Full Methods

Protein expression and purification

Human SIRT1 was expressed in *E. coli* BL21Start™ (DE3) (Invitrogen) as an N-terminal fusion to a hexa-histidine affinity tag. The protein was purified by Ni^{2+}-chelate chromatography followed by size exclusion and ion exchange chromatography. The resulting protein was typically >95% pure as assessed by SDS-PAGE. The Human SIRT1-E5c and SIRT1-A,B,D,E,F,G,H were expressed with a hexa-histidine affinity tag at the C-terminus. The truncated proteins were expressed in *E. coli* BL21Start™ (DE3) and purified by Ni^{2+}-chelate chromatography. SIRT1-E5c was purified with an additional ion exchange step. SIRT1-E5c protein was used for ITC studies because it expresses at high levels in *E. coli,* is highly soluble, retains full catalytic activity and can be fully activated by small molecule SIRT1 activators.

SIRT1 Fluorescence Polarization Assay and HTS

In the SIRT1 FP assay, SIRT1 activity was monitored using a 20 amino acid peptide (Ac-Glu-Glu-Lys(biotin)-Gly-Gln-Ser-Thr-Ser-Ser-His-Ser-Lys(Ac)-Nle-Ser-Thr-Glu-Gly–Lys(MR121 or Tamra)-Glu-Glu-NH_2) derived from the sequence of p53. The peptide was N-terminally linked to biotin and C-terminally modified with a fluorescent tag. The reaction for monitoring enzyme activity was a coupled enzyme assay where the first reaction was the deacetylation reaction catalyzed by SIRT1 and the second reaction was cleavage by trypsin at the newly exposed lysine residue. The reaction was stopped and streptavidin was added in order to accentuate the mass differences between substrate and product. In total, 290,000 compounds were screened and 127 hits were confirmed. The sensitivity of the FP assay allowed identification of compounds that exhibited low level activation of SIRT1 (≥17% activation at 20 μM) producing multiple classes of activators representing distinct structural classes.

The fluorescence polarization reaction conditions were as follows: 0.5 μM peptide substrate, 150 μM βNAD^+, 0-10 nM SIRT1, 25 mM Tris-acetate pH 8, 137 mM Na-Ac,

2.7 mM K-Ac, 1 mM Mg-Ac, 0.05% Tween-20, 0.1% Pluronic F127, 10 mM $CaCl_2$, 5 mM DTT, 0.025% BSA, and 0.15 mM nicotinamide. The reaction was incubated at 37°C and stopped by addition of nicotinamide, and trypsin was added to cleave the deacetylated substrate. This reaction was incubated at 37°C in the presence of 1 μM streptavidin. Fluorescent polarization was determined at excitation (650 nm) and emission (680 nm) wavelengths.

SIRT1 Mass Spectrometry Assay

The mass spectrometry based assay utilizes the same peptide as described for the HTS assay. The mass spectrometry assay was conducted as follows: 0.5 μM peptide substrate, 120 μM βNAD^+, 10 nM SIRT1, and reaction buffer (50 mM Tris-acetate pH 8, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 5 mM DTT, 0.05% BSA). Reactions were incubated for 25 minutes at 25°C. Test compounds were added to the reaction or vehicle control, DMSO. After the incubation with SIRT1, 10% formic acid with 50 mM nicotinamide (Sigma) was added to stop the reaction. The mass spectrometry analysis was performed by BioTrove, Inc. Determination of the mass of the substrate peptide allows for precise determination of the degree of acetylation (i.e. substrate) as compared to deacetylated peptide (product).

p53 Deacetylation Assay

Human osteosarcoma cells (U-2 OS) were plated at 1.5 X 10^4 cells per well in 96 well plates. Test compounds and controls (all in 100% DMSO) were added to cell plates after 24 h. To demonstrate the SIRT1-dependence of this assay read-out, replicate plate sets were co-treated with both test compounds and a SIRT1-specific small molecule inhibitor**,** 6-chloro-2,3,4,9-tetrahydro-1-*H*-carbazole-1-carboxamide. After compound addition, p53 expression and acetylation was induced by the addition of doxorubicin (1 μg/ml final concentration) to each well. Following p53 induction, cells were fixed and then permeablized with PBS-0.1% Triton-X-100. Non-specific protein binding was then blocked by addition of a solution of 5% BSA in PBS-0.1% TWEEN 20 (Block Solution). Primary antibodies, anti-p53-acetyl-lysine-382 (rabbit polyclonal; Cell Signaling

Technology) and anti-beta tubulin (mouse monoclonal; Santa Cruz Biotechnology), were diluted 1:400 and 1:1000, respectively, in Block Solution and added to wells for an overnight incubation at 4°C. Wells were then washed in PBS-0.1% TWEEN 20 (Wash Buffer). Secondary antibodies, IR800CW Goat anti-rabbit IgG (Rockland Immunochemicals) and Alexa-Fluor 680 goat anti-mouse IgG (InVitrogen), were diluted in Block Solution and added to wells for a 1 hr, room temperature incubation. Wells were again washed 5 times in Wash Buffer. Plates were then scanned with a Li-Cor Odyssey infrared scanner. Data was extracted using manufacturer's software. Signals for both Ac-Lys382-p53 and beta-tubulin were background corrected using wells incubated with only secondary antibodies. Each well Ac-Lys382-signal was then normalized to its corresponding beta-tubulin signal to correct for differences in cell number. These values were then normalized to vehicle to generate the % acetylated p53 value for each well.

Mechanism of action studies

The effect of test compounds on the Km of human SIRT1 enzyme for acetylated peptide substrate was examined using the SIRT1 mass spectrometry assay described above. Using the cell-free MS assay, the Km of SIRT1 enzyme for peptide substrate was determined at nine concentrations of compound (100, 33, 11, 3.7, 1.2, 0.41, 0.14, 0.046, and 0.015 μM) and also in the presence of DMSO vehicle alone. To determine the Km, the linear deacetylation rate was determined at 12 concentrations of acetylated peptide substrate (50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.098, 0.049, and 0.024 μM) for each of the compound concentrations and for the vehicle control. SIRT1 enzyme, 2 mM NAD^+, and 0-50 μM acetylated peptide substrate were incubated with 0-100 μM compound at 25°C. At 0, 3, 6, 9, 12, 15, 20, and 25 minutes, the reaction was stopped with 10% formic acid with 50 mM nicotinamide and the conversion of substrates to products determined by mass spectrometry.

Isothermal titration Calorimetry (ITC)

The human SIRT1-E5c protein (41 μM; described above), the mass spectrometry peptide substrate (1.0 mM), and SRT1460 (0.84 mM) stock solutions were used for the ITC

experiments. The buffer conditions were 50 mM Tris-HCl (pH 8.0), 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 2 mM TCEP, and 5% glycerol. Titrations were carried out at 26ºC on a VP-ITC (MicroCal, Inc). SRT1460 was selected for these studies because it is soluble in buffer to the millimolar concentrations required in the experiment.

Isobologram studies

The effect of the combination of resveratrol versus SRT1720 and SRT1720 versus SRT1460 was determined using the SIRT1 mass spectrometry assay described above. A concentration matrix of the 2 compounds was created and tested against SIRT1 enzyme. The % conversion of acetylated peptide substrate to deacetylated peptide product was determined at each of the combinations present in the matrix. The resulting Isobologram was used to evaluate the effect of the combination. For the analysis, a plot in Cartesian coordinates of a dose combination that produce the same effect level is the basis for an Isobologram. If two compounds have variable potency, a constant relative potency (R) - which is the amount of compound needed to achieve the same fold activity (e.g. $EC_{1.25}$ for resveratrol vs SRT1720 and $EC_{2.5}$ for SRT1720 vs SRT1460) - is selected for the X and Y intercepts for Isobologram analysis. The concentration of both compounds which corresponds to the respective EC value is used as an intercept on both the X and Y axes. Using these two intercepts, a theoretical line called the line of additivity is drawn between the two points. Experimental data obtained by the logarithmic titration of the two compounds mixed as a dose pair in a matrix which yield the same effect level (EC value), is plotted on the Isobologram. Statistical comparison of the line of additivity and the curve arising from experimental two drug dose combinations indicates if an effect is additive. Points falling below and above the line of additivity are subjected to regression analysis. Experimental data that is higher than the line of additivity is interpreted antagonistic and experimental data that is lower is interpreted as synergistic, and experimental data that fall on the line of additivity is considered additive.

Pharmacokinetics of SRT1720

SRT1720 in vehicle (2% HPMC + 0.2% DOSS) was administered via oral gavage to C57BL/6 male mice (18-22 grams; 3 mice per dose group per time point) at the doses

indicated. For all *in vivo* studies SRT1720 was dosed as the hydrochloride salt. Mice were sacrificed by CO_2 asphyxiation and blood was collected at 5, 30, 120 and 360 minutes after dosing. Blood was collected and plasma was sent to Charles River Labs (CRL) for drug level analysis. To determine oral bioavailability, SRT1720 in vehicle (10% ethanol/ 40% Polyethylene glycol / 50% H2O) was administered into the tail vein of C57BL/6 male mice (18-22 grams; 3 mice per dose group per time point) at the doses indicated. Blood was collected at 5, 30, 120, and 360 minutes and analyzed for drug levels as described above.

SRT1720 was administered via oral gavage to Sprague-Dawley male rats (250 grams; 3 rats per dose group) at 100 mg/kg in vehicle (2% HPMC + 0.2% DOSS). Blood was collected at 0.033, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hours post dose and analyzed for drug levels. To determine oral bioavailability, SRT1720 was administered into the tail vein at 10 mg/kg doses. SRT1720 was administered in 10% ethanol/ 40% Polyethylene glycol / 50% H2O for IV studies. Blood was collected and analyzed as described above.

Diet induced obesity model

Nine week old C57BL/6 male mice (Charles River Labs) were fed a high fat diet (60% calories from fat; Research Diets) until their mean body weight reached approximately 40 g. The mice were then divided into test groups (6-10 per group). SRT1460 (100 mg/kg), SRT1720 (100 mg/kg), SRT501 (500 mg/kg) and rosiglitazone (5 mg/kg) were administered once daily via oral gavage. The vehicle used was 2% HPMC + 0.2% DOSS. Individual mouse body weights were measured twice weekly. At 2, 4, 6, 8 and 10 weeks of dosing a fed blood glucose measure was taken and after 5 weeks of treatment an IPGTT was conducted on all mice from each of the groups. After 10 weeks of treatment, an ITT was conducted. Statistical analysis was completed using the JMP program (Version 6). Data were analyzed by a one way ANOVA with comparison to control using a Dunnett's Test. A p value < 0.05 indicated a significant difference between groups.

Citrate synthase assay

Citrate synthase (CS) activity in skeletal muscle (gastrocnemius) and white adipose tissue (epididymal) was determined after 11 weeks of treatment using the method described by Srere and Moyes[29,30]. The five mice best representing the mean fasting blood glucose level of each group (DIO Vehicle and DIO SRT1720) were selected for this analysis.

_ob/ob_ model

ob/ob mice and a heterozygous *ob/+* mice were received at 6 week of age (Jackson Labs, Bar Harbor, ME). Mice were placed on a high fat diet (60% calories from fat; Research Diets) for a minimum of one week prior to the start of a study and remained on the high fat diet for the duration of the study. After a one week acclimation, all mice were weighed and blood glucose measurements were taken. The average body weight of the *ob/ob* mice used in the study were ~ 40-45 grams. All mice were sorted by body weight and glucose levels and then were allocated into groups. Animals were dosed with either SRT1720 (100 mg/kg), SRT501 (1000 mg/kg), or vehicle (2% HPMC + 0.2% DOSS) once daily by oral gavage. Blood glucose and insulin were determined as described above. Statistical analysis was completed using the JMP program (Version 6). Data were analyzed by a one way ANOVA with comparison to control using a Dunnett's Test. A p value < 0.05 indicated a significant difference between groups.

Zucker _fa/fa_ model

Six week old, male fatty (*fa/fa*) Zucker (ZF) rats (Harlan Sprague Dawley, Inc.) were housed individually under controlled light (12:12 light:dark) and temperature conditions. At 7 weeks of age animals were randomly assigned to receive either the SIRT1 activator (SRT1720) or vehicle (i.e. 2% HPMC + 0.2% DOSS). The drug was administered by oral gavage on a daily basis (between 3-5pm) for 4 weeks and animals had ad libitum access to food and water. All experimental procedures were approved by the Animal Subjects Committee at UCSF according to NIH guidelines.

The night before beginning the drug treatment animals were overnight fasted (12 h), the following morning (Day 1) blood glucose concentration was measured (OneTouch Ultra, LifeScan, Inc), and a blood sample was taken in a heparinized capillary tube from the tail vein. This sample was centrifuged at 13,000 rpm for 5 min and the plasma was

stored at -80°C for analysis. This procedure was subsequently repeated during the first 3 weeks of drug treatment (i.e.; Days 8, 15 and 22). Also, in the afternoon of Day 22, a fed blood glucose measurement was taken from the tail vein.

Zucker *fa/fa* Pyruvate Tolerance Test

On Day 22, after the overnight fast (and blood collection), rats were injected (1g/kg body weight) intraperotineal with sodium pyruvate (0.36g/mL), diluted in 0.9% NaCl. Blood glucose (tail vein) was measured (OneTouch Ultra, LifeScan, Inc) at 0, 30, 40, 50, 60 and 90 min after injection.

Zucker *fa/fa* Oral Glucose Tolerance Test

On Day 26 of treatment, after a short fast (5h), rats were orally gavaged (1g/kg body weight) with dextrose (Hospira, Inc). Blood glucose (tail vein) was measured (OneTouch Ultra, LifeScan, Inc) at 0, 15, 30, 60 and 90 min, and additional blood was also collected in a heperanized tube at 0, 15, 30 and 60 min. These samples were centrifuged at 13,000RPM for 5 min and the plasma was stored at -80°C for analysis of plasma insulin concentration.

Hyperinsulinemic-euglycemic clamp

Five days before conducting clamp experiments, animals (10 weeks of age) were chronically cannulated under single-dose anesthesia (42mg/kg ketamine HCl, 5mg/kg xylazine, 0.75mg/kg acepromazine maleate; administered intramuscularly) in the jugular vein for infusion of glucose, tracer, and insulin (dual cannula internal diameter [ID] 0.03cm; Dow Corning Silastic, Midland, MI) and in the carotid artery (Intramedic polyethylene tubing PE-50; Clay Adams, Becton Dickinson, Sparks, MD) for blood sampling. All cannulae were tunneled subcutaneously, exteriorized at the back of the neck, and were encased in an infusion harness (Cat# CIH105; Instech Laboratories, Inc.).

The evening before the hyperinsulinemic-euglycemic clamp animals were fasted overnight for 8 hours. Ninety minutes before the clamp, animals were weighed, and infusion lines were connected. Throughout the clamp animals were unrestrained in their standard rat cage, and had free access to water. The two infusion lines were connected

from the infusion pumps (KDS101; KD Scientific, Inc.) to a low-torque dual channel quartz-lined swivel (Cat# 375/D/22QM; Instech Laboratories, Inc.), which was then connected to the jugular vein cannulas. This set-up allowed unrestrained movement in all directions. At -60 min a priming dose of 7.5 μCi of D-[3-^{3}H]glucose (PerkinElmer, Inc.) was administered into the carotid, and a constant-rate (0.167 μCi/min; 16.7μL/min) infusion of D-[3-^{3}H]glucose was started at –70min. Blood samples (25μL in duplicate) were taken at -10min and 0min for measurement of tracer specific activity and basal hepatic glucose production. At time 0 min (i.e.; after 60 min of tracer equilibration), glucose (variable infusion, 50% dextrose; Hospira, Inc) and tracer-plus-insulin (25 mU/kg/min; Humulin® R; Eli Lilly and Company) infusions were started simultaneously. Small blood samples (5μL) were drawn from the carotid artery at 10-min intervals and immediately analyzed for glucose (OneTouch Ultra, LifeScan, Inc). The glucose infusion was adjusted to maintain blood glucose at 100 mg/dL. Blood samples (25μL in duplicate) were taken at 110min and 120min for measurement of tracer specific activity. In addition, larger blood samples (150 μL) were taken at 0 min and 120min for determination of insulin and free fatty acid (FFA) concentrations. All blood samples were immediately centrifuged (13,000g for 5 min), and plasma was stored at –80°C for subsequent analysis.

SIRT1 small molecule modulators

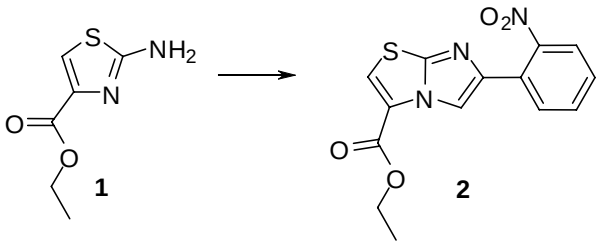
Resveratrol was obtained from Sigma-Aldrich. Test compounds, SRT1460, SRT1720, and SRT2183 were synthesized at Sirtris Pharmaceuticals as part of the SAR studies we have conducted to identify potent well tolerated SIRT1 activators. The inhibitor, 6-chloro-2,3,4,9-tetrahydro-1-*H*-carbazole-1-carboxamide, was synthesized at Sirtris Pharmaceuticals and has been previously described[18].

General Techniques: Reactions were run under an inert atmosphere (N_2). Commercial reagents were used as purchased. Compounds **2-7** were prepared to previously described methods (see Nunes, J.J., Milne, J., Bemis, J., Xie, R., Vu, C.V., Ng, P.Y., Disch, J.S., US Patent Application Publication US2007/0037865 A1, February 15, 2007.) Microwave reactions were performed using a Biotage Initiator instrument.

NMR spectra were obtained using a Varian Unity 300 and Bruker AVANCE III 400 instruments. Melting points were determined by differential scanning calorimetry (TA Instruments DSCQ200). Analytical HPLC was performed on an Agilent 1100 Series HPLC equipped with a 3.5 um Eclipse XDB-C18 (4.6 mm x 100 mm) column with the following conditions: MeCN/H_2O, modified with 0.1 % Formic acid mobile phase. Gradient elution: 5% hold (2 min), 5% to 95% gradient (11 min), 95% to 5% gradient (0.3 min), 5% hold (2.7 min), 15 min. total run time. Flow rate: 0.8 ml/min. Medium pressure chromatography was performed on an ISCO Combiflash R_f system using a pentane/ethyl acetate solvent system. High resolution mass spectra were obtained with an Agilent 6210 Time-of-Flight LC/MS by flow-injection (solvents are Water and ACN with 0.1% Formic Acid).

Compound **2** was prepared as described in Nunes, J.J., Milne, J., Bemis, J., Xie, R., Vu, C.V., Ng, P.Y., Disch, J.S., US Patent Application Publication US2007/0037865 A1, February 15, 2007.

Preparation of 6-(2-nitrophenyl)-imidazo[2,1-*b*]thiazole-3-carboxylic acid ethyl ester (2):

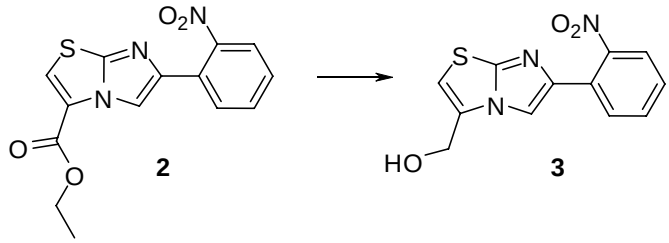


A solution of ethyl 2-aminothiazole-4-carboxylate (**1**) (2.1g, 12 mmol) in methyl ethyl ketone (25 mL) and 2-bromo-2'-nitroacetophenone (3.0 g, 12 mmol) was stirred under reflux for 18 hours, cooled to room temperature and filtered. The filtrate was concentrated *in vacuo* to afford 3.10 g (79% yield) of 6-(2-nitro-phenyl)-imidazo[2,1-*b*]thiazole-3-carboxylic acid ethyl ester (**2**). ESI-MS calculated for $C_{14}H_{11}N_3O_4S$ $[M+H]^+$

318.1, found: 318.0; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.39 p.p.m. (br s, 1 H), 8.31 (br s, 1 H), 7.92 (d, 1 H, *J* = 8 Hz), 7.82 (d, 1 H, *J* = 7 Hz), 7.3-7.7 (m, 2 H), 4.4 (q, 2 H, *J* = 7 Hz), 1.37 (t, 3 H, *J* = 7 Hz).

Compound **3** was prepared as described in Nunes, J.J., Milne, J., Bemis, J., Xie, R., Vu, C.V., Ng, P.Y., Disch, J.S., US Patent Application Publication US2007/0037865 A1, February 15, 2007.

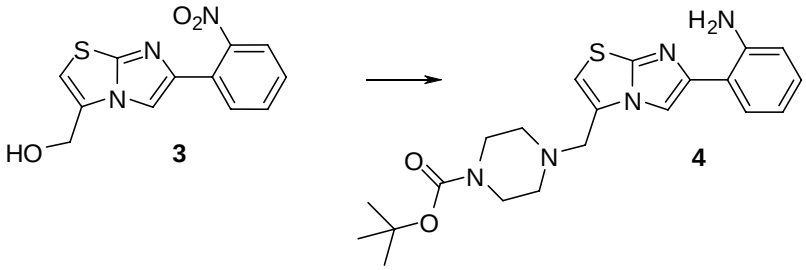
Preparation of [6-(2-nitrophenyl)imidazo[2,1-*b*]thiazol-3-yl]methanol (3):



A solution of 6-(2-nitrophenyl)imidazo[2,1-*b*]thiazole-3-carboxylic acid ethyl ester (**2**) (14.50 g, 46 mmol) in THF (100 mL) and water (100 mL) containing NaOH (7.3 g, 4 eq) was stirred at room temperature for 18 hours and then concentrated *in vacuo*. The aqueous layer was washed once with CH_2Cl_2 and then acidified with 6 N HCl. The solids were collected by filtration and dried to afford 7.4 g of the acid intermediate. This material (7.4 g, 26 mmol) was dissolved in anhydrous THF (200 mL) containing N-methylmorpholine (2.8 mL, 26 mmol) and cooled to 0 °C. Isobutyl chloroformate (3.35 mL, 26 mmol) was added and the reaction mixture was stirred in the ice bath for 3 hours. $NaBH_4$ (0.97 g, 25.6 mmol) was added as a solution in water (30 mL). The reaction mixture was stirred at 0 °C for 45 min, warmed to room temperature and concentrated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and concentrated to afford the crude product. Purification by chromatography afforded 5.20 g (74% yield) of [6-(2-nitrophenyl)imidazo[2,1-b]thiazol-3-yl]methanol (**3**). ESI-MS calculated for $C_{12}H_9N_3O_3S$ $[M+H]^+$ 276.0, found: 276.0; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.14 p.p.m. (br s, 1 H), 7.2-7.9 (m, 4 H), 7.16 (br s, 1 H), 5.65 (t, 1 H, *J* = 7 Hz), 4.6 (m, 2 H).

Compound **4** was prepared as described in Nunes, J.J., Milne, J., Bemis, J., Xie, R., Vu, C.V., Ng, P.Y., Disch, J.S., US Patent Application Publication US2007/0037865 A1, February 15, 2007.

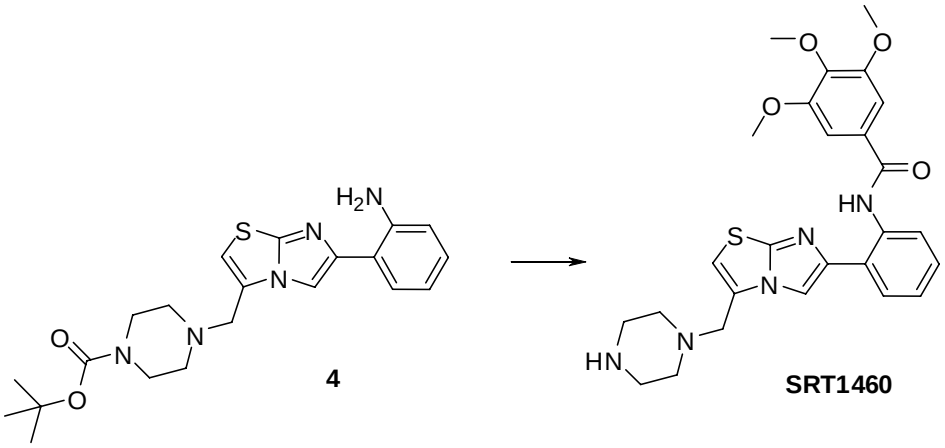
Preparation of 4-[6-(2-aminophenyl)imidazo[2,1-*b*]thiazol-3-ylmethyl]piperazine-1-carboxylic acid *tert*-butyl ester (4):



A solution of [6-(2-nitrophenyl)imidazo[2,1-*b*]thiazol-3-yl]methanol (**3**) (1.0 g, 3.6 mmol) and Et_3N (0.51 mL, 3.64 mmol) in CH_2Cl_2 (100 mL) at 0 °C was treated with methanesulfonyl chloride (0.28 mL, 3.7 mmol) and the resulting reaction mixture was allowed to warm to room temperature and stirred for 15 min. The reaction was quenched with brine and extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to afford the mesylate intermediate. This material was dissolved in CH_3CN (4 mL) containing Et_3N (0.51 mL, 3.6 mmol) and Boc-piperazine (680 mg, 3.6 mmol). The resulting solution was stirred at room temperature for 1 day. The reaction mixture was concentrated and the resulting residue was partitioned between CH_2Cl_2 and water. The organic layer was dried (Na_2SO_4) and concentrated to afford crude product. This material dissolved in MeOH (6 mL) and water (1 mL) containing sodium hydrosulfide hydrate (200 mg). The resulting reaction mixture was stirred under reflux for 24 hours, cooled to room temperature and concentrated. The residue was diluted with water (2 mL) and extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and concentrated to afford 0.90 g (60% yield) of 4-[6-(2-amino-phenyl)-imidazo[2,1-*b*]thiazol-3-ylmethyl]-piperazine-1-carboxylic acid tert-butyl ester (**4**). ESI-MS calculated for $C_{21}H_{27}N_5O_2S$ $[M+H]^+$ 414.2, found: 414.1; ^1H NMR (300 MHz,

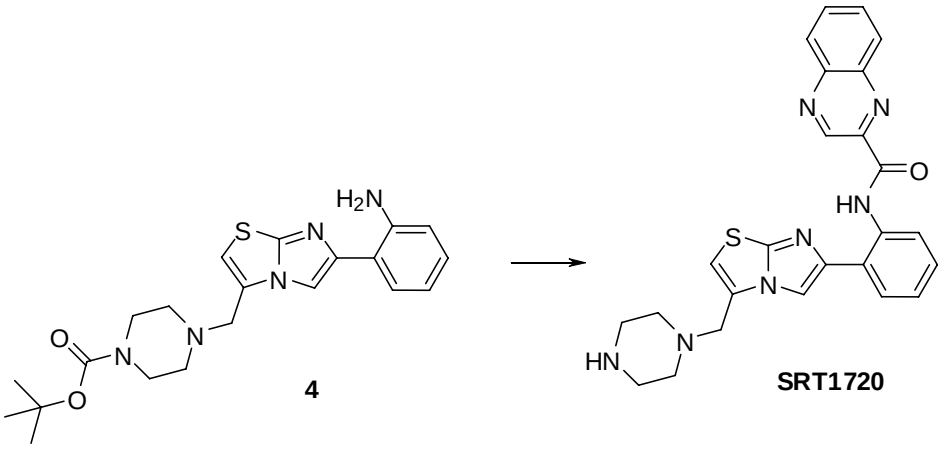
DMSO-d_6): δ 9.2 p.p.m. (br s, 1 H), 8.7 (br s, 1 H), 8.15 (s, 1 H), 8.10 (s, 1 H), 6.8-7.8 (m, 4 H), 6.16 (br s, 2 H), 3.72 (br s, 2 H), 1.39 (br s, 9 H).

Preparation of 3,4,5-trimethoxy-N-(2-(3-(piperazin-1-ylmethyl)imidazo[2,1-*b*]thiazol-6-yl)phenyl)benzamide (SRT1460):



A solution of 4-[6-(2-aminophenyl)imidazo[2,1-*b*]thiazol-3-ylmethyl]piperazine-1-carboxylic acid *tert*-butyl ester (**4**) (300 mg. 0.73 mmol) was dissolved in pyridine (5 mL) and treated with of 3,4,5-trimethoxybenzoyl chloride (167 mg, 0.73 mmol). The reaction mixture was heated in a microwave reactor (160^o x 10 min), cooled to room temperature and concentrated *in vacuo*. The resulting crude product was purified by chromatography (gradient elution, CH_2Cl_2 to 95% CH_2Cl_2, 4% MeOH and 1% Et_3N). The purified product was then treated with a solution containing 25% TFA in CH_2Cl_2 (2mL) for 2 hours, concentrated and the resulting residue was triturated with Et_2O to afford 335 mg (65% yield) of **SRT1460** as the TFA salt. The HCl salt of **SRT1460** was prepared by the same method as the HCl salt of **SRT1720**. m.p.: 193.5 °C (HCl salt); HRMS calculated for $C_{26}H_{29}N_5O_4S$ $[M+H]^+$ 508.2018; found: 508.2039; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.9 p.p.m. (br s, 1 H), 9.0 (br s, 1 H), 8.7-7.10 (m, 7 H), 8.5 (s, 1 H), 4.0 (br s, 9 H), 3.8 (m, 2 H), 3.2-2.8 (m, 8 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 47.56 p.p.m., 50.01, 56.15, 60.16, 104.68, 111.52, 120.70, 120.93, 123.63, 126.89, 128.13, 130.13, 136.01, 140.54, 144.43, 147.75, 152.86, 164.09.

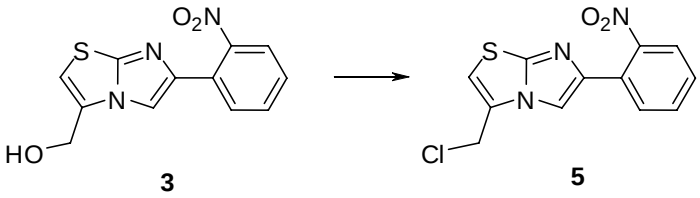
Preparation of *N*-(2-(3-(piperazin-1-ylmethyl)imidazo[2,1-*b*]thiazol-6-yl)phenyl)quinoxaline-2-carboxamide (SRT1720):



A solution of 4-[6-(2-aminophenyl)imidazo[2,1-*b*]thiazol-3-ylmethyl]piperazine-1-carboxylic acid tert-butyl ester (**4**) (250 mg, 0.60 mmol) in pyridine (3 mL) was treated with (117 mg, 0.61 mmol) of 2-quinoxaloyl chloride. The reaction was carried out according to the procedure described for **SRT1460**. *N*-(2-(3-(piperazin-1-ylmethyl)imidazo[2,1-*b*]thiazol-6-yl)phenyl)quinoxaline-2-carboxamide (**SRT1720)** was obtained as the TFA salt (245 mg, 57% yield). The corresponding HCl salt of **SRT1720** could then be prepared by dissolving the TFA salt in water and neutralized with NaHCO3. The resulting aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The resulting residue was taken up in 50% aqueous CH_3CN and 1 mL of 3 N HCl was added. The mixture was then lyophilized to obtain **SRT1720** as the HCl salt. m.p.: *dec*. (HCl salt), 221.4 °C (freebase); HRMS calculated for $C_{25}H_{23}N_7OS$ $[M+H]^+$ 470.1763; found: 470.1753; ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.9 p.p.m. (br s, 1 H), 9.8 (br s, 1 H), 9.6 (br s, 1 H) 8.9 – 7.2 (m, 11 H), 4.8 (br s, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 47.49 p.p.m., 49.88, 111.45, 120.47, 121.84, 124.02, 127.04, 128.10, 129.20, 129.23, 131.39, 132.15, 135.39, 139.54, 143.03, 143.80, 144.36, 144.62, 147.76, 161.57.

Compound **5** was prepared as described in Nunes, J.J., Milne, J., Bemis, J., Xie, R., Vu, C.V., Ng, P.Y., Disch, J.S., US Patent Application Publication US2007/0037865 A1, February 15, 2007.

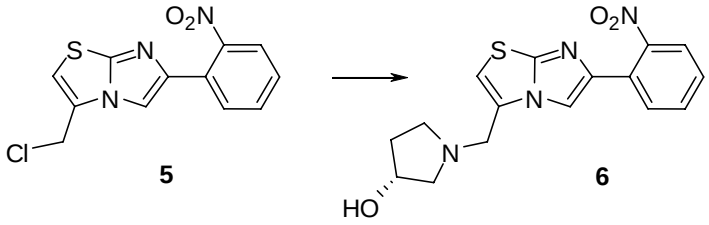
Preparation of 3-(chloromethyl)-6-(2-nitrophenyl)imidazo[2,1-*b*]thiazole (5):



To a solution of (6-(2-nitrophenyl)imidazo[2,1-*b*]thiazol-3-yl)methanol (**3)** (165 g, 0.6 mol) in dichloromethane (1.65 L) was added $SOCl_2$ (400 mL, 5.5 mol) at room temperature. DMF (0.3 mL) was added and the mixture was stirred at 30 °C for 2 h. The reaction mixture was cooled to 0 °C, filtered, dried and concentrated *in vacuo* to give 174 g (99% yield) of 3-(chloromethyl)-6-(2-nitrophenyl)imidazo[2,1-*b*]thiazole (**5**) as a white solid. This material was used without additional purification.

Compound **6** was prepared as described in Nunes, J.J., Milne, J., Bemis, J., Xie, R., Vu, C.V., Ng, P.Y., Disch, J.S., US Patent Application Publication US2007/0037865 A1, February 15, 2007.

Preparation of (*R*)-1-((6-(2-nitrophenyl)imidazo[2,1-*b*]thiazol-3-yl)methyl)pyrrolidin-3-ol (6):

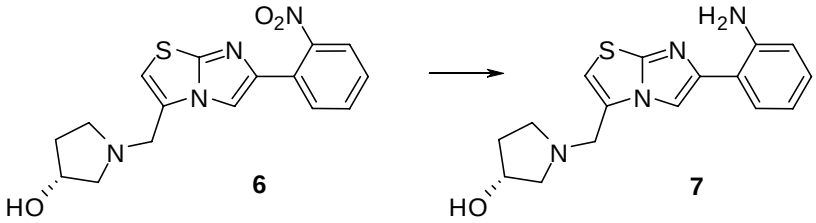


To a solution of 3-(chloromethyl)-6-(2-nitrophenyl)imidazo[2,1-*b*]thiazole (**5**) (38.9 g; 0.13 mol) in DMF (270 mL), was added (*R*)-pyrrolidine-3-ol (15 g, 0.17 mol), K_2CO_3 (39.3 g, 0.28 mol, 3.0 eq.) and catalytic amount of NaI. The resulting mixture was stirred at room temperature overnight, filtered through celite, and concentrated *in vacuo*. The

residue was purified by chromatography (eluted with CH_2Cl_2/MeOH = 40:1) to give 24.4 g (75% yield) of (*R*)-1-((6-(2-nitrophenyl)imidazo[2,1-*b*]thiazol-3-yl)methyl)pyrrolidin-3-ol (**6**) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.92 p.p.m. (1H, dd, *J* = 8.0, 1.2 Hz), 7.83 (1H, s), 7.66 (1H, dd, *J* = 8.0, 1.2 Hz), 7.58 (1H, dt, *J* = 7.6, 1.2 Hz), 7.41 (1H, dt, *J* = 8.0, 1.2 Hz), 6.65 (1H, s), 4.38 (1H, m), 3.77 (2H, d, *J* = 0.8 Hz), 2.89 (1H, m), 2.66-2.68 (2H, m), 2.42 (1H, m), 2.22 (1H, m), 1.80 (1H, m).

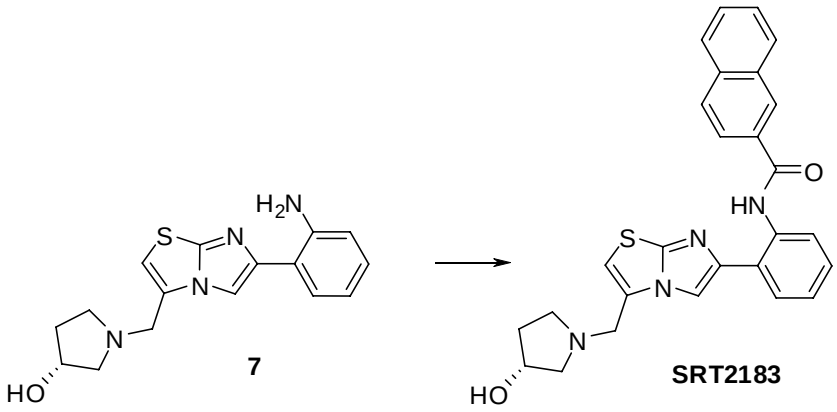
Compound **7** was prepared as described in Nunes, J.J., Milne, J., Bemis, J., Xie, R., Vu, C.V., Ng, P.Y., Disch, J.S., US Patent Application Publication US2007/0037865 A1, February 15, 2007.

Preparation of (*R*)-1-((6-(2-aminophenyl)imidazo[2,1-*b*]thiazol-3-yl)methyl)pyrrolidin-3-ol (7):



A solution of (*R*)-1-((6-(2-nitrophenyl)imidazo[2,1-*b*]thiazol-3-yl)methyl)pyrrolidin-3-ol (**6)** (18.2 g, 53 mmol) in ethanol (260 mL) was treated with 10% Pd/C (wet, 3.0 g). The atmosphere was evacuated from the reaction vessel and backfilled with H_2 (1 atm). The mixture was stirred over H_2 (1 atm) for 60 h. The reaction mixture was filtered through celite to remove the catalyst and concentrated under *in vacuo* to afford 15.6 g (94% yield) of (*R*)-1-((6-(2-aminophenyl)imidazo[2,1-*b*]thiazol-3-yl)methyl)pyrrolidin-3-ol (**7**) as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.77 p.p.m. (1H, s), 7.45 (1H, dd, *J* = 5.6, 1.2 Hz), 7.08 (1H, dt, *J* = 5.6, 1.6 Hz), 6.71-6.75 (2H, m), 6.59 (1H, s), 4.32 (1H, m), 3.72 (2H, d, *J* = 0.8 Hz), 3.43 (2H, s), 2.84 (1H, m), 2.63 (2H, m), 2.42 (1H, m), 2.14 (1H, m), 1.73 (1H, m).

Preparation of (*R*)-*N*-(2-(3-((3-hydroxypyrrolindin-1-yl)methyl)imidazo[2,1-*b*]thiazol-6-yl)phenyl)-2-naphthamide (SRT2183):



A mixture of (*R*)-1-((6-(2-aminophenyl)imidazo[2,1-*b*]thiazol-3-yl)methyl)pyrrolidin-3-ol (**7**) (2.0 g, 6.4 mmol), 2-naphthalene carboxylic acid (2.19 g, 12.7 mmol), HATU (4.84 g, 12.7 mmol) and DIPEA (3.29 g, 25.4 mmol) in DMF (50 mL) was stirred at room temperature overnight. Water (250 mL) was added and a yellow precipitate formed and was collected by filtration. A mixture of the yellow solid, $LiOH{\cdot}H_2O$ (1.33 g, 31.8 mmol), THF (40 mL), CH_3OH (40 mL), H_2O (15 mL) was stirred at 40 °C overnight. The mixture was concentrated *in vacuo* to remove the CH_3OH and THF. The resulting suspension was filtered. The collected solid was washed with ethyl acetate to give crude product as a yellow solid (1.2 g**).** The crude material was stirred 4N HCl/CH_3OH (6 mL) at room temperature overnight. The resulting solid was collected by filtration, washed with CH_3OH and dried *in vacuo* to give 1.23 g (95% yield) of (*R*)-*N*-(2-(3-((3-hydroxypyrrolindin-1-yl)methyl)imidazo[2,1-*b*]thiazol-6-yl)phenyl)-2-naphthamide (**SRT2183**) hydrochloride salt as a white solid. m.p.: 233.6 °C (HCl salt); $[a]_D^{25}$ = -10.7 º, measured in methanol with C= 0.96 (9.6 mg/mL); HRMS calculated for $C_{27}H_{24}N_4O_2S$ $[M+H]^+$ 469.1693; found: 469.1696; ^1H NMR (DMSO-d_6, 400 MHz): δ 13.0 p.p.m. (1H, s) 11.3-11.8 (1H, m), 8.96 (1H, m), 8.71-8.75 (2H, m), 8.05-8.14 (4H, m), 7.68-7.86 (4H, m), 7.40 (1H, t, *J* = 7.6 Hz), 7.23 (1H, t, *J* = 7.6 Hz), 4.81 (2H, m), 4.44 (1H, m), 3.10-3.40 (4H, m), 1.87-2.32 (2H, m); ^{13}C NMR (100 MHz, DMSO-d_6): δ p.p.m. 33.10, 48.01, 48.53, 51.85, 60.39, 68.14, 111.33, 111.43, 118.58, 118.79, 120.96,

123.65, 123.91, 127.06, 127.48, 127.74, 128.04, 128.17, 128.46, 128.86, 132.20, 132.22, 134.37, 136.13, 144.42, 147.72, 164.50.

References

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