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A Convenient Synthesis of a Selective Gelatinase Inhibitor as an Antimetastatic Agent

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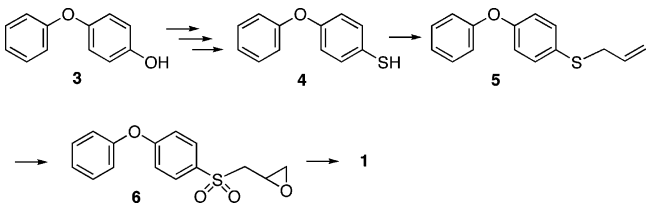
Abstract: Compound **1**, 2-(4-phenoxyphenylsulfonylmethyl)thiirane, is a potent and selective inhibitor for human gelatinases (*J. Am. Chem. Soc.* **2000**, *122*, 6799−6800), enzymes implicated in a number of diseases, including cancer. This compound is showing excellent promise in animal trials in a number of disease models. Large quantities of this compound were necessary for these studies. A convenient four-step synthetic route for compound **1** is described herein. The synthesis is amenable to scale-up to tens of grams and gives an overall yield of 57% for this important compound.

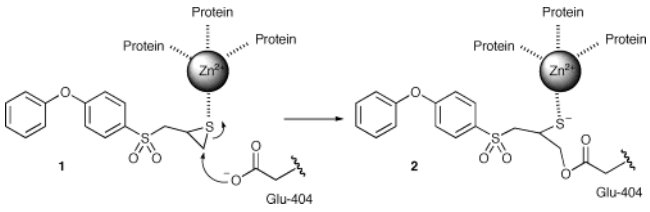
The extracellular matrix (ECM) is the environment that surrounds cells. The health of ECM is critical for the well-being of the organism in normal physiological processes. Matrix metalloproteinases (MMPs) constitute a family of 27 enzymes that have the ability to alter the ECM by their functions.[1] The various functions of MMPs have not been elucidated, which is an area of active current research. The functions of these enzymes are strictly regulated under normal physiological processes. When the cellular regulation is lost, the levels of activities of these enzymes are elevated, an event that causes a number of pathological conditions that include cancer growth, tumor metastasis and angiogenesis, arthritis, connective tissue diseases, inflammation, and cardiovascular, neurological, and autoimmune diseases.[2]

Two of the MMPs, MMP-2 and MMP-9, also known as gelatinases A and B, have been implicated in a number of these pathological conditions. They have been the targets of many inhibitor design programs.[3] Unfortunately, the known inhibitors for these enzymes are generally broad-spectrum in that they also inhibit other classes of MMPs. This broad breadth of activity may be at the root of some of the difficulties that these MMP inhibitors have suffered in clinical trials recently.[4]

SCHEME 1



We described recently a mechanism-based inhibitor that was high selectivity for gelatinase inhibition.[5] Compound **1** is the prototype of this type of inhibitor. The biphenyl moiety fits in the deep hydrophobic pocket in the active sites of gelatinases, and the inhibitor interacts with the active site zinc ion through the thiirane moiety. This interaction activates the thiirane for nucleophilic attack by the active site glutamate in these enzymes, resulting in species **2**, which is the irreversibly inhibited enzyme.[5,6] The mode of inhibition is at the root of the selectivity seen with the inhibitor for gelatinases, as described in detail earlier.[5]



Whereas virtually all MMP inhibitors have been designed as zinc ion chelators, the principles behind the design of inhibitor **1** and its unique mechanism of action were points of departure. Furthermore, selectivity in targeting gelatinases provided a unique opportunity in exploring the roles of gelatinases in disease processes.

This inhibitor is currently undergoing trials in several animal models for diseases involving excessive gelatinase activities, including models for cancer metastasis. These studies necessitated the development of a new high-yielding synthetic route for compound **1** that was amenable to scales in quantities of tens of grams, which is the subject of the present report.

The previous synthesis started from the commercially available *p*-phenoxyphenol (**3**) to give the thiol derivative **4** (Scheme 1). This transformation took place in three steps, with one step requiring heating at 260 °C, as described by Newman and Karnes for a related system.[7] Oxidation of **5** to **6** required 7 days and proceeded in

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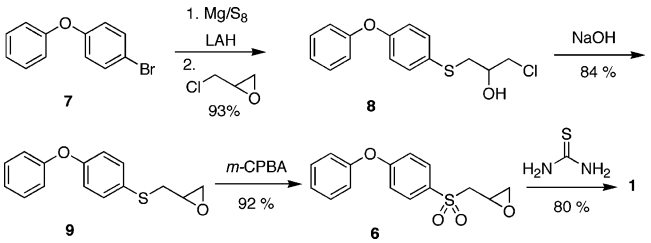
SCHEME 2



modest yield. The oxirane to thiirane conversion with ammonium thiocyanate (**6** to **1**) gave a poor yield of only 14%. The synthesis was accomplished in 5% overall yield. The approach was useful in preparing research quantities of the desired inhibitor for in vitro studies, but it clearly was not amenable to large-scale preparation needed for animal studies.

The new synthesis commences from the commercially available 4-bromophenyl phenyl ether (**7**). The lithium salt of *p*-phenoxythiophenol was generated in situ from **7** by the use of metallic magnesium and sulfur in the presence of lithium aluminum hydride, which was in turn allowed to react with epichlorohydrin to give the key intermediate, 1-chloro-3-(4-phenoxy-phenylsulfanyl)propan-2-ol (**8**). This one-pot reaction obviated the need for handling of thiol **4**, which is both noxious and prone to air oxidation. The formation of epoxide **9** from derivative **8** under basic condition proceeded well in good yield. Subsequently, the sulfide was oxidized by the reaction of *m*-CPBA to give **6** in 92% yield. The exchange of the oxygen in the oxirane **6** with a sulfur to give the desired thiirane **1** with the use of thiourea gave 80% yield.

The number of steps in the synthesis of **1** was reduced to four, and the overall total yield was increased from 5% in the previous synthesis to 57% by the approach depicted in Scheme 2. On somewhat less tangible issues, the reagents in this synthetic sequence were cheaper and the reactions required shorter durations. The present synthesis is practical and can be carried out in substantially larger scale. This synthesis has made the animal studies possible.

Experimental Section

1-Chloro-3-(4-phenoxyphenylsulfanyl)propan-2-ol (8). The procedure was adapted from that reported by Szajnman et al.[8] Metallic magnesium (9.0 g, 370 mmol) was added to a solution of 4-bromophenyl phenyl ether (92.6 g, 372 mmol) in anhydrous THF (500 mL) with vigorous stirring at room temperature. The reaction mixture was refluxed under argon until the metal was dissolved. The solution was cooled to 0 °C, and sulfur (12.6 g, 393 mmol) was added. The mixture was stirred at room temperature for 4 h, at which time lithium aluminum hydride (4 g) was added portionwise to the solution at 0 °C. The solution was allowed to warm to room temperature, and the mixture was stirred for 2 h, followed by quenching by the addition of ethyl acetate (4.4 mL, 45 mmol) at 0 °C. The mixture was poured portionwise into a cooled solution of epichlorohydrin (68 g, 740 mmol) in dichloromethane (700 mL). The mixture was stirred at room temperature for 1 h after the last portion of epichlorohydrin was added. The mixture was partitioned between dichloromethane and an aqueous saturated solution of sodium potassium tartrate (2 × 500 mL), and the organic layer was washed with water (2 × 500 mL), dried ($MgSO_4$), and filtered through Celite. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (hexane/EtOAc = 6:1−3:1) to afford **8** (101 g, 93%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.4 (m, 2H), 7.4 (m, 2H), 7.2 (m, 1H), 7.1 (m, 2H), 7.0 (m, 2H), 3.9 (m, 1H, C*H*OH), 3.7 (m, 2H, CH_2Cl), 3.1 (ddd, J = 44.5, 14.0 Hz, 5.5 Hz, 2H, SCH_2CH), 2.7 (d, J = 5.0 Hz, 1H, O*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.4, 156.7, 133.4, 130.1, 128.0, 124.0, 119.5, 119.6, 69.6, 48.2, 39.9; HRMS calcd for $C_{15}H_{15}ClO_2S$ (M^+) 294.0481, found 294.0461.

2-(4-Phenoxyphenylsulfanylmethyl)oxirane (9). To a solution of compound **8** (55 g, 187 mmol) in anhydrous *p*-dioxane (250 mL) was added sodium hydroxide (28 g, 700 mmol). The reaction mixture was stirred at room temperature for 1 h, and a catalytic amount of sodium hydride (0.1 g, 4 mmol) was added. The reaction mixture was stirred at room temperature overnight and then was filtered through Celite. The filtrate was concentrated under reduced pressure, yielding the title compound (40.6 g, 84%) as an oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.5 (m, 2H), 7.4 (m, 2H), 7.2 (m, 1H), 7.0 (m, 2H), 6.9 (m, 2H), 3.2 (m, 1H), 3.1 (dd, J = 14.0, 4.0 Hz, 1H), 2.9 (ddd, J = 14.0, 6.0, 1.5 Hz, 1H), 2.8 (m, 1H), 2.5 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.2, 156.8, 133.7, 130.0, 128.7, 123.9, 119.5, 119.4, 51.3, 47.6, 38.2; HRMS calcd for $C_{15}H_{14}O_2S$ (M^+) 258.0715, found 258.0739.

2-(4-Phenoxyphenylsulfonylmethyl)oxirane (6). To a solution of compound **9** (35 g, 135 mmol) in dichloromethane (650 mL) was added a solution of *m*-chloroperoxybenzoic acid (62.8 g, 77%, 280 mmol) in dichloromethane (350 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h and was then warmed to room temperature overnight. The suspension was filtered, and the filtrate was washed with aqueous sodium thiosulfate (2 × 600 mL, 10% w/v), followed by aqueous sodium bicarbonate (2 × 500 mL, 5% w/v), followed by brine (500 mL). The organic layer was dried over magnesium sulfate and was concentrated. The product was purified by silica gel chromatography (hexane/EtOAc = 3:1−3:2) to yield the title compound as an oil (35.9 g, 92%). The ^{1}H and ^{13}C NMR spectra and mass spectrum were identical to the reported values.[5]

2-(4-Phenoxyphenylsulfonylmethyl)thiirane (1). To a solution of compound **6** (21 g, 72 mmol) in anhydrous methanol (700 mL) was added thiourea (12 g, 158 mmol). The reaction mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure. The residue was partitioned between ethyl acetate (600 mL) and water (400 mL), the organic layer was washed with brine (400 mL) and dried ($MgSO_4$), and the suspension was filtered. The solvent was evaporated from the filtrate, and the residue was purified by column chromatography (hexane/EtOAc = 3:1−2:1). The desired product was crystallized as white needles from ethyl acetate/hexane (17.8 g, 80%). The ^{1}H and ^{13}C NMR spectra and mass spectrum were identical to the reported values.[5]

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Supporting Information Available: NMR spectra for the synthetic molecules. This material is available free of charge via the Internet at http://pubs.acs.org.

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