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Synthesis, Kinetic Characterization and Metabolism of Diastereomeric 2-(1-(4-Phenoxyphenylsulfonyl)ethyl)thiiranes as Potent Gelatinase and MT1-MMP Inhibitors

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Abstract

Gelatinases (MMP-2 and MMP-9) have been implicated in a number of pathological conditions, including cancer and cardiovascular disease. Hence, small molecule inhibitors of these enzymes are highly sought for use as potential therapeutic agents. 2-(4-Phenoxyphenylsulfonylmethyl)thiirane (SB-3CT) has previously been demonstrated to be a potent and selective inhibitor of gelatinases, however, it is rapidly metabolized because of oxidation at the *para* position of the phenoxy ring and at the α -position to the sulfonyl group. α -Methyl variants of SB-3CT were conceived to improve metabolic stability and as mechanistic probes. We describe herein the synthesis and evaluation of these structural variants as potent inhibitors of gelatinases. Two (compounds 5b and 5d) among the four synthetic stereoisomers were found to exhibit slow-binding inhibition of gelatinases and MMP-14 (MT1-MMP), which is a hallmark of the mechanism of this class of inhibitors. The ability of these compounds to inhibit MMP-2, MMP-9, and MMP-14 could target cancer tissues more effectively. Metabolism of the newly synthesized inhibitors showed that both oxidation at the α -position to the sulfonyl group and oxidation at the *para* position of the terminal phenyl ring were prevented. Instead oxidation on the thiirane sulfur is the only biotransformation pathway observed for these gelatinase inhibitors.

Keywords

enzyme inhibition; gelatinase; metabolism

Matrix metalloproteinases (MMPs) constitute a family of 26 zinc-dependent endopeptidases in humans, which are involved in restructuring of the extracellular matrix and processing of multiple bioactive proteins (1). The full scope of their functions is not understood, but they perform critical roles in development and tissue remodeling (2). To maintain tissue homeostasis, MMP activity is strictly regulated at multiple levels. However, deregulation of MMP activity is characteristic of a variety of pathological conditions (3,4). Gelatinases A and B, also known as MMP-2 and MMP-9, respectively, constitute a subclass of MMPs that have emerged as important targets for intervention in several pathological conditions, including cancer and neurological and cardiovascular diseases (5,6). Selective targeting of gelatinases

over those of the other members of the MMP family has long been sought, but few such examples exist (7,8).

Previously, we described the design, synthesis, and biological effects of inhibition of gelatinases by 2-(4-phenoxyphenylsulfonylmethyl)thiirane (compound **1**, also known as SB-3CT). This inhibitor exhibits high potency and selectivity in inhibition of gelatinases *in vitro* (9) and *in vivo* in animal models of cancer metastases (10–12) and stroke (13). An investigation of the metabolism of this compound revealed that it was extensively and rapidly metabolized (14,15), despite the fact that the compound exhibited *in vivo* activity. Compound **1** had low metabolic stability, with 0.5% of the compound remaining following a 15-min incubation with rat liver microsomes (16). Metabolic stability describes the rate and extent to which a molecule is metabolized. A compound that is rapidly and extensively metabolized is referred to as having a low degree of metabolic stability. Low metabolic stability can contribute to undesirable pharmaceutical properties, such as low oral efficacy and/or short duration of action, and can reduce the efficacy of an otherwise potent molecule. Therefore, the ability to evaluate metabolic stability of analogs early in the discovery process improves the odds of selecting a lead with good *in vivo* activity. The major metabolic pathways of **1** observed *in vitro* resulted from oxidation at the α -position to the sulfonyl group resulting in the formation of the sulfinic acid **2**, oxidation at the *para*-position of the phenoxy group (compound **3**) and oxidation at the thiirane ring sulfur (compound **4**) (14). The hydroxylated metabolite **3** was, incidentally, a more potent gelatinase inhibitor than the parent compound **1** (14). Evaluation of the metabolic stability of **3** revealed that 30% of the compound remained after 15-min incubation with rat liver microsomes, with oxidation α to the sulfonyl as the primary pathway of metabolism.

We undertook in this study to prepare analogs of **1** (compounds **5**) with the intention of minimizing metabolism. Reducing the degree of metabolism increases bioavailability and prolongs half-life, which in turn allows lower and less frequent dosing. Our approach was to methylate one of the P450-mediated oxidation sites of the molecule α to the sulfonyl moiety. As disclosed herein, blocking this position by methylation abrogated hydroxylation not only at that site, but also at the terminal phenyl ring. Instead, the only metabolic pathway observed for these compounds was *S*-oxidation of the thiirane moiety.

Methods and Materials

Chemicals and reagents

All organic reagents were purchased from either Sigma-Aldrich Chemical Company (St Louis, MO, USA) or Acros Organics (Geel, Belgium), unless otherwise stated. All reactions were performed under an atmosphere of nitrogen, unless noted otherwise. ^1H and ^{13}C NMR spectra were recorded on a Varian INOVA-500 (Varian Inc., Palo Alto, CA, USA) spectrometer. Thin-layer chromatography was performed with Whatman reagents 0.25 mm silica gel 60-F plates. Flash chromatography was carried out with silica gel 60, 230–400 mesh (0.040–0.063 mm particle size) purchased from EM Science (Gibbstown, NJ, USA). Human recombinant MMP-2 and MMP-9 were prepared as described previously (14). Human recombinant active MMP-7 and catalytic domains of MMP-3 and MMP-14 were purchased from EMD Biosciences (La Jolla, CA, USA). The catalytic domain of human recombinant MMP-1 was from Biomol International (Plymouth Meeting, PA, USA). Fluorogenic substrates were purchased from Peptides International (Louisville, KY, USA), (MOCacPLGL(Dpa)AR-NH₂, MOCacRKPVE(Nva)WRK(Dnp)-NH₂), and (MOCacKPLGL(Dpa)AR-NH₂) were obtained from R&D Systems (Minneapolis, MN, USA). Inhibitor stock solutions (10 mM) were prepared in DMSO. The methodology for enzyme inhibition and assays was the same as reported previously (17). Substrate hydrolysis was measured with a Varian Cary Eclipse fluorescence spectrophotometer. Rat liver microsomes were purchased from BD Biosciences (Woburn, MA, USA).

Microsomal incubations

Incubations consisted of rat liver microsomes (0.5 mg), NADPH (0.5 mM), and 50 μM compound in potassium phosphate buffer (50 μM , pH 7.4) at 37 °C for 20 min in a volume of 500 μL . The reaction was terminated by addition of one volume of acetonitrile. The precipitated protein was centrifuged (3 min, 10 500 $\times$ g) and the supernatant was analyzed by reversed-phase HPLC with UV detection and LC/MS/MS.

HPLC chromatography

The HPLC system consisted of a Perkin-Elmer series 200 quaternary pump (Perkin-Elmer Corp., Norwalk, CT, USA), a Perkin-Elmer UV detector series 200, and a Perkin-Elmer auto sampler series 200. Samples were analyzed on a YMC 5 μm basic 4.6 mm i.d. $\times$ 15 cm column (YMC Inc., Wilmington, NC, USA) connected to a YMC 5 μm basic 4.0 mm i.d. $\times$ 2 cm guard cartridge. The mobile phase consisted of elution at 1 mL/min with 90% A/10% B for 5 min, followed by a 20-min linear gradient to 10% A/90% B, then 10% A/90% B for 10 min (A = 0.1% TFA/water, B = 0.1% TFA/acetonitrile). Effluent was monitored by UV detection at 245 nm.

Mass spectrometry

All mass spectrometric experiments were performed with a Micromass (Beverly, MA, USA) Quattro-LC triple quadrupole (Q1-q-Q3) instrument running MASSLYNX 4.0 software. The Quattro-LC was connected to a Waters (Milford, MA, USA) 2690 HPLC equipped with a Waters 2690 autosampler and a Waters 996 photodiode array detector. For LC/MS and LC/MS/MS experiments, the eluent flow rate of 1 mL/min was reduced by 50% prior to entering the ion source region with a custom-built flow splitter, which consisted of a zero dead volume PEEK tee with identical lengths and diameters of tubing on each outlet. The Quattro-LC Z-spray interface was operated in the ESI mode with the following parameters: capillary voltage = 2.8–3.0 kV, cone voltage = 20–35 V, source temperature = 125–150 °C, desolvation temperature = 150–250 °C, desolvation gas (N₂) flow rate = 430–550 L/h. For MS/MS experiments, the mass spectrometer tune was optimized by directly infusing a solution of compound **1**. Collisionally activated dissociation was employed for all MS/MS experiments by admitting argon gas into the collision cell (q). For product ion scans, argon gas was admitted into q until the parent signal intensity was reduced by ~50% (i.e., single collision conditions), then collision energy (voltage offset between Q1 and Q3) was increased to 10–50 V. The resolving powers of both Q1 and Q3 were reduced from a setting of 15 to 12 to maximize signal intensities, and MS/MS spectra were acquired in the continuum mode of data acquisition at a rate of ≥400 a.m.u/seconds.

S-4-Phenoxyphenyl ethanethioate (6)

The procedure was the same as that reported by Lim *et al.* (18) except that acetyl chloride was used instead of epichlorohydrin. Purification of the crude product by column chromatography (hexanes/EtOAc = 8/1) afforded **6** in 66% yield. ¹H NMR (500 MHz, CDCl₃) δ 7.34–7.42 (m, 4H), 7.15–7.21 (m, 1H), 7.08 (d, *J* = 8.0 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 2H), 2.43 (s, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 194.8, 159.1, 156.2, 136.3, 130.1, 124.3, 121.4, 120.0, 119.0, 30.2; HRMS (FAB) calcd for C₁₄H₁₂O₂S (M⁺) 244.0558, found 244.0544.

(S)-1-(But-3-yn-2-ylsulfonyl)-4-phenoxybenzene ((S)-9)

(*R*)-But-3-yn-2-yl methanesulfonate ((*R*)-**7**) was separately prepared from (*R*)-3-butyn-2-ol. To a cooled solution of (*R*)-3-butyn-2-ol (1.0 g, 14 mmol) in CH₂Cl₂ (30 mL) was added TEA (2.0 mL, 14 mmol). Mesyl chloride (1.0 mL, 14 mmol) was added dropwise and the resulting mixture was stirred under an atmosphere of nitrogen for 1 h in an ice-water bath. It was filtered over silica gel and the solvent was removed *in vacuo*. The mixture was diluted with ether and the solution was filtered through a layer of anhydrous MgSO₄. The solvent was evaporated to afford (*R*)-**7** in quantitative yield. This was used in the next step without further purification.

Compound **6** (1.7 g, 7.0 mmol) was dissolved in 1:1 MeCN/MeOH (30 mL). Potassium carbonate (1.5 g, 11 mmol) was added and the mixture was stirred under an atmosphere of nitrogen for 1 h at room temperature and then cooled in an ice-water bath. Compound (*R*)-**7** (1.0 g, 6.8 mmol) – as prepared above – was added dropwise and the mixture was stirred for 3 h in the ice-water bath. The suspension was filtered and the solvent was evaporated. The concentrate was diluted with water and extracted with EtOAc. The combined organic extracts were dried over anhydrous MgSO₄ and concentrated *in vacuo*. The product (i.e., (*S*)-**8**) was carried on to the next step without further purification.

The sample (*S*)-**8** was dissolved in CH₂Cl₂ (30 mL) and cooled in an ice-water bath. It was treated with *m*-CPBA (3.3 g, 15 mmol) and the resulting white suspension was stirred at room temperature for 1 h. The mixture was filtered and the filtrate was washed with saturated Na₂S₂O₃, followed by saturated NaHCO₃. The organic layer was separated and the aqueous layer was extracted with CH₂Cl₂. The combined organic extracts were dried over anhydrous MgSO₄ and concentrated *in vacuo*. Purification of the product by silica gel chromatography

(hexanes/CH₂Cl₂ = 2/3) gave the title compound as a white solid in 67% yield (1.3 g). Enantiomeric excess was determined by NMR in the presence of a chiral shifting reagent, europium tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate], Eu(hfc)₃. The NMR sample was prepared by adding 1 mg of (*S*)-**9** and 1 mg of Eu(hfc)₃ in 0.7 mL CDCl₃. >90% ee; [α]_D²⁵ -22.8 (*c* 0.33, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.87–7.92 (m, 2H), 7.41–7.46 (m, 2H), 7.23–7.28 (m, 1H), 7.06–7.12 (m, 4H), 3.93 (qd, *J* = 2.5, 7.1 Hz, 1H), 2.41 (d, *J* = 2.4 Hz, 1H), 1.60 (d, *J* = 7.2 Hz, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 163.0, 154.7, 132.1, 130.2, 129.2, 125.2, 120.5, 117.1, 77.5, 75.7, 53.9, 15.1; HRMS (FAB) calcd for C₁₆H₁₅O₃S (*M* + H⁺) 287.0742, found 287.0742.

(*R*)-1-(But-3-yn-2-ylsulfonyl)-4-phenoxybenzene ((*R*))-9)

The synthesis was carried out as described for (*S*)-**9**, using (*S*)-**7**. The ¹H and ¹³C spectra were identical to those of (*S*)-**9**. >90% ee; [α]_D²⁵ +22.8 (*c* 0.33, CHCl₃); HRMS (ESI) calcd for C₁₆H₁₄NaO₃S (*M* + Na⁺) 309.0556, found 309.0538.

(*S*)-1-(But-3-en-2-ylsulfonyl)-4-phenoxybenzene ((*S*))-10)

Compound (*S*)-**9** (1.3 g, 4.6 mmol) was dissolved in EtOAc (30 mL), and was treated with 5% palladium–barium sulfate (0.13 g) and five drops of quinoline. The mixture was stirred under an atmosphere of hydrogen for 3.5 h at room temperature. It was filtered through a layer of Celite and the solution was concentrated under reduced pressure. Column chromatography on silica gel (hexanes/CH₂Cl₂ = 1/7) of the crude compound gave 1.2 g (92%) of the title

compound. [α]_D²⁵ +8.8 (*c* 0.33, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.74–7.79 (m, 2H), 7.39–7.45 (m, 2H), 7.21–7.27 (m, 1H), 7.02–7.10 (m, 4H), 5.83 (ddd, *J* = 7.8, 10.1, 17.4 Hz, 1H), 5.26–5.32 (m, 1H), 5.13 (dd, *J* = 1.1, 17.2 Hz, 1H), 3.70 (quin, 1H), 1.45 (d, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 162.7, 155.1, 131.8, 131.5, 130.4, 125.3, 121.9, 120.6, 117.4, 64.5, 13.3; HRMS (FAB) calcd for C₁₆H₁₇O₃S (*M* + H⁺) 289.0898, found 289.0882.

(*R*)-1-(But-3-en-2-ylsulfonyl)-4-phenoxybenzene ((*R*))-10)

The synthesis was carried out as described for (*S*)-**10**, using (*R*)-**9**. The ¹H and ¹³C spectra were identical to those of (*S*)-**10**. [α]_D²⁵ -9.0 (*c* 0.35, CHCl₃); HRMS (ESI) calcd for C₁₆H₁₆NaO₃S (*M* + Na⁺) 311.0712, found 311.069.

(2*R*)-2-(1*R*-(4-Phenoxyphenylsulfonyl)ethyl)oxirane (11a) and (2*S*)-2-(1*S*-(4-phenoxyphenylsulfonyl)ethyl)oxirane (11b)

A solution of compound (*S*)-**10** (1.1 g, 3.8 mmol) in CH₂Cl₂ (40 mL) was cooled in an ice-water bath and was treated with *m*-CPBA (8.8 g, 39 mmol, 77%). The mixture was stirred for 1 h in the ice-water bath, warmed to room temperature and stirred for 6 days. The suspension was filtered over silica gel and the filtrate was washed with saturated Na₂S₂O₃, followed by saturated NaHCO₃. The organic layer was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. Purification by silica gel chromatography (hexanes/EtOAc = 5/1) gave 0.55 g of **11a** and 0.26 g of **11b** in 70% overall yield. Enantiomeric excess was determined by NMR in the presence of Eu(hfc)₃. The NMR sample was prepared by adding 1 mg of **11a** and 1 mg of Eu

(hfc)₃ in 0.7 mL CDCl₃. For **11a**: >90% ee; [α]_D²⁵ +6.5 (*c* 0.37, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.81–7.85 (m, 2H), 7.41–7.46 (m, 2H), 7.23–7.28 (m, 1H), 7.07–7.11 (m, 4H), 3.14 (ddd, *J* = 2.6, 3.9, 6.8 Hz, 1H), 2.84 (quin, 1H), 2.77–2.80 (m, 1H), 2.45 (dd, *J* = 2.6, 4.6 Hz, 1H), 1.43 (d, *J* = 7.0 Hz, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 162.9, 154.6, 131.2, 130.5, 130.2, 125.3, 120.5, 117.4, 62.9, 50.5, 46.6, 10.9; HRMS (FAB) calcd for C₁₆H₁₇O₄S (*M* + H⁺)

305.0848, found 305.0850. For **11b**: >90% ee; [α]_D²⁵ +6.9 (*c* 0.33, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.84–7.88 (m, 2H), 7.40–7.45 (m, 2H), 7.22–7.26 (m, 1H), 7.07–7.11 (m, 4H), 3.24

(ddd, $J = 2.6, 4.1, 7.0$ Hz, 1H), 3.00 (quin, $J = 7.2$ Hz, 1H), 2.75–2.79 (m, 1H), 2.46 (dd, $J = 2.6, 4.8$ Hz, 1H), 1.37 (d, $J = 7.2$ Hz, 3H); ^{13}C NMR (126 MHz, CDCl_3) δ 162.8, 154.7, 131.2, 130.2, 125.2, 120.5, 117.4, 62.6, 50.0, 44.2, 9.3; HRMS (FAB) calcd for $\text{C}_{16}\text{H}_{17}\text{O}_4\text{S}$ ($\text{M} + \text{H}^+$) 305.0848, found 305.0856.

(2S)-2-(1R-(4-Phenoxyphenylsulfonyl)ethyl)oxirane (11c) and (2R)-2-(1R-(4-phenoxyphenylsulfonyl)ethyl)oxirane (11d)

The synthesis was carried out as described for **11a** and **11b**, using (*R*)-**10**. The ^1H and ^{13}C spectra of **11c** and **11d** were identical to those of **11a** and **11b**, respectively. For **11c**: >90% ee; $[\alpha]_{\text{D}}^{25} -6.2$ (c 0.36, CHCl_3); HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{16}\text{NaO}_4\text{S}$ ($\text{M} + \text{Na}^+$) 327.0662, found 327.0645. For **11d**: >90% ee; $[\alpha]_{\text{D}}^{25} -6.8$ (c 0.35, CHCl_3); HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{16}\text{NaO}_4\text{S}$ ($\text{M} + \text{Na}^+$) 327.0662, found 327.0644.

(2S)-2-(1S-(4-Phenoxyphenylsulfonyl)ethyl)thiirane (5a)

To a solution of **11a** (0.50 g, 1.6 mmol) in CH_2Cl_2 (5 mL) was added a mixture of thiourea (1.3 g, 16 mmol, 99%) in methanol (15 mL). The resulting mixture was stirred for 24 h at room temperature, after which the solvent was removed under reduced pressure. The residue was partitioned between ethyl ether and water. The organic layer was dried over anhydrous MgSO_4 , and the suspension was filtered. Evaporation of solvent gave the crude product, which was purified by silica gel chromatography (hexanes/EtOAc = 5/1). The desired product was recrystallized from 2-propanol as white crystals in 81% yield (0.43 g). Enantiomeric excess was determined by NMR in the presence of $\text{Eu}(\text{hfc})_3$. The NMR sample was prepared by adding 1 mg of **5a** and 1 mg of $\text{Eu}(\text{hfc})_3$ in 0.7 mL CDCl_3 . >90% ee; $[\alpha]_{\text{D}}^{25} +23.6$ (c 0.37, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 7.83–7.87 (m, 2H), 7.40–7.45 (m, 2H), 7.22–7.26 (m, 1H), 7.07–7.11 (m, 4H), 3.05–3.11 (m, 1H), 2.99 (quin, $J = 7.1$ Hz, 1H), 2.44 (dd, $J = 1.7, 6.5$ Hz, 1H), 2.08 (dd, $J = 1.7, 5.3$ Hz, 1H), 1.44 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (126 MHz, CDCl_3) δ 162.8, 154.8, 131.5, 130.5, 130.2, 125.1, 120.4, 117.6, 64.8, 31.4, 21.0, 11.2; HRMS (FAB) calcd for $\text{C}_{16}\text{H}_{17}\text{O}_3\text{S}_2$ ($\text{M} + \text{H}^+$) 321.0619, found 321.0612.

(2R)-2-(1S-(4-Phenoxyphenylsulfonyl)ethyl)thiirane (5b)

The synthesis was carried out as described for **5a**, using **11b**, but the reaction mixture was stirred for 4 days at room temperature. The desired product was recrystallized from 2-propanol as white crystals in 72% yield. Enantiomeric excess was determined by NMR in the presence of $\text{Eu}(\text{hfc})_3$. The NMR sample was prepared by adding 1 mg of **5b** and 1 mg of $\text{Eu}(\text{hfc})_3$ in 0.7 mL CDCl_3 . >90% ee; $[\alpha]_{\text{D}}^{25} +22.7$ (c 0.37, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 7.82–7.86 (m, 2H), 7.42–7.47 (m, 2H), 7.24–7.29 (m, 1H), 7.087.12 (m, 4H), 2.96 (dt, $J = 5.6, 9.5$ Hz, 1H), 2.60 (dq, $J = 6.9, 9.5$ Hz, 1H), 2.55 (dd, $J = 1.8, 6.0$ Hz, 1H), 2.24 (dd, $J = 1.9, 5.3$ Hz, 1H), 1.51 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (126 MHz, CDCl_3) δ 163.2, 154.9, 131.6, 130.5, 125.5, 120.7, 117.7, 67.0, 34.0, 26.5, 14.9; HRMS (FAB) calcd for $\text{C}_{16}\text{H}_{17}\text{O}_3\text{S}_2$ ($\text{M} + \text{H}^+$) 321.0619, found 321.0633.

(2R)-2-(1R-(4-Phenoxyphenylsulfonyl)ethyl)thiirane (5c)

The synthesis was carried out as described for **5a**, using **11c**. The ^1H and ^{13}C spectra of **5c** were identical to those of **5a**. >90% ee; $[\alpha]_{\text{D}}^{25} -23.1$ (c 0.33, CHCl_3); HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{16}\text{NaO}_3\text{S}_2$ ($\text{M} + \text{Na}^+$) 343.0433, found 343.0424.

(2S)-2-(1R-(4-Phenoxyphenylsulfonyl)ethyl)thiirane (5d)

The synthesis was carried out as described for **5b**, using **11d**. The ^1H and ^{13}C spectra of **5d** were identical to those of **5b**. >90% ee; $[\alpha]_{\text{D}}^{25} -22.0$ (c 0.33, CHCl_3); HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{16}\text{NaO}_3\text{S}_2$ ($\text{M} + \text{Na}^+$) 343.0433, found 343.0419.

(2R)-2-(1S-(4-Phenoxyphenylsulfonyl)ethyl)thiirane S-oxide (12)

A solution of compound **5b** (31 mg, 97 μmol) in CH_2Cl_2 (3 mL) was cooled in an ice-water bath and treated with *m*-CPBA (22 mg, 98 μmol , 77%). After the mixture was stirred for 5 min, the solvent was evaporated. The mixture was purified by silica gel chromatography (EtOAc) to give **12** (5.0 mg, 15%). ^1H NMR (500 MHz, CDCl_3) δ 7.80–7.88 (m, 2H), 7.40–7.49 (m, 2H), 7.24–7.32 (m, 1H), 7.09–7.16 (m, 4H), 2.94–3.01 (m, 1H), 2.82–2.92 (m, 1H), 2.35–2.45 (m, 2H), 1.60 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (126 MHz, CDCl_3) δ 163.6, 131.6, 130.5, 129.9, 125.6, 120.8, 118.1, 58.6, 47.0, 37.8, 10.6; HRMS (FAB) calcd for $\text{C}_{16}\text{H}_{17}\text{O}_4\text{S}_2$ ($\text{M} + \text{H}^+$) 337.0568, found 337.0579.

4-(But-3-en-2-ylsulfonyl)phenol (15)

A solution of 4-hydroxythiophenol (0.54 g, 4.3 mmol) in MeCN (20 mL) was treated with racemic **7** (0.70 g, 4.7 mmol). TEA (0.60 mL, 4.3 mmol) was added dropwise and the mixture was stirred under an atmosphere of nitrogen for 2 h at room temperature. After the solvent was removed, the crude compound was taken up into CH_2Cl_2 and water. Layers were separated and the aqueous layer was extracted with CH_2Cl_2 . The combined organic extracts were dried over anhydrous MgSO_4 and concentrated to dryness in vacuo. The resulting residue was carried on to the next reaction by dissolving it in CH_2Cl_2 (20 mL). *m*-CPBA (2.1 g, 9.4 mmol, 77%) was added at ice-water temperature and the mixture was stirred for 30 min. The suspension was filtered and the filtrate was purified by silica gel chromatography (hexanes/EtOAc = 1/1) to give 0.50 g (55%) of 4-(but-3-en-2-ylsulfonyl)phenol as a solid. It was then dissolved in EtOAc (20 mL) and treated with 5% palladium–barium sul-fate (50 mg) and five drops of quinoline. The mixture was stirred under an atmosphere of hydrogen for 2 h at room temperature. The catalyst was filtered and the filtrate was evaporated to dryness. The residue was purified by silica gel chromatography (hexanes/EtOAc = 1/1) to give the title compound in 92% yield (0.46 g). ^1H NMR (500 MHz, CDCl_3) δ 7.65–7.75 (m, 2H), 6.91–7.01 (m, 2H), 5.81 (ddd, $J = 7.9, 10.0, 17.4$ Hz, 1H), 5.29 (d, $J = 10.4$ Hz, 1H), 5.13 (d, $J = 17.1$ Hz, 1H), 3.70 (quin, $J = 7.1$ Hz, 1H), 1.43 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR (126 MHz, CDCl_3) δ 161.1, 131.9, 131.3, 127.8, 122.2, 115.9, 64.6, 13.3; HRMS (FAB) calcd for $\text{C}_{10}\text{H}_{12}\text{O}_3\text{S}$ ($\text{M} + \text{H}^+$) 213.0585, found 213.0576.

(4-(4-(But-3-en-2-ylsulfonyl)phenoxy)phenoxy)(tert-butyl)dimethylsilane (16)

The procedure was adapted from that reported by Evans *et al.* (19). To a round bottom flask was added activated powdered 4 Å molecular sieves, compound **15** (0.20 g, 0.94 mmol), the boronic acid **14** (0.47 g, 1.9 mmol), $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ (0.19 g, 0.95 mmol), CH_2Cl_2 (15 mL), and TEA (0.40 mL, 2.8 mmol). The mixture was stirred under an atmosphere of nitrogen for 1 h. The solvent was evaporated and the product was purified by silica gel chromatography (hexanes/EtOAc = 5/1) to give **16** in 51% yield (0.20 g). ^1H NMR (500 MHz, CDCl_3) δ 7.73–7.77 (m, 2H), 6.98–7.02 (m, 2H), 6.93–6.97 (m, 2H), 6.86–6.90 (m, 2H), 5.79–5.88 (m, 1H), 5.29 (dt, $J = 0.9, 10.3$ Hz, 1H), 5.14 (dt, $J = 1.0, 17.3$ Hz, 1H), 3.66–3.74 (m, 1H), 1.44 (d, $J = 7.0$ Hz, 3H), 1.01 (s, 9H), 0.23 (s, 6H); ^{13}C NMR (126 MHz, CDCl_3) δ 163.5, 153.2, 148.6, 131.7, 131.6, 129.8, 121.9, 121.6, 116.6, 64.5, 25.9, 18.4, 13.3, -4.2; HRMS (FAB) calcd for $\text{C}_{28}\text{H}_{30}\text{O}_4\text{SSi}$ ($\text{M} + \text{H}^+$) 419.1712, found 419.1714.

tert-Butyldimethyl(4-(oxiran-2-yl)ethylsulfonyl)phenoxy) phenoxy)silane (17a and 17b)

A solution of compound **16** (0.15 g, 0.36 mmol) in CH_2Cl_2 (15 mL) was cooled in an ice-water bath and was treated with *m*-CPBA (0.40 g, 1.8 mmol, 77%). The mixture was stirred for 1 h in an ice-water bath, warmed to room temperature and stirred for 6 days. The suspension was filtered over silica gel and the filtrate was washed with saturated $\text{Na}_2\text{S}_2\text{O}_3$, followed by saturated NaHCO_3 . The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by silica gel chromatography (hexanes/EtOAc = 5/1) gave 32 mg of **17a** and 30 mg of **17b** in 40% overall yield. For **17a**: ^1H NMR (500 MHz, CDCl_3) δ 7.79–7.83 (m, 2H), 7.02–7.05 (m, 2H), 6.94–6.99 (m, 2H), 6.87–6.90 (m, 2H), 3.14 (ddd, J = 2.4, 4.0, 6.8 Hz, 1H), 2.77–2.86 (m, 2H), 2.46 (dd, J = 2.6, 4.6 Hz, 1H), 1.43 (d, J = 7.0 Hz, 3H), 1.01 (s, 9H), 0.23 (s, 6H); ^{13}C NMR (126 MHz, CDCl_3) δ 163.9, 153.3, 148.4, 131.4, 130.2, 122.0, 121.6, 117.0, 63.2, 50.8, 47.0, 25.8, 18.4, 11.2, -4.2; HRMS (FAB) calcd for $\text{C}_{22}\text{H}_{30}\text{O}_5\text{SSi}$ (M^+) 434.1583, found 434.1588. For **17b**: ^1H NMR (500 MHz, CDCl_3) δ 7.83–7.86 (m, 2H), 7.02–7.05 (m, 2H), 6.95–6.98 (m, 2H), 6.86–6.90 (m, 2H), 3.25 (dd, J = 2.3, 4.9 Hz, 1H), 3.01 (quin, J = 7.1 Hz, 1H), 2.77 (dd, J = 4.1, 4.7 Hz, 1H), 2.47 (dd, J = 2.6, 4.8 Hz, 1H), 1.37 (d, J = 7.2 Hz, 3H), 1.01 (s, 9H), 0.23 (s, 6H); ^{13}C NMR (126 MHz, CDCl_3) δ 163.8, 153.3, 148.5, 131.4, 130.9, 122.0, 121.6, 117.0, 62.8, 50.3, 44.4, 25.9, 18.4, 9.6, -4.2; HRMS (FAB) calcd for $\text{C}_{22}\text{H}_{30}\text{O}_5\text{SSi}$ (M^+), found 434.1579.

tert-Butyldimethyl(4-(4-(S)-thiiran-2-yl)ethylsulfonyl)phenoxy) phenoxy)silane (18a)

To a solution of **17a** (15 mg, 35 μmol) in CH_2Cl_2 (1 mL) was added a mixture of thiourea (14 mg, 0.18 mmol, 99%) in methanol (4 mL). The resulting mixture was stirred for 24 h at room temperature, after which, the solvent was removed under reduced pressure. The residue was partitioned between ethyl ether and water. The organic layer was dried over anhydrous MgSO_4 , and the suspension was filtered. Evaporation of solvent gave the crude product, which was purified by silica gel chromatography (hexanes/EtOAc = 6/1) to yield 12 mg (77%) of **18a**. ^1H NMR (500 MHz, CDCl_3) δ 7.81–7.85 (m, 2H), 7.03–7.06 (m, 2H), 6.95–6.99 (m, 2H), 6.87–6.90 (m, 2H), 3.09 (td, J = 5.4, 6.9 Hz, 1H), 2.99 (quin, J = 7.1 Hz, 1H), 2.44 (dd, J = 1.7, 6.5 Hz, 1H), 2.08 (dd, J = 1.7, 5.3 Hz, 1H), 1.44 (d, J = 7.0 Hz, 3H), 1.01 (s, 9H), 0.23 (s, 6H); ^{13}C NMR (126 MHz, CDCl_3) δ 163.8, 153.3, 148.6, 131.6, 130.2, 121.9, 121.6, 117.1, 65.0, 31.7, 25.9, 21.2, 18.4, 11.3, -4.2; HRMS (FAB) calcd for $\text{C}_{22}\text{H}_{30}\text{O}_4\text{S}_2\text{Si}$ (M^+) 450.1355, found 450.1331.

tert-Butyldimethyl(4-(4-(S)-thiiran-2-yl)ethylsulfonyl) phenoxy)phenoxy)silane (18b)

The synthesis was carried out as described for **18a**, using **17b**, but the reaction mixture was stirred for 4 days at room temperature. Purification by silica gel chromatography (hexanes/EtOAc = 6/1) gave **18b** in 64% yield. ^1H NMR (500 MHz, CDCl_3) δ 7.80–7.86 (m, 2H), 7.03–7.09 (m, 2H), 6.95–7.01 (m, 2H), 6.88–6.93 (m, 2H), 2.97 (dt, J = 5.4, 9.6 Hz, 1H), 2.54–2.65 (m, 2H), 2.25 (dd, J = 1.3, 5.3 Hz, 1H), 1.52 (d, J = 6.4 Hz, 3H), 1.03 (d, J = 0.8 Hz, 9H), 0.25 (d, J = 0.8 Hz, 6H); ^{13}C NMR (126 MHz, CDCl_3) δ 163.9, 153.4, 148.5, 131.5, 130.1, 121.9, 121.6, 117.1, 67.0, 34.0, 26.5, 25.9, 18.4, 15.0, -4.2. HRMS (FAB) calcd for $\text{C}_{22}\text{H}_{30}\text{O}_4\text{S}_2\text{Si}$ (M^+) 450.1355, found 450.1349.

4-(4-Thiiran-2-yl)ethylsulfonyl)phenoxy)phenol (13a)

To a solution of compound **18a** (10 mg, 22 μmol) in methanol (5 mL) was added a few drops of conc. HCl. After the mixture was stirred for 2 h at room temperature, the solvent was evaporated *in vacuo*. The crude product was taken up into water and EtOAc. Layers were separated and the aqueous layer was extracted with EtOAc. The organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by silica gel chromatography (hexanes/EtOAc = 1/1) gave **13a** in 96% yield (7.2 mg). ^1H NMR (500 MHz, CDCl_3) δ 7.81–7.86 (m, 2H), 7.02–7.07 (m, 2H), 6.97–7.01 (m, 2H), 6.87–6.92 (m, 2H), 5.05 (s, 1H), 3.08

(td, $J = 5.9, 6.6$ Hz, 1H), 2.99 (t, $J = 7.2$ Hz, 1H), 2.45 (dd, $J = 1.8, 6.4$ Hz, 1H), 2.08 (dd, $J = 1.8, 5.2$ Hz, 1H), 1.44 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (126 MHz, CDCl_3) δ 163.9, 153.3, 148.2, 131.6, 130.2, 122.2, 117.0, 117.0, 65.1, 31.6, 21.2, 11.4; HRMS (FAB) calcd for $\text{C}_{16}\text{H}_{17}\text{O}_4\text{S}_2$ ($\text{M} + \text{H}^+$) 337.0563, found 337.0564.

4-(4-Thiiran-2-yl)ethylsulfonyl)phenoxy)phenol (**13b**)

The synthesis was carried out as described for **13a**, using **18b**. Purification by silica gel chromatography (hexanes/EtOAc = 1/1) gave **13b** in 96% yield. ^1H NMR (500 MHz, CDCl_3) δ 7.79–7.84 (m, 2H), 7.03–7.07 (m, 2H), 6.96–7.01 (m, 2H), 6.88–6.92 (m, 2H), 4.99 (s, 1H), 2.95 (dt, $J = 5.7, 9.6$ Hz, 1H), 2.60 (dq, $J = 7.0, 9.4$ Hz, 1H), 2.54 (dd, $J = 1.9, 6.1$ Hz, 1H), 2.24 (dd, $J = 1.9, 5.3$ Hz, 1H), 1.50 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR (126 MHz, CDCl_3) δ 163.9, 153.3, 148.1, 131.5, 130.1, 122.3, 117.0, 67.0, 34.0, 26.5, 14.9; HRMS (FAB) calcd for $\text{C}_{16}\text{H}_{17}\text{O}_4\text{S}_2$ ($\text{M} + \text{H}^+$) 337.0563, found 337.0564.

Crystals growth and analysis

Crystals of suitable size for single-crystal X-ray diffraction analysis were obtained by diffusion of hexane into a diethyl ether solution of compound **11a** at room temperature overnight. Crystals were examined under a light hydrocarbon oil. The datum crystal was cut from a larger needle. It was then affixed to a 0.2-mm Mitegen loop mounted atop a tapered copper mounting-pin and transferred to the 100 K nitrogen stream of a Bruker SMART Apex diffractometer (Bruker AXS Inc., Madison, WI, USA) equipped with an Oxford Cryosystems 700 (Oxford Cryosystems Ltd, Oxford, UK) series low-temperature apparatus. Cell parameters were determined using reflections harvested from three orthogonal sets of $30\ 0.5^\circ\ \phi$ scans. The orientation matrix derived from this was passed to COSMO^a to determine the optimum data collection strategy. Minimum fourfold redundancy was achieved using a combination of ω and ϕ scan series. Data were measured to $0.81\ \text{\AA}$. Cell parameters were refined using reflections with $I \geq 10\sigma(I)$ harvested from the entire data collection. All data were corrected for Lorentz and polarization effects and runs were scaled using SADABS^b. The structures were solved using direct methods. All non-hydrogen atoms were assigned after the initial solution. Hydrogens were placed at calculated geometries and allowed to ride on the position of the parent atom. Hydrogen thermal parameters were set to $1.2\times$ the equivalent isotropic U of the parent atom, $1.5\times$ for methyl hydrogens. Non-hydrogen atoms were refined with parameters for anisotropic thermal motion.

The same methodology was performed to elucidate the crystal structures of **11b**, **5a**, and **5b**. An analysis of the crystal for compound **5a** at 100 K showed a phase transition resulting in the destruction of the samples, thus data were collected at room temperature rather than at cryogenic temperatures. Crystallographic data (excluding structure factors) for compounds **5a**, **5b**, **11a**, and **11b** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers 737463, 737464, 737465, and 737466. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44-1223-336033 or by deposit@ccdc.cam.ac.uk].

Results and Discussion

In our previous studies, SB3-CT was found to be a highly potent and selective inhibitor of gelatinases (9,20). However, we found that it is rapidly metabolized to the sulfinic acid (**2**) and to the *p*-hydroxy derivative (**3**) because of oxidation at the α -position to the sulfonyl group (and the attendant fragmentation) and at the *para* position of the terminal phenyl ring,

^aCOSMO (2004). Madison, WI: Bruker-AXS.

^bSheldrick G.M. (2004) SADABS. Germany: University of Göttingen.

respectively (14,15). We showed that *p*-hydroxylation results in a metabolite that is a more potent gelatinase inhibitor than the parent SB-3CT, however α -oxidation produces a metabolite that is not an inhibitor of gelatinases (14). In this study, we systematically α -methylated the sulfonyl group in an attempt to block oxidation at this position and thus maintain inhibitor potency. In addition, reducing the degree of metabolism has several advantages, including increasing bioavailability and prolonging half-life, thereby allowing lower and less frequent dosing.

Synthesis of isomers of **5**

The four isomers of **5** were synthesized starting from optically pure 3-butyne-2-ol enantiomers. Basic methanolysis of thioacetate **6** yielded the corresponding thiolate, which was allowed to react with mesylate (*R*)-**7** to give (*S*)-**8** (Figure 1). This entailed a nucleophilic attack by the thiolate at the electrophilic carbon of the mesylate, leading to an inversion of configuration. After oxidation to the sulfone using *m*-CPBA, partial hydrogenation of alkyne to alkene over Pd–BaSO₄ gave (*S*)-**10**. Standard epoxidation using *m*-CPBA produced the diastereomeric oxiranes **11a** and **11b** (*R_f* = 0.5 and 0.4 in hexanes/EtOAc = 2/1), which were separated by flash chromatography. Conversion of the isolated oxiranes to thiiranes **5a** and **5b**, by treatment with thiourea involved an inversion of configuration. Assignment of the stereochemistry of the separated oxiranes and thiiranes was carried out by X-ray crystallography (*vide infra*). The thiiranes **5c** and **5d** were accessed according to the same procedure, using (*S*)-**7** as the starting mesylate.

We add that alternative routes to the optically active thiiranes were explored prior to using the chiral synthetic scheme outlined in Figure 1, which lead to success. One initial approach was to generate racemic **10** from the direct reaction of **6** with 3-chlorobut-1-ene, but because of regioselectivity issues, we opted to prepare racemic **10** from 3-butyne-2-ol using the same methodology as in Figure 1. Racemic **10** could then be subjected to Sharpless asymmetric dihydroxylation reaction (21) and the resulting diol could be converted to the epoxide under Mitsunobu conditions (22). The installation of chirality by Sharpless dihydroxylation, however, did not give good ee values. Another approach was hydrolytic kinetic resolution of the racemic terminal epoxides **11** catalyzed by chiral (salen)Co^{III} complexes (23). Although this method afforded both the recovered unreacted epoxide and the 1,2-diol product in enantioenriched forms, it was limited by low yield and the need for a long reaction time. At the end, careful considerations of yields, reaction times and stereo-selection prompted us to pursue the chemistry outlined in Figure 1.

The optical purities of the synthesized compounds were determined by NMR in the presence of the chiral shift reagent europium tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate], Eu(hfc)₃. ¹H NMR invariably revealed the presence of only one enantiomer in each case, hence, we can indicate that the ee values for each isomer should be at the minimum 90%.

The absolute stereochemistry of the synthesized oxiranes and thiiranes was assigned by X-ray crystallography (Figure 2). The X-ray crystal structure of one TLC spot revealed it to be **11a** (Figure 2B). The absolute stereochemistry was determined by comparison of the intensities of the Friedel pairs of reflections (Flack parameter = 0.054 (13); a value of 0 indicates the correct enantiomorph of the space group and chirality of the molecule) and by comparison of the known chirality of the mesylate precursor. The X-ray crystal structure of the other TLC spot was due to **11b** [Figure 2C, Flack parameter = 0.041 (11)]. It is known that conversion of the oxiranes to the corresponding thiiranes using thiourea involves an inversion of stereochemistry (24, 25). This was confirmed by obtaining the X-ray structures of the two thiiranes prepared from the two chromatographically separated oxiranes. The product of thiirane formation reaction from **11a** proved to be **5a** [Figure 2D, Flack parameters = 0.043 (19)]. The same inversion of

stereochemistry at the thiirane was observed for the crystal structure of **5b** [Figure 2E, Flack parameter = 0.044 (13)], which was prepared from **11b**.

Inhibition of MMPs

Of the four synthetic diastereomers of **5**, the **5b** and **5d** enantiomers showed slow-binding inhibition of gelatinases, with inhibition constants (K_i) that are in the nanomolar range for MMP-2 (0.18 ± 0.03 and $0.39 \pm 0.03 \mu\text{M}$, respectively) and in the low micromolar for MMP-9 (3.5 ± 0.2 and $3.3 \pm 0.2 \mu\text{M}$, respectively). The second-order rate constants for the onset of slow-binding inhibition (k_{on}) are rapid (10^2 to 10^4 M/second) and the first-order rate constants for the reversal of the process (k_{off}) are slow (10^{-4} to $10^{-3}/\text{second}$). For comparison, SB-3CT is an order of magnitude more potent against MMP-2 ($K_i = 13.9 \text{ nM}$) and a five to sixfold better inhibitor of MMP-9 ($K_i = 0.6 \mu\text{M}$) (9). However, compounds **5b** and **5d** were >50-fold more metabolically stable than SB-3CT. As mentioned earlier, low metabolic stability can significantly reduce the efficacy of an otherwise potent compound, decreasing bioavailability and shortening the half-life, which in turn requires higher and more frequent dosing. Thus, by maximizing metabolic stability the likelihood of clinical success is improved. The slow-binding behavior appears to require *anti* arrangement of the hydrogen α to the sulfone and the thiirane sulfur of the inhibitor. On the contrary, compounds **5a** and **5c** were merely linear competitive inhibitors of gelatinases (with K_i values of 7.2 ± 0.1 and $4.1 \pm 0.1 \mu\text{M}$ against MMP-2, respectively). Inhibition of MMPs by the enantiomers that exhibited the slow-binding behavior (also seen with SB-3CT) was investigated next. The kinetic parameters for the inhibition of MMPs are shown in Table 1. No significant activity against MMP-1, MMP-3, and MMP-7 was observed up to $60 \mu\text{M}$. The two enantiomers inhibit each gelatinase equally well, although they are more potent in inhibition of MMP-2. However, we note that in addition to inhibition of gelatinases, the slow-binding kinetic profile was extended to include MMP-14, with K_i values of 0.74 ± 0.17 and $2.1 \pm 0.2 \mu\text{M}$ for **5b** and **5d**, respectively. MMP-14 is known to activate MMP-2 (26), thus inhibition of MMP-14 will provide two levels of control on the activity of MMP-2. MMP-14 has been shown to be essential for invasion of cancer cells (27) and the gelatinases are known to be involved in cancer invasion and angiogenesis (28). Thus, compounds **5b** and **5d** could target the MMP-14/MMP-2/MMP-9 axis in cancer tissues in a more effective manner.

In vitro metabolism and metabolite identification

The *in vitro* metabolism of the active diastereomer **5b** was investigated using rat liver microsomes. The HPLC chromatogram of **5b** following incubation with rat liver microsomes showed one significant metabolite, designated as **M**, as well as the parent compound itself (Figure 3A). The two peaks flanking **M** were determined to be background peaks and not any metabolite. The presence of **5b** in rat liver microsomes was confirmed by HPLC retention time comparison to the authentic synthetic standard (Figure 3B) and by product ion mass spectrometry.

Analysis of **M** by LC/MS indicated oxidation of the parent compound, as depicted by the protonated molecular ion at m/z 337, which was 16 a.m.u higher than that of **5b**. The selected ion chromatogram (Figure 3C) showed only one peak, which suggested that only one compound with an $m/z = 337$ was produced as metabolite. This ruled out the possibility of sulfinic acid **2**, the product resulting from the oxidation at the α -position to the sulfonyl group, as the metabolite. Hence, methylation α to the sulfone eliminated the possibility of this transformation, as was our intent. MS/MS analysis showed cleavage of the sulfonyl–methylthiirane linkage at m/z 233 and 87, with subsequent loss of SO from the ion at m/z 233 to give a fragment at m/z 185 (Figure 4A). Cleavage of the phenoxyphenyl–sulfonyl linkage resulted in an ion at m/z 169. These data indicated that the terminal phenyl ring and the middle phenyl ring were intact, and that oxidation of the sulfur in the thiirane ring was the most likely

transformation to account for the increase in mass by 16 a.m.u. However, an increase of 16 a.m.u. for the fragment at m/z 87 was not observed. We had seen this previously for the sulfoxide metabolite of SB-3CT (compound **4**) (14). As the fragment at m/z 185 could be obtained by hydroxylation of the terminal phenyl ring (m/z 169 + 16), we set out to prepare the authentic thiirane sulfoxide (compound **12**) and *p*-hydroxy derivative of **5b** (compound **13b**) to confirm the identity of the metabolite.

The *S*-oxide **12** was produced after treatment of **5b** with an equivalent of *m*-CPBA. Compound **12** should exist in two diastereomeric forms, as was seen in the ¹H NMR spectrum of the crude product taken immediately after the reaction. However, only one was isolable; the other diastereomer decomposed to the corresponding alkene. Thiirane *S*-oxides are known to readily decompose to form olefins and sulfur monoxide (29,30). The synthesis of the *p*-hydroxy derivative **13b** was initiated by copper-mediated arylation of racemic phenolic **15** using boronic acid **14** (18) to give **16** (Figure 5). Compound **15** was separately prepared by *S*-alkynylation of 4-hydroxythiophenol using racemic **7**, and then oxidation to the sulfone using *m*-CPBA followed by partial catalytic hydrogenation. The succeeding steps involved epoxidation of the alkene to produce oxiranes **17a** and **17b** (which were separable by chromatography), conversion to the thiirane and desilylation of the phenolic alcohol to afford diastereomers **13a** and **13b**.

Comparison of HPLC retention times and MS/MS analyses of the authentic synthetic standards **12** and **13b** (Figures 3 and 4) confirmed the identity of the metabolite. Compound **12** had the identical HPLC retention time (Figure 3B) and MS/MS spectrum (Figure 4B) as the metabolite. In contrast, the MS/MS spectrum of compound **13b** showed cleavage of the sulfonyl–methylthiirane linkage at m/z 249 and 87, and cleavage of the phenoxyphenyl–sulfonyl bond at m/z 185 (Figure 4C). These data identified **M** as the sulfoxide of compound **5b** (compound **12**). It must be noted that only one diastereomer of **12** was observed as the metabolite in the chromatogram, and its retention time matched that of the isolated synthetic standard. Because the other diastereomer readily decomposes, as was observed in the synthetic preparation, the possibility that it could also form – but experiences decomposition – during metabolism could not be discounted.

The presence of the sulfoxide as the only metabolite of compound **5** indicated that α -methylation with suitable stereochemistry abrogated oxidation at both the α -position to the sulfonyl group as well as the *para*-position of the terminal phenyl ring. Interestingly, oxidation of the methyl group itself was not observed. The reason for the absence of hydroxylation at the *para*-position of the terminal ring of these methylated thiiranes is not immediately obvious. However, the most likely explanation for this outcome is that the methylated derivatives can no longer fit in the active site of the P450 enzyme that performs this transformation.

The *S*-oxide metabolite was tested for any inhibitory activities toward MMPs. When compared with the inhibition results of compound **5b** (Table 1), compound **12** was shown to be a poorer inhibitor by at least three orders of magnitude (K_i of 20 μ M against MMP-2, and MMP-9 and MMP-14 inhibition at a fixed 60 μ M inhibitor concentration were 30% and 0%, respectively). The *p*-hydroxy derivative, compound **13b**, was however found to exhibit a similar inhibition profile as **5b** and **5d**. The inhibition constants of **13b** are in the nanomolar range for MMP-2 ($K_i = 0.22 \pm 0.09 \mu$ M) and in the low micromolar for MMP-9 and MMP-14 ($K_i = 1.9 \pm 0.7$ and

2.1 $\pm$ 0.7 μ M, respectively). Marginal to no inhibition against MMP-1, MMP-3, and MMP-7 was observed at inhibitor concentrations of up to 60 μ M.

Conclusions

The metabolism of compound **5** was significantly less extensive than that of SB-3CT, a potent and selective gelatinase inhibitor. Introduction of a methyl group α to the sulfonyl moiety prevented oxidation at that position, as well as hydroxylation of the terminal phenyl ring. The ability of compounds **5b** and **5d** to inhibit both gelatinases and MMP-14 activities could result in effective targeting of the MMP-14/MMP-2/MMP-9 axis, which has been shown to be important for cancer progression. Compounds **5b** and **5d** would appear to be potent and selective for gelatinases and are poised for utilization in animal models of disease involving these enzymes.

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Abbreviations

a.m.u.	atomic mass units
CAD	collisionally activated dissociation
<i>m</i> -CPBA	<i>meta</i> -chloroperoxybenzoic acid
DMSO	dimethylsulfoxide
ESI	electrospray ionization
FAB	fast atom bombardment
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
LC	liquid chromatography
MMP	matrix metalloproteinase
MS	mass spectrometry
MOCac	(7-methoxycoumarin-4-yl)acetyl
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
TEA	triethylamine
TFA	trifluoroacetic acid
UV	ultraviolet

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Figure 1.
Synthetic scheme for the preparation of compounds **5**.

Figure 2.
The X-ray structures of the oxiranes **11a**, **11b**, and thiiranes **5a**, **5b**, in stereoview (left) and the ORTEP diagram (right) shown at 50% probability level (**5a**, shown at 30% probability level). Ar, 4-phenoxyphenyl group.

Figure 3.

HPLC chromatogram of (A) compound **5b** following 20-min incubation with rat liver microsomes (B) synthetic standards, and (C) selected ion chromatogram (SIC) using $m/z = 337$ ($M + H^+$). HPLC conditions: YMC 5 μ m basic 4.6 mm i.d. $\times$ 15 cm column, 1 mL/min, UV detection at 245 nm, 5-min 90% A/10% B, 20-min linear gradient to 10% A/90% B, 5-min 10% A/90% B (A = 0.1% TFA/water, B = 0.1% TFA/acetonitrile).

Figure 4.
Product ion mass spectra of (A) metabolite **M** (B) synthetic standard **12**, and (C) compound **13b**.

Figure 5.
Synthetic scheme for the preparation of *p*-hydroxy **13**.

Table 1

Kinetic parameters for inhibition of MMPs by the synthetic inhibitors

Enzyme	5b			5d		
	$10^{-3} k_{on}$ (M/second)	$10^4 k_{off}$ (second)	K_i (μM)	$10^{-3} k_{on}$ (M/second))	$10^4 k_{off}$ (second)	K_i (μM)
MMP-2	3.3 ± 0.1	5.9 ± 1.0	0.18 ± 0.03	2.1 ± 0.1	8.1 ± 0.6	0.39 ± 0.03
MMP-9	0.26 ± 0.01	9.0 ± 0.6	3.5 ± 0.2	0.22 ± 0.01	7.2 ± 0.4	3.3 ± 0.2
MMP-14 _{cat}	1.2 ± 0.1	8.9 ± 2.1	0.74 ± 0.17	0.46 ± 0.01	9.5 ± 0.9	2.1 ± 0.2
MMP-1 _{cat}	na	na	NI	na	na	NI
MMP-3 _{cat}	na	na	8% Inhibition at $60 \mu M$	na	na	9% Inhibition at $60 \mu M$
MMP-7	na	na	NI	na	na	NI

NI, not inhibiting up to $60 \mu M$.
na, not applicable.