Supporting Information

Discovery of pro-soft drug modulators of sphingosine-1-phosphate receptor 1 (S1PR1) as topical tool compounds

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Biology Experimental

S1PR β-Arrestin Assay

The example below is for S1PR1. The same assay conditions were used for selectivity assessment of S1PR2-4, but with a CHO-K1 EDG1 β-arrestin cell line that stably expresses β-arrestin 2 and the relevant S1PR isoform.

β-arrestin recruitment assays were carried out using the human PathHunter CHO-K1 EDG1 β-arrestin cell line (DiscoveRx Corporation) in a chemi-luminescence detection assay. This cell line stably expresses β-arrestin 2 and S1PR1 fused to complementing portions of β-galactosidase (enzyme accepter and “pro-link”, respectively), which associate upon β-arrestin recruitment to form functional β-galactosidase enzyme. Cells were grown to 80% confluency in growth medium (F12 nutrient HAMS supplemented with 10% heat-inactivated USA FBS, 1% L-glutamax, 800 µg/ml geneticin and 300 µg/ml hygromycin). Cells were harvested from the flask using enzyme free cell dissociation buffer (Gibco) and washed from flasks with Optimem solution (Gibco). Cells were then centrifuged at 1000 rpm for 2-3 min and re-suspended in assay buffer (prepared from Sigma kit H1387 supplemented with 20 ml/L HEPES, 4.7 ml/L NaHCO3, 0.1% pluronic acid F-68 solution, 0.1% BSA and adjusted to pH 7.4 using sodium hydroxide at 1x106 cells/ml. Cells were dispensed into assay plates containing compounds (100nl/well of a solution of test compound in 100% DMSO) at 1x104 cells/well and incubated at 37ºC/5% CO2 for 90 minutes followed by 15 min at room temperature. 5 µL detection mix (1 part Galacton Star, 5 parts Emerald II, 19 parts Assay Buffer; DiscoveRx) were added per well and the plates incubated at room temperature for 60 minutes. Luminescence was quantified using a Viewlux plate reader.

All values reported correspond to n of 2.

DMPK Experimental

CHI logD

The LogD_{7.4} of a compound describes the affinity of a substance for lipids at pH 7.4 (lipophilicity).

One experimental methodology used to evaluate the LogD_{7.4} for new chemical entities exploits the direct relationship that is observed between lipophilicity and binary solvent partitioning. Using a fast gradient, reverse-phase HPLC method to determine the chromatographic hydrophobicity index, (CHI) as devised by Camurri and Zaramella¹ and Valko et al.² By plotting the retention time of a set of reference compounds against known CHI values, the CHI value of test compounds can be calculated according to their retention time. Test compounds were prepared as 0.5 mM solutions in 50:50 acetonitrile:water and analysed by reversed-phase HPLC-UV (wavelength 254 nm) using a Phenomenex Luna C18 100Å 150x4.6 mm 5 micron column with a gradient of aqueous phase (50 mM ammonium acetate (pH 7.4)) and mobile phase (acetonitrile).

All values reported correspond to n of 1.
1. Camurri, G. and A. Zaramella, High-throughput liquid chromatography/mass spectrometry method for the determination of the chromatographic hydrophobicity index. Anal Chem., 2001. 73(15): p. 3716-22.

2. Valko, K., et al., Fast gradient HPLC method to determine compounds binding to human serum albumin. Relationships with octanol/water and immobilized artificial membrane lipophilicity. J Pharm Sci., 2003. 92(11): p. 2236-48.

Kinetic Solubility
The aqueous solubility of the test compounds was measured using laser nephelometry. Compounds were subject to serial dilution from 10 mM to 0.5 mM in DMSO. An aliquot was then mixed with MilliQ water to obtain an aqueous dilution plate with a final concentration range of 250 – 12 µM, with a final DMSO concentration of 2.5%. Triplicate aliquots were transferred to a flat bottomed polystyrene plate which was immediately read on the NEPHELOstar (BMG Lab Technologies). The amount of laser scatter caused by insoluble particulates (relative nephelometry units, RNU) was plotted against compound concentration using a segmental regression fit, with the point of inflection being quoted as the compounds aqueous solubility (µM). All values reported correspond to n of 1.

pKa
Sirius T3 (Sirius Analytical Inc, UK) instrument has been used for pKₐ determination of the compounds. The pKₐ determination is based on acid-base titration and the protonation/deprotonation of the molecule is measured either by UV spectroscopy or potentiometrically. The pKₐ value is calculated from the pH where the 50-50% of the protonated and unprotonated form of the molecules are present. The UV-metric method provides pKₐ results for samples with chromophores whose UV absorbance changes as a function of pH. It typically requires 5 µl of a 10 mM solution of the samples and the UV absorbance is monitored over 54 pH values in a buffered solution in about 5 min. When the ionization centre is far from the UV chromophore pH-metric method based on potentiometric acid-base titration is used. The pH of each point in the titration curve is calculated using equations that contain pKₐ, and the calculated points are fitted to the measured curve by manipulating the pKₐ. The pKₐ that provides the best fit is taken to be the measured pKₐ. Usually 0.5 – 1 mg of solid material is required for the measurements. When the compound precipitates at some point during the pH titration co-solvent method using methanol is applied using various concentration of co-solvent. The pKₐ in water is calculated using the Yasuda-Shedlovsky extrapolation method. All values reported correspond to n of 1.

Human Liver Microsome (HLM) stability experiments
Test compound (0.5 µM) was incubated with female CD1 mouse liver microsomes (Xenotech LLC TM; 0.5 mg/mL 50 mM potassium phosphate buffer, pH 7.4) and the reaction started with addition of excess NADPH (8 mg/mL 50 mM potassium phosphate buffer, pH 7.4). Immediately, at time zero, then at 3, 6, 9, 15 and 30 min an aliquot (50 µL) of the incubation mixture was removed and mixed with acetonitrile (100 µL) to stop the reaction. Internal standard was added to all samples, the samples centrifuged to sediment precipitated protein and the plates then sealed prior to UPLC-MSMS analysis using a Quattro Premier XE (Waters Corporation, USA). XLfit (IDBS, UK) was used to calculate
the exponential decay and consequently the rate constant (k) from the ratio of peak area of test compound to internal standard at each timepoint. The rate of intrinsic clearance (CLi) of each test compound was then calculated using the following calculation:

$$\text{CLi (mL/min/g liver)} = k \times V \times \text{Microsomal protein yield}$$

Where V (mL/mg protein) is the incubation volume/mg protein added and microsomal protein yield is taken as 52.5 mg protein per g liver. Verapamil (0.5 μM) was used as a positive control to confirm acceptable assay performance. Experiments were performed using a single timecourse experiment. All values reported correspond to n of 1.

Human Liver Hepatocyte (HLH) stability experiments

Cryopreserved vials of human cryopreserved hepatocytes, supplied by Life Technologies, were thawed according to manufacturer’s instructions and cells resuspended in Williams Medium E (WME) containing cell maintenance supplement pack (CM4000, Life Technologies). Hepatocytes were incubated in suspension (0.5 million cells/mL) in 48 well non-collagen coated cell culture plates for 10 minutes at 37 °C, 5% CO₂. Upon addition of an equal volume of supplemented WME containing 1 μM test compound, an aliquot of incubation solution was removed to acetonitrile containing internal standard (final concentration 0.5 μM test compound and a cell density of 0.25 million cells/mL). Similarly, aliquots were removed at 3, 6, 9, 15, 30, 45, 60, 90 and 120 minutes. 100 μL of 80:20 water:acetonitrile was added to all samples and the analysis plate was centrifuged for 10 min at room temperature prior to injection and analysis of samples by UPLC-MS/MS. The response (area ratio of test compound to internal standard) was plotted against time using an exponential decay model and rate of disappearance calculated. All values reported correspond to n of 1.

Human Skin S9 stability experiments

An incubation mix was prepared containing 50 mM potassium phosphate buffer, pH 7.4), 0.3 mg/mL human skin S9 (Sekisui Xenotech), NADPH (final concentration 0.8 mg/mL), UDPGA (final concentration 0.16 mg/mL) and warmed to 37 °C for 5 minutes. The reaction was initiated upon addition of test compound (final concentration 0.5 μM). Immediately, at time zero, then at 3, 6, 15, 30, 60, 120 and 180 minutes, an aliquot (50 μL) of the incubation mixture was removed and mixed with acetonitrile (100 μL) to terminate the reaction. Internal standard was added to all samples, the samples centrifuged to sediment precipitated protein and the plates then sealed prior to UPLCMSMS analysis using a Quattro Premier XE (Waters corporation, USA).

Grafit (Erithacus Ltd) was used to calculate the exponential decay and consequently the rate constant (k) from the ratio of peak area of test compound to internal standard at each timepoint. The half life (T₁/₂) of each test compound was determined using the following equation:

$$T_{1/2} = \frac{0.693}{k}$$

All values reported correspond to n of 1.

In vivo pharmacokinetics

All regulated procedures on living animals in the University of Dundee were carried out under the authority of a project licence issued by the Home Office under the Animals (Scientific Procedures) Act 1986, as amended in 2012 (and in compliance with EU Directive EU/2010/63). Licence
applications will have been approved by the University’s Ethical Review Committee (ERC) before submission to the Home Office. The ERC has a general remit to develop and oversee policy on all aspects of the use of animals on University premises and is a sub-committee of the University Court, its highest governing body.

A 22.5 µL single dose of compound (10a) at a 1% w/v concentration in a propylene glycol:ethanol 7:3 formulation was applied topically to a 1.5 cm² area of shaved skin on female Balb/c mice. The skin was shaved 24 h prior to treatment. Two hours post application (n=3 mice) or eight hours post application (n=3 mice) a blood sample was taken from each mouse and diluted with nine volumes of sterile water. The mice were then humanely killed. The exposed skin at the application site on each mouse was cleaned with vehicle to remove excess dose and the skin dissected. The diluted blood samples and skin samples were stored frozen prior to analysis of compound 10a levels by UPLC/MS/MS.

Blood concentrations for (Z,Z)-10a

| Time-point (h) | Concentration (ng/mL) | Scaled concentration (ng/mL) | Mean concentration (ng/mL) |
|---------------|-----------------------|------------------------------|---------------------------|
|               | Mouse 1 | Mouse 2 | Mouse 3 | Mouse 1 | Mouse 2 | Mouse 3 | Mouse 1 | Mouse 2 | Mouse 3 |
| 2h-post application | < LLoQ | < LLoQ | < LLoQ | < LLoQ | < LLoQ | < LLoQ | < LLoQ | < LLoQ |

LLoQ = 1 ng/mL
Scaled LLoQ = dilution factor * LLoQ = 10*1 = 10 ng/mL
HLoQ= 1000 ng/mL

Skin concentrations for (Z,Z)-10a

| Time (h) | Skin weight mg | Solvent volume µL | Total Homog Vol (µL) | scaled measured conc ng/µL | ng/mg skin | average ng/mg per time |
|----------|----------------|------------------|---------------------|---------------------------|-------------|------------------------|
| Mouse1 2 | 104.9 | 944.1 | 1.049 | 743.4 | 7.434 | 3.6 |
| Mouse2 2 | 91.5 | 923.5 | 0.915 | 244.5 | 2.445 |
| Mouse3 2 | 65.7 | 591.3 | 0.657 | 102.8 | 1.028 |
| Mouse4 8 | 111.9 | 1007.1 | 1.119 | 146 | 1.46 |
| Mouse5 8 | 77.26 | 695.34 | 0.7726 | 164.6 | 1.646 |
| Mouse6 8 | 100.44 | 903.96 | 1.0044 | 304.2 | 3.042 |

LLoQ = 2 ng/mL
Scaled LLoQ = dilution factor * LLoQ = 10*2 = 20 ng/mL
HLoQ= 1000 ng/mL
Blood concentrations for 9k

| Time-point (h) | Concentration (ng/mL) | Scaled concentration (ng/mL) | Mean concentration (ng/mL) |
|---------------|-----------------------|------------------------------|---------------------------|
| Mouse 1       | Mouse 2               | Mouse 3                      | Mouse 1                   | Mouse 2            | Mouse 3            |
| 2h-post application | < LLoQ < LLoQ < LLoQ | < LLoQ < LLoQ < LLoQ | < LLoQ < LLoQ < LLoQ |

| Time-point (h) | Concentration (ng/mL) | Scaled concentration (ng/mL) | Mean concentration (ng/mL) |
|---------------|-----------------------|------------------------------|---------------------------|
| Mouse 4       | Mouse 5               | Mouse 6                      | Mouse 4                   | Mouse 5            | Mouse 6            |
| 8h-post application | < LLoQ < LLoQ < LLoQ | < LLoQ < LLoQ < LLoQ | < LLoQ < LLoQ < LLoQ |

LLoQ = 1 ng/mL
Scaled LLoQ = dilution factor * LLoQ = 10*1 = 10 ng/mL
HLoQ = 1000 ng/mL

Skin concentrations for 10a

| Time (h) | skin weight mg | solvent volume µL | Total Homog Vol (mL) | scaled measured conc-ng/mL | ng/mg skin | average per time |
|----------|----------------|-------------------|----------------------|---------------------------|------------|-----------------|
| Mouse1   | 2              | 104.9             | 944.1                | 1.049                     | 723.5      | 7.24            |
| Mouse2   | 2              | 91.5              | 823.5                | 0.915                     | 617.4      | 61.7            |
| Mouse3   | 2              | 65.7              | 591.3                | 0.657                     | 7941.5     | 79.4            |
| Mouse4   | 8              | 111.9             | 1007.1               | 1.119                     | 2561.5     | 25.8            |
| Mouse5   | 8              | 77.26             | 695.34               | 0.773                     | 3289.5     | 32.9            |
| Mouse6   | 8              | 100.44            | 903.96               | 1.094                     | 5090       | 50.9            |

LLoQ = 1 ng/mL
Scaled LLoQ = dilution factor * LLoQ = 10*1 = 10 ng/mL
HLoQ = 1000 ng/mL

GSH method

Test compound (10 µM) was incubated with 0.5 mg/mL human liver microsomes (Mixed Gender, Life Technologies) in 50 mM potassium phosphate buffer, pH 7.4 in the presence of either NADPH (8mg/mL) alone, glutathione alone or NADPH plus glutathione. Reactions are initiated by addition of 5 µL of 1 mM test compound (final concentration 10 µM, 1% solvent). Immediately, an aliquot (100 µL) of the incubation mixture was removed and mixed with acetonitrile (200 µL) to stop the reaction. A further 100 µL aliquot is removed at 90 minutes. After addition of 100 µL of water, the samples were centrifuged to sediment precipitated protein and the plates then sealed prior to analysis using a Xevo QTof Quadrupole Time-of-Flight Mass Spectrometer (Waters corporation, USA). Detection of metabolites is performed by analysis with MetabolynxXS and by manual data
searching. Elemental composition of the parent ion and MS/MS analysis is used to determine the nature of the GSH adduct. Incubations with Clozapine were performed to provide a positive control. All values reported correspond to $n$ of 1.

Chemistry Experimental

General information
Reactions using microwave irradiation were carried out in a Biotage Initiator microwave. Normal phase TLC was carried out on pre-coated silica plates (Kieselgel 60 F254, BDH) with visualization via UV light (UV 254/365 nm) and/or ninhydrin solution. Flash chromatography was performed using CombiFlash Companion Rf (Teledyne ISCO) and prepakced silica gel columns purchased from Grace Davison Discovery Science or Silicycle. Mass-directed preparative HPLC separations were performed using a Waters HPLC (2545 binary gradient pumps, 515 HPLC make-up pump, 2767 sample manager) connected to a Waters 2998 photodiode array and a Waters 3100 mass detector. Preparative HPLC separations were performed with a Gilson HPLC (321 pumps, 819 injection module, 215 liquid handler/injector) connected to a Gilson 155 UV/vis detector. On both instruments, HPLC chromatographic separations were conducted using Waters XBridge C18 columns, 19 mm × 100 mm, 5 μm particle size, using 0.1% ammonia in water (solvent A) and acetonitrile (solvent B) as mobile phase. NMR spectra were recorded on a Bruker Avance DPX 500 spectrometer (1H at 500 MHz and 13C at 125 MHz) or a Bruker Avance III HD Ascend 400 (1H at 400 MHz), both using automatic tuning and matching. Chemical shifts (δ) are expressed in ppm recorded using the residual solvent as the internal reference in all cases. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), or a combination thereof. Coupling constants (J) are quoted to the nearest 0.5 Hz. LCMS analysis and chromatographic separation were conducted with a Bruker MicroTOF mass spectrometer (Bruker S) or an Agilent (Agilent) Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LC/MS, where both instruments were connected to an Agilent diode array detector. Alternatively a Bruker MicroTOF II focus mass spectrometer (Bruker N) connected in parallel to dionex ultimate 3000 RSLC system with diode array detector was used. The columns used were a Waters XBridge (50 mm × 2.1 mm, 3.5 μm particle size) or Waters XSelect (30 mm × 2.1 mm, 2.5 μm particle size) and the compounds were eluted with a gradient of 5–95% acetonitrile/water + 0.1% ammonia. All compounds for in vitro experiments displayed >90% purity by LCMS. High resolution mass spectrometry (HRMS) was performed using a Bruker MicroTof mass spectrometer. Unless otherwise stated herein reactions have not been optimized. Solvents and reagents were purchased from commercial suppliers and used without further purification. Dry solvents were purchased in Sure/Seal bottles stored over molecular sieves. Ponesimod was prepared following Bolli et. al.\textsuperscript{12}

Experimental procedures and analysis for compounds 4a-h, 9a-l and 10a-i

\textbf{(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one 4a}

\[\text{\begin{align*}
\text{\(N\)} & \quad \text{\(S\)} \\
\text{\(O\)} & \quad \text{\(H\)} \\
\text{\(C\)} & \quad \text{\(Cl\)}
\end{align*}}\]

Sodium acetate (5.5 g, 67 mmol) was dissolved in AcOH (75 ml) and the 3-chloro-4-hydroxy-benzaldehyde (5.2 g, 33.5 mmol) was added to the stirred solution. The mixture was stirred for 3 hours
until a homogeneous solution was obtained. In a separate flask, the 1-isothiocyanato-2-methyl-benzene (5 g, 33.5 mmol) was dissolved in DCM under nitrogen and n-propylamine (2 g, 33.5 mmol) added dropwise at room temperature (exotherm to 35 °C). The mixture was stirred for 15 minutes at room temperature then cooled in an ice-salt bath to -2 °C. The addition funnel was rinsed with DCM and bromoacetyl bromide (6.8 g, 33.5 mmol) added dropwise at such a rate as to maintain the temperature below 5 °C over 15 minutes. The mixture was stirred for 15 minutes with cooling before again rinsing the addition funnel with DCM and the dropwise addition of pyridine (5.4 g, 68.7 mmol), again keeping the temperature below 5 °C. After the addition was complete, the resulting suspension was stirred with cooling for 45 minutes then allowed to warm to room temperature. The mixture was stirred a further 60 minutes. The mixture was then heated to 60 °C and approximately 45 ml of DCM distilled off under a stream of nitrogen. The AcOH solution of the benzaldehyde and sodium acetate was added in a steady stream and the resulting yellow suspension heated overnight at 60 °C. Approximately 25 ml of solvent was removed under vacuum. The remaining mixture was heated at 60 °C and water (40 ml) added dropwise over 45 minutes. The yellow suspension was stirred for 30 minutes with heating, then allowed to cool to room temperature. The mixture was filtered and the collected pale yellow solid washed with 2:1 AcOH/H2O (25 ml) followed by water (25 ml). The solid was dried at the pump then at 50 °C under vacuum to give (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one as a pale yellow solid (10.7 g; 80% yield).

$^1$H NMR (500 MHz, DMSO-d$_6$) δ: 11.01 (s, 1H), 7.71 (d, J=2.1 Hz, 1H), 7.68 (s, 1H), 7.53 (dd, J=2.2, 8.6 Hz, 1H), 7.39 - 7.31 (m, 3H), 7.27 (d, J=7.5 Hz, 1H), 7.16 (d, J=8.5 Hz, 1H), 3.39-3.23 (m, 2H), 2.09 (s, 3H), 1.55 - 1.49 (m, 2H), 0.85 (t, J=7.4 Hz, 3H). $^{13}$C NMR (125 MHz, DMSO-d$_6$) δ: 165.32, 154.79, 146.58, 135.83, 134.74, 132.09, 130.63, 129.84, 129.07, 129.04, 128.54, 126.83, 125.74, 120.55, 118.56, 117.27, 54.29, 23.38, 17.08, 11.68. HPLC $t_R$ (Bruker S, basic, 7.5 minutes): 3.20 minutes, 100%; Calculated for C$_{20}$H$_{19}$ClN$_2$O$_2$S 386.1; found MS: m/z 387.1 [M+H]$^+$. HRMS: Calculated for C$_{20}$H$_{19}$ClN$_2$O$_2$S 386.0929, found 387.0941 [M+H]$^+$. 
$4a^1$H NMR (500 MHz, DMSO-$d_6$)
4a $^{13}$C NMR (125 MHz, DMSO-d$_6$):

4a HMBC (DMSO-d$_6$): $^1$H-$^{13}$C coupling of H$_9$-C$_3$ (6.4 Hz)

4a NOESY (DMSO-d$_6$): $^1$H-$^{13}$C coupling of H$_9$-C$_3$ (6.4 Hz)

2-chloro-N-(2-methoxyphenyl)acetamide
To a solution of 2-methoxyaniline (1 g, 8 mmol) in THF (35 ml) was added triethylamine (1.2 g, 12 mmol) and the reaction cooled to -70 °C. To this was added 2-chloroacetyl chloride (0.9 g, 8 mmol) dropwise and the reaction slowly warmed to room temperature. The reaction was diluted with water, extracted with ethyl acetate and the organics dried (MgSO$_4$), filtered and concentrated to give 2-chloro-N-(2-methoxyphenyl)acetamide as a red oil (1.6 g, 98% yield).

$^1$H NMR (500 MHz, DMSO-d$_6$) δ: 9.48 (s, 1H), 7.98 (d, J=7.9 Hz, 1H), 7.14 - 7.10 (m, 1H), 7.08 (d, J=7.0 Hz, 1H), 6.95 - 6.92 (m, 1H), 4.39 (s, 2H), 3.86 (s, 3H).

(2Z)-3-(2-methoxyphenyl)-2-propylimino-thiazolidin-4-one 2b

To a solution of 2-chloro-N-(2-methoxyphenyl)acetamide (0.8 g, 4.0 mmol) in DMF (15 ml) was added 1-isothiocyanatopropane (405 mg, 4.0 mmol). 60% Sodium hydride (160 mg, 4.0 mmol) was added in three portions, 20 minutes apart and the reaction stirred at room temperature overnight. The reaction was quenched with water, acidified with 2 M HCl and washed with ethyl acetate. The aqueous was then basified with saturated sodium bicarbonate and extracted with ethyl acetate. The organics were dried (MgSO$_4$), filtered and concentrated to give (2Z)-3-(2-methoxyphenyl)-2-propylimino-thiazolidin-4-one as a yellow oil (350 mg, 33% yield).

$^1$H NMR (400 MHz, CDCl$_3$) δ: 7.43 - 7.38 (m, 1H), 7.19 (dd, J=1.7, 7.7 Hz, 1H), 7.08 - 7.02 (m, 2H), 3.99 (dd, J=16.8, 27.0 Hz, 2H), 3.82 (s, 3H), 3.31 - 3.22 (m, 2H), 1.62 (s, 2H), 0.89 (dd, J=7.4, 7.4 Hz, 3H).

(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(2-methoxyphenyl)-2-propylimino-thiazolidin-4-one 4b

To a solution of (2Z)-3-(2-methoxyphenyl)-2-propylimino-thiazolidin-4-one (106 mg, 0.40 mmol) in acetic acid (1 ml) was added 3-chloro-4-hydroxy-benzaldehyde (63 mg, 0.40 mmol) and sodium
acetate (66 mg, 0.80 mmol) and heated overnight at 85 °C. The reaction was cooled to room
temperature, diluted with DCM and washed with water followed by sat sodium bicarbonate. The
organics were collected via phase separator, concentrated and purified by column (0-100% ethyl
acetate in heptane) to give (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(2-methoxyphenyl)-
2-propylimino-thiazolidin-4-one (65 mg, 38.2% yield) as a yellow solid. ¹H NMR (400 MHz, DMSO-d₆)
δ: 11.00 (s, 1H), 7.70 (d, J=2.2 Hz, 1H), 7.64 (s, 1H), 7.51 (dd, J=2.2, 8.7 Hz, 1H), 7.47 - 7.42 (m, 1H),
7.28 (dd, J=1.7, 7.7 Hz, 1H), 7.20 - 7.14 (m, 2H), 7.08 - 7.03 (m, 1H), 3.75 (s, 3H), 3.33 (s, 2H), 1.54 -
1.48 (m, 2H), 0.84 (t, J=7.3 Hz, 3H). HPLC tᵣ (Agilent, acidic, 3.5 minutes): 1.79 minutes, 100%;
Calculated for C₂₀H₁₉ClN₂O₃S, 402.1, found MS: m/z 403.1 [M+H]⁺. HRMS: Calculated for C₂₀H₁₉ClN₂O₃S,
402.0805, found 403.0892 [M+H]⁺.

(2Z)-3-isopropyl-2-propylimino-thiazolidin-4-one 2c

To a solution of propan-2-amine (1 g, 17 mmol) in DCM (25 ml) was added 1-isothiocyanato-propane
(1.7 g, 17 mmol) and stirred at room temperature for 1.5 hours. The reaction was cooled to 0 °C on an
ice bath and to this was added dropwise addition of 2-bromoacetyl bromide (3.4 g, 17 mmol). After
15 minutes pyridine (2.7 g, 34 mmol) was added and stirred at 0 °C for 20 minutes before warming to
room temperature. The reaction was quenched with water, the organics collected via phase separator
and concentrated. The product was purified by flash chromatography using ISCO combiflash on silica
gel (40 g) eluting with ethyl acetate/heptane gradient (0-100%). Fractions corresponding to product
were combined and concentrated to afford a mixture of the two regio-isomers (2Z)-3-isopropyl-2-
propylimino-thiazolidin-4-one and (2Z)-2-isopropylimino-3-propyl-thiazolidin-4-one (2.8 g, 84.7% yield).

HPLC tᵣ (Agilent, acidic, 3.5 minutes): 1.24 minutes, 60%; Calculated for C₉H₁₆ClN₂O₂S, 200.1, found MS:
m/z 201.2 [M+H]⁺ and 1.48 minutes, 40%; MS: m/z 201.2 [M+H]⁺.

(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-isopropyl-2-propylimino-thiazolidin-4-one 4c

To a solution of a mixture of (2Z)-2-isopropylimino-3-propyl-thiazolidin-4-one (200 mg, 0.99
mmol) and (2Z)-3-isopropyl-2-propylimino-thiazolidin-4-one (200 mg, 0.99 mmol) in acetic acid (5
ml) was added 3-chloro-4-hydroxy-benzaldehyde (313 mg, 1.99 mmol) and sodium acetate (328 mg,
3.99 mmol). The reaction was heated at 85 °C overnight. The reaction was cooled to room
temperature, diluted with ethyl acetate and washed with water followed by sat sodium bicarbonate solution. The organics were dried, filtered and concentrated. The product was purified by flash chromatography using ISCO combiflash on silica gel (24 g) eluting with ethyl acetate/ heptane gradient (0-60%). Fractions were combined and concentrated to a yellow solid. The regioisomers were separated and purified by reverse phase preparative HPLC (Gilson acidic 60-90% gradient). Fractions were concentrated on the genevac overnight to afford \( (2Z,5Z)\)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-isopropyl-2-propylimino-thiazolidin-4-one (48 mg, 13.5% yield).

\[ ^1H \text{ NMR (400 MHz, DMSO-} d_6 \text{)} \delta: 11.00 - 11.00 (m, 1H), 7.63 (d, J=2.2 Hz, 1H), 7.55 (s, 1H), 7.45 (dd, J=2.2, 8.6 Hz, 1H), 7.11 (d, J=8.5 Hz, 1H), 4.83 - 4.75 (m, 1H), 2.52 - 2.50 (m, 2H), 1.66 (q, J=7.0 Hz, 2H), 1.41 (d, J=6.9 Hz, 6H), 0.95 (t, J=7.3 Hz, 3H). \]

HPLC \( t_R \) (Agilent, acidic, 3.5 minutes): 2.03 minutes, 100%; Calculated for C\(_{16}\)H\(_{19}\)ClN\(_2\)O\(_2\)S 338.1, found MS: m/z 339.1 [M+H]+. HRMS: Calculated for C\(_{16}\)H\(_{19}\)ClN\(_2\)O\(_2\)S 338.0856, found 339.0938 [M+H]+.

\( (2Z)\)-2-propylimino-3-tetrahydropyran-4-yl-thiazolidin-4-one \( 2d \)

To a solution of tetrahydropyran-4-amine (1.0 g, 9.9 mmol) in DCM (15 ml) was added 1-isothiocyanatopropane (1.0 g, 9.9 mmol) and stirred at room temperature for 1.5 hours. The reaction was cooled to 0 °C on an ice bath and 2-bromoacetyl bromide (2.0 g, 9.9 mmol) was added dropwise. After 15 minutes pyridine (1.6 g, 20 mmol) was added and stirring continued at 0 °C for 20 minutes. The reaction was quenched with water, the organics were collected via phase separator and concentrated. The product was purified by flash chromatography using ISCO combiflash on silica gel (40 g) eluting with ethyl acetate/ heptane gradient (0-100%) to afford a mixture of regioisomers \( (2Z)\)-2-propylimino-3-tetrahydropyran-4-yl-thiazolidin-4-one and \( (2Z)\)-3-propyl-2-tetrahydropyran-4-ylimino-thiazolidin-4-one as a yellow oil (1.4 g, 59.7% yield).

HPLC \( t_R \) (Agilent, acidic, 3.5 minutes): 1.22 minutes, Calculated for C\(_{11}\)H\(_{18}\)N\(_2\)O\(_2\)S 242.1, found 51%; MS: m/z 243.2 [M+H]+ and 1.35 minutes, 49%; MS: m/z 243.1 [M+H]+.

\( (2Z,5Z)\)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-propylimino-3-tetrahydropyran-4-yl-thiazolidin-4-one \( 4d \)
To a solution of a mixture of (2Z)-3-propyl-2-tetrahydropyran-4-ylimino-thiazolidin-4-one (200 mg, 0.99 mmol) and (2Z)-2-propylimino-3-tetrahydropyran-4-yl-thiazolidin-4-one (242 mg, 0.99 mmol) in acetic acid (5 ml) was added 3-chloro-4-hydroxy-benzaldehyde (313 mg, 1.99 mmol) and Sodium acetate (328 mg, 3.99 mmol). The reaction was heated at 85 °C overnight. The reaction was cooled to room temperature, washed with water followed by saturated sodium bicarbonate solution and the organics dried with a phase separator and concentrated. The product was purified by flash chromatography using ISCO combiflash on silica gel (40 g) eluting with ethyl acetate/ heptane gradient (0-100%). Fractions corresponding to product were combined and concentrated to a yellow solid. The regioisomers were separated and purified by reverse phase preparative HPLC (Gilson acidic 50-80% gradient) and the fractions were concentrated on the genevac overnight to afford (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-propylimino-3-tetrahydropyran-4-yl-thiazolidin-4-one (47 mg, 11.7% yield).

\(^1\)H NMR (400 MHz, DMSO-d\(_6\)) \(\delta: 11.07 - 11.05 (m, 1H), 7.64 (d, J=2.2 Hz, 1H), 7.58 (s, 1H), 7.45 (dd, J=2.2, 8.6 Hz, 1H), 7.10 (d, J=8.5 Hz, 1H), 4.67 - 4.59 (m, 1H), 3.95 (dd, J=4.2, 11.3 Hz, 2H), 3.41 - 3.31 (m, 2H), 2.65 - 2.54 (m, 2H), 2.52 - 2.50 (m, 2H), 1.69 - 1.63 (m, 2H), 1.53 - 1.49 (m, 2H), 0.95 (t, J=7.3 Hz, 3H). HPLC \(t_\text{R}\) (Agilent, acidic, 3.5 minutes): 1.86 minutes, 100%; Calculated for C\(_{18}\)H\(_{21}\)ClN\(_2\)O\(_3\)S 380.1, found MS: \(m/z\) 381.1 \[M+H\]^+.

\((2Z)-3-(1,1-dioxothian-4-yl)-2-propylimino-thiazolidin-4-one 2e\)

To a solution of 1,1-dioxothian-4-amine (1.0 g, 6.7 mmol) in DCM (15 ml) was added 1-isothiocyanatopropane (0.68 g, 6.7 mmol) and stirred at room temperature for 1.5 hours. The reaction was cooled to 0 °C on an ice bath and to this was added 2-bromoacetyl bromide (1.4 g, 6.7 mmol) dropwise. After 15 minutes pyridine (1.0 g, 13.4 mmol) was added and stirring continued at 0 °C for a further 20 minutes before warming to room temperature. The reaction was quenched with water. Then the organics collected via phase separator and concentrated. The product was purified by flash chromatography using ISCO combiflash on silica gel (40 g) eluting with ethyl acetate/ heptane gradient (0-100%). Fractions were combined and concentrated to afford an off-white solid with (2Z)-3-(1,1-dioxothian-4-yl)-2-propylimino-thiazolidin-4-one as the major regiosiomer in the mixture (0.46 g, 23.6% yield).

HPLC \(t_\text{R}\) (Agilent, acidic, 3.5 minutes): 1.21 minutes, Calculated for C\(_{11}\)H\(_{18}\)N\(_2\)O\(_3\)S\(_2\) 290.1, found 23%; MS: \(m/z\) 291.1 \[M+H\]^+.

\((2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(1,1-dioxothian-4-yl)-2-propylimino-thiazolidin-4-one 4e\)
To a solution of (2Z)-3-{(1,1-dioxothian-4-yl)-2-propylimino-thiazolidin-4-one (340 mg, 1.47 mmol) and (2Z)-2-{(1,1-dioxothian-4-yl)imino-3-propyl-thiazolidin-4-one (115 mg, 0.50 mmol) in acetic acid (5 ml) was added 3-chloro-4-hydroxy-benzaldehyde (463 mg, 2.95 mmol) and sodium acetate (485 mg, 5.91 mmol). The reaction was heated at 85 °C overnight. Ethyl acetate was added and the precipitate collected via vacuum filtration to afford (2Z,5Z)-5-{[3-chloro-4-hydroxy-phenyl)methylene]-3-(1,1-dioxothian-4-yl)-2-propylimino-thiazolidin-4-one (400 mg, 60% yield).

¹H NMR (500 MHz, DMSO-d₆) δ: 11.04 (bs, 1H), 7.41 - 7.38 (m, 2H), 7.16 (d, J=9.0 Hz, 1H), 6.48 (d, J=7.9 Hz, 1H), 4.80 - 4.73 (m, 1H), 3.40 (t, J=13.0 Hz, 2H), 3.28 (dd, J=6.6, 6.6 Hz, 2H), 3.15 - 3.04 (m, 4H), 1.87 (d, J=12.5 Hz, 2H), 1.67 - 1.61 (m, 2H), 0.97 (dd, J=7.3, 7.3 Hz, 3H). HPLC tᵣ (Agilent, acidic, 3.5 minutes): 1.67 minutes, 100%; Calculated for C₁₈H₂₁ClN₂O₄S₂ 428.1, found MS: m/z 429.1 [M+H]⁺. HRMS: Calculated for C₁₈H₂₁ClN₂O₄S₂ 428.0631, found 429.0715 [M+H]⁺.

(2Z)-3-{(1-methyl-2-oxo-4-piperidyl)-2-propylimino-thiazolidin-4-one 2f

To a solution of 4-amino-1-methyl-piperidin-2-one dihydrochloride (1.0 g, 5.0 mmol) in DCM (25 ml) was added triethylamine (1.0 g, 10 mmol) followed by 1-isothiocyanatopropane (0.5 g, 5.0 mmol) and stirred at room temperature for 90 minutes. The reaction was cooled to 0 °C and 2-bromoacetyl bromide (1.0 g, 5.0 mmol) was added dropwise. After 15 minutes pyridine (0.8 g, 10 mmol) was added and slowly warmed to room temperature overnight. The reaction was quenched with water and the organics collected via phase separator. The organics were concentrated and purified by flash chromatography using ISCO combiflash on silica gel (24 g) eluting with ethyl acetate/ heptane gradient (0-100%) to afford a mixture of (2Z)-3-{(1-methyl-2-oxo-4-piperidyl)-2-propylimino-thiazolidin-4-one and (2Z)-2-{[1-methyl-2-oxo-4-piperidyl]imino]-3-propyl-thiazolidin-4-on (0.25 g, 18.7% yield).

HPLC tᵣ (Bruker, basic, 7.5 minutes): 3.0 minutes, Calculated for C₁₂H₁₉N₃O₂S 269.1, found 18%; MS: m/z 270.1 [M+H]⁺ and 3.2 minutes, 82%; MS: m/z 270.1 [M+H]⁺.

(2Z,5Z)-5-{[3-chloro-4-hydroxy-phenyl)methylene]-3-{(1-methyl-2-oxo-4-piperidyl)-2-propylimino-thiazolidin-4-one 4f
To a solution of (2Z)-3-(1-methyl-2-oxo-4-piperidyl)-2-propylimino-thiazolidin-4-one (250 mg, 0.92 mmol) in Acetic acid (5 ml) was added Sodium acetate (152 mg, 1.85 mmol) and 3-chloro-4-hydroxy-benzaldehyde (145 mg, 0.92 mmol). The reaction was heated at 85 °C overnight. The reaction was concentrated under reduced pressure and the residue suspended in water. The precipitate was collected via vacuum filtration and dried in a vacuum oven overnight. The product was triturated in ether and filtered under vacuum to give (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(1-methyl-2-oxo-4-piperidyl)-2-propylimino-thiazolidin-4-one (78 mg, 19.6% yield).

\[ ^1H \text{NMR (400 MHz, DMSO-d}_6) \delta: 8.05 (dd, J=5.7, 5.7 \text{ Hz, 1H}), 7.72 (s, 1H), 7.52 (d, J=2.6 \text{ Hz, 1H}), 7.27 (dd, J=2.5, 9.1 Hz, 1H), 6.30 (d, J=9.1 Hz, 1H), 4.66 - 4.66 (m, 1H), 3.78 - 3.75 (m, 2H), 3.39 (s, 3H), 3.04 - 2.98 (m, 2H), 2.47 (d, J=3.0 Hz, 2H), 2.17 - 2.14 (m, 2H), 1.41 (dd, J=7.3, 14.4 Hz, 2H), 0.84 (t, J=7.4 Hz, 3H). \]

HPLC \( t_R \) (Agilent, acidic, 7.5 minutes): 2.48 minutes, 100%; Calculated for C_{19}H_{22}ClN_{3}O_{3}S 407.1, found MS: \( m/z \) 408.1 [M+H]^+.

(2Z)-3-cyclopropyl-2-propylimino-thiazolidin-4-one 2g

To a solution of cyclopropanamine (1.0 g, 18 mmol) in DCM (25 ml) was added 1-isothiocyanatopropane (1.8 g, 18 mmol) and stirred at room temperature for 1.5 hours. The reaction was cooled on an ice bath to 0 °C and to this was added 2-bromoacetyl bromide (3.5 g, 18 mmol) dropwise. After 15 minutes pyridine (2.8 g, 35 mmol) was added and stirring continued at 0 °C for 20 minutes before warming to room temperature. The reaction was quenched with water, the organics were collected via phase separator and concentrated. The product was purified by flash chromatography using ISCO combiflash on silica gel (40 g) eluting with ethyl acetate/ heptane gradient (0-100%). Fractions were combined and concentrated separately to afford (2Z)-3-cyclopropyl-2-propylimino-thiazolidin-4-one (564 mg, 14.6% Yield).

\[ ^1H \text{NMR (400 MHz, CDCl}_3) \delta: 3.75 (s, 2H), 3.27 (t, J=6.9 \text{ Hz, 2H}), 2.70 - 2.65 (m, 1H), 1.69 (dd, J=7.1, 14.3 \text{ Hz, 2H}), 1.05 - 0.91 (m, 7H). \]

HPLC \( t_R \) (Agilent, acidic, 3.5 minutes): 0.55 minutes, 100%; Calculated for C_{9}H_{14}N_{2}OS 198.1, found MS: \( m/z \) 199.1 [M+H]^+.

(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-cyclopropyl-2-propylimino-thiazolidin-4-one 4g
To a solution of (2Z)-3-cyclopropyl-2-propylimino-thiazolidin-4-one (200 mg, 1.00 mmol) in acetic acid (5 mL) was added 3-chloro-4-hydroxy-benzaldehyde (316 mg, 2.01 mmol) and Sodium acetate (331 mg, 4.03 mmol). The reaction was heated at 85 °C overnight. The reaction was diluted with ethyl acetate and washed with water followed by saturated sodium bicarbonate. The organics were dried via phase separator and concentrated to a yellow oil. The product was purified by flash chromatography using ISCO combiflash on silica gel (24 g) eluting with ethyl acetate/ heptane gradient (0-100%). Fractions were combined and concentrated to a yellow solid which was suspended in MeCN/ Water (1:1) formed a precipitate which was collected by vacuum filtration to afford (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-cyclopropyl-2-propylimino-thiazolidin-4-one (115 mg, 32% Yield).

¹H NMR (400 MHz, DMSO-d₆) δ: 10.95 - 10.94 (m, 1H), 7.63 (d, J=2.2 Hz, 1H), 7.54 (s, 1H), 7.45 (dd, J=2.2, 8.7 Hz, 1H), 7.11 (d, J=8.5 Hz, 1H), 2.80 - 2.73 (m, 1H), 2.55 - 2.50 (m, 2H), 1.65 (q, J=7.1 Hz, 2H), 0.97 - 0.89 (m, 7H). HPLC tᵣ (Agilent, acidic, 3.5 minutes): 1.64 minutes, 100%; Calculated for C₁₆H₁₇ClN₂O₂S 336.1, found MS: m/z 337.1 [M+H]+.

(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(2-hydroxyphenyl)-2-propylimino-thiazolidin-4-one 4h

To a solution of (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(2-hydroxyphenyl)-2-propylimino-thiazolidin-4-one (50 mg, 0.12 mmol) in DCM (1 ml) was cooled to -70 °C under nitrogen and 1M tribromoborane (155 mg, 0.62 mmol) added dropwise. After 1 hr the reaction was quenched with 0.5 ml water and concentrated. 50% ACN/Water was added and the precipitate was collected via vacuum filtration to afford (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(2-hydroxyphenyl)-2-propylimino-thiazolidin-4-one (20.5 mg, 40% Yield).

¹H NMR (500 MHz, DMSO-d₆) δ: 11.01 - 10.98 (m, 1H), 9.81 - 9.81 (m, 1H), 7.71 (d, J=2.1 Hz, 1H), 7.65 (s, 1H), 7.52 (dd, J=2.1, 8.6 Hz, 1H), 7.29 - 7.25 (m, 1H), 7.16 (d, J=8.2, 8.2 Hz, 2H), 6.97 (d, J=8.1 Hz, 1H), 6.89 (t, J=7.6 Hz, 1H), 3.32 (d, J=2.7 Hz, 2H), 1.56 - 1.50 (m, 2H), 0.85 (dd, J=7.4, 7.4 Hz, 3H). HPLC tᵣ (Agilent, acidic, 3.5 minutes): 1.65 minutes, 100%; Calculated for C₁₉H₁₇ClN₂O₃S 388.1, found MS: m/z 389.1 [M+H]+. HRMS: Calculated for C₁₉H₁₇ClN₂O₃S 388.0648, found 389.0733 [M+H]+.

(2Z)-3-(o-tolyl)-2-(oxetan-3-ylimino)thiazolidin-4-one 7a
A solution of oxetan-3-amine (0.98 g, 13 mmol) in dry DCM (25 ml) under nitrogen had 1-isothiocyanato-2-methyl-benzene (2.0 g, 13 mmol) added over 15 minutes. Stirred at room temperature for 1 hour. Cooled to 0 °C then pyridine (2.1 g, 27 mmol) was added in a single portion. After stirring for 5 minutes, 2-bromoacetyl bromide (2.7 g, 13 mmol) was added dropwise over 15 minutes at 0 °C. Warmed to room temperature and stirred for 2 hours. 10 ml of water was added and passed through a phase separator. Concentrated to dryness and purified by column (25-100% ethyl acetate in heptane) to give (2Z)-3-(o-tolyl)-2-(oxetan-3-ylimino)thiazolidin-4-one as an orange oil that solidified on standing for 48 hours (1.4 g, 34% yield).

¹H NMR (400 MHz, CDCl₃) δ: 7.39 - 7.33 (m, 3H), 7.14 (d, J=7.5 Hz, 1H), 4.92 - 4.85 (m, 2H), 4.67 - 4.58 (m, 3H), 4.04 (s, 2H), 2.20 (s, 3H). HPLC tₘ (Agilent, acidic, 3.5 minutes): 1.16 minutes, 100%; Calculated for C₁₃H₁₄N₂O₂S 262.1, found MS: m/z 263.1 [M+H]⁺.

(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(o-tolyl)-2-(oxetan-3-ylimino)thiazolidin-4-one 9a

A solution of (2Z)-3-(o-tolyl)-2-(oxetan-3-ylimino)thiazolidin-4-one (300 mg, 1.1 mmol), 3-chloro-4-hydroxy-benzaldehyde (188 mg, 1.2 mmol) and sodium acetate (187 mg, 2.3 mmol) was heated at 100 °C for 24 hours. Purified by column using 25-100% ethyl acetate in heptane to give 150 mg of a crude yellow solid containing two compounds by NMR. Further purified by mass directed autoprep (5-95% ACN gradient over 15 minutes, 0.1% ammonia) to give (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(o-tolyl)-2-(oxetan-3-ylimino)thiazolidin-4-one as a yellow solid (30 mg; 6% yield).

¹H NMR (400 MHz, CDCl₃) δ: 10.02 (bs, 1H), 7.71 (s, 1H), 7.56 (d, J=2.1 Hz, 1H), 7.45 - 7.35 (m, 4H), 7.21 (d, J=7.6 Hz, 1H), 7.16 (d, J=8.4 Hz, 1H), 4.99 - 4.92 (m, 2H), 4.78 - 4.65 (m, 3H), 2.22 (s, 3H). HPLC tₘ (BrukerN, acidic, 7.5 minutes): 3.50 minutes, 100%; Calculated for C₂₀H₁₇ClN₂O₂S 400.1, found MS: m/z 401.1 [M+H]⁺. HRMS: Calculated for C₂₀H₁₇ClN₂O₂S 400.0721, found 401.0744 [M+H]⁺.

(2Z)-2-(3,3-difluorocyclobutyl)imino-3-(o-tolyl)thiazolidin-4-one 7b
DCM (12 ml) was added to (3,3-difluorocyclobutyl)ammonium chloride (1.0 g, 7.0 mmol) under nitrogen. TEA (0.9 g, 7.0 mmol) was added at room temperature, followed by addition of 1-isothiocyanato-2-methyl-benzene (1040 mg, 7.0 mmol) dropwise. Reaction was stirred at room temperature for 1 hour. Cooled in ice-bath and 2-bromoacetyl bromide (1.4 g, 7.0 mmol) added dropwise over 15 minutes. Stirred at 0 °C for 15 minutes, then pyridine (1.1 g, 14 mmol) added dropwise over 15 minutes. The resultant suspension was stirred at 0 °C for 1 hour and then warmed to room temperature for another hour. 10 ml water added and passed through phase separator. Concentrated and purified by column (0-50% ethyl acetate in heptane) to give (2Z)-2-(3,3-difluorocyclobutyl)imino-3-(o-tolyl)thiazolidin-4-one (1.5 g, 66% yield) as a yellow oil. The undesired regioisomer (2Z)-3-(3,3-difluorocyclobutyl)-2-(o-tolylimino)thiazolidin-4-one (350 mg, 18% yield) was also isolated from this column.

\[ ^1H \text{NMR (400 MHz, CDCl}_3\text{): } \delta: 7.39 - 7.30 (m, 3H), 7.12 (d, J=7.8 Hz, 1H), 4.04 (s, 2H), 4.01 - 3.79 (m, 1H), 3.00 - 2.87 (m, 2H), 2.66-2.41 (m, 2H), 2.17 (s, 3H). \]

HPLC \( t_R \) (Agilent, acidic, 7.5 minutes): 3.59 minutes, 100%; Calculated for C_{13}H_{14}N_{2}O_{2}S 296.1, found MS: m/z 297.1 [M+H]^+. HRMS: Calculated for C_{14}H_{14}F_{2}N_{2}OS 296.0868, found 297.0894 [M+H]^+.

(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-(3,3-difluorocyclobutyl)imino-3-(o-tolyl)thiazolidin-4-one 9b

(2Z)-2-(3,3-difluorocyclobutyl)imino-3-(o-tolyl)thiazolidin-4-one (200 mg, 0.67 mmol) was dissolved in ethyl acetate before the addition of 3-chloro-4-hydroxy-benzaldehyde (105 mg, 0.67 mmol) and sodium acetate (132 mg, 1.61 mmol). The resulting solution was heated to 85 °C and allowed to stir for 16 hours. The reaction mixture was allowed to cool to room temperature, then poured into water and extracted with ethyl acetate. The combined organic extracts were washed with sat. NaHCO\textsubscript{3}, dried over magnesium sulphate and concentrated under vacuum. The crude material was triturated with DCM and the solid was collected by filtration to afford (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-(3,3-difluorocyclobutyl)imino-3-(o-tolyl)thiazolidin-4-one as a yellow solid (256 mg; 83% yield).

\[ ^1H \text{NMR (500 MHz, DMSO-d}_6\text{): } \delta: 11.11 - 11.01 (m, 1H), 7.74 (d, J=2.1 Hz, 1H), 7.71 (s, 1H), 7.55 (dd, J=2.2, 8.6 Hz, 1H), 7.40 - 7.38 (m, 2H), 7.37 - 7.29 (m, 2H), 7.16 (d, J=8.4 Hz, 1H), 4.00 - 3.94 (m, 1H), \]
3.14 - 3.02 (m, 2H), 2.53 - 2.50 (m, 2H), 2.09 (s, 3H). HPLC t_R (Agilent, acidic, 3.5 minutes): 1.86 minutes, 100%; Calculated for C_{21}H_{17}N_{2}F_{2}O_{2}S 434.1, found MS: m/z 435.0 [M+H]^+.

(2Z)-2-(2-methoxyethylimino)-3-(o-tolyl)thiazolidin-4-one 7c

1-isothiocyanato-2-methyl-benzene (2.0 g, 13 mmol) in DCM (40 ml) under nitrogen had dropwise addition of 2-methoxyethanamine (1.0 g, 13 mmol) over 5 minutes. Stirred at room temperature for 30 minutes. Cooled in ice-bath and 2-bromoacetyl bromide (2.7 g, 13 mmol) added dropwise over 15 minutes. Stirred at 0 °C for 15 minutes, then pyridine (2.1 g, 27 mmol) was added dropwise over 10 minutes. The resultant suspension was stirred at 0 °C for 1 hour, then warmed to room temperature for another hour. 10 ml water added and passed through phase separator. Concentrated and purified by column (0-50% ethyl acetate in heptane) to give (2Z)-2-(2-methoxyethylimino)-3-(o-tolyl)thiazolidin-4-one (3.1 g, 80% yield) as a yellow oil. The undesired regioisomer (2Z)-3-(2-methoxyethyl)-2-(o-tolylimino)thiazolidin-4-one (0.45 g, 11% yield) was also isolated from this column.

^1H NMR (400 MHz, CDCl_3) δ: 7.39-7.29 (m, 3H), 7.15 (d, J=7.2 Hz, 1H), 4.03 (s, 2H), 3.55 - 3.49 (m, 4H), 3.35 (s, 3H), 2.20 (s, 3H). HPLC t_R (Agilent, acidic, 7.5 minutes): 2.58 minutes, 100%; Calculated for C_{13}H_{17}N_{2}O_{2}S 264.1, found MS: m/z 265.1 [M+H]^+.

(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-(2-methoxyethylimino)-3-(o-tolyl)thiazolidin-4-one 9c

(2Z)-2-(2-methoxyethylimino)-3-(o-tolyl)thiazolidin-4-one (198 mg, 0.75 mmol) was dissolved in ethyl acetate before the addition of 3-chloro-4-hydroxy-benzaldehyde (117 mg, 0.75 mmol) and sodium acetate (132 mg, 1.61 mmol). The resulting solution was heated to 85 °C and allowed to stir for 16 hours. The reaction mixture was allowed to cool to room temperature, then poured into water and extracted with ethyl acetate. The combined organic extracts were washed with saturated NaHCO_3, dried over magnesium sulphate and concentrated under vacuum. DCM was added to the crude reaction mixture. The resulting precipitate was collected by filtration and dried under vacuum to afford (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-(2-methoxyethylimino)-3-(o-tolyl)thiazolidin-4-one (56 mg, 17% yield) as a yellow solid.
¹H NMR (500 MHz, DMSO-d₆) δ: 11.07 - 11.04 (m, 1H), 7.72 (d, J=2.1 Hz, 1H), 7.68 (s, 1H), 7.53 (dd, J=2.1, 8.6 Hz, 1H), 7.40 - 7.37 (m, 2H), 7.36 - 7.30 (m, 1H), 7.27 (d, J=7.5 Hz, 1H), 7.16 (d, J=8.5 Hz, 1H), 3.53 - 3.43 (m, 4H), 3.22 (s, 3H), 2.09 (s, 3H). HPLC tᵣ (Agilent, acidic, 7.5 minutes): 3.75 minutes, 100%; Calculated for C₂₀H₁₉ClN₂O₃ 402.1, found MS: m/z 403.1 [M+H]⁺.

(2Z)-2-(3-fluoropropylimino)-3-(o-tolyl)thiazolidin-4-one 7d

DCM (5 ml) was added to 3-fluoropropylammonium chloride (250 mg, 2.2 mmol) under nitrogen. TEA (284 mg, 2.2 mmol) was added at room temperature, followed by addition of 1-isothiocyanato-2-methyl-benzene (328 mg, 2.2 mmol) dropwise. The reaction was stirred at room temperature for 0.5 hour. Cooled in ice-bath and 2-bromoacetyl bromide (444 mg, 2.2 mmol) was added dropwise over 15 minutes. Stirred at 0 °C for 15 minutes, then pyridine (348 mg, 4.4 mmol) was added dropwise over 15 minutes. The resultant suspension was stirred at 0 °C for 1 hour, then warmed to room temperature for another hour. 5 ml water added and passed through phase separator. Concentrated and purified by column (0-50% ethyl acetate in heptane) to give (2Z)-2-(3-fluoropropylimino)-3-(o-tolyl)thiazolidin-4-one (500 mg, 78% yield) as a yellow oil which solidified on standing at room temperature.

¹H NMR (400 MHz, CDCl₃) δ: 7.35 (s, 2H), 7.34 - 7.29 (m, 1H), 7.13 (d, J=7.2 Hz, 1H), 4.44 (td, J=5.8, 47.2 Hz, 2H), 4.04 (s, 2H), 3.42 (td, J=3.6, 7.3 Hz, 2H), 2.19 (s, 3H), 2.02 - 1.88 (m, 2H). HPLC tᵣ (Agilent, acidic, 3.5 minutes): 1.27 minutes, 100%; Calculated for C₁₃H₁₆FN₂OS 266.1, found MS: m/z 267.10 [M+H]⁺. HRMS: Calculated for C₁₃H₁₆FN₂OS 266.0962, found 267.0973 [M+H]⁺.

(2Z,5Z)-5-[[3-chloro-4-hydroxy-phenyl]methylene]-2-(3-fluoropropylimino)-3-(o-tolyl)thiazolidin-4-one 9d

(2Z)-2-(3-fluoropropylimino)-3-(o-tolyl)thiazolidin-4-one (200 mg, 0.75 mmol) was dissolved in ethyl acetate before the addition of 3-chloro-4-hydroxy-benzaldehyde (117 mg, 0.75 mmol) and sodium acetate (132 mg, 1.6 mmol). The resulting solution was heated to 85 °C and allowed to stir for 16 hours. The reaction mixture was allowed to cool to room temperature, then poured into water and extracted with ethyl acetate. The combined organic extracts were washed with sat. NaHCO₃, dried
over magnesium sulphate and concentrated under vacuum. DCM was added to the crude material. The resulting precipitate was collected by filtration and dried under vacuum to afford (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-(3-fluoropropylimino)-3-(o-tolyl)thiazolidin-4-one (96 mg, 30% yield) as a yellow solid. 

¹H NMR (500 MHz, DMSO-d₆) δ: 11.56 (br s, 1H), 7.70 (d, J=1.8 Hz, 1H), 7.68 (s, 1H), 7.51 (dd, J=1.5, 8.4 Hz, 1H), 7.40 - 7.36 (m, 2H), 7.36 - 7.27 (m, 2H), 7.12 (d, J=8.5 Hz, 1H), 4.42 (dt, J=5.8, 47.5 Hz, 2H), 3.44 (td, J=3.3, 6.6 Hz, 2H), 2.09 (s, 3H), 1.94 - 1.82 (m, 2H). HPLC tₚ (Agilent, acidic, 7.5 minutes): 3.94 minutes, 100%; Calculated for C₂₀H₁₈ClFN₂O₂S 404.1, found MS: m/z 405.1 [M+H⁺].

(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-(2-methoxyethylimino)-3-(o-tolyl)thiazolidin-4-one 9e

A solution of (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-(2-methoxyethylimino)-3-(o-tolyl)thiazolidin-4-one (40 mg, 0.1 mmol) in DCM (1 ml) was cooled to -78 °C. 0.5 ml of a 1 M solution of tribromoborane (0.5 mmol) in DCM was added and stirred at -78 °C for three hours. The reaction was warmed to 0 °C and the reaction continued for 3 hours. Poured onto 50 ml ice-water and diluted with 50 ml DCM. The pH was adjusted from 1 to 7 with solid NaHCO₃. Extracted with 2 x 50 ml DCM. Combined organics dried over sodium sulphate, filtered and concentrated and purified by column (25-100% ethyl acetate in heptane) to give (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-(2-methoxyethylimino)-3-(o-tolyl)thiazolidin-4-one (40 mg, 35%) as a light yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ: 11.01 (s, 1H), 7.70 (d, J=2.2 Hz, 1H), 7.66 (s, 1H), 7.52 (dd, J=2.1, 8.6 Hz, 1H), 7.40-7.21 (m, 4H), 7.15 (d, J=8.5 Hz, 1H), 4.65 (t, J=5.4, 1H), 3.54 - 3.37 (m, 4H), 2.08 (s, 3H), HPLC tₚ (BrukerN, acidic, 7.5 min): 3.10 minutes, 95%; Calculated for C₁₉H₁₈ClN₂O₃S 388.1, found MS: m/z 389.1 [M+H⁺]. HRMS: Calculated for C₁₉H₁₈ClN₂O₃S 388.0722, found 389.0705 [M+H⁺].

(2Z,5Z)-5-[(3-bromo-4-hydroxy-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one 9f

A mixture of (2Z,5Z)-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (200 mg; 0.8 mmol), 4-hydroxy-3-bromo-benzaldehyde (163 mg; 0.8 mmol) and sodium acetate (132 mg; 1.6 mmol in acetic acid (2 ml) were heated overnight at 60 °C. The mixture was concentrated to dryness. Purified by column (0-20% ethyl acetate in heptane) to give (2Z,5Z)-5-[(3-bromo-4-hydroxy-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (150 mg, 37% yield) as a pale yellow solid.
\( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \): 7.73 (d, J=2.1 Hz, 1H), 7.68 (s, 1H), 7.49 (dd, J=2.1, 8.6 Hz, 1H), 7.38 - 7.33 (m, 3H), 7.21 (d, J=7.1 Hz, 1H), 7.13 (d, J=8.4 Hz, 1H), 5.93 (s, 1H), 3.46 - 3.36 (m, 2H), 2.21 (s, 3H), 1.71-1.59 (2H, m), 0.94 (t, J=7.4 Hz, 3H). HPLC \( t_R \) (BrukerN, acidic, 7.5 minutes): 4.20 minutes, 100%; Calculated for C\(_{20}\)H\(_{20}\)BrN\(_2\)O\(_2\)S 388.1, found 430.0 MS: m/z 431.0 [M+H]. HRMS: Calculated for C\(_{20}\)H\(_{20}\)BrN\(_2\)O\(_2\)S 430.0423, found 431.0436.

(2Z,5Z)-5-[[4-hydroxy-3-(trifluoromethyl)phenyl]methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one 9g

![Chemical Structure of 9g](image)

A solution of (2Z)-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (85 mg, 0.34 mmol), 4-hydroxy-3-(trifluoromethyl)benzaldehyde (65 mg, 0.34 mmol) and sodium acetate (56 mg, 0.68 mmol) in Acetic acid (1 ml) was heated to 60 °C overnight. Temperature was increased to 80 °C for 4 hours. Diluted with 5 ml water. 20 ml DCM added and passed through phase separator. Solvent removed and mixture purified by column (10-20% ethyl acetate in heptane) to give a yellow solid. Suspended in 10 ml 1:1 DCM:heptane and pale yellow solid isolated by filtration to give (2Z,5Z)-5-[[4-hydroxy-3-(trifluoromethyl)phenyl]methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (65 mg, 41% yield).

\( ^1H \) NMR (400 MHz, DMSO-d\(_6\)) \( \delta \): 11.38 - 11.38 (m, 1H), 7.89 (d, J=1.9 Hz, 1H), 7.81 (dd, J=2.0, 8.7 Hz, 1H), 7.77 (s, 1H), 7.40 - 7.37 (m, 2H), 7.36 - 7.26 (m, 2H), 7.22 (d, J=8.5 Hz, 1H), 3.38-3.23 (m, 2H), 2.09 (s, 3H), 1.56 - 1.47 (m, 2H), 0.84 (t, J=7.4 Hz, 3H). HPLC \( t_R \) (Agilent, acidic, 7.5 minutes): 4.27 minutes, 90%; Calculated for C\(_{21}\)H\(_{20}\)F\(_3\)N\(_2\)O\(_2\)S 420.1, found MS: m/z 421.1 [M+H]. HRMS: Calculated for C\(_{21}\)H\(_{20}\)F\(_3\)N\(_2\)O\(_2\)S 420.1192, found 421.1193.

2-hydroxy-5-[[2Z]-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]benzonitrile 9h

![Chemical Structure of 9h](image)

A suspension of (2Z,5Z)-5-[[3-bromo-4-hydroxy-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (45 mg, 0.10 mmol), dicyanozinc (12 mg, 0.10 mmol) in DMA (0.5 ml) had nitrogen gas bubbled through for 10 minutes. Tetrakis(triphenylphosphine)palladium(0) (24 mg, 0.02 mmol) was then added and the tube sealed and heated in a microwave to 100 °C for 1.5 hours. Additional dicyanozinc (36 mg, 0.30 mmol) was added and the reaction heated at 100 °C for 12 hours. Diluted with 5 ml water. 20 ml DCM added and passed through phase separator. Solvent removed and mixture...
purified by column to give 2-hydroxy-5-[(Z)-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-
ylidene]methyl]benzonitrile as a pale yellow solid (3 mg; 7% yield).

¹H NMR (400 MHz, DMSO-d₆) δ: 11.93 (s, 1H), 8.02 (d, J=2.4 Hz, 1H), 7.90 (dd, J=2.3, 8.9 Hz, 1H), 7.76 (s, 1H), 7.45 - 7.43 (m, 2H), 7.41 - 7.31 (m, 2H), 7.26 (d, J=8.8 Hz, 1H), 3.49-3.25 (2H, m), 2.15 (s, 3H), 1.61 - 1.54 (m, 2H) 0.90 (t, J=7.4 Hz, 3H). HPLC tR (Agilent, acidic, 7.5 minutes): 3.92 minutes, 100%; Calculated for C₂₁H₁₉N₃O₂S 377.1, found MS: m/z = 378.1 (100%) [M+H]+.

(2Z,5Z)-5-[(5-hydroxy-2-pyridyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one 9i

5-hydroxypyridine-2-carbaldehyde (50 mg, 0.4 mmol), sodium acetate (66 mg, 0.8 mmol) and (2Z)-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (100 mg, 0.4 mmol) in acetic acid (2ml) was heated to 60 °C overnight. Concentrated to dryness then diluted with 5 ml water. 20 ml DCM added and passed through phase separator. Purified by column (10-50% EtOAc in heptane) to give (2Z,5Z)-5-[(5-hydroxy-2-pyridyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one as a yellow solid (65 mg, 43% yield).

¹H NMR (400 MHz, DMSO-d₆) δ: 11.53 (s, 1H), 8.38 (d, J=2.8 Hz, 1H), 7.73 - 7.70 (m, 2H), 7.38 - 7.22 (m, 5H), 3.35 - 3.29 (m, 2H), 2.08 (s, 3H), 1.55 - 1.46 (m, 2H), 0.84 (t, J=7.3 Hz, 3H). HPLC tR (BrukerN, acidic, 7.5min): 3.40 minutes, 100%; Calculated for C₁₉H₁₉N₂O₂S 353.1, found MS: m/z 354.1 [M+H]+. HRMS: Calculated for C₁₉H₁₉N₂O₂S 353.1271; found 354.1291 [M+H]+.

(2Z,5Z)-5-[(4-hydroxyphenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one 9j

A mixture of (2Z)-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (100 mg, 0.4 mmol), 4-hydroxybenzaldehyde (50 mg, 0.4 mmol) and sodium acetate (66 mg, 0.8 mmol in acetic acid (1 ml) were heated overnight at 60 °C. The mixture was concentrated under vacuum to by approximately 50% then suspended in water and the solid isolated by filtration. Purified by column (0-20% ethyl acetate in heptane) to give (2Z,5Z)-5-[(4-hydroxyphenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one as a pale yellow solid (80 mg; 54% yield).

¹H NMR (400 MHz, CDCl₃) δ: 7.77 (s, 1H), 7.48 (d, J=8.5 Hz, 2H), 7.34 - 7.29 (m, 3H), 7.24 - 7.18 (m, 1H), 6.86 (d, J=8.7 Hz, 2H), 6.01 (s, 1H), 3.46 - 3.36 (m, 2H), 2.22 (s, 3H), 1.71 - 1.58 (m, 2H), 0.94 (t, J=7.4 Hz, 3H). HPLC tR (BrukerN, acidic, 7.5 minutes): 5.30 minutes, 100%; Calculated for C₂₀H₂₀N₂O₂S 352.1, found MS: m/z 353.1 [M+H]⁺. HRMS: Calculated for C₂₀H₂₀N₂O₂S 352.1318; found 353.1328 [M+H]⁺.
(2Z,5Z)-5-[(4-hydroxy-3-methyl-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one 9k

A mixture of (2Z)-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (1 g, 4 mmol), 4-hydroxy-3-methyl-benzaldehyde (0.55 g, 4 mmol) and sodium acetate (0.66 g, 8 mmol in acetic acid (10 ml)) were heated overnight at 60 °C. The mixture was concentrated under vacuum by approximately 50% then suspended in water and the solid isolated by filtration. Purified by column (0-20% ethyl acetate in heptane) to give (2Z,5Z)-5-[(4-hydroxy-3-methyl-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one as a pale yellow solid (0.65 g; 44% yield).

¹H NMR (500 MHz, DMSO-d₆) δ: 10.16 (s, 1H), 7.61 (s, 1H), 7.41 (s, 1H), 7.39-7.34 (m, 3H), 7.29-7.32 (m, 1H), 7.24 (d, J=7.7 Hz, 1H), 6.99-6.94 (d, J=7.7 Hz, 1H), 3.34-3.24 (m, 2H), 2.18 (s, 3H), 2.05 (s, 3H), 1.56-1.46 (m, 2H), 0.82 (t, J=7.3 Hz, 3H).

¹³C NMR (125 MHz, DMSO-d₆) δ: 165.57, 157.70, 147.08, 135.84, 134.85, 133.10, 130.60, 130.12, 129.48, 129.10, 128.96, 126.79, 125.08, 124.32, 116.44, 115.38, 54.20, 23.40, 17.09, 15.98, 11.69. HPLC tᵣ (Agilent, acidic, 7.5 minutes): 4.12 minutes, 100%; HRMS: Calculated for C₂₁H₂₂N₂O₂S 366.1475, found 367.1476 [M+H]+.

9k ¹H NMR (500 MHz, DMSO-d₆)
9k $^{13}$C NMR (125 MHz, DMSO-d$_6$)

$^{1}$H-$^{13}$C coupling 6.3 Hz

9k HMBC (DMSO-d$_6$): $^{1}$H-$^{13}$C coupling of H$^{3}$-C$^{3}$ (6.3 Hz)
A solution of 5-hydroxypyridine-2-carbaldehyde (50 mg, 0.4 mmol), 4-hydroxy-3-isopropylbenzaldehyde (66 mg, 0.4 mmol) and (2Z,5Z)-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (100 mg, 0.4 mmol) in acetic acid (2 mL) was heated to 60 °C for 72 hours. Concentrated to dryness then diluted with 5 ml of water. 20 ml of DCM was added and the mixture passed through a phase separator. The mixture was concentrated and purified by column (10-50% ethyl acetate in heptane) to give an impure yellow oil. Further purified by mass directed autoprep (20-95% ACN gradient over 15 minutes, 0.1% formic acid) to give (2Z,5Z)-5-[(4-hydroxy-3-isopropyl-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one as a yellow solid (40 mg, 24% yield).

¹H NMR (400 MHz, DMSO-d₆) δ: 10.20 - 10.19 (m, 1H), 7.68 (s, 1H), 7.48 (d, J=2.2 Hz, 1H), 7.40 - 7.37 (m, 3H), 7.36 - 7.30 (m, 1H), 7.26 (d, J=7.4 Hz, 1H), 6.97 (d, J=8.3 Hz, 1H), 3.35 - 3.21 (m, 3H), 2.09 (s, 3H), 1.55 - 1.48 (m, 2H), 1.21 (d, J=6.9 Hz, 6H), 0.84 (t, J=7.4 Hz, 3H). HPLC tᵣ (BrukerN, acidic, 7.5 minutes): 5.00 minutes, 100%; Calculated for C₂₃H₂₆N₂O₂S 394.2, found MS: m/z 395.2 [M+H]^+. HRMS: Calculated for C₂₃H₂₆N₂O₂S 394.1788; found 395.1800 [M+H]^+. 
(2Z,5Z)-5-[(4-hydroxy-3-methyl-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (100 mg, 0.27 mmol) was suspended in DCM (2 ml) and DMAP (2 mg, 0.02 mmol) added, followed by dropwise addition of Et$_3$N (33 mg, 0.33 mmol), whereupon a homogeneous yellow solution was obtained. The acetyl chloride (24 mg, 0.30 mmol) was added dropwise (some fuming observed). The resulting amber solution was stirred overnight. The mixture was evaporated and purified on silica, eluting with 100:0 to 70:30 heptane/ethyl acetate to give [2-methyl-4-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] acetate (74 mg, 65%) as a white solid.

$^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$: 7.72 (s, 1H), 7.61 (s, 1H), 7.56-7.55 (d, J=7.2 Hz, 1H), 7.39-7.37 (m, 2H), 7.33-7.30 (m, 1H), 7.25 (t, J=8.5 Hz, 2H), 3.36-3.25 (m, 2H), 2.33 (s, 3H), 2.19 (s, 3H), 2.09 (s, 3H), 1.54-1.48 (m, 2H), 0.83 (t, J=7.5 Hz, 3H) $^{13}$C NMR (125 MHz, DMSO-d$_6$) $\delta$: 168.8, 165.2, 150.1, 146.6, 135.8, 134.7, 132.8, 131.3, 131.1, 130.6, 129.1, 129.0, 128.7, 128.4, 126.9, 123.2, 121.1, 54.3, 23.4, 20.6, 17.1, 15.8, 11.7. HPLC $t_R$ (Agilent, acidic, 7.5 minutes): 4.47 minutes, 100%; Calculated for C$_{23}$H$_{24}$N$_2$O$_3$S 408.2, found MS: $m/z$ 409.2 [M+H]$^+$. 

[2-methyl-4-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] acetate 10a
**10a** $^1$H NMR (500 MHz, DMSO-d$_6$)

![1H NMR spectrum of 10a](image)

**10a** $^{13}$C NMR (125 MHz, DMSO-d$_6$)

![13C NMR spectrum of 10a](image)

**10a** HMBC (DMSO-d$_6$): $^1$H-$^{13}$C coupling of H$^9$-C$^3$ (6.3 Hz)

![HMBC spectrum of 10a](image)
10a NOESY (DMSO-d$_6$)

[2-bromo-4-[(Z)-(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] acetate

10b

1H NMR (400 MHz, DMSO-d$_6$) $\delta$: 8.07 (d, J=2.1 Hz, 1H), 7.80 - 7.75 (m, 2H), 7.51 (d, J=8.4 Hz, 1H), 7.41 - 7.39 (m, 2H), 7.38 - 7.30 (m, 2H), 2.53 - 2.51 (m, 2H), 2.39 (s, 3H), 2.11 (s, 3H), 1.57 - 1.49 (m, 2H), 0.85 (t, J=7.4 Hz, 3H). HPLC t$_R$ (Agilent, acidic, 3.1 minutes): 2.06 minutes, 100%. Calculated for C$_{22}$H$_{21}$BrN$_2$O$_3$S 472.0, found MS: m/z 473.1 [M+H]$^+$. 
Decomposition study when stored in DMSO-d$_6$ solution for 28 days. Hydrolysis monitored by following acetic acid CH$_3$ peak at 1.91 ppm. 6.3% reduction at day 28.

Blue = day 1. Red = day 7. Green = day 14. Purple = day 21. Orange = day 28.

[2-trifluoromethyl-4-[[Z]-[[2Z]-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] acetate 10c

(2Z,5Z)-5-[[4-hydroxy-3-(trifluoromethyl)-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (50 mg, 0.12 mmol) was suspended in DCM (1.5 ml) and DMAP (0.7 mg, 0.006 mmol) added, followed by dropwise addition of Et$_3$N (14 mg, 0.14 mmol), whereupon a homogeneous yellow solution was obtained. The acetyl chloride (10 mg, 0.13 mmol) was added dropwise (some fuming observed). The resulting amber solution was stirred overnight. The mixture was evaporated and purified on silica, eluting with 100:0 to 70:30 heptane/ethyl acetate to give [2-trifluoromethyl-4-[[Z]-[[2Z]-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] acetate (16 mg; 29%) as a white solid.
¹H NMR (400 MHz, DMSO-d₆) δ: 8.15 (d, J=2.0 Hz, 1H), 8.05 (dd, J=2.2, 8.6 Hz, 1H), 7.91 (s, 1H), 7.66 (d, J=8.5 Hz, 1H), 7.41 - 7.31 (m, 4H), 2.53 - 2.51 (m, 2H), 2.38 (s, 3H), 2.12 (s, 3H), 1.57 - 1.49 (m, 2H), 0.85 (t, J=7.3 Hz, 3H). HPLC tᵣ (Agilent, acidic, 3.1 min): 2.09, 100%; Calculated for C₂₃H₂₁F₃N₂O₃S 462.1, found MS: m/z 463.2 [M+H]+.

Decomposition study when stored in DMSO solution for 28 days. Hydrolysis monitored by following acetic acid CH₃ peak at 1.91 ppm. 1.4% reduction at day 28.

[2-methyl-4-[(Z)-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl]-3-hydroxypropanoate 10f

[2-methyl-4-[(Z)-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] 3-methoxypropanoate (53 mg, 0.12 mmol) was dissolved in CHCl₃ (2 ml) under nitrogen and TMSI (99 mg, 0.50 mmol) added dropwise. The resulting yellow solution was heated at 60 ºC overnight. A further 0.07 ml of TMSI was added and heating continued for a further 3 hours. The mixture was cooled and washed with water. The aqueous phase was extracted with DCM and the combined organics evaporated. Purified on silica, eluting with 100:0 to 50:50 heptane/ethyl acetate to give [2-methyl-4-
[(Z)-(Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl[phenyl]-3-hydroxypropanoate (24 mg, 45%) as a white solid.

^1^H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\): 7.76 (s, 1H), 7.50 - 7.46 (m, 2H), 7.39 - 7.34 (m, 3H), 7.24 - 7.17 (m, 2H), 4.09 - 4.01 (m, 2H), 3.45 - 3.34 (m, 2H), 2.92 (t, \(J=5.6\) Hz, 2H), 2.30 (s, 3H), 2.22 (s, 3H), 1.67 - 1.57 (m, 2H), 0.94 (t, \(J=7.3\) Hz, 3H). HPLC \(t_R\) (BrukerS, basic, 7.5min): 4.40, 100%; Calculated for C\textsubscript{24}H\textsubscript{26}N\textsubscript{2}O\textsubscript{4}S\textsubscript{4} 438.2, found MS: \(m/z\) 439.2 [M+H]^+.

[2-methyl-4-[(Z)-(Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] 3-methoxypropanoate 10g

![Chemical structure](image)

(2Z,5Z)-5-[(4-hydroxy-3-methyl-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (200 mg, 0.54 mmol) was suspended in DCM (4 ml) and DMAP (4 mg, 0.04 mmol) added, followed by dropwise addition of Et\textsubscript{3}N (66 mg, 0.66 mmol), whereupon a homogeneous yellow solution was obtained. The 3-methoxypropanoyl chloride (74 mg, 0.60 mmol) was added dropwise (some fuming observed). The resulting amber solution was stirred overnight. The mixture was evaporated and purified on silica, eluting with 100:0 to 70:30 heptane/ethyl acetate to give [2-methyl-4-[(Z)-(Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] 3-methoxypropanoate (186 mg, 75%) as a white solid.

^1^H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\): 7.76 (s, 1H), 7.49 - 7.45 (m, 2H), 7.39 - 7.34 (m, 3H), 7.23 - 7.17 (m, 2H), 3.84 (t, \(J=6.2\) Hz, 2H), 3.45 (s, 3H), 3.44 - 3.34 (m, 2H), 2.90 (t, \(J=6.2\) Hz, 2H), 2.30 (s, 3H), 2.22 (s, 3H), 1.67 - 1.61 (m, 2H), 0.94 (t, \(J=7.3\) Hz, 3H). HPLC \(t_R\) (Agilent, acidic, 7.5 minutes): 5.22 minutes, 100%; Calculated for C\textsubscript{25}H\textsubscript{28}N\textsubscript{2}O\textsubscript{4}S 452.2, found MS: \(m/z\) 453.1 [M+H]^+.

[2-methyl-4-[(Z)-(Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] 2-methylpropanoate 10h

![Chemical structure](image)

(2Z,5Z)-5-[(4-hydroxy-3-methyl-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (100 mg, 0.27 mmol) was suspended in DCM (2 ml) and DMAP (2 mg, 0.02 mmol) added, followed by dropwise addition of Et\textsubscript{3}N (33 mg, 0.33 mmol), whereupon a homogeneous yellow solution was obtained. The 2-methylpropanoyl chloride (32 mg, 0.30 mmol) was added dropwise (some fuming observed). The resulting amber solution was stirred overnight. The mixture was evaporated and purified on silica, eluting with 100:0 to 70:30 heptane/ethyl acetate to give [2-methyl-4-[(Z)-(Z)-3-
(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene][methyl][phenyl] 2-methylpropanoate (96 mg, 78%) as a colourless gum.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.76 (s, 1H), 7.47 (dd, $J$=2.3, 4.3 Hz, 2H), 7.38 - 7.34 (m, 3H), 7.22 (d, $J$=7.3 Hz, 1H), 7.15 (d, $J$=9.0 Hz, 1H), 3.46 - 3.34 (m, 2H), 2.94 - 2.85 (m, 1H), 2.28 (s, 3H), 2.22 (s, 3H), 1.67 - 1.61 (m, 2H), 1.39 (d, $J$=7.0 Hz, 6H), 0.94 (t, $J$=7.4 Hz, 3H). HPLC $t_R$ (Agilent, acidic, 7.5 minutes): 4.87 minutes, 100%; Calculated for C$_{25}$H$_{28}$N$_2$O$_3$S 436.2, found MS: m/z 437.2 [M+H]$^+$. 

[2-methyl-4-[[Z]-[(Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene][methyl][phenyl]] 2,2-dimethylpropanoate 10i

A solution of (2Z,5Z)-5-[(4-hydroxy-3-methyl-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (50 mg, 0.14 mmol), DCC (34 mg, 0.17 mmol) and DMAP (3 mg, 0.02 mmol) in DMF (0.5 ml) had 2,2-dimethylpropanoic acid (90 mg, 0.88 mmol) added and stirred at 40 °C overnight. Filtered and purified by mass directed autoprep (50-95% ACN gradient over 15 minutes, 0.1% formic acid) to give [2-methyl-4-[[Z]-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene][methyl][phenyl]] 2,2-dimethylpropanoate (27 mg, 42% yield) as a white solid.

$^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 7.73 (s, 1H), 7.63 (s, 1H), 7.58 (d, $J$=8.3 Hz, 1H), 7.41 - 7.37 (m, 2H), 7.35 - 7.28 (m, 2H), 7.24 (d, $J$=8.4 Hz, 1H), 3.42-3.24 (m, 2H), 2.20 (s, 3H), 2.10 (s, 3H), 1.55 - 1.50 (m, 2H), 1.36 (s, 9H), 0.84 (t, $J$=7.2 Hz, 3H). HPLC $t_R$ (Agilent, acidic, 7.5 minutes): 5.19 minutes, 100%; Calculated for C$_{26}$H$_{31}$N$_2$O$_3$S 450.2, found MS: m/z 451.2 [M+H]$^+$. HRMS: Calculated for C$_{26}$H$_{31}$N$_2$O$_3$S 450.2050, found 451.2052 [M+H]$^+$. 

Characterisation of ponesimod 
(Z)-5-((Z)-3-chloro-4-((R)-2,3-dihydroxypropoxy)benzylidene)-2-(propylimino)-3-(o-tolyl)thiazolidin-4-one

Synthesised as shown in Bolli et al.\textsuperscript{12}

$^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$: 7.78 (d, $J$=2.0 Hz, 1H), 7.71 (s, 1H), 7.64 (dd, $J$=1.9, 8.6 Hz, 1H), 7.37-7.29 (m, 4H), 7.27 (d, $J$=7.6 Hz, 2H), 5.04 (d, $J$=5.1 Hz, 1H), 4.72 (t, $J$=5.6 Hz, 1H), 4.16-4.03 (m, 2H), 3.84 (m, 1H), 3.53-3.46 (m, 2H), 3.36-3.25 (m, 2H), 2.12 (s, 3H), 1.54-1.48 (m, 2H), 0.87 (t, $J$=7.2 Hz, 3H). $^{13}$C NMR (125 MHz, DMSO-d$_6$) $\delta$: 165.2, 155.2, 146.4, 135.8, 134.8, 134.7, 131.6, 130.6, 130.0, 129.1, 129.0,
128.1, 126.9, 128.8, 122.1, 119.7, 114.2, 70.5, 69.4, 62.2, 54.3, 23.4, 17.1, 11.7. HPLC tR (Agilent, acidic, 7.5min): 3.83, 100%; Calculated for C_{23}H_{25}ClN_{2}O_{4}S 460.1, found MS: m/z = 461.1 (100%) [M+H]^+.

**Ponesimod** ¹H NMR (500 MHz, DMSO-d$_6$)
Ponesimod $^{13}$C NMR (125 MHz, DMSO-$d_6$)

$^1$H-$^{13}$C coupling 6.4 Hz

Ponesimod HMBC (DMSO-$d_6$): $^1$H-$^{13}$C coupling of H$^9$-C$^3$ (6.4 Hz)
We conducted NMR studies of compound 9h after incubation in DMSO-d$_6$ for 6 months. HMBC experiments measuring the three bond coupling constant between H$_9$-C$_3$ and H$_9'$-C$_3'$ were analysed and confirmed that the double bond in the major component (68%) had a coupling constant of 6.4 Hz indicating a Z arrangement, while in the minor component (32%) the coupling was measured at 11.9 Hz indicating an E arrangement.
(Z,Z)-9h 68%, (Z,E)-9h 32% $^1$H NMR (500 MHz, DMSO-d$_6$)
(Z,Z)-9h 68%, (Z,E)-9h 32% HSQC (Blue) H MBC (Red) (DMSO-d$_6$): (Z,Z)-9h $^1$H-$^{13}$C coupling of H$_9$-C$_3$ (6.4 Hz), (Z,E)-9h $^1$H-$^{13}$C coupling of H$_9$'-C$_3$' (11.9 Hz).

(Z,Z)-9h 68%, (Z,E)-9h 32% HMBC (DMSO-d$_6$) expansion: (Z,Z)-9h $^1$H-$^{13}$C coupling of H$_9$-C$_3$ (6.4 Hz), (Z,E)-9h $^1$H-$^{13}$C coupling of H$_9$'-C$_3$' (11.9 Hz).
Proposed catalysed mechanism for tautomerisation of \((Z,Z)-9h\) to \((Z,E)-9h\)

We hypothesised that the isomerisation could occur as a result of resonance from a phenol oxanion lone pair onto the 5-position of the thiazolidin-4-one ring (step B), that is able to stabilise negative charges. The hypothetical resonance form has a single bond at the 5-position of the thiazolidin-4-one ring. This would allow rotation of the benzylic group (step C), which could then be in the E arrangement when the double bond is reformed in step D.