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

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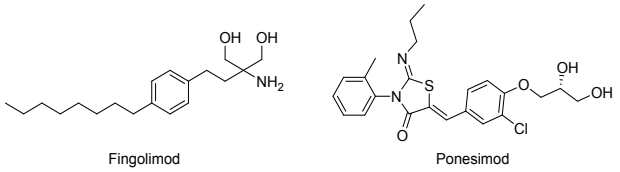
KEYWORDS. S1PR, soft-drug, plasma stability, topical tool compound.

ABSTRACT: In order to study the role of S1PRs in inflammatory skin disease, S1PR modulators are dosed orally and topically in animal models of disease. The topical application of S1PR modulators in these models may however lead to systemic drug concentrations, which can complicate interpretation of the observed effects. We set out to design soft drug S1PR modulators as topical tool compounds to overcome this limitation. A fast follower approach starting from the drug ponesimod allowed the rapid development of an active phenolic series of soft drugs. The phenols were however chemically unstable. Protecting the phenol as an ester removed the instability and provided a compound that is converted by enzymatic hydrolysis in the skin to the phenolic soft drug species. In simple formulations, topical dosing of these S1PR modulators to mice led to micromolar skin concentrations but no detectable blood concentrations. These topical tools will allow researchers to investigate the role of S1PR in skin, without involvement of systemic S1PR biology.

Sphingosine-1-phosphate receptor (S1PR) agonists, such as fingolimod and ponesimod (Figure 1), initially activate S1P receptors, but subsequently trigger receptor internalisation and down regulation of signalling; shutting down the sphingosine-1-phosphate signalling pathway. Fingolimod was approved in 2010 for the treatment of relapsing/remitting multiple sclerosis and is the only S1PR agonist approved to date.[1] It is efficacious at low doses (0.5 mg/day) and at low steady state systemic concentrations (C_{max} 3.1 ng/mL). Recently the potential for the S1PR pathway to be of therapeutic use in the treatment of a range of diverse inflammatory skin diseases has emerged.[2-6] Some studies have explored the skin biology of S1PR agonists by topical application of these compounds in various animal models of diseases such as atopic dermatitis,[4] allergic dermatitis[5] and psoriasis.[6] Topical application can however also lead to systemic effects. Following penetration through the stratum corneum, drugs will eventually distribute into the vasculature. If the rate of absorption exceeds the rate of elimination, topical dosing will lead to systemic drug exposure. Topical dosing of potent drugs, such as fingolimod, may lead to sufficient systemic drug concentrations to elicit measureable biological effects, complicating the interpretation of such studies.



Fingolimod Ponesimod

Figure 1. Selected S1PR modulators.

In order to remove the potential for systemic exposure, we decided to develop a soft drug S1PR modulator.[7,8] Soft drugs are locally active, in this case in the skin, but are designed to undergo rapid systemic metabolism to metabolites, which are either inactive or rapidly cleared from systemic circulation.[9] Due to ease of access of the diseased organ, many dermatological diseases are ideally suited to treatment with topical soft drugs, which can safely engage biological targets, previously shown to lead to adverse side effects, following oral dosing.

In their paper describing ponesimod's discovery, Bolli et al. disclosed that phenols, such as compound **4a**, although active were unsuitable for progression, as an oral drug, due to high clearance in both *in vitro* and *in vivo* experiments.[10] The authors speculated that the high clearance may be due to the fact that phenols are well-known substrates for phase 2 metabolism conjugating enzymes.[10] Glucuronidation is a common phase II metabolism pathway that covalently conjugates glucuronic acid, in a base-catalysed process from UDPGA (uridine-50-diphosphoglucuronic acid) to lipophilic substrates via UGT enzymes (uridine-50-diphosphoglucuronosyl transferases).[11] Sulfation, another common phase II metabolism pathway, covalently links a substrate to a sulfo group (SO3), usually derived from 3'-phosphoadenosine-5'-phosphosulfate (PAPS), via sulfotransferase enzymes.[12] As the glucuronide and sulfate metabolites are highly polar, and therefore water-soluble, they subsequently undergo renal or biliary elimination. Due to their affinity for phase II metabolism, phenols are commonly used motifs when designing soft drugs.[13,14] There is little evidence of clinically relevant drug-related inhibition of glucuronidation or sulfation, so the risk of drug-drug interactions is considered to be low.[15] Accordingly we set out to utilise phase II metabolism pathways as the major routes of clearance for our S1PR agonist soft drugs.

Although **4a** had been shown to be rapidly cleared, which was confirmed in our hands (Table 1), the compound displayed poor aqueous solubility. Aqueous solubility is an important parameter for topically applied drugs as it can support use in a higher water content formulations, such as a creams, which may be preferred by patients over oily formulations like ointments. We therefore set out to improve the aqueous solubility of **4a**.

Keeping the 3-chloro-4-hydroxybenzylidene motif from **4a** constant we synthesised a series of phenols with different substituents to replace the 2-tolyl **4a** motif with aromatic or

aliphatic groups (Scheme 1). Using Method A the appropriate aniline was reacted with 2-chloroacetyl chloride to give the corresponding 2-chloro-*N*-phenylacetamide, which was condensed with 1-isothiocyanatopropane to give the required thiazolidinone core **2a,b**. Subsequent condensation with 3-chloro-4-hydroxybenzaldehyde **3**, generated compounds **4a,b**. Compounds **4c-g** with aliphatic R^1 groups used Method B, where amines **1c-g** were reacted with 1-isothiocyanatopropane, then with 2-bromoacetyl bromide in the same reaction vessel. The resulting thiazolidinone cores **2c-g** were condensed with 3-chloro-4-hydroxybenzaldehyde **3** and the products **4c-g** obtained using preparatory HPLC. **4h** was prepared by BBr_3 demethylation of the anisole **4b** to give the corresponding phenol.

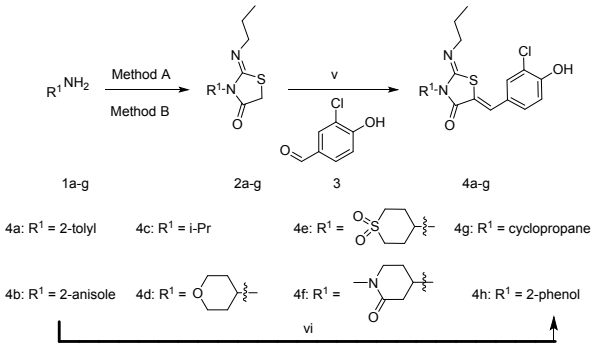
Compounds **9a-9e** replaced the *n*-propyl group of **4a** with several small *N*-linked aliphatic substituents, while compounds **9f-l** looked at effects of substituents on the 4-hydroxybenzylidene group (Scheme 2). The appropriately substituted thiazolidin-4-one core **7a-d** was synthesised utilising a one-pot, two step reaction. Alkyl amines were reacted with 1-isothiocyanato-2-methylbenzene **5** to give the resulting thiourea **6a-d** which was condensed with 2-bromoacetyl bromide, followed by addition of pyridine to furnish the desired thiazolidin-4-one. The thiazolidin-4-one cores **6a-d** were condensed with the 4-hydroxybenzaldehyde **3** to give **9a-d**. **9e** was synthesised by treatment of **9c** with BBr_3. **2a** was reacted with **8f**,**g**,**i-l** to furnish products **9f**,**g**,**i-l**. **9h** was synthesised using a Negishi coupling with dicyanozinc and palladium tetrakis from **9f**.

The configuration of the double bonds in ponisimod and **4a** were determined by X-ray crystallography.[10] The HMBC and NOESY data of ponesimod and **4a** were compared with **9k** and **10a** (see supporting information). The HMBC data for the alkene proton to the carbonyl carbon (H^9-C^3 or $H^{9'}$-$C^{3'}$) in all cases was consistent and suggested a *Z* double bond arrangement of the alkene bond (the size of the ^1H-^{13}C coupling constant was estimated to be 6-7 Hz). The only cross peaks observed in the NOESY experiments were between the 2-tolyl and imine groups. These weak signals between the respective methyl groups (see supporting information) were also observed for ponesimod, **4a**, **9k** and **10a**. It may be expected that if the imine was in the *E* configuration that there would have been cross peaks observed between the methyl of the 2-tolyl group and the NCH_2 protons of the imine group, however this was not observed. Taken together, the data was consistent with the *Z* configuration observed using X-ray crystallography but did not confirm it. Based on the analysis of analogous compounds **4a-h**, **9a-l** and **10a-i** were assigned to the *Z*,*Z*-isomer, unless stated otherwise.

Compounds **4c-h** and **9a-e** were designed to improve solubility by reducing logD or aromatic ring count.[16] Although the CHIlogD values were lower or equivalent for **4d**, **4e**, **4g** and **9a-e**, the compounds did not show an improvement in aqueous solubility (Table 1). Reducing the aromatic ring count in **4c-e** and **4g** also failed to improve aqueous solubility, while **4f** gave an improvement in aqueous solubility 250 µM possibly due to a 3-log unit reduction in CHIlogD, but had a pIC_{50} of <6.0. The addition of a 2-phenol group into the R^1 position, compound **4h**, lowered the CHIlogD by 1.1 units and improved aqueous solubility to 220 µM. Two of the changes to the 2-propylimino group showed an improvement in aqueous solubility. **9a** with a 2-oxetan-3-ylimino group moderately increased solubility to 150 µM, compared to 79 µM for **4a**. **9e** gave an improvement in aqueous solubility (>250 µM) presumably due to the addition of the polar hydroxyl-group and the commensurate reduction in CHIlogD, but unfortunately the compound had a pIC_{50} of <6.0. The fact that **9a** improves aqueous solubility and was equipotent identifies the 2-oxetan-3-ylimino group as a potentially useful change to incorporate in the design of future compounds.

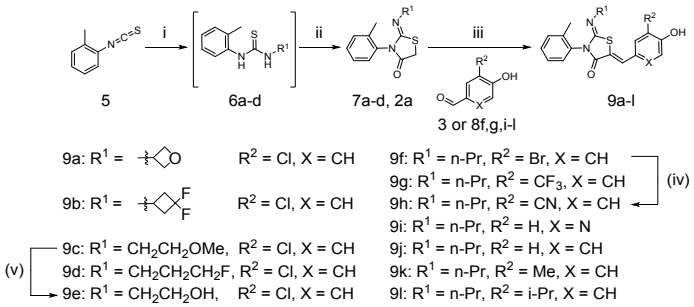
Having examined two of the vectors off the thiazolidin-4-one core we turned our attention to the benzylidene substituent to optimise activity, aqueous solubility and hepatic metabolism. For reason of synthetic expediency, we kept the 2-tolyl and *n*-propyl groups in place with the intention of combining the optimum substituents in subsequent design rounds. We therefore synthesised a series of phenols (**9f-9l**) using the method shown in Scheme 2. **9f-9l** contained a range of electron withdrawing and donating groups ortho to the 4-phenol of the benzylidene substituent. **9f**, **9g** and **9i-9k** were largely equipotent to **4a**, while **9h** and **9l** had a pIC_{50} of <6.0, presumably in the case of **9l** due to increased steric bulk (Table 2). The trifluoromethyl group of **9g** had low aqueous solubility, while **9f** and **9h-9l** had acceptable solubility.

Scheme 1.



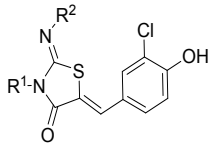
Method A (i) 2-chloroacetyl chloride, TEA, THF, -78 ˚C to RT, 2h (ii) 1-isothiocyanatopropane, NaH, DMF, RT, 16h. Method B (iii) 1-isothiocyanatopropane, CH_2Cl_2, RT, 2h (iv) 2-bromoacetyl bromide, pyridine, CH_2Cl_2, 0 ˚C to RT, 1h (v) NaOAc, AcOH, 65 ˚C, 16h (vi) BBr_3, CH_2Cl_2, -70 ˚C to 0 ˚C, 3h.

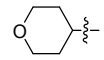
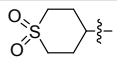
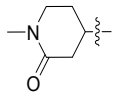
Scheme 2.



(i) R_2NH_2, CH_2Cl_2, RT, 1h (ii) 2-bromoacetyl bromide, pyridine, CH_2Cl_2, 0 ˚C to RT, 2h (iii) NaOAc, AcOH, 65 ˚C, 16h (iv) dicyanozinc, $Pd(PPh_3)_4$, DMA 100 ˚C, 1.5h. (v) BBr_3, DCM, -78 ˚C, 3h then 0 ˚C, 3h.

Table 1. Optimisation of the thiazolidinone core.

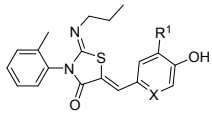


Compound	R^1	R^2	CHIlogD[b]	Kinetic Solubility (μM)[c]	H S1PR1 pIC_{50}[d]
4a	2-tolyl	*n*-Pr	3.8	79	7.4
4b	2-anisole	*n*-Pr	3.3	79	7.2
4c	*i*-Pr	*n*-Pr	>4.3	20	6.8
4d		*n*-Pr	3.7	70	7.4
4e		*n*-Pr	2.6	75	6.7
4f[a]		*n*-Pr	0.6	>250	<6.0
4g	cyclopropane	*n*-Pr	3.4	79	6.2
4h	2-phenol	*n*-Pr	2.7	220	6.6
9a	2-tolyl		2.6	150	7.3
9b	2-tolyl		3.6	79	6.7
9c	2-tolyl	CH_2CH_2OMe	2.9	110	6.3
9d	2-tolyl	$CH_2CH_2CH_2F$	3.4	20	7.6
9e	2-tolyl	CH_2CH_2OH	1.9	>250	<6.0

[a]racemic mixture. [b]Reverse-phase HPLC method to determine the chromatographic hydrophobicity index (CHI): n of 1. [c]The aqueous kinetic solubility of the test compounds was measured using laser nephelometry: n of 1. [d]Human S1PR1 activity was measured using a human PathHunter β-Arrestin recruitment assay. All pIC_{50}s reported in this table correspond to $n \geq 2$, reported as their geometric mean.

As soft drugs must be rapidly cleared systemically and phenols commonly undergo phase 2 metabolism, we used human hepatocytes (H Heps) to study this potential route of metabolism. We sought to obtain clearance rates of greater than 85% human liver blood flow (>4.8 mL/min/g); data shown in Table 2. We then measured intrinsic clearance in human liver microsomes (HLM) to determine if phase 1 metabolism was contributing to the observed intrinsic clearance in hepatocytes. As glucuronidation is a base-catalyzed process, where conserved carboxylate and histidine residues facilitate the deprotonation of the phenol, we expected to see an effect of the pK_a of the phenolic hydrogen on the rate of hepatic clearance.[14] We explored the effect of the phenol pK_a on hepatic clearance with a set of *ortho*-substituents and a *meta*-pyridine (Table 2). Electron-withdrawing groups did reduce the pK_a of the phenol **4a** and **9f-i** have increased hepatic clearance rates vs unsubstituted **9j**. However, weakly electron-donating groups demonstrated an even greater increase in hepatic clearance rates (**9k** and **9l**) despite the expected increase in pKa. For this phenolic scaffold *ortho*-substituents led to an increase in glucuronidation rate in all cases and was independent of phenolic pK_a.

Table 2. Effect of substituents on the phenol.



Compound	R^1	X	H S1PR1 pIC_{50}[a]	Kinetic Solubility (μM)[b]	pK_a[c]	HLM Cl[d]	H Heps Cl[e]	Stability[f]	CHI logD[g]
4a	Cl	CH	7.4	79	6.8	1.6	8.4	6	3.8
9f	Br	CH	7.7	79	6.4	1.6	6.0	16	3.9
9g	CF_3	CH	7.5	14	6.6	1.0	4.2	10	3.8
9h	CN	CH	<6.0	220	-	-	<0.5	20	-

9i	H	N	7.0	110	7.1	2.8	7.9	4	3.0
9j	H	CH	7.1	79	8.3	2.4	1.7	0	3.5
9k	Me	CH	7.6	78	8.5	2.8	31	3	3.8
9l	*i*-Pr	CH	<6.0	79	8.6	-	12	3	4.3

[a]Human S1PR1 activity was measured using a human PathHunter β-Arrestin recruitment assay. All pIC_{50}s reported in this table correspond to n ≥ 2, reported as their geometric mean. [b]The aqueous kinetic solubility of the test compounds was measured using laser nephelometry: n=1. [c]pK_a was determined using a potentiometric fast UV-metric titration method: n=1. [d]Intrinsic clearance in human liver microsomes (mL/min/g): n=1. [e]Intrinsic clearance in human liver hepatocytes (mL/min/g): n=1. [f]% decrease in purity when stored in DMSO solution for 28 days: n=1. [g]Reverse-phase HPLC method to determine the chromatographic hydrophobicity index (CHI): n=1.

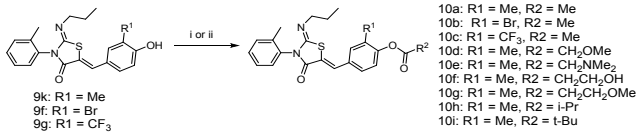
Although several compounds shown in Table 2 satisfy the rapid clearance requirements of a soft drug and retain primary activity, none were suitable for progression into *in vivo* studies due to chemical stability liabilities. Analysis of the originally pure compounds shown in Table 1, after being in DMSO solution for 28 days showed a range of purities. Compounds with electron-withdrawing groups *ortho* to the phenol (**4a**, **9f-h**) were the least stable with a 6-20% impurity formed over 28 days. Compounds with neutral or donating groups in the *ortho* position (**9j-l**) were more stable, in some cases giving compounds which were stable over a 28 day period (**9j**). The instability in solution represented a major development hurdle as topical drugs are usually stored in solution or suspensions (cream, ointment, paste, lotion or gels), rather than in solid form, as is the case for oral drugs. We turned our attention to identifying the impurity and preventing its formation.

We conducted NMR studies of compound **9h** after incubation in DMSO-d_6 for 6 months (see supporting information for HMBC and NOESY spectra). In that time the impurity had increased from 20% to 32% of the mixture based on the integration of H^{18} vs $H^{18'}$ in the ^{1}H NMR spectrum. The NOESY spectrum of the mixture indicated no changes in the arrangement of the imine (no correlation was observed between H^{11}-H^{19} or $H^{11'}$-$H^{19'}$). 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.

As we expected them to be significantly less active due to the orientation of the phenol group, no examples of (*Z*,*E*) compounds were isolated.

The association between electron withdrawing groups and the rate of the isomerisation could be explained by the requirement for a base catalysed isomerisation mechanism (see supporting information for proposed mechanism). We hypothesised that protecting the phenol via alkylation (as in ponesimod) or acylation would remove the ability of the conjugated pi-system to isomerise the double bond from *Z* to *E*. To test this theory we synthesised compounds **10a-i** via esterification of the parent phenol (Scheme 3). Reaction of phenol **9k**,**f**,**g** with the corresponding acid chloride gave compounds **10a-h**. Reaction of phenol **9k** with dimethylpropanoic acid and DCC gave compound **10i**.

Scheme 3.



(i) Acid chloride (1eq), DMAP (0.05 eq), TEA (1.2eq) in CH_2Cl_2 at rt, 16h (ii) 2,2-dimethylpropanoic acid (1eq), DCC (1.2eq), DMAP (0.2eq), DMF, 40 ˚C, 16h.

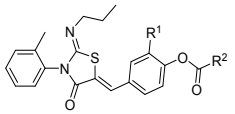
We were delighted to discover that acylation blocked isomerisation and **10a**,**f** shown in Table 3 did not isomerise after being in a DMSO solution for 28 days. Electron-withdrawing groups at R^1 (**10b**,**c**) did lead to a slight decrease in purity (6.3 and 1.4% respectively) over the 28 day duration of this experiment, but the degradation product was due to hydrolysis of the ester to the phenol rather than isomerisation of the double bond. Decomposition studies used ^{1}H-NMR to monitor the increase in the acetic acid methyl group peak over 28 days (see supporting information). **10a** showed no hydrolysis or isomerisation. However, the chemical stability was poor when heteroatoms were alpha to the carbonyl of the acetate group **10d**,**e** and these compound degraded on standing within 24h, preventing full characterisation. Chemically, instability was not an issue when the heteroatoms were in the beta position **10f**,**g**.

We expected the ester to be unstable in skin, which would liberate the phenol to engage the receptor in the target tissue. Determining skin stability using human skin S9 fraction (Table 3) demonstrated compounds **10a-c**,**f**,**g** underwent rapid metabolism. Bulking out the ester with *i*-Pr **10h** or *t*-Bu **10i** gave longer half-lives as expected.

Unfortunately acylation of **9k** led to a decrease in aqueous solubility, for example **10a** (39 μM), **9k** (79 μM). This could be improved by adding polar groups as in **10f** (79 μM), however solubility only improved to the level of phenol **4a**, and is still lower than what is desirable in a topical drug. Our identification of the propensity for the phenol series to isomerise meant they were unsuitable for development as tool compounds or potential drugs. The acylated series does not have the desired solubility of a potential drug, but is suitable for use as a topical tool.

10a was selected for further study, due its ease of synthesis, stability to degradation in solution and instability in skin.

Table 3. Effect of substitution at R^1 and R^2 on skin S9 and chemical stability.



Compound	R^1	R^2	H Skin S9 (half-life min)[b]	Stability (% decrease)[c]
Ponesimod	Cl	-	>180	0

10a	Me	Me	7.3	0
10b	Br	Me	8.8	6.3
10c	CF_3	Me	8.3	1.4
10d	Me	CH_2OMe	-	-[a]
10e	Me	CH_2NMe_2	-	-[a]
10f	Me	CH_2CH_2OH	8.2	0
10g	Me	CH_2CH_2OMe	4.8	-
10h	Me	*i*-Pr	21	-
10i	Me	*t*-Bu	>180	-

[a]Unstable after 24h in DMSO solution: n=1. [b]Stability measured in skin S9 over 180 mins in the presence of enzymatic cofactors: n=1. [c]% loss in purity when stored in DMSO solution for 28 days: n=1.

The selectivity of (*Z*,*Z*)-**10a** and (*Z*,*Z*)-**9k** across S1PR1-4 was determined (Table 4). As with ponesimod,[10] both (*Z*,*Z*)-**10a** and (*Z*,*Z*)-**9k** were most active against S1PR1, with >40-fold and >80-fold selectivity respectively over the other S1PR isoforms measured. (*Z*,*Z*)-**10a** and (*Z*,*Z*)-**9k** were equipotent. The reactivity of the exocyclic double bond of (*Z*,*Z*)-**10a** was examined using a glutathione trapping experiment in human liver microsomes; no evidence of glutathione adducts or derivatives was observed (see supporting information).

Table 4. Selectivity against S1PR1-4.[a]

Compound	S1PR1 pIC_{50}	S1PR2 pIC_{50}	S1PR3 pIC_{50}	S1PR4 pIC_{50}
Ponesimod[b]	8.2	<5.0	7.0	6.0
10a	7.6	<5.0	6.0	<5.0
9k	8.0[c]	<5.0	6.1	<5.0

[a]S1PR1-4 activity was measured using a human PathHunter β-Arrestin recruitment assay (n=2). [b]S1PR1-4 activity reported in the literature using a GTPγS assay.[9] [c]The potency of **9k** on S1PR1 slightly shifted to a higher value in this experiment, which is independent to the experiments performed to establish the SAR (Table 2).

To demonstrate (*Z*,*Z*)-**10a** is a suitable tool for *in vivo* experiments, a topical pharmacokinetic experiment in mice (see supporting information) using 22.5 µL of 1% propylene glycol/ethanol 7/3 formulation was carried out. At 2 and 8 h time points, (*Z*,*Z*)-**10a** concentrations in blood were below the lower limit of quantification (LLoQ). (*Z*,*Z*)-**10a** concentrations in blood were 8.8 µM (2h) and 4.9 µM (8h). (*Z*,*Z*)-**9k** concentrations were below the LLoQ in blood at both time points and 134 µM (2h) and 101 µM (8h) in the skin. (*Z*,*Z*)-**9k** is present in the skin of mice at >10,000-fold above the IC_{50} demonstrating that the modulator is likely to be present at sufficient concentration to inhibit local S1PR1. (*Z*,*Z*)-**10a** is also present in the skin of mice at >350-fold above the IC_{50} and will also be able to locally inhibit S1PR1.

Metabolite identification of (*Z*,*Z*)-**10a** using incubation with human skin S9 fraction confirmed that the expected phenol (*Z*,*Z*)-**9k** was obtained after hydrolysis of the ester group: no other metabolites were observed (Figure 2a). Based on the stability of (*Z*,*Z*)-**9k** in DMSO over 28 days (Table 2) it is likely this hydrolysis is enzymatically driven. We then performed metabolite identification studies using (*Z*,*Z*)-**9k** in human hepatocytes to confirm the routes of clearance of our S1PR1 modulators. As before (*Z*,*Z*)-**9k** isomerises into (*Z*,*E*)-**9k** in solution; Figure 2b shows the disappearance of parent phenol (both (*Z*,*Z*)-**9k** orange and (*Z*,*E*)-**9k** green isomeric forms) and identifies the glucuronide conjugation product, hydroxylation products and hydroxylation with sulfation metabolites.

In conclusion, we have used a fast follower approach to identify several highly cleared and active phenolic S1PR1 modulators. Many of the phenol soft drugs were unstable in solution due to isomerisation. We were able to prevent this isomerisation by acylation of the phenol, to deliver chemically stable chemical tools. The strategy underpinning our S1PR1 soft drug modulators is illustrated in Figure 2c. When (*Z*,*Z*)-**10a** is applied to the skin of mice it should be enzymatically hydrolysed to give (*Z*,*Z*)-**9k**. At this point **9k** can bind to S1PR1 in the epidermis causing receptor internalisation and degradation. (*Z*,*Z*)-**9k** will also start to slowly isomerise to (*Z*,*E*)-**9k**. The mixture of isomers of **9k** would then enter the blood stream and be distributed to the liver, where it would be rapidly metabolised and cleared. The hepatic intrinsic clearance rate for phenol (*Z*,*Z*)-**9k**, 31 mL/min/g (Table 2), would correspond to 97% liver blood flow if there is a good *in vitro* to *in vivo* correlation, predicting that a single pass through the liver could eliminate the majority of the drug, greatly reducing the risk of systemic on-target toxicities, which to date have limited the use of S1PR modulators.

(*Z*,*Z*)-**10a** provides the community with a valuable new tool that will enable targeted studies of S1PR biology in skin, lung or other suitable tissues.

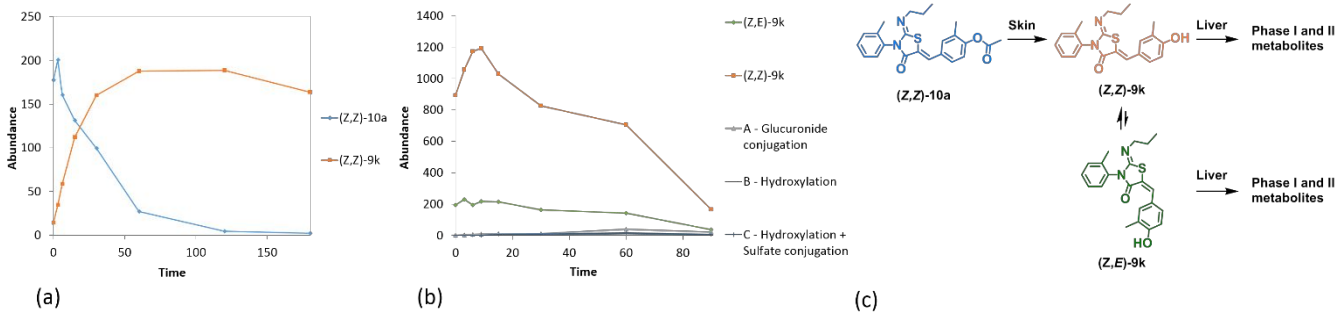


Figure 2. (a) Metabolite identification of (*Z*,*Z*)-**10a** in human skin S9 fraction (n=1). (b) Metabolite identification of (*Z*,*Z*)-**9k** and (*Z*,*E*)-**9k** in human hepatocytes (n=1). (c) Depiction of the enzymatic hydrolysis of (*Z*,*Z*)-**10a** and the hepatic metabolism of (*Z*,*Z*)-**9k** and (*Z*,*E*)-**9k.**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:
Experimental and characterization data for all new Compounds and all biological and DMPK methods (PDF).

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

S1PR, sphingosine-1-phosphate receptor; IL, interleukin; PASI, psoriasis area and severity index; UDPGA, uridine-50-diphosphoglucuronic acid; UGT uridine-50-diphosphoglucuronosyl transferase; HPLC, high pressure liquid chromatography; rt, room temperature; TEA, triethylamine; THF, tetrahydrofuran DMF, dimethyl formamide; CHI, chromatographic hydrophobicity index; HLM, human liver microsomes; DMSO, dimethylsulfoxide; Hz, hertz; DMAP, dimethylaminopyridine; DCC, N,N'-Dicyclohexylcarbodiimide; DMA, Dimethylacetamide; HMBC, Heteronuclear Multiple Bond Correlation; HSQC, Heteronuclear Single Quantum Correlation; NOESY, Nuclear Overhauser Effect Spectroscopy.

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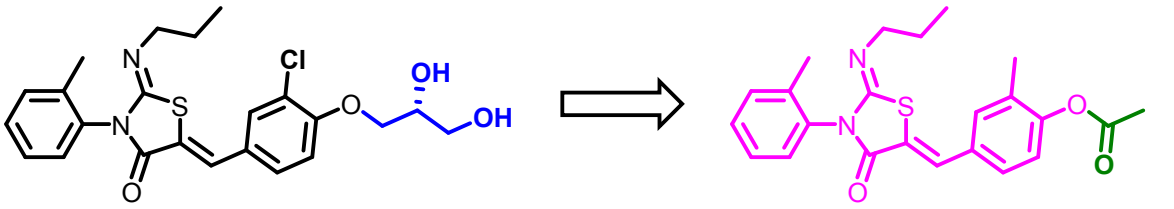

Ponesimod
pIC_{50} 8.0

Soft-drug group (phenol) rapidly cleared in human hepatocytes

Ester rapidly cleared in human skin S9 to active phenol (pIC_{50} 7.6)

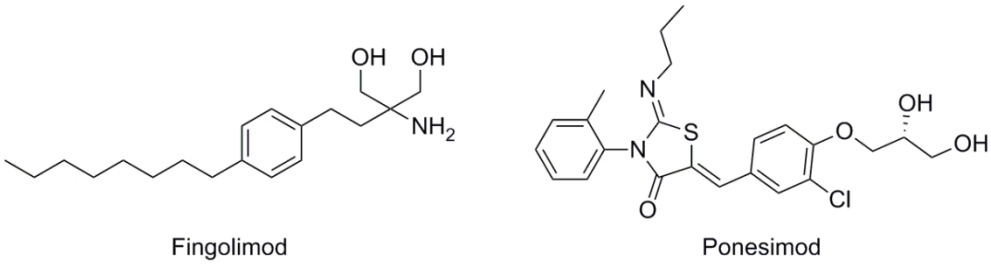

Figure 1

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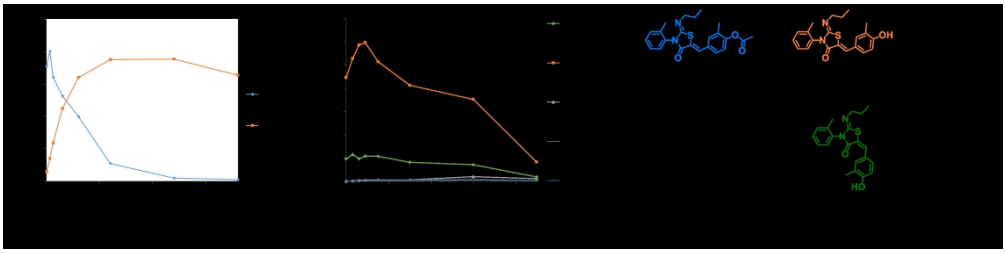

Figure 2

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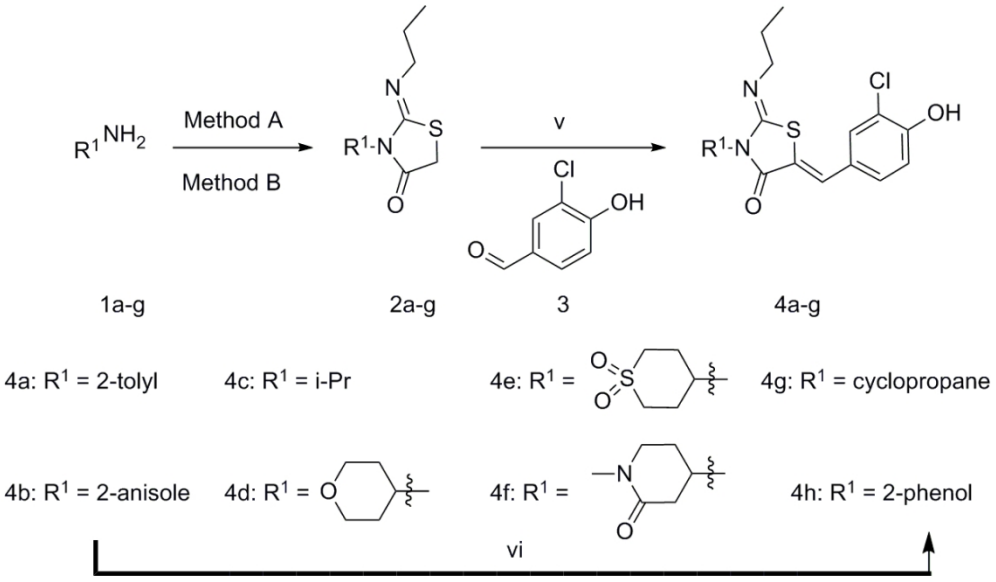

Scheme 1

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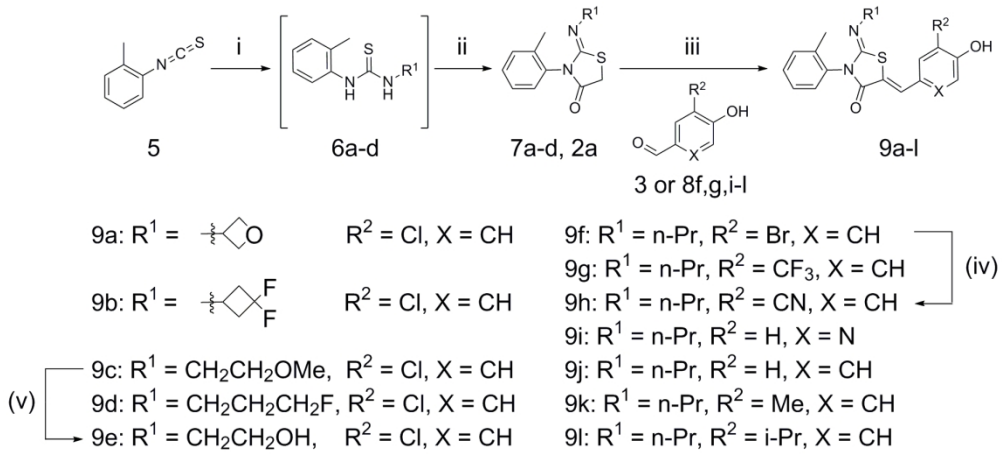

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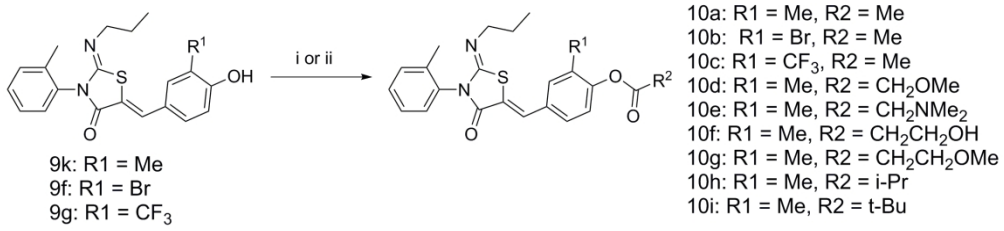

Scheme 3

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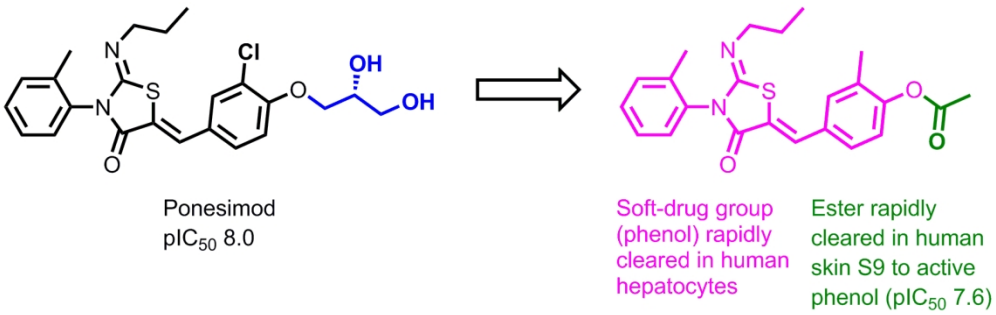

Table of contents graphic

155x49mm (300 x 300 DPI)