs, suggesting that progression of GIN to adenoma is either delayed or blocked. As shown in Figure 6, there was a trend toward higher GIN:adenoma ratio with sulindac and ($\pm$)-**4a** treatment, although there were no significant differences between treatment groups.

Conclusion

In summary, optimization of the regioselective, copper-catalyzed opening of acylnitroso HDA adducts allowed the synthesis of iron-chelating inhibitors of 5-lipoxygenase. A set of inhibitors with varying IBGs, including hydroxamates, *N*-hydroxycarbamates and *N*-hydroxyureas, was synthesized. An inhibitor lacking in the ability to chelate iron was also synthesized. Testing of the compounds in a cellular assay provided evidence of inhibition of translocation of 5-LOX to the perinuclear membrane for two of the compounds. This mode of action has not yet been described for compounds inhibiting 5-LOX. It was also demonstrated that the IBG is essential for inhibition. Further testing of the lead compound *in vivo* yielded promising results in a colorectal cancer model. The *in vivo* results, coupled with the potential for a new mode of action, warrant further exploration of this class of 5-LOX inhibitors. A manuscript reporting on the synthesis of zone 2 inhibitors will be forthcoming.⁶²

Experimental

General Information

Tetrahydrofuran (THF) and diethyl ether were distilled from sodium metal/benzophenone ketyl. Methylene chloride (CH_2Cl_2), acetonitrile (CH_3CN), 1,4-dioxane and triethylamine (TEA) were distilled from CaH_2 . *N,N*-Dimethylformamide (DMF) was distilled from CaH_2 and stored over 3 Å molecular sieves. All other purchased reagents were of reagent grade quality and were used without further purification. Compounds **2**, ($\pm$)-**3a** and **b** were synthesized according to published procedures.¹⁸ Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. All NMR spectra were recorded at 300 MHz, 500 MHz, or 600 MHz on a Varian Utility Plus spectrometer. Chemical shifts are reported as δ in ppm from the residual solvent peak (CDCl_3 ^1H 7.26, ^{13}C 77.00 or d_6 -DMSO ^1H 2.50, ^{13}C 39.51). Mass spectrometric analysis was performed on a JEOL AX505HA, JEOL JMS-GCmate or Micromass ZQ (LC/MS) mass spectrometer. Analytical TLC was carried out using Merck aluminum-backed 0.2 mm silica gel 60 F-254 plates. Column chromatography was conducted using Merck silica gel 60 (230–400) meshes. All reactions were periodically monitored by TLC and worked up after complete consumption of starting materials unless specified otherwise.

Methyl hydroxy((1*S**,2*S**)-2-(3-phenoxybenzyl)cyclopent-3-enyl)carbamate (($\pm$)-**4a**)

To a flame-dried flask charged with freshly ground magnesium (112 mg, 4.61 mmol) and diethyl ether (10 mL) were added **2** (1.20 g, 4.57 mmol) and a crystal of HgCl_2 . The flask was immersed in a water bath at 40 °C and stirred until bubbling ceased. A second flame-dried flask was charged with ($\pm$)-**3a** (500 mg, 2.5 mmol), CuCN (113 mg, 1.26 mmol), and diethyl ether (50 mL). To the second flask was added the Grignard solution over 3 hours *via* syringe pump. After complete addition, the reaction was stirred for 1 hour and quenched with satd. NH_4Cl (50 mL). The phases were separated and the aqueous phase was extracted with diethyl ether (50 mL). The combined organic phases were washed with brine, dried with anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. Flash chromatography (hexanes : EtOAc 4 : 1) gave ($\pm$)-**4a** (950 mg, 2.8 mmol, 74%) as a colorless oil. ^1H NMR (300 MHz, CDCl_3): δ 1.49 (s, 9H), 2.59 (m, 3H), 2.92 (dd, J = 5.4, 13.5 Hz, 1H), 3.31 (bs, 1H), 4.47 (m, 1H), 5.59 (m, 1H), 5.68 (m, 1H), 6.85–7.45 (m, 9H); ^{13}C NMR (75 MHz, CDCl_3): δ 28.59, 34.65, 40.11, 48.90, 63.64, 82.15, 116.82, 118.90, 120.10, 123.30, 124.47, 129.03, 129.74, 129.97, 132.82, 142.48, 157.07, 157.27, 157.50; HRMS [FAB, MH^+] calcd for $\text{C}_{20}\text{H}_{21}\text{NO}_4$ 340.1549, found 340.1550.

Methyl (1*S**,2*S**)-2-benzylcyclopent-3-enyl(hydroxy)carbamate (($\pm$)-**4b**)

To a flame-dried flask charged with freshly ground magnesium (128 mg, 5.28 mmol) and diethyl ether (10 mL) were added benzyl bromide (826 mg, 4.83 mmol) and a crystal of HgCl_2 . The flask was immersed in a water bath at 40 °C and stirred until the magnesium was consumed. A flame-dried flask fitted with an addition funnel was charged with ($\pm$)-**3a** (500 mg, 3.2 mmol), CuCN (28.6 mg, 0.32 mmol), and diethyl ether (20 mL). The Grignard solution was transferred *via* cannula to the addition funnel and added dropwise over 45 minutes. After complete addition, the addition funnel was rinsed with diethyl ether (10 mL), the rinse transferred to the reaction and the reaction was allowed to warm to room temperature and stirred for 1 hour. The reaction was quenched with satd. NH_4Cl (20 mL), the phases separated, and the aqueous phase was extracted with diethyl ether (20 mL). The combined organic phases were washed with brine, dried with anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. Flash chromatography (hexanes : EtOAc 3 : 1) gave ($\pm$)-**4b** (250 mg, 1.01 mmol, 31%) as a colorless oil. ^1H NMR (300 MHz, CDCl_3): δ 2.47 – 2.60 (m, J = 8.79, 8.79, 7.57, 1.71 Hz, 1 H) 2.60 – 2.74 (m, J = 8.79 Hz, 2 H) 2.87 (dd, J = 13.43, 6.35 Hz, 1 H) 3.24 – 3.48 (m, 1 H) 3.70 (s, 3 H) 4.43 – 4.61 (m, 1 H) 5.54 – 5.76

(m, 2 H) 7.12 – 7.39 (m, 5 H) 7.58 (br. s., 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 34.78, 40.32, 48.88, 53.59, 63.77, 126.24, 128.48, 128.77, 129.30, 133.05, 140.33, 157.96; HRMS [FAB, MH^+] calcd for $\text{C}_{14}\text{H}_{18}\text{NO}_3$ 248.1287, found 248.1275. IR (thin film) 3269 (br), 2920, 1695, 1452, 1393, 1109 cm^{-1} .

***tert*-Butyl (1 S^* ,2 S^*)-2-benzylcyclopent-3-enyl(hydroxy)carbamate (($\pm$)-4c)**

A flame-dried flask was charged with ($\pm$)-4c (1 g, 5 mmol), CuCN (227 mg, 2.54 mmol), and diethyl ether (35 mL) and cooled to -78°C . Benzylmagnesium chloride (1.0M in Et_2O , 10.14 mL, 10.14 mmol) was added over 90 minutes *via* syringe pump. After complete addition, the reaction was allowed to warm to room temperature and stirred for 1 hour. The reaction was quenched with satd. NH_4Cl (20 mL), the phases separated, and the aqueous phase was extracted with diethyl ether (20 mL). The combined organic phases were washed with brine, dried with anh. Na_2SO_4 , filtered, and concentrated under reduced pressure. The residue was triturated in hexanes and filtration gave ($\pm$)-4c (1.43 g, 4.94 mmol, 97%) as a white powder after recrystallization from EtOAc /hexanes. ^1H NMR (300 MHz, CDCl_3) δ 1.48 (s, 9 H) 2.39 – 2.55 (m, 1 H) 2.55 – 2.75 (m, 2 H) 2.92 (dd, $J=13.48$, 5.78 Hz, 1 H) 3.18 – 3.39 (m, $J=10.38$, 6.31, 4.28, 2.14, 2.14 Hz, 1 H) 4.46 (ddd, $J=8.99$, 6.85 Hz, 1 H) 5.51 – 5.73 (m, 2 H) 6.78 (br. s., 1 H) 7.22 (s, 5 H); ^{13}C NMR (75 MHz, CDCl_3) δ 28.58, 34.66, 40.35, 49.08, 63.59, 82.21, 126.24, 128.53, 128.84, 129.37, 133.03, 140.34, 156.95; HRMS [FAB, MH^+] calcd for $\text{C}_{17}\text{H}_{24}\text{NO}_3$ 290.1756, found 290.1753. IR (thin film) 3216 (br), 2976, 2927, 1686, 1395, 1105 cm^{-1} .

***tert*-Butyl hydroxy((1 S^* ,2 S^*)-2-(3-phenoxybenzyl)cyclopent-3-enyl)carbamate (($\pm$)-4d)**

To a flame-dried flask charged with freshly ground magnesium (142 mg, 5.83 mmol) and diethyl ether (10 mL) were added 2 (1.60 g, 6.08 mmol) and a crystal of HgCl_2 . The flask was immersed in a water bath at 40°C and stirred until the magnesium was consumed. A second flame-dried flask was charged with ($\pm$)-3a (1 g, 5 mmol), CuCN (227 mg, 2.54 mmol), and diethyl ether (30 mL) and cooled to -78°C . To the second flask was added the Grignard solution over 90 minutes *via* syringe pump. After complete addition, the reaction was allowed to warm to room temperature and stirred for 1 hour. The reaction was quenched with satd. NH_4Cl (30 mL), the phases separated, and the aqueous phase was extracted with diethyl ether (30 mL). The combined organic phases were washed with brine, dried with anh. Na_2SO_4 , filtered, and concentrated under reduced pressure. Flash chromatography (hexanes : EtOAc 85 : 15) gave ($\pm$)-4d (1.51 g, 3.96 mmol, 78%) as a clear, colorless, glassy oil. ^1H NMR (300 MHz, CDCl_3) δ 1.47 (s, 9 H) 2.40 – 2.54 (m, 1 H) 2.54 – 2.63 (m, 1 H) 2.54 – 2.71 (m, 1 H) 2.91 (dd, $J=13.48$, 5.78 Hz, 1 H) 3.21 – 3.36 (m, 1 H) 4.45 (ddd, $J=8.88$, 6.96, 6.85 Hz, 1 H) 5.53 – 5.63 (m, 1 H) 5.63 – 5.71 (m, 1 H) 6.80 – 7.40 (m, 9 H); ^{13}C NMR (75 MHz, CDCl_3) δ 28.59, 34.66, 40.12, 48.90, 63.65, 82.15, 116.82, 118.91, 120.09, 123.30, 124.47, 129.02, 129.74, 129.97, 132.82, 142.47, 157.06, 157.27; HRMS [FAB, MH^+] calcd for $\text{C}_{23}\text{H}_{27}\text{NO}_4$ 318.1940, found 381.1938. IR (thin film) 3223 (br), 3057, 2976, 2928, 1686, 1582, 1487, 1393, 1368, 1305, 1252, 1164, 1106 cm^{-1} .

***N*-((1 S^* ,2 S^*)-2-(3-Phenoxybenzyl)cyclopent-3-enyl)hydroxylamine (($\pm$)-5b)**

Compound ($\pm$)-4d (1.00 g, 2.6 mmol), Et_3SiH (1.67 mL, 10.5 mmol), and CH_2Cl_2 (30 mL) were combined in a round-bottom flask and cooled in an ice/brine bath. TFA (1.95 mL, 26.2 mmol) was added and the reaction was stirred for 2 hours. The reaction was diluted with CH_2Cl_2 (20 mL) and washed with satd. NaHCO_3 (2×40 mL). The organic phase was dried with anh. Na_2SO_4 , filtered, and concentrated *in vacuo* to give the title compound (700 mg, 2.50 mmol, 95%) as a yellow oil which was used without further purification. ^1H NMR (300 MHz, CDCl_3) δ 2.17 – 2.32 (m, 1 H) 2.47 – 2.76 (m, 3 H) 2.77 – 2.90 (m, 1 H) 3.49 (ddd,

$J=7.24, 3.53, 3.35$ Hz, 1 H) 5.54 – 5.63 (m, 1 H) 5.63 – 5.72 (m, 1 H) 6.81 – 6.91 (m, 2 H) 6.91 – 7.04 (m, 3 H) 7.04 – 7.15 (m, 1 H) 7.19 – 7.40 (m, 3 H).

***N*-((1*S*^{*},2*S*^{*})-2-Benzylcyclopent-3-enyl)hydroxylamine ((±)-5a)**

The procedure was the same as that described for (±)-**5b**. (±)-**4c** (400 mg, 1.382 mmol) gave (±)-**5a** (173 mg, 0.914 mmol, 66%) as a brown oil. ¹H NMR (300 MHz, CDCl₃) δ 2.19 – 2.36 (m, 1 H) 2.49 – 2.66 (m, 2 H) 2.71 (dd, $J=7.65, 3.35$ Hz, 2 H) 2.80 – 2.94 (m, 1 H) 3.52 (ddd, $J=7.42, 3.59, 3.35$ Hz, 1 H) 5.57 – 5.65 (m, 1 H) 5.65 – 5.74 (m, $J=5.80, 2.15, 2.00$, 2.00 Hz, 1 H) 7.15 – 7.37 (m, 5 H).

***N*-((1*S*^{*},2*S*^{*})-2-Benzylcyclopent-3-enyl)hydroxylammonium *p*-toluenesulfonate ((±)-5a Tosylate)**

Compound (±)-**4c** (95 mg, 0.33 mmol) was subjected to the conditions described above. The crude residue was dissolved in diethyl ether (5 mL) and treated with a saturated solution of anhydrous *p*-toluenesulfonic acid in diethyl ether (3 mL). The solution was stirred for 5 minutes at which point a white precipitate formed. The precipitate was filtered and washed with diethyl ether to yield **5a** (70 mg, 0.19 mmol, 56%) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 2.39 (s, 3 H) 2.56 (dd, $J=13.70, 9.42$ Hz, 1 H) 2.59 – 2.76 (m, 2 H) 2.92 (dd, $J=13.27, 5.56$ Hz, 1 H) 3.37 (br. s., 1 H) 3.74 – 3.88 (m, 1 H) 5.48 – 5.56 (m, 1 H) 5.56 – 5.63 (m, 1 H) 7.04 – 7.12 (m, 2 H) 7.16 – 7.24 (m, 5 H) 7.74 (d, $J=8.13$ Hz, 2 H) 10.78 (dd, 2 H).

***N*-Hydroxy-*N*-((1*S*^{*},2*S*^{*})-2-(3-phenoxybenzyl)cyclopent-3-enyl)formamide ((±)-6a)**

Compound (±)-**5b** (150 mg, 0.53 mmol) and 2,2,2-trifluoroethyl formate (341 mg, 2.66 mmol) were dissolved in MTBE (3 mL) and heated to reflux for 5 hours. The solution was concentrated *in vacuo* and the residue was purified by flash chromatography (hexanes : EtOAc 7:3) to give (±)-**6a** (42 mg, 0.14 mmol, 25%) as a yellow semi-solid. ¹H NMR (500 MHz, CDCl₃) δ 2.50 – 2.62 (m, 1 H) 2.67 (dd, $J=13.56, 7.58$ Hz, 1 H) 2.75 (dd, $J=13.56, 7.58$ Hz, 1 H) 2.76 – 2.84 (m, 1 H) 3.30 – 3.43 (m, 1 H) 3.96 (ddd, $J=8.92, 6.60$ Hz, 1 H) 5.60 – 5.66 (m, 1 H) 5.70 (dq, $J=6.23, 2.18$ Hz, 1 H) 6.82 – 6.90 (m, 2 H) 6.93 (d, $J=7.58$ Hz, 1 H) 6.97 – 7.04 (m, 2 H) 7.11 (t, $J=7.38$ Hz, 1 H) 7.25 (t, 1 H) 7.29 – 7.38 (m, 2 H) 7.51 (s, 1 H) 9.36 (br. s., 1 H); ¹³C NMR (126 MHz, CDCl₃) δ 35.47, 39.93, 48.51, 64.34, 117.10, 119.16, 119.76, 123.51, 124.15, 128.71, 130.03, 132.73, 141.33, 155.77, 157.40, 157.68; HRMS [FAB, MH⁺] calcd for C₁₉H₁₉NO₃ 310.1443, found 310.1459. IR (thin film) 3058 (br), 2922, 1667, 1581, 1486, 1251, 1213 cm⁻¹.

***N*-Hydroxy-*N*-((1*S*^{*},2*S*^{*})-2-(3-phenoxybenzyl)cyclopent-3-enyl)acetamide ((±)-6b)**

Compound (±)-**5b** (175 mg, 0.62 mmol) and Et₃N (436 μL, 3.1 mmol) were dissolved in CH₂Cl₂ (5 mL) and acetic anhydride (147 μL, 1.55 mmol) was added slowly. The reaction was stirred at room temperature overnight at which point TLC (hexanes : EtOAc 4:1) shows two products. The solution was concentrated *in vacuo* and the residue was dissolved in THF (3 mL) and treated with a 1M NaOH solution (2 mL). The reaction was monitored by TLC (hexanes : EtOAc 7:3) until the two products from the first step coalesced (*ca.* 2h). The solution was acidified with a 10% citric acid solution and extracted into EtOAc (2 × 10mL). The organic layers were combined, washed with brine, dried with anh. Na₂SO₄, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexanes : EtOAc 7:3) to give (±)-**6b** (120 mg, 0.371 mmol, 60%) as a reddish-brown oil. ¹H NMR (300 MHz, DMSO-*d*₆) δ 1.97 (s, 3 H) 2.30 – 2.45 (m, 2 H) 2.54 (br. s., 1 H) 2.76 (dd, $J=13.51, 5.38$ Hz, 1 H) 3.02 – 3.21 (m, 1 H) 4.74 – 4.94 (m, 1 H) 5.48 – 5.57 (m, 1 H) 5.60 – 5.69 (m, 1 H) 6.76 – 6.85 (m, 1 H) 6.86 – 6.92 (m, 1 H) 6.93 – 7.04 (m, 3 H) 7.06 – 7.16 (m, 1 H) 7.28 (t, $J=7.89$ Hz, 1 H) 7.33 – 7.43 (m, 2 H) 9.55 (s, 1 H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ

20.78, 34.09, 38.72, 39.76, 47.98, 58.83, 116.36, 118.24, 119.62, 123.14, 124.46, 128.90, 129.65, 129.97, 132.50, 142.17, 156.23, 156.95; HRMS [FAB, MH⁺] calcd for C₂₀H₂₁NO₃ 324.1600, found 324.1614. IR (thin film) 3200 (br), 3061, 2918, 1582, 1487, 1445, 1250, 1213, 1163 cm⁻¹.

***N*-Hydroxy-*N*-((1*S**,2*S**)-2-(3-phenoxybenzyl)cyclopent-3-enyl)benzamide ((±)-6c)**

Compound (±)-**5b** (100 mg, 0.35 mmol) and Et₂*i*-PrN (75 μL, 0.427 mmol) were dissolved in CH₂Cl₂ (3 mL) and benzyl chloride (45 μL, 0.39 mmol) was added slowly. The reaction was stirred at room temperature for 4 hours at which point TLC (hexanes : EtOAc 4:1) shows two products. The solution was concentrated *in vacuo* and the residue was dissolved in THF (1 mL) and treated with a 1M NaOH solution (1 mL). The reaction was monitored by TLC (hexanes : EtOAc 4:1) until the two products from the first step coalesced (*ca.* 2h). The solution was acidified with a 10% citric acid solution and extracted into EtOAc (2×10mL). The organic layers were washed with brine, dried with anh. Na₂SO₄, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexanes : EtOAc 4:1) to give (±)-**6c** (72 mg, 0.19 mmol, 52%) as an orange oil. ¹H NMR (600 MHz, CDCl₃) δ 2.49 (dd, *J*=13.94, 8.07 Hz, 1 H) 2.53 (ddd, *J*=16.73, 8.80, 2.05 Hz, 1 H) 2.62 (dd, *J*=13.65, 6.90 Hz, 1 H) 2.78 – 2.83 (m, *J*=16.73, 6.35, 2.19, 2.19, 2.19 Hz, 1 H) 3.50 – 3.56 (m, *J*=12.51, 8.03, 2.20, 2.20 Hz, 1 H) 4.34 (dd, *J*=8.22, 6.46 Hz, 1 H) 5.59 (dq, *J*=6.16, 2.05 Hz, 1 H) 5.67 (dq, *J*=6.24, 2.23 Hz, 1 H) 6.67 (t, *J*=1.91 Hz, 1 H) 6.70 (d, *J*=7.63 Hz, 1 H) 6.83 (ddd, *J*=8.07, 2.49, 0.88 Hz, 1 H) 6.97 – 6.99 (m, 2 H) 7.11 (tt, *J*=7.37, 1.14 Hz, 1 H) 7.17 (t, *J*=7.92 Hz, 1 H) 7.33 – 7.36 (m, 4 H) 7.39 – 7.42 (m, 2 H) 7.46 – 7.49 (m, 1 H) 8.47 (br. s., 1 H); ¹³C NMR (151 MHz, CDCl₃) δ 35.77, 39.39, 48.48, 64.36, 116.72, 118.73, 119.48, 123.16, 123.83, 127.71, 128.29, 128.64, 129.63, 129.71, 130.85, 132.60, 141.09, 157.13, 157.17; HRMS [FAB, MH⁺] calcd for C₂₅H₂₃NO₃ 386.1756, found 386.1744. IR (thin film) 3200 (br), 3059, 2920, 1718, 1580, 1486, 1446, 1252, 1214 cm⁻¹.

4-(Hydroxy((1*S,2*S**)-2-(3-phenoxybenzyl)cyclopent-3-enyl)amino)-4-oxobutanoic acid ((±)-6d)**

Compound (±)-**5b** (150 mg, 0.53 mmol) and succinic anhydride (53 mg, 0.53 mmol) were dissolved in CH₂Cl₂ (3 mL) and stirred at room temperature for 4 hours. The solution was concentrated *in vacuo* and the residue was dissolved in THF (1 mL) and treated with a 1M NaOH solution (1 mL). The solution was acidified with a 10% citric acid solution and extracted into EtOAc (2×10mL). The organic layers were washed with brine, dried with anh. Na₂SO₄, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexanes : EtOAc 3:2) to give (±)-**6d** (21 mg, 0.055 mmol, 10%) as a yellow semi-solid. ¹H NMR (500 MHz, DMSO-*d*₆) δ 2.00 – 2.10 (m, 1 H) 2.26 (t, *J*=6.98, 6.18 Hz, 2 H) 2.40 (d, *J*=6.98, 6.18 Hz, 2 H) 2.51 – 2.58 (m, 1 H) 2.73 – 2.81 (m, 2 H) 3.95 – 4.03 (m, *J*=7.58, 7.58, 5.88, 5.68 Hz, 1 H) 5.50 – 5.58 (m, 1 H) 5.62 – 5.68 (m, 1 H) 6.81 (dd, *J*=7.98, 2.39 Hz, 1 H) 6.83 – 6.87 (m, 1 H) 6.94 – 7.01 (m, 3 H) 7.12 (t, *J*=7.38 Hz, 1 H) 7.28 (t, *J*=7.88 Hz, 1 H) 7.38 (t, *J*=7.78 Hz, 2 H) 7.99 (d, *J*=7.78 Hz, 1 H); ¹H NMR (500 MHz, CDCl₃) δ 2.13 (ddd, *J*=17.40, 4.54, 1.99 Hz, 1 H) 2.35 – 2.46 (m, 2 H) 2.59 – 2.64 (m, *J*=12.96, 8.17 Hz, 1 H) 2.63 – 2.69 (m, 2 H) 2.71 – 2.80 (m, 2 H) 2.83 (dd, *J*=12.96, 6.18 Hz, 1 H) 4.22 – 4.29 (m, 1 H) 5.59 – 5.61 (m, 1 H) 5.68 – 5.71 (m, 1 H) 5.87 (d, *J*=7.98 Hz, 1 H) 6.83 – 6.86 (m, 2 H) 6.93 (d, *J*=7.58 Hz, 1 H) 6.99 (d, *J*=7.58 Hz, 2 H) 7.10 (t, *J*=7.48 Hz, 1 H) 7.21 – 7.26 (m, 1 H) 7.30 – 7.39 (m, 2 H); ¹³C NMR (126 MHz, CDCl₃) δ 30.14, 30.97, 39.68, 40.06, 54.49, 55.12, 116.91, 118.95, 119.78, 123.40, 124.32, 129.31, 129.86, 129.99, 132.92, 142.22, 157.32, 157.53, 172.26, 176.05; HRMS [FAB, MH⁺] calcd for C₂₂H₂₃NO₅ 382.1654, found 382.1657. IR (thin film) 3200 (br), 3059, 2923, 2852, 1713, 1581, 1250, 1213, 1163 cm⁻¹.

1-Hydroxy-1-((1S*,2S*)-2-(3-phenoxybenzyl)cyclopent-3-enyl)urea ((±)-7a)

Compound (±)-**5b** (100 mg, 0.355 mmol) and trimethylsilylisocyanate (41 mg, 0.335 mmol) were dissolved in anhydrous THF (2 mL) and allowed to stir at room temperature for 12 hours. The reaction was concentrated and the residue purified by flash chromatography to give (±)-**7a** (55 mg, 0.17 mmol, 48%) as a yellow semi-solid. ¹H NMR (600 MHz, CDCl₃) δ 2.46 – 2.52 (m, 3 H) 2.88 (dd, *J*=13.50, 5.28 Hz, 1 H) 3.08 – 3.23 (m, 1 H) 4.66 (q, *J*=7.43 Hz, 1 H) 5.50 (br. s., 2 H) 5.51 – 5.57 (m, 1 H) 5.61 (dt, *J*=4.11, 2.05 Hz, 1 H) 6.79 (dd, *J*=7.78, 2.20 Hz, 1 H) 6.88 (d, *J*=2.05 Hz, 1 H) 6.92 (d, *J*=7.63 Hz, 1 H) 6.95 – 7.00 (m, 2 H) 7.04 – 7.11 (m, *J*=7.41, 7.41, 1.17, 1.03 Hz, 1 H) 7.19 (t, *J*=7.78 Hz, 1 H) 7.27 – 7.35 (m, 2 H) 8.31 (br. s., 1 H); ¹³C NMR (151 MHz, CDCl₃) δ 34.16, 40.15, 48.81, 62.81, 116.45, 118.57, 119.79, 123.08, 124.27, 128.91, 129.50, 129.71, 132.54, 142.53, 156.85, 157.31, 162.05; HRMS [FAB, MH⁺] calcd for C₁₉H₂₀N₂O₃ 325.1552, found 325.1542.

1-Hydroxy-3-methyl-1-((1S*,2S*)-2-(3-phenoxybenzyl)cyclopent-3-enyl)urea ((±)-7b)

Compound (±)-**5b** (150 mg, 0.53 mmol) and methyl isocyanate (33 mg, 0.59 mmol) were dissolved in acetonitrile (3 mL) and stirred at room temperature overnight. The solution was concentrated and the residue was purified by flash chromatography (hexanes : EtOAc 1:1) to give (±)-**7b** (36 mg, 0.11 mmol, 20%) as a yellow semi-solid. ¹H NMR (500 MHz, CDCl₃) δ 2.43 – 2.54 (m, 3 H) 2.78 (d, *J*=4.79 Hz, 3 H) 2.92 (dd, *J*=13.46, 5.08 Hz, 1 H) 3.04 – 3.13 (m, 1 H) 4.61 – 4.72 (m, 1 H) 5.52 (dq, *J*=6.13, 1.94 Hz, 1 H) 5.59 – 5.68 (m, 1 H) 5.95 – 6.04 (m, 1 H) 6.80 (dd, *J*=8.08, 1.69 Hz, 1 H) 6.83 – 6.88 (m, 1 H) 6.93 (d, *J*=7.58 Hz, 1 H) 6.95 – 7.00 (m, 2 H) 7.03 (br. s., 1 H) 7.06 – 7.12 (m, 1 H) 7.21 (t, *J*=7.88 Hz, 1 H) 7.28 – 7.36 (m, 2 H); ¹³C NMR (126 MHz, CDCl₃) δ 27.01, 34.08, 40.55, 49.17, 64.20, 116.65, 118.81, 119.93, 123.37, 124.65, 129.38, 129.73, 129.99, 132.77, 143.01, 157.05, 157.54, 162.04; HRMS [FAB, MH⁺] calcd for C₂₀H₂₂N₂O₃ 339.1709, found 339.1725. IR (thin film) 3190 (br), 2922, 1641, 1581, 1534, 1486, 1251, 1214, 1162 cm⁻¹.

1-Hydroxy-3,3-dimethyl-1-((1S*,2S*)-2-(3-phenoxybenzyl)cyclopent-3-enyl)urea ((±)-7c)

Compound (±)-**5b** (150 mg, 0.53 mmol) and Et₂*i*-PrN (113 μL, 0.64 mmol) were dissolved in CH₂Cl₂ (4 mL) and *N,N*-dimethylcarbamoyl chloride (63 mg, 0.59 mmol) was added slowly. The reaction was stirred at room temperature for 4 hours and concentrated *in vacuo*. The residue was purified by flash chromatography (hexanes : EtOAc 3:2) to give (±)-**7c** (81 mg, 0.23 mmol, 43%) as a yellow viscous oil. ¹H NMR (500 MHz, CDCl₃) δ 2.42 – 2.49 (m, *J*=17.02, 8.70, 2.26 Hz, 1 H) 2.57 – 2.63 (m, 1 H) 2.61 (dd, *J*=13.36, 7.98 Hz, 1 H) 2.74 (dd, *J*=13.46, 6.88 Hz, 1 H) 2.84 (s, 6 H) 3.27 – 3.32 (m, 1 H) 3.78 (ddd, *J*=8.62, 5.36 Hz, 1 H) 5.61 (dddd, *J*=6.11, 2.09 Hz, 1 H) 5.68 (dddd, *J*=6.13, 2.14 Hz, 1 H) 6.55 (br. s., 1 H) 6.82 – 6.89 (m, 2 H) 6.95 (d, *J*=7.58 Hz, 1 H) 6.98 – 7.03 (m, 2 H) 7.09 – 7.13 (m, 1 H) 7.25 (t, *J*=7.78 Hz, 1 H) 7.34 (dd, *J*=8.57, 7.38 Hz, 2 H); ¹³C NMR (126 MHz, CDCl₃) δ 33.51, 37.23, 40.28, 48.42, 67.34, 116.52, 118.71, 119.58, 123.14, 124.03, 128.77, 129.53, 129.68, 132.85, 142.03, 157.11, 165.73; HRMS [FAB, MH⁺] calcd for C₂₁H₂₄N₂O₃ 353.1865, found 353.1861. IR (thin film) 3270 (br), 2923, 1581, 1487, 1444, 1396, 1251, 1214, 1163 cm⁻¹.

Benzyl hydroxy((1S*,2S*)-2-(3-phenoxybenzyl)cyclopent-3-enyl)carbamate ((±)-7d)

Compound (±)-**5b** (100 mg, 0.36 mmol) and Et₂*i*-PrN (75 μL, 0.43 mmol) were dissolved in CH₂Cl₂ (3 mL) and benzyl chloroformate (55 μL, 0.39 mmol) was added slowly. The reaction was stirred at room temperature for 4 hours at which point TLC (hexanes : EtOAc 4:1) shows two products. The solution was concentrated *in vacuo* and the residue was dissolved in THF (1 mL) and treated with a 1M NaOH solution (1 mL). The reaction was monitored by TLC (hexanes : EtOAc 4:1) until the two products from the first step coalesced (*ca.* 2h). The solution was acidified with a 10% citric acid solution and extracted

into EtOAc (2x10mL). The organic layers were washed with brine, dried with anhydrous Na_2SO_4 , and concentrated *in vacuo*. The residue was purified by flash chromatography (hexanes : EtOAc 4:1) to give ($\pm$)-**7d** (95 mg, 0.23 mmol, 64%) as a clear, pale yellow oil. ^1H NMR (600 MHz, CDCl_3) δ 2.42 (dddd, $J=16.43, 8.80, 2.05$ Hz, 1 H) 2.49 – 2.55 (m, 1 H) 2.54 (dd, $J=13.65, 8.36$ Hz, 1 H) 2.71 (dd, $J=13.65, 6.31$ Hz, 1 H) 3.15 – 3.22 (m, $J=10.69, 6.44, 4.37, 2.16, 2.16$ Hz, 1 H) 4.45 (ddd, $J=8.80, 6.90$ Hz, 1 H) 5.05 (d, $J=12.32$ Hz, 1 H) 5.09 (d, $J=12.32$ Hz, 1 H) 5.50 (dq, $J=6.16, 2.05$ Hz, 1 H) 5.58 (dq, $J=6.24, 2.23$ Hz, 1 H) 5.99 (br. s., 1 H) 6.74 – 6.76 (m, $J=7.92, 2.35, 0.88$ Hz, 1 H) 6.77 (t, $J=1.76$ Hz, 1 H) 6.82 (d, $J=7.92$ Hz, 1 H) 6.89 – 6.92 (m, 2 H) 7.02 (tt, $J=7.45, 1.06$ Hz, 1 H) 7.11 (t, $J=7.78$ Hz, 1 H) 7.23 – 7.31 (m, 7 H); ^{13}C NMR (151 MHz, CDCl_3) δ 34.47, 39.86, 48.47, 63.39, 68.10, 116.62, 118.66, 119.59, 123.06, 124.02, 128.16, 128.37, 128.58, 128.68, 129.48, 129.69, 132.60, 135.76, 141.96, 156.74; HRMS [FAB, MH^+] calcd for $\text{C}_{26}\text{H}_{25}\text{NO}_4$ 415.1784, found 415.1768. IR (thin film) 3267 (br), 3062, 2921, 1696, 1581, 1486, 1445, 1250, 1214, 1100 cm^{-1} .

Methyl (1S*,2S*)-2-(3-phenoxybenzyl)cyclopent-3-enylcarbamate (($\pm$)-**8**)

Compound ($\pm$)-**4a** (100 mg, 0.3 mmol) in MeOH (2 mL) was added to a solution of TiCl_3 (757 mg, 1.47 mmol; 10% wt in 20–30% wt hydrochloric acid) and saturated aqueous Rochelle salt (4 mL). The pH was adjusted to 6.5 with the addition of 1M aqueous HCl or 10% aqueous NaHCO_3 as needed for 2 hours. The reaction was filtered through Celite and concentrated *in vacuo*. The residue was purified by flash chromatography to give ($\pm$)-**8** (40 mg, 0.12 mmol, 42%) as a clear film. ^1H NMR (600 MHz, CDCl_3) δ 2.10 – 2.20 (m, 1 H) 2.59 (dd, $J=13.21, 8.80$ Hz, 1 H) 2.69 – 2.81 (m, 2 H) 2.84 – 2.94 (m, 1 H) 3.65 (s, 3 H) 4.03 (br. s., 1 H) 4.73 (br. s., 1 H) 5.57 – 5.61 (m, 1 H) 5.64 – 5.72 (m, 1 H) 6.82 – 6.89 (m, 2 H) 6.93 – 6.97 (m, 1 H) 6.98 – 7.02 (m, 2 H) 7.08 – 7.12 (m, 1 H) 7.23 – 7.27 (m, 1 H) 7.31 – 7.37 (m, 2 H); ^{13}C NMR (151 MHz, CDCl_3) δ 29.70, 39.67, 39.74, 51.96, 54.35, 56.22, 116.65, 118.65, 119.55, 123.03, 124.08, 128.93, 129.54, 129.68, 132.59, 142.11, 157.03; HRMS [FAB, MH^+] calcd for $\text{C}_{20}\text{H}_{21}\text{NO}_3$ 324.1600, found 324.1607.

Cellular Enzyme Translocation Study

All measurements were performed by using a four channel Zeiss LSM 510 laser scanning confocal microscope. A 63 $\times$, 1.2 numerical aperture water immersion objective was used for all experiments. For *in vivo* measurements of 5-LOX activity, transient transfection of GFP-tagged 5-LO wild type and mutants were used. 5-LOX activity was determined by DPPP (Diphenyl-1-pyrenylphosphene) fluorescence probe. Expression levels of the protein were monitored with 488Ar-Ne laser line. Cells with similar expression level were selected based the GFP signal. DPPP was excited with 345 UV laser and emission was measured at 390 nm band pass filter. 30 min prior to imaging, transient transfected cells were treated with 1.5 μM (DPPP) in the presence of DMEM medium. Cells were then washed with Hank's Balance Salt Solution (HBSS) containing 1 mM EGTA. 5-LOX activation was induced by the addition of 2–10 μM ionomycin and 5 μM exogenous arachidonic acid or ionomycin+AA +inhibitor, and DPPP signal was monitored. In control experiments, non-transfected cells were treated with ionomycin and arachidonic acid and DPPP signal was monitored with the time. Signal saturation was achieved upon addition of 1 μM cumene hydroperoxide to the cells to determine relative activity. Regions of interest on the perinuclear membrane were defined, and the fluorescence intensity at given time was measured for at least five cells and averaged (F). The fluorescence intensity at saturation level (Fmax) was determined together with the control cell (Fmin). 5-LOX activity was then calculated by the following formula: Activity=(F-Fmin)/(Fmax-Fmin). Data was analyzed by Zeiss biophysical software package. Five sets of data were used from different transfected cells and activity profile was plotted versus time. The translocation pattern of the protein was monitored using signal from EGFP which was detected from 505 nm long pass filter.

In Vivo Tumor Inhibition Study

Animals—All animal use protocols were approved by the University of Notre Dame Institutional Animal Care and Use Committee. Male C57Bl6/J APC^(Min/+) mice were purchased from Jackson Laboratories (Bar Harbor, Maine). Mice were housed in individually ventilated cages with hardwood chip bedding, and provided ad libitum access to fresh water and food (Teklad 2014 Rodent Maintenance Diet, Teklad, Inc., Madison, WI). Mice were acclimated for 7 days prior to initiation of experimental use.

Study design—At 60 days of age the mice were randomized into groups of six to receive either vehicle (olive oil), 0.10 mg of sulindac, 1.0 mg of sulindac, 0.10 mg of (±)-**4a**, or 1.0 mg of (±)-**4a** daily, divided between two daily doses. Each dose was administered as a 0.25 ml bolus given perorally via a 3 ½ French tomcat catheter (Sherwood Medical, St. Louis, Missouri). Mice were dosed for 30 days and euthanized by carbon dioxide narcosis at 90 days of age. Gross necropsy was performed and the intestinal tracts were incised along their lengths and fixed in formalin. The number of grossly visible tumors was determined for each intestinal tract using a dissecting microscope (40 × magnification). Tissues were then processed and embedded in paraffin. Serial sections of rolled intestine were cut and stained with Hematoxylin-eosin (H & E) for pathological evaluation by light microscopy.

Histological classification of intestinal lesions was performed using an established system (Boivin, 2003). Lesions were classified as either gastrointestinal intraepithelial neoplasia (GIN); adenoma; or carcinoma. GIN lesions were distinguished by elongation and crowding of crypt cells having hyperchromatic nuclei and varying degrees of nuclear atypia. Adenomas were characterized by pronounced cytologic atypia with a large portion of the lesion composed of branching tubules within the lamina propria or finger-like processes covered by lamina propria covered by epithelium, often pedunculated. Carcinomas were characterized by neoplastic glandular epithelium penetrating through the muscularis mucosa. The number of each type of lesion was enumerated for each intestinal tract; and as a means to evaluate progression of neoplasia from GIN to adenoma, the mean ratio of GIN:adenoma was calculated for each group and compared between groups.

Acknowledgments

We would like to thank Dr. Bill Boggess (University of Notre Dame) and Nonka Sevova (University of Notre Dame) for mass spectroscopic analyses, and Dr. Jaroslav Zajicek (University of Notre Dame) for NMR assistance. We acknowledge the NIH (GM068012 at Notre Dame and GM52598 at UIC) for support of this work.

Notes and references

1. Charlier C, Michaux C. Eur. J. Med. Chem. 2003; 38:645–659. [PubMed: 12932896]
2. Kontogiorgis CA, Hadjipavlou-Litini DJ. Curr. Med. Chem. 2002; 9:89. [PubMed: 11860351]
3. Allison M, Howatson A, Torrance C, Lee F, Russell R. N. Engl. J. Med. 1992; 327:749–754. [PubMed: 1501650]
4. Salinas G, Rangasetty UC, Uretsky BF, Birnbaum Y. J. Cardiovasc. Pharmacol. Ther. 2007; 12:98–111. [PubMed: 17562780]
5. Hinz B, Brune K. Trends in Pharmacol. Sci. 2008; 29:391–397. [PubMed: 18606461]
6. Young RN. Euro. J. Med. Chem. 1999; 34:671–685.
7. Peters-Golden M, Henderson WR Jr. N. Engl. J. Med. 2007; 357:1841–1854. [PubMed: 17978293]
8. Rubin P, Mollison KW. Prostaglandins. 2007; 83:188–197.
9. Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, Ichimasa M, Sugamura K, Nakamura M, Takano S, Nagata K. J. Exp. Med. 2001; 193:255–262. [PubMed: 11208866]

10. Matsuoka T, Hirata M, Tanaka H, Takahashi Y, Murata T, Kabashima K, Sugimoto Y, Kobayashi T, Ushikubi F, Aze Y, Eguchi N, Urade Y, Yoshida N, Kimura K, Hizoguchi A, Honda Y, Nagai H, Narumiya S. *Science*. 2000; 287:2013. [PubMed: 10720327]
11. Barnes PJ. *Nat. Rev. Drug Discov*. 2004; 3:831–844. [PubMed: 15459674]
12. Barnes PJ. *Br. J. Pharmacol*. 2006; 147(Suppl 1):S297–S303. [PubMed: 16402117]
13. Heath CW Jr, Thun MJ, Greenberg ER, Levin B, Marnett LJ. *Cancer*. 1994; 74:2885–2888. [PubMed: 7954252]
14. Marnett LJ. *Cancer Res*. 1992; 52:5575–5589. [PubMed: 1394181]
15. Steele VE, Holmes CA, Hawk ET, Kopelovich L, Lubet RA, Crowell JA, Sigman CC, Kelloff GJ. *Cancer Epidemiol. Biomarkers Prev*. 1999; 8:467–483. [PubMed: 10350444]
16. Shureiqi I, Lippman SM. *Cancer Res*. 2001; 61:6307–6312. [PubMed: 11522616]
17. Vogt PF, Miller MJ. *Tetrahedron*. 1998; 54:1317–1348.
18. Surman MD, Mulvihill MJ, Miller MJ. *J. Org. Chem*. 2002; 67:4115–4121. [PubMed: 12054945]
19. Bäckvall JE, Sellen M, Grant B. *J. Am. Chem. Soc*. 1990; 112:6615–6621.
20. Bäckvall JE, Sellen M. *J. Chem. Soc. Chem. Commun*. 1987:827–829.
21. Bäckvall J, Persson ESM, Bombrun A. *J. Org. Chem*. 1994; 59:4126–4130.
22. Persson E, Bäckvall J. *Acta Chem. Scand*. 1995; 49:899.
23. Charlier C, Henichart JP, Durant F, Wouters J. *J. Med. Chem*. 2006; 49:186–195. [PubMed: 16392803]
24. Greene, TW.; Wuts, PGM. *Protective Groups in Organic Synthesis*. Wiley-Interscience; 1999. p. 779
25. Inglesi M, Inglesi M. *Farmaco*. 1990; 45:1327. [PubMed: 2090142]
26. Bernhart C, Bernhart C. *Euro. J. Med. Chem*. 1976; 11:369.
27. Sparks SM, Vargas JD, Shea KJ. *Org. Lett*. 2000; 2:1473–1475. [PubMed: 10814476]
28. Yoda R, Yoda R. *Chem. Pharm. Bull*. 1994; 42:686.
29. Olah GA, Narang SC, Gupta BGB, Malhotra R. *J. Org. Chem*. 1979; 44:1247–1251.
30. Phillips OA, Udo EE, Ali AAM, Al-Hassawi N. *Bioorg. Med. Chem*. 2003; 11:35–41. [PubMed: 12467705]
31. Zayia GH. *Org. Lett*. 1999; 1:989–991.
32. Hill DR, Hsiao C, Kurukulasuriya R, Wittenberger SJ. *Org. Lett*. 2002; 4:111–113. [PubMed: 11772103]
33. Satoh Y, Powers C, Toledo LM, Kowalski TJ, Peters PA, Kimble EF. *J. Med. Chem*. 1995; 38:68–75. [PubMed: 7837242]
34. Mattingly PG, Miller MJ. *J. Org. Chem*. 1980; 45:410–415.
35. Gennari C, Schimperna G, Venturini I. *Tetrahedron*. 1988; 44:4221–4232.
36. Axelrod, B.; Cheesbrough, TM.; Laakso, S. [53] Lipoxygenase from soybeans : EC 1.13.11.12 Linoleate:oxygen oxidoreductase. Lowenstein, John M., editor. Vol. Vol. Volume 71. Academic Press; 1981. p. 441-451. *Methods in Enzymology*
37. Kristie D, Thompson JE. *Phytochemistry*. 1989; 28:2577–2581.
38. Waslidge NB, Hayes DJ. *Anal. Biochem*. 1995; 231:354–358. [PubMed: 8594985]
39. Auerbach BJ, Kiely JS, Cornicelli JA. *Anal. Biochem*. 1992; 201:375–380. [PubMed: 1632527]
40. Broadwater, J.; Khine, HH.; Woodruff, K. U.S. Patent Application 20,090,253,158. 2009 Oct 8.
41. Kratky D, Lass A, Abuja PM, Esterbauer H, Kühn H. *Biochim. Biophys. Acta, Mol. Cell Biol Lipids*. 1999; 1437:13–22.
42. Anthon GE, Barrett DM. *J. Agric. Food Chem*. 2001; 49:32–37. [PubMed: 11170556]
43. Pufahl RA, Kasten TP, Hills R, Gierse JK, Reitz BA, Weinberg RA, Masferrer JL. *Anal. Biochem*. 2007; 364:204–212. [PubMed: 17376394]
44. Cho YS, Kim HS, Kim CH, Cheon HG. *Anal. Biochem*. 2006; 351:62–68. [PubMed: 16442488]
45. Bou R, Codony R, Tres A, Decker EA, Guardiola F. *Anal. Biochem*. 2008; 377:1–15. [PubMed: 18358821]
46. Robinson BS, Rathjen DA, Trout NA, Easton CJ, Ferrante AJ. *Immunol*. 2003; 171:4773–4779.

47. Carter G, Young P, Albert D, Bouska J, Dyer R, Bell R, Summers J, Brooks D. *J Pharmacol Exp Ther.* 1991; 256:929–937. [PubMed: 1848634]
48. Bell R, Bouska J, Malo P, Lanni C, Harris R, Otis E, Stewart A, Brooks D, Carter G. *J. Pharmacol. Exp. Ther.* 1995; 272:724–731. [PubMed: 7853186]
49. Brooks CD, Stewart AO, Basha A, Bhatia P, Ratajczyk JD, Martin JG, Craig RA, Kolasa T, Bouska JB, Lanni C. *J. Med. Chem.* 1995; 38:4768–4775. [PubMed: 7490726]
50. Chen X, Zhang Y, Funk CD. *J. Biol. Chem.* 1998; 273:31237–31244. [PubMed: 9813031]
51. Woods JW, Coffey MJ, Brock TG, Singer II, Peters-Golden M. *J. Clin. Invest.* 1995; 95:2035–2046. [PubMed: 7738170]
52. Murphy RC, Gijón MA. *Biochem J.* 2007; 405:379–395. [PubMed: 17623009]
53. Moser AR, Pitot HC, Dove WF. *Science.* 1990; 247:322–324. [PubMed: 2296722]
54. Su L, Kinzler KW, Vogelstein B, Preisinger AC, Moser AR, Luongo C, Gould KA, Dove WF. *Science.* 1992; 256:668–670. [PubMed: 1350108]
55. Gutierrez LS, Suckow M, Lawler J, Ploplis VA, Castellino FJ. *Carcinogenesis.* 2003; 24:199–207. [PubMed: 12584168]
56. Bertagnolli MM. *Ann. N. Y. Acad. Sci.* 1999; 889:32–44. [PubMed: 10668480]
57. Paoni NF, Feldman MW, Gutierrez LS, Ploplis VA, Castellino FJ. *Physiol. Genomics.* 2003; 15:228–235. [PubMed: 13130079]
58. Narayanan BA, Narayanan NK, Pittman B, Reddy BS. *Clin. Cancer Res.* 2004; 10:7727–7737. [PubMed: 15570007]
59. Jacoby RF, Marshall DJ, Newton MA, Novakovic K, Tutsch K, Cole CE, Lubet RA, Kelloff GJ, Verma A, Moser AR, Dove WF. *Cancer Res.* 1996; 56:710–714. [PubMed: 8631000]
60. Giardiello FM, Hamilton SR, Krush AJ, Piantadosi S, Hyland LM, Celano P, Booker SV, Robinson CR, Offerhaus GJA. *N. Engl. J. Med.* 1993; 328:1313–1316. [PubMed: 8385741]
61. Guillen-Ahlers H, Buechler SA, Suckow MA, Castellino FJ, Ploplis VA. *Carcinogenesis.* 2008; 29:1421–1427. [PubMed: 18499699]
62. Bolger J, Miller M. Manuscript submitted for review.

Fig. 1.
Inhibitors of the 5-LOX pathway

Fig. 2.

Zone 1

Zone 3

Zone 2

Fig. 3.

Fig. 4. Cells transfected with GFP-tagged 5-lipoxygenase after treatment with A) Zileuton and B) (±)-4a. Green fluorescence shows the intracellular location of 5-LOX. 5-LOX is localized in both the cytoplasm and the nucleoplasm of COS-7 cells in the resting state but translocates to the either side of the nuclear membrane upon cell activation. In A, translocation of 5-LOX to the nuclear membrane is complete by 32 seconds, while after 55 seconds 5-LOX remains in the cytoplasm and the nucleoplasm in B.

Fig. 5.
Effect of Sulindac and (±)-**4a** on gastrointestinal tumor numbers in APC^(Min/+) mice

Fig. 6.
GIN:Adenoma ratio in APC^(Min/+) mice treated with Sulindac and (±)-4a

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

Table 1

Regioselective Opening of HDA Cycloadducts

Entry	R ¹	time	R ²	mol% CuCN	Solvent	°C	Yield
1	Me	45	Bn	10	Et ₂ O	25	31
2	<i>t</i> -Bu	180	Bn	10	Et ₂ O	25	50
3	<i>t</i> -Bu	180	Bn	20	Et ₂ O	25	58
4	<i>t</i> -Bu	180	Bn	50	Et ₂ O	25	73
5	Me	180	3-PhOBn	20	Et ₂ O	25	74
6	Me	180	3-PhOBn	50	Et ₂ O	25	79
7	<i>t</i> -Bu	180	3-PhOBn	20	Et ₂ O	25	27
8	<i>t</i> -Bu	180	3-PhOBn	50	Et ₂ O	25	67
9	<i>t</i> -Bu	5	Bn	10	THF	-78	62
10	<i>t</i> -Bu	180	Bn	50	Et ₂ O	-78	97
11	<i>t</i> -Bu	180	3-PhOBn	50	Et ₂ O	78	78

Table 2

Removal of Carbamate

Entry		Conditions	Result
1	(±)-4b	LiOH/THF/H ₂ O	nr
2	(±)-4b	NaOH/THF/H ₂ O	nr
3	(±)-4b	Cs ₂ CO ₃ /MeOH/H ₂ O	nr
4	(±)-4b	HBr/AcOH	Complex mixture
5	(±)-4b	Conc. HCl/EtOAc	nr
6	(±)-4c	TFA/CH ₂ Cl ₂	Complex mixture
7	(±)-4c	HCl/EtOAc	Complex mixture
8	(±)-4c	TFA/2eq anisole/CH ₂ Cl ₂	55% ^a
9	(±)-4c	TFA/2eq Et ₃ SiH/CH ₂ Cl ₂	66%
10	(±)-4d	TFA/4eq Et ₃ SiH/CH ₂ Cl ₂	95%
11	(±)-4c	TMSCl/NaI/CH ₃ CN	Complex mixture

^a Isolated as the tosylate salt.

Table 3

Inhibition of enzymatic activity and translocation

Compound	Inhibition of	
	Enzyme Activity ^a	Membrane Translocation
(±)- 4a	na ^b	Yes
(±)- 6a	+	No
(±)- 6b	+	No
(±)- 6c		not tested
(±)- 6d	–	No
(±)- 7a	na	Yes
(±)- 7b	+	No
(±)- 7c	+++	No
(±)- 7d		not tested
(±)- 8	–	No
Zileuton	++	No

^a –/+/+/+++ qualitative inhibition of lipid peroxide production in reference to Zileuton.

^b not applicable as the compounds showed extensive, multifaceted inhibition (see text)