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Synthesis and pharmacological evaluation of novel *N*-aryl-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamides as TRPM8 antagonists

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ABSTRACT

A novel series of *N*-aryl-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamides was identified as transient receptor potential melastatin 8 (TRPM8) channel blockers through analogue-based rational design, synthesis and screening. Details of the synthesis, effect of aryl groups and their substituents on in-vitro potency were studied. The effects of selected functional groups on the 4-position of the chromene ring were also studied, which showed interesting results. The 4-hydroxy derivatives showed excellent potency and selectivity. Optical resolution and screening of alcohols revealed that (*R*)-(−)-isomers were in general more potent than the corresponding (*S*)-(+)-isomers. The isomer (*R*)-(−)-**10e** (IC_{50}: 8.9 nM) showed a good pharmacokinetic profile upon oral dosing at 10 mg/kg in Sprague–Dawley (SD) rats. The compound (*R*)-(−)-**10e** also showed excellent efficacy in relevant rodent models of neuropathic pain.

1. Introduction

Transient receptor potential (TRP) ion channels comprise of an extensive family of nonselective cation channels that are activated by a wide variety of stimuli.[1,2] More than 30 members of the TRP channels have been identified thus far in mammals with a wide range of expression profiles. Based on their amino acid sequence and structural similarity, they are categorized into seven groups, namely TRP canonical (C), TRP vanilloid (V), TRP melastatin (M), TRP polycystin (P), TRP mucolipin (ML), TRP ankyrin (A), and TRP nompc (N).[3,4] All these channels were first discovered in *Drosophila melanogaster* as light activated ion channels. TRP channels are known to be activated by a wide range of physical, mechanical and chemical stimuli.[5] A growing body of evidence suggests that certain members of the TRP family play critical roles in the transduction of temperature and pain sensation.[6] The melastatin subgroup consists of eight channels namely TRPM 1–8. Among the super-family of TRP channels, TRPM8, TRPA1 and TRPV1 share significant sequence homology and appear to reproduce the broad spectrum of temperature sensation, though they differ in their anatomical distribution and the stimuli to which they respond.[7] The transient receptor potential melastatin 8 (TRPM8) and transient receptor potential ankyrin 1 (TRPA1) have been implicated in cold sensation, while the heat-sensitive transient receptor potential vanilloid 1 (TRPV1) is activated by noxious heat above 42 °C.[8]

TRPM8 is expressed in the small dorsal root ganglia (DRG) neurons and in neurons from trigeminal ganglia in the periphery. It is a calcium (Ca^{2+})-permeable, nonselective cation channel and is of special interest as this polymodal receptor responds to both temperature changes and to several chemicals. TRPM8 is activated at temperatures ranging from 8 to 26 °C.[9–11] It is also activated by several exogenous ligands, such as menthol, icilin, eucalyptol, geraniol and linalool. The therapeutic cooling sensation elicited by these chemicals is due to the activation of TRPM8 channels.[9,11] TRPM8 can sense temperature changes in the range of both innocuous cold (15–28 °C) and noxious cold (<15 °C) and the role of TRPM8 in skin cold sensation has been demonstrated using TRMP8 null mice.[8,12,13] Recent studies revealed that intravenous administration of certain known TRPM8 antagonists induces hypothermia and decrease in deep body temperature.[14]

Cold allodynia is a pathological pain state induced by innocuous cold. The pain is intractable and often occurs in patients with complex regional pain syndrome.[15] Approximately 80% of these patients suffer from cold allodynia. Cold allodynia is also present in other disease conditions such as diabetic neuropathy and fibromyalgia.[16,17] Thus, the presence of cold allodynia in a wide range of clinical disorders provides a strong rationale for the development

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of TRPM8 antagonists as a novel antihyperalgesic or antiallodynic agent.[18,19] Several patents and publications have recently been reported in the literature exploring the potential therapeutic use of TRPM8 antagonists.[20–26]

The two well-studied urea derivatives *N*-(4-*tert*-butylphenyl)-4-(3-chloropyridine-2-yl)tetrahydropyrazine-1(2*H*)-carboxamide (BCTC 1) and capsazepine 2 (Fig. 1) are known to inhibit rodent TRPM8 with low potency and are nonselective.[27,28] BCTC is also a potent inhibitor of TRPV1 (IC_{50}: 34.9 ± 19 nM), thus limiting its potential therapeutic use as a selective TRPM8 antagonist. Certain other urea derivatives are also reported in the literature as TRPM8 antagonists. However, their TRPV1 activity had not been discussed.[24] Based on the above literature, we designed urea derivatives of spiro[chromene-2,4′-piperidine] as potential TRPM8 antagonists.

In this paper, we describe design, synthesis and structure–activity relationship (SAR) analysis of a novel series of spiro[chromene-2,4′-piperidine]-1′-carboxamides as TRPM8 antagonists. The study resulted in identification of (4*R*)-(−)-8-chloro-4-hydroxy-*N*-[4-(trifluoromethoxy)phenyl]-3,4-di-hydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (−)-**10e**, as a potent, highly selective and orally available TRPM8 antagonist which shows efficacy in various rodent models of neuropathic pain.

2. Results and discussion

2.1. Chemistry

The synthesis of urea derivatives of 3,4-dihydrospiro[chromene-2,4′-piperidine] **8a**–**m** is described in Scheme 1. Thus, appropriately substituted 2-hydroxy-acetophenone **3** was reacted with *N*-BOC-4-piperidone **4** in the presence of pyrrolidine in methanol to give the spiro derivatives **5a**–**f** in good to excellent yield.[29–32] This well-known reaction proceeds via an enamine intermediate of **3** with pyrrolidine, which adds to the carbonyl group of piperidone **4** and cyclizes through the phenolic oxygen to form the spirocyclic compound.[33] Sodium borohydride reduction of **5** gave alcohol **6** which on treatment with triethylsilane in refluxing trifluoroacetic acid (TFA) resulted in reductive deoxygenation and concomitant deprotection of the *N*-BOC group.[34] The crude amine, isolated after basification of the TFA salt, was treated with ~10% (w/v) HCl in EtOAc to give **7** as its hydrochloride salt. The amine hydrochloride **7** was coupled with various phenyl aryl carbamates in the presence of excess triethylamine in dimethyl sulfoxide to afford urea derivatives **8a**–**m** in excellent yield.[35,36]

The synthesis of 4-oxo derivatives **9a**–**f** and 4-hydroxy derivatives (±)-**10a**–**f** is given in Scheme 2. The ketones **9a**–**f** were prepared by direct coupling of free amine derived from **5c** to **5e** with appropriately substituted aryl phenylcarbamate in the presence of triethylamine in dimethyl sulfoxide at room temperature. The racemic alcohols **10a**–**f** were prepared by carbonyl group reduction of the corresponding 4-oxochromene **9a**–**f** using sodium borohydride in ethanol.[29]

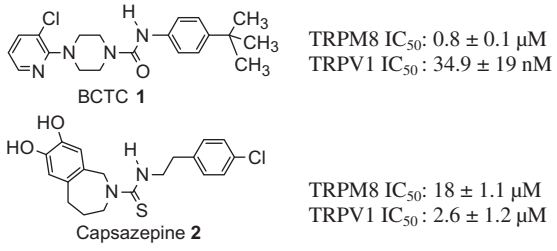
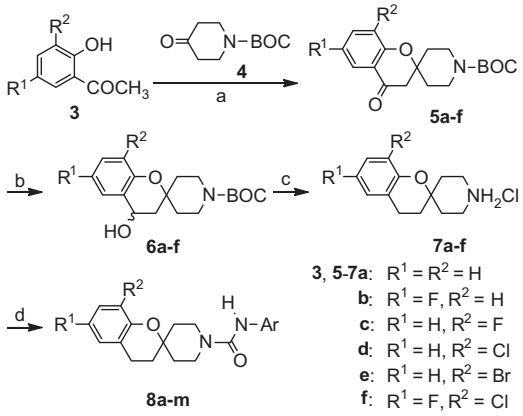
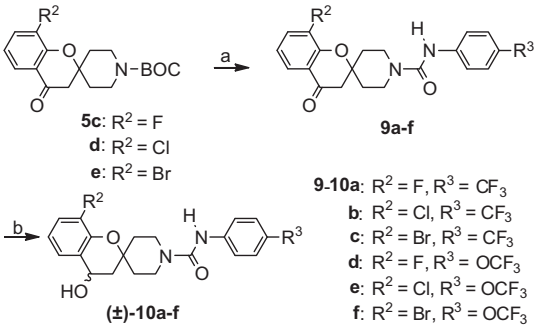


Figure 1. Structures of BCTC **1** and capsazepine **2**.

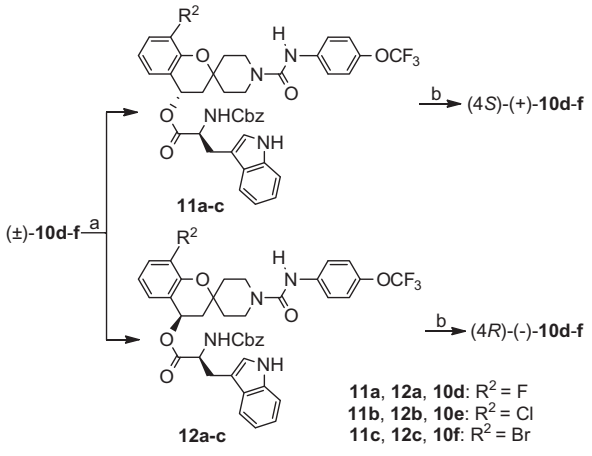


Scheme 1. Reagents and conditions: (a) pyrrolidine, MeOH, RT, 24 h; (b) $NaBH_4$, EtOH, RT, 1 h.; (c) (i) triethylsilane, TFA, reflux, 18 h; (ii) ~10% (w/v) HCl in EtOAc, 0 °C–RT, 2 h; (d) $ArNHCO_2Ph$, Et_3N, DMSO, RT, 2 h.



Scheme 2. Reagents and conditions: (a) (i) ~10% (w/v) HCl in EtOAc; (ii) R^3-$C_6H_4NHCO_2Ph$, Et_3N, DMSO, RT, 2 h. (b) $NaBH_4$, EtOH, 0 °C–RT, 1 h.

It was considered important to resolve the alcohol (±)-**10** into individual isomers as a part of this study. Initial attempts to prepare the chiral alcohols via asymmetric reduction of ketone **9** using (*R*)-2-methyl-CBS-oxazaborolidine–borane complex failed to give the expected alcohol. Attempted asymmetric reduction using (−)-DIP-chloride also failed to give the alcohol **10**. This was probably due to the presence of urea moiety in the ketone substrate. A conventional approach of optical resolution via the diasteromeric esters worked extremely well and is depicted in Scheme 3. The



Scheme 3. Reagents and conditions: (a) Cbz-L-Trp-OH, EDCI, DMAP, CH_2Cl_2, RT, 18 h. (b) $LiOH{\cdot}H_2O$, THF–MeOH, water, RT, 1 h.

esterification of racemic spirocyclic alcohol (±)-**10** with (2*S*)-2-{[(benzyloxy)-carbonyl]amino}-3-(1*H*-indol-3-yl)propanoic acid (Cbz-L-Trp-OH) using *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) as a coupling agent in the presence of 4-(dimethylamino)pyridine (DMAP) in dichloromethane gave a diastereomeric mixture of esters **11** and **12**.[37] The mixture of esters was separated by flash silica gel column chromatography to yield the less polar isomer **11** followed by the more polar isomer **12**. Compounds **11a–c** and **12a–c** were found to be enantiomerically and diasteriomerically pure (>98%) based on chiral HPLC analysis. Hydrolysis of the less polar diastereomers **11a–c** with aqueous lithium hydroxide yielded corresponding optically pure alcohols (+)-**10d–f**.

Similarly, hydrolysis of the more polar diastereomers **12a–c** yielded corresponding optically pure alcohols (−)-**10d–f** in good yields. Stereochemical descriptors (*R* or *S*) of (+)-**10d–f** and (−)-**10d–f** were assigned by correlating the sign of optical rotation of these compounds with that of known chromene alcohol derivatives prepared through enantoselective reduction of the corresponding ketones using a chiral oxazaborolidine–borane complex.[38]

2.2. In-vitro pharmacology

The urea derivatives **8a–m** (Scheme 1) and **9a–f**, (±)-**10a–f** (Scheme 2), were prepared as part of a structure–activity relationship (SAR) analysis and were subjected to in-vitro hTRPM8 assay using recombinant Chinese hamster ovary (CHO) cells. Tables 1–2 show SAR of these series of compounds. The compound **8a** (Table 1) bearing no substitution on both aryl rings showed poor TRPM8 antagonistic activity (IC_{50}: 2423 nM). Introduction of an appropriate substituent on the aryl ring seemed to be essential to achieve good potency. A trifluoromethyl group at 4-position of the aryl ring as in the case of **8b** resulted in nearly nine-fold improvement in potency. An additional fluorine substituent at 6-position of the chromene ring as in the case of **8c** resulted in significant loss of potency. A fluorine, chlorine or a bromine substituent at the 8-position of the chromene ring in addition to the trifluoromethyl group at 4-position of the phenyl ring resulted in excellent potency (compounds **8d–f**). The 8-chloro derivative **8g** with a trifluoromethoxy group at 4-position of the phenyl ring also retained good potency. Compound **8h** with an electron-withdrawing cyano group on the phenyl ring showed significant loss in potency. A methyl substitution at 4-position of the phenyl ring as in the case of **8i** showed six-fold loss in potency compared to the corresponding trifluoromethyl derivative **8e**. An additional chlorine substituent on the phenyl ring as in the case of **8j** resulted in partial loss of potency. Di-substitution on the left-hand side of the molecule as in the case of **8k** also resulted in a loss of potency. The thiazole derivative **8l** and benzothiazole derivative **8m** showed only moderate potency.

The in-vitro screening results of keto compounds **9a–f** and the corresponding alcohols (±)-**10a–f** are given in Table 2. The keto compounds **9a–c** with trifluoromethyl group at the 4-position of the phenyl ring showed moderate potency. Introduction of a trifluoromethoxy group at the 4-position of the phenyl ring as in the case of **9d–f** resulted in improved potency. The conversion of the hydrogen bond acceptor group (carbonyl) at 4-position to a donor group (hydroxyl) significantly and consistently improved potency (compounds (±)-**10a–f**).

The effect of absolute stereochemistry of the hydroxyl group on the hTRPM8 in-vitro potency of **10d–f** is depicted in Table 3. The inhibitory potency of individual isomers (+)-**10**–f and (−)-**10d–f** on related channels such as TRPV1, TRPV3, TRPV4 and TRPA1 was also studied and the results are shown in the Table 3. Interestingly, the optical antipodes showed significant differences in their inhibitory potency on TRPM8 channel and (*R*)-isomers of **10d–f** were more potent than the corresponding (*S*)-isomers. A similar but opposite trend was observed in the case of TRPV1 and the (*S*)-isomers of **10d–f** were more potent than the corresponding (*R*)-isomers. Both (*R*)-(−)-**10d–f** and (*S*)-(+)-**10d–f** showed very good selectivity over TRPV3, TRPV4 and TRPA1 and absolute stereochemistry did not play any significant role on selectivity.

2.3. Metabolic stability and PK profile of (*R*)-(−)-10e

The in-vitro metabolic stability of (−)-**10e** was determined by measuring the disappearance of (−)-**10e** in liver microsomal incubations at 37 °C for 60 min. Compound (−)-**10e** showed moderate to high stability across the species with 81%, 79%, 42%, 72% and 67% remaining in the male CD1 mouse, male Wistar rat, male Beagle dog, male Cynomolgus monkey and pooled human liver microsomes, respectively.

A single dose pharmacokinetic (PK) profile of (−)-**10e** was examined in male Sprague Dawley (SD) rats at 10 mg/kg dose (Fig. 2). Following an oral administration, quantifiable levels of (−)-**10e** were found up to 8 h post dose in all the animals. The compound (−)-**10e** showed rapid absorption with a median T_{max} of 1 h post dose. A mean C_{max} of 1088 ng/mL and mean AUC_{0-t} of 4978 ng/h/mL were observed at 10 mg/kg oral dose.

2.4. In-vivo pharmacology

To establish correlation between in-vitro potency, pharmacokinetics (PK) and pharmacodynamics (PD), (−)-**10e** was also screened

Table 1
In-vitro hTRPM8 activity of **8a–m**[a]

Compd.	R^1	R^2	Ar	hIC_{50} (nM)
8a	H	H	Ph	2423.0
8b	H	H	4-CF_3Ph	280.7
8c	F	H	4-CF_3Ph	666.9
8d	H	F	4-CF_3Ph	97.3
8e	H	Cl	4-CF_3Ph	48.5
8f	H	Br	4-CF_3Ph	18.9
8g	H	Cl	4-OCF_3Ph	41.1
8h	H	Cl	4-CNPh	543.7
8i	H	Cl	4-CH_3Ph	289.6
8j	H	Cl	2-Cl, 4-CF_3Ph	177.8
8k	F	Cl	4-CF_3Ph	457.2
8l	H	Cl	4-CF_3, 1,3-thiazol-2-yl	265.8
8m	H	Cl	6-F, 1,3-benzothiazol-2-yl	113.1

[a] IC_{50} values were determined by radiometric $^{45}Ca^{+2}$ uptake assay from at least two independent experiments using six point concentration dose–response curves.

Table 2
In-vitro hTRPM8 activity of **9a–f** and (±)-**10a–f**[a]

Compd.	R^2	R^3	hIC_{50} (nM)
9a	F	CF_3	295.2
9b	Cl	CF_3	230.4
9c	Br	CF_3	656.9
9d	F	OCF_3	182.1
9e	Cl	OCF_3	111.0
9f	Br	OCF_3	107.3
(±)-**10a**	F	CF_3	90.1
(±)-**10b**	Cl	CF_3	65.9
(±)-**10c**	Br	CF_3	44.5
(±)-**10d**	F	OCF_3	22.5
(±)-**10e**	Cl	OCF_3	13.3
(±)-**10f**	Br	OCF_3	19.5

[a] IC_{50} values were determined by radiometric $^{45}Ca^{+2}$ uptake assay from at least two independent experiments using six point concentration dose–response curves.

Table 3
hTRPM8 activity and selectivity of optical isomers (*S*)-(+)-**10d–f** and (*R*)-(−)-**10d–f**

Compd.	IC_{50} (nM)		% Inhibition at 1.0 μM		
	hTRPM8[a]	hTRPV1[a]	hTRPV3	hTRPV4	hTRPA1
(*S*)-(+)-**10d**	94.0	266.3	23.2	6.6	2.8
(*R*)-(−)-**10d**	9.5	841.6	26.4	12.5	7.8
(*S*)-(+)-**10e**	46.3	217.8	17.7	12.8	15.9
(*R*)-(−)-**10e**	8.9	>1000	27.7	38.8	23.5
(*S*)-(+)-**10f**	60.0	412.9	8.0	3.3	4.3
(*R*)-(−)-**10f**	16.6	>1000	16.8	16.3	1.6

[a] TRPM8 and TRPV1 IC_{50} values were determined by radiometric $^{45}Ca^{+2}$ uptake assay from at least two independent experiments using six point concentration dose–response curves. TRPV3, TRPV4 and TRPA1 assays were run in duplicate and the results are expressed as percentage inhibition at 1.0 μM concentration of the compounds.

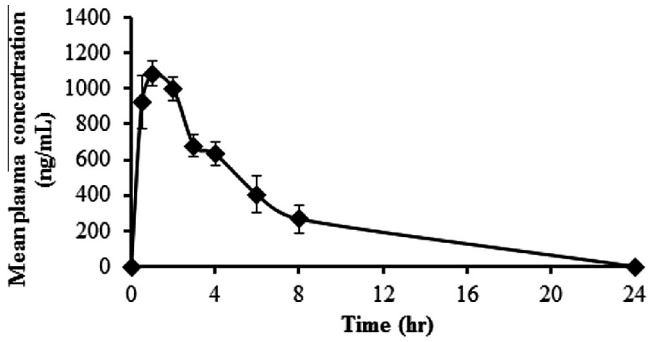


Figure 2. Pharmacokinetic profile of (*R*)-(−)-**10e** in male SD rats (mean ± SD of three animals per time point).

in rat TRPM8 channels using $^{45}Ca^{2+}$ uptake assay. It showed an IC_{50} of 16.5 nM, which is close to the human potency of 8.9 nM (Table 3). TRPM8 is considered as the predominant mammalian cold sensor in the induction of hypersensitivity to cold stimuli.[12,13,39] The in-vivo efficacy of compound (−)-**10e** was evaluated in three rodent models of neuropathic pain and the results are given below.

First, the compound (−)-**10e** was evaluated in acetone spray induced cold allodynia model in SD rats that had undergone chronic constriction injury (CCI) neuropathic surgical procedure, using capsazepine as a reference compound.[28] Compound (−)-**10e** (10 mg/kg, po) significantly inhibited the cold allodynia induced by acetone spray in rats, with CCI nerve injury, with a maximal efficacy of 74% reversal observed at 2 h post administration. Capsazepine (10 mg/kg, ip) also produced a moderate efficacy of 44% inhibition of cold-allodynia (Fig. 3).

The compound (−)-**10e** was then evaluated in a behavioral assay of icilin-induced wet dog shakes (WDS) in C57/BL6 mice to measure responses to sensation of intense cold.[40] Icilin a TRPM8 agonist, was used in the experiment to induce WDS in mice.[41,42] The compound (−)-**10e** (3, 10 and 30 mg/kg, po) was evaluated in this study. The top dose, 30 mg/kg, significantly inhibited WDS like cold hypersensitivity in mice with a maximal of 72% efficacy. The standard comparator drug pregabalin (30 mg/kg, po) produced a significant 57% inhibition of icilin-induced WDS behavior (Fig. 4).

Finally, the compound (−)-**10e** was evaluated in oxaliplatin-induced peripheral neuropathic pain in C57/BL6 mice. Oxaliplatin, a platinum-based chemotherapeutic drug, is known to induce significant cold hypersensitivity presumably due to increase in TRPM8 expression.[43–45] Compound (−)-**10e** (3, 10 and 30 mg/kg, po) produced a dose-dependent inhibition of nocifensive paw licking in oxaliplatin-induced cold allodynia in mice with a maximal of 100% effect observed at 30 mg/kg dose. Pregabalin (30 mg/kg, po) also produced a significant 69% efficacy (Fig. 5).

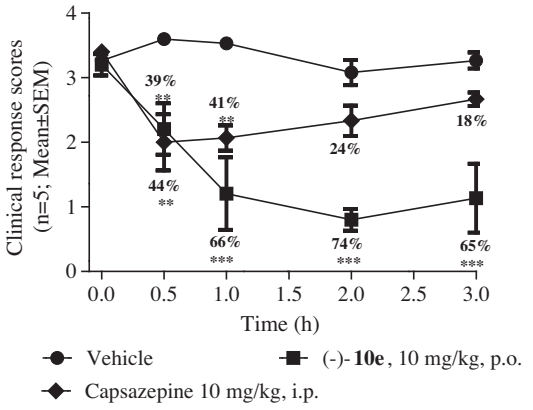


Figure 3. Effect of (−)-**10e** on acetone spray induced cold allodynia in rats with CCI. Clinical scoring pattern—0: no response; 1: brisk withdrawal or flicking of paw; 2: repeated flicking of paw; 3: repeated flicking and licking of paw (<5 s); 4: repeated flicking and licking of paw (>5 s). $^{**}p < 0.01$, $^{***}p < 0.001$, treatment groups vs. vehicle group, two-way ANOVA, followed by Bonferroni's test.

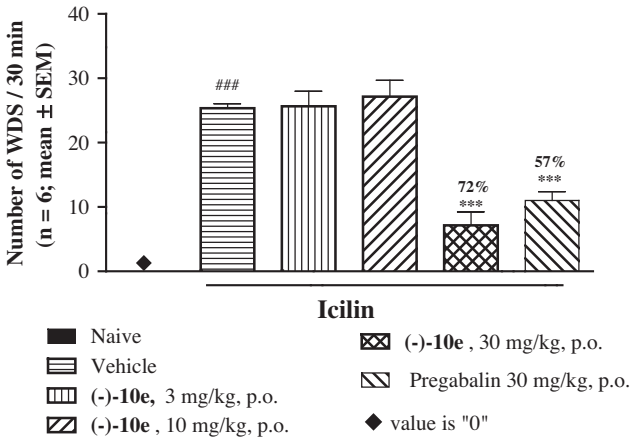


Figure 4. Effect of (−)-**10e** on icilin-induced WDS in C57BL/6J mice. Percent inhibition is given above the respective bar. $^{###}p < 0.001$, vehicle group versus naïve group, $^{***}p < 0.001$ vehicle group versus treated groups, one-way ANOVA, followed by Bonferroni's test.

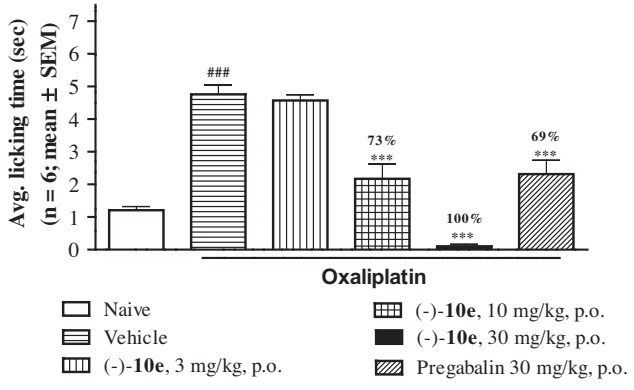


Figure 5. Effect of (−)-**10e** on oxaliplatin-induced cold allodynia in male C57BL/6J mice. Percent inhibition is given above the respective bar. $^{###}p < 0.001$, vehicle group versus naïve group, $^{***}p < 0.001$ vehicle group versus treated groups, one-way ANOVA, followed by Bonferroni's test.

Thus, the compound (−)-**10e** produced significant efficacy in various preclinical rodent models of cold allodynia/hypersensitivity.

3. Conclusion

In summary, several novel, structurally diverse urea derivatives of 3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidines] were synthesized and evaluated as TRPM8 antagonists. A detailed SAR analysis revealed that introduction of a hydroxyl group at the 4-position of the dihydrochromene ring resulted in improved potency. Optical resolution of the chiral alcohols and re-screening of the individual isomers demonstrated that the (*R*)-(−)-isomers were three to four-fold more potent than the corresponding (*S*)-(+)-isomers. The selected compound (−)-**10e** showed excellent potency, metabolic stability and favorable pharmacokinetics properties. From the rodent models of neuropathic pain explored in the present work, we believe that (−)-**10e** has potential utility in the management of pain. Further work is in progress to understand the effect of (−)-**10e** on body temperature in relevant preclinical models.

4. Experimental

4.1. Chemistry

Melting points are uncorrected. Infrared spectra were recorded on Perkin Elmer spectrum one FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 MHz FT NMR spectrometer in either $CDCl_3$ or DMSO-d_6 as specified using tetramethylsilane as internal standard. Chemical shifts are quoted in ppm (δ) relative to TMS (^{1}H) using residual protonated solvent as internal standard. Routine mass spectra (MS) were recorded on a Thermo Finnigan model LCQ Deca XP Max spectrometer using ESI or APCI source at positive or negative polarity mode by direct infusion method. High-resolution mass spectra (HRMS) of all test compounds were measured on a Thermo Scientific LTQ Orbitrap Discovery MS system coupled with a LQT Tune Plus (version 2.5.5 SPI) software operating in a positive electron spray ionization (ESI) mode. Optical purity of all chiral test compounds was measured on a Chiralpak IA or Chiralpak AD-H Diacel column. Optical rotations were measured on a Jasco P-1030 polarimeter. All test compounds used in biological assay were greater than 98% pure as determined by HPLC on a Shimadzu or Waters system using PDA detector.

All chemicals, reagents and solvents were procured from commercial sources and were used as received. Petroleum ether (PE) refers to the fraction boiling in the 40–60 °C range. *tert*-Butyl-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate derivatives **5** were synthesized according to literature procedures starting from commercially available 2-hydroxyacetophones **3** and *N*-BOC-4-piperidone **4**.[34] 3,4-Dihydrospiro[chromene-2,4′-piperidine]hydrochloride **7** was prepared from **5** by carbonyl group reduction and reductive deoxygenation by following a literature procedure.[34] All aryl phenyl carbamates used for the preparation of urea derivatives were prepared by the reaction of appropriate aromatic or hetero-aromatic amines with phenyl chloroformate in the presence of pyridine in tetrahydrofuran by the following literature procedure.[35]

4.1.1. General procedure for the synthesis of 4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidines] 5a–f

A solution of appropriate 2-hydroxyacetophone derivative **3** (100 mmol), *N*-*tert*-butoxycarbonyl-4-piperidone **4** (19.92 g, 100 mmol) and pyrrolidine (8.21 mL, 100 mmol) in methanol (150 mL) was stirred at room temperature for 18–24 h. Upon completion of the reaction (monitored by TLC), the mixture was concentrated under vacuum and the brown residue thus obtained was partitioned between EtOAc (500 mL) and 1 N hydrochloric acid (500 mL). The layers were separated. The aqueous layer was extracted with EtOAc (2 × 250 mL). The combined organic extracts were successively washed with water (2 × 250 mL), saturated $NaHCO_3$ solution (250 mL) and brine (200 mL). The residue obtained after evaporation of the solvent was purified by flash silica gel column chromatography using 20–30% EtOAc in petroleum ether (PE) as eluent.

4.1.1.1. *tert*-Butyl 4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 5a. White solid; yield 76%; mp 91–92 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.39 (s, 9H), 1.57–1.65 (m, 2H), 1.85–1.90 (m, 2H), 2.84 (s, 2H), 3.01–3.19 (m, 2H), 3.69–3.73 (m, 2H), 7.02–7.09 (m, 2H), 7.58 (t, J = 7.5 Hz, 1H), 7.72 (d, J = 7.8 Hz, 1H); APCI-MS (m/z) 218 [M+H–CO_2, –$(CH_3)_2$C=CH].

4.1.1.2. *tert*-Butyl 6-fluoro-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 5b. White solid; yield 51%; mp 117–119 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.39 (s, 9H), 1.56–1.66 (m, 2H), 1.84–1.89 (m, 2H), 2.86 (s, 2H), 3.10 (br s, 2H), 3.68–3.73 (m, 2H), 7.11–7.17 (m, 1H), 7.41–7.50 (m, 2H); APCI-MS (m/z) 236 [(M+H)–CO_2,–$(CH_3)_2$C=CH].

4.1.1.3. *tert*-Butyl 8-fluoro-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 5c. Off-white solid; yield 88%; mp 98–100 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40 (s, 9H), 1.58–1.71 (m, 2H), 1.87–1.98 (m, 2H), 2.92 (s, 2H), 3.05–3.13 (m, 2H), 3.69–3.76 (m, 2H), 7.00–7.08 (m, 1H), 7.52–7.60 (m, 2H); APCI-MS (m/z) 236 [(M+H)–CO_2, –$(CH_3)_2$C=CH].

4.1.1.4. *tert*-Butyl 8-chloro-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 5d. Off-white solid; yield 85%; mp 117–118 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.39 (s, 9H), 1.60–1.69 (m, 2H), 1.86–1.95 (m, 2H), 2.92 (s, 2H), 2.99–3.18 (m, 2H), 3.79–3.85 (m, 2H), 7.07 (t, J = 7.8 Hz, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H); APCI-MS (m/z) 352 $[M+H]^+$.

4.1.1.5. *tert*-Butyl 8-bromo-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 5e. Off-white solid; yield 77%; mp 127–128 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.59–1.68 (m, 2H), 1.82–1.94 (m, 2H), 2.91 (s, 2H), 3.01–3.21 (m, 2H), 3.80–3.86 (m, 2H), 7.01 (t, J = 7.8 Hz, 1H), 7.74 (d, J = 7.5 Hz, 1H), 7.90 (d, J = 7.5 Hz, 1H); APCI-MS (m/z) 396 $[M+H]^+$.

4.1.1.6. *tert*-Butyl 8-chloro-6-fluoro-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 5f. Off-white solid; yield 78%; mp 131–133 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.56–1.66 (m, 2H), 2.00–2.11 (m, 2H), 2.75 (s, 2H), 3.19–3.32 (m, 2H), 3.90–4.10 (m, 2H), 7.36 (dd, J = 7.5, 3.0 Hz, 1H), 7.47 (dd, J = 7.8, 3.0 Hz, 1H); APCI-MS (m/z) 370 $[M+H]^+$.

4.1.2. General procedure for the synthesis of 4-hydroxy-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylates 6a–f

To a stirred solution of appropriate spiroketone **5** (10 mmol) in ethanol (30 mL) was added sodium borohydride (378 mg, 10 mmol) portion wise and the mixture was further stirred at RT for 1 h. The reaction mixture was diluted with water (300 ml) and extracted with EtOAc (3 × 200 mL). The combined extracts were washed with water (200 mL) and brine (100 mL). The solvent was evaporated under reduced pressure and the residue obtained was dried under vacuum to give alcohols **6a–f**.

4.1.2.1. *tert*-Butyl 4-hydroxy-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 6a. White semisolid; yield 91%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.39 (s, 9H), 1.48–1.52 (m, 1H), 1.55–1.63 (m, 2H), 1.69–1.77 (m, 2H), 2.05–2.11 (m, 1H), 3.10–3.20 (m, 2H), 3.66 (br s, 2H), 4.67 (q, J = 7.2 Hz, 1H), 5.36 (d, J = 6.0 Hz, 1H), 6.76 (d, J = 7.8 Hz, 1H), 6.88 (t, J = 7.2 Hz, 1H), 7.12

(t, J = 7.8 Hz, 1H), 7.40 (d, J = 7.8 Hz, 1H); APCI-MS (m/z) 220 [(M+H)–CO_2, –$(CH_3)_2C$=CH].

4.1.2.2. *tert*-Butyl 6-fluoro-4-hydroxy-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 6b. Colorless liquid; yield 90%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.39 (s, 9H), 1.47–1.58 (m, 1H), 1.62–1.69 (m, 2H), 1.70–1.77 (m, 2H), 2.06–2.12 (m, 1H), 3.06 (br s, 1H), 3.18 (br s, 1H), 3.70 (br s, 2H), 4.62–4.69 (m, 1H), 5.50 (d, J = 6.3 Hz, 1H), 6.76–6.81 (m, 1H), 6.93–7.00 (m, 1H), 7.12–7.18 (m, 1H); APCI-MS (m/z) 238 [(M+H)–CO_2, –$(CH_3)_2C$=CH].

4.1.2.3. *tert*-Butyl 8-fluoro-4-hydroxy-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 6c. White semisolid; yield 89%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40 (s, 9H), 1.54–1.62 (m, 1H), 1.67–1.74 (m, 2H), 1.79–1.84 (m, 2H), 2.10–2.15 (m, 1H), 3.17 (br s, 2H), 3.68 (br s, 2H), 4.70 (q, J = 7.8 Hz, 1H), 5.51 (d, J = 6.0 Hz, 1H), 6.82–6.89 (m, 1H), 7.03–7.10 (m, 1H), 7.21 (d, J = 7.8 Hz, 1H); APCI-MS (m/z) 238 [(M+H)–CO_2, –$(CH_3)_2C$=CH].

4.1.2.4. *tert*-Butyl 8-chloro-4-hydroxy-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 6d. White semisolid; yield 84%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40 (s, 9H), 1.51–1.71 (m, 3H), 1.77–1.81 (m, 2H), 2.09–2.16 (m, 1H), 3.02–3.16 (m, 2H), 3.69–3.78 (m, 2H), 4.68–4.75 (m, 1H), 5.53 (d, J = 6.8 Hz, 1H), 6.90 (t, J = 7.8 Hz, 1H), 7.28 (d, J = 6.9 Hz, 1H), 7.37 (d, J = 6.9 Hz, 1H); APCI-MS (m/z) 254 [(M+H)–CO_2, –$(CH_3)_2C$=CH].

4.1.2.5. *tert*-Butyl 8-bromo-4-hydroxy-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 6e. Off-white semisolid; yield 82%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40 (s, 9H), 1.62–1.66 (m, 4H), 1.82–1.88 (m, 2H), 2.88–2.96 (m, 2H), 3.77–3.83 (m, 2H), 4.70–4.76 (m, 1H), 5.53 (d, J = 5.4 Hz, 1H, exchangeable with D_2O), 6.85 (t, J = 7.2 Hz, 1H), 7.42 (t, J = 7.8 Hz, 2H); APCI-MS (m/z) 298 [(M+H)–CO_2, –$(CH_3)_2C$=CH].

4.1.2.6. *tert*-Butyl 8-chloro-6-fluoro-4-hydroxy-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate 6f. White semisolid; yield 87%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40 (s, 9H), 1.59–1.67 (m, 2H), 1.71–1.83 (m, 2H), 2.11–2.17 (m, 2H), 3.00–3.15 (m, 2H), 3.76 (br s, 2H), 4.70 (t, J = 7.2 Hz, 1H), 5.68 (d, J = 6.3 Hz, 1H), 7.16–7.22 (m, 1H), 7.29–7.33 (m, 1H); APCI-MS (m/z) 272 [(M+H)–CO_2, –$(CH_3)_2C$=CH].

4.1.3. General procedure for the synthesis of 3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]hydrochlorides (7a–f)

To a stirred solution of alcohol **6a**–**f** (9.0 mmol) in TFA (30 mL) was added triethylsilane (5.75 mL, 36 mmol) and the mixture was refluxed for 18 h. The reaction mixture was concentrated under reduced pressure to result a viscous residue. The residue was dissolved in water (100 mL) and neutralized with saturated $NaHCO_3$ solution. The mixture was extracted with EtOAc (2 × 200 mL). The combined extracts were washed with water (100 mL) and brine (100 mL). The residue obtained after evaporation of the solvent was dissolved in minimum quantity of EtOAc. Excess of ~10% (w/v) HCl in EtOAc (300 mL) was added under stirring. The white solid separated out was collected by filtration.

4.1.3.1. 3,4-Dihydrospiro[chromene-2,4′-piperidine]hydro-chloride 7a. White solid; yield 82%; mp 220–222 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80–1.88 (m, 6H), 2.72–2.76 (m, 2H), 3.00–3.08 (m, 2H), 3.11–3.15 (m, 2H), 6.80–6.86 (m, 2H), 7.02–7.11 (m, 2H), 9.11 (br s, 2H); ESI-MS (m/z) 204 [M+H]$^+$.

4.1.3.2. 6-Fluoro-3,4-dihydrospiro[chromene-2,4′-piperidine]hydrochloride 7b. Off-white solid; yield 88%; mp 231–233 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78–1.85 (m, 6H), 2.72–2.77 (m, 2H), 3.04 (br s, 2H), 3.13 (br s, 2H), 6.80–6.86 (m, 1H), 6.90–6.97 (m, 2H), 9.21 (br s, 2H); ESI-MS (m/z) 222 [M+H]$^+$.

4.1.3.3. 8-Fluoro-3,4-dihydrospiro[chromene-2,4′-piperidine]hydrochloride 7c. Off-white solid; yield 83%; mp 209–211 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.85–1.95 (m, 6H), 2.78 (t, J = 6.9 Hz, 2H), 3.04 (br s, 2H), 3.17–3.23 (m, 2H), 6.78–6.85 (m, 1H), 6.93 (d, J = 7.2 Hz, 1H), 7.00–7.06 (m, 1H), 9.06 (br s, 2H); APCI-MS (m/z) 222 [M+H]$^+$.

4.1.3.4. 8-Chloro-3,4-dihydrospiro[chromene-2,4′-piperidine]hydrochloride 7d. Off-white solid; yield 85%; mp 221–223 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.84–1.90 (m, 6H), 2.77–2.81 (m, 2H), 2.98–3.06 (m, 2H), 3.19–3.25 (m, 2H), 6.85 (t, J = 7.8 Hz, 1H), 7.09 (d, J = 7.8 Hz, 1H), 7.25 (d, J = 7.8 Hz, 1H), 9.30 (br s, 2H); APCI-MS (m/z) 238 [M+H]$^+$.

4.1.3.5. 8-Bromo-3,4-dihydrospiro[chromene-2,4′-piperidine]hydrochloride 7e. Off-white solid; yield 86%; mp 231–232 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80–1.88 (m, 6H), 2.78–2.82 (m, 2H), 3.01–3.12 (m, 2H), 3.20–3.29 (m, 2H), 6.81 (t, J = 7.8 Hz, 1H), 7.13 (d, J = 7.8 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 8.99 (br s, 2H); APCI-MS (m/z) 282 [M+H]$^+$.

4.1.3.6. 8-Chloro-6-fluoro-3,4-dihydrospiro[chromene-2,4′-piperidine]hydrochloride 7f. Off-white solid; yield 71%; mp >250 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.44–1.51 (m, 2H), 1.58–1.64 (m, 2H), 1.70–1.78 (m, 2H), 2.68–2.86 (m, 6H), 7.01 (dd, J = 8.4, 2.4 Hz, 1H), 7.20 (dd, J = 8.4, 2.4 Hz, 1H), 8.35 (br s, 2H); APCI-MS (m/z) 256 [M+H]$^+$.

4.1.4. General procedure for the synthesis of urea derivatives 8a–m

To a stirred solution of 3,4-dihydrospiro[chromene-2,4′-piperidine]hydrochloride **7a**–**f** (0.50 mmol) in anhydrous DMSO (4 mL) was added appropriate phenyl aryl carbamate (0.55 mmol) followed by triethylamine (0.35 mL, 2.50 mmol). The resulting mixture was stirred for 2–4 h (monitored by TLC) at room temperature. On completion of the reaction, the mixture was diluted with EtOAc (200 mL) and washed with water (2 × 50 mL), followed by brine (50 mL). The residue obtained after evaporation of the solvent was purified by silica gel column chromatography using 10–20% EtOAc in PE as eluent to obtain the product as white to off-white solid.

4.1.4.1. *N*-Phenyl-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8a). Reaction of **7a** (150 mg, 0.625 mmol) with phenyl phenylcarbamate (147 mg, 0.688 mmol) gave 151 mg (75%) of the product as a white solid. Mp 167–168 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.55–1.62 (m, 2H), 1.69–1.74 (m, 2H), 1.78–1.83 (m, 2H), 2.72–2.76 (m, 2H), 3.17–3.27 (m, 2H), 3.83–3.90 (m, 2H), 6.78–6.83 (m, 2H), 6.91 (t, J = 7.5 Hz, 1H), 7.05–7.09 (m, 2H), 7.21 (t, J = 7.5 Hz, 2H), 7.45 (d, J = 7.8 Hz, 2H), 8.52 (s, 1H); HRMS (ESI) calcd for $C_{20}H_{22}N_2O_2$, [M+H]$^+$, 323.1754; found, 323.1752.

4.1.4.2. *N*-[4-(Trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8b). Reaction of **7a** (50 mg, 0.208 mmol) with phenyl [4-(trifluoromethyl)-phenyl]carbamate (64 mg, 0.229 mmol) gave 63 mg (77%) of the product as a white solid. Mp 158–160 °C; ^{1}H NMR

(300 MHz, DMSO-d_6) δ 1.57–1.64 (m, 2H), 1.70–1.83 (m, 4H), 2.74 (t, J = 5.7 Hz, 2H), 3.20–3.26 (m, 2H), 3.86–3.82 (m, 2H), 6.78–6.84 (m, 2H), 7.04–7.10 (m, 2H), 7.57 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 8.93 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{21}F_3N_2O_2$, $[M+H]^+$ 391.1627; found, 391.1625.

4.1.4.3. 6-Fluoro-*N*-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8c).

Reaction of **7b** (50 mg, 0.194 mmol) with phenyl[4-(trifluoromethyl)phenyl]carbamate (60 mg, 0.213 mmol) gave 51 mg (65%) of the product as a white solid. Mp 162–164 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56–1.63 (m, 2H), 1.69–1.81 (m, 4H), 2.74 (t, J = 6.6 Hz, 2H), 3.21–3.28 (m, 2H), 3.86–3.91 (m, 2H), 6.78–6.82 (m, 1H), 6.89–6.95 (m, 2H), 7.57 (d, J = 8.1 Hz, 2H), 7.68 (d, J = 8.7 Hz, 2H), 8.93 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{20}F_4N_2O_2$, $[M+H]^+$, 409.1533; found, 409.1530.

4.1.4.4. 8-Fluoro-*N*-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8d).

Reaction of **7c** (50 mg, 0.194 mmol) with phenyl[4-(trifluoromethyl)phenyl]carbamate (60 mg, 0.213 mmol) gave 53 mg (67%) of the product as a white solid. Mp 163–165 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.57–1.68 (m, 2H), 1.74–1.85 (m, 4H), 2.78 (br s, 2H), 3.20–3.26 (m, 2H), 3.87–3.93 (m, 2H), 6.78–6.83 (m, 1H), 6.92 (d, J = 7.8 Hz, 1H), 6.97–7.05 (m, 1H), 7.58 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 8.95 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{20}F_4N_2O_2$, $[M+H]^+$, 409.1533; found, 409.1530.

4.1.4.5. 8-Chloro-*N*-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8e).

Reaction of **7d** (500 mg, 1.823 mmol) with phenyl[4-(trifluoromethyl)phenyl]carbamate (564 mg, 2.006 mmol) gave 627 mg (81%) of the product as a white solid. Mp 158–159 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.61–1.69 (m, 2H), 1.84–1.94 (m, 4H), 2.80–2.86 (m, 2H), 3.42–3.50 (m, 2H), 3.90–3.97 (m, 2H), 6.60 (br s, 1H), 6.80 (t, J = 7.8 Hz, 1H), 6.98 (d, J = 7.5 Hz, 1H), 7.20 (d, J = 7.8 Hz, 1H), 7.47–7.55 (m, 4H); HRMS (ESI) calcd for $C_{21}H_{20}ClF_3N_2O_2$, $[M+H]^+$, 425.1238; found, 425.1230.

4.1.4.6. 8-Bromo-*N*-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8f).

Reaction of **7e** (200 mg, 0.628 mmol) with phenyl[4-(trifluoromethyl)phenyl]carbamate (194 mg, 0.690 mmol) gave 224 mg (76%) of the product as a white solid. Mp 159–160 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.59–1.66 (m, 2H), 1.73–1.86 (m, 4H), 2.74–2.81 (m, 2H), 3.18–3.28 (m, 2H), 3.98–4.05 (m, 2H), 6.79 (t, J = 7.8 Hz, 1H), 7.11 (d, J = 7.2 Hz, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.58 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 8.96 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{20}BrF_3N_2O_2$, $[M+H]^+$, 469.0733; found, 469.0729.

4.1.4.7. 8-Chloro-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8g).

Reaction of **7d** (100 mg, 0.364 mmol) with phenyl[4-(trifluoromethoxy)phenyl]carbamate (119 mg, 0.401 mmol) as described gave 140 mg (87%) of the product as a white solid. Mp 139–140 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.62–1.70 (m, 2H), 1.72–1.80 (m, 2H), 1.82–1.87 (m, 2H), 2.75–2.81 (m, 2H), 3.17–3.24 (m, 2H), 3.94–4.00 (m, 2H), 6.83 (t, J = 7.8 Hz, 1H), 7.07 (d, J = 7.5 Hz, 1H), 7.20–7.29 (m, 3H), 7.56 (d, J = 8.9 Hz, 2H), 8.76 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{20}ClF_3N_2O_3$, $[M+H]^+$, 441.1187; found, 441.1187.

4.1.4.8. 8-Chloro-*N*-(4-cyanophenyl)-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8h).

Reaction of **7d** (100 mg, 0.364 mmol) with phenyl(4-cyanophenyl)carbamate (96 mg, 0.401 mmol) gave 107 mg (77%) of the product as a white solid. Mp >250 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60–1.67 (m, 2H), 1.73–1.86 (m, 4H), 2.75–2.84 (m, 2H), 3.15–3.26 (m, 2H), 3.95–4.03 (m, 2H), 6.83 (t, J = 7.2 Hz, 1H), 7.07 (d, J = 7.0 Hz, 1H), 7.23 (d, J = 7.5 Hz, 1H), 7.64–7.70 (m, 4H), 9.05 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{20}ClN_3O_2$, $[M+H]^+$, 382.1316; found, 382.1316.

4.1.4.9. 8-Chloro-*N*-(4-methylphenyl)-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8i).

Reaction of **7d** (100 mg, 0.364 mmol) with phenyl(4-methylphenyl) carbamate (91 mg, 0.401 mmol) gave 111 mg (82%) of the product as a white solid. Mp 194–196 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56–1.64 (m, 2H), 1.70–1.86 (m, 4H), 2.22 (s, 3H), 2.79 (t, J = 6.3 Hz, 2H), 3.14–3.22 (m, 2H), 3.92–3.97 (m, 2H), 6.83 (t, J = 7.8 Hz, 1H), 7.01–7.08 (m, 3H), 7.23 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 8.1 Hz, 2H), 8.45 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{23}Cl_1N_2O_2$, $[M+H]^+$, 371.1520; found, 371.1519.

4.1.4.10. 8-Chloro-*N*-[2-chloro-4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8j).

Reaction of **7d** (75 mg, 0.273 mmol) with phenyl[2-chloro-4-(trifluoromethyl)phenyl]carbamate (95 mg, 0.301 mmol) gave 102 mg (81%) of the product as a white solid. Mp 157–158 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60–1.79 (m, 4H), 1.80–1.89 (m, 2H), 2.77–2.82 (m, 2H), 3.20–3.31 (m, 2H), 3.93–3.99 (m, 2H), 6.84 (t, J = 7.8 Hz, 1H), 7.08 (d, J = 7.2 Hz, 1H), 7.24 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.79–7.84 (m, 2H), 8.49 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{19}Cl_2F_3N_2O_2$, $[M+H]^+$, 459.0848; found, 459.0849.

4.1.4.11. 8-Chloro-6-fluoro-*N*-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8k).

Reaction of **7f** (100 mg, 0.342 mmol) with phenyl[4-(trifluoromethyl)phenyl]carbamate (106 mg, 0.376 mmol) gave 103 mg (68%) of the product as a white solid. Mp 159–160 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.59–1.68 (m, 2H), 1.71–1.77 (m, 2H), 1.82–1.87 (m, 2H), 2.75–2.82 (m, 2H), 3.16–3.24 (m, 2H), 3.94–4.03 (m, 2H), 7.01 (dd, J = 5.3, 2.4 Hz, 1H), 7.25 (dd, J = 5.4, 2.4 Hz, 1H), 7.58 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 8.97 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{19}ClF_4N_2O_2$, $[M+H]^+$, 443.1143; found, 443.1144.

4.1.4.12. 8-Chloro-*N*-[4-(trifluoromethyl)-1,3-thiazol-2-yl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8l).

Reaction of **7d** (250 mg, 0.911 mmol) with phenyl[4-(trifluoromethyl)-1,3-thiazol-2-yl]carbamate (289 mg, 1.002 mmol) gave 197 mg (50%) of the product as a white solid. Mp 96–97 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.63–1.76 (m, 4H), 1.80–1.86 (m, 2H), 2.75–2.81 (m, 2H), 3.19–3.26 (m, 2H), 4.03–4.10 (m, 2H), 6.84 (t, J = 7.8 Hz, 1H), 7.07 (d, J = 7.5 Hz, 1H), 7.24 (d, J = 7.2 Hz, 1H), 7.80 (s, 1H), 11.42 (br s, 1H); HRMS (ESI) calcd for $C_{18}H_{17}ClF_3N_3O_2S$, $[M+H]^+$, 432.0754; found, 432.0753.

4.1.4.13. 8-Chloro-*N*-(6-fluoro-1,3-benzothiazol-2-yl)-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (8m).

Reaction of **7d** (75 mg, 0.273 mmol) with phenyl(6-fluoro-1,3-benzothiazol-2-yl)carbamate (87 mg, 0.301 mmol) gave 105 mg (89%) of the product as a white solid. Mp 188–189 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.64–1.78 (m, 4H), 1.80–1.87 (m, 2H), 2.72–2.80 (m, 2H), 3.22–3.28 (m, 2H), 4.05–4.11 (m, 2H), 6.84 (t, J = 7.5 Hz, 1H), 7.08 (d, J = 7.5 Hz, 1H), 7.21–7.26 (m, 2H), 7.60–7.66 (m, 1H), 7.78–7.84 (m, 1H), 11.30 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{19}ClFN_3O_2S$, $[M+H]^+$, 432.0943; found, 432.0934.

4.1.5. General procedure for the synthesis of urea derivatives 9a–f

To a stirred suspension of *tert*-butyl 4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxylate **5c–e** (10 mmol) in ethyl acetate (10 mL) was added excess of ~10% (w/v) HCl in EtOAc (300 mL) and the mixture was stirred at room temperature for 2 h. Ethyl acetate was evaporated under reduced pressure to afford the amine hydrochloride (yield ~100%) as a white solid, which was used as such for the coupling reaction.

To a stirred solution of above amine hydrochloride (10 mmol) in anhydrous DMSO (30 mL), was added appropriate phenyl aryl carbamate (11 mmol) followed by triethylamine (6.97 mL, 50 mmol). The resulting mixture was stirred at room temperature until completion of reaction (2–4 h). The mixture was diluted with EtOAc (300 mL) and washed with water (2 × 100 mL), followed by brine (100 mL). The residue obtained after evaporation of the solvent was purified by silica gel column chromatography using 10–20% EtOAc in PE as eluent to afford the products **9a–f** as white to off-white solid.

4.1.5.1. 8-Fluoro-4-oxo-*N*-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (9a).

Reaction of 8-fluoro-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]hydrochloride (300 mg, 1.104 mmol), prepared by deprotection of **5c**, with phenyl[4-(trifluoromethyl)phenyl]carbamate (342 mg, 1.214 mmol) gave 443 mg (95%) of the product as a white solid. Mp 186–188 °C; IR (KBr) 3370, 1691, 1655, 1492, 1338, 1223, 1100 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.74–1.81 (m, 2H), 1.93–2.01 (m, 2H), 2.96 (s, 2H), 3.15–3.26 (m, 2H), 3.90–3.98 (m, 2H), 7.02–7.10 (m, 1H), 7.52–7.60 (m, 4H), 7.64–7.70 (m, 2H), 8.97 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{18}F_4N_2O_3$, $[M+H]^+$, 423.1326; found, 423.1322.

4.1.5.2. 8-Chloro-4-oxo-*N*-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (9b).

Reaction of 8-chloro-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]hydrochloride (500 mg, 1.735 mmol), prepared by deprotection of **5d**, with phenyl[4-(trifluoromethyl)phenyl]carbamate (537 mg, 1.908 mmol) gave 639 mg (84%) of the product as a white solid. Mp 203–204 °C; IR (KBr) 3449, 3373, 1690, 1654, 1597, 1337, 1100, 840 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.70–1.80 (m, 2H), 1.92–2.02 (m, 2H), 2.96 (s, 2H), 3.15–3.25 (m, 2H), 3.97–4.05 (m, 2H), 7.09 (t, J = 7.8 Hz, 1H), 7.59 (d, J = 8.7 Hz, 2H), 7.66–7.80 (m, 4H), 8.98 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{18}ClF_3N_2O_3$, $[M+H]^+$, 439.1030; found, 439.1028.

4.1.5.3. 8-Bromo-4-oxo-*N*-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (9c).

Reaction of 8-bromo-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]hydrochloride (500 mg, 1.503 mmol), prepared by deprotection of **5e**, with phenyl[4-(trifluoromethyl)phenyl]carbamate (465 mg, 1.654 mmol) gave 603 mg (83%) of the product as a white solid. Mp 233–235 °C; IR (KBr) 3321, 1693, 1647, 1596, 1542, 1315, 1226, 1120, 835 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.68–1.77 (m, 2H), 1.92–2.00 (m, 2H), 2.94 (s, 2H), 3.15–3.26 (m, 2H), 3.98–4.06 (m, 2H), 7.02 (t, J = 7.8 Hz, 1H), 7.58 (d, J = 8.7 Hz, 2H), 7.67 (d, J = 8.7 Hz, 2H), 7.75 (d, J = 7.2 Hz, 1H), 7.91 (d, J = 7.2 Hz, 1H), 8.98 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{18}BrF_3N_2O_3$, $[M+H]^+$, 483.0525; found, 483.0525.

4.1.5.4. 8-Fluoro-4-oxo-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (9d).

Reaction of 8-fluoro-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]hydrochloride (300 mg, 1.104 mmol), prepared by deprotection of **5c**, with phenyl[4-(trifluoromethoxy)phenyl]carbamate (361 mg, 1.214 mmol) gave 407 mg (84%) of the product as a white solid. Mp 168–170 °C; IR (KBr) 3299, 1697, 1640, 1489, 1427, 1254, 1153 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.72–1.81 (m, 2H), 1.93–2.00 (m, 2H), 2.96 (s, 2H), 3.15–3.25 (m, 2H), 3.88–3.95 (m, 2H), 7.01–7.09 (m, 1H), 7.23 (d, J = 8.4 Hz, 2H), 7.53–7.63 (m, 4H), 8.78 (s, 1H); HRMS (ESI) calcd for $C_{21}H_{18}F_4N_2O_4$, $[M+H]^+$, 439.1275; found, 439.1271.

4.1.5.5. 8-Chloro-4-oxo-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (9e).

Reaction of 8-chloro-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]hydrochloride (2.0 g, 6.941 mmol), prepared by the deprotection of **5d**, with phenyl[4-(trifluoromethoxy)phenyl]carbamate (2.27 g, 7.635 mmol) gave 2.75 g (87%) of the product as a white solid. Mp 152–153 °C; IR (KBr) 3336, 1638, 1534, 1417, 1282, 1159 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.73–1.77 (m, 2H), 1.94–1.98 (m, 2H), 2.95 (s, 2H), 3.14–3.22 (m, 2H), 3.97–4.01 (m, 2H), 7.11 (t, J = 7.8 Hz, 1H), 7.23 (d, J = 8.7 Hz, 2H), 7.55 (d, J = 8.7 Hz, 2H), 7.72 (d, J = 7.2 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H), 8.78 (s, 1H); HRMS (ESI) calcd for $C_{21}H_{18}C_{l1}$-$F_3N_2O_4$, $[M+H]^+$, 455.098; found, 455.0977.

4.1.5.6. 8-Bromo-4-oxo-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (9f).

Coupling reaction of 8-bromo-4-oxo-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]hydrochloride (600 mg, 1.804 mmol), prepared by the deprotection of **5e**, with phenyl[4-(trifluoromethoxy)phenyl]carbamate (590 mg, 1.984 mmol) gave 847 mg (94%) of the product as a white solid. Mp 176–178 °C; IR (KBr) 3421, 1638, 1534, 1438, 1281, 1160 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.68–1.76 (m, 2H), 1.92–2.00 (m, 2H), 2.95 (s, 2H), 3.12–3.26 (m, 2H), 3.98–4.06 (m, 2H), 7.03 (t, J = 7.5 Hz, 1H), 7.23 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.7 Hz, 2H), 7.75 (d, J = 7.8 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 8.78 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{18}BrF_3N_2O_4$, $[M+H]^+$, 499.0474; found, 499.0472.

4.1.6. General procedure for the synthesis of alcohols (±)-10a–f

To a stirred solution of appropriate 4-oxo derivative **9a–f** (2.0 mmol) in ethanol (10 mL) was added $NaBH_4$ (76 mg, 2.0 mmol) portion-wise at room temperature. After 1 h, the mixture was diluted with water (100 mL) and extracted with EtOAc (2 × 200 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL). The solvent was evaporated and the residue obtained was purified on a short length silica gel column using 40–50% EtOAc in PE to yield the product as a white solid.

4.1.6.1. (±)-8-Fluoro-4-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (10a).

White solid; yield 94%; mp 140–141 °C; IR (KBr) 3336, 1650, 1482, 1327, 1223, 1114, cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.66–1.79 (m, 3H), 1.86–1.92 (m, 2H), 2.13–2.20 (m, 1H), 3.12–3.23 (m, 2H), 3.86–3.92 (m, 2H), 4.70–4.76 (m, 1H), 5.50–5.56 (m, 1H), 6.85–6.91 (m, 1H), 7.04–7.12 (m, 1H), 7.21–7.26 (m, 1H), 7.58 (d, J = 7.8 Hz, 2H), 7.68 (d, J = 7.8 Hz, 2H), 8.94 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{20}F_4N_2O_3$, $[M+H]^+$, 425.1482; found, 425.1479.

4.1.6.2. (±)-8-Chloro-4-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (10b).

White solid; yield 80%; mp 152–154 °C; IR (KBr) 3402, 1650, 1453, 1326, 1235, 1114, cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.61–1.78 (m, 3H), 1.81–1.93 (m, 2H), 2.12–2.19 (m, 1H), 3.10–3.17 (m, 2H), 3.93–3.99 (m, 2H), 4.70–4.76 (m, 1H),

5.55 (d, J = 5.7 Hz, 1H), 6.92 (t, J = 7.8 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.58 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 8.95 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{20}ClF_3N_2O_3$, $[M+H]^+$, 441.1187; found, 441.1184.

4.1.6.3. (±)-8-Bromo-4-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (10c). White solid; yield 87%; mp 159–161 °C; IR (KBr) 3397, 1651, 1559, 1326, 1235, 1114, cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.64–1.78 (m, 3H), 1.80–1.93 (m, 2H), 2.11–2.18 (m, 1H), 3.10–3.17 (m, 1H), 3.27–3.35 (m, 1H), 3.93–4.01 (m, 2H), 4.70–4.77 (m, 1H), 5.55 (d, J = 6.6 Hz, 1H), 6.86 (t, J = 7.8 Hz, 1H), 7.43 (t, J = 6.9 Hz, 2H), 7.58 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 8.94 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{20}BrF_3N_2O_3$, $[M+H]^+$, 485.0682; found, 485.0680.

4.1.6.4. (±)-8-Fluoro-4-hydroxy-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (10d). White solid; yield 93%; mp 143–144 °C; IR (KBr) 3348, 1644, 1511, 1260, 1200, 735, cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.65–1.78 (m, 3H), 1.82–1.90 (m, 2H), 2.10–2.20 (m, 1H), 3.10–3.25 (m, 2H), 3.84–3.90 (m, 2H), 4.70–4.75 (m, 1H), 5.52 (d, J = 6.0 Hz, 1H), 6.85–6.91 (m, 1H), 7.04–7.11 (m, 1H), 7.20–7.26 (m, 3H), 7.56 (d, J = 8.4 Hz, 2H), 8.74 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{20}F_4N_2O_4$, $[M+H]^+$, 441.1432; found, 441.1428.

4.1.6.5. (±)-8-Chloro-4-hydroxy-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (10e). White solid; yield 87%; mp 150–152 °C; IR (KBr) 3333, 1644, 1510, 1453, 1238, 1159, 964 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.60–1.77 (m, 3H), 1.81–1.92 (m, 2H), 2.12–2.19 (m, 1H), 3.09–3.16 (m, 1H), 3.21–3.29 (m, 1H), 3.88–3.96 (m, 2H), 4.71–4.77 (m, 1H), 5.54 (d, J = 6.3 Hz, 1H), 6.91 (t, J = 7.8 Hz, 1H), 7.23 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 7.2 Hz, 1H), 7.39 (d, J = 7.2 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 8.75 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{20}ClF_3N_2O_4$, $[M+H]^+$, 457.1136; found, 457.1133.

4.1.6.6. (±)-8-Bromo-4-hydroxy-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (10f). White solid; yield 81%; mp 159–161 °C; IR (KBr) 3340, 1643, 1510, 1447, 1238, 1160, 964 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.63–1.77 (m, 3H), 1.82–1.91 (m, 2H), 2.10–2.17 (m, 1H), 3.09–3.17 (m, 2H), 3.95–4.04 (m, 2H), 4.70–4.78 (m, 1H), 5.54 (d, J = 6.0 Hz, 1H), 6.86 (t, J = 7.2 Hz, 1H), 7.23 (d, J = 8.4 Hz, 2H), 7.39–7.47 (m, 2H), 7.56 (d, J = 8.4 Hz, 2H), 8.75 (br s, 1H); HRMS (ESI) calcd for $C_{21}H_{20}BrF_3N_2O_4$, $[M+H]^+$, 501.0631; found, 501.0630.

4.1.7. General procedure for synthesis of L-tryptophan esters 11a–c and 12a–c

To a stirred solution of (±)-**10a**–**f** (1.5 mmol) and Cbz-L-Trp-OH (609 mg, 1.8 mmol) in dichloromethane (20 ml) were added EDCI (345 mg, 1.8 mmol) and DMAP (220 mg, 1.8 mmol) at room temperature. The mixture was stirred at room temperature for 18 h. The solvent was evaporated under vacuum and the residue thus obtained was diluted with water (250 mL) and EtOAc (250 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined EtOAc extracts were washed with water (100 mL), dried (Na_2SO_4) and evaporated to afford a ~1:1 mixture of diastereomers **11a**–**c** and **12a**–**c**.

4.1.7.1. (4*S*)-8-Fluoro-1′-{[4-(trifluoromethoxy)phenyl]carbamoyl}-3,4-dihydrospiro[chromene-2,4′-piperidin]-4-yl *N*-[(benzyloxy)carbonyl]-L-tryptophanate (11a) and (4*R*)-8-fluoro-1′-{[4-(trifluoromethoxy)phenyl]carbamoyl}-3,4-dihydrospiro[chromene-2,4′-piperidin]-4-yl *N*-[(benzyloxy) carbonyl]-L-tryptophanate (12a). EDCI mediated esterification of (±)-**10d** (320 mg, 0.726 mmol) with Cbz-L-Trp-OH (295 mg, 0.871 mmol) afforded 575 mg of a mixture of **11a** and **12a**. Flash silica gel chromatography (30% EtOAc in PE) afforded 205 mg (37%) of **11a** as a white solid; mp 105–106 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 1.63–1.76 (m, 3H), 1.85–2.00 (m, 2H), 2.20–2.29 (m, 1H), 3.06–3.27 (m, 5H), 3.79–3.91 (m, 2H), 4.25–4.36 (m, 1H), 4.97 (s, 2H), 5.80–5.86 (m, 1H), 6.47 (d, J = 7.8 Hz, 1H), 6.70–6.80 (m, 1H), 6.95 (t, J = 7.2 Hz, 1H), 7.08 (t, J = 7.8 Hz, 1H), 7.13–7.30 (m, 8H), 7.37 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.53 (d, J = 8.7 Hz, 2H), 7.88 (d, J = 7.8 Hz, 1H), 8.77 (br s, 1H), 10.89 (br s, 1H); HRMS (ESI) calcd for $C_{40}H_{36}F_4N_4O_7$, $[M+H]^+$, 761.2592; found, 761.2599. HPLC: Chiralpak IA (Daicel), 250 × 4.6 mm, 5 μm, eluting with hexane/EtOH, 70:30, 99.17%; retention time: 14.5 min. Further elution with increasing amounts of EtOAc in PE gave 166 mg (30%) of **12a** as a white solid, mp 102–103 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 1.17–1.60 (m, 3H), 1.68–1.73 (m, 2H), 1.97–2.03 (m, 1H), 3.00–3.23 (m, 5H), 3.72–3.80 (m, 1H), 3.84–3.91 (m, 1H), 4.23–4.32 (m, 1H), 5.01 (s, 2H), 5.84–5.90 (m, 1H), 6.79–7.02 (m, 4H), 7.16–7.33 (m, 9H), 7.49 (d, J = 7.2 Hz, 1H), 7.59 (d, J = 8.7 Hz, 2H), 7.95 (d, J = 7.2 Hz, 1H), 8.75 (br s, 1H), 10.90 (br s, 1H); HRMS (ESI) calcd for $C_{40}H_{36}F_4N_4O_7$, $[M+H]^+$, 761.2592; found, 761.2598. HPLC: Chiralpak IA (Daicel), 250 × 4.6 mm, 5 μm, eluted with hexane/EtOH, 70:30, 99.69%, retention time: 10.4 min.

4.1.7.2. (4*S*)-8-Chloro-1′-{[4-(trifluoromethoxy)phenyl]carbamoyl}-3,4-dihydrospiro[chromene-2,4′-piperidin]-4-yl *N*-[(benzyloxy)carbonyl]-L-tryptophanate (11b) and (4*R*)-8-chloro-1′-{[4-(trifluoromethoxy)phenyl]carbamoyl}-3,4-dihydrospiro[chromene-2,4′-piperidin]-4-yl *N*-[(benzyloxy)carbonyl]-L-tryptophanate (12b). EDCI mediated esterification of (±)-**10e** (2.10 g, 4.596 mmol) with Cbz-L-Trp-OH (1.86 g, 5.515 mmol) yielded 3.51 g of a mixture of **11b** and **12b**. Flash silica gel chromatography (eluent, 30% EtOAc in PE) afforded 1.54 g (43%) of **11b** as a white solid. Mp 114–116 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 1.58–1.80 (m, 3H), 1.92–2.05 (m, 2H), 2.21–2.30 (m, 1H), 3.04–3.34 (m, 5H), 3.85–4.01 (m, 2H), 4.24–4.31 (m, 1H), 4.97 (s, 2H), 5.80–5.86 (m, 1H), 6.59 (d, J = 7.8 Hz, 1H), 6.78 (t, J = 7.8 Hz, 1H), 6.96 (t, J = 7.5 Hz, 1H), 7.06 (t, J = 7.8 Hz, 1H), 7.17–7.39 (m, 9H), 7.47 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 8.7 Hz, 2H), 7.89 (d, J = 6.9 Hz, 1H), 8.77 (s, 1H), 10.89 (br s, 1H); HRMS (ESI) calcd for $C_{40}H_{36}ClF_3N_4O_7$, $[M+H]^+$, 777.2297; found, 777.2303. HPLC: Chiralpak IA (Daicel), 250 × 4.6 mm, 5 μm, eluting with hexane/EtOH, 70:30, 98.97%; retention time: 14.6 min. Further elution with increasing amounts of EtOAc in PE gave 1.14 g (32%) of **12b** as a white solid. Mp 113–115 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 1.00–1.23 (m, 2H), 1.38–1.44 (m, 1H), 1.52–1.59 (m, 1H), 1.66–1.79 (m, 2H), 1.89–1.99 (m, 1H), 2.98–3.23 (m, 4H), 3.78–3.85 (m, 1H), 3.93–4.01 (m, 1H), 4.23–4.32 (m, 1H), 5.00 (s, 2H), 5.85–5.90 (m, 1H), 6.89–7.03 (m, 4H), 7.10 (d, J = 7.8 Hz, 1H), 7.17–7.31 (m, 7H), 7.40 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.59 (d, J = 8.7 Hz, 2H), 7.94 (d, J = 6.9 Hz, 1H), 8.75 (br s, 1H), 10.90 (br s, 1H); HRMS (ESI) calcd for $C_{40}H_{36}ClF_3N_4O_7$, $[M+H]^+$, 777.2297; found, 777.2302. HPLC: Chiralpak IA (Daicel), 250 × 4.6 mm, 5 μm, eluting with hexane/EtOH, 70:30, 99.75%; retention time: 11.1 min.

4.1.7.3. (4*S*)-8-Bromo-1′-{[4-(trifluoromethoxy)phenyl]carbamoyl}-3,4-dihydrospiro[chromene-2,4′-piperidin]-4-yl *N*-[(benzyloxy)carbonyl]-L-tryptophanate (11c) and (4*R*)-8-bromo-1′-{[4-(trifluoromethoxy)phenyl]carbamoyl}-3,4-dihydrospiro[chromene-2,4′-piperidin]-4-yl *N*-[(benzyloxy) carbonyl]-L-tryptophanate (12c). EDCI mediated esterification of (±)-**10f** (660 mg, 1.316 mmol) with Cbz-L-Trp-OH (534 mg, 1.579 mmol)

yielded 722 mg of ~1:1 mixture of **11c** and **12c**. Flash silica gel column chromatography (eluent, 30% EtOAc in PE) afforded 389 mg (36%) **11c** as a white solid. Mp 109–110 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56–1.79 (m, 3H), 1.86–1.98 (m, 2H), 2.21–2.30 (m, 1H), 3.06–3.23 (m, 5H), 3.89–4.02 (m, 2H), 4.23–4.33 (m, 1H), 4.97 (s, 2H), 5.78–5.85 (m, 1H), 6.62 (d, J = 7.2 Hz, 1H), 6.73 (t, J = 7.8 Hz, 1H), 6.96 (t, J = 7.2 Hz, 1H), 7.07 (t, J = 7.8 Hz, 1H), 7.17–7.38 (m, 8H), 7.46–7.57 (m, 4H), 7.88 (d, J = 6.9 Hz, 1H), 8.77 (br s, 1H), 10.89 (br s, 1H); HRMS (ESI) calcd for $C_{40}H_{36}BrF_3N_4O_7$, $[M+H]^+$, 821.1792; found, 821.1801. HPLC: Chiralpak IA (Daicel), 250 × 4.6 mm, 5 μm, eluting with *n*-hexane/EtOH, 60:40, 98.46%; retention time: 9.8 min. Further elution of the column gave 324 mg (30%) of **12c** as a white solid. Mp 113–114 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.07–1.15 (m, 1H), 1.39–1.44 (m, 1H), 1.51–1.59 (m, 1H), 1.65–1.75 (m, 2H), 2.98–3.25 (m, 5H), 3.79–3.86 (m, 1H), 3.95–4.02 (m, 1H), 4.24–4.31 (m, 1H), 5.00 (s, 2H), 5.84–5.90 (m, 1H), 6.86 (t, J = 7.8 Hz, 1H), 6.90–7.04 (m, 3H), 7.10–7.20 (m, 2H), 7.23–7.33 (m, 7H), 7.48 (d, J = 7.8 Hz, 1H), 7.53–7.63 (m, 3H), 7.94 (d, J = 6.6 Hz, 1H), 8.75 (br s, 1H), 10.89 (br s, 1H); HRMS (ESI) calcd for $C_{40}H_{36}BrF_3N_4O_7$, $[M+H]^+$, 821.1792; found, 821.1802. HPLC: Chiralpak IA (Daicel), 250 × 4.6 mm, 5 μm, eluting with *n*-hexane/EtOH, 60:40, 100%; retention time: 8.6 min.

4.1.8. General procedure for the hydrolysis of esters: synthesis of optically pure alcohols (+)-(10d–f) and (−)-(10d–f)

To a solution of tryptophan ester **11a–c** or **12a–c** (0.5 mmol) in mixture of THF (10 mL), MeOH (4 mL), and water (4 mL) was added LiOH·H_2O (42 mg, 1.0 mmol). The reaction mixture was stirred at room temperature for 1 h. The solvents were evaporated under reduced pressure. The residue thus obtained was diluted with water (100 mL) and acidified with 1.0 N hydrochloric acid to pH 4.0. The mixture was extracted with EtOAc (2 × 200 mL) and the combined extracts were washed with water (100 mL) and dried over anhydrous Na_2SO_4. The residue obtained after evaporation of the solvent was purified by silica gel column chromatography using 40–50% ethyl acetate in PE to give optically pure alcohols.

4.1.8.1. (4*S*)-(+)-8-Fluoro-4-hydroxy-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (+)-(10d). LiOH mediated hydrolysis of **11a** (170 mg, 0.223 mmol) as described gave 83 mg (84%) of the product as a white solid. Mp 144–146 °C; ^{1}H NMR and IR spectra were identical to that of (±)-**10d**. HPLC: Chiralpak IA (Daicel), 250 × 4.6 mm, 5 μm, eluting with hexane/isopropanol, 90:10, 99.03%, retention time: 40.7 min; specific optical rotation: $[\alpha]_D^{25}$: +8.11° (*c* 0.1, $CHCl_3$).

4.1.8.2. (4*S*)-(+)-8-Chloro-4-hydroxy-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (+)-(10e). LiOH mediated hydrolysis of **11b** (1.50 g, 1.930 mmol) as described gave 785 mg (89%) of the product as a white solid. Mp 154-156 °C; ^{1}H NMR and IR spectra were identical to that of (±)-**10e**. HPLC: Chiralpak AD-H (Daicel), 250 × 4.6 mm, 5 μm, eluting with hexane/isopropanol, 80:20, 98.42%; retention time: 15.7 min; Specific optical rotation: $[\alpha]_D^{25}$: +3.72° (*c* 0.1, $CHCl_3$).

4.1.8.3. (4*S*)-(+)-8-Bromo-4-hydroxy-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (+)-(10f). LiOH mediated hydrolysis of **11c** (360 mg, 0.438 mmol) as described gave 176 mg (80%) of the product as a white solid. Mp 164–166 °C; ^{1}H NMR and IR spectra were identical to that of (±)-**10f**. HPLC: Chiralpak AD-H (Daicel) 250 × 4.6 mm, 5 μm, eluting with hexane/isopropanol, 80:20, 98.28%, retention time: 17.2 min; Specific optical rotation: $[\alpha]_D^{25}$: + 4.41° (*c* 0.1, $CHCl_3$).

4.1.8.4. (4*R*)-(−)-8-Fluoro-4-hydroxy-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (−)-(10d). LiOH mediated hydrolysis of **12a** (145 mg, 0.190 mmol) as described above afforded 68 mg (81%) of the product as a white solid. Mp 145–146 °C; ^{1}H NMR and IR spectra were identical to that of (±)-**10d**. HPLC: Chiralpak IA (Daicel), 250 × 4.6 mm, 5 μm, eluting with hexane/isopropanol, 90:10, 98.20%, retention time: 35.7 min; Specific optical rotation: $[\alpha]_D^{25}$: –7.79° (*c* 0.1, $CHCl_3$).

4.1.8.5. (4*R*)-(−)-8-Chloro-4-hydroxy-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro[chromene-2,4′-piperidine]-1′-carboxamide (−)(−)-(10e). LiOH mediated hydrolysis of **12b** (975 mg, 1.254 mmol) as described afforded 499 mg (87%) of the product as a white solid. Mp 149–150 °C; ^{1}H NMR and IR spectra were identical to that of (±)-**10e**. ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.9, 22.0, 30.9, 33.1, 35.6, 39.7, 40.3, 60.7, 75.4, 120.2 (q, J = 253.0 Hz, O*C*F_3), 120.5, 120.6 (2C), 121.1 (2C), 127.1, 128.5 (d, J = 11.4 Hz, *C*–OCF_3), 139.9, 142.5, 147.6, 154.7; HPLC: Chiralpak AD-H (Daicel) 250 × 4.6 mm, 5 μm, eluting with hexane/isopropanol, 80:20, 98.36%, retention time: 9.1 min; Specific optical rotation: $[\alpha]_D^{25}$: −3.27° (*c* 0.1, $CHCl_3$).

4.1.8.6. (4*R*)-(−)-8-Bromo-4-hydroxy-*N*-[4-(trifluoromethoxy)phenyl]-3,4-dihydro-1′*H*-spiro-[chromene-2,4′-piperidine]-1′-carboxamide (−)-(10f). LiOH mediated hydrolysis of **12c** (300 mg, 0.365 mmol) as described afforded 130 mg (71%) of the product as a white solid. Mp 161–162 °C; ^{1}H NMR and IR spectra were identical to that of (±)-**10f**. HPLC: Chiralpak AD-H (Daicel) 250 × 4.6 mm, 5 μm, eluting with hexane/isopropanol 80:20, 98.52%; retention time: 9.7 min; Specific optical rotation: $[\alpha]_D^{25}$: −5.02° (*c* 0.1, $CHCl_3$).

4.2. Biology

4.2.1. Screening of TRPM8 antagonists using ^{45}calcium uptake assay

Radiometric calcium influx assay was performed according to reported protocol.[46,47] Chinese hamster ovary (CHO) cells stably expressing human TRPM8 were generated in-house and maintained in F-12 Dulbecco's Modified Eagle Medium (DMEM F-12) supplemented with 10% FBS and G418. The cells were cultured at 37 °C in humidified air containing 5% CO_2. A 10 mM stock of compound (−)-**10e** was prepared in DMSO. Subsequent dilutions from the stock solution were made in drug dilution buffer (DDB) (DMEM F-12 containing 1.8 mM $CaCl_2$). Cells were seeded in 96-well plates at a density of 0.03 × 106 cells/well, and cultured overnight at 37 °C in the presence of 5% CO_2. Before the assay, the cells were washed twice with assay buffer (DMEM F12 with 0.1% BSA and 1.8 mM $CaCl_2$). The assay was carried out at 25 °C in a total volume of 200 μL. Cells were treated with test compounds for 10 min. $^{45}Ca^{+2}$ solution was added at a final concentration of 5 μCi/mL followed by the addition of icilin as an agonist. After 4 min of agonist treatment, the drug was washed out and the wells were rinsed thrice with wash buffer (145 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose and 0.1% BSA). The cells were lysed in lysis buffer (50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% deoxycholate and 0.1% SDS) on a shaker. Radioactivity in cell lysates was measured as counts per minute (cpm) using TopCount liquid scintillation counter (Perkin Elmer). IC_{50} values were calculated from concentration response curve by nonlinear regression analysis using GraphPad Prism 3.0 (GraphPad Software Inc.).

4.2.2. Selectivity screening of TRPM8 antagonists using ^{45}calcium uptake assay

Radiometric calcium assay was performed as described above with some minor modifications. CHO cells stably expressing human TRPA1, TRPV1, TRPV3 or TRPV4 were generated in-house and maintained in DMEM F-12 supplemented with 10% FBS and G418. All the cell types were cultured at 37 °C in humidified air containing 5% CO_2. Before the assay, the cells were washed twice with assay buffer. The assay was carried out in total volume of 200 μL at 30 °C for TRPV1, 32 °C for TRPV3 and 25 °C for TRPV4 and TRPA1. The cells were treated with Compound (−)-**10e** for 10 min. $^{45}Ca^{+2}$ solution was added at a final concentration of 5 μCi/mL followed by the addition of respective agonist (allyl isothiocyanate for TRPA1, capsaicin for TRPV1, 2-aminoethoxydiphenyl borate (2-APB) for TRPV3 and 4α-phorbol 12,13-didecanoate (4αPDD) for TRPV4). After 2–5 min of agonist treatment, the drug was washed out and wells were rinsed thrice with wash buffer. The cells were lyzed in lysis buffer on a shaker. Radioactivity in cell lysates was measured as counts per minute (cpm) using TopCount liquid scintillation counter (Perkin Elmer).

4.2.3. Metabolic stability test of (4*R*)-(−)-10e

The in-vitro metabolic stability of (−)-**10e** was determined using liver microsomes from male CD1 mouse, male Wistar rat, male Beagle dog, male Cynomolgus monkey and pooled human liver microsomes obtained from Xenotech (USA). A 5.0 mM stock of (−)-**10e** was prepared in DMSO from which a working solution of 0.1 mM was prepared in DMSO. A 5.0 μL aliquot of the working solution, 25 μL of 20 mg/mL of protein (liver microsomes) was spiked into 445 μL of KH_2PO_4 buffer (pH 7.4). The reaction was initiated by the addition of a 25 μL aliquot of 40 mM NADPH (reduced nicotinamide adenine dinucleotide phosphate) in water to get a final concentration of 2.0 mM of NADPH, 1.0 μM of test item and 1.0 mg/mL of protein. The reaction mixture was incubated at 37 °C for 60 min and terminated by the addition of 3 mL of TBME (*tert*-butyl methyl ether). For the 0 min samples, the reaction was terminated with 3 mL of TBME prior to the addition of NADPH. A 25 μL aliquot of a 10.0 μg/mL internal standard solution in methanol was spiked in both 0 h and 60 min samples. The samples were processed by vortex mixing for 5 min and centrifuging at 1000*g* for 5 min. The clear supernatant liquid was separated and dried under nitrogen. The residue was reconstituted by adding a 500 μL of reconstitution solvent (9:1 v/v mixture of acetonitrile and 2.0 mM ammonium acetate in water) and ~5 μL sample was injected into the LC/MS/MS system. The peak area ratios of test item to internal standard were determined.

Based on the comparison of area ratios of test item to internal standard in the 60 min samples relative to the 0 min samples, the percent metabolized and percent remaining of the test compound were estimated.

4.2.4. Single dose oral PK study of (4*R*)-(−)-10e in SD rats

Three male SD (Sprague Dawley) rats weighing between 180−220 g were used for the study. The animals were fasted overnight before dosing and food was administered 4 h post dosing. Water was removed just before dosing and was provided ad libitum 2 h post dosing. Compound (−)-**10e** was formulated using 1% Tween 80® and 0.5% (w/v) methyl cellulose suspension in water (qs). The suspension was administered by oral gavage and blood samples were collected at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 and 24.0 h post dose into tubes containing K_2EDTA as anticoagulant. The blood samples were centrifuged at 1000*g* for 10 min and plasma was separated. The plasma samples, standard and quality control samples were processed using liquid–liquid extraction after the addition of an internal standard and the concentrations were estimated using an achiral LC/MS/MS method. The plasma concentration versus time data was subjected to pharmacokinetic analysis using Pharsight Winnonlin® version 5.2 Software for estimation of pharmacokinetic parameters including C_{max}, AUC_{0-t} and T_{max}.

4.2.5. Chronic constriction injury (CCI) acetone test for (4*R*)-(−)-10e

Male SD rats weighing 160–200 g were used for the study. Chronic constriction injury (CCI) of the sciatic nerve was performed under aseptic conditions. The rats were anesthetized with an intraperitoneal injection of ketamine and xylazine (40 and 8 mg/kg, respectively) combination. The sciatic nerve on the left side was exposed through a mid-thigh incision and separation of the heads of the biceps femoris muscle. Proximal to the sciatic trifurcation, approximately 7 mm of nerve was freed, and four loose ligatures of 4–0 chromic gut were placed around the sciatic nerve. The muscle and skin were then closed in two layers and covered with iodine solution. A single experimenter performed all the sciatic nerve ligations to maintain the uniformity. After seven days of the ligation, the animals were taken for the experiment.[48,49]

On the day of the experiment, animals were acclimatized for 15 min in the experimental plexiglass chamber containing sieving iron wire mesh (0.5 cm^2 pore size) in the false bottom of the chamber. Throughout the experimental period, ambient temperature was maintained at 21 ± 0.5 °C. After acclimatization period, 0.5% MC (methyl cellulose), capsazepine (10 mg/kg, ip) and compound (−)-**10e** (10 mg/kg, po) were administered and acetone (100 μl) was applied as a spray on the ipsilateral paw and the response was observed and recorded for 2 min. This procedure was repeated 3–5 times with a 5 min interval between each application. Cold pain was measured by number of flinches and frequency of paw licking measured in a 2 min interval at 0, 0.5, 1.0, 2.0 and 3.0 h post treatment. Based on the flinching and licking, clinical scores were assigned and correlated for the determination of cold pain intensity.[50]

4.2.6. Icilin induced wet dog shake test for (4*R*)-(−)-10e

Healthy, male C57/BL6 mice (20–30 g) were used for the study. Icilin was dissolved in 20% DMSO and 1% Tween 80 in distilled water and injected ip in a volume of 10 ml/kg. The experiments were performed during the light phase between 9 am and 3 pm. Each animal was acclimatized in bell jar (22 × 18 × 25 cm; L × W × H) for 10 min for three consecutive days before icilin administration. The animals were weighed, grouped and icilin (30 mg/kg, ip) was administered to all groups except the naïve groups. Compound (−)-**10e** (3, 10 and 30 mg/kg, po) and pregabalin (30 mg/kg, po) were administered 30 and 60 min prior to icillin injection, respectively. The naïve and vehicle groups received 0.5% MC as vehicle. Incillin-induced cold pain was estimated by the number of WDS during a 30 min period post-icillin injection.

4.2.7. Oxaliplatin-induced peripheral neuropathic pain test for (4*R*)-(−)-10e

Healthy, male C57/BL6 mice (20–30 g) were used for the study. Oxaliplatin was dissolved in 5% glucose in distilled water and injected ip in a volume of 10 ml/kg. Oxaliplatin is known to increase TRPM8 mRNA expression in dorsal root ganglia that reaches the peak on day 3. Hence, in the current study, animals were tested for cold allodynia 3 days post oxaliplatin (3 mg/kg, ip) injection. Acetone (50 μl) was sprayed on the sub plantar region of the right paw with the help of a microsyringe sprayer and immediately the animal was kept in a chamber to record licking time during a 60 s observation period. This procedure was repeated three times with a 15 min interval between each application. The naïve and vehicle groups received 0.5% MC as vehicle. Compound (−)-**10e** (3, 10 and 30 mg/kg, po) and pregabalin (30 mg/kg, po) were administered 30 and 60 min prior to the acetone spray, respectively.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.08.031.

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