Biological Assays

AT1 and AT2 Radioligand Binding Assays

These in vitro assays were used to assess the ability of test compounds to bind to the AT1 and the AT2 receptors.

Membrane Preparation From Cells Expressing Human AT1 or AT2 Receptors

Chinese hamster ovary (CHO-K1) derived cell lines stably expressing the cloned human AT1 or AT2 receptors, respectively, were grown in HAM's-F12 medium supplemented with 10% fetal bovine serum, 10 µg/ml penicillin/streptomycin, and 500 µg/ml geneticin in a 5% CO2 humidified incubator at 37 °C. AT2 receptor expressing cells were grown in the additional presence of 100 nM PD123,319 (AT2 antagonist). When cultures reached 80-95% confluence, the cells were washed thoroughly in PBS and lifted with 5 mM EDTA. Cells were pelleted by centrifugation and snap frozen in MeOH-dry ice and stored at -80 °C until further use.

For membrane preparation, cell pellets were resuspended in lysis buffer (25 mM Tris/HCl pH 7.5 at 4 °C, 1 mM EDTA, and one tablet of Complete Protease Inhibitor Cocktail Tablets with 2 mM EDTA per 50 mL buffer (Roche cat.# 1697498, Roche Molecular Biochemicals, Indianapolis, IN)) and homogenized using a tight-fitting Dounce glass homogenizer (10 strokes) on ice. The homogenate was centrifuged at 1000 x g, the supernatant was collected and centrifuged at 20,000 x g. The final pellet was resuspended in membrane buffer (75 mM Tris/HCl pH 7.5, 12.5 mM MgCl2, 0.3 mM EDTA, 1 mM EGTA, 250 mM sucrose at 4 °C) and homogenized by extrusion through a 20G gauge needle. Protein concentration of the membrane suspension was determined by the method described in Bradford (1976) Anal Biochem. 72:248-54. Membranes were snap frozen in MeOH-dry ice and stored at -80 °C until further use.

Ligand Binding Assay to Determine Compound Affinities for the Human AT1 and AT2 Angiotensin Receptors

Binding assays were performed in 96-well Acrowell filter plates (Pall Inc., cat.# 5020) in a total assay volume of 100 µL with 0.2 µg membrane protein for membranes containing the human AT1 receptor, or 2 µg membrane protein for membranes containing the human AT2 receptor in assay buffer (50 mM Tris/HCl pH 7.5 at 20 °C, 5 mM MgCl2, 25 µM EDTA, 0.025% BSA). Saturation binding studies for determination of Kd values of the ligand were done using N-terminally Europium-labeled angiotensin-II ([Eu]AngII, H-(Eu-N1)-Ahx-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH; PerkinElmer, Boston, MA). Displacement assays for determination of pKi values of test compounds were done with [Eu]AngII at 2 nM and 11 different concentrations of drug ranging from 1 pM to 10 µM. Drugs were dissolved to a concentration of 1 mM in DMSO and from there serially diluted into assay buffer. Non-specific binding was determined in the presence of 10 µM unlabeled angiotensin-II. Assays were incubated for 120 minutes in the dark, at room temperature or 37 °C, and binding reactions were terminated by rapid filtration through the Acrowell filter plates followed by three washes with 200 µL ice cold wash buffer (50 mM Tris/HCl pH 7.5 at 4 ºC, 5 mM MgCl2) using a Waters filtration manifold. Plates were tapped dry and incubated with 50 µl DELFIA Enhancement Solution (PerkinElmer cat.# 4001-0010) at room temperature for 5 minutes on a shaker. Filter-bound [Eu]AngII was quantitated immediately on a Fusion plate reader (PerkinElmer) using Time Resolved Fluorescence (TRF). Binding data were analyzed by nonlinear regression analysis with the GraphPad Prism Software package (GraphPad Software, Inc., San Diego, CA) using the 3-parameter model

for one-site competition. The BOTTOM (curve minimum) was fixed to the value for nonspecific binding, as determined in the presence of 10 µM angiotensin II. Ki values for drugs were calculated from observed IC50 values and the Kd value of [Eu]AngII according to the Cheng-Prusoff equation described in Cheng et al. (1973) Biochem Pharmacol. 22(23):3099-108. Selectivities of test compounds for the AT1 receptor over the AT2 receptor were calculated as the ratio of AT2Ki/AT1Ki. Binding affinities of test compounds were expressed as negative decadic logarithms of the Ki values (pKi).

In this assay, a higher pKi value indicates that the test compound has a higher binding affinity for the receptor tested. Exemplary compounds of the invention that were tested in this assay, typically were found to have a pKi at the AT1 receptor greater than or equal to about 5.0. For example, the compound of Example 1 was found to have a pKi value greater than about 7.0.

Quantitation of Inhibitor Potencies (IC50) at Human and Rat NEP, and Human ACE

The inhibitory activities of compounds at human and rat NEP and human ACE were determined using in vitro assays as described below.

Extraction of NEP Activity from Rat Kidneys

Rat NEP was prepared from the kidneys of adult Sprague Dawley rats. Whole kidneys were washed in cold PBS and brought up in ice-cold lysis buffer (1% Triton X-114, 150 mM NaCl, 50 mM Tris pH 7.5; Bordier (1981) J. Biol. Chem. 256: 1604-1607) in a ratio of 5 mL of buffer for every gram of kidney. Samples were homogenized using a polytron hand held tissue grinder on ice. Homogenates were centrifuged at 1000 x g in a swinging bucket rotor for 5 minutes at 3 °C. The pellet was resuspended in 20 mL of ice cold lysis buffer and incubated on ice for 30 minutes. Samples (15-20 mL) were then layered onto 25 mL of ice-cold cushion buffer (6% w/v sucrose, 50 mM pH 7.5 Tris, 150 mM NaCl, 0.06%, Triton X-114), heated to 37 °C for 3-5 minutes and centrifuged at 1000 x g in a swinging bucket rotor at room temperature for 3 minutes. The two upper layers were aspirated off, leaving a viscous oily precipitate containing the enriched membrane fraction. Glycerol was added to a concentration of 50% and samples were stored at -20 °C. Protein concentrations were quantitated using a BCA detection system with BSA as a standard.

Enzyme Inhibition Assays

Recombinant human NEP and recombinant human ACE were obtained commercially (R&D Systems, Minneapolis, MN, catalog numbers 1182-ZN and 929-ZN, respectively). The fluorogenic peptide substrate Mca-BK2 (Mca-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(Dnp)-OH; Johnson et al. (2000) Anal. Biochem. 286: 112-118) was used for the human NEP and ACE assays, and Mca-RRL (Mca-DArg-Arg-Leu-(Dnp)-OH; Medeiros et al. (1997) Braz. J. Med. Biol. Res. 30:1157-1162) was used for the rat NEP assay (both from Anaspec, San Jose, CA).

The assays were performed in 384-well white opaque plates at room temperature using the respective fluorogenic peptides at a concentration of 10 µM in assay buffer (50 mM Tris/HCL at 25 °C, 100 mM NaCl, 0.01% Tween-20, 1 µM Zn, 0.025% BSA). Human NEP and human ACE were used at concentrations that resulted in quantitative proteolysis of 5 µM of Mca-BK2 within 20 minutes at room temperature. The rat NEP enzyme preparation was used at a concentration that yielded quantitative proteolysis of 3 µM of Mca-RRL within 20 minutes at room temperature.

Compounds were serially diluted in assay buffer containing 400 µM of tris(2-carboxyethyl)phosphine hydrochloride (Thermo Scientific, Rockford, IL) (TCEP) to reduce potential disulfides. Assays were started by adding 25 µL of enzyme to 12.5 µL of serially diluted test compound (10 µM to 20 pM). Inhibitors were allowed to equilibrate with the enzyme for 10 minutes before 12.5 µL of the fluorogenic substrates were added to initiate the reaction. Reactions were terminated by the addition of 10 µL of 3.6% glacial acetic acid after 20 minutes of incubation. Plates were read on a fluorometer with excitation and emission wavelengths set to 320 nm and 405 nm, respectively.

Raw data (relative fluorescence units) were normalized to % activity from the average high readings (no inhibition, 100% enzyme activity) and average low readings (full inhibition, highest inhibitor concentration, 0% enzyme activity) using three standard NEP and ACE inhibitors, respectively. Nonlinear regression of the normalized data was performed using a one site competition model (GraphPad Software, Inc., San Diego, CA). Data were reported as pIC50 values.

Exemplary compounds of the invention that were tested in this assay, typically were found to have a pIC50 for the NEP enzyme greater than or equal to about 5.0. For example, the compound of Example 1 has a pIC50 value greater than or equal to about 7.0.

Pharmacodynamic (PD) assay for ACE, AT1, and NEP Activity in Anesthetized Rats

Male, Sprague Dawley, normotensive rats were anesthetized with 120 mg/kg (i.p.) of inactin. Once anesthetized, the jugular vein, carotid artery (PE 50 tubing) and bladder (URI-1 urinary silicone catheter) were cannulated and a tracheotomy was performed (Teflon Needle, size 14 gauge) to faciliate spontaneous respiration. The animals were then allowed a 60 minute stablization period and kept continuously infused with 5mL/kg/h of saline (0.9%) throughout, to keep them hydrated and ensure urine production. Body temperature was maintained throughout the experiment by use of a heating pad. At the end of the 60 minute stabilization period, the animals were dosed intravenously (i.v.) with two doses of angiotensin (AngI, 1.0 µg/kg, for ACE inhibitor activity; AngII, 0.1 µg/kg, for AT1 receptor antagonist activity) at 15 minutes apart. At 15 minutes post-second dose of angiotensin (AngI or AngII), the animals were treated with vehicle or test compound. Five minutes later, the animals were additionally treated with a bolus i.v. injection of atrial natriuretic peptide (ANP; 30 µg/kg). Urine collection (into pre-weighted eppendorf tubes) was started immediately after the ANP treatment and continued for 60 minutes. At 30 and 60 minutes into urine collection, the animals were re-challenged with angiotensin (AngI or AngII). Blood pressure measurements were done using the Notocord system (Kalamazoo, MI). Urine samples were frozen at -20 °C until used for the cGMP assay. Urine cGMP concentrations were determined by Enzyme Immuno Assay using a commercial kit (Assay Designs, Ann Arbor, Michigan, Cat. No. 901-013). Urine volume was determined gravimetrically. Urinary cGMP output was calculated as the product of urine output and urine cGMP concentration. ACE inhibition or AT1 antagonism was assessed by quantifying the % inhibition of pressor response to AngI or AngII, respectively. NEP inhibition was assessed by quantifying the potentiation of ANP-induced elevation in urinary cGMP output.

In Vivo Evaluation of Antihypertensive Effects in the Conscious SHR Model of Hypertension

Spontaneously hypertensive rats (SHR, 14-20 weeks of age) were allowed a minimum of 48 hours acclimation upon arrival at the testing site. Seven days prior to testing, the animals were either placed on a restricted low-salt diet with food containing 0.1% of sodium for sodium depleted SHRs (SD-SHR) or were placed on a normal diet for sodium repleted SHRs (SR-SHR). Two days prior to testing, the animals were surgically implemented with catheters into a carotid artery and the jugular vein (PE50 polyethylene

tubing) connected via a PE10 polyethylene tubing to a selected silicone tubing (size 0.020 ID x 0.037 OD x 0.008 wall) for blood pressure measurement and test compound delivery, respectively. The animals were allowed to recover with appropriate post operative care.

On the day of the experiment, the animals were placed in their cages and the catheters were connected via a swivel to a calibrated pressure transducer. After 1 hour of acclimation, a baseline measurement was taken over a period of at least five minutes. The animals were then dosed i.v. with vehicle or test compound in ascending cumulative doses every 60 minutes followed by a 0.3 mL saline to clear the catheter after each dose. Data was recorded continuously for the duration of the study using Notocord software (Kalamazoo, MI) and stored as electronic digital signals. In some studies, the effects of a single intravenous or oral (gavage) dose were monitored for at least 6 hours after dosing. Parameters measured were blood pressure (systolic, diastolic and mean arterial pressure) and heart rate.

In Vivo Evaluation of Antihypertensive Effects in the Conscious DOCA-Salt Rat Model of Hypertension

CD rats (male, adult, 200-300 grams, Charles River Laboratory, USA) were allowed a minimum of 48 hours acclimation upon arrival at the testing site before they were placed on a high salt diet. One week after the start of the high salt diet, a DOCA-salt pellet (100 mg, 21 days release time, Innovative Research of America, Sarasota, FL) was implanted subcutaneously and unilateral nephrectomy was performed. On 16 or 17 days post DOCA-salt pellet implantation, animals were implanted surgically with catheters into a carotid artery and the jugular vein with a PE50 polyethylene tubing, which in turn was connected via a PE10 polyethylene tubing to a selected silicone tubing (size 0.020 ID x 0.037 OD x 0.008 wall) for blood pressure measurement and test compound delivery, respectively. The animals were allowed to recover with appropriate post operative care. On the day of the experiment, each animal was kept in its cage and connected via a swivel to a calibrated pressure transducer. After 1 hour of acclimation, a baseline measurement was taken over a period of at least five minutes. The animals were then dosed i.v. with a vehicle or test compound in escalating cumulative doses every 60 minutes followed by 0.3 mL of saline to flush the catheter after each dose. In some studies, the effects of a single intravenous or oral (gavage) dose was tested and monitored for at least 6 hours after dosing. Data was recorded continuously for the duration of the study using Notocord software (Kalamazoo, MI) and stored as electronic digital signals. Parameters measured were blood pressure (systolic, diastolic and mean arterial pressure) and heart rate. For cumulative and single dosing, the percentage change in mean arterial pressure (MAP, mmHg) or heart rate (HR, bpm) was determined as previously described.

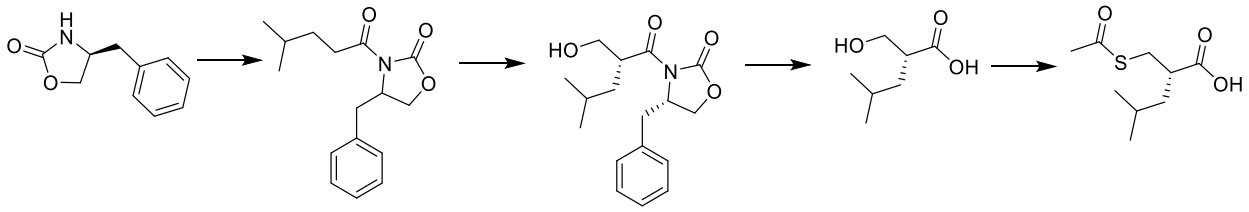
Synthetic Procedures

Unless noted otherwise, all materials, such as reagents, starting materials and solvents, were purchased from commercial suppliers (such as Sigma-Aldrich, Fluka Riedel-de Haën, and the like) and were used without further purification.

Reactions were run under nitrogen atmosphere, unless noted otherwise. The progress of reactions were monitored by thin layer chromatography (TLC), analytical high performance liquid chromatography (anal. HPLC), and mass spectrometry, the details of which are given in specific examples. Solvents used in analytical HPLC were as follows: solvent A was 98% water/2% MeCN /1.0 mL/L TFA; solvent B was 90% MeCN/10% water/1.0 mL/L TFA.

Reactions were worked up as described specifically in each preparation or example; commonly reaction mixtures were purified by extraction and other purification methods such as temperature-, and solvent-dependent crystallization, and precipitation. In addition, reaction mixtures were routinely purified by preparative HPLC, typically using Microsorb C18 and Microsorb BDS column packings and conventional eluents. Characterization of reaction products was routinely carried out by mass and ^{1}H-NMR spectrometry. For NMR measurement, samples were dissolved in deuterated solvent (CD_3OD, $CDCl_3$, or DMSO-d_6), and ^{1}H-NMR spectra were acquired with a Varian Gemini 2000 instrument (400 MHz) under standard observation conditions. For compound **35**, NMR were recorded in DMSO-d6 on a Bruker 600 MHz Ultra Shieldplus instrument. Mass spectrometric identification of compounds was typically conducted using an electrospray ionization method (ESMS) with an Applied Biosystems (Foster City, CA) model API 150 EX instrument or an Agilent (Palo Alto, CA) model 1200 LC/MSD instrument. High resolution mass spectrometry (HRMS) was performed on a ThermoFinnigan (Thermo Electronics, San Jose, CA) LTQ Orbitrap XL mass spectrometer equipped with Fourier Transform (FT) and Linear Ion Trap (LIT) mass analyzers.

***(S)*-2-Acetylsulfanylmethyl-4-methylpentanoic Acid**



Step 1

Isocaproic acid (10.0 g, 86.1 mmol) was dissolved in methylene chloride (30.0 mL, 468.0 mmol), and thionyl chloride (18.8 mL, 258 mmol) was added. The mixture was stirred at room temperature overnight, then evaporated to dryness. *(S)*-4-Benzyl-2-oxazolidinone (15.1 g, 85.0 mmol) was dissolved in THF (200 mL, 2.5 mol), cooled at -78 °C under nitrogen, and stirred for 10 minutes. 1.6 M of *n*-butyllithium in hexane (53.1 mL) was added dropwise and stirred for 15 minutes. 4-4-Methylpentanoyl chloride (12.6 g, 93.5 mmol) was added dropwise, stirred for 30 minutes at -78 °C, then warmed to 0 °C for 2 hours. 150 mL of saturated $NaHCO_3$ was added and the mixture was warmed to room temperature for 30 minutes. The mixture was extracted with DCM, washed with Na_2CO_3 (5%) and saturated aqueous NaCl, dried over $MgSO_4$, filtered and concentrated. Excess oxazolidinone was removed using hexanes to provide *(S)*-4-benzyl-3-(4-methylpentanoyl)oxazolidin-2-one (14.5 g, 52.7 mmol, 61%). ^{1}H-NMR (DMSO) δ (ppm): 0.85 (dd, 6H), 1.40-1.60 (m, 3H), 2.7-3 (m, 4H), 4.15 (t, 1H), 4.28 (t, 1H), 4.60 (m, 1H), 7.15-7.35 (m, 5H).

Step 2

(S)-4-benzyl-3-(4-methylpentanoyl)oxazolidin-2-one (14.5 g, 52.7 mmol) was dissolved in DCM (151 mL, 2.4 mol) and stirred at 0 °C under nitrogen. 1 M titanium tetrachloride in DCM (48.6 mL) was added and stirred for 15 minutes. DIPEA (8.9 mL, 51.0 mmol) was added dropwise at 0 °C and the mixture was stirred for 75 minutes. 1,3,5-Trioxane (4.6 g, 51.0 mmol) in DCM (30 mL) was then added. After 10 minutes a second equivalent of 1 M titanium tetrachloride in DCM (48.6 mL) was added and the mixture stirred at 0 °C for 5 hours. The reaction was then quenched with 250 mL of saturated ammonium chloride. Water and DCM were added, and the aqueous phase was extracted twice more with DCM. The organic layers were combined, dried over $MgSO_4$, filtered and concentrated. The resulting material was purified by silica gel chromatography (0-60% EtOAc:hexanes) to provide *(S)*-4-benzyl-3-(*(R)*-2-hydroxymethyl-4-methylpentanoyl)oxazolidin-2-one (13.9 g, 45.5 mmol, 86%).

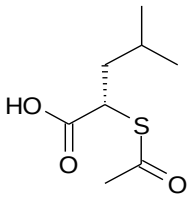
Step 3

(S)-4-benzyl-3-(*(R)*-2-hydroxymethyl-4-methylpentanoyl)oxazolidin-2-one (13.9 g, 45.5 mmol) was dissolved in THF (200 mL, 2 mol) and stirred at 0 °C. 9 M hydrogen peroxide in water (46.3 mL) was added, followed by dropwise addition of 1.5 M lithium hydroxide monohydrate in water (54.4 mL). The mixture was then warmed to room temperature and stirred for 2.5 hours. Potassium hydroxide (4.6 g, 81.6 mmol) was added and the mixture was heated at 60 °C for 30 minutes and then cooled at room temperature. To this was added a solution of sodium sulfite (10 g in 200 mL water) followed by water and chloroform (200 mL of each). The aqueous layer was extracted twice more with $CHCl_3$ (150 mL), acidified and extracted with EtOAc. The organic layer was then washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and evaporated to provide *(R)*-2-hydroxymethyl-4-methylpentanoic acid (5.4 g, 37 mmol, 81%).

Step 4

Triphenylphosphine (19.5 g, 74.3 mmol) was dissolved in THF (200 mL, 2 mol) and cooled at 0 °C. Diisopropyl azodicarboxylate (14.6 mL, 74.3 mmol) was added dropwise and the mixture stirred for 10 minutes at 0 °C. *(R)*-2-hydroxymethyl-4-methylpentanoic acid (5.4 g, 37 mmol) and thioacetic acid (8.0 mL, 111 mmol) were dissolved in THF (20 mL) and added dropwise to the reaction. After the addition, the mixture was removed from the ice bath and stirred at room temperature. The mixture was stirred for 3.5 hours, concentrated to approximately a third of the volume, and then partitioned between EtOAc and saturated $NaHCO_3$. The organic layer was extracted three times more with saturated $NaHCO_3$ and the combined aqueous extracts were washed twice with $CHCl_3$, acidified with 1N HCl and extracted three times with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and evaporated to provide (S)-2-acetylsulfanylmethyl-4-methylpentanoic acid as a clear oil (2.49g, 12.2 mmol, 33%). ^{1}H-NMR (DMSO) δ (ppm): 0.8 (dd, 6H), 1.25 (m, 1H), 1.42 (m, 1H), 1.55 (m, 1H), 2.28(s, 3H), 2.45 (m, 1H), 2.86 (m, 1H), 3.0 (m, 1H), 12.3 (br s, 1H).

***(S)*-2-Acetylsulfanyl-4-methylpentanoic acid**



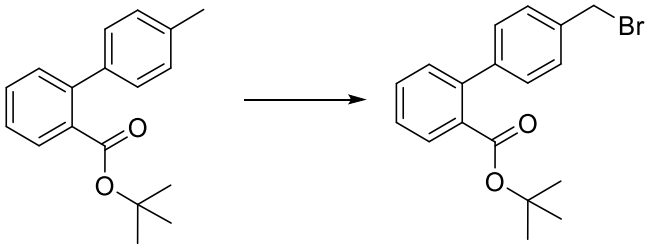
Step 1

D-Leucine (9.9 g, 75.2 mmol) was dissolved in 3.0 M of HBr in water (120 mL) and cooled to 0 °C. A solution of $NaNO_2$ (8.3 g, 120 mmol) in water (20 mL, 100 mmol) was added. The reaction was stirred at 0 °C for 2.5 hours and then extracted twice with EtOAc, washed twice with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and concentrated to afford 12.6 g (64.1 mmol, 85%) of *(R)*-2-bromo-4-methylpentanoic acid as a pale yellow oil. This was taken on to the next step without further purification. ^{1}H-NMR (DMSO) δ (ppm): 4.31 (1H, t), 1.75 (2H, m) 1.65 (1H, m) 0.82 (6H, dd).

Step 2

(R)-2-Bromo-4-methylpentanoic acid (12.5 g, 64.1 mmol), potassium thioacetate (11.0 g, 96.1 mmol), and DMF (100 mL, 1.0 mol) were combined and the mixture stirred at room temperature for 1 hour. Water (100 mL) was then added, and the mixture was extracted three times with EtOAc, washed twice with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and concentrated. The product was purified by silica gel chromatography (0-40% EtOAc:hexanes with 5% acetic acid) to yield 6.8 g (35.7 mmol, 58%) of the title compound as a pale yellow oil. ^{1}H-NMR (DMSO) δ (ppm): 3.96 (1H, t), 2.45 (3H, s), 1.70 (1H, m), 1.55 (1H, m), 1.42 (1H, m), 0.84 (6H, dd).

4'-Bromomethylbiphenyl-2-carboxylic acid *t*-butyl ester



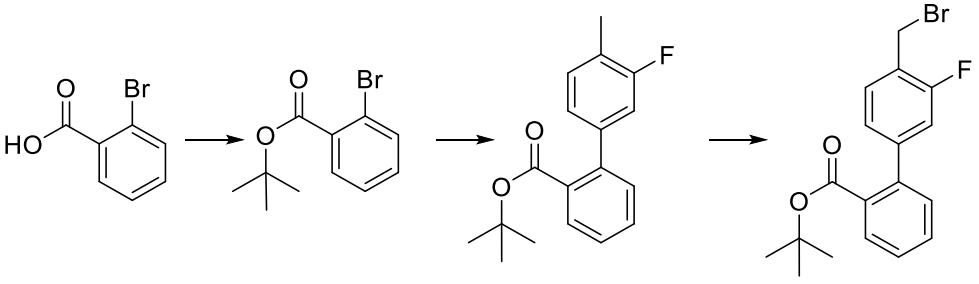
Step 1

A solution of 4'-methylbiphenyl-2-carboxylic acid (Boron Molecular Inc.,48.8 g, 230 mmol) and thionyl chloride (150 mL) was stirred at room temperature. After 5.5 hours, the mixture was concentrated *in vacuo*. Excess thionyl chloride was removed by co-distillation with toluene to afford a yellow solid (52.6 g). The material was then dissolved in THF (500 mL) and cooled to 0 °C. Potassium *t*-butoxide (15.0 g, 130 mmol) was added portion wise, followed by addition of 1M solution of potassium *t*-butoxide in THF (250 mL). Additional solid potassium *t*-butoxide (21.4 g, 100 mmol) was added and the mixture was stirred at 0 °C for 1.5 hours. The mixture was then partitioned between EtOAc and water, and the organic layer was washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered and concentrated to afford 4'-methylbiphenyl-2-carboxylic acid *t*-butyl ester as a yellow oil (62.3 g, 230 mmol, 100%), which was used directly in the next step.

Step 2

Benzoyl peroxide (3.9 g, 16.0 mmol) was added to a solution of intermediate (3a) (62 g, 230 mmol) and NBS (41.2 g, 230 mmol) in benzene (800 mL). The mixture was then heated at reflux. After 4.5 hours, benzoyl peroxide (1 g) was added, followed by NBS (16 g, 66.0 mmol) 30 minutes later. The mixture was stirred for a total of 6 hours, cooled, filtered, and concentrated *in vacuo*. The resulting residue was then crystallized from diethyl ether and hexane at 4 °C overnight to give the title compound as a pale yellow solid (40.7 g, 117 mmol, 51%). ^{1}H-NMR (DMSO): δ (ppm) 1.1 (s, 9H), 4.6 (s, 2H), 7.1-7.6 (m, 8H).

4'-Bromomethyl-3'-fluorobiphenyl-2-carboxylic acid *t*-butyl ester



Step 1

To a solution of 1.0 M DCC in methylene chloride (505 mL, 505 mmol, 1.0 eq) cooled at 0°C was added 2-bromobenzoic acid (14a) (101.6 g, 505 mmol, 1.0 eq) followed by DMAP (5.7 g, 470 mmol, 0.09 eq) and *t*-butyl alcohol (53.2 mL, 560 mmol, 1.1 eq). The mixture was stirred at room temperature for 18 hours and was then filtered. The organic was washed with 400 mL saturated $NaHCO_3$, saturated aqueous NaCl, dried

over $MgSO_4$, filtered and concentrated to produce tert-butyl 2-bromobenzoate as an oil (141.8 g, 551 mmol, apparent yield >100%) which was used directly in the next step.

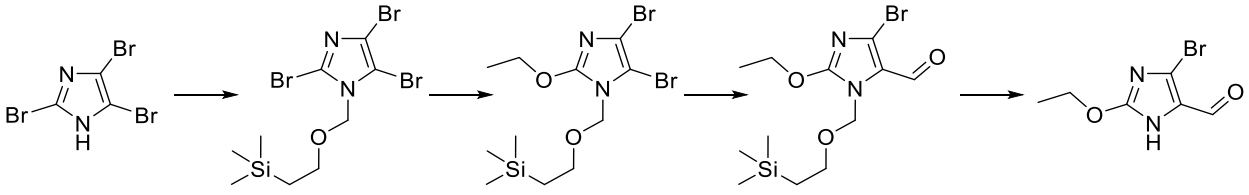
Step 2

tert-butyl 2-bromobenzoate (111.2 g, 433 mmol 1.0 eq) and 3-fluoro-4-methylphenylboronic acid (73.2 g, 476 mmol, 1.1 eq) were suspended in isopropyl alcohol (370 mL). A 2.0 M solution of sodium carbonate in water (370 mL) was added and the mixture was degassed under nitrogen. Tetrakis(triphenylphosphine)palladium(0) (5.0 g, 4.3 mmol, 0.01 eq) was then added and the mixture was stirred at 90 °C for 46 hours. The mixture was cooled to room temperature, diluted with 800 mL EtOAc, and the layers were separated. The organic was washed with saturated aqueous NaCl and concentrated under reduced pressure. The recovered oil was purified by silica gel chromatography (4-6% EtOAc:hexanes) to yield tert-butyl 3'-fluoro-4'-methyl-[1,1'-biphenyl]-2-carboxylate as an oil (97.4 g, 340 mmol, 78%).

Step 3

tert-Butyl 3'-fluoro-4'-methyl-[1,1'-biphenyl]-2-carboxylate (89.8 g, 314 mmol, 1.0 eq) was dissolved in CCl_4 (620 mL) and was degassed under nitrogen. NBS (55.8 g, 314 mmol, 1.0 eq) was added, followed by benzoyl peroxide (1.5 g, 6.3 mmol, 0.02 eq) and the mixture was heated at 90 °C under nitrogen for 16 hours. The reaction was cooled in an ice bath, filtered, and concentrated under reduced pressure. The recovered oil was triturated with 150 mL of 3% EtOAc:hexanes. The solution was chilled at -20 °C for 2 hours, then filtered and washed with 200 mL cold 3% EtOAc:hexanes solution to yield 4'-bromomethyl-3'-fluorobiphenyl-2-carboxylic acid *t*-butyl ester as an off white solid (88.9 g, 243 mmol, 77%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.3 (m, 9H), 4.6 (s, 2H), 7.0-7.1 (m, 2H), 7.3 (dd, 1H), 7.4 (m, 1H), 7.5 (m, 1H), 7.8 (dd, 1H).

5-Bromo-2-ethoxy-3H-imidazole-4-carbaldehyde



Step 1

2,4,5-Tribromo-1H-imidazole (98.7 g, 324 mmol, 1.0 eq) was dissolved into 1.20 L of methylene chloride and cooled to 0 °C. To this was added DIPEA (62 mL, 360 mmol, 1.1 eq) followed by the slow addition of [β-(trimethylsilyl)ethoxy]methyl chloride (60.2 mL, 340 mmol, 1.05 eq). The solution was slowly warmed to room temperature. After 2 hours the mixture was washed 2x with 600mL of 1M H_3PO_4/saturated aqueous NaCl (1/10 mL). The organic layer was dried over $MgSO_4$, and evaporated to dryness, yielding 2,4,5-tribromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazole as faint yellow liquid that solidified on standing (137 g, 315 mmol, 97%).

Step 2

2,4,5-Tribromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazole (130 g, 290 mmol, 1.0 eq) was dissolved into 650 mL anhydrous ethanol. To this was slowly added potassium tert-butoxide (98.6 g, 879 mmol, 3.0 eq) and the mixture was heated to reflux for 16 hours. The mixture was then cooled to room temperature,

filtered and concentrated. The resulting oil was dissolved into 800 mL EtOAc and washed with 400 mL saturated $NaHCO_3$. The layers were separated and the organic was washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered and concentrated, yielding 4,5-dibromo-2-ethoxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazole as a brown oil (115.3 g, 288 mmol, 99%). MS *m/z*: [M + H^+] calcd for $C_{11}H_{20}Br_2N_2O_2Si$, 398.9 found 401.2.

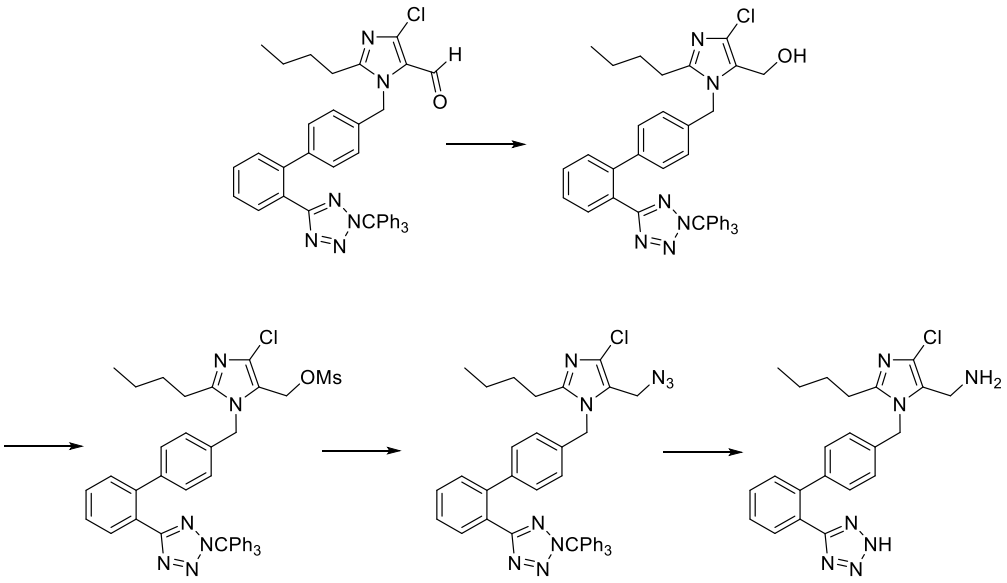
Step 3

4,5-dibromo-2-ethoxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazole (69.5 g, 174 mmol, 1.0 eq) was dissolved in 600 mL of anhydrous THF and cooled to -78 °C under nitrogen. A 2.5 M solution of n-butyllithium in hexanes (72.9 mL, 180 mmol, 1.05 eq) was added dropwise and the mixture was stirred at -78 °C for 10 minutes. DMF (40 mL, 520 mmol, 3.0 eq) was then added and the mixture was stirred at -78 °C for 15 minutes and was then warmed to room temperature. The reaction was quenched with 10 mL water, diluted with 600 mL EtOAc and was washed with 100mL water, saturated aqueous NaCl, dried over $MgSO_4$ and concentrated under reduced pressure. The recovered material was purified by silica gel chromatography (15-30% EtOAc:hexanes) to produce 4-bromo-2-ethoxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazole-5-carbaldehyde as a pale yellow oil (45 g, 129 mmol, 74%).

Step 4

4-bromo-2-ethoxy-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazole-5-carbaldehyde (105.8 g, 303 mmol, 1.0 eq) was cooled at 0 °C in ice. TFA (300 mL) was added and the mixture was stirred at 0 °C for 15 minutes, then warmed to room temperature. After 90 minutes the mixture was concentrated under reduced pressure and redissolved in 700 mL EtOAc. The organic was washed 2x 600 mL saturated bicarbonate, saturated aqueous NaCl, dried over $MgSO_4$, and concentrated under reduced pressure to produce a yellow solid. The material was suspended in 300 mL hexane and stirred at 0 °C for 30 minutes. The material was filtered and the solid was washed with 150 mL of cold hexane to yield 5-bromo-2-ethoxy-3H-imidazole-4-carbaldehyde as a pale white solid (61.2 g, 279 mmol, 92%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.4 (m, 3H), 4.5 (m, 2H), 5.2 (s, 1H), 9.2 (d, 1H).

Synthesis of Intermediate A



Step 1

To an EtOH (500 mL) solution of 2-butyl-5-chloro-3-[2'-(1-trityl-1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazole-4-carbaldehyde[14] (41.0 g, 61.8 mmol) was added solid $NaBH_4$ (2.8 g, 74.1 mmol). The mixture was stirred at room temperature for 1 hour. After cooling to 0 °C, the reaction was quenched by dropwise addition of a 50/50 solution of acetic acid/water until no effervescence was observed. EtOAc (500 mL) was added and the organic was washed three times with saturated aqueous NaCl (100 mL). Solvent was removed and purification was achieved by silica gel chromatography (50:50 EtOAc:hexanes) using an isocratic gradient to provide 2-butyl-5-chloro-3-[2'-(1-trityl-1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-yl}-methanol as a white solid (37 g, 55.6 mmol, 90%). MS m/z: [M + H+] calcd for $C_{41}H_{37}ClN_6O$, 665.28; found 665.3. ^{1}H-NMR (CD_3OD) δ (ppm): 0.79 (m, 3H), 1.20 (m, 2H), 1.46 (m, 2H), 2.45 (m, 2H), 4.30 (s, 2H), 5.19 (s, 2H), 6.83-6.90 (m, 8H), 7.05 (d, 2H), 7.23-7.33 (m, 10H), 7.53 (m, 2H), 7.81 (d, 1H).

Step 2

To a DCM (200 mL) solution of 2-butyl-5-chloro-3-[2'-(1-trityl-1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-yl}-methanol (13.6 g, 20.4 mmol), cooled to -78 °C, was added methane sulfonyl chloride (6.3 mL, 81.6 mmol). The mixture was stirred at -78 °C for 15 minutes. A saturated $NaHCO_3$ solution (100 mL) and EtOAc (500 mL) were added to the cooled mixture, which was then allowed to reach room temperature. The organic was washed an additional three times with saturated aqueous NaCl (100 mL). After drying over sodium sulfate and filtration, the removal of solvent provided intermediate methanesulfonic acid 2-butyl-5-chloro-3-[2'-(1-trityl-1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-ylmethyl ester as a yellow oil (15 g, 20 mmol, 98%) which due to its reactive nature was used directly in the next step.

Step 3

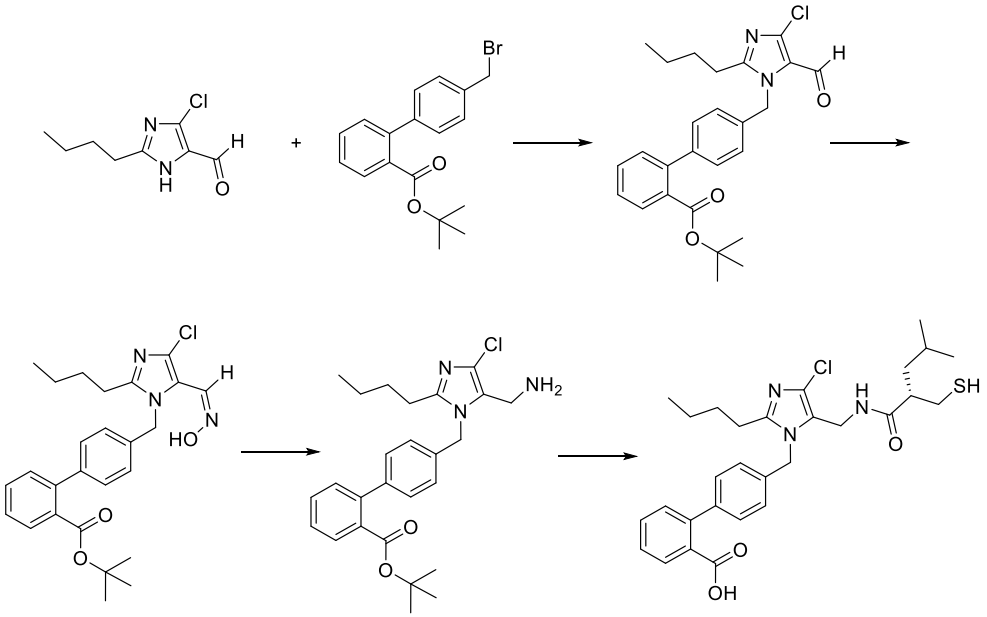
To a dimethyl sulfoxide (100 mL) solution of 2-butyl-5-chloro-3-[2'-(1-trityl-1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-ylmethyl ester (15 g, 20 mmol) was added sodium azide (3.9 g, 60 mmol). The mixture was stirred at room temperature for 20 minutes. EtOAc (500 mL) and saturated aqueous NaCl (100 mL) were added and the layers were separated, retaining the organic layer. The organic was washed

an additional three times with saturated aqueous NaCl (100 mL). After drying over sodium sulfate and filtration, the removal of solvent provided intermediate 5-[4'-(5-azidomethyl-2-butyl-4-chloro-imidazol-1-ylmethyl)-biphenyl-2-yl]-1-trityl-1H-tetrazole as a yellow solid (12 g, 17 mmol, 85%), which was used directly in the next step. MS m/z: [M + H+] calcd for $C_{41}H_{36}ClN_9$, 690.29; found 690.5.

Step 4

To a MeOH (150 mL) solution of 5-[4'-(5-azidomethyl-2-butyl-4-chloro-imidazol-1-ylmethyl)-biphenyl-2-yl]-1-trityl-1H-tetrazole (12 g, 17 mmol), was added palladium on carbon (6.0 g; 10% Pd w/w). The solution was first degassed and backfilled with nitrogen gas (1 atm) followed by additional degassing and backfilling with hydrogen gas (1 atm). The solution was then stirred at room temperature for 2-3 hours at which the azide reduction was 50% complete. Additional palladium on carbon (1.0 g; 10% Pd w/w) was added and the solution stirred under hydrogen. After 1.5 hours the reaction was complete, the mixture was degassed and backfilled with nitrogen prior to filtration through Celite. The filtrate was reduced to a volume of 50 mL. Into the filtrate was slowly added 6N HCl (150 mL) and the solution was stirred for 4 hours and then evaporated to dryness. Purification was accomplished by preparative HPLC (reverse phase) to provide 2-butyl-5-chloro-3-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-3H-imidazol-4-yl}methylamine•2[$C_2HF_3O_2$] as a white solid (4.0 g, 6.1 mmol, 36%). MS m/z: [M + H+] calcd for $C_{22}H_{24}ClN_7$, 422.19; found. 422.0. ^{1}H-NMR (CD_3OD) δ (ppm): 0.89 (m, 3H), 1.35 (m, 2H), 1.59 (m, 2H), 2.88 (m, 2H), 4.20 (s, 2H), 5.58 (s, 2H), 7.10 (d, 2H), 7.19 (d, 2H), 7.52-7.67 (m, 4H).

Synthesis of Compound 7



Step 1

2-Butyl-5-chloro-3H-imidazole-4-carbaldehyde (9.9 g, 53.3 mmol), 4'-bromomethylbiphenyl-2-carboxylic acid *t*-butyl ester (18.5 g, 53.3 mmol), and K_2CO_3 (7.4 g, 53.3 mmol) were combined in DMF (200 mL) and stirred at room temperature overnight. The reaction was quenched with water, extracted with EtOAc, washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered and concentrated. The crude mixture was purified by flash chromatography (0-40% EtOAc:hexanes) to yield 21.5 g (47.4 mmol, 89%) of 4'-(2-butyl-4-chloro-5-formylimidazol-1-ylmethyl)biphenyl-2-carboxylic acid *t*-butyl ester.

Step 2

4'-(2-Butyl-4-chloro-5-formylimidazol-1-ylmethyl)biphenyl-2-carboxylic acid *t*-butyl ester (12.1 g, 26.7 mmol) and NH_2OH•HCl (2.4 g, 34.7 mmol) were combined in 80 mL water and 160 mL pyridine and stirred at room temperature overnight. Water (80 mL) was added and the mixture was stirred for 1 hour. The precipitate was filtered and dried to yield 11.7 g (25.3 mmol, 95%) of 4'-[2-butyl-4-chloro-5-(hydroxyiminomethyl)imidazol-1-ylmethyl]biphenyl-2-carboxylic acid t-butyl ester which was used in the next step without further purification.

Step 3

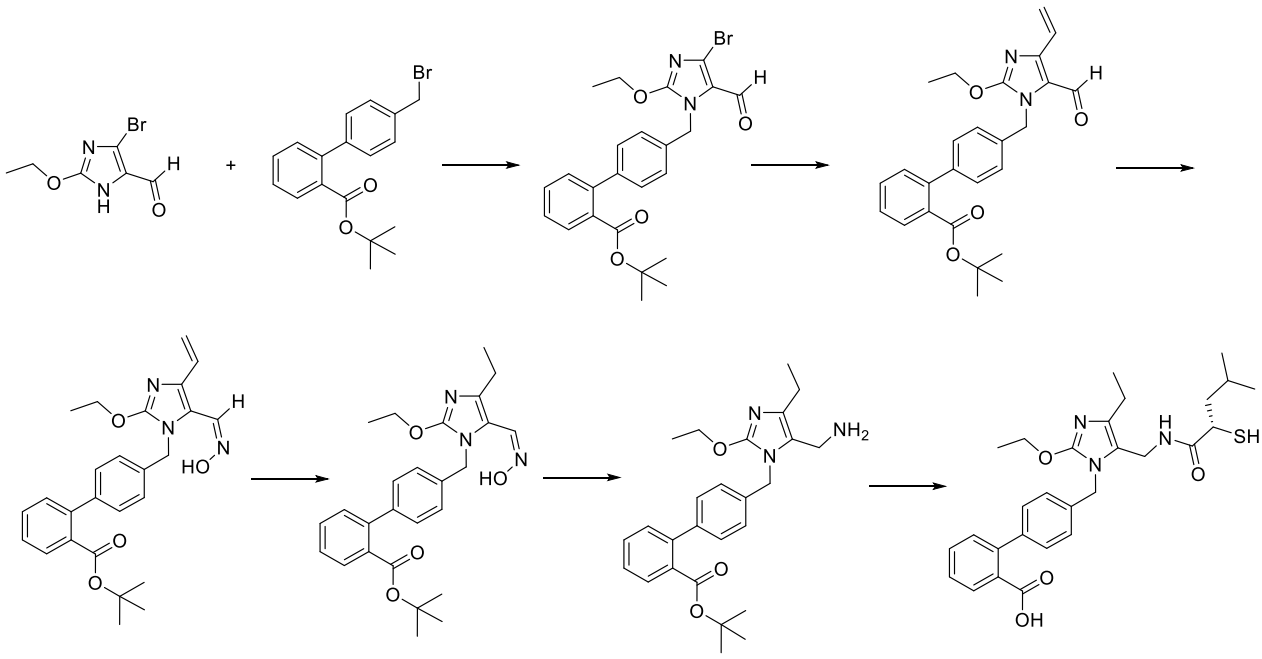
4'-[2-Butyl-4-chloro-5-(hydroxyiminomethyl)imidazol-1-ylmethyl]biphenyl-2-carboxylic acid *t*-butyl ester (8.8 g, 19 mmol), $NaBH_3CN$ (5.3 g, 84.6 mmol) and NH_4OAc (3.2 g, 41.4 mmol) were dissolved in MeOH (100 mL) and cooled to 0 °C. After 15 minutes, $TiCl_3$ (8.70 g, 56.4 mmol) was added in three portions, and the mixture was stirred at 0 °C for 10 minutes. The mixture was then removed from the ice bath and allowed to warm to room temperature, while stirring, for 3 hours. The mixture was cooled to 0 °C, the reaction quenched with NH_4OH (100 mL), and the mixture stirred for 15 minutes. The resulting titanium salt precipitant was filtered off and rinsed with MeOH multiple times. The filtrate was concentrated. The resulting residue was taken up in a 3:1 mixture of $CHCl_3$ and isopropanol. Saturated aqueous NaCl and

saturated aqueous $NaHCO_3$ were added. The product was extracted twice with a 3:1 mixture of $CHCl_3$ and isopropanol, washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered and concentrated to yield 8.2 g (18.6 mmol, 98%) of 4'-(5-aminomethyl-2-butyl-4-chloroimidazol-1-ylmethyl)biphenyl-2-carboxylic acid t-butyl ester.

Step 4

(S)-2-Acetylsulfanylmethyl-4-methylpentanoic acid (371 mg, 1.8 mmol) and HATU (691 mg, 1.8 mmol) was dissolved in DMF (5 mL, 60 mmol) and stirred at room temperature for 15 minutes. DIPEA (0.3 mL, 2 mmol) was then added and the mixture was stirred for 1 hour. 4'-(5-Aminomethyl-2-butyl-4-chloroimidazol-1-ylmethyl)biphenyl-2-carboxylic acid *t*-butyl ester (750 mg, 1.7 mmol) and DIPEA (0.3 mL, 2 mmol) in DMF (2 mL) was added and the mixture was stirred at room temperature for 4 hours. The reaction was quenched with water and the mixture extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered and concentrated. The reaction was purified by silica gel chromatography (1:1 EtOAc:hexanes) to obtain the acetylsulfanyl ester intermediate. This intermediate was dissolved in DCM:TFA (1:1) (3 mL each) and stirred at room temperature for 3.5 hours and then evaporated to dryness. This intermediate was redissolved in 1:1 MeOH:NaOH (1N) (2 mL each) and stirred at room temperature under nitrogen for 1 hour, the reaction quenched with acetic acid, and the mixture concentrated. The resulting material was purified by preparative HPLC (30-70%) to yield 360 mg (0.55 mmol, 32%) of (S)-4'-((2-butyl-4-chloro-5-((2-(mercaptomethyl)-4-methylpentanamido)methyl)-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid•$[C_2HF_3O_2]$. MS *m/z*: $[M + H^+]$ calcd for $C_{29}H_{36}ClN_3O_3S$, 542.22; found, 542.4. ^{1}H-NMR (DMSO) δ (ppm): 8.4 (1H, t), 7.69 (1H, d), 7.53 (1H, t), 7.42 (1H, t), 7.33 (1H, d), 7.27 (2H, d), 7.01 (2H, d), 5.23 (2H, s), 4.18 (2H, m), 2.35 (1H, m), 2.05 (1H, t), 1.45 (2H, m) 1.38 (2H, m) 1.2 (3H, m), 0.77 (9H, m).

Synthesis of Compound 33



Step 1

5-Bromo-2-ethoxy-3H-imidazole-4-carbaldehyde (3.0 g, 13.7 mmol), 4'-bromomethylbiphenyl-2-carboxylic acid *t*-butyl ester (4.8 g, 13.7 mmol), and potassium carbonate (1.9 g, 13.7 mmol), were dissolved in DMF (60 mL, 780 mmol), and the mixture was stirred at room temperature overnight. The reaction was quenched with water, and the mixture was extracted with EtOAc, washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and concentrated. The resulting material was purified by silica gel chromatography (0-30% EtOAc:hexanes) to yield 4'-(4-bromo-2-ethoxy-5-formylimidazol-1-ylmethyl)biphenyl-2-carboxylic acid *t*-butyl ester (5.6 g, 4.8 mmol, 35%). MS m/z: [M+H$^+$] calcd for $C_{24}H_{25}BrN_2O_4$, 485.1; found 485.3. ^{1}H-NMR ($CDCl_3$) δ (ppm): 9.58 (1H, s), 7.80 (1H, d), 7.50 (1H, t), 7.40 (1H, t), 7.30 (5H, m), 5.42 (2H, s), 4.57 (2H, q), 1.46 (3H, t), 1.18 (9H, s).

Step 2

4'-(4-bromo-2-ethoxy-5-formylimidazol-1-ylmethyl)biphenyl-2-carboxylic acid *t*-butyl ester (5.6 g, 11.5 mmol), tetrakis(triphenylphosphine) palladium(0) (2.1 g, 1.8 mmol), and (2-ethenyl)tri-n-butyltin (13.5 mL, 46.2 mmol) were dissolved in DMF (70 mL, 900 mmol), and heated at 90 °C for 1.5 hours. The mixture was cooled to room temperature and 300 mL of EtOAc was added. The organic layer was washed using a 20% potassium fluoride solution (2 x 100 mL) and saturated aqueous NaCl. The resulting material was purified by silica gel chromatography (0-60% EtOAc:hexanes) to yield 4'-(2-ethoxy-5-formyl-4-vinylimidazol-1-ylmethyl)biphenyl-2-carboxylic acid *t*-butyl ester (4.3 g, 10 mmol, 87%). MS m/z: [M+H$^+$] calcd for $C_{26}H_{28}N_2O_4$, 433.2; found 433.4.

Step 3

4'-(2-ethoxy-5-formyl-4-vinylimidazol-1-ylmethyl)biphenyl-2-carboxylic acid *t*-butyl ester (4.3 g, 10 mmol) was dissolved in pyridine (40 mL, 500 mmol). Hydroxylamine hydrochloride (1.7 g, 25 mmol) was added, followed by the addition of water (20 mL, 1000 mmol). The mixture was stirred at room temperature overnight. Water (30 mL) was added and the mixture was stirred for 20 minutes. The precipitant was filtered off and dried to yield 4'-[2-ethoxy-5-(hydroxyiminomethyl)-4-vinylimidazol-1-ylmethyl]biphenyl-2-carboxylic acid *t*-butyl ester (3.5 g, 7.7mmol, 77%). MS m/z: [M+H+] calcd for $C_{26}H_{29}N_3O_4$, 448.2; found 448.3.

Step 4

4'-[2-ethoxy-5-(hydroxyiminomethyl)-4-vinylimidazol-1-ylmethyl]biphenyl-2-carboxylic acid *t*-butyl ester (3.5 g, 7.7 mmol) was dissolved in ethanol (100 mL, 2 mol). Pearlman's Catalyst, wet (0.1:0.4:0.5, palladium hydroxide:carbon black:water, 660 mg) was added. The mixture was degassed and stirred at room temperature under hydrogen for 3 hours. The palladium was filtered off and the solute was concentrated to yield 4'-[2-ethoxy-4-ethyl-5-(hydroxyiminomethyl)imidazol-1-ylmethyl]biphenyl-2-carboxylic acid *t*-butyl ester (3.3 g, 7.3 mmol, 95%). MS m/z: [M+H+] calcd for $C_{26}H_{31}N_3O_4$, 450.2; found 450.3.

Step 5

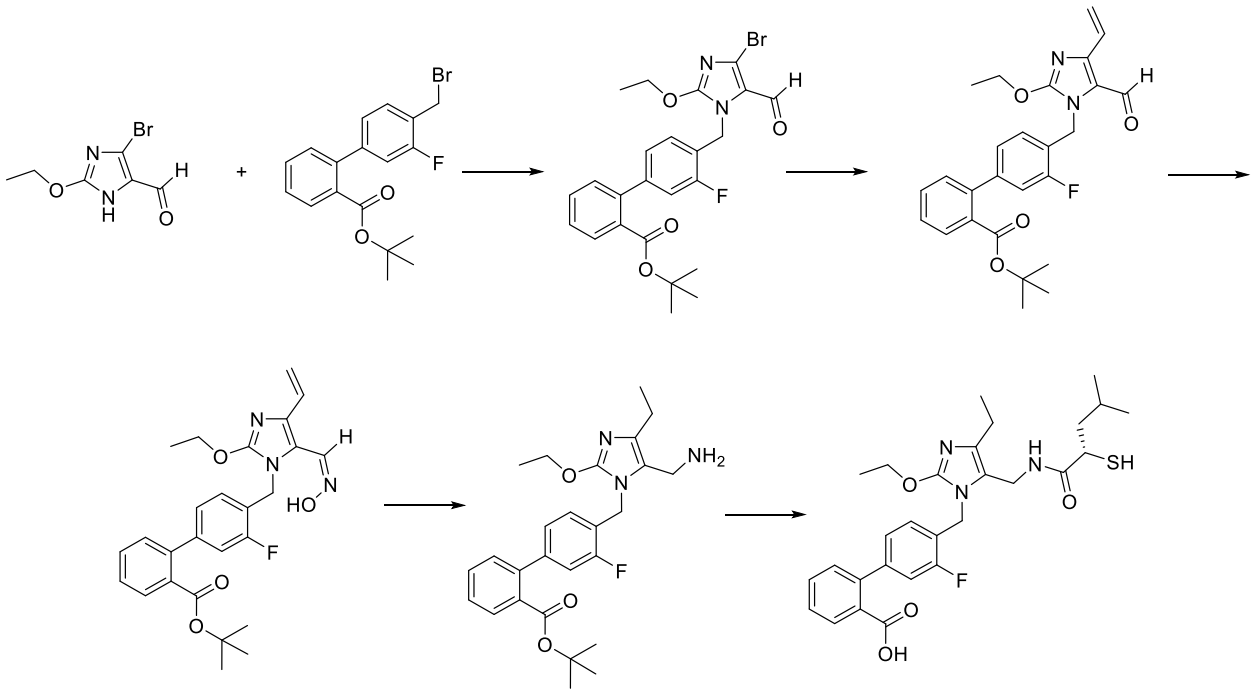
4'-[2-ethoxy-4-ethyl-5-(hydroxyiminomethyl)imidazol-1-ylmethyl]biphenyl-2-carboxylic acid *t*-butyl ester (3.5 g, 7.7 mmol), $NaBH_3CN$ (1.9 g, 30.5 mmol) and ammonium acetate (1150 mg, 14.9 mmol) were dissolved in MeOH (30 mL, 700 mmol). The mixture was cooled at 0 °C and stirred for 15 minutes before titanium(III) chloride (3.1 g, 20.3 mmol) was added. The mixture was stirred at 0 °C for 10 minutes then warmed to room temperature and stirred for 3 hours. Ammonium hydroxide (75 ml) was added and the mixture was stirred at room temperature overnight. 75 mL of saturated sodium bicarbonate was then added. The resulting mixture was extracted with DCM, and the organic layer was dried over $MgSO_4$, filtered, and concentrated to obtain 4'-(5-aminomethyl-2-ethoxy-4-ethylimidazol-1-ylmethyl)-biphenyl-2-carboxylic acid t-butyl ester (2.5 g, 5.7 mmol, 75%). MS m/z: [M+H+] calcd for $C_{26}H_{33}N_3O_3$, 436.3; found 436.6.

Step 6

(S)-2-Acetylsulfanylmethyl-4-methylpentanoic acid (1.0 g, 5.1 mmol) was dissolved in DMF (50 mL, 600 mmol). HATU (1.8 g, 4.8 mmol) and DIPEA (800 µL, 4.6 mmol) were added and the mixture was stirred at room temperature for 20 minutes. 4'-(5-Aminomethyl-2-ethoxy-4-ethylimidazol-1-ylmethyl)biphenyl-2-carboxylic acid *t*-butyl ester (2.0 g, 4.6 mmol) in DMF (25 mL, 300 mmol) with DIPEA (800 µL, 4.6 mmol) was added and the mixture was stirred at room temperature overnight. The reaction was quenched with water, and the mixture was extracted with EtOAc, washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and concentrated. This intermediate was taken up in DCM:TFA (4 mL each), stirred at room temperature for 4 hours, then concentrated to dryness. The residue was dissolved in 1:1 MeOH:1N NaOH (5 mL each) and stirred at room temperature under nitrogen for 1 hour before the reaction was quenched with acetic acid and the mixture concentrated. The residue was purified by preparative HPLC (10-70% MeCN in water w/ 0.05% TFA) to obtain (S)-4'-((2-ethoxy-4-ethyl-5-((2-mercapto-4-methylpentanamido)methyl)-1H-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid•[$C_2HF_3O_2$] (822 mg, 1.3 mmol, 28%). MS m/z: [M+H+] calcd for $C_{29}H_{37}N_3O_4S$, 524.3; found 524.4. ^{1}H-NMR (DMSO) δ

(ppm): 8.33 (1H, s), 7.65 (1H, d), 7.49 (1H, t), 7.38 (1H, t), 7.24 (3H, m), 7.16 (2H, d), 5.05 (2H, s), 4.41 (2H, d), 4.10 (2H, m), 2.41 (3H, m), 2.29 (1H, m), 2.06 (1H, t), 1.20 (8H, m), 0.74 (6H, dd).

Synthesis of Compound 35



Step 1

5-Bromo-2-ethoxy-3H-imidazole-4-carbaldehyde (15.0 g, 68.5 mmol), 4'-bromomethyl-3'-fluorobiphenyl-2-carboxylic acid *t*-butyl ester (25.0 g, 68.5 mmol) and potassium carbonate (9.5 g, 68.5 mmol) were dissolved in DMF (958 mL, 12.4 mol) and was stirred at room temperature for 2 hours. The reaction was quenched with water, and the mixture extracted with EtOAc, washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The mixture was purified by silica gel chromatography (0-30% EtOAc:hexanes) to yield 4'-(4-bromo-2-ethoxy-5-formylimidazol-1-ylmethyl)-3'-fluorobiphenyl-2-carboxylic acid t-butyl ester (25g, 49.5 mmol, 72%). MS m/z: [M+H$^+$] calcd for $C_{24}H_{24}BrFN_2O_4$, 503.1; found 503.2. ^{1}H-NMR ($CDCl_3$) δ (ppm): 9.57 (1H, s), 7.80 (1H, d), 7.48 (2H, m), 7.27 (1H, s), 7.11 (1H, t), 7.01 (2H, d), 5.47 (2H, s), 4.33 (2H, q), 1.41 (3H, t), 1.24 (9H, s).

Step 2

4'-(4-bromo-2-ethoxy-5-formylimidazol-1-ylmethyl)-3'-fluorobiphenyl-2-carboxylic acid t-butyl ester (15a) (11.0 g, 21.8 mmol) was dissolved in 1,2-dimethoxyethane (100 mL, 1 mol). Tetrakis(triphenylphosphine)palladium(0) (252 mg, 218 µmol) was added and the mixture was stirred under nitrogen for 20 minutes. Water (48 mL, 2.6 mol), 2,4,6-trivinylcyclotriboroxane pyridine complex (2.1 g, 8.7 mmol) and potassium carbonate (3.0 g, 21.8 mmol) were then added and the mixture was heated at 90 °C under nitrogen. After 2 hours, the mixture was cooled to room temperature, diluted with EtOAc, washed with water and saturated aqueous NaCl, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The mixture was purified by silica gel chromatography (0-50% EtOAc:hexanes) to yield 4'-(2-ethoxy-5-formyl-4-vinylimidazol-1-ylmethyl)-3'-fluorobiphenyl-2-carboxylic acid *t*-butyl ester: (9.8 g, 21.7

mmol, 99%). MS m/z: [M+H$^+$] calcd for $C_{26}H_{27}FN_2O_4$, 451.2; found 451.0. ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.17 (1H, s), 7.79 (1H, d), 7.48 (1H, t), 7.40 (1H, t), 7.28 (1H, s), 7.01(2H, m), 6.86 (1H, t), 6.67 (1H, m), 5.95 (1H, d), 5.41 (2H, s), 5.27 (1H, d), 4.48 (2H, q), 1.38 (3H, t), 1.25 (9H, s).

Step 3

4'-(2-ethoxy-5-formyl-4-vinylimidazol-1-ylmethyl)-3'-fluorobiphenyl-2-carboxylic acid *t*-butyl ester (19.5 g, 43.4 mmol) was dissolved in pyridine (100 mL, 1 mol). Hydroxylamine hydrochloride (9.0 g, 130 mmol) was added, followed by water (50 mL), and the mixture was stirred at room temperature overnight. Water (100 mL) was then added and the mixture was stirred for 20 minutes. The precipitant was filtered off and dried to yield 4'-[2-ethoxy-5-(hydroxyiminomethyl)-4-vinylimidazol-1-ylmethyl]-3'-fluorobiphenyl-2-carboxylic acid *t*-butyl ester: (13.5 g, 29 mmol, 67%). MS m/z: [M+H$^+$] calcd for $C_{26}H_{28}FN_3O_4$, 466.2; found 466.4. ^{1}H-NMR ($CDCl_3$) δ (ppm): 9.78 (1H, s), 7.81 (1H, d), 7.48 (2H, m), 7.26 (1H, s), 7.0 (4H, m), 6.20 (1H, d), 5.53 (1H, d), 5.50 (2H, s), 4.55 (2H, q), 1.43 (3H, t), 1.25 (9H, s).

Step 4

4'-[2-ethoxy-5-(hydroxyiminomethyl)-4-vinylimidazol-1-ylmethyl]-3'-fluorobiphenyl-2-carboxylic acid *t*-butyl ester (4.0 g, 8.6 mmol) was dissolved in ethanol (250 mL, 4.3 mol) and sulfuric acid (0.50 mL, 9.4 mmol), and subjected to sonication. Once fully dissolved, the mixture was added to 10% Pd/C, Degussa type, wet 50% (0.05:0.45:0.5, palladium:carbon black:water, 3.0 g, 1.4 mmol) in 20 ml of ethanol. The solution was degassed and stirred at room temperature under hydrogen for 5 hours. The palladium was filtered off and the mixture was concentrated to yield 4'-(5-aminomethyl-2-ethoxy-4-ethylimidazol-1-ylmethyl)-3'-fluorobiphenyl-2-carboxylic acid *t*-butyl ester (3.3 g, 7.3 mmol, 85%). MS m/z: [M+H$^+$] calcd for $C_{26}H_{32}FN_3O_3$, 454.3; found 454.2. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.82 (1H, d), 7.50 (1H, t), 7.42 (1H, t) 7.35 (1H, d), 7.27 (1H, d), 7.12 (1H, s), 7.04 (1H, d), 5.34 (1H, br), 4.65 (2H, s), 4.40 (2H, q), 4.12 (2H, q), 2.70 (1H, br), 1.50 (3H, t), 1.28 (9H, s), 1.17 (3H, t).

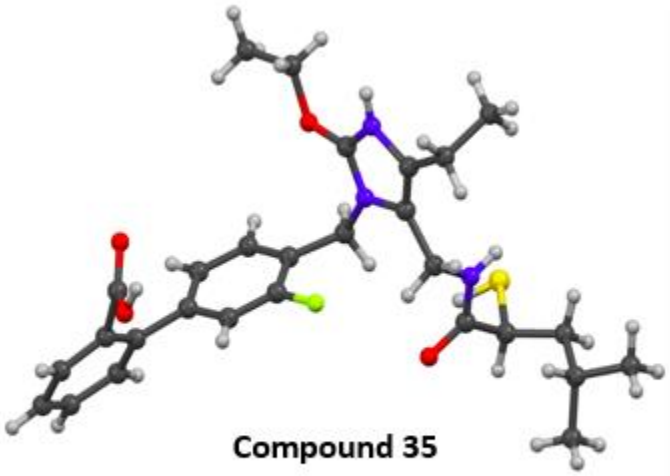
Step 5

(S)-2-Acetylsulfanyl-4-methylpentanoic acid (877 mg, 4.6 mmol) was dissolved in DMF (20 mL, 300 mmol). HATU (1.8 g, 4.6 mmol) and DIPEA (0.8 mL, 4.6 mmol) were added and the mixture was stirred at room temperature for 30 minutes. 4'-(5-Aminomethyl-2-ethoxy-4-ethylimidazol-1-ylmethyl)-3'-fluorobiphenyl-2-carboxylic acid *t*-butyl ester (1.9 g, 4.2 mmol) in DMF (20 mL, 300 mmol) with DIPEA (0.8 mL, 4.6 mmol) was added, and the mixture was stirred at room temperature for 2 hours. The reaction was quenched with water, extracted with EtOAc, washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered and concentrated. The mixture was purified by silica gel chromatography (0-50% EtOAc:hexanes) to obtain to obtain the acetylsulfanyl ester intermediate (2.4 g, 3.8mmol, 83%). MS m/z: [M+H$^+$] calcd for $C_{34}H_{44}FN_3O_5S$, 626.3; found 626.2. ^{1}H-NMR ($CDCl_3$): 7.80 (1H, d), 7.48 (1H, t), 7.40 (1H, t), 7.25 (1H, d), 7.06 (2H, d), 6.88 (1H, t), 6.08 (1H, t), 4.96 (2H, s), 4.38 (2H, q), 4.22 (2H, m), 3.86 (1H, t), 2.50 (2H, q), 2.32 (3H, s), 1.82 (1H, m), 1.64 (1H, m), 1.52 (1H, m), 1.34 (3H, t), 1.26 (9H, s), 1.18 (3H, t), 0.87 (6H, dd). The intermediate was dissolved in DCM:TFA (1:1) (5 mL each) and stirred at room temperature for 3 hours, then concentrated. The residue was dissolved in MeOH (20 ml), and 10N NaOH (2ml) was added. The mixture was stirred at room temperature for 25 minutes under nitrogen before the reaction was quenched with acetic acid and the mixture concentrated. The resulting material was purified by preparative HPLC (10-70%) to obtain the title compound as a TFA salt (1.6 g; 2.5mmol, 66%). MS m/z: [M+H$^+$] calcd for $C_{28}H_{34}FN_3O_4S$, 528.2; found 528.2. HRMS Molecular Formula $C_{28}H_{35}FN_3O_4S$, Mass Found

528.2332 [M+H$^+$], Mass Required 528.2327 [M+H$^+$]; ^{1}H-NMR (600 MHz, DMSO) δ (ppm): 0.80 (d, *J* = 6.6 Hz), 0.85 (d, *J* = 6.6 Hz), 1.13 (t, *J* = 7.2 Hz), 1.23 (t, *J* = 7.2 Hz), 1.40 (ddd, *J* = 13.8, 7.8, 6.6 Hz), 1.55 (s, *J* = 6.6 Hz), 1.63 (ddd, *J* = 13.2, 8.3, 6.6 Hz), 2.44 (q, *J* = 7.2 Hz), 2.64 (d, *J* = 9.6 Hz), 3.31 (ddd, *J* = 9.3, 8.2, 7.4 Hz), 4.15 (d, *J* = 5.4 Hz), 4.28 (q, *J* = 8.4 Hz), 5.0 (s), 6.86 (t, *J* = 7.8 Hz), 7.11 (d, *J* = 7.8 Hz), 7.18 (d, *J* = 10.8 Hz), 7.39 (d, *J* = 7.8 Hz), 7.48 (t, *J* = 7.8 Hz), 7.56 (t, *J* = 7.8 Hz), 7.76 (*J* = 7.8 Hz), 8.26 (t, *J* = 4.8 Hz); ^{13}C NMR (150 MHz, DMSO) δ (ppm) 14.6, 14.7, 22.1, 22.1, 25.7, 31.5, 38.7, 39.3, 44.6, 115.2, 118.0, 19.9, 64.9, 123.2, 124.7, 127.8, 127.8, 129.3, 130.5, 131.0, 132.1, 135.7, 139.4, 142.3, 151.4, 159.1, 169.2, 172.1.

Crystal Structure Determination of Compound 35

Crystals of **compound 35** hemi-1,5-naphthalnedisulfonate salt were obtained from slow crystallization in isopropanol and water. Single crystal X-ray data were collected at 120K using Mo-Ka radiation on a Nonius Kappa-CCD diffractometer equipped with Oxford Cryostream Liquid Nitrogen Cooler. Data reduction was performed using HKL Scalepack and cell parameters were obtained using Denzo and Scalepak. The structure was solved using direct methods by SHELXS97. The structure was refined by least square full matrix refinement using SHELXL97. All H-atoms were found on the Fourier difference map and refined isotropically. The absolute configuration (*S*) was determined using Flack parameters based on anomalous dispersion effect.



Rat Pharmacokinetics of Compound 35

Compound **35** contains a sulfhydryl moiety which can form disulfide linkages in vivo. To accurately quantify the body burden of drug-related material, as well as the concentration of **35**, two bioanalytical methods were employed. To measure the concentration of **35** plus any disulfide products, blood was treated with dithiothreitol (DTT) to reduce all disulfide bonds. The concentration of **35** and all associated disulfide products is referred to as “total reducible”. ATo quantify the levels of circulating **35**, blood was treated with a sulfhydryl derivatizing agent, methyl acrylate (MA). The concentration of **35** as the free sulfhydryl is referred to as “monomer”.

Non-Compartmental Composite Pharmacokinetic Parameters of **35** Following Intravenous or Oral Administration to Male Rats

	Total Reducible		Monomer	
Route, Dose (mg/kg)	IV, 1	PO, 10	IV, 1	PO, 10
AUC_{0-24} (µg.hr/mL)	0.612	1.40	0.362	0.357
CL (L/hr/kg)	1.49	NA	2.76	NA
Vss (L/kg)	9.14	NA	0.320	NA
T½ (hr)	10.9	5.18	1.74	2.96
C_{max} (µg/mL)	NA	0.578	NA	0.501
T_{max} (hr)	NA	0.5	NA	0.5
%F	NA	22.2%	NA	9.86%
Monomer:Total C_{max}	NA	86.6%		
Monomer:Total AUC	59.2%	26.2%		

N=6 rats in a composite design; blood samples were split and analyzed for both total reducible (DTT) and monomer (MA)