

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 acceptor 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 μ g/ml geneticin and 300 μ g/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 37°C/5% CO₂ for 90 minutes followed by 15 min at room temperature. 5 μ L 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 μ M, 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 (μ M).

All values reported correspond to n of 1.

pKa

Sirius T3 (Sirius Analytical Inc, UK) instrument has been used for pK_a determination of the compounds. The pK_a determination is based on acid-base titration and the protonation/deprotonation of the molecule is measured either by UV spectroscopy or potentiometrically. The pK_a 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 pK_a results for samples with chromophores whose UV absorbance changes as a function of pH. It typically requires 5 μ l 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 pK_a, and the calculated points are fitted to the measured curve by manipulating the pK_a. The pK_a that provides the best fit is taken to be the measured pK_a. 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 pK_a 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 μ M) 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 μ L) of the incubation mixture was removed and mixed with acetonitrile (100 μ L) 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 UPLCMSMS 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:

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

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 μ M) 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 μ M test compound, an aliquot of incubation solution was removed to acetonitrile containing internal standard (final concentration 0.5 μ M 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 μ L 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 μ M). Immediately, at time zero, then at 3, 6, 15, 30, 60, 120 and 180 minutes, an aliquot (50 μ L) of the incubation mixture was removed and mixed with acetonitrile (100 μ L) 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 ($T_{1/2}$) of each test compound was determined using the following equation:

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

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 cm^2 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	
2h-post application	< 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 = 1 ng/mL

Scaled LLoQ = dilution factor * LLoQ = $10 \times 1 = 10$ ng/mL

HLoQ= 1000 ng/mL

Skin concentrations for (Z,Z)-**10a**

	Time (h)	skin weight mg	solvent volume μL	Total Homog Vol (mL)	scaled measured conc ng/mL	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	823.5	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	2.0
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 = $10 \times 2 = 20$ 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

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 = 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 uL	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	49
Mouse2	2	91.5	823.5	0.915	6174	61.7	
Mouse3	2	65.7	591.3	0.657	7941.5	79.4	
Mouse4	8	111.9	1007.1	1.119	2581.5	25.8	37
Mouse5	8	77.26	695.34	0.773	3289.5	32.9	
Mouse6	8	100.44	903.96	1.004	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 MetabolyNXS 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.

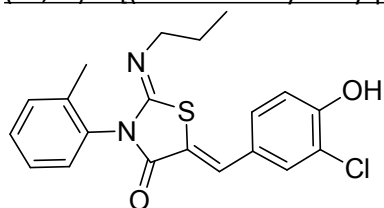
Chemistry Experimental

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 prepacked 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.*¹²

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

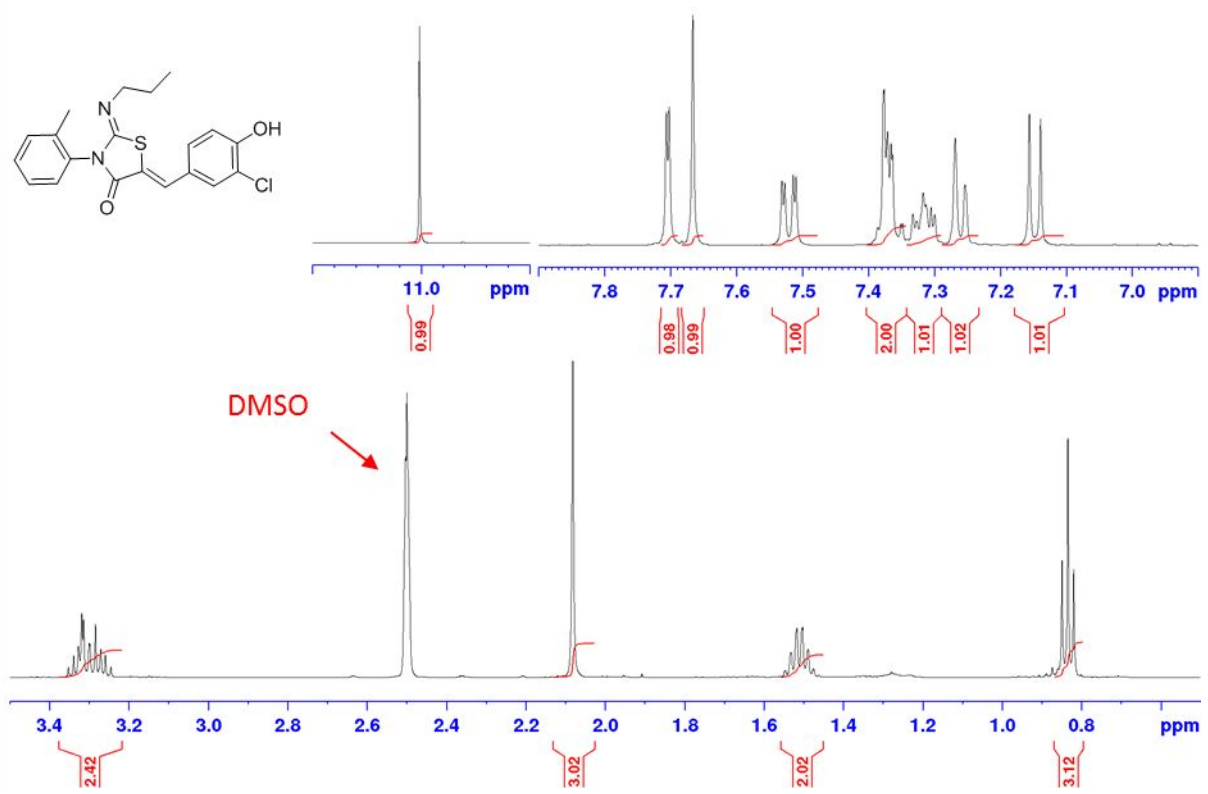
(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one **4a**



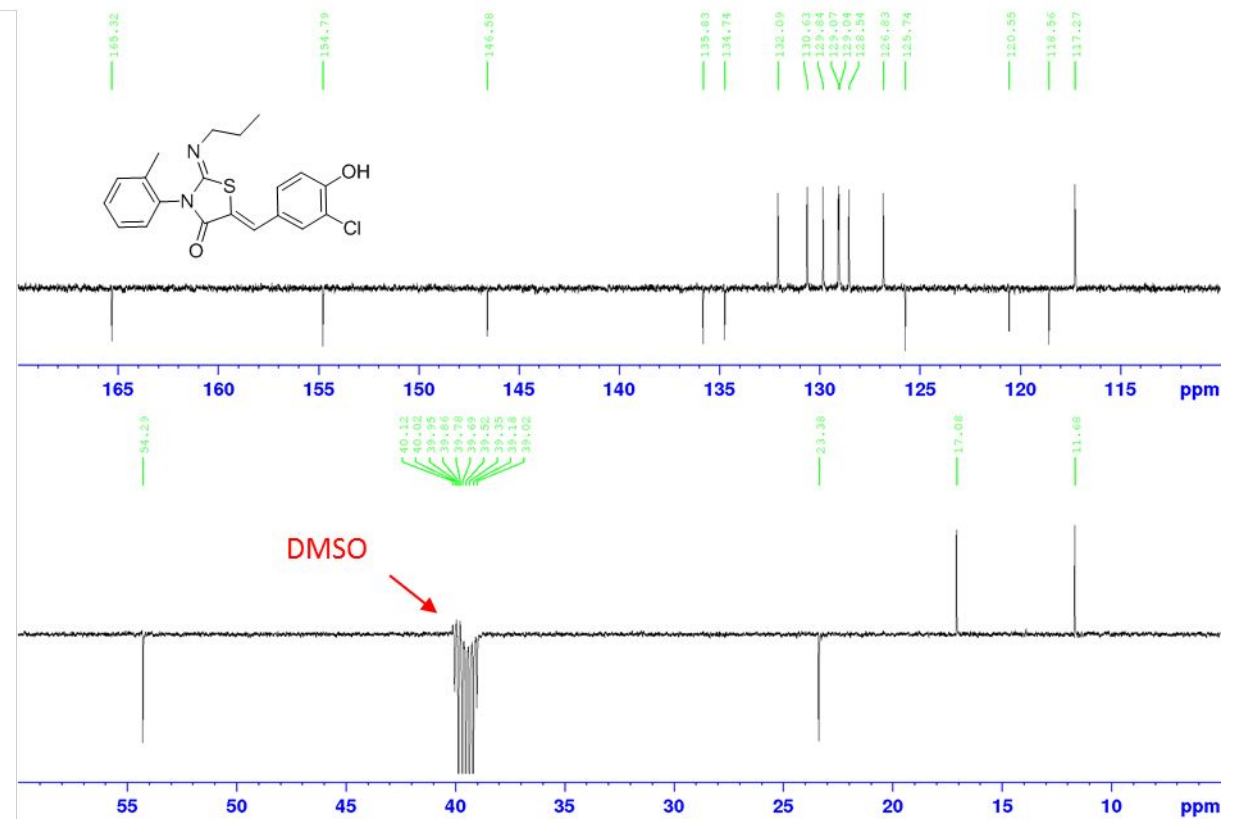
Sodium acetate (5.5 g, 67 mmol) was dissolved in AcOH (75 ml) and the 3-chloro-4-hydroxy-benzaldehyde (5.2 g, 33.5 mmol) was added to the stirred solution. The mixture was stirred for 3 hours

until a homogeneous solution was obtained. In a separate flask, the 1-isothiocyanato-2-methylbenzene (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-hydroxyphenyl)methylene]-3-(*o*-tolyl)-2-propylimino-thiazolidin-4-one as a pale yellow solid (10.7 g; 80% yield).

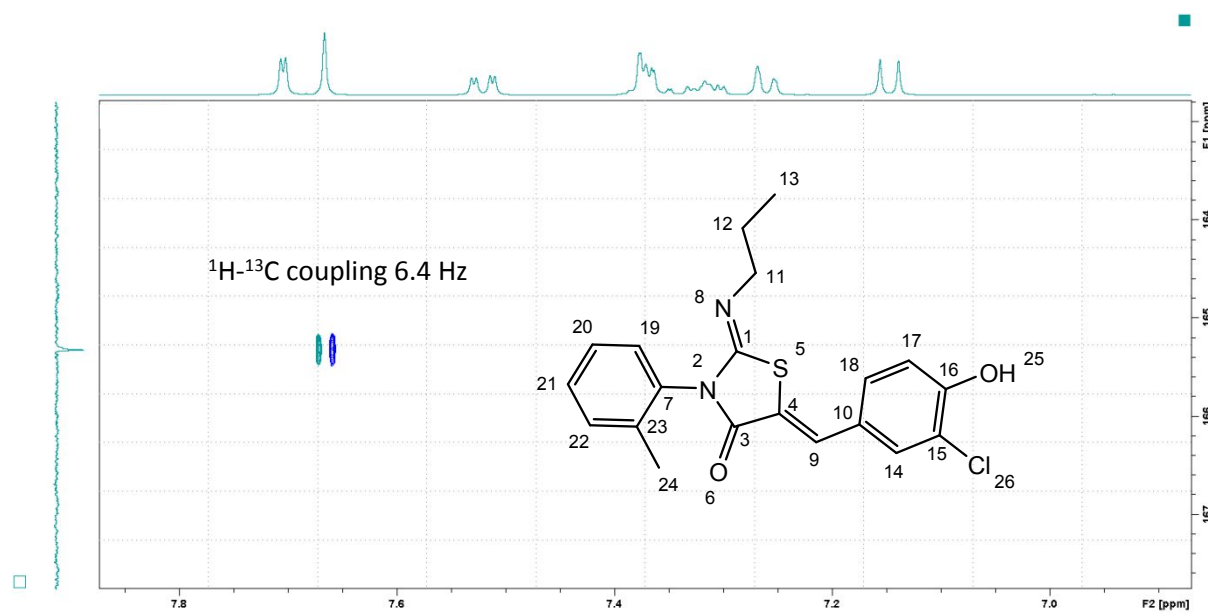
¹H NMR (500 MHz, DMSO-*d*₆) δ: 11.01 (s, 1H), 7.71 (d, *J*=2.1 Hz, 1H), 7.68 (s, 1H), 7.53 (dd, *J*=2.2, 8.6 Hz, 1H), 7.39 - 7.31 (m, 3H), 7.27 (d, *J*=7.5 Hz, 1H), 7.16 (d, *J*=8.5 Hz, 1H), 3.39-3.23 (m, 2H), 2.09 (s, 3H), 1.55 - 1.49 (m, 2H), 0.85 (t, *J*=7.4 Hz, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ: 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 C₂₀H₁₉ClN₂O₂S 386.1; found MS: *m/z* 387.1 [M+H]⁺. HRMS: Calculated for C₂₀H₁₉ClN₂O₂S 386.0929, found 387.0941 [M+H]⁺.



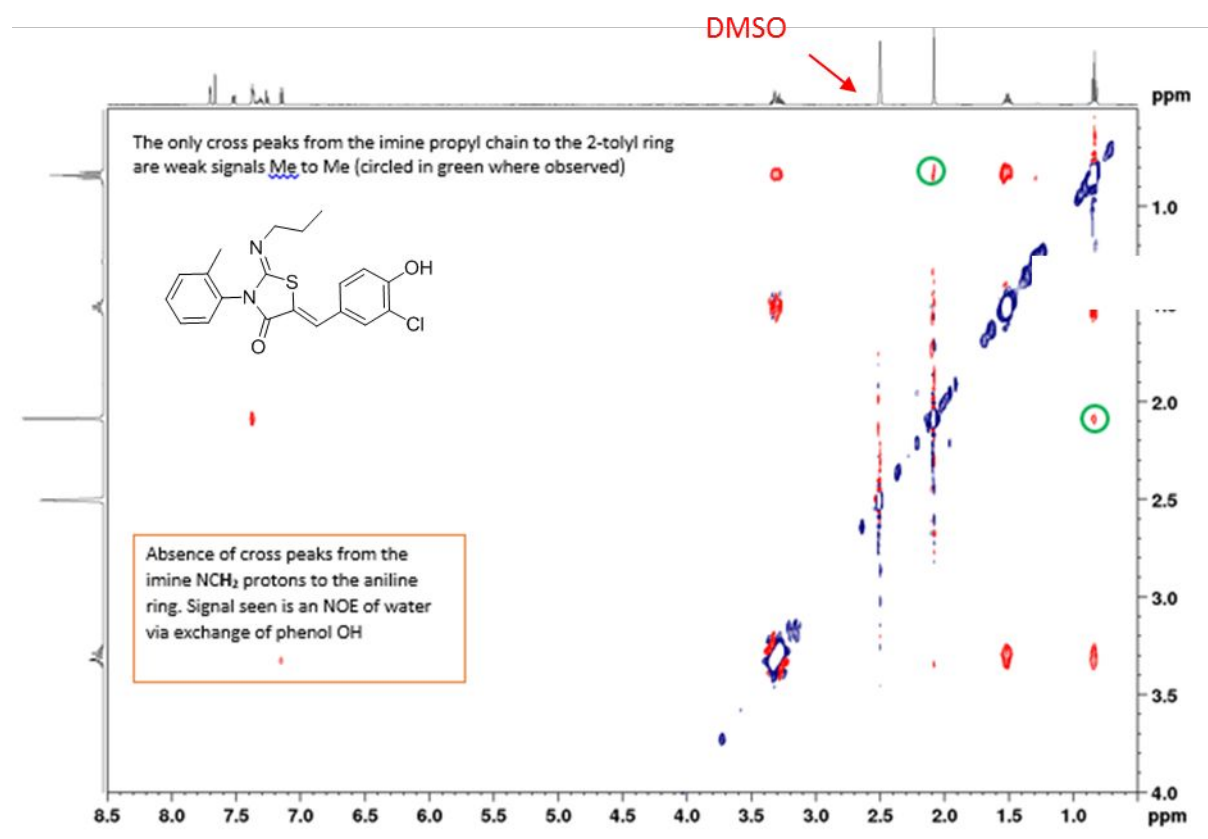
4a ¹H NMR (500 MHz, DMSO-d₆)



4a ^{13}C NMR (125 MHz, DMSO-d_6):

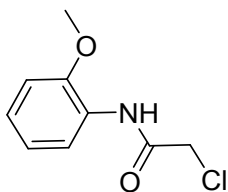


4a HMBC (DMSO-d_6): ^1H - ^{13}C coupling of H^9 - C^3 (6.4 Hz)



4a NOESY (DMSO-d_6)

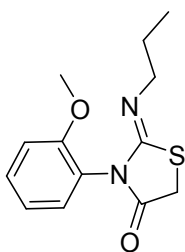
2-chloro-N-(2-methoxyphenyl)acetamide



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 $-70\text{ }^{\circ}\text{C}$. 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).

^1H 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).

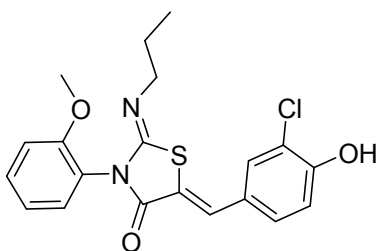
(2Z)-3-(2-methoxyphenyl)-2-propylimino-thiazolidin-4-one **2b**



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).

^1H 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).

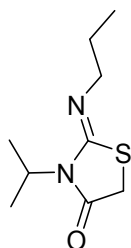
(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(2-methoxyphenyl)-2-propylimino-thiazolidin-4-one **4b**



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 t_R (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]⁺.

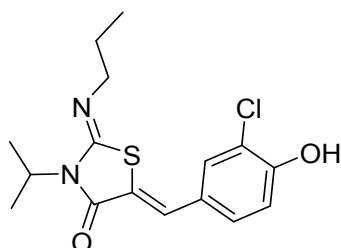
(2Z)-3-isopropyl-2-propylimino-thiazolidin-4-one 2c



To a solution of propan-2-amine (1 g, 17 mmol) in DCM (25 ml) was added 1-isothiocyanatopropane (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 t_R (Agilent, acidic, 3.5 minutes): 1.24 minutes, 60%; Calculated for C₉H₁₆ClN₂O₃S, 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

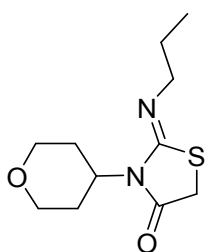


To a solution of a mixture of (2Z)-2-isopropylimino-3-propyl-thiazolidin-4-one (200 mg, 0.99 mmol) and (2Z)-3-isopropyl-2-propylimino-thiazolidin-4-one (200 mg, 0.99 mmol) in acetic acid (5 ml) was added 3-chloro-4-hydroxy-benzaldehyde (313 mg, 1.99 mmol) and sodium acetate (328 mg, 3.99 mmol). The reaction 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 NMR (400 MHz, DMSO- d_6) δ : 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 $\text{C}_{16}\text{H}_{19}\text{ClN}_2\text{O}_2\text{S}$ 338.1, found MS: m/z 339.1 $[\text{M}+\text{H}]^+$. HRMS: Calculated for $\text{C}_{16}\text{H}_{19}\text{ClN}_2\text{O}_2\text{S}$ 338.0856, found 339.0938 $[\text{M}+\text{H}]^+$.

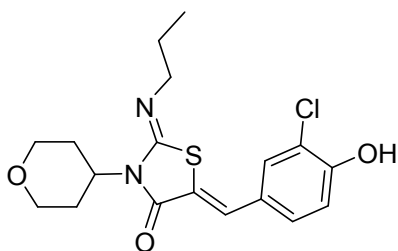
(2Z)-2-propylimino-3-tetrahydropyran-4-yl-thiazolidin-4-one **2d**



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 $\text{C}_{11}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$ 242.1, found 51%; MS: m/z 243.2 $[\text{M}+\text{H}]^+$ and 1.35 minutes, 49%; MS: m/z 243.1 $[\text{M}+\text{H}]^+$.

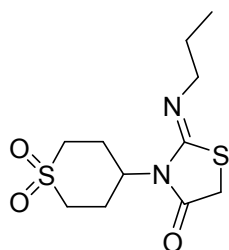
(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-propylimino-3-tetrahydropyran-4-yl-thiazolidin-4-one **4d**



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).

¹H NMR (400 MHz, DMSO-d₆) δ: 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_R (Agilent, acidic, 3.5 minutes): 1.86 minutes, 100%; Calculated for C₁₈H₂₁ClN₂O₃S 380.1, found MS: m/z 381.1 [M+H]⁺. HRMS: Calculated for C₁₈H₂₁ClN₂O₃S 380.0961, found 381.1046 [M+H]⁺.

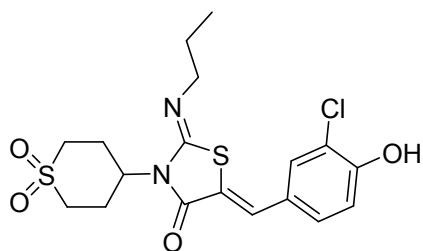
(2Z)-3-(1,1-dioxothian-4-yl)-2-propylimino-thiazolidin-4-one 2e



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 regioisomer in the mixture (0.46 g, 23.6% yield).

HPLC t_R (Agilent, acidic, 3.5 minutes): 1.21 minutes, Calculated for C₁₁H₁₈N₂O₃S₂ 290.1, found 23%; MS: m/z 291.1 [M+H]⁺. 1.28 minutes, 77%; MS m/z 291.1 [M+H]⁺.

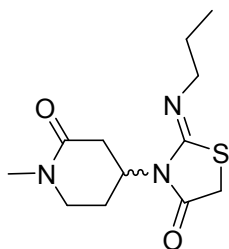
(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(1,1-dioxothian-4-yl)-2-propylimino-thiazolidin-4-one 4e



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 t_R (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]⁺.

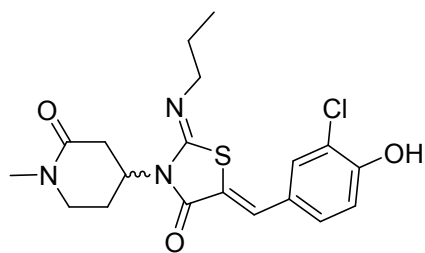
(2Z)-3-(1-methyl-2-oxo-4-piperidyl)-2-propylimino-thiazolidin-4-one **2f**



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 t_R (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]⁺.

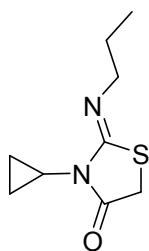
(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(1-methyl-2-oxo-4-piperidyl)-2-propylimino-thiazolidin-4-one **4f**



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-hydroxybenzaldehyde (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 NMR (400 MHz, DMSO-d₆) δ: 8.05 (dd, J=5.7, 5.7 Hz, 1H), 7.72 (s, 1H), 7.52 (d, J=2.6 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 C₁₉H₂₂ClN₃O₃S 407.1, found MS: m/z 408.1 [M+H]⁺.

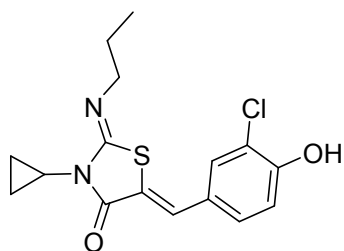
(2Z)-3-cyclopropyl-2-propylimino-thiazolidin-4-one 2g



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 NMR (400 MHz, CDCl₃) δ: 3.75 (s, 2H), 3.27 (t, J=6.9 Hz, 2H), 2.70 - 2.65 (m, 1H), 1.69 (dd, J=7.1, 14.3 Hz, 2H), 1.05 - 0.91 (m, 7H). HPLC t_R (Agilent, acidic, 3.5 minutes): 0.55 minutes, 100%; Calculated for C₉H₁₄N₂OS 198.1, found MS: m/z 199.1 [M+H]⁺.

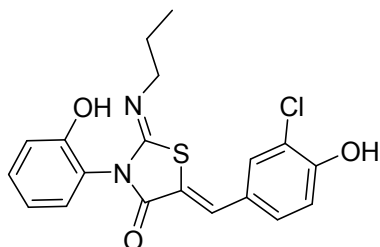
(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-cyclopropyl-2-propylimino-thiazolidin-4-one 4g



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 t_R (Agilent, acidic, 3.5 minutes): 1.64 minutes, 100%; Calculated for C₁₆H₁₇ClN₂O₂S 336.1, found MS: m/z 337.1 [M+H]⁺.

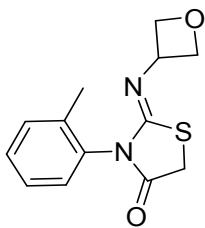
(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(2-hydroxyphenyl)-2-propylimino-thiazolidin-4-one 4h



To a solution of (2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-3-(2-methoxyphenyl)-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 (dd, 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 t_R (Agilent, acidic, 3.5 minutes): 1.65 minutes, 100%; Calculated for C₁₉H₁₇ClN₂O₃S 338.1, found MS: m/z 339.1 [M+H]⁺. HRMS: Calculated for C₁₉H₁₇ClN₂O₃S 338.0648, found 339.0733 [M+H]⁺.

(2Z)-3-(o-tolyl)-2-(oxetan-3-ylimino)thiazolidin-4-one 7a

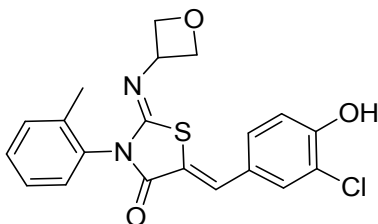


A solution of oxetan-3-amine (0.98 g, 13 mmol) in dry DCM (25 ml) under nitrogen had 1-isothiocyanato-2-methyl-benzene (2.0 g, 13 mmol) added over 15 minutes. Stirred at room temperature for 1 hour. Cooled to 0 °C then pyridine (2.1 g, 27 mmol) was added in a single portion. After stirring for 5 minutes, 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 t_R (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

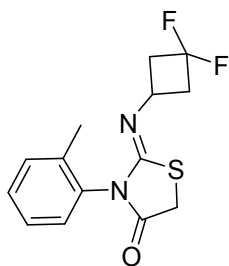
9a



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 t_R (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]⁺.

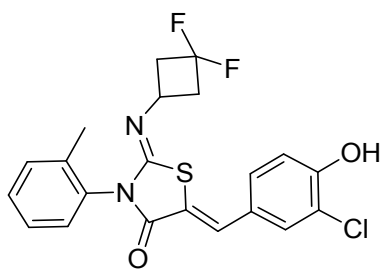
(2Z)-2-(3,3-difluorocyclobutyl)imino-3-(o-tolyl)thiazolidin-4-one **7b**



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 NMR (400 MHz, CDCl₃) δ: 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 C₁₃H₁₄N₂O₂S 296.1, found MS: m/z 297.1 [M+H]⁺. HRMS: Calculated for C₁₄H₁₄F₂N₂OS 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**

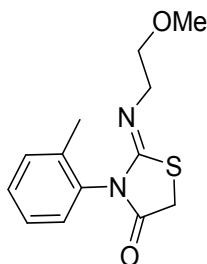


(2Z)-2-(3,3-difluorocyclobutyl)imino-3-(o-tolyl)thiazolidin-4-one (200 mg, 0.67 mmol) was dissolved in ethyl acetate before the addition of 3-chloro-4-hydroxy-benzaldehyde (105 mg, 0.67 mmol) and sodium acetate (132 mg, 1.61 mmol). The resulting solution was heated to 85 °C and allowed to stir for 16 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. NaHCO₃, 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 NMR (500 MHz, DMSO-d₆) δ: 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 $C_{21}H_{17}N_2F_2O_2S$ 434.1, found MS: m/z 435.0 $[M+H]^+$.

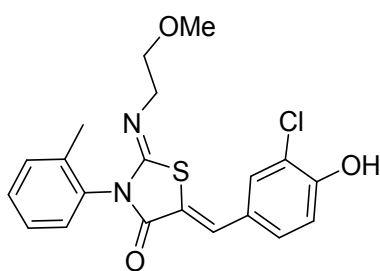
(2Z)-2-(2-methoxyethylimino)-3-(o-tolyl)thiazolidin-4-one 7c



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 $C_{13}H_{17}N_2O_2S$ 264.1, found MS: m/z 265.1 $[M+H]^+$.

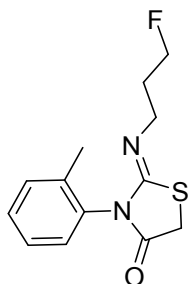
(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-(2-methoxyethylimino)-3-(o-tolyl)thiazolidin-4-one 9c



(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-d_6) 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 t_R (Agilent, acidic, 7.5 minutes): 3.75 minutes, 100%; Calculated for $\text{C}_{20}\text{H}_{19}\text{ClN}_2\text{O}_3\text{S}$ 402.1, found MS: m/z 403.1 $[\text{M}+\text{H}]^+$.

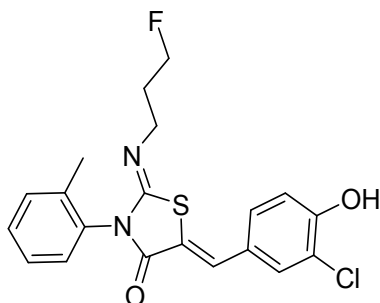
(2Z)-2-(3-fluoropropylimino)-3-(o-tolyl)thiazolidin-4-one 7d



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, CDCl_3) 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 t_R (Agilent, acidic, 3.5 minutes): 1.27 minutes, 100%; Calculated for $\text{C}_{13}\text{H}_{16}\text{FN}_2\text{OS}$ 266.1, found MS: m/z 267.10 $[\text{M}+\text{H}]^+$. HRMS: Calculated for $\text{C}_{13}\text{H}_{16}\text{FN}_2\text{OS}$ 266.0962, found 267.0973 $[\text{M}+\text{H}]^+$.

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

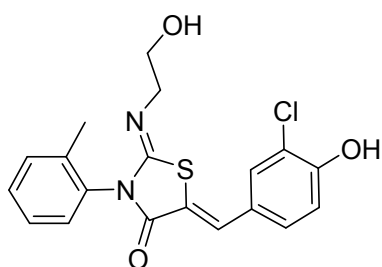


(2Z)-2-(3-fluoropropylimino)-3-(o-tolyl)thiazolidin-4-one (200 mg, 0.75 mmol) was dissolved in ethyl acetate before the addition of 3-chloro-4-hydroxy-benzaldehyde (117 mg, 0.75 mmol) and sodium acetate (132 mg, 1.6 mmol). The resulting solution was heated to 85°C and allowed to stir for 16 hours. The 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. NaHCO_3 , 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 t_R (Agilent, acidic, 7.5 minutes): 3.94 minutes, 100%; Calculated for C₂₀H₁₈ClFN₂O₂S 404.1, found MS: m/z 405.1 [M+H]⁺.

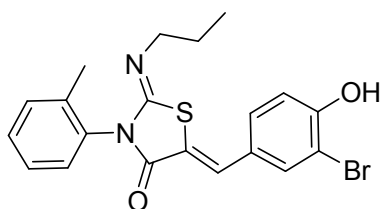
(2Z,5Z)-5-[(3-chloro-4-hydroxy-phenyl)methylene]-2-(2-methoxyethylimino)-3-(o-tolyl)thiazolidin-4-one 9e



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 t_R (BrukerN, acidic, 7.5min): 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]⁺.

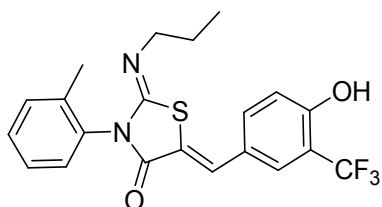
(2Z,5Z)-5-[(3-bromo-4-hydroxy-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one 9f



A mixture of (2Z)-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (200 mg; 0.8 mmol), 4-hydroxy-3-bromobenzaldehyde (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) δ : 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 $\text{C}_{20}\text{H}_{20}\text{BrN}_2\text{O}_2\text{S}$ 388.1, found 430.0 MS: m/z 431.0 $[\text{M}+\text{H}]^+$. HRMS: Calculated for $\text{C}_{20}\text{H}_{20}\text{BrN}_2\text{O}_2\text{S}$ 430.0423, found 431.0436.

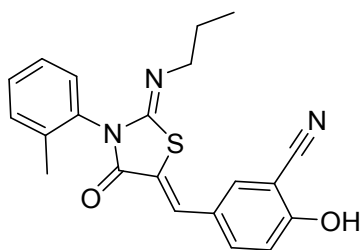
(2Z,5Z)-5-[[4-hydroxy-3-(trifluoromethyl)phenyl]methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one 9g



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, $\text{DMSO}-d_6$) δ : 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 $\text{C}_{21}\text{H}_{20}\text{F}_3\text{N}_2\text{O}_2\text{S}$ 420.1, found MS: m/z 421.1 $[\text{M}+\text{H}]^+$. HRMS: Calculated for $\text{C}_{21}\text{H}_{20}\text{F}_3\text{N}_2\text{O}_2\text{S}$ 420.1192, found 421.1193.

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

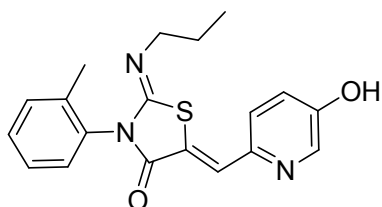


A suspension of (2Z,5Z)-5-[(3-bromo-4-hydroxy-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (45 mg, 0.10 mmol), dicyanozinc (12 mg, 0.10 mmol) in DMA (0.5 ml) had nitrogen gas bubbled through for 10 minutes. Tetrakis(triphenylphosphine)palladium(0) (24 mg, 0.02 mmol) was then added and 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- d_6) 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 t_R (Agilent, acidic, 7.5 minutes): 3.92 minutes, 100%; Calculated for $\text{C}_{21}\text{H}_{19}\text{N}_3\text{O}_2\text{S}$ 377.1, found MS: $m/z = 378.1$ (100%) $[\text{M}+\text{H}]^+$.

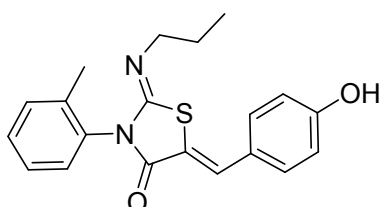
(2Z,5Z)-5-[(5-hydroxy-2-pyridyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one 9i



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- d_6) 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 t_R (BrukerN, acidic, 7.5min): 3.40 minutes, 100%; Calculated for $\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_2\text{S}$ 353.1, found MS: m/z 354.1 $[\text{M}+\text{H}]^+$. HRMS: Calculated for $\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_2\text{S}$ 353.1271; found 354.1291 $[\text{M}+\text{H}]^+$.

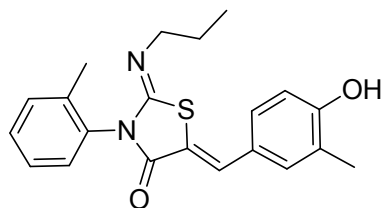
(2Z,5Z)-5-[(4-hydroxyphenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one 9j



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).

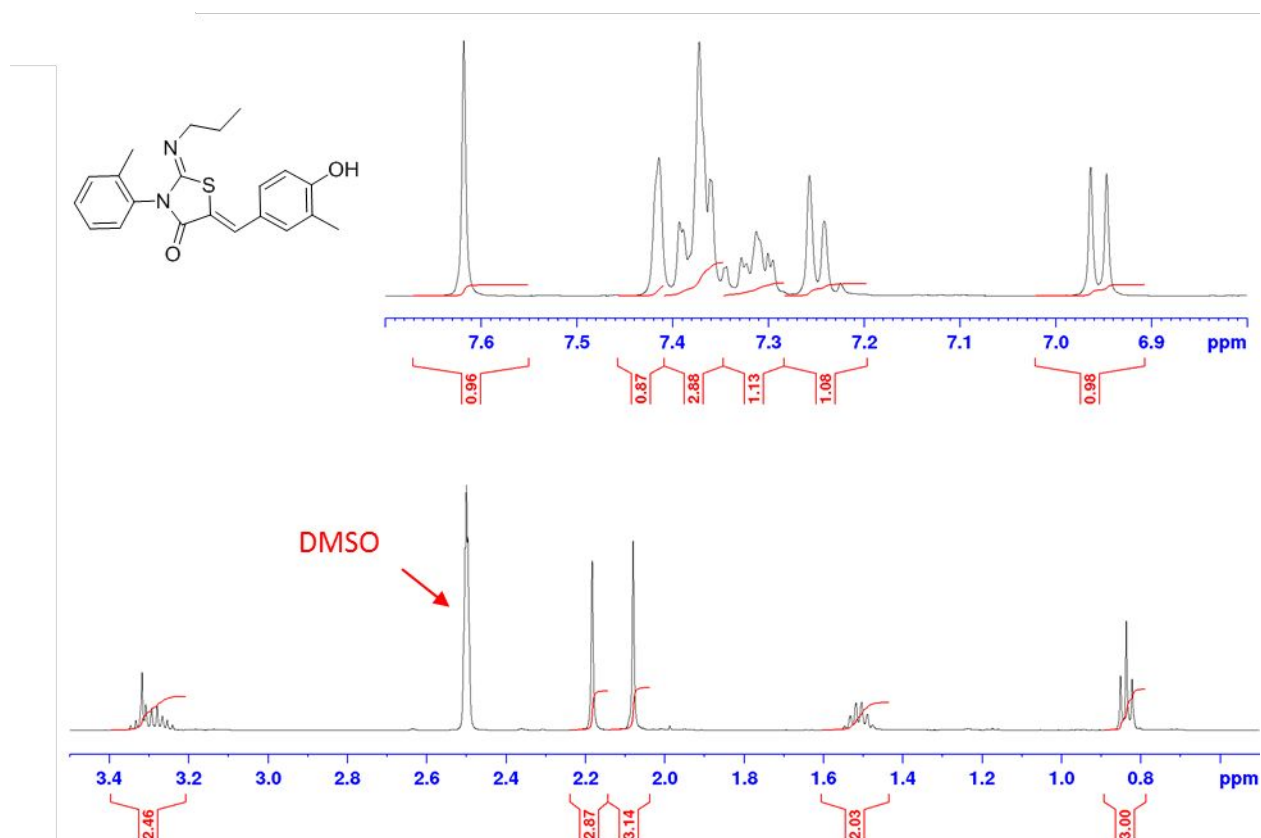
^1H NMR (400 MHz, CDCl_3) δ : 7.77 (s, 1H), 7.48 (d, $J=8.5$ Hz, 2H), 7.34 - 7.29 (m, 3H), 7.24 - 7.18 (m, 1H), 6.86 (d, $J=8.7$ Hz, 2H), 6.01 (s, 1H), 3.46 - 3.36 (m, 2H), 2.22 (s, 3H), 1.71 - 1.58 (m, 2H), 0.94 (t, $J=7.4$ Hz, 3H). HPLC t_R (BrukerN, acidic, 7.5 minutes): 5.30 minutes, 100%; Calculated for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_2\text{S}$ 352.1, found MS: m/z 353.1 $[\text{M}+\text{H}]^+$. HRMS: Calculated for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_2\text{S}$ 352.1318; found 353.1328 $[\text{M}+\text{H}]^+$.

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

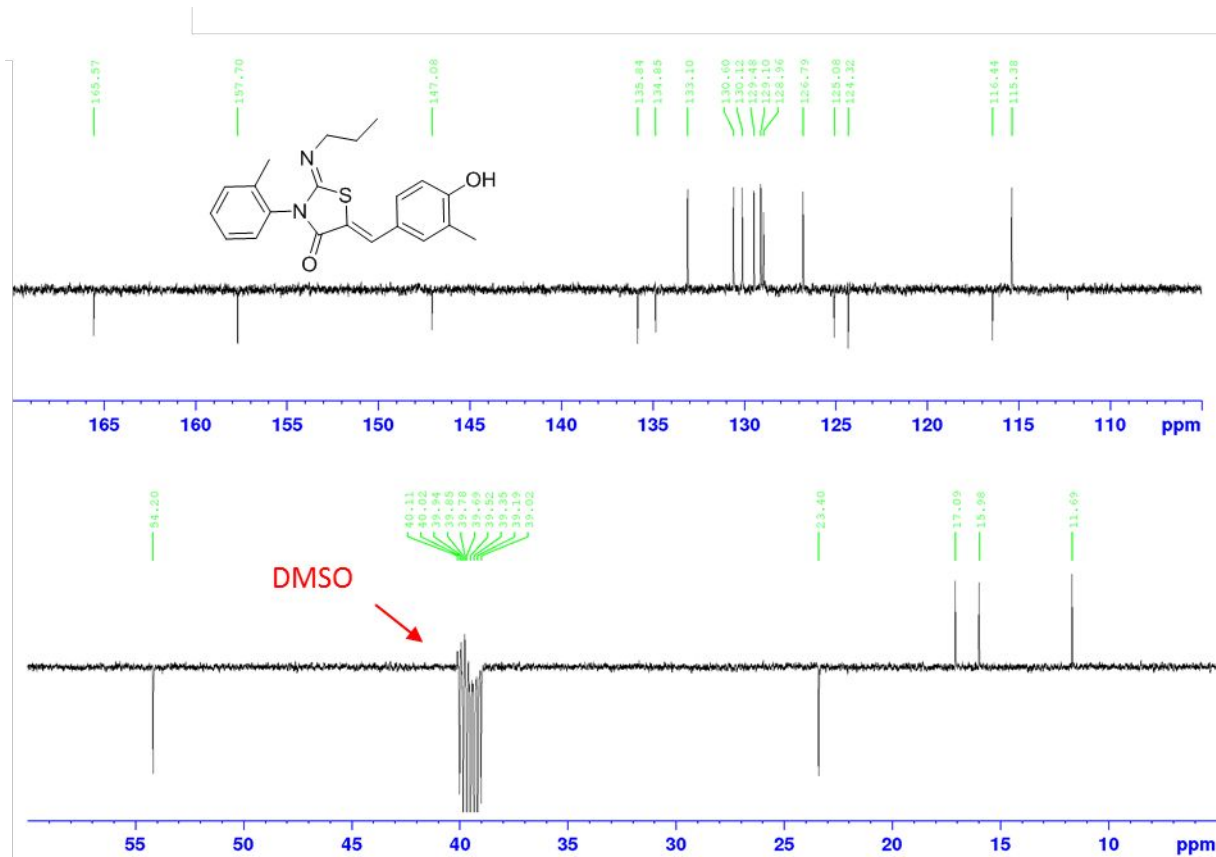


A mixture of (2Z)-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (1 g, 4 mmol), 4-hydroxy-3-methylbenzaldehyde (0.55 g, 4 mmol) and sodium acetate (0.66 g, 8 mmol in acetic acid (10 ml)) were heated overnight at 60 °C. The mixture was concentrated under vacuum by approximately 50% then suspended in water 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 t_R (Agilent, acidic, 7.5 minutes): 4.12 minutes, 100%; HRMS: Calculated for C₂₁H₂₂N₂O₂S 366.1475, found 367.1476 [M+H]⁺.

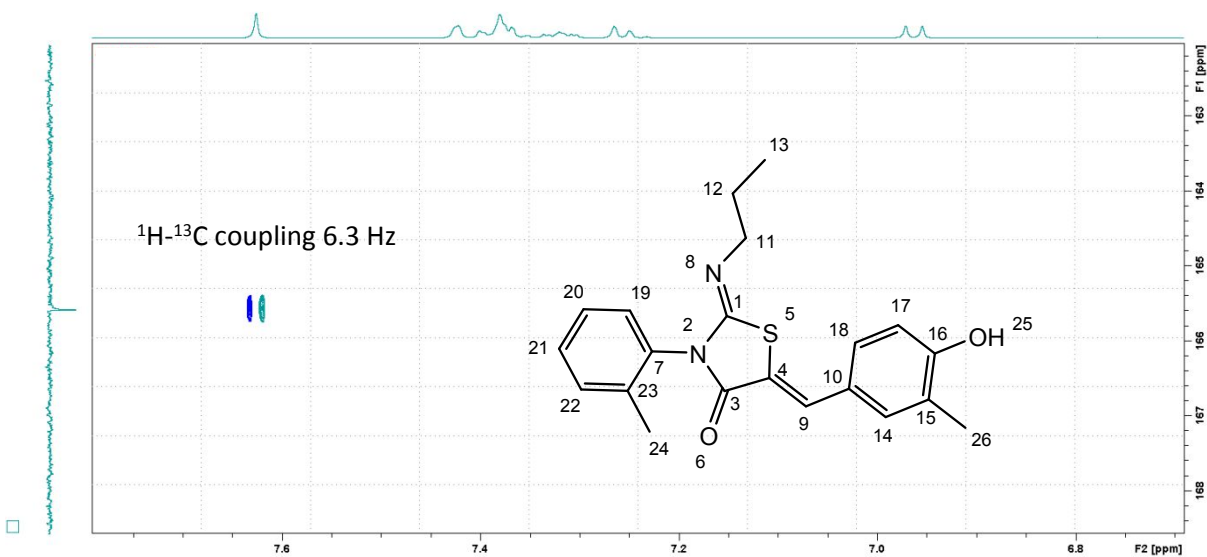


9k ¹H NMR (500 MHz, DMSO-d₆)

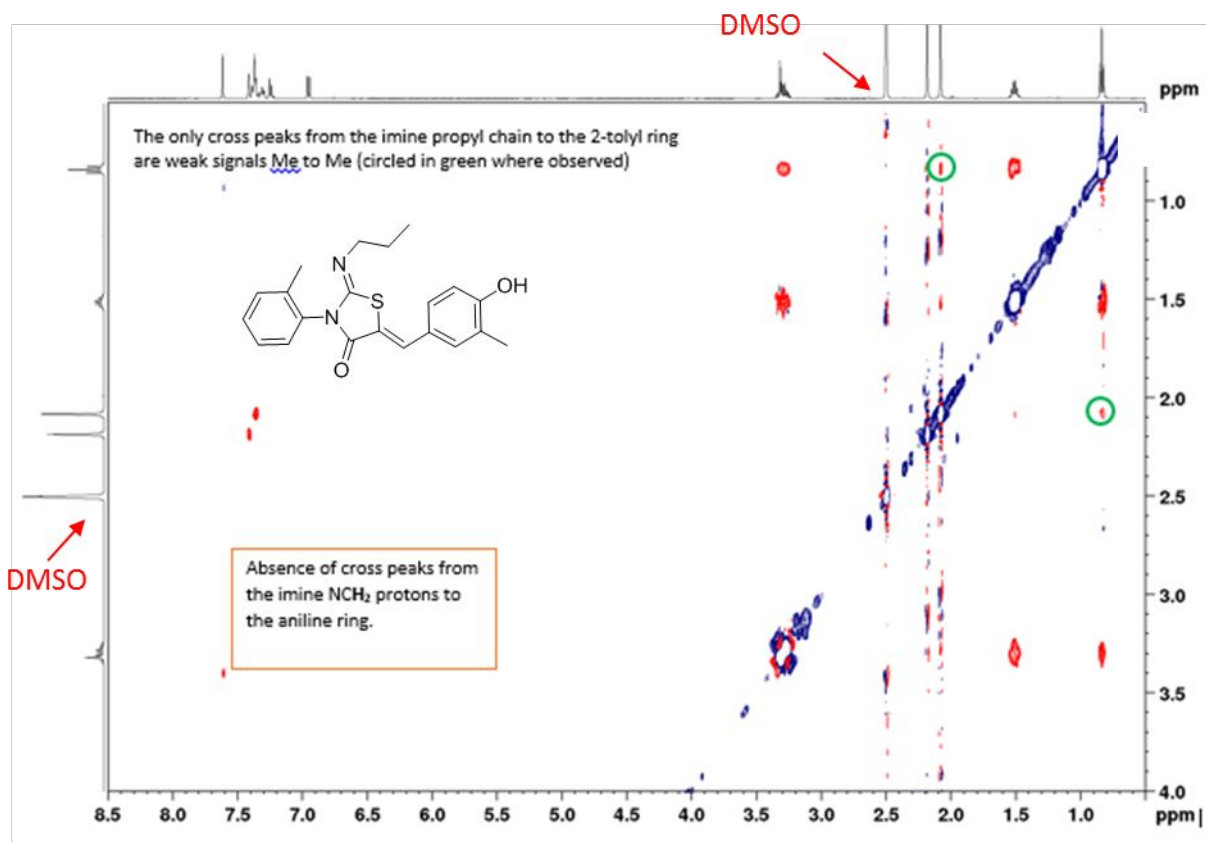


9k ¹³C NMR (125 MHz, DMSO-d₆)

□

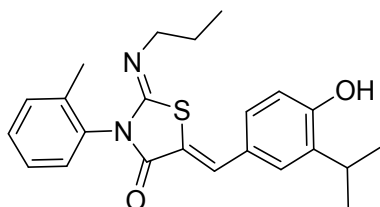


9k HMBC (DMSO-d₆): ¹H-¹³C coupling of H⁹-C³ (6.3 Hz)



9k NOESY (DMSO-d₆)

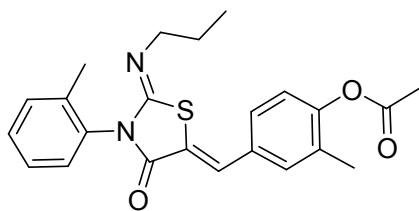
(2Z,5Z)-5-[(4-hydroxy-3-isopropyl-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one **9l**



A solution of 5-hydroxypyridine-2-carbaldehyde (50 mg, 0.4 mmol), 4-hydroxy-3-isopropylbenzaldehyde (66 mg, 0.4 mmol) and (2Z)-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).

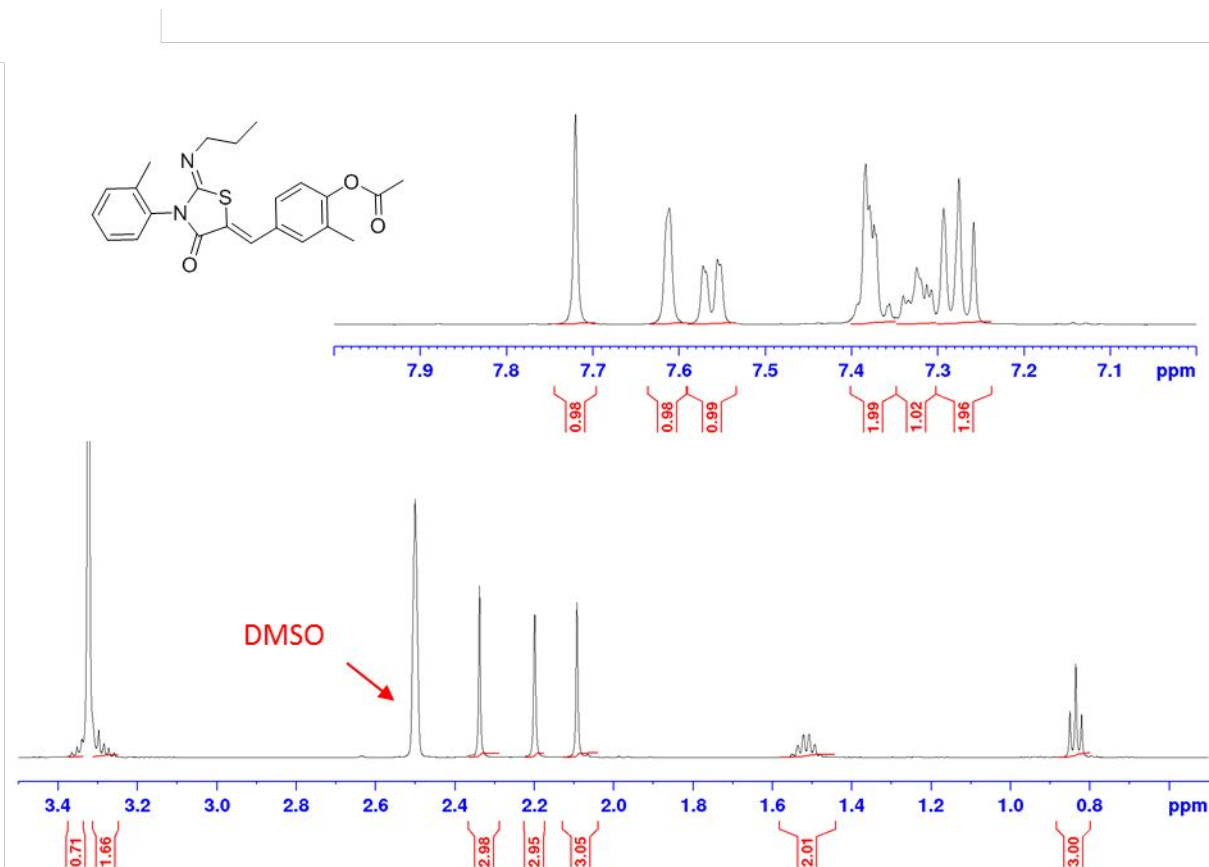
¹H NMR (400 MHz, DMSO-d₆) δ: 10.20 - 10.19 (m, 1H), 7.68 (s, 1H), 7.48 (d, J=2.2 Hz, 1H), 7.40 - 7.37 (m, 3H), 7.36 - 7.30 (m, 1H), 7.26 (d, J=7.4 Hz, 1H), 6.97 (d, J=8.3 Hz, 1H), 3.35 - 3.21 (m, 3H), 2.09 (s, 3H), 1.55 - 1.48 (m, 2H), 1.21 (d, J=6.9 Hz, 6H), 0.84 (t, J=7.4 Hz, 3H). HPLC t_R (BrukerN, 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]⁺.

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

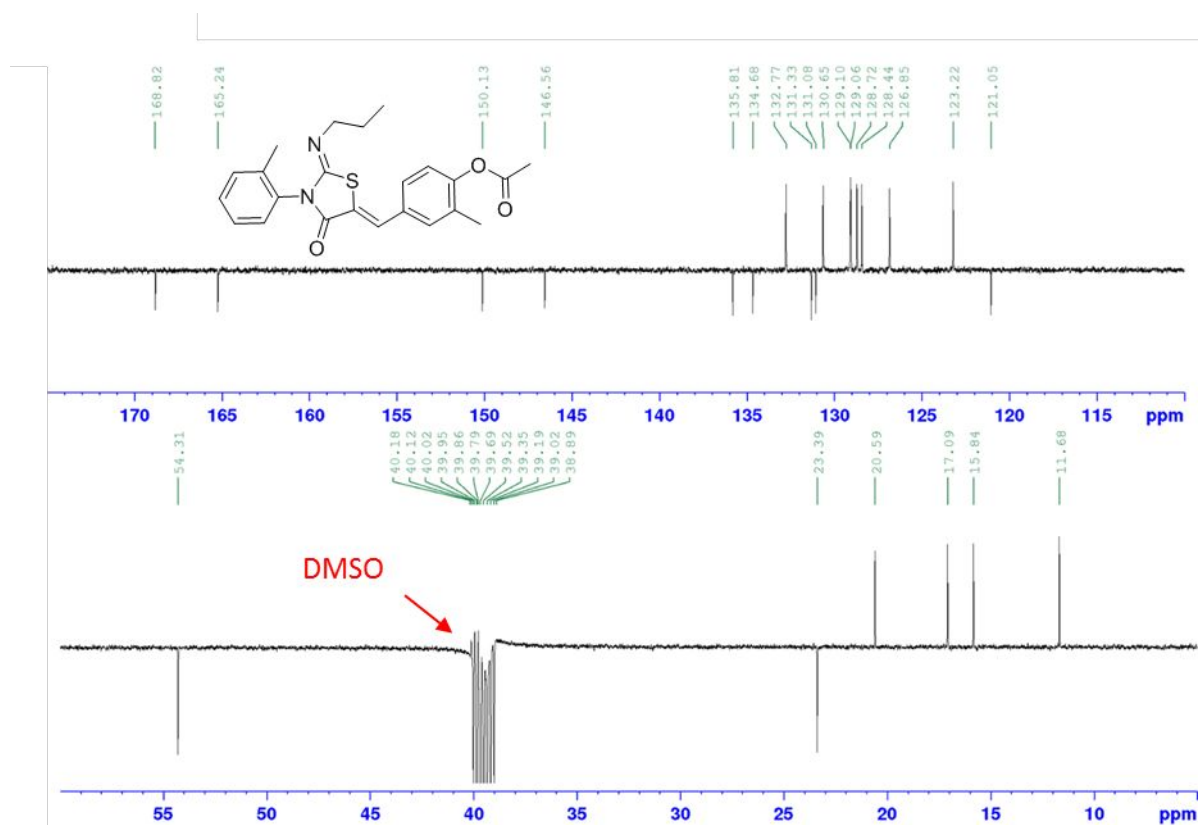


(2Z,5Z)-5-[[4-(3-hydroxy-3-methyl-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (100 mg, 0.27 mmol) was suspended in DCM (2 ml) and DMAP (2 mg, 0.02 mmol) added, followed by dropwise addition of Et₃N (33 mg, 0.33 mmol), whereupon a homogeneous yellow solution was obtained. The acetyl 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-[(Z)-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] acetate (74 mg, 65%) as a white solid.

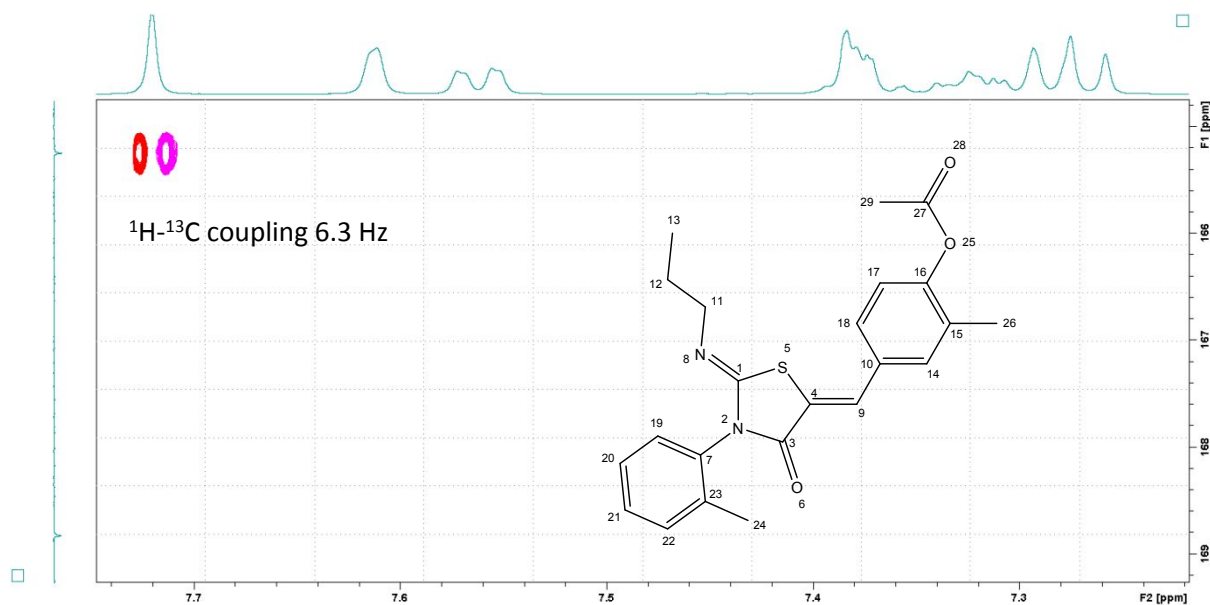
¹H NMR (500 MHz, DMSO-d₆) δ: 7.72 (s, 1H), 7.61 (s, 1H), 7.56-7.55 (d, J=7.2 Hz, 1H), 7.39-7.37 (m, 2H), 7.33-7.30 (m, 1H), 7.25 (t, J=8.5 Hz, 2H), 3.36-3.25 (m, 2H), 2.33 (s, 3H), 2.19 (s, 3H), 2.09 (s, 3H), 1.54-1.48 (m, 2H), 0.83 (t, J=7.5 Hz, 3H) ¹³C NMR (125 MHz, DMSO-d₆) δ: 168.8, 165.2, 150.1, 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 C₂₃H₂₄N₂O₃S 408.2, found MS: m/z 409.2 [M+H]⁺.



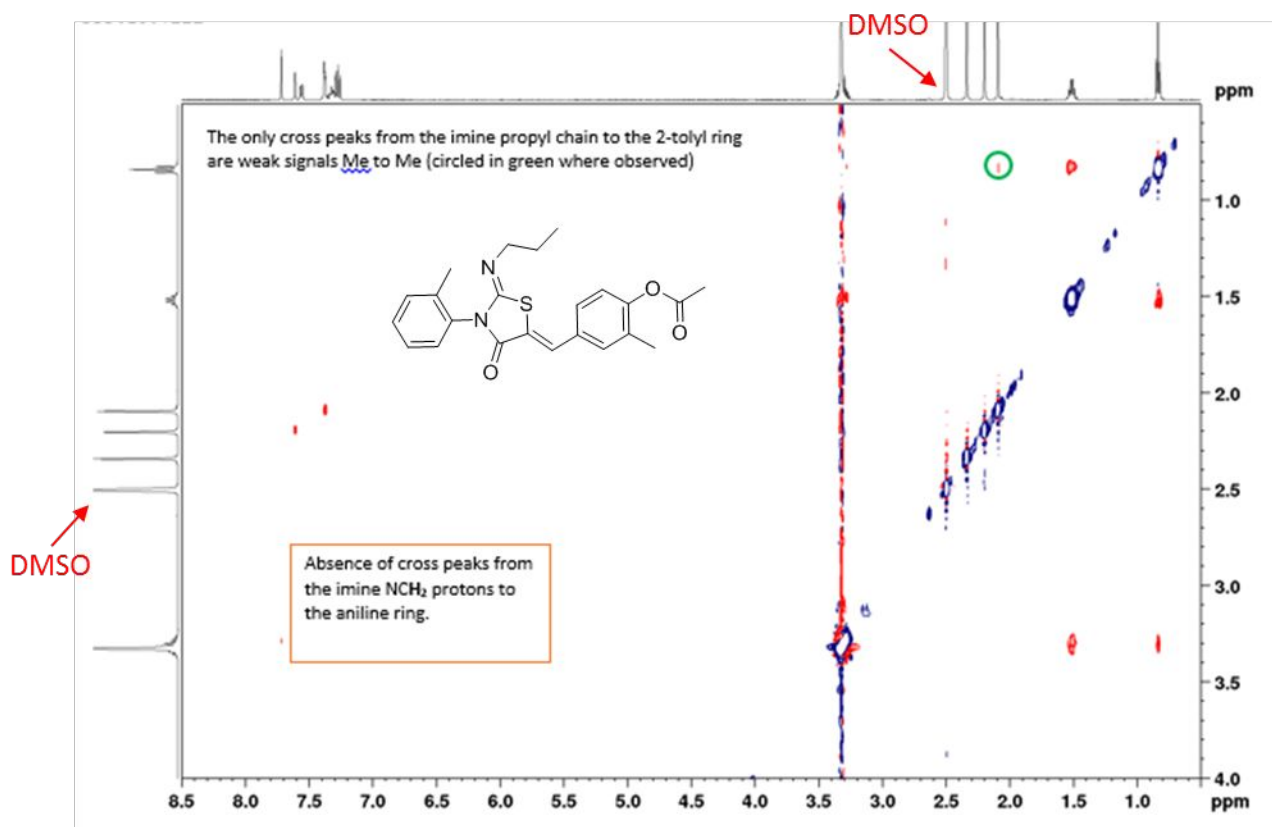
10a ^1H NMR (500 MHz, DMSO- d_6)



10a ^{13}C NMR (125 MHz, DMSO- d_6)

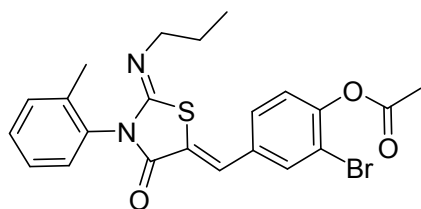


10a HMBC (DMSO- d_6): ^1H - ^{13}C coupling of H^9 - C^3 (6.3 Hz)



10a NOESY (DMSO-d₆)

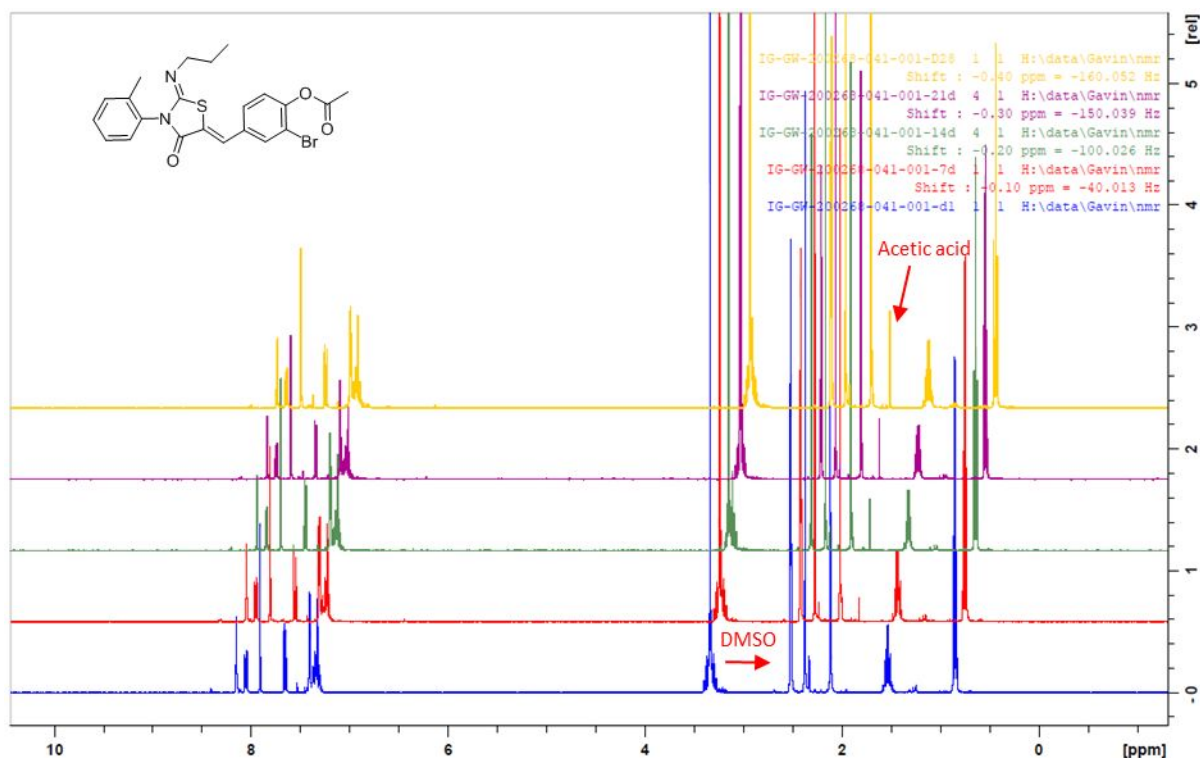
[2-bromo-4-[(Z)-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] acetate
10b



(2Z,5Z)-5-[[4-hydroxy-3-bromo-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (30 mg, 0.12 mmol) was suspended in DCM (1 ml) and DMAP (0.5 mg; 0.005 mmol) added, followed by dropwise addition of Et₃N (8.4 mg, 0.14 mmol), whereupon a homogeneous yellow solution was obtained. The acetyl chloride (6 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-bromo-4-[(Z)-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] acetate (17 mg; 51%) as a white solid.

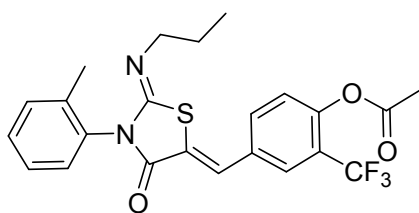
¹H NMR (400 MHz, DMSO-d₆) δ: 8.07 (d, J=2.1 Hz, 1H), 7.80 - 7.75 (m, 2H), 7.51 (d, J=8.4 Hz, 1H), 7.41 - 7.39 (m, 2H), 7.38 - 7.30 (m, 2H), 2.53 - 2.51 (m, 2H), 2.39 (s, 3H), 2.11 (s, 3H), 1.57 - 1.49 (m, 2H), 0.85 (t, J=7.4 Hz, 3H). HPLC t_R (Agilent, acidic, 3.1 minutes): 2.06 minutes, 100%. Calculated for C₂₂H₂₁BrN₂O₃S 472.0, found MS: m/z 473.1 [M+H]⁺.

Decomposition study when stored in DMSO-d₆ solution for 28 days. Hydrolysis monitored by following acetic acid CH₃ 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.

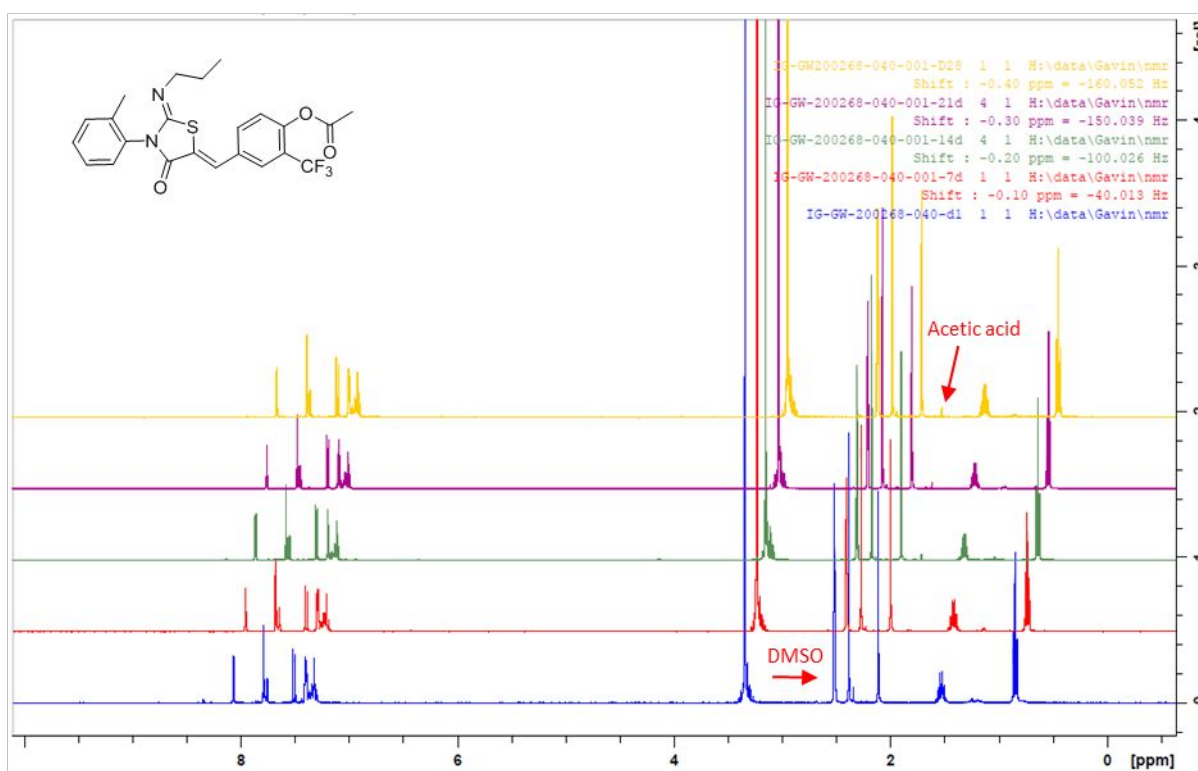
[2-trifluoromethyl-4-[(Z)-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] acetate **10c**



(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 Et₃N (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.

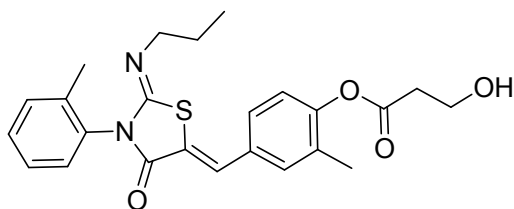
^1H NMR (400 MHz, DMSO-d_6) δ : 8.15 (d, $J=2.0$ Hz, 1H), 8.05 (dd, $J=2.2, 8.6$ Hz, 1H), 7.91 (s, 1H), 7.66 (d, $J=8.5$ Hz, 1H), 7.41 - 7.31 (m, 4H), 2.53 - 2.51 (m, 2H), 2.38 (s, 3H), 2.12 (s, 3H), 1.57 - 1.49 (m, 2H), 0.85 (t, $J=7.3$ Hz, 3H). HPLC t_R (Agilent, acidic, 3.1 min): 2.09, 100%; Calculated for $\text{C}_{23}\text{H}_{21}\text{F}_3\text{N}_2\text{O}_3\text{S}$ 462.1, found MS: m/z 463.2 $[\text{M}+\text{H}]^+$.

Decomposition study when stored in DMSO solution for 28 days. Hydrolysis monitored by following acetic acid CH_3 peak at 1.91 ppm. 1.4% reduction at day 28.



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

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

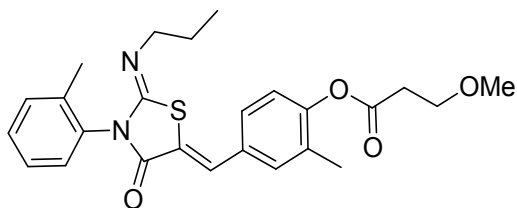


[2-methyl-4-[(Z)-[(Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] 3-methoxypropanoate (53 mg, 0.12 mmol) was dissolved in CHCl_3 (2 ml) under nitrogen and TMSI (99 mg, 0.50 mmol) added dropwise. The resulting yellow solution was heated at 60°C overnight. A further 0.07 ml of 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)-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl]-3-hydroxypropanoate (24 mg, 45%) as a white solid.

¹H NMR (500 MHz, CDCl₃) δ: 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 C₂₄H₂₆N₂O₄S 438.2, found MS: m/z 439.2 [M+H]⁺.

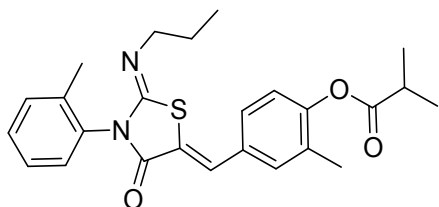
[2-methyl-4-[(Z)-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] 3-methoxypropanoate 10g



(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 Et₃N (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)-[(2Z)-3-(o-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] 3-methoxypropanoate (186 mg, 75%) as a white solid.

¹H NMR (500 MHz, CDCl₃) δ: 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 C₂₅H₂₈N₂O₄S 452.2, found MS: m/z 453.1 [M+H]⁺.

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

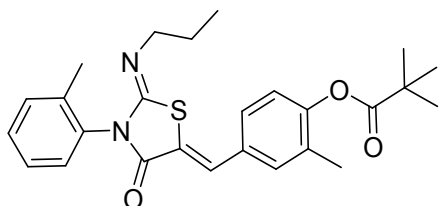


(2Z,5Z)-5-[(4-hydroxy-3-methyl-phenyl)methylene]-3-(o-tolyl)-2-propylimino-thiazolidin-4-one (100 mg, 0.27 mmol) was suspended in DCM (2 ml) and DMAP (2 mg, 0.02 mmol) added, followed by dropwise addition of Et₃N (33 mg, 0.33 mmol), whereupon a homogeneous yellow solution was obtained. The 2-methylpropanoyl 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)-[(2Z)-3-

(*o*-tolyl)-4-oxo-2-propylimino-thiazolidin-5-ylidene]methyl]phenyl] 2-methylpropanoate (96 mg, 78%) as a colourless gum.

¹H NMR (500 MHz, CDCl₃) δ: 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 C₂₅H₂₈N₂O₃S 436.2, found MS: m/z 437.2 [M+H]⁺.

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



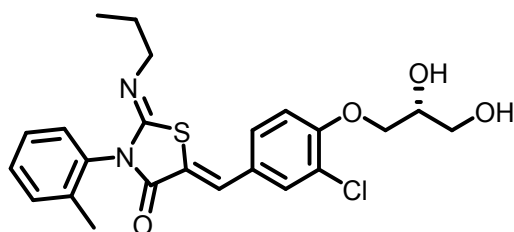
A solution of (2Z,5Z)-5-[(4-hydroxy-3-methyl-phenyl)methylene]-3-(*o*-tolyl)-2-propylimino-thiazolidin-4-one (50 mg, 0.14 mmol), DCC (34 mg, 0.17 mmol) and DMAP (3 mg, 0.02 mmol) in DMF (0.5 ml) had 2,2-dimethylpropanoic acid (90 mg, 0.88 mmol) added and stirred at 40 °C overnight. Filtered and purified 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.

¹H NMR (400 MHz, DMSO-d₆) δ: 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=7.3 Hz, 3H). HPLC t_R (Agilent, acidic, 7.5 minutes): 5.19 minutes, 100%; Calculated for C₂₆H₃₁N₂O₃S 450.2, found MS: m/z 451.2 [M+H]⁺. HRMS: Calculated for C₂₆H₃₁N₂O₃S 450.2050, found 451.2052 [M+H]⁺.

Characterisation of ponesimod

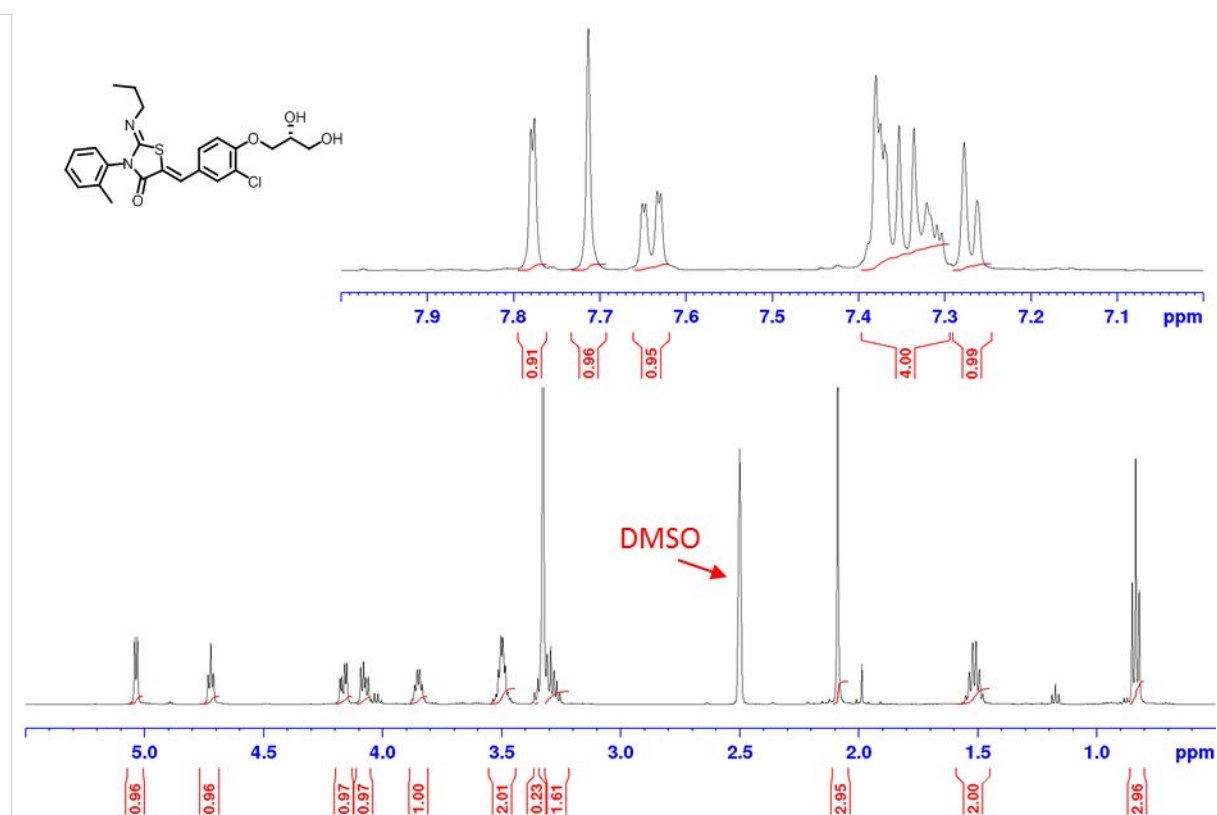
(Z)-5-((Z)-3-chloro-4-((R)-2,3-dihydroxypropoxy)benzylidene)-2-(propylimino)-3-(*o*-tolyl)thiazolidin-4-one

Synthesised as shown in Bolli *et al.*¹²

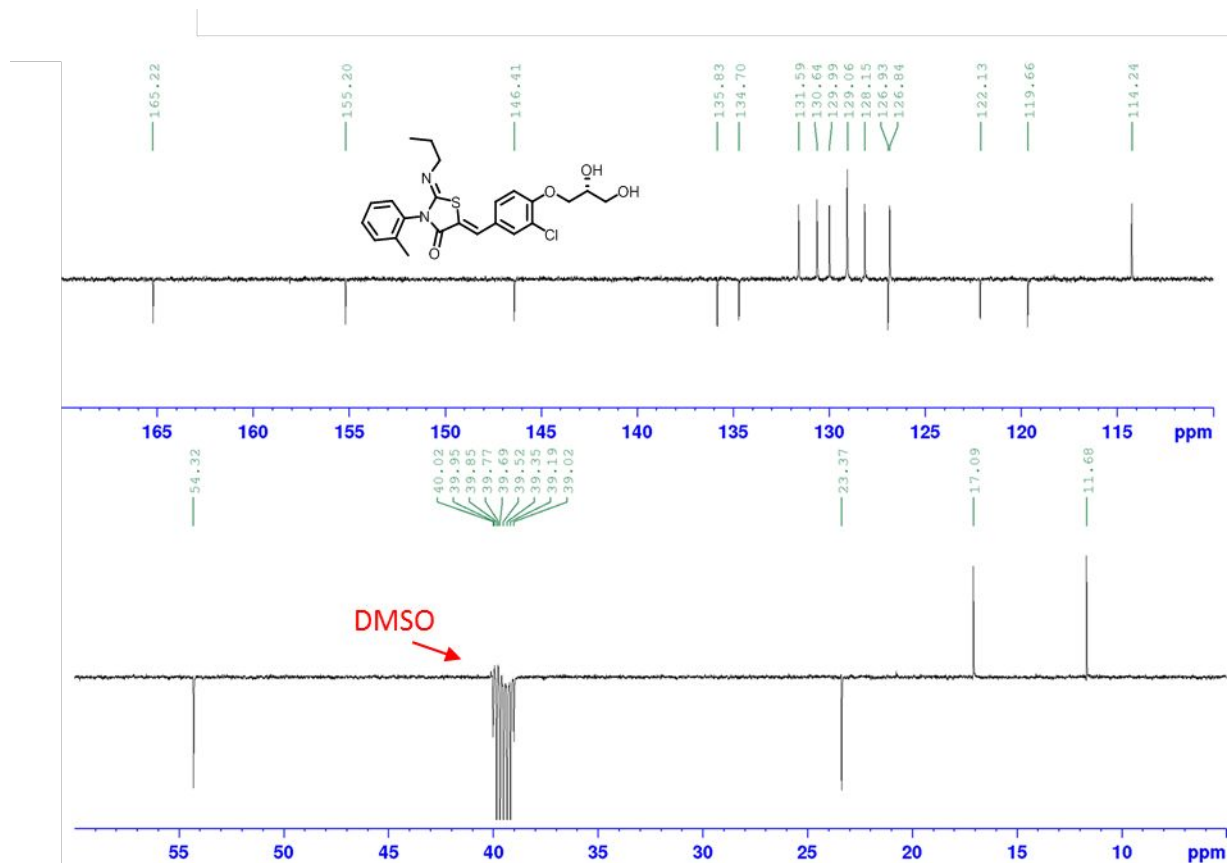


¹H NMR (500 MHz, DMSO-d₆) δ: 7.78 (d, J=2.0 Hz, 1H), 7.71 (s, 1H), 7.64 (dd, J=1.9, 8.6 Hz, 1H), 7.37-7.29 (m, 4H), 7.27 (d, J=7.6 Hz, 2H), 5.04 (d, J=5.1 Hz, 1H), 4.72 (t, J=5.6 Hz, 1H), 4.16-4.03 (m, 2H), 3.84 (m, 1H), 3.53-3.46 (m, 2H), 3.36-3.25 (m, 2H), 2.12 (s, 3H), 1.54-1.48 (m, 2H), 0.87 (t, J=7.2 Hz, 3H). ¹³C NMR (125 MHz, DMSO-d₆) δ: 165.2, 155.2, 146.4, 135.8, 134.7, 131.6, 130.6, 130.0, 129.1, 129.0,

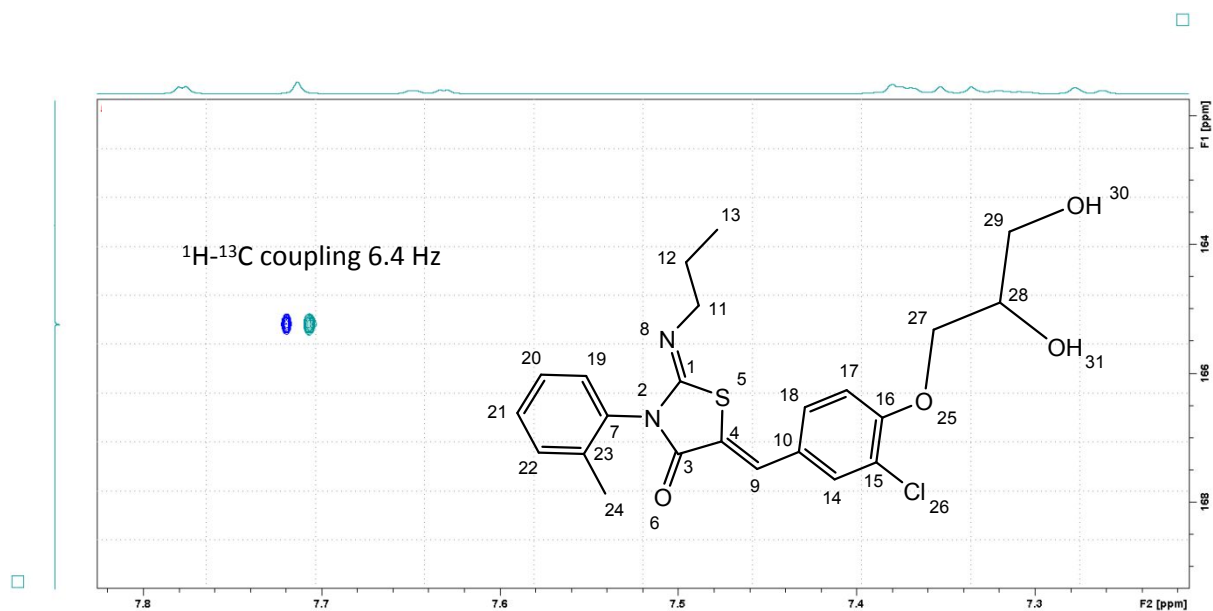
128.1, 126.9, 128.8, 122.1, 119.7, 114.2, 70.5, 69.4, 62.2, 54.3, 23.4, 17.1, 11.7. HPLC tR (Agilent, acidic, 7.5min): 3.83, 100%; Calculated for C₂₃H₂₅ClN₂O₄S 460.1, found MS: *m/z* = 461.1 (100%) [M+H]⁺.



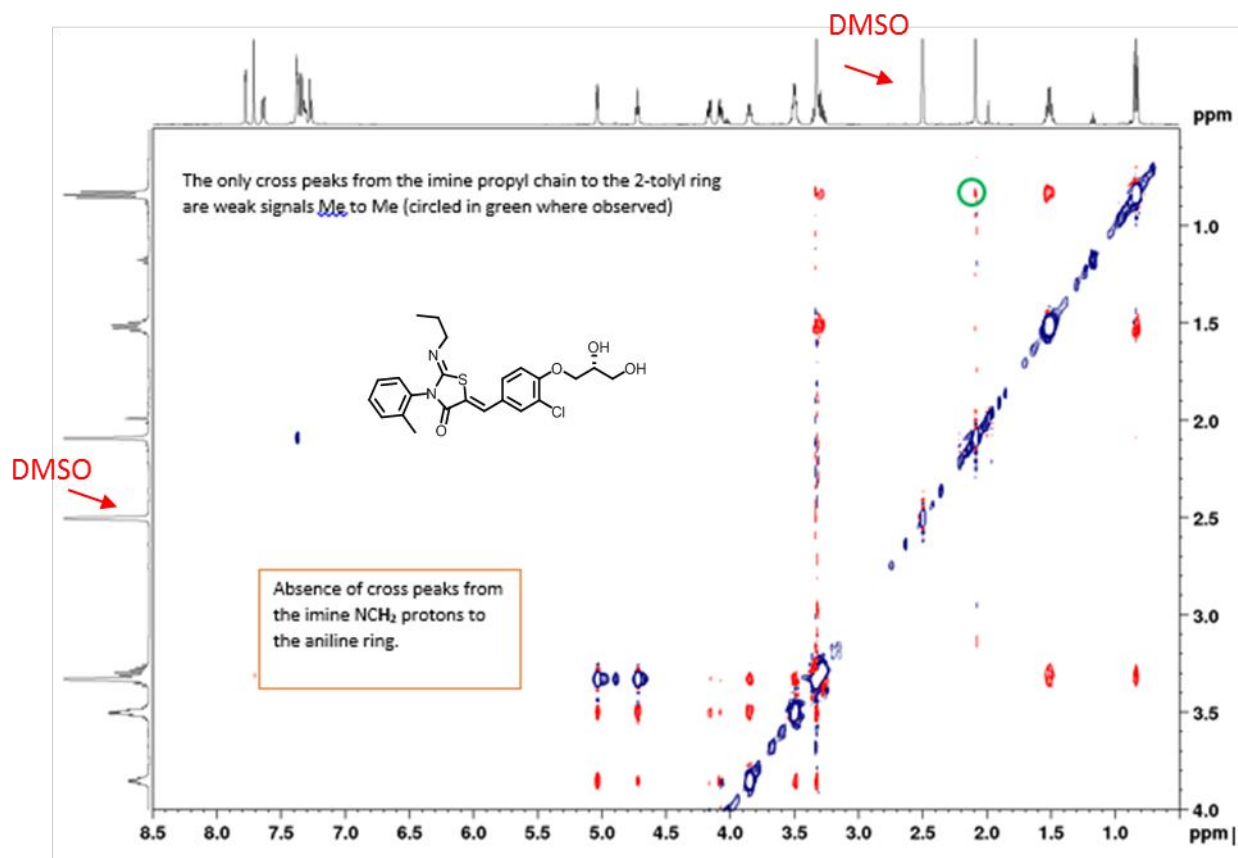
Ponesimod ¹H NMR (500 MHz, DMSO-d₆)



Ponesimod ^{13}C NMR (125 MHz, DMSO-d_6)



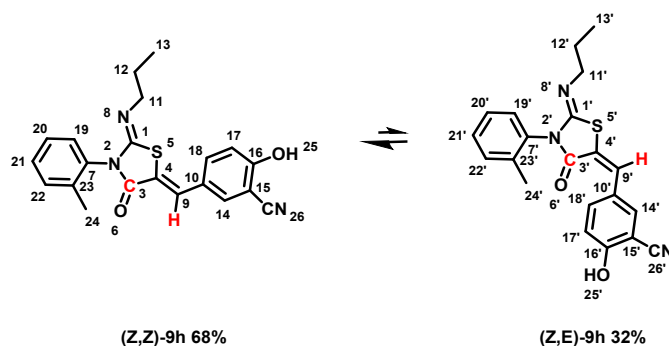
Ponesimod HMBC (DMSO-d_6): ^1H - ^{13}C coupling of H^9 - C^3 (6.4 Hz)

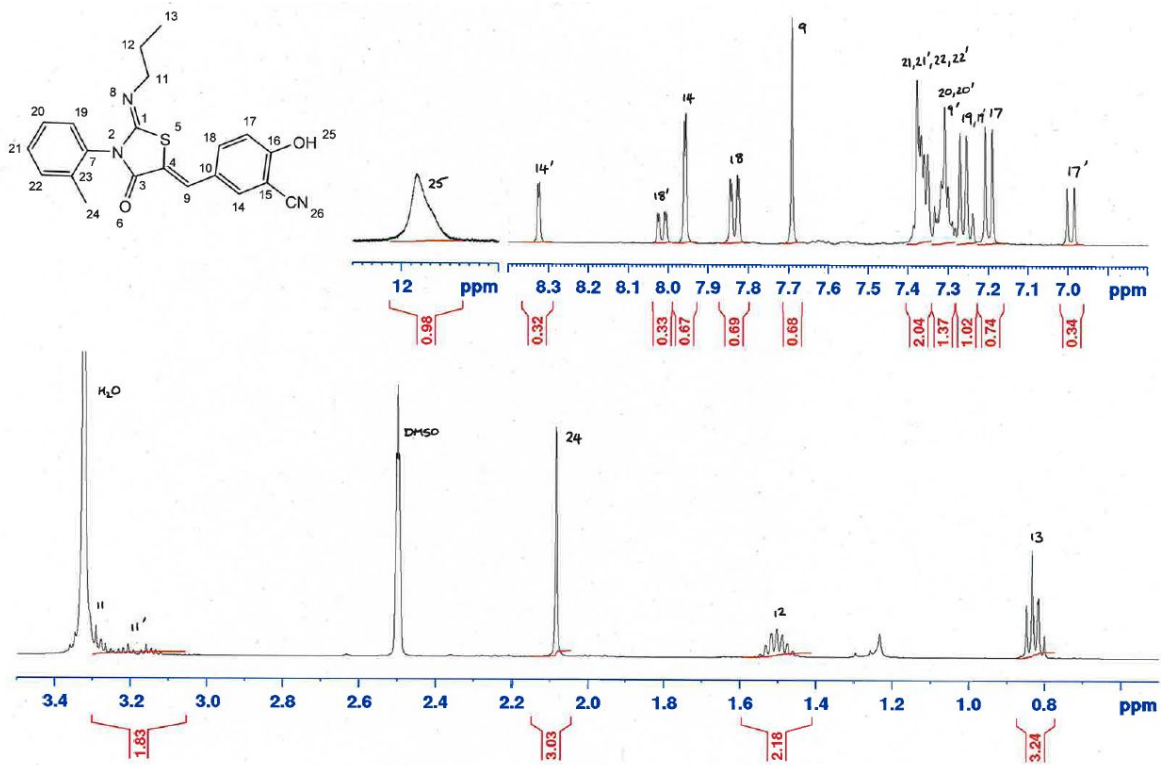


Ponesimod NOESY (DMSO-d₆)

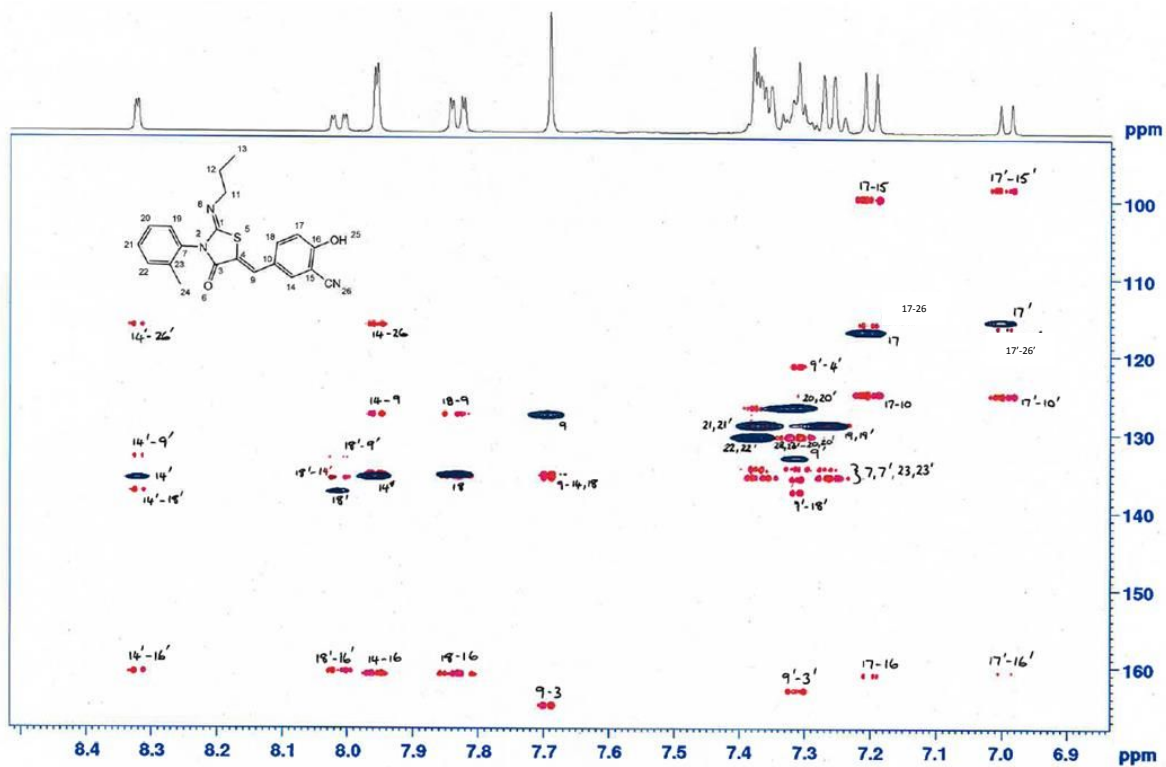
NMR spectra of tautomer mixture of (Z,Z)-**9h** (Z,E)-**9h**

We conducted NMR studies of compound **9h** after incubation in DMSO-d₆ for 6 months. HMBC experiments measuring the three bond coupling constant between H⁹-C³ 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.

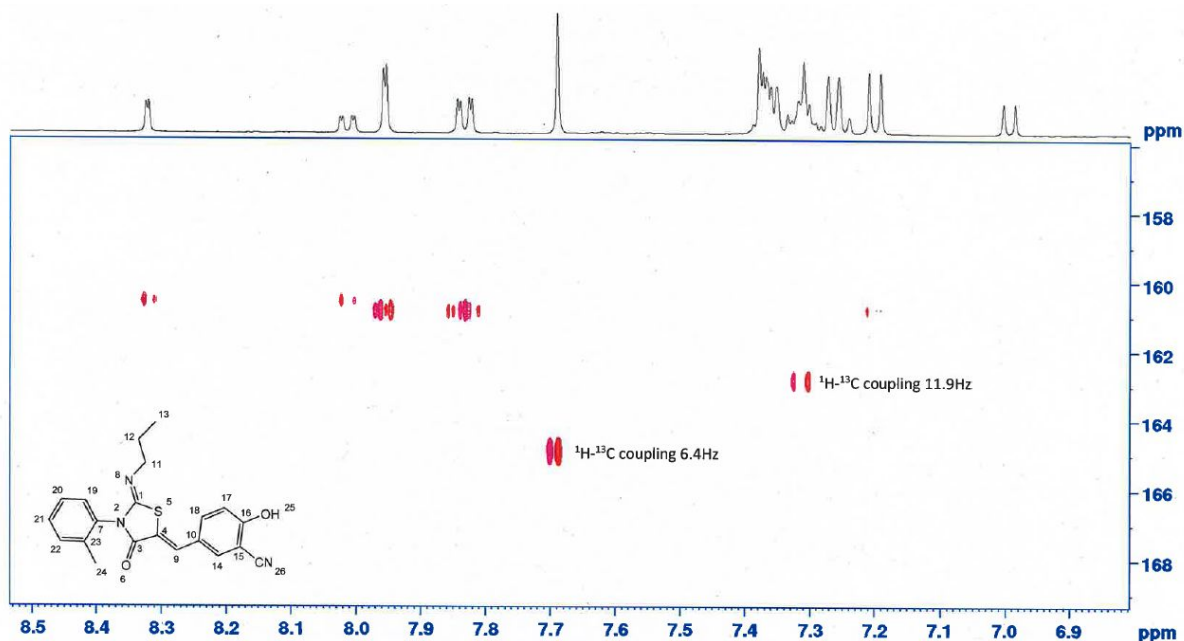




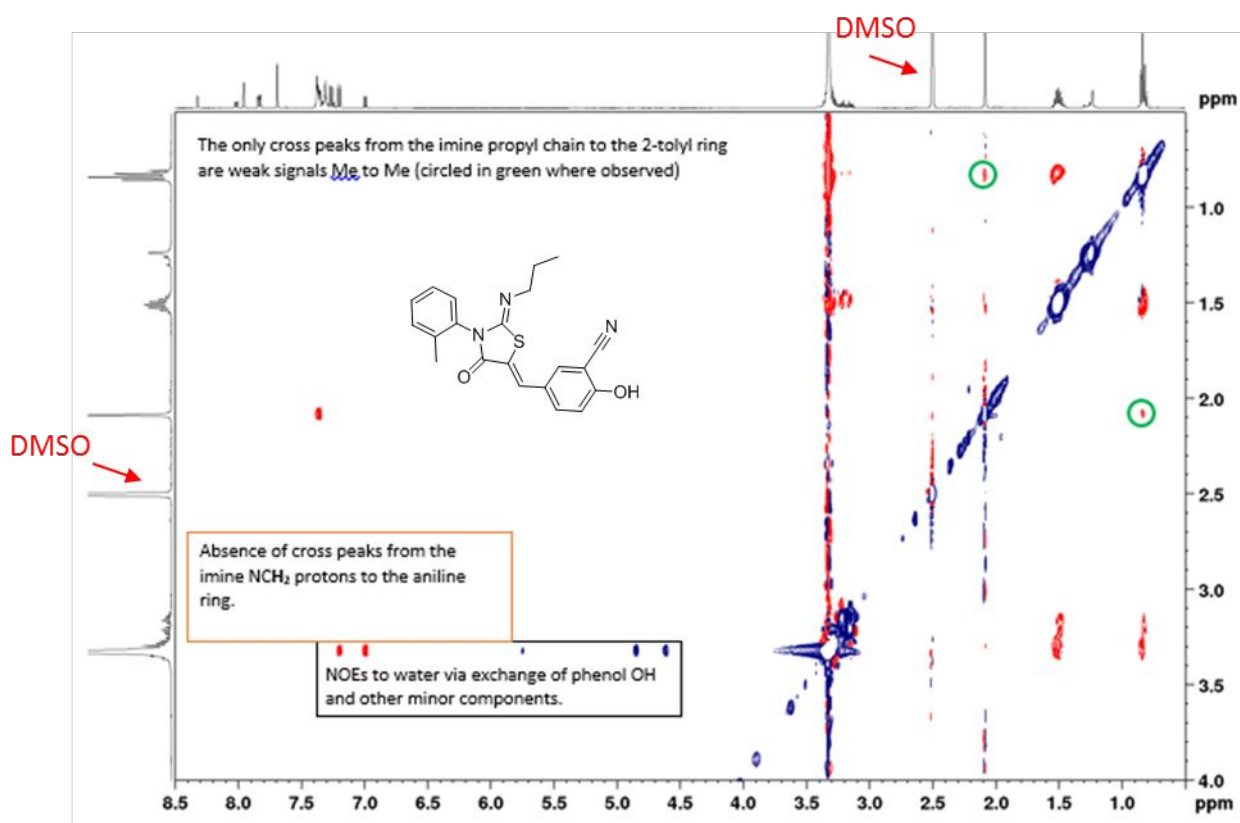
(Z,Z)-9h 68%, (Z,E)-9h 32% ¹H NMR (500 MHz, DMSO-d₆)



(Z,Z)-**9h** 68%, (Z,E)-**9h** 32% HSQC (Blue) H₁ MBC (Red) (DMSO-d₆): (Z,Z)-**9h** ¹H-¹³C coupling of H⁹-C³ (6.4 Hz), (Z,E)-**9h** ¹H-¹³C coupling of H⁹-C^{3'} (11.9 Hz).



(Z,Z)-**9h** 68%, (Z,E)-**9h** 32% HMBC (DMSO-d₆) expansion: (Z,Z)-**9h** ¹H-¹³C coupling of H⁹-C³ (6.4 Hz), (Z,E)-**9h** ¹H-¹³C coupling of H⁹-C^{3'} (11.9 Hz).



(*Z,Z*)-**9h** 68%, (*Z,E*)-**9h** 32% NOESY (DMSO- d_6)

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**

