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Article

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Development of a Novel Human Parathyroid Hormone Receptor 1 (hPTHR1) Agonist (CH5447240), a Potent and Orally Available Small-Molecule for Treatment of Hypoparathyroidism

Yoshikazu Nishimura, Toru Esaki, Yoshiaki Isshiki, Naoki Okamoto, Yoshiyuki Furuta, Tomoya Kotake, Yoshiaki Watanabe, Masateru Ohta, Toshito Nakagawa, Hiroshi Noda, Masaru Shimizu, Hitoshi Saito, Tatsuya Tamura, Haruhiko Sato*

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ABSTRACT

During the course of derivatization of HTS hit **4a**, we have identified a novel small-molecule hPTHR1 agonist, 1-(3,5-dimethyl-4-(2-((2-((1*R*,4*R*)-4-methylcyclohexyl)-4-oxo-1,3,8-triazaspiro[4.5]dec-1-en-8-yl)sulfonyl)ethyl)phenyl)-1-methylurea (CH5447240, **14l**). Compound **14l** exhibited a potent *in vitro* hPTHR1 agonist effect with EC_{20} of 3.0 nM and EC_{50} of 12 nM and showed excellent physicochemical properties, such as high solubility in fasted state simulated intestinal fluid and good metabolic stability in human liver microsomes. Importantly, **14l** showed 55% oral bioavailability, and a significantly elevated serum calcium level in hypocalcemic model rats.

INTRODUCTION

Hypoparathyroidism is an endocrine disorder most commonly seen after surgical removal of a parathyroid gland in patients with thyroid cancer, and is also associated with hereditary or acquired abnormalities, such as autoimmunity, DiGeorge syndrome, mitochondrial dysfunction, and activating mutations of the calcium-sensing receptor. It is caused by insufficient production of parathyroid hormone (PTH) from the parathyroid glands or by tissue insensitivity to PTH, a polypeptide containing 84 amino acids that is secreted by the parathyroid glands to

maintain serum calcium and phosphate levels through the PTH type 1 receptor (PTHR1) in bone and kidney[1,2]. Hypoparathyroidism is characterized by hypocalcemia and hyperphosphatemia and the clinical signs of hypocalcemia-associated hypoparathyroidism are tetany, seizures, mental disturbances, congestive heart failure, or stridor, while hyperphosphatemia can contribute to major long-term complications, such as ectopic mineralization in the soft tissues (kidney, brain, eye and vascular)[3-5].

To maintain the serum calcium level of hypoparathyroidism patients, high-dose oral calcium and active vitamin D have conventionally been used. However, long-term vitamin D therapy causes not only marked swings in blood calcium (hypercalcemia and hypocalcemia) but also hypercalciuria, resulting in nephrolithiasis and impaired renal function[6,7]. In contrast, PTH can maintain serum calcium level without hypercalciuria[8,9], and recombinant human PTH(1-84) was recently launched as an alternative option for patients with hypoparathyroidism whose calcium level cannot be controlled on calcium and vitamin D[10].

However, because of its peptidic nature, recombinant PTH is subcutaneously injected. Although some oral deliveries of PTH have been investigated, no successful formulation has been reported so far. Therefore, the advent of orally available small-molecule hPTHR1 agonists is still eagerly anticipated. At the time of writing, two small-molecule hPTHR1 antagonists, compound **1** (SW106)[11,12] and compound **2**[13], have been reported, and only one small-molecule agonist, compound **3** (AH3960)[12,14], although its clinical development has not been reported.

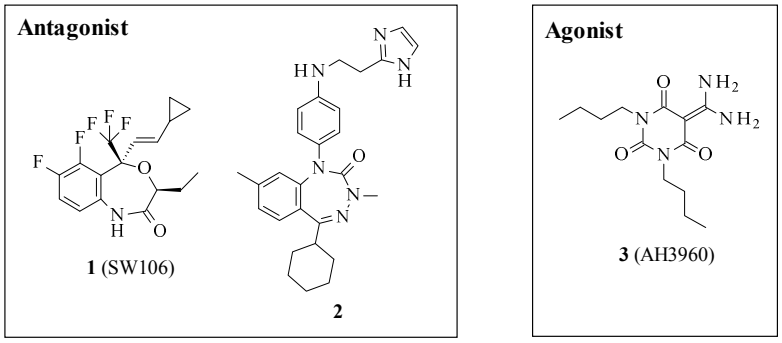


Figure 1. Small-molecule PTHR1 Ligands.

Recently we have reported an orally-active small molecule PTHR1 agonist, 1-{3,5-dimethyl-4-[2-({4-oxo-2-[4-(trifluoromethoxy)phenyl]-1,3,8-triazaspiro[4.5]dec-1-en-8-yl}sulfonyl)ethyl]phenyl}-5,5-dimethylimidazolidine-2,4-dione (PCO371), and its potential application for hypoparathyroidism[15]. In this report, we explain the optimization of an HTS hit **4a** into compound **14l** (CH5447240), which became the lead compound in the discovery of PCO371, and outline its characterization as a highly orally active PTHR1 agonist.

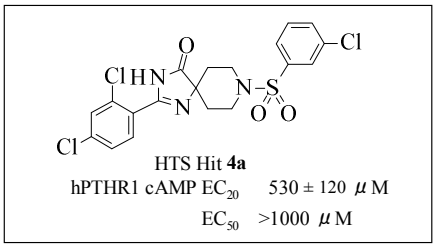
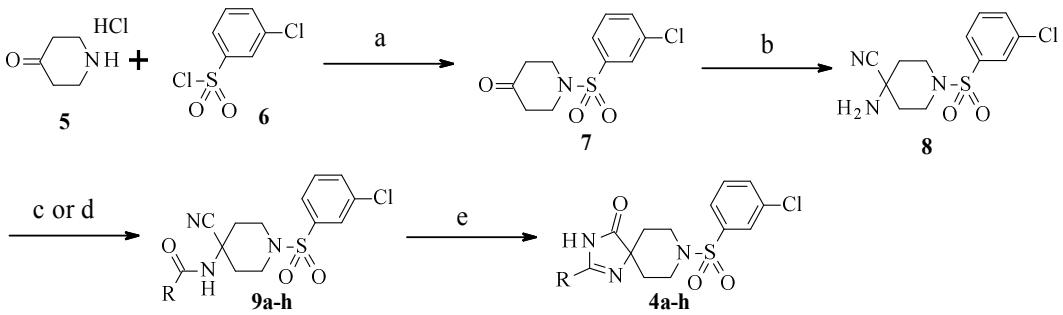


Figure 2. Structure and hPTHR1 agonistic activity of HTS hit **4a**.

CHEMISTRY

Novel hPTHR1 small-molecule agonists reported herein were synthesized as shown in Schemes 1-4. The synthesis of 3-chlorophenyl sulfonamides **4a-h** started from the reaction of piperidin-4-one hydrochloride (**5**) and 3-chlorobenzenesulfonyl chloride (**6**), as shown in Scheme 1. The resulting ketone **7** was converted to aminonitrile **8** by Strecker synthesis. Compound **8** was then reacted with various acyl chlorides or carboxylic acids to give **9a-h**. Finally, compounds **9a-h** were treated with hydrogen peroxide under basic conditions to afford spiro-imidazolone derivatives **4a-h**.

Scheme 1. Synthesis of 3-chlorobenzenesulfonamide derivatives



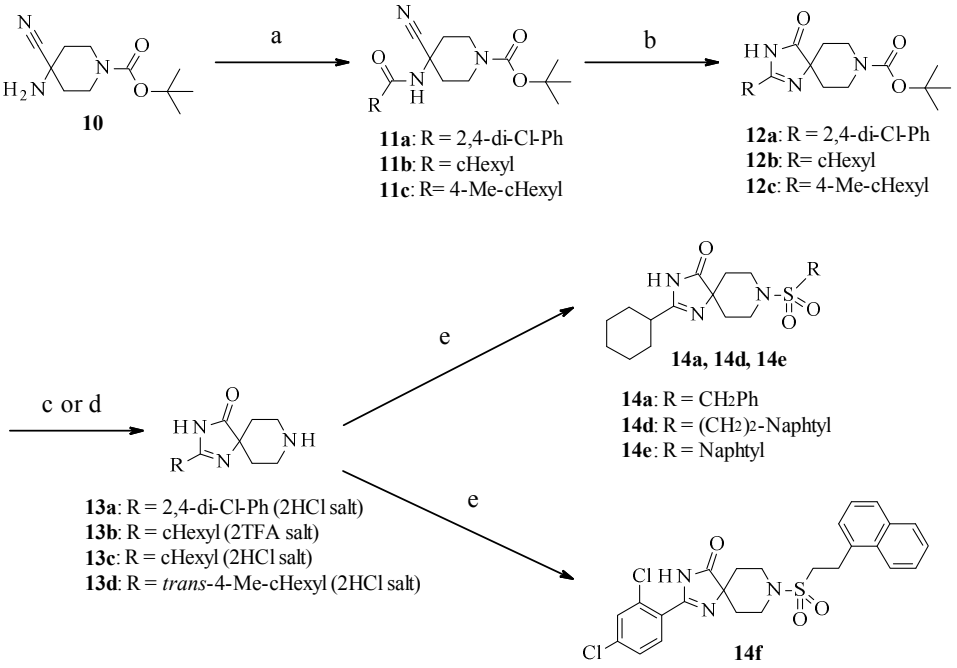
9a: R = 2,4-di-Cl-Ph
9b: R = 4-Cl-Ph
9c: R = 2-Cl-Ph
9d: R = Ph
9e: R = 4-CO_2Me-Ph
9f: R = 4-Py
9g: R = *c*Hex
9h: R = *trans*-4-Me-cHex

4a: R = 2,4-di-Cl-Ph
4b: R = 4-Cl-Ph
4c: R = 2-Cl-Ph
4d: R = Ph
4e: R = 4-CO_2H-Ph
4f: R = 4-Py
4g: R = *c*Hex
4h: R = *trans*-4-Me-cHex

Reagents and conditions: (a) $K_2CO_3/CHCl_3$-H_2O, room temperature, 72%. (b) KCN, NH_4OAc, MeOH, room temperature, 86%. (c) R-COCl, Na_2CO_3, CH_2Cl_2, room temperature, 44%–90%. (d) (1*R*,4*R*)-4-methylcyclohexane-1-carboxylic acid, HATU, DIPEA, DMF, room temperature, 90%. (e) 30% H_2O_2 aq., 6M NaOH aq., EtOH, 80ºC, 45%–76%.

Compounds **14a** and **14d-f** were prepared as shown in Scheme 2. Initially, aminonitrile **10** was reacted with acid chlorides to afford amide nitriles **11a-c**. Cyclization of **11a-c** to **12a-c**, followed by the deprotection of BOC group under acidic conditions**,** gave piperidines **13a-d**. Acyl piperidines **14a** and **14d-f** were finally obtained by coupling of sulfonyl chlorides with amine **13a-d**.

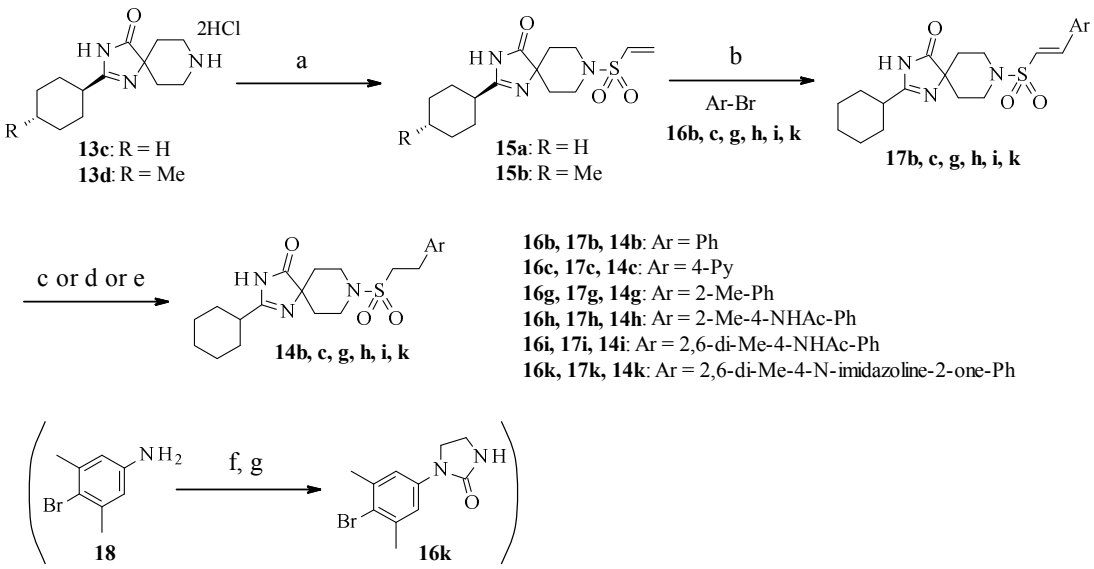
Scheme 2. Synthesis of sulfonylpiperidines



Reagents and conditions: (a) R-COCl, Et_3N, DMAP, CH_2Cl_2, room temperature, 30%–71%. (b) 30% H_2O_2 aq., 6M NaOH aq., EtOH, 80ºC, 43%–86%. (c) 4M HCl-dioxane, room temperature, 99%–100%. (d) TFA, CH_2Cl_2, room temperature, 92%. (e) R-SO_2Cl, Et_3N, CH_2Cl_2, room temperature, 39%–90%.

Arylethylsulfonamides **14b-c**, **14g-i**, and **14k** were prepared by following the synthetic route depicted in Scheme 3. Piperidines **13c-d** were reacted with 2-chloroethane-1-sulfonyl chloride to give vinylsulfonamides **15a-b**. Heck reaction of **15a** with aryl bromides afforded *trans*-ethenesulfonamides **17b-c**, **17g-i**, and **17k**, followed by hydrogenation of olefin to give arylethylsulfonamides **14b-c**, **14g-i,** and **14k**.

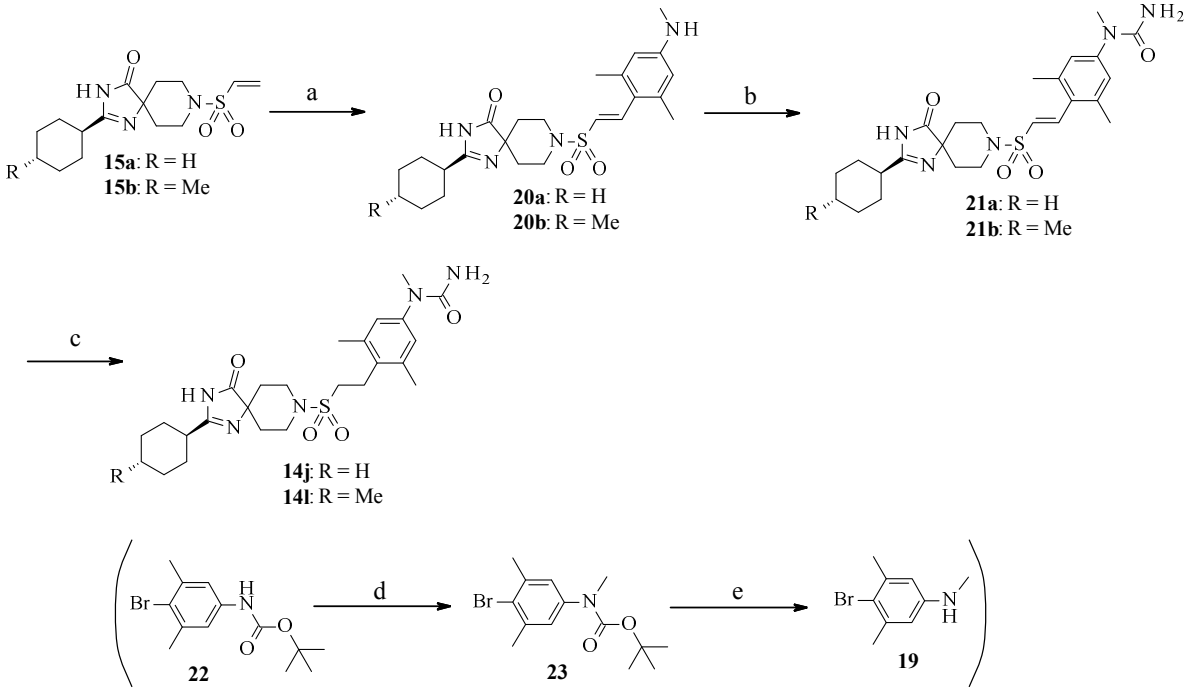
Scheme 3. Synthesis of arylethylsulfonamides via Heck reaction



Reagents and conditions: (a) 2-chloroethane-1-sulfonyl chloride, Et_3N, CH_2Cl_2, room temperature, 66%–80%. (b) ArBr, $Pd(dba)_2$, $(t\text{-}Bu)_3P\text{-}HBF_4$, methyl dicyclohexylamine, NMP, 110ºC, 51%–87%. (c) 10% Pd/C, H_2, EtOH-AcOEt-AcOH, room temperature, 68%–75%. (d) 10% Pd/C, H_2, EtOH-DMF, 30 bar, 40ºC (H-Cube®), 63%–66%. (e) 20% $Pd(OH)_2$/C, H_2, MeOH, room temperature, 41%–78%. (f) 2-chloroethyl isocyanate, DMF, room temperature. (g) NaH, DMF, room temperature, 81% from **18**.

The synthetic route of *N*-methylurea derivatives **14j** and **14l** is illustrated in Scheme 4. Heck reaction of vinylsulfonamides **15a-b** with aryl bromide **19** gave trans-olefins **20a-b**. *N*-Methylurea derivatives **21a-b** were prepared by the reaction of **20a-b** with sodium cyanate under acidic conditions. Finally hydrogenation of the olefin moiety of **21a-b** afforded **14j** and **14l**. Aryl bromide reagent **19** was prepared by methylation and deprotection from **22**.

Scheme 4. Synthesis of *N*-methylurea derivatives



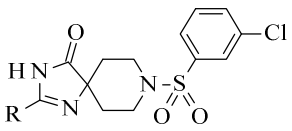
Reagents and conditions: (a) 4-bromo-*N*,3,5-trimethylaniline (**19**), $Pd(dba)_2$, $(t\text{-}Bu)_3P\text{-}HBF_4$, methyl dicyclohexylamine, NMP, 110ºC, 77%–79%. (b) NaOCN, AcOH, CH_2Cl_2, room temperature, 82%–97%. (c) 20% $Pd(OH)_2/C$, H_2, MeOH, room temperature, 62%–80%. (d) MeI, NaH, DMF, room temperature, 93%. (e) TFA, CH_2Cl_2, room temperature, 99%.

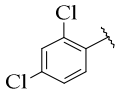
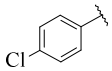
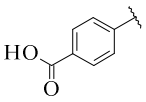
RESULTS AND DISCUSSION

With the aim of identifying novel orally available small-molecule hPTHR1 agonists, we conducted an HTS campaign on more than one million compounds in the Chugai-Roche library using a sensitive cell-based assay[16] and have identified spiro-imidazolone derivative **4a** as a hit. hPTHR1 agonistic activity was evaluated in a cAMP production assay in HKRK-B7 cells that stably express hPTHR1 LLC-PK1 cells. Parent LLC-PK1 cells without hPTHR1 were used as a counter assay. Hit compound **4a** shows the cAMP activity in HKRK-B7 cells expressing hPTHR1, whereas no cAMP activity was observed in LLC-PK1 cells without hPTHR1. To enhance the activity of agonist **4a**, of which the EC_{20} value was in the sub-millimolar range, we conducted structure-activity relationship (SAR) studies focusing on the two phenyl rings and the sulfonyl linker. The first SAR studies, focusing on the

phenyl ring at the 2-position of spiro-imidazolone, are shown in Table 1. Compounds **4a-h** were tested *in vitro* on hPTHR1-expressing HKRK-B7 cells, and 1 mM of each of those compounds produced various extents of cAMP activity. In comparison with **4a,** mono chlorophenyl derivatives **4b** and **4c** produced similar agonistic activity with values of 22% and 18%, respectively. Surprisingly, the agonistic activity of non-substituted phenyl derivative **4d** was almost completely undetected, and moreover, the hydrophilic derivatives **4e-f** were also inactive. On the other hand, cyclohexyl derivatives **4g-h** showed more potent agonistic activity than **4a**. From these results, hydrophobic substituents on the imidazolone moiety were suggested to be important for the PTHR1 agonistic activity. These findings focused our next efforts on derivatization at the phenylsulfonyl moiety of **4g**. Because the derivatized compounds had weak PTHR1 agonistic activity at the beginning of the hit to lead optimization, the agonistic activity at 1 mM concentration and the EC_{20} value were used as indicators for structural modifications.

Table 1. SAR of 2-substituted imidazolone derivatives



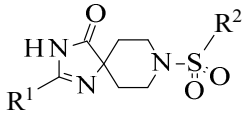
No.	R	cAMP Production at 1 mM (%)*[1]
4a		23 ± 2.6
4b		22*[2]
4c		18*[2]
4d		1.9*[2]
4e		1.3*[2]

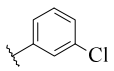
4f		1.0*[2]
4g		45 ± 3.6
4h		35 ± 5.9

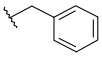
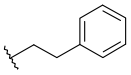
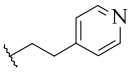
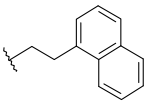
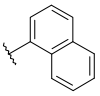
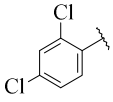
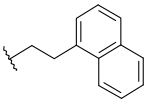
*[1] Values for cAMP production at 1 mM are the ratio of cAMP production stimulated by a concentration of 1 mM of compound to the maximal cAMP production in HKRK-B7 cells[6], which was based on cAMP production stimulated by 100 nM hPTH(1-34). Values represent the mean ± SEM of two experiments (n=2), with each experiment performed in duplicate, except for values marked *[2], which are from one experiment (n=1) performed in duplicate. All compounds showed no response in LLC-PK1 cells without hPTHR1.

For SAR analysis of the phenylsulfonyl moiety, compounds **14a-f** were evaluated as hPTHR1 agonists by the cAMP assay. Although benzyl derivative **14a** did not show any agonistic activity, phenethyl derivative **14b** showed similar agonistic activity to **4g**. Importantly, naphthylethyl derivative **14d** showed 35 times more potent agonistic activity (EC_{20}=15 μM) than **4g**; however, pyridylethyl derivative **14c** was inactive. When compared to **14d**, the activity levels of **14e** and **14f** were 10 times less effective, with EC_{20} values of 220 and 150 μM, respectively. Solubility and human liver microsome (hLM) stability of **14d** were measured and revealed **14d** to be poorly water-soluble and metabolically unstable (Table 3). These drawbacks were suggested to be due to the existence of a hydrophobic naphthyl ring.

Table 2. SAR of piperidine substituent R^2



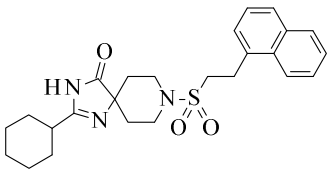
No.	R^1	R^2	hPTHR1 cAMP EC_{20} (μM)*[1]
4g			140 ± 18

14a			>1000*[2]
14b			240 ± 87
14c			>1000*[2]
14d			15 ± 0.8
14e			220 ± 110
14f			150 ± 69

[*1] Values for EC_{20} represent the concentration of a compound that produces 20% of the maximal cAMP production in HKRK-B7 cells[6], and represent the mean ± SEM of two experiments (n=2), with each experiment performed in duplicate, except for values marked [*2], which are from one experiment (n=1) performed in duplicate. The EC_{20} value of PTH(1-34) was 0.2 ± 0.05 nM. All compounds showed no response in LLC-PK1 cells without hPTHR1.

Therefore, we concentrated on modifying the naphthyl moiety in the next SAR analysis. Our specific concept in the subsequent derivatization was to replace the naphthalene ring with a phenyl ring, having a hydrophilic substituent, to improve the water-solubility and metabolic stability.

Table 3. Profile of compound **14d**



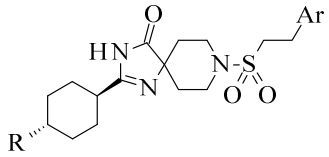
hPTHR1 EC_{20} (μM)	15 ± 0.8
hPTHR1 EC_{50} (μM)	>1000

Solubility (PBS) (μg/ml)	BLQ*
Solubility (FaSSIF) (μg/ml)	3.1
Human LM CL (μL/min/mg)	300

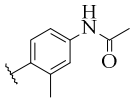
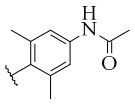
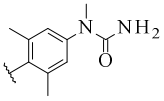
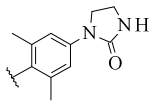
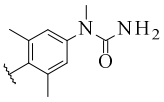
*BLQ means below-the-limit-of-quantitation. Intrinsic clearance (CL) was identified using hLM with NADPH *in vitro*.

For this purpose, derivatives **14g-l** were tested in a solubility assay and an *in vitro* metabolic stability assay, in addition to the hPTHR agonist assay, as shown in Table 4. Although insertion of acetamide at 4-position on the phenyl ring improved hPTHR1 agonistic activity, solubility, and hLM metabolic stability in the absence of NADPH compared to a non-substituted phenyl ring, the hLM clearance of acetanilides **14h** and **14i** was higher than that of **14g**. We considered that hydrolysis of acetanilide is one of the major metabolic pathways in hLM, so to improve metabolic stability, acetanilide was changed to urea. **14j** and **14l** showed good hLM stability with or without NADPH condition and potent agonist activity with EC_{20} values of 8.1 and 3.0 μM, respectively. These results suggested the importance of carbonyl oxygen for agonistic activity (**14h-j**, and **14l**) and the inadequate size of the cyclic urea in **14k**. Except for **14k**, most derivatives showed the expected improved solubility in sodium phosphate buffer (PBS) and fasted state simulated intestinal fluid (FaSSIF) assays. Compared with **14d,** all tested compounds showed enhanced stability in metabolic stability assays.

Table 4. SAR of arylethylsulfonamide derivatives



No.	R	Ar	hPTHR1 (μM)		Solubility (μg/ml)		hLM CL (μL/min/mg)	
			EC_{20}*[1]	EC_{50}*[1]	PBS	FaSSIF	NADPH(+)	NADPH(-)
14g	H		30 ± 11	>1000	11	15	100	0

14h	H		11 ± 1.8	50 ± 20	280	400	46	33
14i	H		6.4 ± 0.6	26 ± 3.9	8.0	38	39	18
14j	H		8.1 ± 1.0	67 ± 5.8	290	450	23	0
14k	H		240[*2]	>1000	BLQ*[3]	BLQ*[3]	62	3.4
14l	Me (CH5447240)		3.0 ± 0.2	12 ± 1.0	99	250	45	0.14

*[1] Values for EC_{20} and EC_{50} represent the concentration of a compound that produces, respectively, 20% and 50% of the maximal cAMP production in HKRK-B7 cells[6] and are the mean of two experiments (n=2), which were each performed in duplicate, except for values marked *[2], which are from one experiment (n=1) performed in duplicate. EC_{20} and EC_{50} values of PTH(1-34) were 0.6 ± 0.1 nM and 2.5 ± 0.4 nM, respectively. All compounds showed no response in LLC-PK1 cells without hPTHR1. *[3] BLQ means below-the-limit-of-quantitation.

Based on the well-balanced *in vitro* profiles of **14l**, its pharmacokinetics (PK) was studied in female rats. In this study **14l** exhibited good exposure with bioavailability of 55% (Table 5). The *in vitro* hPTHR1 agonistic activity and PK profile of **14l** encouraged us to perform an *in vivo* pharmacological assay, which tested the compound's ability to restore the serum calcium level in a thyroparathyroidectomized (TPTX) rat model (Figure 3). The results showed that **14l** exhibits PTH-like calcemic and hypophosphatemic activity by oral administration. Furthermore, **14l** at 19 and 60 mg/kg dose-dependently increased serum calcium levels and decreased serum phosphorus levels with more prolonged profiles than those of subcutaneous (sc) hPTH(1-34). It is likely that the prolonged duration of calcemic activity of **14l** in rats can be attributed to better bioavailability, lower clearance and longer half-life than hPTH(1-34). A previous study showed that sc administration of PTH(1-34) in rats gave Tmax of 15 min and $T_{1/2}$ of

30 min[17], and these are shorter than those of **14l**. Bone anabolic activity of **14l** has not been tested in vivo; however, **14l** has a longer PK profile than PTH(1-34), so it is unlikely to exert anabolic activity on bone, which requires a sharp and transient PK profile [18, 19, 20, 21]. In a competition assay, membranes prepared from COS-7 cells expressing human PTHR1 were used with a radiolabelled tracer of ^{125}I-[Aib^{1,3},Nle^{8},Gln^{10},Har^{11},Ala^{12},Trp^{14},Tyr^{15}]–PTH(1–15), which interacts mainly with the transmembrane domain of PTHR1[15]. **14l** at 300 μM inhibited 75% of the binding by the radio-labeled tracer, but the IC_{50} value of **14l** was 93.7 μM (compared to 0.08 nM for hPTH(1-34)), which was almost eight times weaker than its cAMP agonistic activity (EC_{50}=12.0 μM). The binding affinity of **14l** was assessed from the displacement of a PTH peptide tracer, whereas the cAMP agonistic activity of **14l** was assessed from the accumulation of intracellular cAMP when a phosphodiesterase inhibitor, IBMX, was added. These different assay methods may partly explain the discrepancy between the values for binding affinity and cAMP agonistic activity of **14l** *in vitro*. For the preliminary safety assessment, an inhibition assay of the human ether-a-go-go–related gene channel was conducted, using an automated patch clamp platform[22], and in this assay **14l** showed low inhibitory activity with 10% inhibition at 10 μM.

Table 5. PK parameters of **14l** in female rats at the 3 mg/kg dose

Route	$T_{1/2}$ (h)	Tmax (h)	Cmax (ng/mL)	AUCinf (ng*h/mL)	CL (mL/h/kg)	Vss (L/kg)	F (%)
i.v.	1.1	–	–	4820	624	0.395	–
p.o.	1.0	1.0	1360	2650	1140	–	55.0

Plasma concentrations were measured by LC-MS/MS. PK parameters were calculated by noncompartmental analysis using Watson7.1. Data are expressed as mean (n=2).

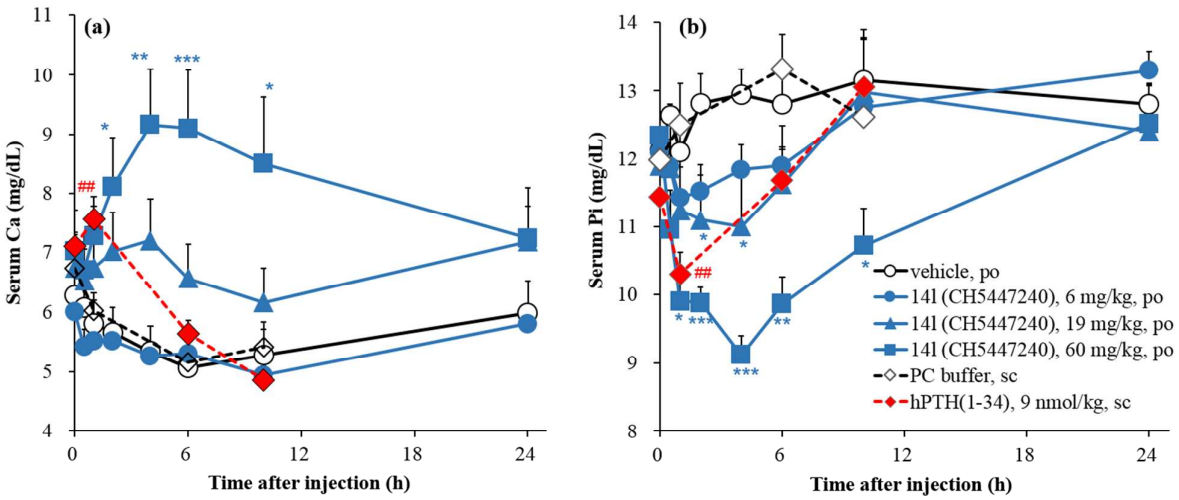


Figure 3. (a) Calcemic and (b) hypophosphatemic effects of **14l** (CH5447240) in TPTX rat model. Treatment began 6 days after TPTX surgery. N = 5, six-week-old rats, Data are given as the mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001 for **14l** versus TPTX with vehicle, or ##p < 0.01 for PTH(1-34) versus PC buffer.

CONCLUSION

We identified a novel small-molecule hPTHR1 agonist **4a** as a hit from an HTS campaign and have successfully optimized **4a** to **14l**, which had agonist activity 100 times more potent than **4a**. **14l** exhibited, orally and dose-dependently, a potent calcium effect in TPTX rats. From these findings, **14l** was considered to be a candidate drug for patients with hypoparathyroidism, and being orally available, was thought to have great benefit for patients who have to take drugs over a long period. However, studies of **14l** in hLM detected a GSH-trapped reactive metabolite, and a program of further optimization study was needed before the clinical candidate PCO371 could be obtained.

EXPERIMENTAL SECTION

Chemistry. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Silica gel chromatography purification was performed using prepacked silica gel cartridges (Biotage, Shoko Scientific). Reverse-phase column chromatography purification was performed using Wakosil 25C18 (Wako Pure Chemical Industries). Nuclear magnetic resonance (NMR) spectra were determined with a Varian MR-400 spectrometer (400 MHz, Agilent). Chemical shifts are reported in parts per million (ppm, δ units). The following NMR abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs = broad singlet. High resolution mass spectrometric analysis was performed on a Xevo G2-S Tof instrument (Waters). All biologically tested compounds were determined as ≥95% pure by UHPLC analysis measured at 230 nm. The UHPLC analysis was carried out on a Waters ACQUITY UPLC I-Class system including a ACQUITY UPLC PDA. Method: The gradient was 5% B to 100% B for 0–1.0 min; 100% B (1.0 mL/min flow rate) for 1.0–1.4 min. Mobile Phase A: 10 mM AcONH4 in water. Mobile Phase B: methanol. Column: Ascentis Express C18 column (2.1 mmI.D. × 50 mm). Column temperature: 35 °C.

8-((3-Chlorophenyl)sulfonyl)-2-(2,4-dichlorophenyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (4a). To a stirred mixture of 2,4-dichloro-*N*-(1-((3-chlorophenyl)sulfonyl)-4-cyanopiperidin-4-yl)benzamide (40.5 mg, 0.086 mmol) (**9a**) in EtOH (430 µl), 6 mol/L NaOH (143 µl, 0.857 mmol) and 30% aq. H_2O_2 (61 µl, 0.60 mmol) were added at 0ºC. The mixture was stirred at 80ºC for 2 h. This mixture was evaporated and EtOAc and H_2O were added. The organic layer was washed with H_2O and sat. aq. NaCl, and dried over anhyd. Na_2SO_4, and evaporated *in vacuo*. The mixture was purified by column chromatography (silica gel, EtOAc-Hexane) to afford **4a** (20.9 mg, 51%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 8.55 (1H, brs), 7.82 (1H, d, *J* = 8.4 Hz), 7.79 (1H, dd, *J* = 1.7, 1.7 Hz), 7.70-7.67

(1H, m), 7.60-7.57 (1H, m), 7.49 (1H, dd, *J* = 8.4, 8.4 Hz), 7.48 (1H, d, *J* = 2.0 Hz), 7.36 (1H, dd, *J* = 8.4, 2.0 Hz), 3.78-3.73 (2H, m), 3.12-3.05 (2H, m), 2.17-2.10 (2H, m), 1.72-1.66 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 184.6, 156.4, 138.7, 138.6, 135.5, 133.0, 132.8, 132.1, 130.5, 130.5, 128.1, 127.6, 126.2, 125.7, 67.7, 42.1, 32.3. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{17}Cl_3N_3O_3S$ (M+H), 472.0056; Found 472.0050. Purity 99%, RT 1.00 min.

2-(4-Chlorophenyl),-8-((3-chlorophenyl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (4b). 4b was prepared from **9b** (39.1 mg, 0.089 mmol) using a procedure similar to that described for the preparation of **4a**. (White solid, 17.7 mg, 45%) ^{1}H-NMR (DMSO-d_6) δ: 11.67 (1H, brs), 7.90 (2H, d, *J* = 7.8 Hz), 7.88-7.85 (1H, m), 7.84 (1H, dd, *J* = 1.7, 1.7 Hz), 7.81-7.78 (1H, m), 7.74 (1H, dd, *J* = 7.8, 7.8 Hz), 7.58 (2H, d, *J* = 7.8 Hz), 3.66-3.60 (2H, m), 2.94-2.88 (2H, m), 1.87-1.79 (2H, m), 1.63-1.57 (2H, m). ^{13}C-NMR (DMSO-d_6) δ: 186.1, 157.9, 137.9, 136.5, 134.1, 133.2, 131.6, 128.8, 128.7, 127.2, 126.8, 126.1, 67.4, 42.1, 31.8. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{18}Cl_2N_3O_3S$ (M+H), 438.0446; Found 438.0436. Purity 99%, RT 1.00 min.

2-(2-Chlorophenyl)-8-((3-chlorophenyl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (4c). 4c was prepared from **9c** (80 mg, 0.183 mmol) using a procedure similar to that described for the preparation of **4a**. (White solid, 29 mg, 36%) ^{1}H-NMR ($CDCl_3$) δ: 8.48 (1H, brs), 7.85-7.82 (1H, m), 7.80-7.78 (1H, m), 7.70-7.67 (1H, m), 7.59-7.56 (1H, m), 7.49 (1H, dd, *J* = 7.9, 7.9 Hz), 7.46-7.44 (2H, m), 7.39-7.35 (1H, m), 3.79-3.73 (2H, m), 3.13-3.06 (2H, m), 2.18-2.11 (2H, m), 1.73-1.68 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 184.6, 157.3, 138.7, 135.5, 133.0, 132.8, 132.1, 131.1, 130.7, 130.5, 127.8, 127.6, 127.6, 125.7, 67.6, 42.1, 32.3. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{18}Cl_2N_3O_3S$ (M+H), 438.0446; Found 438.0449. Purity 99%, RT 0.93 min.

8-((3-Chlorophenyl)sulfonyl)-2-phenyl-1,3,8-triazaspiro[4.5]dec-1-en-4-one (4d). 4d was prepared from **9d** (41.9 mg, 0.104 mmol) using a procedure similar to that described for the preparation of **4a**. (White solid, 22.1 mg, 53%) ^{1}H-NMR (DMSO-d_6) δ: 11.62 (1H, brs), 7.91-7.85 (3H, m), 7.84 (1H, dd, *J* = 1.7, 1.7 Hz), 7.82-7.79 (1H, m), 7.74 (1H, dd, *J* = 7.8, 7.8 Hz), 7.58-7.53 (1H, m), 7.51-7.46 (2H, m), 3.66-3.60 (2H, m), 2.97-2.89 (2H, m), 1.87-1.80 (2H, m), 1.62-1.55 (2H, m). ^{13}C-NMR (DMSO-d_6) δ: 186.2, 158.7, 137.9, 134.1, 133.2, 131.7, 131.6, 128.6, 128.3, 126.8, 126.8, 126.1, 67.3, 42.1, 31.8. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{19}ClN_3O_3S$ (M+H), 404.0836; Found 404.0832. Purity 100%, RT 0.93 min.

4-(8-((3-Chlorophenyl)sulfonyl)-4-oxo-1,3,8-triazaspiro[4.5]dec-1-en-2-yl)benzoic acid (4e). 4e was prepared from **9e** (29.9 mg, 0.065 mmol) using a procedure similar to that described for the preparation of **4a**. (White solid, 14.1 mg, 49%) ^{1}H-NMR (DMSO-d_6) δ: 13.3 (1H, brs), 11.73 (1H, brs), 8.05-7.98 (4H, m), 7.89-7.85 (1H, m), 7.84 (1H, dd, *J* = 1.7, 1.7 Hz), 7.82-7.79 (1H, m), 7.74 (1H, dd, *J* = 7.8, 7.8 Hz), 3.67-3.61 (2H, m), 2.96-2.89 (2H, m), 1.88-1.81 (2H, m), 1.65-1.59 (2H, m). ^{13}C-NMR (DMSO-d_6) δ: 186.0, 166.6, 158.3, 137.9, 134.1, 133.6, 133.2, 131.9, 131.6, 129.5, 127.1, 126.8, 126.1, 67.6, 42.1, 31.7. HRMS (ESI-TOF) *m/z* calcd for $C_{20}H_{19}ClN_3O_5S$ (M+H), 448.0734; Found 448.0734. Purity 99%, RT 0.75 min.

8-((3-Chlorophenyl)sulfonyl)-2-(pyridin-4-yl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (4f). 4f was prepared from **9f** (60 mg, 0.148 mmol) using a procedure similar to that described for the preparation of **4a**. (light yellow solid, 41 mg, 68%) ^{1}H-NMR ($CDCl_3$) δ: 10.30 (1H, brs), 8.81 (2H, dd, *J* = 4.5, 1.7 Hz), 7.82 (1H, dd, *J* = 1.7, 1.7 Hz), 7.73-7.70 (3H, m), 7.65-7.62 (1H, m), 7.53 (1H, dd, *J* = 7.9, 7.9 Hz), 3.84-3.79 (2H, m), 3.11-3.03 (2H, m),

2.24-2.17 (2H, m), 1.69-1.64 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 187.5, 156.6, 150.9, 138.7, 135.5, 135.3, 133.1, 130.5, 127.6, 125.7, 120.5, 69.6, 42.2, 32.3. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{18}ClN_4O_3S$ (M+H), 405.0788; Found 405.0787. Purity 95%, RT 0.86 min.

8-((3-Chlorophenyl)sulfonyl)-2-cyclohexyl-1,3,8-triazaspiro[4.5]dec-1-en-4-one (4g). 4g was prepared from **9g** (46.1 mg, 0.112 mmol) using a procedure similar to that described for the preparation of **4a**. (White solid, 35.1 mg, 76%) ^{1}H-NMR ($CDCl_3$) δ: 8.60 (1H, brs), 7.78 (1H, dd, *J* = 1.8, 1.8 Hz), 7.69-7.65 (1H, m), 7.59-7.55 (1H, m), 7.48 (1H, dd, *J* = 7.9, 7.9 Hz), 3.73-3.68 (2H, m), 3.05-2.97 (2H, m), 2.42-2.35 (1H, m), 2.07-1.98 (2H, m), 1.89-1.78 (4H, m), 1.74-1.68 (1H, m), 1.57-1.50 (2H, m), 1.40-1.20 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.5, 165.0, 138.7, 135.4, 132.9, 130.4, 127.6, 125.6, 67.4, 42.1, 39.4, 32.3, 29.7, 25.6, 25.4. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{25}ClN_3O_3S$ (M+H), 410.1305; Found 410.1301. Purity 99%, RT 0.97 min.

8-((3-Chlorophenyl)sulfonyl)-2-((1*R*,4*R*)-4-methylcyclohexyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (4h). 4h was prepared from **9h** (46.4 mg, 0.109 mmol) using a procedure similar to that described for the preparation of **4a**. (White solid, 33.2 mg, 72%) ^{1}H-NMR ($CDCl_3$) δ: 8.63 (1H, brs), 7.72 (1H, dd, *J* = 1.7, 1.7 Hz), 7.63-7.60 (1H, m), 7.53-7.50 (1H, m), 7.42 (1H, dd, *J* = 7.8, 7.8 Hz), 3.68-3.63 (2H, m), 2.99-2.91 (2H, m), 2.30-2.22 (1H, m), 2.01-1.93 (2H, m), 1.86-1.80 (2H, m), 1.77-1.71 (2H, m), 1.50-1.44 (2H, m), 1.37-1.27 (3H, m), 0.98-0.87 (2H, m), 0.86 (3H, d, *J* = 6.6 Hz). ^{13}C-NMR ($CDCl_3$) δ: 186.6, 165.1, 138.8, 135.4, 132.9, 130.5, 127.6, 125.6, 67.4, 42.1, 39.3, 34.1, 32.3, 32.0, 29.7, 22.4. HRMS (ESI-TOF) *m/z* calcd for $C_{20}H_{27}ClN_3O_3S$ (M+H), 424.1461; Found 424.1456. Purity 98%, RT 1.01 min.

1-((3-Chlorophenyl)sulfonyl)piperidin-4-one (7). To a stirred mixture of piperidin-4-one hydrochloride (**5**) (0.5 g, 3.69 mmol) and K_2CO_3 (1.27 g, 9.22 mmol) in $CHCl_3$ (9 ml) and H_2O (9 ml) was added 3-chlorobenzenesulfonyl chloride (**6**) (0.57 ml, 4.06 mmol) at room temperature. The mixture was stirred at room temperature for 12 h under nitrogen atmosphere. The reaction mixture was diluted with CH_2Cl_2 and separated. The organic layer was washed with H_2O and sat. aq. NaCl, and dried over anhyd. Na_2SO_4, and evaporated *in vacuo*. The residue was triturated with EtOAc-Hexane to afford **7** (728 mg, 72%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 7.80-7.78 (1H, m), 7.71-7.67 (1H, m), 7.62-7.59 (1H, m), 7.51 (1H, dd, *J* = 7.9, 7.9 Hz), 3.44 (4H, t, *J* = 6.2 Hz), 2.57 (4H, t, *J* = 6.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 205.2, 138.5, 135.8, 133.5, 130.8, 127.6, 125.7, 46.0, 40.8.

4-amino-1-((3-chlorophenyl)sulfonyl)piperidine-4-carbonitrile (8). To a stirred mixture of 1-((3-chlorophenyl)sulfonyl)piperidin-4-one (**7**) (700 mg, 2.56 mmol) and ammonium acetate (217 mg, 2.81 mmol) in MeOH (7 ml) was added KCN (167 mg, 2.56 mmol) at room temperature. The mixture was stirred at room temperature for 5 h under nitrogen atmosphere. $NaHCO_3$ (107 mg, 1.279 mmol) was added at 0ºC and the mixture was evaporated. The residue was diluted with CH_2Cl_2 and H_2O and separated. The organic layer was washed with H_2O and sat. aq. NaCl, and dried over anhyd. Na_2SO_4, and evaporated *in vacuo*. The mixture was purified by column chromatography (silica gel, EtOAc-Hexane) to afford **8** (657 mg, 86%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 7.76-7.75 (1H, m), 7.66-7.63 (1H, m), 7.62-7.58 (1H, m), 7.50 (1H, dd, *J* = 7.9, 7.9 Hz), 3.62-3.55 (2H, m), 2.96-2.88 (2H, m), 2.13-2.06 (2H, m), 1.88-1.81 (2H, m), 1.75 (2H, brs). ^{13}C-NMR ($CDCl_3$) δ: 138.2, 135.8, 133.4, 130.7, 127.7, 125.6, 122.7, 48.9, 42.4, 36.5.

2,4-dichloro-*N*-(1-((3-chlorophenyl)sulfonyl)-4-cyanopiperidin-4-yl)benzamide (9a). To a stirred mixture of 4-amino-1-((3-chlorophenyl)sulfonyl)piperidine-4-carbonitrile (**8**) (50 mg, 0.167 mmol) and DIPEA (35 µl, 0.20 mmol) in CH_2Cl_2 (834 µl) was added 2,4-dichlorobenzoyl chloride (26 µl, 0.18 mmol) at 0ºC. The mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with CH_2Cl_2, quenched by H_2O and separated. The organic layer was washed with H_2O and sat. aq. NaCl, and dried over anhyd. Na_2SO_4, and evaporated *in vacuo*. The mixture was purified by column chromatography (silica gel, EtOAc-Hexane) to afford **9a** (67.7 mg, 86%) as a white solid. ^{1}H-NMR (DMSO-d_6) δ: 9.13 (1H, s), 7.92-7.89 (1H, m), 7.86 (1H, dd, *J* = 1.8, 1.8 Hz), 7.83-7.80 (1H, m), 7.77 (1H, dd, *J* = 7.8, 7.8 Hz), 7.73 (1H, d, *J* = 1.8 Hz), 7.54 (1H, dd, *J* = 8.3, 1.8 Hz), 7.47 (1H, d, *J* = 8.3 Hz), 3.21-3.10 (4H, m), 2.40-2.32 (2H, m), 2.30-2.22 (2H, m). ^{13}C-NMR (DMSO-d_6) δ: 165.6, 136.8, 135.2, 134.3, 134.3, 133.5, 131.6, 131.0, 130.5, 129.2, 127.5, 127.0, 126.3, 119.1, 48.4, 41.7, 32.3. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{17}Cl_3N_3O_3S$ (M+H), 472.0056; Found 472.0050.

4-Chloro-*N*-(1-((3-chlorophenyl)sulfonyl)-4-cyanopiperidin-4-yl)benzamide (9b). 9b was prepared from **8** (50 mg, 0.167 mmol) and 4-chlorobenzoyl chloride using a procedure similar to that described for the preparation of **9a**. (White solid, 54.8 mg, 75% yield) ^{1}H-NMR (DMSO-d_6) δ: 8.89 (1H, s), 7.92-7.89 (1H, m), 7.87-7.80 (4H, m), 7.77 (1H, dd, *J* = 7.8, 7.8 Hz), 7.62-7.58 (2H, m), 3.58-3.51 (2H, m), 2.87-2.80 (2H, m), 2.50-2.44 (2H, m), 2.17-2.09 (2H, m). ^{13}C-NMR (DMSO-d_6) δ: 165.9, 137.0, 136.9, 134.3, 133.5, 131.9, 131.6, 129.6, 128.5, 126.9, 126.2, 118.9, 49.2, 42.3, 32.8. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{18}Cl_2N_3O_3S$ (M+H), 438.0446; Found 438.0446.

2-Chloro-*N*-(1-((3-chlorophenyl)sulfonyl)-4-cyanopiperidin-4-yl)benzamide (9c). 9c was prepared from **8** (50 mg, 0.167 mmol) and 2-chlorobenzoyl chloride using a procedure similar to that described for the preparation of **9a**. (White solid, 58.6 mg, 80% yield) ^{1}H-NMR (DMSO-d_6) δ: 9.03 (1H, s), 7.89-7.86 (1H, m), 7.82 (1H, dd, *J* = 1.7, 1.7 Hz), 7.79-7.76 (1H, m), 7.73 (1H, dd, *J* = 7.7, 7.7 Hz), 7.48-7.46 (2H, m), 7.40-7.37 (2H, m), 3.19-3.06 (4H, m), 2.34-2.23 (4H, m). ^{13}C-NMR (DMSO-d_6) δ: 166.4, 136.6, 135.4, 134.2, 133.4, 131.5, 131.3, 129.6, 129.5, 129.0, 127.2, 126.9, 126.2, 119.2, 48.1, 41.5, 32.1. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{16}Cl_2N_3O_3S$ (M-H), 436.0290; Found 436.0285.

***N*-(1-((3-chlorophenyl)sulfonyl)-4-cyanopiperidin-4-yl)benzamide (9d). 9d** was prepared from **8** (50 mg, 0.167 mmol) and benzoyl chloride using a procedure similar to that described for the preparation of **9a**. (White solid, 59.8 mg, 89%). ^{1}H-NMR (DMSO-d_6) δ: 8.81 (1H, s), 7.92-7.89 (1H, m), 7.88-7.86 (1H, m), 7.84-7.74 (4H, m), 7.64-7.59 (1H, m), 7.54-7.49 (2H, m), 3.54-3.48 (2H, m), 2.90-2.83 (2H, m), 2.50-2.45 (2H, m), 2.20-2.12 (2H, m). ^{13}C-NMR (DMSO-d_6) δ: 167.0, 136.9, 134.3, 133.5, 133.3, 132.0, 131.6, 128.3, 127.7, 127.0, 126.2, 119.1, 49.0, 42.3, 32.7. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{19}ClN_3O_3S$ (M+H), 404.0836; Found 404.0827.

Methyl 4-((1-((3-chlorophenyl)sulfonyl)-4-cyanopiperidin-4-yl)carbamoyl)benzoate (9e). 9e was prepared from **8** (50 mg, 0.167 mmol) and methyl 4-(chlorocarbonyl)benzoate using a procedure similar to that described for the preparation of **9a**. (White solid, 38.5 mg, 50% yield) ^{1}H-NMR (DMSO-d_6) δ: 9.00 (1H, s), 8.06-8.03 (2H, m), 7.92-7.89 (2H, m), 7.88-7.85 (1H, m), 7.83 (1H, dd, *J* = 1.7, 1.7 Hz), 7.80-7.77 (1H, m), 7.73 (1H, dd, *J* = 7.8, 7.8 Hz), 3.89 (3H, s), 3.55-3.50 (2H, m), 2.84-2.76 (2H, m), 2.50-2.42 (2H, m), 2.14-2.07 (2H, m). ^{13}C-NMR

(DMSO-d_6) δ: 166.2, 165.6, 137.3, 137.0, 134.3, 133.5, 132.4, 131.7, 129.1, 128.1, 126.9, 126.2, 118.8, 52.5, 49.3, 42.4, 32.7. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{21}ClN_3O_5S$ (M+H), 462.0890; Found 462.0884.

***N*-(1-((3-Chlorophenyl)sulfonyl)-4-cyanopiperidin-4-yl)isonicotinamide (9f). 9f** was prepared from **8** (50 mg, 0.167 mmol) and 4-pyridinecarbonyl chloride using a procedure similar to that described for the preparation of **9a**. (White solid, 29.4 mg, 44%) ^{1}H-NMR (DMSO-d_6) δ: 9.13 (1H, s), 8.78 (2H, dd, *J* = 4.4, 1.6 Hz), 7.92-7.89 (1H, m), 7.87 (1H, dd, *J* = 1.7, 1.7 Hz), 7.84-7.80 (1H, m), 7.77 (1H, dd, *J* = 7.8, 7.8 Hz), 7.73 (2H, dd, *J* = 4.4, 1.6 Hz), 3.59-3.54 (2H, m), 2.88-2.80 (2H, m), 2.52-2.45 (2H, m), 2.18-2.10 (2H, m). ^{13}C-NMR (DMSO-d_6) δ: 165.5, 150.3, 140.2, 137.0, 134.3, 133.5, 131.7, 126.9, 126.2, 121.5, 118.7, 49.3, 42.3, 32.7. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{18}ClN_4O_3S$ (M+H), 405.0788; Found 405.0785.

***N*-(1-((3-Chlorophenyl)sulfonyl)-4-cyanopiperidin-4-yl)cyclohexanecarboxamide (9g). 9g** was prepared from **8** (50 mg, 0.167 mmol) and cyclohexylcarbonyl chloride using a procedure similar to that described for the preparation of **9a**. (White solid, 61.7 mg, 90%) ^{1}H-NMR (DMSO-d_6) δ: 8.19 (1H, s), 7.87-7.84 (1H, m), 7.80 (1H, dd, *J* = 1.7, 1.7 Hz), 7.77-7.74 (1H, m), 7.71 (1H, dd, *J* = 7.6, 7.6 Hz), 3.19-3.13 (2H, m), 2.95-2.87 (2H, m), 2.27-2.20 (2H, m), 2.12-1.99 (3H, m), 1.69-1.52 (5H, m), 1.28-1.10 (5H, m). ^{13}C-NMR (DMSO-d_6) δ: 175.6, 136.7, 134.3, 133.5, 131.6, 126.9, 126.3, 119.5, 47.7, 43.3, 41.8, 32.5, 28.8, 25.3, 25.0. HRMS (ESI-TOF) m/z calcd for $C_{19}H_{25}ClN_3O_3S$ (M+H), 410.1305; Found 410.1295.

(1*R*, 4*R*)-*N*-(1-((3-Chlorophenyl)sulfonyl)-4-cyanopiperidin-4-yl)-4-methylcyclohexane-1-carboxamide (9h). To a stirred mixture of 4-amino-1-((3-chlorophenyl)sulfonyl)piperidine-4-carbonitrile (**8**) (50 mg, 0.167 mmol),

(1*R*, 4*R*)-4-methylcyclohexane-1-carboxylic acid (26 mg, 0.183 mmol) and DIPEA (35 µl, 0.200 mmol) in DMF (834 µl) at 0ºC was added HATU (76 mg, 0.200 mmol). The mixture was stirred at room temperature for 12 h. The reaction mixture was quenched by H_2O. The mixture was extracted with EtOAc. The organic layer was washed with H_2O and sat. aq. NaCl, and dried over anhyd. Na_2SO_4, and evaporated *in vacuo*. The mixture was purified by column chromatography (silica gel, EtOAc-Hexane) to afford **9h** (63.4 mg, 90%) as a white solid. ^{1}H-NMR (DMSO-d_6) δ: 8.26 (1H, s), 7.91-7.88 (1H, m), 7.83 (1H, dd, *J* = 1.7, 1.7 Hz), 7.81-7.77 (1H, m), 7.75 (1H, dd, *J* = 7.6, 7.6 Hz), 3.24-3.18 (2H, m), 2.97-2.90 (2H, m), 2.31-2.23 (2H, m), 2.10-2.01 (3H, m), 1.72-1.58 (4H, m), 1.35-1.23 (3H, m), 0.92-0.82 (2H, m), 0.88 (3H, d, *J* = 6.4 Hz). ^{13}C-NMR (DMSO-d_6) δ: 175.7, 136.7, 134.3, 133.5, 131.6, 126.9, 126.3, 119.4, 47.7, 43.2, 41.8, 33.8, 32.5, 31.5, 28.8, 22.5. HRMS (ESI-TOF) *m/z* calcd for $C_{20}H_{27}ClN_3O_3S$ (M+H), 424.1461; Found 424.1454.

***tert*-Butyl 4-Cyano-4-(2,4-dichlorobenzamido)piperidine-1-carboxylate (11a).** To a stirred mixture of *tert*-butyl 4-amino-4-cyanopiperidine-1-carboxylate (**10**) (12.2 g, 54.2 mmol) and triethylamine (15.1 ml, 108 mmol) in CH_2Cl_2 (200 ml) at room temperature was added 2,4-dichlorobenzoyl chloride (8.4 ml, 59.9 mmol). Then dimethylaminopyridine (331 mg, 2.71 mmol) was added. The mixture was stirred at room temperature for 1 h. This mixture was quenched by H_2O. The mixture was extracted with CH_2Cl_2. The organic layer was washed with H_2O and sat. aq. NaCl, and dried over anhyd. Na_2SO_4, and evaporated *in vacuo*. The mixture was triturated with EtOAc-Hexane to afford **11a** (6.54 g, 30%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 7.74 (1H, d, *J* = 8.4 Hz), 7.44 (1H, d, *J* = 2.0 Hz), 7.36 (1H, dd, *J* = 8.4, 2.0 Hz), 6.55 (1H, brs), 4.01-3.90 (2H, m), 3.40-3.31 (2H, m), 2.53-2.44 (2H, m), 1.97-1.88 (2H, m), 1.47 (9H, s). ^{13}C-NMR ($CDCl_3$) δ: 164.6, 154.4, 138.1, 132.2, 131.5, 131.5, 130.3, 128.1,

118.3, 80.7, 51.0, 39.9, 34.7, 28.5. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{20}Cl_2N_3O_3$ (M-H), 396.0882; Found 396.0881.

***tert*-Butyl 4-Cyano-4-(cyclohexanecarboxamido)piperidine-1-carboxylate (11b). 11b** was prepared from **10** (8.42 g, 25.1 mmol) and cyclohexylcarbonyl chloride using a procedure similar to that described for the preparation of **11a**. (White solid, 5.33 g, 63%) ^{1}H-NMR ($CDCl_3$) δ: 5.59 (1H, brs), 4.01-3.87 (2H, m), 3.30-3.20 (2H, m), 2.47-2.32 (2H, m), 2.14-2.07 (1H, m), 1.87-1.64 (7H, m), 1.49-1.41 (2H, m), 1.46 (9H, s), 1.30-1.20 (3H, m). ^{13}C-NMR ($CDCl_3$) δ: 175.7, 154.4, 118.8, 80.5, 50.2, 45.3, 40.0, 34.8, 29.5, 28.5, 25.7, 25.6. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{28}N_3O_3$ (M-H), 334.2131; Found 334.2136.

***tert*-Butyl 4-Cyano-4-(4-methylcyclohexane-1-carboxamido)piperidine-1-carboxylate (11c). 11c** was prepared from **10** (4.07 g, 18.1 mmol) and 4-methylcyclohexane carbonyl chloride (cis-trans mixture) using a procedure similar to that described for the preparation of **11a**. (White solid, 4.46 g, 71%, cis : trans = 4 : 6) ^{1}H-NMR ($CDCl_3$) δ: 5.54 (0.4H, brs, cis), 5.50 (0.6H, brs, trans), 4.00-3.90 (2H, m), 3.30-3.22 (2H, m), 2.44-2.36 (2H, m), 2.30-2.25 (0.4H, m, cis), 2.06-1.98 (0.6H, m, trans), 1.88-1.30 (11H, m), 1.46 (9H, s), 0.94 (1.2H, d, J = 6.7 Hz, cis), 0.89 (1.8H, d, J = 6.5 Hz, trans).

***tert*-Butyl 2-(2,4-Dichlorophenyl)-4-oxo-1,3,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (12a).** To a stirred mixture of *tert*-butyl 4-cyano-4-(2,4-dichlorobenzamido)piperidine-1-carboxylate (**11a**) (6.22 g, 18.1 mmol) in EtOH (100 ml), 6 mol/L aq. NaOH (36 ml) and 30% aq. H_2O_2 (16 ml) were added at room temperature. The mixture was stirred at 80ºC for 4 h. This mixture was evaporated, and EtOAc and H_2O were added. The organic

layer was washed with H_2O and sat.aq.NaCl, and dried over anhyd. Na_2SO_4, and evaporated *in vacuo*. The mixture was purified by column chromatography (silica gel, EtOAc-Hexane) to afford **12a** (3.11 g, 43%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 8.84 (1H, brs), 7.93 (1H, d, *J* = 8.4 Hz), 7.50 (1H, d, *J* = 2.0 Hz), 7.40 (1H, dd, *J* = 8.4, 2.0 Hz), 4.12-3.95 (2H, m), 3.48-3.37 (2H, m), 1.98-1.88 (2H, m), 1.59-1.52 (2H, m), 1.48 (9H, s). ^{13}C-NMR ($CDCl_3$) δ: 185.7, 156.1, 154.9, 138.5, 133.0, 132.3, 130.6, 128.2, 126.7, 79.9, 69.1, 39.8, 32.5, 28.6. HRMS (ESI-TOF) m/z calcd for $C_{18}H_{20}Cl_2N_3O_3$ (M-H), 396.0882; Found 396.0876.

***tert*-Butyl 2-Cyclohexyl-4-oxo-1,3,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (12b). 12b** was prepared from **11b** (5.33 g, 15.9 mmol) using a procedure similar to that described for the preparation of **12a**. (White solid, 4.43 g, 83%) ^{1}H-NMR ($CDCl_3$) δ: 8.74 (1H, brs), 4.01-3.89 (2H, m), 3.45-3.36 (2H, m), 2.48-2.41 (1H, m), 1.96-1.91 (2H, m), 1.86-1.69 (5H, m), 1.49-1.24 (7H, m), 1.47 (9H, s). ^{13}C-NMR ($CDCl_3$) δ: 187.4, 164.4, 154.8, 79.6, 68.6, 40.1, 39.2, 32.4, 29.7, 28.5, 25.7, 25.5. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{28}N_3O_3$ (M-H), 334.2131; Found 334.2129.

***tert*-Butyl 2-((1*R*,4*R*)-4-Methylcyclohexyl)-4-oxo-1,3,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (12c). 12c** was prepared from **11c** (4.46 g, 12.8 mmol) using a procedure similar to that described for the preparation of **12a**. (White solid, 3.83 g, 86%) ^{1}H-NMR ($CDCl_3$) δ: 8.78 (1H, brs), 4.03-3.87 (2H, m), 3.46-3.34 (2H, m), 2.42-2.33 (1H, m), 1.99-1.92 (2H, m), 1.86-1.76 (5H, m), 1.49-1.41 (4H, m), 1.47 (9H, s), 1.08-0.96 (2H, m), 0.93 (3H, d, *J* = 6.5 Hz). ^{13}C-NMR ($CDCl_3$) δ: 187.4, 164.5, 154.8, 79.6, 68.6, 40.0, 39.2, 34.2, 32.4, 32.0, 29.7, 28.5, 22.4. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{30}N_3O_3$ (M-H), 348.2287; Found 348.2290.

2-(2,4-Dichlorophenyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one dihydrochloride (13a). The mixture of *tert*-butyl 2-(2,4-dichlorophenyl)-4-oxo-1,3,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (**12a**) (30 mg, 0.075 mmol) and 4 mol/L HCl-dioxane (377 µl, 1.51 mmol) was stirred at room temperature for 2 h. This mixture was evaporated to afford **13a** (28 mg, 100%) as a white solid. ^{1}H-NMR (CD_3OD) δ: 7.85 (1H, d, J = 8.4 Hz), 7.84 (1H, d, J = 2.0 Hz), 7.67 (1H, dd, J = 8.4, 2.0 Hz), 3.68-3.61 (2H, m), 3.54-3.47 (2H, m), 2.40-2.33 (2H, m), 2.24-2.17 (2H, m). ^{13}C-NMR (CD_3OD) δ: 179.6, 167.5, 142.3, 135.3, 134.0, 132.2, 129.6, 123.5, 65.2, 40.5, 29.7. HRMS (ESI-TOF) *m/z* calcd for $C_{13}H_{14}Cl_2N_3O$ (M+H), 298.0514; Found 298.0516.

2-Cyclohexyl-1,3,8-triazaspiro[4.5]dec-1-en-4-one bis(2,2,2-trifluoroacetate) (13b). To a mixture of *tert*-butyl 2-cyclohexyl-4-oxo-1,3,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (**12b**) (4.43 g, 13.2 mmol) in CH_2Cl_2 (60 ml) was added trifluoroacetic acid (20 ml) at room temperature. The mixture was stirred at room temperature for 5 h and evaporated. The residue was triturated with CH_2Cl_2 to afford **13b** (5.66 g, 92%) as a white solid. ^{1}H-NMR (DMSO-d_6) δ: 8.82 (1H, brs), 8.62 (1H, brs), 3.40-3.33 (2H, m), 3.22-3.11 (2H, m), 2.54-2.47 (1H, m), 1.99-1.86 (4H, m), 1.79-1.62 (5H, m), 1.50-1.39 (2H, m), 1.35-1.13 (3H, m). ^{13}C-NMR (DMSO-d_6) δ: 182.4, 172.4, 158.3 (q, J = 35.1 Hz), 115.9 (q, J = 293.2 Hz), 63.7, 37.9, 28.5, 28.4, 25.0, 24.7. HRMS (ESI-TOF) *m/z* calcd for $C_{13}H_{22}N_3O$ (M+H), 236.1763; Found 236.1766.

2-Cyclohexyl-1,3,8-triazaspiro[4.5]dec-1-en-4-one dihydrochloride (13c). 13c was prepared from **12b** (43.1 mg, 0.128 mmol) using a procedure similar to that described for the preparation of **13a**. (White solid, 39.8 mg, 100%) ^{1}H-NMR (CD_3OD) δ: 3.66-3.60 (2H, m), 3.58-3.51 (2H, m), 2.97-2.88 (1H, m), 2.41-2.28 (4H, m),

2.16-2.09 (2H, m), 1.99-1.93 (2H, m), 1.87-1.80 (1H, m), 1.68-1.58 (2H, m), 1.55-1.30 (3H, m). ^{13}C-NMR (CD_3OD) δ: 180.4, 178.0, 63.0, 40.2, 39.7, 29.9, 29.8, 29.4, 26.2. HRMS (ESI-TOF) *m/z* calcd for $C_{13}H_{22}N_3O$ (M+H), 236.1763; Found 236.1772.

2-((1*R*,4*R*)-4-Methylcyclohexyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one dihydrochloride (13d). 13d was prepared from **12c** (100 mg, 0.286 mmol) using a procedure similar to that described for the preparation of **13a**. (White solid, 91.1 mg, 99%) ^{1}H-NMR (DMSO-d_6) δ: 9.46 (2H, brs), 3.56-3.48 (2H, m), 3.41-3.35 (2H, m), 2.82-2.75 (1H, m), 2.19-2.00 (6H, m), 1.85-1.79 (2H, m), 1.75-1.65 (2H, m), 1.47-1.36 (1H, m), 1.06-0.96 (2H, m), 0.93 (3H, d, *J* = 6.5 Hz). ^{13}C-NMR (DMSO-d_6) δ: 177.9, 177.2, 62.8, 38.3, 37.3, 33.1, 31.0, 28.1, 27.8, 22.1. HRMS (ESI-TOF) *m/z* calcd for $C_{14}H_{24}N_3O$ (M+H), 250.1919; Found 250.1942.

8-(Benzylsulfonyl)-2-cyclohexyl-1,3,8-triazaspiro[4.5]dec-1-en-4-one (14a). To a stirred mixture of 2-cyclohexyl-1,3,8-triazaspiro[4.5]dec-1-en-4-one bis(2,2,2-trifluoroacetate) **13b** (60 mg, 0.129 mmol) and triethylamine (63 µl, 0.452 mmol) in CH_2Cl_2 (1.5 ml) was added benzylsulfonyl chloride (30 mg, 0.157 mmol) at 0ºC. The mixture was stirred at room temperature for overnight. This mixture was evaporated and the residue was purified by column chromatography (silica gel, EtOAc-hexane) to afford **14a** (19.5 mg, 39%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 8.02 (1H, brs), 7.45-7.35 (5H, m), 4.22 (2H, s), 3.60-3.53 (2H, m), 3.30-3.23 (2H, m), 2.45-2.37 (1H, m), 1.95-1.81 (6H, m), 1.76-1.70 (1H, m), 1.50-1.25 (7H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.7, 165.0,

131.5, 129.6, 129.4, 129.3, 68.2, 57.8, 42.6, 39.8, 33.3, 30.3, 26.3, 26.1. HRMS (ESI-TOF) *m/z* calcd for $C_{20}H_{28}N_3O_3S$ (M+H), 390.1851; Found 390.1852. Purity 100%, RT = 0.90 min.

2-Cyclohexyl-8-(phenethylsulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (14b). To a stirred solution of (*E*)-2-cyclohexyl-8-(styrylsulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (**17b**) (28.2 mg, 0.070 mmol) in EtOH (1.0 ml), AcOEt (0.5 ml) and AcOH (0.15 ml) were added 10% Pd/C (10 mg) and this mixture was stirred at room temperature for 2 days under hydrogen atmosphere. The mixture was filtered and evaporated. The residue was purified by column chromatography (silica gel, hexane-EtOAc) to afford **14b** (19.3 mg, 68%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 7.87 (1H, brs), 7.35-7.30 (2H, m), 7.28-7.24 (1H, m), 7.24-7.20 (2H, m), 3.77-3.71 (2H, m), 3.43-3.36 (2H, m), 3.22-3.12 (4H, m), 2.45-2.38 (1H, m), 2.00-1.90 (4H, m), 1.86-1.80 (2H, m), 1.75-1.70 (1H, m), 1.62-1.54 (2H, m), 1.45-1.20 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.1, 164.8, 138.5, 129.2, 128.7, 127.3, 67.7, 51.3, 42.0, 39.5, 33.0, 30.1, 29.7, 26.0, 25.8. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{30}N_3O_3S$ (M+H), 404.2008; Found 404.2021. Purity 98%, RT 0.95 min.

2-Cyclohexyl-8-((2-(pyridin-4-yl)ethyl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (14c). 14c was prepared from **17c** (20 mg, 0.050 mmol) using a procedure similar to that described for the preparation of **14b**. (White solid, 15 mg, 75%) ^{1}H-NMR ($CDCl_3$) δ: 8.57-8.55 (2H, m), 7.82 (1H, brs), 7.18-7.15 (2H, m), 3.78-3.72 (2H, m), 3.44-3.38 (2H, m), 3.22-3.12 (4H, m), 2.46-2.38 (1H, m), 2.05-1.90 (4H, m), 1.86-1.80 (2H, m), 1.76-1.70 (1H, m), 1.65-1.55 (2H, m), 1.46-1.20 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 185.6, 164.5, 150.2, 147.1, 123.7, 67.3, 49.7,

41.7, 39.2, 32.6, 29.7, 28.7, 25.6, 25.4. HRMS $C_{20}H_{29}N_4O_3S$ (ESI-TOF) *m/z* calcd for 405.1960 (M+H),; Found 405.1963. Purity 95%, RT 0.78 min.

2-Cyclohexyl-8-((2-(naphthalen-1-yl)ethyl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (14d). 14d was prepared from **13b** (1.00 g, 2.16 mmol) and 1-naphthylethylsulfonyl chloride using a procedure similar to that described for the preparation of **14a**. (White solid, 877 mg, 90%) ^{1}H-NMR ($CDCl_3$) δ: 8.97 (1H, brs), 8.03 (1H, d, *J* = 8.4 Hz), 7.88 (1H, d, *J* = 8.4 Hz), 7.78 (1H, d, *J* = 7.8 Hz), 7.60-7.55 (1H, m), 7.54-7.49 (1H, m), 7.42 (1H, t, *J* = 7.8 Hz), 7.39-7.37 (1H, m), 3.82-3.75 (2H, m), 3.65-3.60 (2H, m), 3.43-3.37 (2H, m), 3.32-3.28 (2H, m), 2.44-2.36 (1H, m), 2.03-1.95 (2H, m), 1.93-1.87 (2H, m), 1.83-1.77 (2H, m), 1.72-1.65 (1H, m), 1.60-1.54 (2H, m), 1.45-1.18 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 187.3, 165.4, 134.5, 134.3, 131.7, 129.4, 128.2, 127.0, 126.9, 126.2, 125.9, 123.3, 68.0, 50.4, 42.1, 39.6, 32.9, 29.9, 26.9, 25.9, 25.7. HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{32}N_3O_3S$ (M+H), 454.2164; Found 454.2169. Purity 100%, RT 1.04 min.

2-Cyclohexyl-8-(naphthalen-1-ylsulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (14e). **14e** was prepared from **13c** (15 mg, 0.049 mmol) and naphthalene-1-sulphonyl chloride using a procedure similar to that described for the preparation of **14a**. (White solid, 13.8 mg, 67%) ^{1}H-NMR ($CDCl_3$) δ: 8.75 (1H, d, *J* = 8.6 Hz), 8.39 (1H, brs), 8.23 (1H, d, *J* = 6.4 Hz), 8.07 (1H, d, *J* = 8.1 Hz), 7.93 (1H, d, *J* = 8.1 Hz), 7.68-7.51 (3H, m), 3.84-3.76 (2H, m), 3.28-3.19 (2H, m), 2.40-2.31 (1H, m), 2.00-1.91 (2H, m), 1.89-1.75 (4H, m), 1.73-1.65 (1H, m), 1.54-1.46 (2H, m), 1.40-1.16 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.4, 164.7, 134.5, 134.4, 133.6, 130.2, 129.0, 128.9, 128.1, 126.9, 125.2,

124.1, 67.7, 41.5, 39.3, 32.5, 29.6, 25.6, 25.4. HRMS (ESI-TOF) *m/z* calcd for $C_{23}H_{28}N_3O_3S$ (M+H), 426.1851; Found 426.1850. Purity 98%, RT 1.00 min.

2-(2,4-Dichlorophenyl)-8-((2-(naphthalen-1-yl)ethyl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (14f). **14f** was prepared from **13a** (15 mg, 0.040 mmol) and 1-naphthylethylsulfonyl chloride using a procedure similar to that described for the preparation of **14a**. (White solid, 18.8 mg, 90%) ^{1}H-NMR ($CDCl_3$) δ: 8.62 (1H, s), 8.05 (1H, d, *J* = 8.6 Hz), 7.94-7.87 (2H, m), 7.79 (1H, d, *J* = 7.8 Hz), 7.61-7.34 (6H, m), 3.88-3.80 (2H, m), 3.67-3.61 (2H, m), 3.52-3.43 (2H, m), 3.36-3.29 (2H, m), 2.14-2.04 (2H, m), 1.77-1.69 (2H, m). ^{13}C-NMR ($CDCl_3$) δ: 184.7, 156.3, 138.6, 134.1, 134.0, 132.8, 132.2, 131.3, 130.5, 129.1, 128.1, 127.9, 126.7, 126.6, 126.2, 125.9, 125.6, 123.0, 67.8, 50.2, 41.7, 32.6, 26.66. HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{24}Cl_2N_3O_3S$ (M+H), 516.0916; Found 516.0914. Purity 100%, RT 1.08 min.

2-Cyclohexyl-8-((2-methylphenethyl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (14g). The solution of (*E*)-2-cyclohexyl-8-((2-methylstyryl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (**17g**) (25 mg, 0.060 mmol) in EtOH-DMF (4:1, 8.5ml) was pumped through the 10% Pd-C cartridge (CatCart^{TM}) at a flow rate of 2.0 mL/min, at 40ºC, under 30 bar H_2 pressure using a continuous hydrogenation reactor (H-Cube®). The reaction mixture was evaporated and purified by column chromatography (silica gel, hexane-EtOAc) to afford **14g** (15.9 mg, 63%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 8.89 (1H, brs), 7.18-7.14 (4H, m), 3.81-3.74 (2H, m), 3.43-3.37 (2H, m), 3.14 (4H, s), 2.47-2.39 (1H, m), 2.35 (3H, s), 2.02-1.90 (4H, m), 1.86-1.80 (2H, m), 1.75-1.69 (1H, m), 1.60-1.54 (2H, m), 1.47-1.20 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 187.3, 165.2, 136.7, 136.3, 131.0, 129.1, 127.5, 126.8, 68.0, 50.0, 42.1,

39.6, 33.0, 29.9, 27.1, 26.0, 25.8, 19.5. HRMS (ESI-TOF) *m/z* calcd for $C_{22}H_{32}N_3O_3S$ (M+H), 418.2164; Found 418.2172. Purity 98%, RT 0.99 min.

***N*-(4-(2-((2-Cyclohexyl-4-oxo-1,3,8-triazaspiro[4.5]dec-1-en-8-yl)sulfonyl)ethyl)-3-methylphenyl)acetamide (14h). 14h** was prepared from **17h** (30 mg, 0.063 mmol) using a procedure similar to that described for the preparation of **14g**. (white solid, 19.9 mg, 66%) ^{1}H-NMR ($CDCl_3$) δ: 8.37 (1H, brs), 7.34-7.28 (2H, m), 7.16 (1H, brs), 7.09 (1H, d, *J* = 8.2 Hz), 3.77-3.71 (2H, m), 3.43-3.36 (2H, m), 3.10 (4H, s), 2.45-2.38 (1H, m), 2.33 (3H, s), 2.17 (3H, s), 2.00-1.90 (4H, m), 1.85-1.80 (2H, m), 1.75-1.70 (1H, m), 1.61-1.54 (2H, m), 1.46-1.20 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.6, 168.6, 165.0, 137.2, 137.0, 132.7, 129.7, 122.4, 118.3, 67.8, 50.0, 42.1, 39.5, 32.9, 30.0, 26.6, 26.0, 25.8, 25.0, 19.7. HRMS (ESI-TOF) *m/z* calcd for $C_{24}H_{35}N_4O_4S$ (M+H), 475.2379; Found 475.2386. Purity 99%, RT 0.86 min.

***N*-(4-(2-((2-Cyclohexyl-4-oxo-1,3,8-triazaspiro[4.5]dec-1-en-8-yl)sulfonyl)ethyl)-3,5-dimethylphenyl)acetamide (14i).** To a stirred solution of (*E*)-*N*-(4-(2-((2-cyclohexyl-4-oxo-1,3,8-triazaspiro[4.5]dec-1-en-8-yl)sulfonyl)vinyl)-3,5-dimethylphenyl)acetamide (**17i**) (20.0 mg, 0.041 mmol) in MeOH (1.5 ml) was added 20% $Pd(OH)_2$ on carbon (14 mg) and this mixture was stirred at room temperature for 7 h under hydrogen atmosphere. The mixture was filtered and evaporated. The residue was purified by column chromatography (silica gel, hexane-EtOAc) to afford **14i** (15.7 mg, 78%) as a colorless glass. ^{1}H-NMR ($CDCl_3$) δ: 8.95 (1H, s), 7.21 (1H, s), 7.18 (2H, s), 3.79-3.73 (2H, m), 3.44-3.38 (2H, m), 3.15-3.10 (2H, m), 3.01-2.95 (2H, m), 2.46-2.39 (1H, m), 2.33 (6H, s), 2.16 (3H, s), 2.00-1.90 (4H, m), 1.84-1.79

(2H, m), 1.74-1.69 (1H, m), 1.60-1.55 (2H, m), 1.46-1.23 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.8, 168.4, 165.0, 137.1, 136.3, 130.8, 119.9, 67.6, 48.0, 41.8, 39.2, 32.6, 29.6, 25.6, 25.4, 24.6, 22.8, 19.9. HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{37}N_4O_4S$ (M+H), 489.2535; Found 489.2539. Purity 98%, RT 0.89 min.

1-(4-(2-((2-Cyclohexyl-4-oxo-1,3,8-triazaspiro[4.5]dec-1-en-8-yl)sulfonyl)ethyl)-3,5-dimethylphenyl)-1-methylurea (14j). 14j was prepared from **21a** (27 mg, 0.054 mmol) using a procedure similar to that described for the preparation of **14i**. (Colorless glass, 21.8 mg, 80%) ^{1}H-NMR ($CDCl_3$) δ: 9.69 (1H, brs), 6.95 (2H, s), 4.76 (2H, brs), 3.74-3.67 (2H, m), 3.55-3.48 (2H, m), 3.22 (3H, s), 3.22-3.17 (2H, m), 2.98-2.94 (2H, m), 2.48-2.40 (1H, m), 2.36 (6H, s), 1.95-1.82 (6H, m), 1.76-1.68 (3H, m), 1.52-1.22 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 187.2, 165.7, 158.3, 141.7, 138.4, 134.6, 126.8, 67.2, 47.7, 41.5, 39.3, 37.0, 32.4, 29.6, 25.7, 25.5, 22.9, 19.8. HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{38}N_5O_4S$ (M+H), 504.2644; Found 504.2644. Purity 98%, RT 0.88 min.

2-Cyclohexyl-8-((2,6-dimethyl-4-(2-oxoimidazolidin-1-yl)phenethyl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (14k). 14k was prepared from **17k** (30 mg, 0.058 mmol) using a procedure similar to that described for the preparation of **14i**. (Colorless glass, 12.4 mg, 41%) ^{1}H-NMR ($CDCl_3$) δ: 9.02 (1H, brs), 7.22 (2H, s), 5.09 (1H, brs), 3.94-3.89 (2H, m), 3.80-3.73 (2H, m), 3.60-3.54 (2H, m), 3.45-3.38 (2H, m), 3.17-3.11 (2H, m), 3.02-2.96 (2H, m), 2.46-2.38 (1H, m), 2.36 (6H, s), 2.02-1.89 (4H, m), 1.86-1.79 (2H, m), 1.75-1.69 (1H, m), 1.63-1.56 (2H, m), 1.49-1.21 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.8, 165.0, 159.9, 138.4, 136.9, 129.4, 118.1, 67.6, 48.1, 45.4, 41.8, 39.2, 37.6, 32.6, 29.6, 25.7, 25.5, 22.8, 20.1. HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{38}N_5O_4S$ (M+H), 516.2645; Found 516.2655. Purity 99%, RT 0.89 min.

1-(3,5-Dimethyl-4-(2-((2-((1*R*,4*R*)-4-methylcyclohexyl)-4-oxo-1,3,8-triazaspiro[4.5]dec-1-en-8-yl)sulfonyl)ethyl)phenyl)-1-methylurea (14l). 14l was prepared from **21b** (208 mg, 0.403 mmol) using a procedure similar to that described for the preparation of **14i**. (White solid, 130 mg, 62%) ^{1}H-NMR ($CDCl_3$) δ: 9.84 (1H, brs), 6.94 (2H, s), 4.79 (2H, brs), 3.73-3.66 (2H, m), 3.56-3.49 (2H, m), 3.23-3.16 (2H, m), 3.22 (3H, s), 2.99-2.93 (2H, m), 2.40-2.32 (1H, m), 2.35 (6H, s), 1.99-1.94 (2H, m), 1.91-1.79 (4H, m), 1.75-1.67 (2H, m), 1.53-1.35 (3H, m), 1.07-0.95 (2H, m), 0.92 (3H, d, J = 6.5 Hz). ^{13}C-NMR ($CDCl_3$) δ: 187.3, 165.9, 158.4, 141.7, 138.4, 134.6, 126.8, 67.2, 47.6, 41.5, 39.3, 37.0, 34.2, 32.4, 32.0, 29.6, 22.9, 22.4, 19.8. HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{40}N_5O_4S$ (M+H), 518.2801; Found 518.2819. Purity 100%, RT 0.93 min.

2-Cyclohexyl-8-(vinylsulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (15a). To a stirred mixture of 2-cyclohexyl-1,3,8-triazaspiro[4.5]dec-1-en-4-one dihydrochloride (**13c**) (999 mg, 3.24 mmol) and triethylamine (2.7 ml, 19.4 mmol) in CH_2Cl_2 (30 ml) was added 2-chloroethane-1-sulfonyl chloride (440 µl, 4.21 mmol) at 0ºC. The mixture was stirred at 0ºC for 30 min and at room temperature for 30 min. This mixture was quenched by H_2O and diluted with CH_2Cl_2. The organic layer was washed with H_2O and sat. aq. NaCl, and dried over anhyd. Na_2SO_4, and evaporated *in vacuo*. The mixture was triturated with EtOAc-Hexane to afford **15a** (692 mg, 66%) as a white solid. ^{1}H-NMR (DMSO-d_6) δ: 10.88 (1H, brs), 6.92 (1H, dd, *J* = 16.5, 10.0 Hz), 6.23 (1H, d, *J* = 10.0 Hz), 6.17 (1H, d, *J* = 16.5 Hz), 3.55-3.48 (2H, m), 3.10-3.03 (2H, m), 2.42-2.34 (1H, m), 1.89-1.83 (2H, m), 1.80-1.72 (4H, m), 1.69-1.63 (1H, m), 1.50-1.18 (7H, m). ^{13}C-NMR (DMSO-d_6) δ: 186.2, 165.7, 133.2, 129.1, 66.5, 41.6, 38.3, 31.9, 28.8, 25.4, 25.0. HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_{24}N_3O_3S$ (M+H), 326.1538; Found 326.1545.

2-((1R, 4R)-4-Methylcyclohexyl-8-(vinylsulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (15b). 15b was prepared from **13d** (1.00 g, 3.10 mmol) using a procedure similar to that described for the preparation of **15a**. (White solid, 840 mg, 80%) ^{1}H-NMR ($CDCl_3$) δ: 8.82 (1H, brs), 6.48 (1H, dd, *J* = 16.6, 10.0 Hz), 6.26 (1H, d, *J* = 16.6 Hz), 6.03 (1H, d, *J* = 10.0 Hz), 3.69-3.63 (2H, m), 3.28-3.22 (2H, m), 2.40-2.34 (1H, m), 2.03-1.91 (4H, m), 1.86-1.79 (2H, m), 1.61-1.55 (2H, m), 1.48-1.37 (3H, m), 1.07-0.96 (2H, m), 0.93 (3H, d, *J* = 6.6 Hz). ^{13}C-NMR ($CDCl_3$) δ: 186.9, 165.1, 133.0, 128.3, 67.5, 41.6, 39.3, 34.2, 32.3, 32.0, 29.7, 22.4. HRMS (ESI-TOF) *m/z* calcd for $C_{16}H_{26}N_3O_3S$ (M+H), 340.1695; Found 340.1699.

1-(4-Bromo-3,5-dimethylphenyl)imidazolidin-2-one (16k). To a stirred mixture of 4-bromo-3,5-dimethylaniline (**18**) (200 mg, 1.00 mmol) in CH_2Cl_2 (3ml) at 0ºC was added 2-chloroethylisocyanate (0.104 ml, 1.20 mmol) under nitrogen atmosphere. The mixture was stirred at room temperature for 12 h under nitrogen atmosphere. This mixture was evaporated and diluted with DMF (9 ml). Then NaH (60% oil susp.) (48.0 mg, 1.20 mmol) was added at 0°C and the mixture was stirred at room temperature for 2 h under nitrogen atmosphere. The mixture was purified by reverse phase column chromatography (25C18, 0.1% formic acid in CH_3CN-H_2O) to afford **16k** (219 mg, 81%) as a white solid. ^{1}H-NMR (DMSO-d_6) δ: 7.40 (2H, s), 7.00 (1H, brs), 3.83-3.78 (2H, m), 3.41-3.36 (2H, m), 2.33 (6H, s). ^{13}C-NMR (DMSO-d_6) δ: 158.8, 139.6, 137.4, 118.6, 116.9, 44.4, 36.4, 23.7. HRMS (ESI-TOF) *m/z* calcd for $C_{11}H_{14}BrN_2O$ (M+H), 269.0289; Found 269.0287.

(*E*)-2-Cyclohexyl-8-(styrylsulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (17b). The mixture of 2-cyclohexyl-8-(vinylsulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (**15a**) (60 mg, 0.184 mmol), bromobenzene

(**16b**) (25 µl, 0.24 mmol), bis(dibenzylideneacetone)palladium (21 mg, 0.037 mmol), tri-*tert*-butylphosphine tetrafluoroboric acid adduct (11 mg, 0.038 mmol) and methyl dicyclohexylamine (59 µl, 0.28 mmol) in NMP (370 µl) was stirred at 110°C for 1.5 h under nitrogen atmosphere. The reaction mixture was quenched by H_2O. The mixture was extracted with EtOAc. The organic layer was washed with H_2O and sat. aq. NaCl, and dried over anhyd. Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, EtOAc-hexane), then the solid was washed with EtOAc-hexane to afford **17b** (61.0 mg, 83%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 8.59 (1H, brs), 7.51-7.48 (2H, m), 7.48 (1H, d, *J* = 15.5 Hz), 7.44-7.40 (3H, m), 6.72 (1H, d, *J* = 15.5 Hz), 3.75-3.70 (2H, m), 3.29-3.23 (2H, m), 2.45-2.38 (1H, m), 2.06-1.98 (2H, m), 1.93-1.87 (2H, m), 1.84-1.78 (2H, m), 1.73-1.67 (1H, m), 1.62-1.22 (7H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.7, 164.9, 143.1, 132.7, 130.9, 129.1, 128.3, 121.9, 67.6, 41.8, 39.4, 32.5, 29.7, 25.6, 25.4. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{28}N_3O_3S$ (M+H), 402.1851; Found 402.1857.

(*E*)-2-Cyclohexyl-8-((2-(pyridin-4-yl)vinyl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (17c). 17c was prepared from **15a** (60 mg, 0.184 mmol) and 4-bromopyrdine (**16c**) using a procedure similar to that described for the preparation of **17b**. (Colorless glass, 44.4 mg, 60%) ^{1}H-NMR ($CDCl_3$) δ: 8.72 (1H, brs), 8.70 (2H, dd, *J* = 4.5, 1.7 Hz), 7.42 (1H, d, *J* = 15.5 Hz), 7.35 (2H, dd, *J* = 4.5, 1.7 Hz), 6.91 (1H, d, *J* = 15.5 Hz), 3.77-3.72 (2H, m), 3.35-3.27 (2H, m), 2.46-2.39 (1H, m), 2.06-1.99 (2H, m), 1.94-1.88 (2H, m), 1.85-1.79 (2H, m), 1.74-1.68 (1H, m), 1.61-1.55 (2H, m), 1.44-1.20 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.5, 165.1, 150.8, 140.0, 139.9, 127.2, 122.0, 67.4, 41.8, 39.4, 32.4, 29.7, 25.6, 25.4. HRMS (ESI-TOF) *m/z* calcd for $C_{20}H_{27}N_4O_3S$ (M+H), 403.1804; Found 403.1806.

(*E*)-2-Cyclohexyl-8-((2-methylstyryl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (17g). 17g was prepared from **15a** (200 mg, 0.615 mmol) and 2-bromotoluene (**16g**) using a procedure similar to that described for the preparation of **17b**. (Pale yellow solid, 197 mg, 77%) ^{1}H-NMR ($CDCl_3$) δ: 8.60 (1H, brs), 7.74 (1H, d, *J* = 15.4 Hz), 7.50 (1H, d, *J* = 7.6 Hz), 7.32 (1H, dd, *J* = 7.5, 7.6 Hz), 7.27-7.22 (2H, m), 6.62 (1H, d, *J* = 15.4 Hz), 3.76-3.70 (2H, m), 3.29-3.23 (2H, m), 2.46-2.38 (1H, m), 2.43 (3H, s), 2.07-2.00 (2H, m), 1.94-1.88 (2H, m), 1.84-1.79 (2H, m), 1.73-1.68 (1H, m), 1.62-1.54 (2H, m), 1.44-1.20 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 187.0, 165.2, 141.3, 138.1, 132.1, 131.3, 131.0, 127.1, 126.9, 123.2, 67.9, 42.1, 39.7, 32.8, 30.0, 25.9, 25.8, 20.2. HRMS (ESI-TOF) *m/z* calcd for $C_{22}H_{30}N_3O_3S$ (M+H), 416.2008; Found 416.2013.

(*E*)-*N*-(4-(2-((2-Cyclohexyl-4-oxo-1,3,8-triazaspiro[4.5]dec-1-en-8-yl)sulfonyl)vinyl)-3-methylphenyl)aceta mide (17h). 17h was prepared from **15a** (114 mg, 0.350 mmol) and *N*-(4-bromo-3-methylphenyl)acetamide (**16h**) using a procedure similar to that described for the preparation of **17b**. (pale yellow foam, 84.7 mg, 51%) ^{1}H-NMR ($CDCl_3$) δ: 8.80 (1H, brs), 7.65 (1H, d, *J* = 15.4 Hz), 7.47-7.37 (4H, m), 6.56 (1H, d, *J* = 15.4 Hz), 3.74-3.69 (2H, m), 3.29-3.21 (2H, m), 2.46-2.38 (1H, m), 2.40 (3H, s), 2.20 (3H, s), 2.06-1.99 (2H, m), 1.94-1.87 (2H, m), 1.84-1.78 (2H, m), 1.74-1.68 (1H, m), 1.60-1.54 (2H, m), 1.45-1.20 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.8, 168.5, 165.0, 140.2, 140.0, 139.1, 127.7, 127.4, 121.3, 117.5, 67.6, 41.8, 39.4, 32.4, 29.6, 25.6, 25.4, 24.8, 20.0. HRMS (ESI-TOF) *m/z* calcd for $C_{24}H_{33}N_4O_4S$ (M+H), 473.2222; Found 473.2218.

(*E*)-*N*-(4-(2-((2-Cyclohexyl-4-oxo-1,3,8-triazaspiro[4.5]dec-1-en-8-yl)sulfonyl)vinyl)-3,5-dimethylphenyl)ac etamide (17i). 17i was prepared from **15a** (30 mg, 0.092 mmol) and *N*-(4-bromo-3,5-dimethylphenyl)acetamide

(**16i**) using a procedure similar to that described for the preparation of **17b**. (white solid, 39.2 mg, 87%) ^{1}H-NMR (DMSO-d_6) δ: 10.82 (1H, s), 9.94 (1H, s), 7.41 (1H, d, *J* = 15.9 Hz), 7.36 (2H, s), 6.78 (1H, d, *J* = 15.9 Hz), 3.56-3.49 (2H, m), 3.13-3.05 (2H, m), 2.36-2.28 (1H, m), 2.32 (6H, s), 2.04 (3H, s), 1.84-1.69 (6H, m), 1.63-1.58 (1H, m), 1.47-1.15 (7H, m). ^{13}C-NMR (DMSO-d_6) δ: 186.1, 168.4, 165.5, 139.8, 139.6, 137.2, 126.5, 126.1, 118.5, 66.4, 41.7, 38.0, 31.9, 28.6, 25.3, 24.8, 24.0, 21.0. HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{35}N_4O_4S$ (M+H), 487.2379; Found 487.2389.

(*E*)-2-Cyclohexyl-8-((2,6-dimethyl-4-(2-oxoimidazolidin-1-yl)styryl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (17k). 17k was prepared from **15a** (60 mg, 0.184 mmol) and 1-(4-bromo-3,5-dimethylphenyl)imidazolidin-2-one (**16k**) using a procedure similar to that described for the preparation of **17b**. (White solid, 58.5 mg, 62%) ^{1}H-NMR ($CDCl_3$) δ: 9.32 (1H, brs), 7.59 (1H, d, *J* = 15.8 Hz), 7.31 (2H, s), 6.35 (1H, d, *J* = 15.8 Hz), 5.36 (1H, brs), 3.98-3.92 (2H, m), 3.74-3.67 (2H, m), 3.63-3.58 (2H, m), 3.31-3.23 (2H, m), 2.47-2.39 (1H, m), 2.40 (6H, s), 2.07-1.98 (2H, m), 1.95-1.88 (2H, m), 1.85-1.78 (2H, m), 1.74-1.68 (1H, m), 1.64-1.56 (2H, m), 1.48-1.22 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.2, 164.5, 159.3, 140.8, 140.5, 138.0, 126.3, 125.4, 117.4, 67.3, 45.1, 41.7, 39.1, 37.4, 32.4, 29.6, 25.6, 25.4, 21.8. HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{36}N_5O_4S$ (M+H), 514.2488; Found 514.2496.

4-Bromo-*N*,3,5-trimethylaniline (19).

To a stirred mixture of *tert*-butyl (4-bromo-3,5-dimethylphenyl)(methyl)carbamate (**23**) (451 mg, 1.44 mmol) in CH_2Cl_2 (2.2 ml) at 0ºC was added TFA (2.2 ml, 28.7 mmol). The mixture was stirred at room temperature for 4 h under nitrogen atmosphere. This mixture was evaporated. The reaction mixture was diluted with EtOAc and quenched by sat. aq. $NaHCO_3$ (pH 8) and separated. The organic layer was washed with H_2O and sat. aq. NaCl, and dried over anhyd. Na_2SO_4, and evaporated *in vacuo* to afford **19** (304 mg, 99%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 6.36 (2H, s), 2.79 (3H, s), 2.34 (6H, s). ^{13}C-NMR ($CDCl_3$) δ: 147.9, 138.6, 114.6, 112.4, 30.8, 24.0. HRMS (ESI-TOF) *m/z* calcd for $C_9H_{13}BrN$ (M+H), 214.0231; Found 214.0234.

(*E*)-2-Cyclohexyl-8-((2,6-dimethyl-4-(methylamino)styryl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (20a). The mixture of 2-cyclohexyl-8-(vinylsulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (**15a**) (60 mg, 0.184 mmol), 4-bromo-*N*,3,5-trimethylaniline (**19**) (51 mg, 0.24 mmol), bis(dibenzylideneacetone)palladium (21 mg, 0.037 mmol), tri-*tert*-butylphosphine tetrafluoroboric acid adduct (11 mg, 0.038 mmol) and methyl dicyclohexylamine (59 µl, 0.278 mmol) in NMP (370 µl) was stirred at 110ºC for 1.5 h under nitrogen atmosphere. The mixture was purified by reverse phase column chromatography (25C18, 0.1% formic acid in CH_3CN- H_2O) to afford **20a** (65.3 mg, 77%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 8.53 (1H, brs), 7.61 (1H, d, *J* = 15.8 Hz), 6.32 (2H, s), 6.25 (1H, d, *J* = 15.8 Hz), 3.92 (1H, brs), 3.70-3.65 (2H, m), 3.26-3.19 (2H, m), 2.86 (3H, s), 2.45-2.37 (1H, m), 2.37 (6H, s), 2.06-1.98 (2H, m), 1.94-1.88 (2H, m), 1.84-1.78 (2H, m), 1.73-1.67 (1H, m), 1.62-1.54 (2H, m), 1.44-1.20 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 186.8, 164.5, 149.9, 141.2, 139.6, 121.2, 120.3, 112.4, 67.6, 41.8, 39.2, 32.4, 30.2, 29.6, 25.6, 25.4, 22.2. HRMS (ESI-TOF) *m/z* calcd for $C_{24}H_{35}N_4O_3S$ (M+H), 459.2430; Found 459.2422.

8-(((*E*)-2,6-Dimethyl-4-(methylamino)styryl)sulfonyl)-2-((1*R*,4*R*)-4-methylcyclohexyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (20b). 20b was prepared from vinylsulfonamide **15b** (60 mg, 0.177 mmol) and **19** using a procedure similar to that described for the preparation of **20a**. (White solid, 65.9 mg, 79%) ^{1}H-NMR ($CDCl_3$) δ: 8.54 (1H, brs), 7.61 (1H, d, *J* = 15.8 Hz), 6.32 (2H, s), 6.25 (1H, d, *J* = 15.8 Hz), 3.90 (1H, brs), 3.70-3.64 (2H, m), 3.26-3.18 (2H, m), 2.86 (3H, s), 2.37 (6H, s), 2.37-2.29 (1H, m), 2.05-1.98 (2H, m), 1.96-1.90 (2H, m), 1.84-1.77 (2H, m), 1.68-1.53 (3H, m), 1.45-1.36 (2H, m), 1.05-0.94 (2H, m), 0.91 (3H, d, *J* = 6.5 Hz). ^{13}C-NMR ($CDCl_3$) δ: 186.7, 164.7, 149.9, 141.2, 139.6, 121.2, 120.3, 112.4, 67.6, 41.8, 39.1, 34.2, 32.4, 32.0, 30.3, 29.6, 22.4, 22.2. HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{37}N_4O_3S$ (M+H), 473.2586; Found 473.2579.

(*E*)-1-(4-(2-((2-Cyclohexyl-4-oxo-1,3,8-triazaspiro[4.5]dec-1-en-8-yl)sulfonyl)vinyl)-3,5-dimethylphenyl)-1-methylurea (21a). To a stirred mixture of (*E*)-2-cyclohexyl-8-((2,6-dimethyl-4-(methylamino)styryl)sulfonyl)-1,3,8-triazaspiro[4.5]dec-1-en-4-one (**20a**) (48 mg, 0.105 mmol) in AcOH (530 µl) and CH_2Cl_2 (530 µl) was added sodium cyanate (8 mg, 0.123 mmol) at room temperature. The mixture was stirred at room temperature for 11 h. The mixture was purified by reverse phase column chromatography (25C18, 0.1% formic acid in CH_3CN-H_2O) to afford **21a** (43.2 mg, 82%) as a colorless glass. ^{1}H-NMR ($CDCl_3$) δ: 9.92 (1H, brs), 7.54 (1H, d, *J* = 15.9 Hz), 7.02 (2H, s), 6.39 (1H, d, *J* = 15.9 Hz), 4.87 (2H, brs), 3.68-3.60 (2H, m), 3.48-3.40 (2H, m), 3.26 (3H, s), 2.47-2.41 (1H, m), 2.37 (6H, s), 1.97-1.80 (6H, m), 1.78-1.70 (3H, m), 1.52-1.20 (5H, m). ^{13}C-NMR ($CDCl_3$) δ: 187.4, 165.8, 158.1, 143.5, 139.8, 138.7, 131.6, 128.2, 126.6, 67.1, 41.5, 39.3, 37.0, 32.3, 29.5, 25.7, 25.5, 21.2. HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{36}N_5O_4S$ (M+H), 502.2488; Found 502.2502.

1-(3,5-Dimethyl-4-((*E*)-2-((2-((1*R*,4*R*)-4-methylcyclohexyl)-4-oxo-1,3,8-triazaspiro[4.5]dec-1-en-8-yl)sulfonyl)vinyl)phenyl)-1-methylurea (21b). 21b was prepared from **20b** (48.8 mg, 0.103 mmol) using a procedure similar to that described for the preparation of **21a**. (Colorless glass, 51.7 mg, 97%) ^{1}H-NMR ($CDCl_3$) δ: 10.19 (1H, brs), 7.54 (1H, d, *J* = 15.9 Hz), 7.02 (2H, s), 6.39 (1H, d, *J* = 15.9 Hz), 4.91 (2H, brs), 3.67-3.60 (2H, m), 3.47-3.40 (2H, m), 3.25 (3H, s), 2.41-2.32 (1H, m), 2.37 (6H, s), 1.99-1.88 (4H, m), 1.85-1.78 (2H, m), 1.76-1.69 (2H, m), 1.60-1.35 (3H, m), 1.08-0.96 (2H, m), 0.93 (3H, d, *J* = 6.6 Hz). ^{13}C-NMR ($CDCl_3$) δ: 187.6, 166.1, 158.1, 143.4, 139.8, 138.7, 131.6, 128.2, 126.6, 67.1, 41.5, 39.3, 36.9, 34.3, 32.2, 32.0, 29.5, 22.5, 21.2. HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{38}N_5O_4S$ (M+H), 516.2645; Found 516.2643.

***tert*-Butyl (4-Bromo-3,5-dimethylphenyl)(methyl)carbamate (23).**

To a stirred mixture of *tert*-butyl (4-bromo-3,5-dimethylphenyl)carbamate (**22**) (500 mg, 1.67 mmol) in DMF (5.6 ml) was added NaH (60% oil suspension, 80 mg, 2.00 mmol) at 0ºC under nitrogen atmosphere. The mixture was stirred at 0ºC for 5 min , then MeI (208 µl, 3.33 mmol) was added. The mixture was stirred at 0ºC for 5 min and at room temperature for 3 h under nitrogen atmosphere. The reaction mixture was quenched by H_2O and extracted with EtOAc. The organic layer was washed with H_2O and sat.aq.NaCl, and dried over anhyd.Na_2SO_4, and evaporated *in vacuo*. The mixture was purified by column chromatography (silica gel, EtOAc-Hexane) to afford **23** (509 mg, 97%) as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 6.97 (2H, s), 3.21 (3H, s), 2.39 (6H, s), 1.46 (9H, s). ^{13}C-NMR ($CDCl_3$) δ: 154.6, 142.3, 138.4, 125.2, 123.9, 80.4, 37.3, 28.4, 23.9. HRMS (ESI-TOF) *m/z* calcd for $C_{10}H_{12}BrNO_2$ [(M-*tert*-Bu)+H], 258.0129; Found 258.0134.

cAMP production assay. LLC-PK1 cells (ATCC) expressing hPTHR1 (HKRK-B7 cells)[16] were seeded into a 96-well flat-bottomed plate (1.0 x 10^5 cells per 100 μl per well) and incubated overnight. The next day, the culture medium in the wells was discarded, and 50 μl of small-molecule hit compounds or hPTH(1–34) (Peptide Institute, Inc) serially diluted in assay medium (2 mg/ml BSA, 1 mmol/l 3-isobutyl-1-methylxanthine (IBMX), and 2 mmol/l HEPES/McCoy's 5A medium, Life Technologies) was added to each well. The plate was placed in a 37ºC constant-temperature incubator for 20 min to allow the reaction to proceed. After incubation, the liquid in the wells was discarded, the wells were washed with the assay medium (100 μl per well), and the plate was frozen on dry ice. Next, 40 μl of 50 mmol/l HCl was added to each well, and the plate was stored in a freezer set at −20ºC. The cAMP concentrations were measured using a cAMP EIA kit (GE Healthcare) , and cAMP production of each compound was calculated as compared with cAMP production of human PTH(1-34) at 0.1 μmol l^{-1}. EC_{20} and EC_{50} values were determined from a single experiment in duplicate by a non-linear regression Emax model (XLfit, Microsoft Office Excel 2007). Parent LLC-PK1 cells without hPTHR1 were used as a counter assay.

Solubility assay. An aliquot of 50 μL of a 1 mM sample in dimethylsulfoxide (DMSO) was freeze-dried to remove DMSO. To the resulting residue was added 50 mM of PBS or FaSSIF (pH 6.5), which was then irradiated ultrasonically for 10 min, shaken for 2 h, centrifuged for 10 min (3000 rpm), and filtered by Whatman Unifilter. The concentration of the filtrate was analyzed by HPLC-UV based on the calibration curve of each sample. Composition of 50 mM PBS (pH 6.5): 0.05 M of Na_2HPO_4 solution was added to the solution of NaH_2PO_4 (pH 6.5). Composition of FaSSIF: Sodium taurocholate (1.61 g), Lecithin (0.59 g), KH_2PO_4 (3.9 g), KCl (7.7 g) and NaOH (pH 6.5) per 1 L.

LM stability assay. 1 μM of each compound was incubated with human LM (0.5 mg protein/mL) in 50 mM phosphate buffer (pH 7.4) with or without 1 mM NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate) at 37ºC for 30 min. After the enzyme reaction was terminated with the addition of a two-fold volume of acetonitrile followed by addition of same volume of 1μM Warfarin (IS), the reaction mixture was centrifuged at 2000 rpm for 5 min. The resultant supernatant was used as a test sample to measure the stability in human LM by measuring the compound in the sample using liquid chromatography–tandem mass spectrometry (LC–MS/MS).

PK studies. 14l was administered at a dose of 3 mg/kg orally to 5-week-old normal female rats (Crl:CD/SD IGS, CRJ; two rats for each dose group), and blood samples were collected at 15 and 30 min, and 1, 2, 4 and 6 h after administration. Blood samples were also collected 3 min after intravenous administration. The concentrations of plasma **14l** were determined by LC-MS/MS (API-3200 (AB SCIEX), detection limit: 0.3 ng/ml). PK parameters were calculated by a noncompartmental model using Watson7.1.

Single administration study in TPTX rats. Surgical TPTX was carried out in 6-week-old female Crl:CD(SD) rats (Charles River Laboratories). Rats with serum Ca levels of <8.0 mg/dl at 5 days after surgery were used for the experiment. The rats were divided into six groups, each with a similar mean value of serum Ca levels and body weight. TPTX rats were treated once orally (vehicle or **14l** at 6, 19 or 60 mg/kg) or subcutaneously (vehicle or hPTH(1–34) at 9 nmol/kg). Five TPTX rats were assigned to each dose group. The sample size was determined based on previous experiments and a published study on hPTHs and LA-PTH that was conducted in our laboratories[23]. Ten per cent DMSO (Wako Pure Chemical Industries)/10% Kolliphor EL (Sigma-Aldrich) in 10%

hydroxypropyl-bcyclodextrin (HPCD; Nihon Shokuhin Kako)/0.752% glycine (Wako Pure Chemical Industries) buffer was used as vehicle for **14l**, and phosphate-citrate (PC) buffer (pH 6.0) was used as vehicle for hPTH(1–34). Under isoflurane anaesthesia, jugular vein blood was collected immediately before and at 0.5, 1, 2, 4, 6, 10 and 24 h after administration to measure serum Ca (o-CPC method, Wako Pure Chemical Industries) and Pi levels (xanthine oxidase method, Wako Pure Chemical Industries).

Receptor binding assay. Ligand binding to PTHR1 was assessed by the competition method using membranes prepared from COS-7 cells expressing human PTHR1 with ^{125}I-[Aib^{1,3},Nle^{8},Gln^{10},Har^{11},Ala^{12},Trp^{14},Tyr^{15}]–PTH(1–15) as a tracer radioligand[15]. Binding reactions were performed in 96-well vacuum filtration plates and were incubated at room temperature for 90 min. Then the plates were processed by vacuum filtration, and after washing, gamma-rays from the radioligands on the filter were measured. Nonspecific binding was determined in reactions containing an excess ($1x10^{-6}$ M) of unlabeled PTH(1-34). The experiment was performed in triplicate. Curves were fitted to the data using a four-parameter sigmoidal dose-response equation.

ASSOCIATED CONTENT

Supporting Information

The ^{1}H and ^{13}C NMR spectra of biologically tested compounds and the molecular formula strings of synthetic compounds are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s). All authors are employees of Chugai Pharmaceutical Co., Ltd.

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ABREVIATIONS USED

HTS: high throughput screening, BOC: *tert*-butoxycarbonyl, HKRK-B7: hPTHR1 stably expressed in LLCPK1 cells, SAR: structure activity relationship, NADPH: nicotinamide adenine dinucleotide phosphate.

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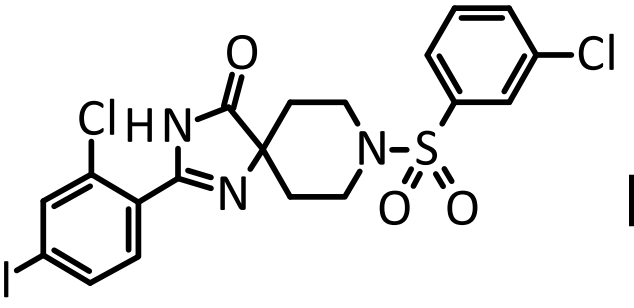
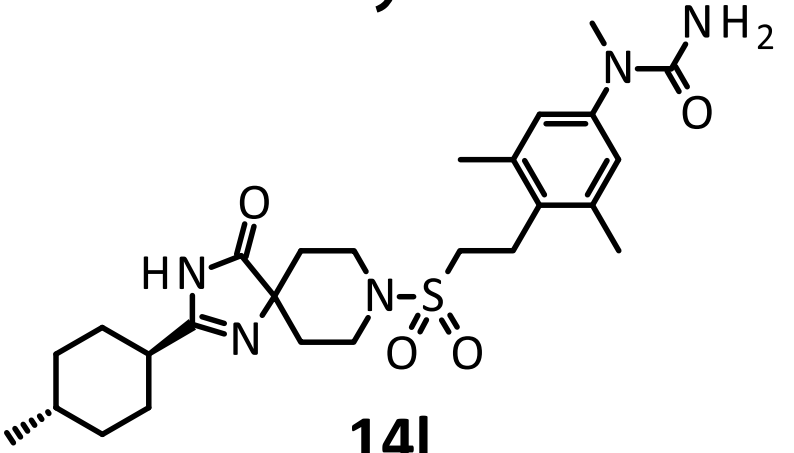
HTS Hit **Orally active PTHR1 agonist**





4a

hPTHR1 cAMP

EC_{20} 530 ± 120 μM

EC_{50} >1,000 μM

14l

hPTHR1 cAMP

EC_{20} 3.0 ± 0.2 μM

EC_{50} 12 ± 1.0 μM

Rat BA (3 mg/kg, po)

55%