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# Discovery of novel *N*-(5-(arylcarbonyl)thiazol-2-yl)amides and *N*-(5-(arylcarbonyl)thiophen-2-yl)amides as potent RORγt inhibitors

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ABSTRACT

Novel series of *N*-(5-(arylcarbonyl)thiazol-2-yl)amides and *N*-(5-(arylcarbonyl)thiophen-2-yl)amides were discovered as potent retinoic acid receptor-related orphan receptor-gamma-t (RORγt) inhibitors. SAR studies of the RORγt HTS hit **6a** led to identification of thiazole ketone amide **8h** and thiophene ketone amide **9g** with high binding affinity and inhibitory activity of Th17 cell differentiation. Compound **8h** showed in vivo efficacy in both mouse experimental autoimmune encephalomyelitis (EAE) and collagen induced arthritis (CIA) models via oral administration.



## 1. Introduction

$CD4^+$ T helper (Th) cells are essential effectors of the immune response and play an important role in inflammation. Th17 cells, a lineage of $CD4^+$ effector T cells characterized by the production of IL-17A and IL-17F, are pathogenic in human autoimmune inflammatory diseases, including multiple sclerosis (MS) and rheumatoid arthritis (RA).[1–5] The presence of IL-17 can be detected in both MS lesions[6,7] and RA synovial fluid.[8,9] Correspondingly, Th17 cells are observed in the infiltrations of mouse experimental autoimmune encephalomyelitis (EAE) CNS and collagen induced arthritis (CIA) inflamed joints. Therapeutic manipulation of Th17 cell activity may have significant implications in the treatment of both autoimmune diseases.

Differentiation and function of Th17 cells are controlled by the transcription factor retinoic acid receptor-related orphan receptor-gamma-t (RORγt).[10,11] It has been shown that the genetic deficiency of RORγt in mice severely impaired Th17 cell differentiation and conferred resistance to EAE.[12] RORγt is a member of the nuclear receptor (NR) superfamily. NRs function by binding to specific *cis*-acting elements on DNA via their DNA-binding domain (DBD). For RORγt, the *cis*-acting element is known as RORE which has been identified in conserved non-coding sequence 2 (CNS2) locating upstream of IL17a in IL17a–IL17f locus. The importance of ROREs for the IL-17a/IL-17f specific genes transcription has been demonstrated.[13] Activity of NRs can be modulated by the binding of ligand to their ligand binding domain (LBD) resulting in the recruitment of transcriptional co-activators (e.g., steroid receptor co-activator 1 or SRC1) or co-repressors (e.g., nuclear receptor co-repressor or NCOR).[10] As far as RORγt is concerned, endogenous hydroxycholesterols were reported to be high-affinity natural ligands for RORγt.[14] The RORγt LBD contains a binding pocket that is suitable for small molecule screening. The development of RORγt modulators has potential utility in controlling the activity of Th17 cells.

A few small molecule inhibitors against RORγt have been reported in literature due to the potential of RORγt as a therapeutic target for the Th17-related autoimmune diseases (Fig. 1).[15–17] digoxin (**1**),[18] SR1001 (**2**)[19] and ursolic acid (**3**)[20] were reported to inhibit RORγt and ameliorate EAE in mice via ip administration. Other small molecular RORγt inhibitors such as SR1555,[21] SR2211 (**4**)[22] and ML209 (**5**)[23] were recently disclosed and showed to suppress Th17 cell differentiation in vitro. However, no small molecule RORγt inhibitors suitable for oral dosing have been reported. In this paper, we report the discovery of novel thiazole/

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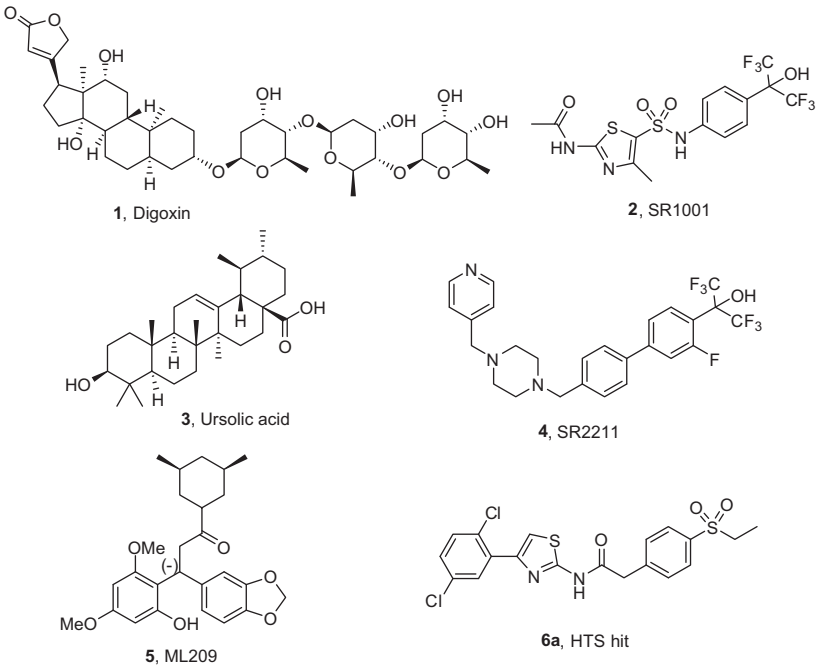



**Figure 1.** Structures of literature RORγt inhibitors (**1**–**5**) and RORγt HTS hit (**6a**).

thiophene ketone amides as potent RORγt inhibitors, of which thiazole ketone amide **8h** demonstrated in vivo efficacy in both EAE and CIA models via oral administration.

## 2. Results and discussion

HTS of the GSK in-house compound collection using a RORγ Fluorescence Resonance Energy Transfer (FRET) assay[24] resulted in the identification of thiazole amide **6a** as a RORγ inhibitor hit with a $pIC_{50}$ of 6.0 (Fig. 1). The binding of **6a** to the RORγt LBD was confirmed with a thermal shift of 7.1 °C in a thermal shift assay[24] and a $pK_i$ of 6.4 in a radioligand binding assay.[25] In the subsequent evaluation in a cell-based assay,[24] compound **6a** was found to inhibit Th17 cell differentiation with 49% of maximum inhibition at 10 μM. On the basis of its in vitro activities, **6a** was used as a chemical starting point for further optimization.

### 2.1. SAR

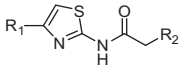
We first explored SAR of the right-hand side (RHS, R2) of the thiazole amide (Table 1). Compounds with RHS alkyl moieties (**6b**–**6d**) were found to be inactive while those with RHS aryls (**6e**–**6g**) started to pick up some RORγt activity. With a sulfonyl group in the *para*-position of the RHS phenyl ring, the RORγt potency improved. Among the sulfones (**6a**, **6h**–**6k**) and sulfonamides (**6l** and **6m**), the ones with straight-chain alkyls showed higher RORγt potency than those with branched alkyls (comparing **6a** with **6j**, and **6l** with **6m**). Fixing the RHS with ethyl sulfonyl, we then explored SAR of the left-hand side (LHS, R1) of the thiazole amide. Similarly, a certain size of LHS moieties such as cyclohexyl (**6p**), phenyl (**6q**) or substituted phenyls (**6r**–**6w**) was needed to maintain RORγt activity. The halogen substitution position on the LHS phenyl ring slightly altered RORγt activity with *ortho* > meta–*para* in general. Among the di-substituted phenyls, 2,3-di-Cl-Ph (**6u**), 2,4-di-Cl-Ph (**6v**) and 2,5-di-Cl-Ph (**6a**) showed better RORγt potency than 2,6-di-Cl-Ph (**6w**), indicating that a certain conformation of the LHS aryl against the central thiazole ring is needed for the best interactions of the receptor and the compound.

In the light of potential conformational effect of LHS moieties on the RORγt activity, we turned our attention to SAR study of 5-substitution on the thiazole ring (Table 2). With increase size of the substituents (**7a**–**7e**), RORγt potency improved in the order of H–Me < Et < *i*-Pr < $CH_2Ph$ < OPh. Tolerance of a heteroatom in the substituent (e.g., O in **7e** vs $CH_2$ in **7d**) encouraged us to install a carbonyl containing moiety in the 5-position of the thiazole ring. Alkyl ketones (**7g**–**7i**) were tolerated and the RORγt potency improved with the increase size of 5-substituents (Me < Et<<Cy–Ph). It was noted that there was 2.4 log unit potency increase from non-substituted thiazole amide **6q** ($pIC_{50}$ = 5.1) to 5-(phenylcarbonyl)thiazole amide **8a** ($pIC_{50}$ = 7.5). Such dramatic potency increase indicates that the conformation of 4-phenyl and 5-phenylcarbonyl on the thiazole ring, mainly enabled by steric hindrance, and their interactions with RORγt LBD play an important role on the RORγt activity.

With above SAR information, we designed and synthesized the compounds with substituents on 4-phenyl (R1) and/or 5-carbonyl-phenyl (R2) of the thiazole amide **8a**, which led to identification of potent RORγt inhibitors (Table 3). Introduction of chloro group at different position of the 4-phenyl ring (**8b**–**8d**) resulted in identification of *m*-Cl-phenyl compound (**8c**) with an increase of RORγt potency. For different substituents in the *meta*-position of the 4-phenyl ring (**8c**, **8e**–**8g**), RORγt potency increased ($CO_2H$ < $CH_2NMe_2$ < CN < Cl) with decreased polarity of the substituents, indicating a hydrophobic interaction existed between the LHS aryl moiety and the hydrophobic pocket of the receptor. Fixing 4-(3-Cl-phenyl) on the thiazole ring and introduction of a chloro or fluoro group at different position of the 5-carbonyl–phenyl ring identified potent thiazole ketone inhibitors (**8h**–**8l**).

Replacement of the thiazole ring with a thiophene ring generally increased RORγt activity (**9a**–**9h**) (Table 3). Thiophene ketone amides have higher c Log *P* and therefore are relatively more hydrophobic than thiazole ketone amides. The binding pocket occupied by the thiazole ring of the ligand is hydrophobic, which may

**Table 1**
SAR of RHS and LHS of the thiazole amide



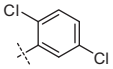
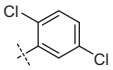
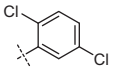
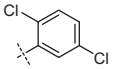
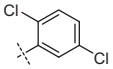
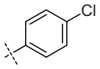
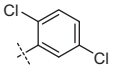
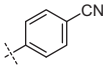
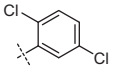
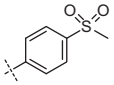
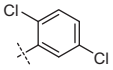
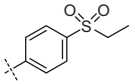
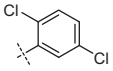
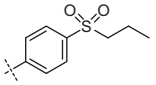
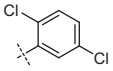
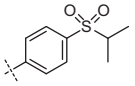
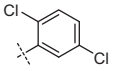
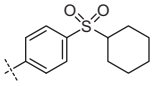
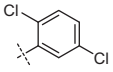
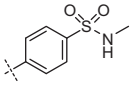
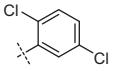
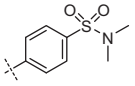
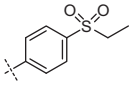
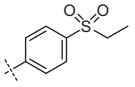
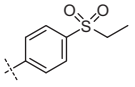
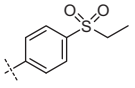
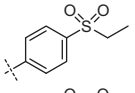
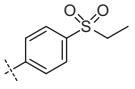
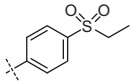
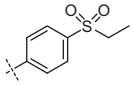
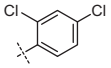
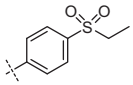
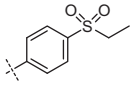
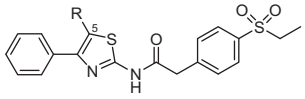

| Compd | R1 | R2 | RORγ FRET $pIC_{50}$[a] (% max inhibition) |
|---|---|---|---|
| **6b** |  | Me | <4.6 |
| **6c** |  |  | <4.6 |
| **6d** |  |  | <4.6 |
| **6e** |  |  | 5.0 (71) |
| **6f** |  |  | 5.1 (80) |
| **6g** |  |  | 5.3 (110) |
| **6h** |  |  | 5.4 (117) |
| **6a** |  |  | 6.0 (116) |
| **6i** |  |  | 5.8 (92) |
| **6j** |  |  | 5.3 (80) |
| **6k** |  |  | <4.6 |
| **6l** |  |  | 5.9 (111) |
| **6m** |  |  | 4.9 (80) |
| **6n** | H |  | <4.6 |
| **6o** | Me |  | <4.6 |
| **6p** |  |  | 5.8 (105) |
| **6q** |  |  | 5.1 (74) |
| **6r** |  |  | 5.7 (116) |
| **6s** |  |  | 5.4 (111) |

**Table 1** (*continued*)

| Compd | R1 | R2 | RORγ FRET $pIC_{50}$[a] (% max inhibition) |
|---|---|---|---|
| **6t** |  |  | 5.4 (100) |
| **6u** |  |  | 6.1 (119) |
| **6v** |  |  | 5.9 (119) |
| **6w** |  |  | 5.2 (120) |

[a] Data are the average of at least two determinations; % max inhibition measured against activation by the surrogate agonist.

**Table 2**
SAR of 5-substitution in the thiazole ring



| Compd | R | RORγ FRET $pIC_{50}$[a] (% max inhibition) |
|---|---|---|
| **6q** | H | 5.1 (74) |
| **7a** | Me | 5.0 (99) |
| **7b** | Et | 5.3 (120) |
| **7c** |  | 5.7 (118) |
| **7d** |  | 6.2 (92) |
| **7e** |  | 6.4 (104) |
| **7f** |  | 5.0 (74) |
| **7g** |  | 5.2 (97) |
| **7h** |  | 5.6 (86) |
| **7i** |  | 7.6 (87) |
| **8a** |  | 7.5 (107) |

[a] Data are the average of at least two determinations; % max inhibition measured against activation by the surrogate agonist.

explain why thiophene ketone amides are somewhat more potent than thiazole ketone amides.

## 2.2. In vitro and in vivo biological evaluations

The binding of the thiazole/thiophene ketone amides to the RORγt LBD was confirmed and their binding affinities were determined by the thermal shift assay as well as the radioligand binding assay. Compounds **8h**, **9g** and **9d** demonstrated thermal shifts of 11.3, 14.8 and 15.3 °C, respectively. In the radioligand binding assay, compounds **8h**, **9g** and **9d** competed against [$^3$H]25-hydroxycholesterol with p$K_i$s of 7.4, 7.5 and 7.6, respectively.

Our next step was to explore our compounds' effects in Th17 cell differentiation system in vitro. Purified CD4+ T cells from mouse were differentiated in Th17 culture conditions in the absence or presence of compounds. Production of IL-17 in the cultures was determined by ELISA. Thiazole ketone amides were found to inhibit the production of IL-17 in mouse generally with $pIC_{50}$s from 5 to 7, about one log unit lower than that obtained by the FRET assay (Table 3). For example, compound **8h** showed a FRET $pIC_{50}$ of 7.8 and a Th17 $pIC_{50}$ of 6.7. Thiophene ketone amides, on the other hand, showed much higher Th17 potency ($pIC_{50}$s from 7 to 8) than the corresponding thiazole ketone amides (comparing **8c** with **9b**, **8h** with **9d**). For example, compound **9g** demonstrated excellent inhibitory activity on Th17 cell differentiation assay with a $pIC_{50}$ of 7.9 as well as in RORγ FRET assay with a $pIC_{50}$ of 7.8. The reason that thiazole ketone amides showed lower Th17 potency than RORγ FRET potency is unclear and cannot be explained by membrane permeability as both thiazole ketone amides and thiophene ketone amides have reasonably good membrane permeability.

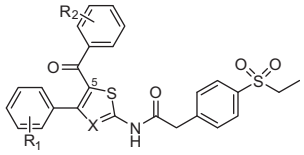
With improved potency, several key compounds were then evaluated for their rodent PK profile (Table 4). Thiazole ketone amide **8h** demonstrated a good PK exposure with oral bioavailability around 48%. Compared to thiazole ketone amides, thiophene ketone amides (e.g., **9d** and **9g**), although having higher RORγ FRET and Th17 potency, showed poor oral exposure with oral bioavailabilities of 3.7% and 4.5%, respectively. Thus, while thiazole ketone amide **8h** was suitable for in vivo pharmacological studies via po administration, thiophene ketone amides **9d** and **9g** could only be evaluated in vivo through ip administration.

With the reasonable oral exposure, we then evaluated **8h** in EAE mice and CIA mice where Th17 cells play a critical role (Fig. 2). Compound **8h** was orally administered twice daily at dose of 100 mg/kg to EAE mice from the day of immunization (Fig. 2A). The treatment resulted in a delay of disease onset and a reduction in clinical severity of EAE in early phase (<20 days). We also examined whether compound **8h** could be effective in treating CIA which also features Th17 pathology. In the CIA model performed on DBA/1 mice where animals were immunized by collagen on day 0 and day 21, compound **8h** was orally administered twice daily at dose of 100 mg/kg to the mice one day before the second collagen immunization (Fig. 2B). The treatment resulted in a delay of disease onset and a reduction in clinical severity of CIA.

## 3. Chemistry

A convergent synthesis of the general structures *N*-(5-(arylcarbonyl)thiazol-2-yl)amides **8** was developed (Scheme 1).[26] Substituted α-bromo ketone **11** was obtained commercially or synthesized through methyl ketone **10**. Protected carbamothioyl

**Table 3**
SAR of thiazole/thiophene ketone amides



| Compd | R1 | R2 | X | RORγ FRET $pIC_{50}$[a] (% max inhibition)[b] | Th17 $pIC_{50}$[a] |
|---|---|---|---|---|---|
| **8a** | H | H | N | 7.5 (107) | 6.5 |
| **8b** | 2-Cl | H | N | 7.4 (90) | 6.4 |
| **8c** | 3-Cl | H | N | 7.9 (100) | 6.9 |
| **8d** | 4-Cl | H | N | 7.4 (102) | 6.1 |
| **8e** | 3-CN | H | N | 7.5 (101) | 6.8 |
| **8f** | $3\text{-}CH_2NMe_2$ | H | N | 6.1 (105) | <5.1 |
| **8g** | $3\text{-}CO_2H$ | H | N | 5.1 (80) | <5 |
| **8h** | 3-Cl | 2-Cl | N | 7.8 (97) | 6.7 |
| **8i** | 3-Cl | 3-Cl | N | 8.0 (85) | 6.6 |
| **8j** | 3-Cl | 4-Cl | N | 7.9 (76) | 6.2 |
| **8k** | 3-Cl | 2-F | N | 7.7 (102) | 6.5 |
| **8l** | 3-Cl | 3-F | N | 7.6 (102) | 6.7 |
| **9a** | H | H | CH | 8.0 (103) | 7.3 |
| **9b** | 3-Cl | H | CH | 7.9 (99) | 8.0 |
| **9c** | 3-CN | H | CH | 7.8 (101) | 7.6 |
| **9d** | 3-Cl | 2-Cl | CH | 8.0 (105) | 7.6 |
| **9e** | 3-Cl | 3-Cl | CH | 8.1 (89) | 7.6 |
| **9f** | 3-Cl | 2-F | CH | 8.2 (106) | 8.0 |
| **9g** | 3-Cl | 3-F | CH | 7.9 (104) | 7.8 |
| **9h** | $3\text{-}CF_3$ | 3-F | CH | 8.0 (104) | 7.9 |

[a] Data are the average of at least two determinations.
[b] % max inhibition measured against activation by the surrogate agonist.

**Table 4**
Mouse PK[a] of the RORγt inhibitors

| Compd | iv | | | | po | | |
|---|---|---|---|---|---|---|---|
| | $t_{1/2}$ (h) | $C_{lb}$ (mL/min/kg) | $V_{ss}$ (L/kg) | $DNAUC_{0\geqslant\infty}$ (ng h/mL) /(mg/kg) | $C_{max}$ (ng/mL) | $DNAUC_{0\geqslant\infty}$ (ng h/mL) /(mg/kg) | $F$ (%) |
| **8h**[b] | 1.0 | 17.8 | 1.24 | 937 | 1547 | 448 | 48 |
| **9d**[c] | 0.79 | 61.4 | 1.85 | 279 | 23.3 | 10 | 3.7 |
| **9g**[c] | 0.65 | 24.0 | 1.10 | 764 | 25.8 | 34 | 4.5 |

[a] Male C57BL/6 mice.
[b] 1 mg/kg (iv) and 10 mg/kg (po).
[c] 1 mg/kg (iv) and 2 mg/kg (po).

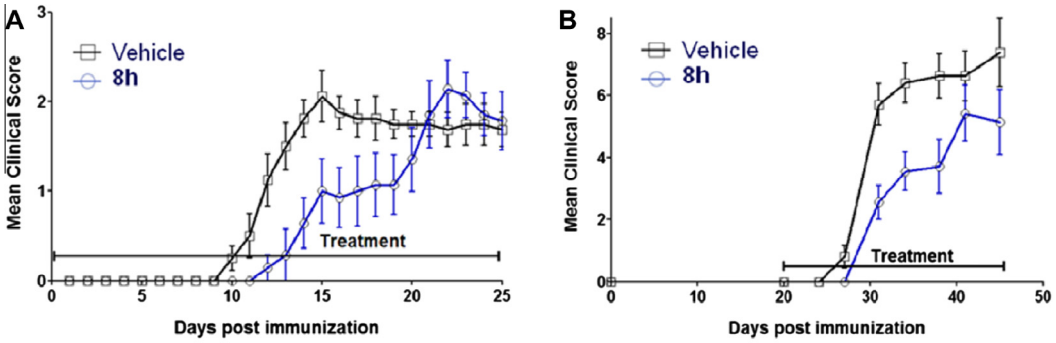
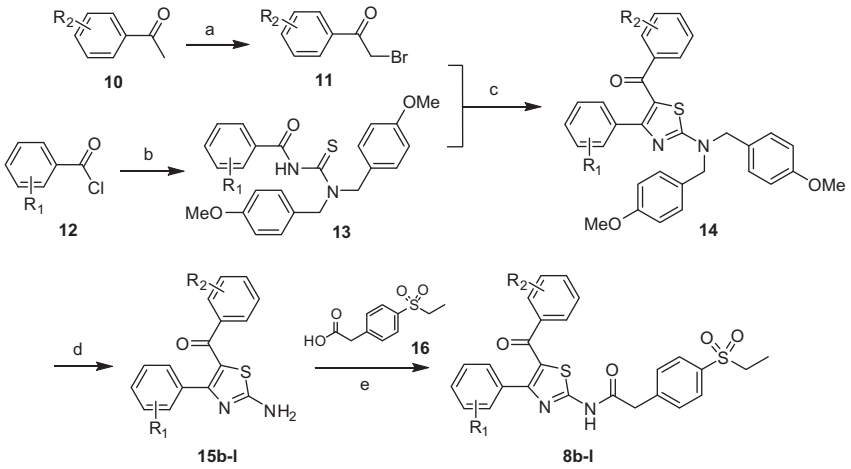


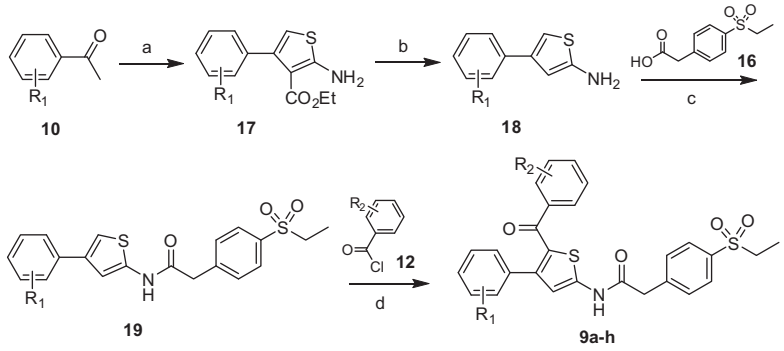

**Figure 2.** (A) Treatment efficacy of compound **8h** in mouse EAE (100 mg/kg, b.i.d, po). (B) Efficacy of compound **8h** in mouse CIA (100 mg/kg, b.i.d., po). Both of EAE and CIA studies have been conducted twice for compound **8h**. The results of the duplicated studies were similar and data of one representative study was showed here.

amide **13** was obtained by condensation of acyl chloride **12**, thiocyanate and bis((4-(methyloxy)phenyl)methyl)amine. Condensation of **11** and **13**, followed by deprotection, afforded the amines **15**, which was then further condensed with acid **16**[26] to produce the desired analogs **8**.

A general synthetic procedure for *N*-(5-(arylcarbonyl)thiophen-2-yl)amides **9** was outlined in Scheme 2.[27] Substituted phenyl methyl ketone **10** was reacted with ethyl 2-cyanoacetate and sulfur in the presence of morpholine to form thiophene ester **17**. Deesterification of **17** provided thiophene amine **18**, which was



**Scheme 1.** Reagents and conditions: (a) $Br_2$, $Et_2O$; (b) bis((4-(methyloxy)phenyl)methyl)amine, $NH_4SCN$, acetone; (c) DMF, 85 °C; (d) TFA, 80 °C; (e) HOBt, EDC, DCM.



**Scheme 2.** Reagents and conditions: (a) ethyl 2-cyanoacetate, sulfur, morpholine, EtOH, reflux; (b) NaOH, ethanol, reflux; (c) EDC, HOBt, DCM; (d) tin(IV) chloride, DCE, reflux.

then coupled with acid **16** to afford thiophene amides **19**. The acylation of **19** with acid chloride **12** catalyzed by $SnCl_4$ produced the desired compounds **9**.

## 4. Conclusions

In summary, we have discovered novel series of *N*-(5-(arylcarbonyl)thiazol-2-yl)amides and *N*-(5-(arylcarbonyl)thiophen-2-yl)amides as potent RORγt inhibitors. SAR studies of the RORγt HTS hit **6a** led to identification of thiazole ketone amide **8h** and thiophene ketone amide **9g** with high binding affinity and inhibitory activity of Th17 cell differentiation. Compound **8h** showed in vivo efficacy in both mouse EAE and CIA models via oral administration. These novel RORγt leads can be used as excellent tool compounds for target validation and related biological studies. Further optimization of the series to improve PK and other developability profile is on-going.

## 5. Experimental

### 5.1. Chemistry

#### 5.1.1. General

Compounds not described below were purchased from commercial vendors or previously reported or reported in Supporting information section. Compound purity was determined using LC–MS analysis. Purification of the compounds was carried out by conventional methods such as chromatography and/or recrystallization using suitable solvents. Chromatographic methods include column chromatography, flash chromatography, and MDAP (mass directed autopurification system). $^1$H NMR spectra were recorded on a Bruker 400 NMR spectrometer operating at 400 MHz. $^{13}$C NMR spectra were recorded at 101 MHz. $CDCl_3$ is deuteriochloroform, and DMSO-$d_6$ is hexadeuteriodimethylsulfoxide. Chemical shifts are given in parts per million ($\delta$) downfield from the internal standard tetramethylsilane (TMS) or the NMR solvent. Abbreviations for NMR data are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, app = apparent, br = broad. J indicates the NMR coupling constant measured in hertz (Hz). High resolution mass spectrometry (HRMS) was operated in positive mode of electrospray ionization (ESI) at an orthogonal acceleration time-off light (oa-TOF) SYNAPT G2 HDMSTM (Waters, Manchester, U.K.). Solutions (500 ng/mL in acetonitrile/$H_2O$ (1:1, v/v)) were introduced via infusion at a flow rate of 5 μL/min to acquire accurate mass. LC–MS (Agilent 1200SL-6110 for acidic LC–MS and Agilent 1200SL-6140 for basic LC–MS) analysis was conducted for all assayed compounds in either acidic or basic conditions. (1) Acidic conditions refer to water containing 0.05% TFA/acetonitrile as mobile phase on an Agilent SB-C18 column (1.8 μm, 4.6 mm × 30 mm) with mass spectra instrument and photodiode array detector. The following conditions were used: a gradient from 5% to 95% in 5 min and held at 95% for 1 min; UV detection at 214 and 254 nm; a flow rate of 1.5 mL/min; full scan; mass range from 100 to 1000 amu (atomic mass unit). (2) Basic conditions refer to water containing 10 mM aqueous $NH_4HCO_3$/

acetonitrile as mobile phase on a Waters XBridge C18 column (3.5 μm, 4.6 mm × 50 mm) with mass spectra instrument and photodiode array detector. The following conditions were used: a gradient from 5% to 95% in 5 min and held at 95% for 1 min; UV detection at 214 and 254 nm; a flow rate of 2 mL/min; full scan; mass range from 100 to 1000 amu. All the assayed compounds possess ⩾95% purity determined using LC–MS analysis. Column chromatography was performed on Isco or Biotage instrument using a prepacked silica gel column, a detector with UV wavelength at 254 nm, and mixed solvents. MDAP equipped with 2489 UV detector, 2767 sample manager, 2545 pump, and 3100 single quadrupole mass spectrometer was performed on Sunfire Prep C18 column (5 μm, 19 mm × 50 mm) using water containing 0.05% TFA/acetonitrile as mobile phase. The following conditions were used: a gradient from 5% to 95% in 15 min and held in 95% for 3 min; a flow rate of 30 mL/min.

#### 5.1.2. *N*-(5-(2-Chlorobenzoyl)-4-(3-chlorophenyl)thiazol-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (8h)

To a solution of 1-(2-chlorophenyl)ethanone (**10b**, 3 g, 19.4 mmol) in $Et_2O$ (30 mL) cooled at 0 °C was added bromine (1.0 mL, 19.4 mmol) dropwise and the resulting mixture was stirred at room temperature for 1.5 h. Solvent was removed in vacuo to afford 2-bromo-1-(2-chlorophenyl)ethanone (**11b**, 4.6 g, 82%) as a brown oil which was used directly in the next step without further purification: MS($ES^+$) *m*/*z* 232.9, 234.9 $(M+H)^+$.

A mixture of 1-[4-(methyloxy)phenyl]methanamine (2.6 g, 19.0 mmol) and 4-(methyloxy)benzaldehyde (3.10 g, 22.7 mmol) in methanol (50 mL) was heated to reflux for 3 h, then cooled to 0 °C, $NaBH_4$ (1.08 g, 28.4 mmol) was added to the reaction portionwise and the resulting mixture was stirred at room temperature overnight. Solvent was removed under reduced pressure and the residue was partitioned between EtOAc and water. The combined organic layers were washed with satd $NaHCO_3$ solution and brine, then dried over anhydrous $Na_2SO_4$. After filtration, solvent was removed in vacuo to afford bis((4-(methyloxy)phenyl)methyl)amine (5.3 g, 93%) as a colorless oil which was used in the next step without further purification: MS($ES^+$) *m*/*z* 258.0 $(M+H)^+$.

To a solution of 3-chlorobenzoyl chloride (**12c**, 2.0 g, 11.4 mmol) in acetone (30 mL) cooled at 0 °C was added ammonium thiocyanate (1.7 g, 22.9 mmol) and the resulting mixture was stirred at this temperature for 1 h. Then bis{[4-(methyloxy)phenyl]methyl}amine (3.5 g, 13.7 mmol) was added at this temperature and stirred for an additional 30 min. The mixture was concentrated under reduced pressure, and then purified directly by chromatography (EtOAc:petroleum ether (PE) = 0–15%) to afford *N*-(bis(4-methoxybenzyl)carbamothioyl)-3-chlorobenzamide (**13c**, 5.3 g, 10.8 mmol, 94%) as a yellow sticky oil: MS($ES^+$) *m*/*z* 455.0 $(M+H)^+$.

A solution of 2-bromo-1-(2-chlorophenyl)ethanone (**11b**, 155 mg, 0.7 mmol) and *N*-(bis(4-methoxybenzyl)carbamothioyl)-3-chlorobenzamide (**13c**, 250 mg, 0.5 mmol) in *N*,*N*-dimethylformamide (DMF) (3 mL) was stirred at 85 °C under nitrogen for 30 min. After cooling to room temperature, the mixture was partitioned between EtOAc and water. The organic layer was washed with brine and dried over anhydrous $Na_2SO_4$. After filtration, solvent was removed in vacuo and the residue was stirred in TFA (4 mL, 51.9 mmol) at 80 °C overnight. Most of TFA was removed under reduced pressure. The residue was neutralized with satd $NaHCO_3$, and then extracted with EtOAc for 3 times. The combined organic layers were washed with brine and dried over anhydrous $Na_2SO_4$. After filtration, the solution was concentrated and further purified by chromatography (EtOAc:PE = 0–50%) to afford [2-amino-4-(3-chlorophenyl)-1, 3-thiazol-5-yl](2-chlorophenyl)methanone (**15h**) (198 mg, 88%) as a yellow solid: MS($ES^+$) *m*/*z* 348.9, 351.0 $(M+H)^+$.

A mixture of **15h** (116 mg, 0.332 mmol), (4-(ethylsulfonyl)phenyl)acetic acid **16** (83 mg, 0.365 mmol), EDC (89 mg, 0.465 mmol) and HOBt (62.8 mg, 0.465 mmol) in dichloromethane (DCM) (5 mL) was stirred at room temperature overnight. Solvent was removed under reduced pressure. The residue was purified by MDAP to afford **8h** (56 mg, 29% yield) as a white solid: $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 13.10 (s, 1H), 7.80 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.32–7.36 (m, 1H), 7.26–7.31 (m, 2H), 7.21-7.26 (m, 3H), 7.13–7.19 (m, 2H), 3.95 (s, 2H), 3.22 (q, *J* = 7.3 Hz, 2H), 1.04 (t, *J* = 7.4 Hz, 3H). $^{13}C$ NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 186.8, 169.9, 161.3, 155.1, 140.4, 138.1, 137.2, 135.5, 132.2, 131.8, 130.5 (2C), 129.7, 129.5, 129.5, 129.4, 129.2, 128.7, 128.0 (2C), 127.8, 126.9, 126.8, 49.2, 41.4, 7.2. HRMS $C_{26}H_{21}N_2O_4S_2Cl_2$ $(M+H)^+$ calcd 559.0320, found 559.0317. LCMS: $t_R$ = 3.50 min, >95%, MS($ES^+$) *m*/*z* 558.9, 560.9 $(M+H)^+$.

#### 5.1.3. *N*-(5-Benzoyl-4-(2-chlorophenyl)thiazol-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (8b)

Compound **8b** (31 mg, 21% yield) was prepared from [2-amino-4-(2-chlorophenyl)-1,3-thiazol-5-yl](phenyl)methanone (70 mg, 0.222 mmol) (**15b**) and **16** (53 mg, 0.233 mmol) in the same manner as described for **8h** as a white solid: $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 13.09 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.49–7.52 (m, 2H), 7.35–7.42 (m, 2H), 7.20–7.29 (m, 5H), 4.03 (s, 2H), 3.29 (q, *J* = 7.2 Hz, 2H), 1.11 (t, *J* = 7.2 Hz, 3H). $^{13}C$ NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 188.9, 170.1, 160.5, 152.4, 141.0, 138.0, 137.7, 134.5, 132.7, 132.5, 132.4, 131.0 (2C), 130.8, 129.6, 129.1 (2C), 128.5 (2C), 128.3 (2C), 127.7, 127.3, 49.7, 41.8, 7.6. HRMS $C_{26}H_{22}N_2O_4S_2Cl$ $(M+H)^+$ calcd 525.0710, found 525.0715. LCMS: $t_R$ = 3.35 min, >95%, MS($ES^+$) *m*/*z* 524.9, 526.9 $(M+H)^+$.

#### 5.1.4. *N*-(5-Benzoyl-4-(3-chlorophenyl)thiazol-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (8c)

Compound **8c** (51 mg, 31% yield) was prepared from [2-amino-4-(3-chlorophenyl)-1, 3-thiazol-5-yl](phenyl)methanone (80 mg, 0.254 mmol) (**15c**) and **16** (61 mg, 0.267 mmol) in the same manner as described for **8h** as a white solid: $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 13.10 (s, 1H), 7.87 (d, *J* = 8.3 Hz, 2H), 7.63 (d, *J* = 8.3 Hz, 2H), 7.58–7.56 (m, 2H), 7.47 (t, *J* = 7.4 Hz, 1H), 7.42 (t, *J* = 1.8 Hz, 1H), 7.35–7.21 (m, 5H), 4.02 (s, 2H), 3.29 (q, *J* = 7.3 Hz, 2H), 1.10 (t, *J* = 7.3 Hz, 3H). $^{13}C$ NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 189.3, 170.1, 160.4, 153.3, 141.0, 138.1, 137.7, 136.5, 133.1, 133.0, 130.9 (2C), 130.2, 129.7, 129.5 (2C), 129.0, 128.7 (2C), 128.5, 128.5 (2C), 125.9, 49.7, 41.8, 7.6. HRMS $C_{26}H_{22}N_2O_4S_2Cl$ $(M+H)^+$ calcd 525.0710, found 525.0710. LCMS: $t_R$ = 3.51 min, >95%, MS($ES^+$) *m*/*z* 524.9, 526.9 $(M+H)^+$.

#### 5.1.5. *N*-(5-Benzoyl-4-(4-chlorophenyl)thiazol-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (8d)

Compound **8d** (30 mg, 21% yield) was prepared from [2-amino-4-(4-chlorophenyl)-1,3-thiazol-5-yl](phenyl)methanone (120 mg, 0.248 mmol) (**15d**) and **16** (62 mg, 0.273 mmol) in the same manner as described for **8h** as a white solid. $^1H$ NMR (600 MHz, DMSO-$d_6$) $\delta$ ppm 13.10 (s, 1H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.57 (d, *J* = 7.2 Hz, 2H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.26–7.31 (m, 4H), 4.01 (s, 2H), 3.28 (q, *J* = 7.2 Hz, 2H), 1.10 (t, *J* = 7.2 Hz, 3H). $^{13}C$ NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 189.3, 170.1, 160.3, 153.7, 141.0, 138.1, 137.7, 133.9, 133.5, 133.1, 131.6 (2C), 130.9 (2C), 129.6 (2C), 128.7 (2C), 128.5 (2C), 128.4 (2C), 125.4, 49.7, 41.8, 7.6. HRMS *m*/*z* (ESI) $C_{26}H_{22}N_2O_4S_2Cl$ $(M+H)^+$ calcd 525.0710, found 525.0716. LCMS: $t_R$ = 3.50 min, >95%, MS($ES^+$) *m*/*z* 525.1, 527.1 $(M+H)^+$.

#### 5.1.6. *N*-(5-Benzoyl-4-(3-cyanophenyl)thiazol-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (8e)

Compound **8e** (42 mg, 35% yield) was prepared from 3-[2-amino-5-(phenylcarbonyl)-1,3-thiazol-4-yl]benzonitrile (70 mg, 0.229 mmol) (**15e**) and **16** (55 mg, 0.241 mmol) in the same

manner as described for **8h** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 13.13 (s, 1H), 7.88 (d, $J$ = 8.3 Hz, 2H), 7.70–7.78 (m, 3H), 7.63 (d, $J$ = 8.3 Hz, 2H), 7.54–7.56 (m, 2H), 7.42–7.48 (m, 2H), 7.28 (t, $J$ = 7.8 Hz, 2H), 4.03 (s, 2H), 3.29 (q, $J$ = 7.3 Hz, 2H), 1.10 (t, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 189.1, 168.5, 146.3, 144.0, 141.5, 138.8, 137.6, 137.3, 134.3, 133.2, 132.2, 131.7, 130.9 (2C), 129.6, 129.3 (2C), 128.4 (2C), 128.3 (2C), 127.7, 118.9, 115.4, 111.5, 49.7, 42.0, 7.7. HRMS $C_{27}H_{22}N_3O_4S_2$ (M+H)$^+$ calcd 516.1052, found 516.1066. LCMS: $t_R$ = 3.20 min, >95%, MS(ES$^+$) $m/z$ 516.1 (M+H)$^+$.

#### 5.1.7. *N*-(5-Benzoyl-4-(3-((dimethylamino)methyl)phenyl) thiazol-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (8f)

Compound **8f** (70 mg, 12% yield) was prepared from (2-amino-4-(3-((dimethylamino)methyl)phenyl) thiazol-5-yl)(phenyl)methanone (260 mg, 0.724 mmol) (**15f**) and **16** (460 mg, 2.015 mmol) in the same manner as described for **8h** as a white solid: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 7.82 (d, $J$ = 8.4 Hz, 2H), 7.63 (d, $J$ = 8.4 Hz, 2H), 7.45 (s, 1H), 7.28–7.44 (m, 4H), 7.13–7.21 (m, 4H), 3.61 (s, 2H), 3.35 (s, 2H), 3.13 (q, $J$ = 7.2 Hz, 2H), 2.20 (s, 6H),1.27 (t, $J$ = 7.2 Hz, 3H). HRMS $C_{29}H_{30}N_3O_4S_2$ (M+H)$^+$ calcd 548.1678, found 548.1685. LCMS: $t_R$ = 2.53 min, >95%, MS(ES$^+$) $m/z$ 547.9 (M+H)$^+$.

#### 5.1.8. 3-(5-Benzoyl-2-(2-(4-(ethylsulfonyl)phenyl)acetamido) thiazol-4-yl)benzoic acid (8g)

Compound **8g** (31 mg, 22% yield) was prepared from 3-[2-amino-5-(phenylcarbonyl)-1,3-thiazol-4-yl]benzoate (80 mg, 0.236 mmol) (**15g**) and **16** (57 mg, 0.248 mmol) in the same manner as described for **8h** with hydrolization of the ester to give a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 13.13 (s, 1H), 13.01 (br, 1H), 8.02 (t, $J$ = 1.5 Hz, 1H), 7.88 (d, $J$ = 8.3 Hz, 2H), 7.79 (d, $J$ = 7.8 Hz, 1H), 7.64 (d, $J$ = 8.3 Hz, 2H), 7.55–7.60 (m, 3H), 7.44 (t, $J$ = 7.4 Hz, 1H),7.24–7.32 (m, 3H), 4.02 (s, 2H), 3.29 (q, $J$ = 7.3 Hz, 2H), 1.10 (t, $J$ = 7.3 Hz, 3H). HRMS $C_{27}H_{23}N_2O_6S_2$ (M+H)$^+$ calcd 535.0998, found 535.1000. LCMS: $t_R$ = 2.91 min, >95%, MS(ES$^+$) $m/z$ 535.0 (M+H)$^+$.

#### 5.1.9. *N*-(5-(3-Chlorobenzoyl)-4-(3-chlorophenyl)thiazol-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (8i)

Compound **8i** (50 mg, 31% yield) was prepared from [2-amino-4-(3-chlorophenyl)-1,3-thiazol-5-yl](3-chlorophenyl)methanone (80 mg, 0.229 mmol) (**15i**) and **16** (55 mg, 0.241 mmol) in the same manner as described for **8h** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 13.15 (s, 1H), 7.88 (d, $J$ = 8.3 Hz, 2H), 7.64 (d, $J$ = 8.3 Hz, 2H), 7.45–7.49 (m, 3H), 7.40 (t, $J$ = 1.8 Hz, 1H), 7.21–7.33 (m, 4H), 4.03 (s, 2H), 3.29 (q, $J$ = 7.3 Hz, 2H), 1.10 (t, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 188.0, 170.2, 161.0, 154.3, 141.0, 139.9, 137.7, 136.5, 133.4, 133.1, 132.4, 130.9 (2C), 130.5, 130.2, 129.8, 129.1 (2C), 128.6, 128.5 (2C), 127.9, 126.1, 49.7, 41.8, 7.6. HRMS $C_{26}H_{21}N_2O_4S_2Cl_2$ (M+H)$^+$ calcd 559.0320, found 559.0318. LCMS: $t_R$ = 3.66 min, >95%, MS(ES$^+$) $m/z$ 558.9, 560.9 (M+H)$^+$.

#### 5.1.10. *N*-(5-(4-Chlorobenzoyl)-4-(3-chlorophenyl)thiazol-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (8j)

Compound **8j** (54 mg, 34% yield) was prepared from [2-amino-4-(3-chlorophenyl)-1,3-thiazol-5-yl](4-chlorophenyl)methanone (80 mg, 0.229 mmol) (**15j**) and **16** (55 mg, 0.241 mmol) in the same manner as described for **8h** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 13.12 (s, 1H), 7.87 (d, $J$ = 8.3 Hz, 2H), 7.62 (d, $J$ = 8.3 Hz, 2H), 7.53–7.56 (m, 2H), 7.40 (t, $J$ = 1.8 Hz, 1H), 7.31–7.35 (m, 4H), 7.24 (t, $J$ = 7.8 Hz, 1H), 4.01 (s, 2H), 3.28 (q, $J$ = 7.3 Hz, 2H), 1.09 (t, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 188.2, 170.2, 160.6, 153.6, 141.0, 137.8, 137.7, 136.8, 136.4, 133.1, 131.3 (2C), 130.9 (2C), 130.3, 129.8, 129.1, 128.7 (2C), 128.6, 128.5 (2C), 125.9, 49.7, 41.8, 7.6. HRMS $C_{26}H_{21}N_2O_4S_2Cl_2$ (M+H)$^+$ calcd 559.0320, found 559.0317. LCMS: $t_R$ = 3.69 min, >95%, MS(ES$^+$) $m/z$ 558.9, 560.9 (M+H)$^+$.

#### 5.1.11. *N*-(4-(3-Chlorophenyl)-5-(2-fluorobenzoyl)thiazol-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (8k)

Compound **8k** (36 mg, 30% yield) was prepared from [2-amino-4-(3-chlorophenyl)-1, 3-thiazol-5-yl](2-fluorophenyl)methanone (70 mg, 0.210 mmol) (**15k**) and **16** (50 mg, 0.221 mmol) in the same manner as described for **8h** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 13.15 (s, 1H), 7.87 (d, $J$ = 7.6 Hz, 2H), 7.63 (d, $J$ = 8.0 Hz, 2H), 7.19–7.47 (m, 5H), 7.21 (t, $J$ = 8.0 Hz, 1H), 7.09–7.13 (m, 1H), 6.97 (t, $J$ = 8.0 Hz, 1H), 4.02 (s, 2H), 3.29 (q, $J$ = 7.2 Hz, 2H), 1.10 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 185.4, 170.3, 161.4, 159.2 (d, $J$ = 249.4 Hz), 155.0, 140.9, 137.7, 136.0, 134.0 (d, $J$ = 8.8 Hz), 132.8, 131.0 (2C), 130.7 (d, $J$ = 2.2 Hz), 130.0, 129.7, 129.2, 128.5 (2C), 128.4, 127.5 (d, $J$ = 7.3 Hz), 127.4, 124.9 (d, $J$ = 3.7 Hz), 116.1 (d, $J$ = 21.3 Hz), 49.7, 41.8, 7.6. HRMS $C_{26}H_{21}N_2O_4S_2ClF$ (M+H)$^+$ calcd 543.0615, found 543.0613. LCMS: $t_R$ = 3.46 min, >95%, MS(ES$^+$) $m/z$ 543.0 (M+H)$^+$.

#### 5.1.12. *N*-(4-(3-Chlorophenyl)-5-(3-fluorobenzoyl)thiazol-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (8l)

Compound **8l** (9 mg, 8% yield) was prepared from [2-amino-4-(3-chlorophenyl)-1,3-thiazol-5-yl](3-fluorophenyl)methanone (70 mg, 0.210 mmol) (**15l**) and **16** (50 mg, 0.221 mmol) in the same manner as described for **8h** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 13.14 (s, 1H), 7.88 (d, $J$ = 8.4 Hz, 2H), 7.63 (d, $J$ = 8.0 Hz, 2H), 7.40 (t, $J$ = 1.6 Hz, 2H), 7.21–7.36 (m, 6H), 4.03 (s, 2H), 3.29 (q, $J$ = 7.2 Hz, 2H), 1.10 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 188.1 (d, $J$ = 2.2 Hz), 170.2, 162.0 (d, $J$ = 247.4 Hz), 160.9, 154.1, 141.0, 140.3 (d, $J$ = 6.6 Hz), 137.7, 136.5, 133.0, 130.9 (2C), 130.8 (d, $J$ = 8.1 Hz), 130.2, 129.8, 129.1, 128.6, 128.5 (2C), 126.0, 125.6 (d, $J$ = 2.9 Hz), 119.7 (d, $J$ = 21.3 Hz), 116.0 (d, $J$ = 22.7 Hz), 49.7, 41.8, 7.6. HRMS $C_{26}H_{21}N_2O_4S_2ClF$ (M+H)$^+$ calcd 543.0615, found 543.0615. LCMS: $t_R$ = 3.53 min, >95%, MS(ES$^+$) $m/z$ 543.0 (M+H)$^+$.

#### 5.1.13. *N*-(4-(3-Chlorophenyl)-5-(3-fluorobenzoyl)thiophen-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (9g)

A mixture of 1-(3-chlorophenyl)ethanone (**10c**, 30 g, 194 mmol), ethyl 2-cyanoacetate (66 g, 582 mmol), sulfur (8 g, 252 mmol) and morpholine (34 g, 388 mmol) in ethanol (340 mL) was heated to reflux and stirred overnight. Solvent was removed, and the residue was purified by flash chromatography (silica gel, EtOAc:PE = 5–10%) to give ethyl 2-amino-4-(3-chlorophenyl)-thiophene-3-carboxylate (**17c**, 15 g, 36% yield) as a yellow solid: MS(ES$^+$) $m/z$ 281.9 (M+H)$^+$.

A solution of KOH (50.8 g, 181 mmol) was added to a solution of **17c** (15.0 g, 45.3 mmol) in ethanol (200 mL). The reaction mixture was heated to reflux for 20 h. After cooling to room temperature, solvent was removed. To the residue was added water (150 mL), and then the solution was acidified to pH ~7 with 4 M HCl, at which point solid precipitated from the solution. The solid was collected by filtration, washed with water, and dried in air to give 2-amino-4-(3-chlorophenyl)thiophene-3-carboxylic acid (11 g, 36.9 mmol) as a beige solid. Then the solid was dissolved in ethanol (150 mL) and 2 M HCl (92 mL, 184 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. Solvent was removed in vacuo, and the residue was triturated with diethyl ether to give 4-(3-chlorophenyl)thiophen-2-amine hydrochloride (**18c**, 7.3 g, 56% yield) as a beige solid: MS(ES$^+$) $m/z$ 209.9 (M+H)$^+$.

To a solution of **18c** (6.0 g, 21.94 mmol), 2-(4-(ethylsulfonyl)phenyl)acetic acid **16** (6.5 g, 28.5 mmol), EDC (6.3 g, 32.9 mmol) and HOBt (4.4 g, 28.5 mmol) in dichloromethane (DCM) (90 mL) was added dropwise diisopropylethylamine

(7.7 mL, 43.9 mmol) at room temperature. The reaction mixture was heated at reflux under nitrogen overnight. The reaction mixture was partitioned between DCM (150 mL) and water (80 mL).The organic phase was washed with water (50 mL × 2), brine (50 mL), dried over anhydrous sodium sulfate, and concentrated. The residue was purified by column chromatography (silica gel, EtOAc:PE = 25–50%) to give *N*-(4-(3-chlorophenyl)thiophen-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (**19c**) (7.0 g, 68% yield) as a pale brown solid: MS($ES^+$) *m*/*z* 419.9 ($M+H^+$).

To a solution of **19c** (200 mg, 0.476 mmol) and 3-fluorobenzoyl chloride (151 mg, 0.953 mmol) in 1,2-dichloroethane (DCE) (16 mL) was added dropwise tin(IV) chloride (1 M in DCM, 0.953 mL, 0.953 mmol). The reaction mixture was heated to reflux overnight. The reaction mixture was diluted with DCM (50 mL), and then washed with water (20 mL). The aqueous phase was extracted with DCM (20 mL). The combined organic phase was dried over anhydrous sodium sulfate. After filtration and concentration, the residue was purified by column chromatography (silica gel, EtOAc:PE = 1:1 to EtOAc:PE:THF = 5:5:2) to give the crude product, which was further purified by MDAP to afford **9g** (15 mg, 6% yield) as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 12.20 (s, 1H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.18–7.25 (m, 8H), 6.86 (s, 1H), 3.97 (s, 2H), 3.32 (q, *J* = 7.6 Hz, 2H), 1.11 (t, *J* = 7.6 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 187.9 (d, *J* = 2.2 Hz), 168.5, 161.8 (d, *J* = 245.8 Hz), 146.6, 145.1, 141.4, 141.1 (d, *J* = 6.6 Hz), 138.0, 137.6, 133.1, 130.9 (2C), 130.5 (d, *J* = 8.1 Hz), 130.2, 129.4, 128.4 (2C), 128.2, 128.1, 127.3, 125.2 (d, *J* = 2.2 Hz), 118.8 (d, *J* = 21.3 Hz), 115.7 (d, *J* = 22.7 Hz), 115.6, 49.7, 42.0, 7.7. HRMS $C_{27}H_{22}NO_4S_2ClF$ $(M+H)^+$ calcd 542.0663, found 542.0665. LCMS: $t_R$ = 3.51 min, >95%. MS($ES^+$) *m*/*z* 541.8, 543.7 $(M+H)^+$.

### 5.1.14. *N*-(5-Benzoyl-4-phenylthiophen-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (9a)

Compound **9a** (68 mg, 50% yield) was prepared from benzoyl chloride (0.061 mL, 0.529 mmol) and 2-(4-(ethylsulfonyl)phenyl)-*N*-(4-phenyl-2-thienyl)acetamide (**19a**) (102 mg, 0.265 mmol) in the same manner as described for **9g** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 7.81 (d, *J* = 8.3 Hz, 2H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.35–7.40 (m, 2H), 7.28 (d, *J* = 7.3 Hz, 1H), 7.05–7.16 (m, 7H), 6.77 (s, 1H), 3.88 (s, 2H), 3.22 (q, *J* = 7.3 Hz, 2H), 1.04 (t, *J* = 7.3 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 189.5, 168.3, 146.1, 145.7, 141.5, 138.8, 137.6, 136.1, 132.1, 130.8 (2C), 129.6 (2C), 129.3 (2C), 128.4 (4C), 128.2 (2C), 128.1, 127.0, 115.5, 49.7, 42.1, 7.6. HRMS $C_{27}H_{24}NO_4S_2$ $(M+H)^+$ calcd 490.1147, found 490.1149. LCMS: $t_R$ = 3.32 min, >95%, MS($ES^+$) *m*/*z* 490.0 $(M+H)^+$.

### 5.1.15. *N*-(5-Benzoyl-4-(3-chlorophenyl)thiophen-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (9b)

Compound **9b** (24 mg, 9% yield) was prepared from benzoyl chloride (134 mg, 0.953 mmol) and **19c** (200 mg, 0.476 mmol) in the same manner as described for **9g** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 1.11 (t, *J* = 7.2 Hz, 3H), 3.30 (q, *J* = 7.2 Hz, 2H), 3.96 (s, 2H), 6.85 (s, 1H), 7.19–7.26 (m, 6H), 7.38–7.47 (m, 3H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.88 (d, *J* = 8.0 Hz, 2H), 12.11 (s, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 189.3, 168.4, 145.9, 144.5, 141.5, 138.9, 138.1, 137.6, 133.0, 132.2, 130.8 (2C), 130.2, 129.4, 129.2 (2C), 128.4 (2C), 128.3 (2C), 128.2, 127.9, 127.5, 115.4, 49.7, 42.0, 7.7. HRMS $C_{27}H_{23}NO_4S_2Cl$ $(M+H)^+$ calcd 524.0757, found 524.0764. LCMS: $t_R$ = 3.48 min, >95%, MS($ES^+$) *m*/*z* 523.9, 525.9 $(M+H)^+$.

### 5.1.16. *N*-(5-Benzoyl-4-(3-cyanophenyl)thiophen-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (9c)

Compound **9c** (57 mg, 33% yield) was prepared from benzoyl chloride (89 mg, 0.633 mmol) and *N*-(4-(3-cyanophenyl)thiophen-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (**19g**) (260 mg, 0.633 mmol) in the same manner as described for **9g** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 12.13 (s, 1H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.55–7.86 (m, 5H), 7.36–7.46 (m, 4H), 7.24 (t, *J* = 7.6 Hz, 2H), 6.88 (s, 1H), 3.96 (s, 2H), 3.29 (q, *J* = 7.2 Hz, 2H), 1.10 (t, *J* = 7.2 Hz, 3H). HRMS $C_{28}H_{23}N_2O_4S_2$ $(M+H)^+$ calcd 515.1099, found 515.1107. LCMS: $t_R$ = 3.18 min, >95%, MS($ES^+$) *m*/*z* 514.9 $(M+H)^+$.

### 5.1.17. *N*-(5-(2-Chlorobenzoyl)-4-(3-chlorophenyl)thiophen-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (9d)

Compound **9d** (1.9 g, 29% yield) was prepared 2-chlorobenzoyl chloride (4.2 g, 23.8 mmol) and **19c** (5.0 g, 11.9 mmol) in the same manner as described for **9g** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 12.16 (s, 1H), 7.86 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.16–7.31 (m, 8H), 6.76 (s, 1H), 3.95 (s, 2H), 3.30 (q, *J* = 7.2 Hz, 2H), 1.11 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 186.4, 168.2, 147.4, 145.6, 140.9, 138.8, 137.1, 136.9, 132.2, 131.2, 130.4 (2C), 129.7, 129.5, 129.2, 129.2, 128.7, 127.9 (2C), 127.7, 127.5, 127.3, 126.7, 115.9, 49.2, 41.5, 7.2. HRMS $C_{27}H_{22}NO_4S_2Cl_2$ $(M+H)^+$ calcd 558.0367, found 558.0378. LCMS: $t_R$ = 3.54 min, >95%, MS($ES^+$) *m*/*z* 557.8, 559.8 $(M+H)^+$.

### 5.1.18. *N*-(5-(3-Chlorobenzoyl)-4-(3-chlorophenyl)thiophen-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (9e)

Compound **9e** (30 mg, 11% yield) was prepared 3-chlorobenzoyl chloride (167 mg, 0.953 mmol) and **19c** (200 mg, 0.476 mmol) in the same manner as described for **9g** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 12.16 (s, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.17–7.38 (m, 8H), 6.85 (s, 1H), 3.96 (s, 2H), 3.30 (q, *J* = 7.2 Hz, 2H), 1.11 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 187.8, 168.5, 146.7, 145.2, 141.4, 140.6, 137.9, 137.6, 133.2, 133.1, 131.6, 130.9 (2C), 130.3, 130.2, 129.4, 128.9, 128.4 (2C), 128.2, 128.1, 127.5, 127.4, 115.6, 49.7, 42.0, 7.7. HRMS $C_{27}H_{22}NO_4S_2Cl_2$ $(M+H)^+$ calcd 558.0367, found 558.0372. LCMS: $t_R$ = 3.66 min, >95%, MS($ES^+$) *m*/*z* 557.8, 559.8 $(M+H)^+$.

### 5.1.19. *N*-(4-(3-Chlorophenyl)-5-(2-fluorobenzoyl)thiophen-2-yl)-2-(4-(ethylsulfonyl)phenyl)acetamide (9f)

Compound **9f** (73 mg, 12% yield) was prepared 2-fluorobenzoyl chloride (574 mg, 3.62 mmol) and **19c** (760 mg, 1.81 mmol) in the same manner as described for **9g** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 12.15 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.31–7.36 (m, 2H), 7.17–7.24 (m, 4H), 7.06 (t, *J* = 7.2 Hz, 1H), 6.93 (t, *J* = 9.6 Hz, 1H), 6.78 (s, 1H), 3.96 (s, 2H), 3.29 (q, *J* = 7.2 Hz, 2H), 1.10 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 185.1, 168.6, 158.9 (d, *J* = 248.6 Hz), 147.4, 145.8, 141.4, 137.6, 137.4, 133.1 (d, *J* = 8.1 Hz), 132.8, 130.9 (2C), 130.3 (d, *J* = 2.9 Hz), 130.0, 129.4, 128.4 (2C), 128.4, 128.3 (d, *J* = 15.4 Hz), 128.1, 128.0, 124.6 (d, *J* = 2.9 Hz), 116.1, 115.8 (d, *J* = 21.3 Hz), 49.7, 42.0, 7.6. HRMS $C_{27}H_{22}NO_4FS_2Cl$ $(M+H)^+$ calcd 542.0663, found 542.0666. LCMS: $t_R$ = 3.45 min, >95%, MS($ES^+$) *m*/*z* 541.8, 543.8 $(M+H)^+$.

### 5.1.20. 2-(4-(Ethylsulfonyl)phenyl)-*N*-(5-(3-fluorobenzoyl)-4-(3-(trifluoromethyl)phenyl)thiophen-2-yl)acetamide (9h)

Compound **9h** (61 mg, 34% yield) was prepared 3-fluorobenzoyl chloride (129 mg, 0.811 mmol) and 2-(4-(ethylsulfonyl)phenyl)-*N*-(4-(3-(trifluoromethyl)phenyl)thiophen-2-yl)acetamide (**19h**) (184 mg, 0.406 mmol) in the same manner as described for **9g** as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 12.15 (s, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.53–7.58 (m, 2H), 7.42–7.48 (m, 2H), 7.14–7.22 (m, 4H), 6.89 (s, 1H), 3.97 (s, 2H), 3.28 (q, *J* = 7.6 Hz, 2H), 1.10 (t, *J* = 7.6 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 187.9 (d, *J* = 2.2 Hz), 168.5, 161.8 (d,

$J$ = 245.0 Hz), 146.8, 145.1, 141.4, 140.9 (d, $J$ = 6.6 Hz), 137.6, 137.0, 133.3, 130.9 (2C), 130.4 (d, $J$ = 8.1 Hz), 129.6, 129.1 (q, $J$ = 32.3 Hz), 128.4 (2C), 127.6, 126.4 (q, $J$ = 4.2 Hz), 125.3 (d, $J$ = 2.2 Hz), 124.8 (q, $J$ = 3.7 Hz), 124.3 (q, $J$ = 274.7 Hz), 118.7 (d, $J$ = 21.3 Hz), 115.8 (d, $J$ = 22.7 Hz), 115.7, 49.7, 42.1, 7.6. HRMS $C_{28}H_{22}NO_4F_4S_2$ $(M+H)^+$ calcd 576.0926, found 576.0934. LCMS: $t_R$ = 3.56 min, >95%, MS($ES^+$) $m/z$ 575.8 $(M+H)^+$.

### 5.2. RORγ FRET assay

The assays were performed in an assay buffer consisting of 50 mM NaF, 50 mM 3-(*N*-morpholino)propanesulfonic acid, pH 7.5, 50 μM 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate, 0.1 mg/mL bovine serum albumin, and 10 mM dithiothreitol in 384-well plates (Greiner 784076, Longwood, FL). The total volume was 10 μL/well. The europium-labeled SRC1 solution was prepared by adding an appropriate amount of biotinylated SRC and europium labeled streptavidin (PerkinElmer Life and Analytical Sciences, Waltham, MA) into assay buffer, with final concentrations of 27 and 3.3 nM, respectively. The allophycocyanin (APC)-labeled-LBD solution was prepared by adding an appropriate amount of biotinylated RORγ-LBD and APC-labeled streptavidin (CR130-100; PerkinElmer Life and Analytical Sciences) at a final concentration of 33 nM each. After 15 min of incubation at room temperature, a 20-fold excess of biotin was added to block the remaining free streptavidin. Equal volumes of europium-labeled SRC- and APC-labeled RORγ-LBD were then mixed with 0.2 μM surrogate agonist *N*-(2-chloro-6-fluorobenzyl)-*N*-((2′-methoxy-[1,1′-biphenyl]-4-yl)methyl)benzenesulfonamide[24] and dispensed into 384-well assay plates at 10 μL volume/well. The 384-well assay plates had 100 nL of test compound in DMSO predispensed into each well. The plates were incubated for 1 h at room temperature and then read on ViewLux (PerkinElmer Life and Analytical Sciences) in LANCE mode configured for europeum-APC labels. Data were collected and analyzed by Activitybase.

### 5.3. RORγt dual FRET assay

*Materials:* RAR-related orphan receptor gamma (RORγ) protein was made at GSK. Proteins were chemically biotinylated using standard methods. Typically proteins have between 1 and 5 biotins. Biotinylated-Peptide was purchased from CPC scientific. Streptavidin-labeled APC (CR130-150) and Eu-W1024 labeled streptavidin (AD0063) were purchased from Perkin Elmer. DMSO was purchased from EMD (MX14561). MOPS (M1254), sodium fluoride (S6521) and CHAPS (C3023) were purchased from Sigma. DTT (F780-01) was purchased from JT Baker. Immunopure D-biotin (29129) was purchased from Pierce. BSA (100350), Frac V, fatty acid free was purchased from Boehringer Mannheim. *Compound preparation:* Compounds were diluted in 100% neat DMSO at 10 mM. The compounds were then dispensed into an intermediate plate (polypropylene Greiner PP V-bottom: 781280) to make serial dilutions in 100% neat DMSO. Approximately 100 nL of the serial dilution was added to the assay plate (Costar 3573) using a Hummingbird (Genomic Solutions). *Stock Buffer:* A 0.5 M solution of MOPS was made by adding 104 g of MOPS to 800 mL $H_2O$ in a graduated cylinder, using a calibrated pH meter, add increasing amounts NaOH to give a final pH of 7.5. This solution was filtered using a Costar 0.2 μm filtering apparatus and stored in the refrigerator until ready to use. *Assay buffer:* Add 100 mL of 10× MOPS stock solution to graduated cylinder bring up to 800 mL. Add 2.09 g of NaF, 0.03 g of CHAPS to the flask, 0.1 g of BSA to the flask. Make sure all components are dissolved. Add $dH_2O$ to give final volume of 1 L. The assay buffer was filtered with a Costar 0.2 μm filtering apparatus. On assay day, DTT was added to the assay buffer to a final concentration of 10 mM. Fresh DTT solid should be used. *Assay:* RORγ were assayed using the generic protocol described. To polypropylene costar conical centrifuge tubes, add assay buffer, an appropriate amount of biotinylated-SRC1(2) from the 1E-4 M (100 μM) stock solution to give a final concentration of 4E-8 M (40 nM). To the above biotinylated SRC1(2) solution, add an appropriate amount of Europium-labeled streptavidin to give a final concentration of 1E-8 M (10 nM). Invert gently to mix. Incubate 15 min at room temperature. At the same time, but in another polypropylene tube add an appropriate amount of biotinylated-ROR protein from the stock solution to give a final concentration of 4E-8 M (40 nM). To the biotinylated-ROR solution, add an appropriate amount of APC-labeled streptavidin to give a final concentration of 2E-8 M (20 nM). Invert gently to mix. Incubate 15 min at room temperature. Following the 15 min incubations, add 20-fold excess biotin from the 1E-2 M (10 mM) stock solution. Invert gently to mix. Incubate 10 min at room temperature. Gently mix the above solutions together to give a final solution containing 20 nM ROR_10 nM APC and 20 nM SRC1(2)_5 nM SA_EU. Incubate 5 min and use a Thermo Combi Multidrop to add 25 μL peptide/ROR solution to assay plates containing 100 nL of test compound. Incubate plates for 1 h at room temperature, then read on ViewLux in Lance mode for EU/APC. *Data analysis:* Raw data was analyzed using ABASE (IBDS) software. The data was normalized initially using the following equation: Normalization = 100 ∗ ((Basal HTRF-value)/(Basal HTRF − Minimal HTRF). The normalized data was then was fit to a 4-parameter logistic equation.

### 5.4. Mouse Th17 cell differentiation assay

CD4+T cells were purified from spleens using anti-CD4 magnetic microbeads (Miltenyi Biotec) and MACS columns (purity was >95%). CD4+ cells were resuspended in RPMI 1640 complete medium and added to 96-well plates pre-coated with anti-mCD3 (5 μg/mL) at $10^5$ cell/well in a total volume of 80 μl. One hundred microliters of a 2× cytokine cocktail and 20 μl of compounds (100×) were added to the well. The final concentrations of antibodies and cytokines (all from R&D Systems, Minneapolis, MN) were as follows: anti-mCD28 (5 μg/mL); anti-mIFN-γ (10 μg/mL); anti-mIL4 (10 μg/mL); mIL-6 (20 ng/mL); hTGF-β1 (5 ng/mL). The culture was incubated in 37 °C for 3 days, and supernatants were collected for enzyme-linked immunosorbent assay (ELISA). The mouse IL-17 ELISA was performed according to the manufacturer's instruction (R&D Systems). The results were analyzed using Prism software with nonlinear regression to determine the $IC_{50}$ value.

### 5.5. Radioligand binding assay

RORγt-LBD protein was incubated with 10 nM 25-[26,27-$^3$H] hydroxycholesterol in the presence of various concentrations of compounds in the assay buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 50 μM CHAPS and 2 mM DTT). After incubation at 37 °C for 60 min, the reaction mixture was immediately terminated by being quickly washed 8 times with ice-cold washing buffer. The results were analyzed using Prism software with nonlinear regression to determine the $IC_{50}$ value, and then the $K_i$ value was obtained through the Cheng–Prusoff equation.

### 5.6. CD-based Tm assay

CD assay was carried out using Jasco J-815 CD spectrometer. The assay concentrations of RORγt-LBD protein and compounds were 4 and 50 μM, respectively. The assay buffer contains PBS pH 7.5 and 0.5% DMSO generated from compound dilution. Protein melting was recorded at 225 nm from 25–70 °C with the ramping rate of 2 °C/min and sampling interval of 1 °C. The Tm value was determined by JASCO Protein Denaturation Analysis Software.

### 5.7. EAE induction and treatment

For EAE induction and treatment, C57BL/6 (B6) mice were purchased from Shanghai SIPPR/BK Experimental Animal Company Ltd (Shanghai, China) and were maintained under pathogen-free conditions. EAE was induced with 300 μg $MOG_{35-55}$ peptide (GL Biochem, Shanghai) emulsified with complete Freund's adjuvant. Pertussis toxin (200 ng, List Biological Laboratories, Campbell, CA) was administrated on the day of immunization and 48 h later. For treatment of EAE, compounds **8h** or vehicle PBS was orally dosed twice daily from day 0 after immunization onward. Mice were examined daily and scored for disease severity using the following standard scale: 0, no clinical signs; 1, limp tail; 2, paraparesis (weakness, incomplete paralysis of one or two hind limbs); 3, paraplegia (complete paralysis of two hind limbs); 4, paraplegia with forelimb weakness or paralysis; 5, moribund or death. Data were expressed as Mean ± S.E.M. of 8 mice.

### 5.8. CIA induction and treatment

For CIA induction and treatment, DBA/1 mouse at age of 6–8 weeks were purchased from Shanghai Laboratory Animal Center and were maintained under pathogen-free conditions. CIA was induced in DBA/1 mouse with 200 μg of bovine type II collagen emulsified with an equal volume of complete Freund's adjuvant. The mice were then boosted with an equal amount of bovine type II collagen emulsified in Freund's incomplete adjuvant on day 21. For CIA treatment, compounds **8h** or vehicle PBS was orally administered at indicated dose twice daily from 2nd immunization onward. Mice were monitored for onset of clinical disease and then the clinical score were evaluated by two evaluator blinded to the treatment groups. The occurrence of arthritis were observed by scoring all paws for severity of erythema and swelling, using score ranging from 0 to 4. 0 = no arthritis, 1 = paws with swelling of 1 joint (wrist/ankle or digit), 2 = swelling of 2 joints or more, 3 = swelling of all joints, and 4 = ankylosed joints. Data were expressed as Mean ± S.E.M of 10 mice.

*Notes:* All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.

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### Supplementary data

Supplementary data (preparation details and spectroscopic data for compounds: **6a**–**w**, **7a**–**i** and **8a**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.12.021.

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