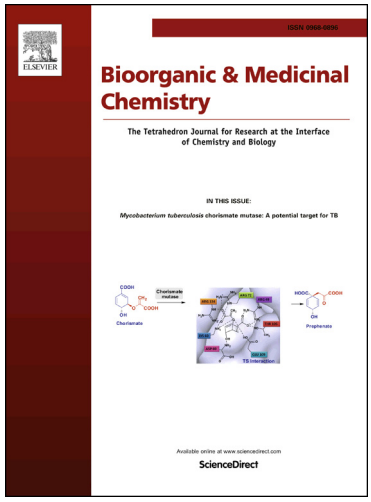
Accepted Manuscript



Pharmacological evaluation of novel (6-aminopyridin-3-yl)(4-(pyridin-2-yl)piperazin-1-yl) methanone derivatives as TRPV4 antagonists for the treatment of pain

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Key words: Transient Receptor Potential Vanilloid 4, TRPV4 antagonist, ion channel, vanilloid receptor, pain

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ABSTRACT

A novel series of (6-aminopyridin-3-yl)(4-(pyridin-2-yl)piperazin-1-yl) methanone derivatives were identified as selective transient receptor potential vanilloid 4 (TRPV4) channel antagonist and showed analgesic effect in Freund's Complete Adjuvant (FCA) induced mechanical hyperalgesia model in guinea pig and rat. Modification of right part based on the compound **16d** which was disclosed in our previous communication led to the identification of compound **26i** as a flagship compound. In this paper, we described the details about design, synthesis and structure-activity relationship (SAR) analysis at right and left part of these derivatives (Figure 1).

1. Introduction

TRPV4 is a cation channel and a member of the TRPV family composed of TRPV1/2, TRPV3, TRPV4 and TRPV5/6[1-6]. TRPV4 was first disclosed as an osmotic-sensitivity receptor activated by osmotic cell swelling and mechanical stimuli[7,8]. Moreover, it was activated by several stimulus including low pH, warm temperature (~35°C), mechano-stimuli, endogenous ligand and some chemical stimulus such as 4-phorbol 12,13-didecanoate (4αPDD)[8-14]. The compound which exhibits high affinity to TRPV4 has been expected a high potential as useful medicine in the therapy and/or prevention of TRPV4 receptor mediated disorder such as heart failure, pulmonary edema, pain, osteoporosis and bladder function[15-23]. Because, TRPV4 is widely expressed such as in epithelial cells of urinary system, airway epithelia of the trachea and the lungs, in the cilia of bronchial epithelium, in the ciliated epithelia of the bile ducts, in the epidermal keratinocytes, in the vascular endothelium, in the inner ear, in osteoblasts of born[5]. Furthermore, there are a lot of reports which suggests the relationship between TRPV4 and pain[24-30]. The activation of the C-fiber by hypotonic stimulation under the inflammatory environment induced by inflammatory mediators is also reported that TRPV4 relates to this activation. Moreover, it is also revealed that TRPV4 is activated by fluid pressure and mechanical stimuli, and TRPV4 relates to hyperalgesia caused by mechanical stimuli, relates to paclitaxel-induced pain[31]. According to these information,

we focused on the TRPV4 as the target of pain and conducted some in vivo validation as same as HTS to afford some hit compounds including bis-thiazole amine. Recently, we reported that novel thiazole derivatives showed more potent antagonism of TRPV4 and showed analgesic effect in FCA induced mechanical hyperalgesia model in previous communication[32]. The further optimization of left and right part around the compound **16d**, the advanced compound **26i** which improved the rat TRPV4 potency was found and moved on the in vivo and selectivity study while maintaining the species differences between human and rat TRPV4 potency. As the result of these studies resulted in identification of **26i** as a potent and highly selective TRPV4 antagonist which shows analgesic effect in FCA induced mechanical hyperalgesia model in guinea pig and rat. In this paper, we report the details of SAR study based on the compound **16d** and their synthesis.

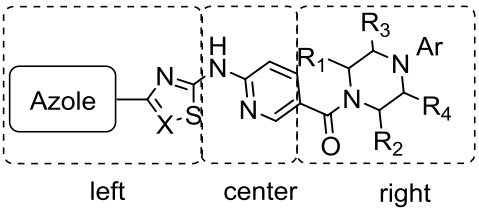
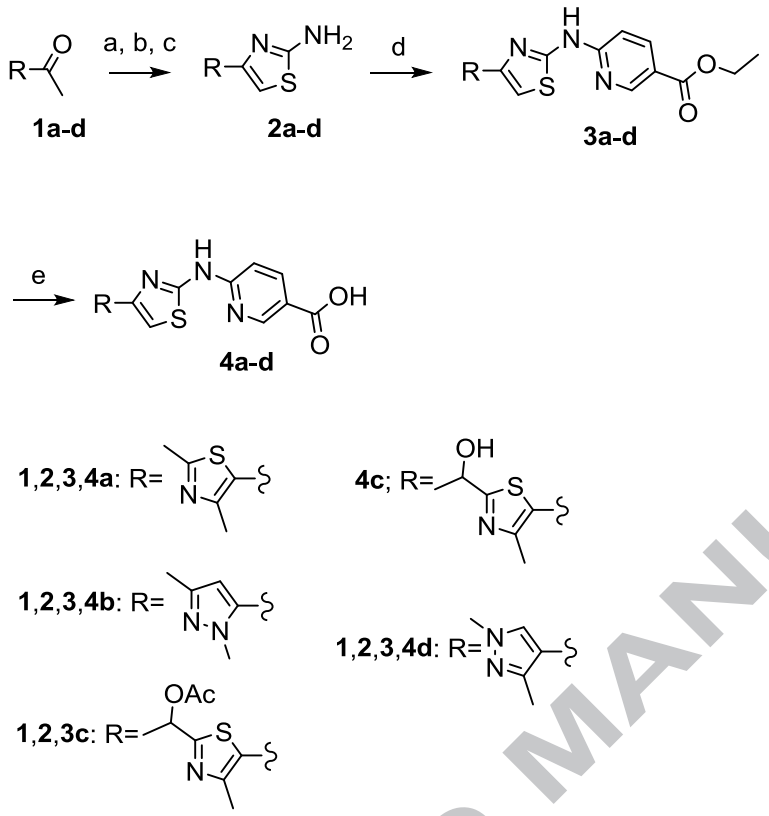


Figure 1. General structure of the SAR compound.

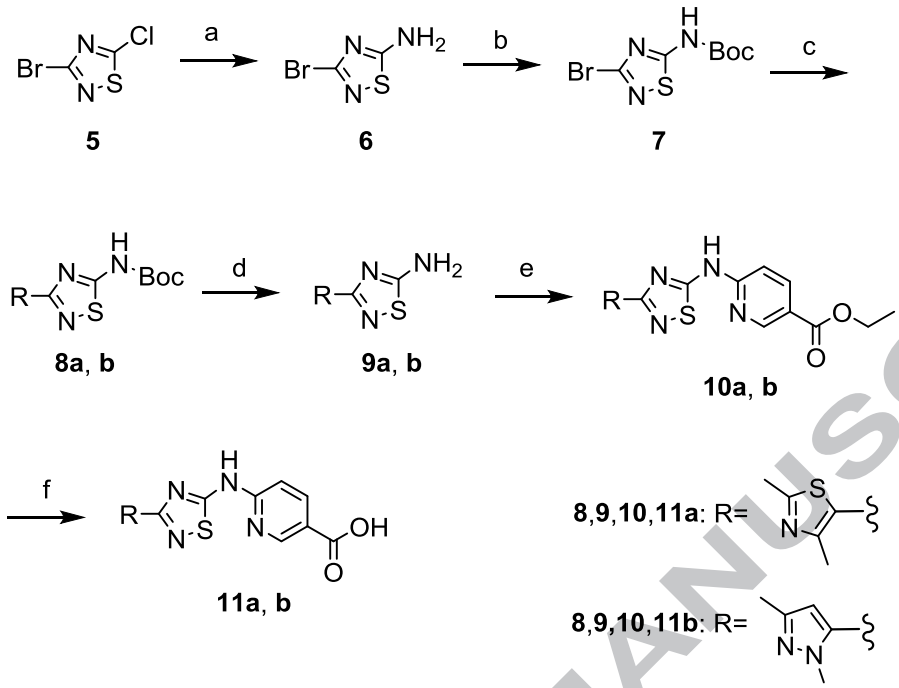
2. Result and discussion

2.1. Chemistry. The key intermediates of carboxylic acid **4a-d** were prepared as described in Scheme 1. After bromination with NBS, the resulting α-bromo ketones were reacted with thiourea to afford the cyclized compounds **2a-d**, respectively. The amines were coupled with ethyl-6-chloronicotinate under Buchwald-Hartwig condition to afford ester **3a-d**, which was then hydrolyzed with sodium hydroxide to afford corresponding carboxylic acid. The synthesis of carboxylic acid **11a** and **11b** was described in Scheme 2. The amination of chloride on thiadiazole with ammonia aqueous solution was given amine **6** followed by protection with Boc group to provide **7**, which was coupled with boronic ester followed by deprotection of Boc group to afford compound **9a** and **9b**, respectively. The next coupling reaction with ethyl-6-chloronicotinate and hydrolysis step was almost same manner with synthesis method from **2a-d** to **4a-d**. The right part including bicyclic amines was synthesized according to Scheme 3. The commercially available Boc protected diazabicyclo[3.2.1]octane was coupled with allyl bromide under Buchwald-Hartwig condition to afford **13a** and **13b**, followed by deprotection of Boc group to provide **14a** and **14b**, respectively. In the case of 2-chloropyrimidine as other end with coupling reaction, the usual alkylation was given **13c** and **13d** which were treated with hydrochloric acid to afford **14c** and **14d**

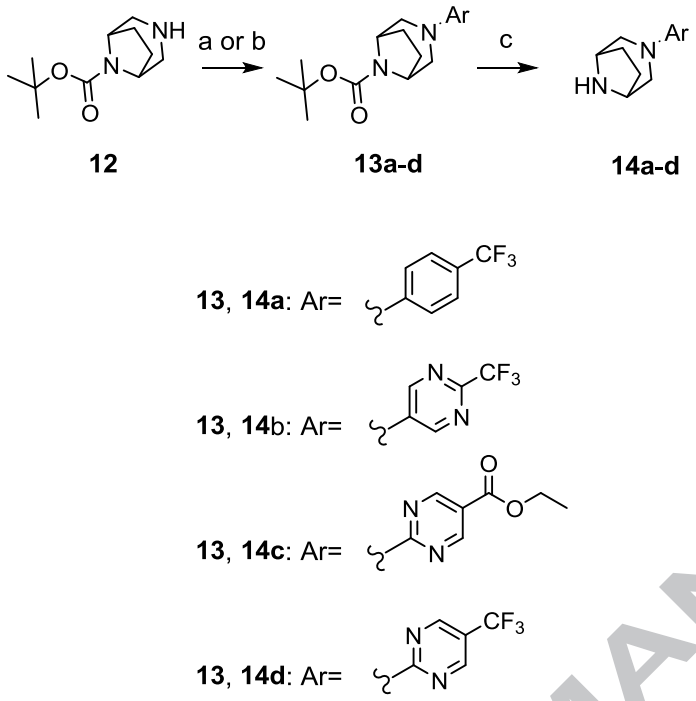
respectively. The resulting carboxylic acids **4a-d**, **11a**, **11b** and amines **14a-d**, commercially available **15** were treated with COMU under basic condition to provide corresponding target compound **16a-d**, **18-22** and intermediate **17**, respectively (Scheme 4). However, intermediate **17d** was not obtained by amidation with corresponding carboxylic acid due to low reactivity of trifluoromethyl piperazine. After acylation with piperazine using acid chloride, the resulting compound **24** was coupled with **2a** to provide **17d** (Scheme 5). After deprotection of Boc group in **17a-j**, the usual alkylation with aryl chloride was provided target compound **26a-i** and **27-35**, respectively (Scheme 6). Conversion of the ester to alcohol **36**, carboxylic acid **37** and amide **38** was shown in Scheme 7, which was prepared by usual and general condition to afford target compounds.



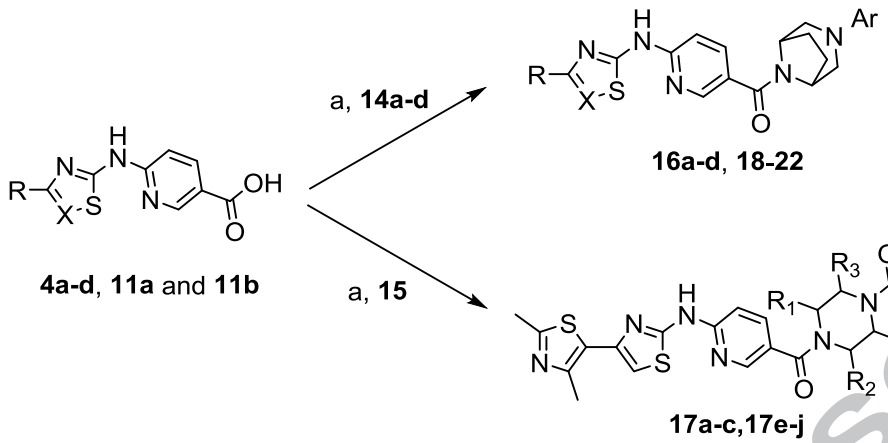
Scheme 1. Synthesis of the carboxylic acid **4a-d**. Reagents and conditions: (a) TMSOTf, Et_3N, THF, 0°C, 2h; (b) NBS, THF, 0°C, 0.5h; (c) thiourea, EtOH, reflux, 1h; (d) ethyl-6-chloronicotinate, sodium carbonate, $Pd(dba)_2$, xantphos, 1,4-dioxane, reflux, 16h; (e) 2N NaOH, EtOH, 80°C, 5h.



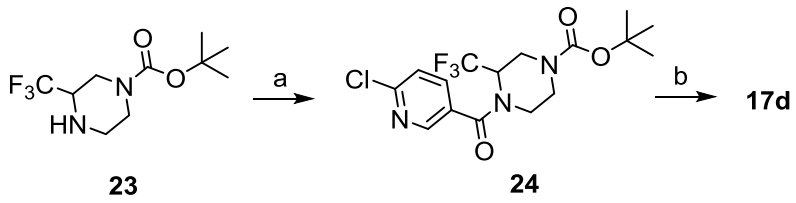
Scheme 2. Synthesis of the the carboxylic acid **11a** and **11b**. Reagents and conditions: (a) 28% ammonia aqueous, EtOH, 70°C, 3h; (b) $(Boc)_2O$, DMAP, THF, 50°C, 1h; (c) 2,4-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole or 1,3-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole, CsF, 1,4-dioxzne, H_2O, 90°C, overnight; (d) 4N HCl in EtOAc, r.t., overnight; (e) ethyl-6-chloronicotinate, potassium phosphate, $Pd_2(dba)_3$, xantphos, 1,4-dioxane, 130°C, 2h, microwave irradiation; (f) 2N NaOH, EtOH, 70°C, 8h.



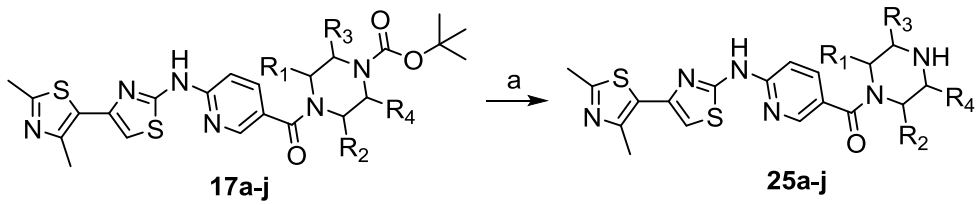
Scheme 3. Synthesis of the intermediate **14a-d**. Reagents and conditions: (a) ArBr, $Pd(dba)_2$, RuPhos, NaO*t*-Pen, 1,4-dioxane, 100°C, 2h, microwave irradiation. For 13a and 13b. (b) 2-chloro-pyrimidine, diisopropyl ethylamine, NMP, 80°C, 5h. For 13c and 13d; (c) 4N HCl in 1,4-dioxane, r.t., overnight.



Scheme 4. Synthesis of the compound **16**-**22**. Reagents and conditions: (a) COMU, Et_3N, DMF, r.t., 1.5h.

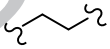


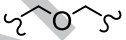
Scheme 5. Synthesis of the intermediate **17d**. Reagents and conditions: (a) 6-chloronicotinoyl chloride , diisopropyl ethylamine, THF/CH_2Cl_2=1/1, 0°C~r.t., 0.5h; (b) **2a**, sodium carbonate, $Pd(dba)_2$, xantphos, 1,4-dioxane, reflux, 16h.

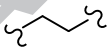


b → **26a-i**, **27-35**

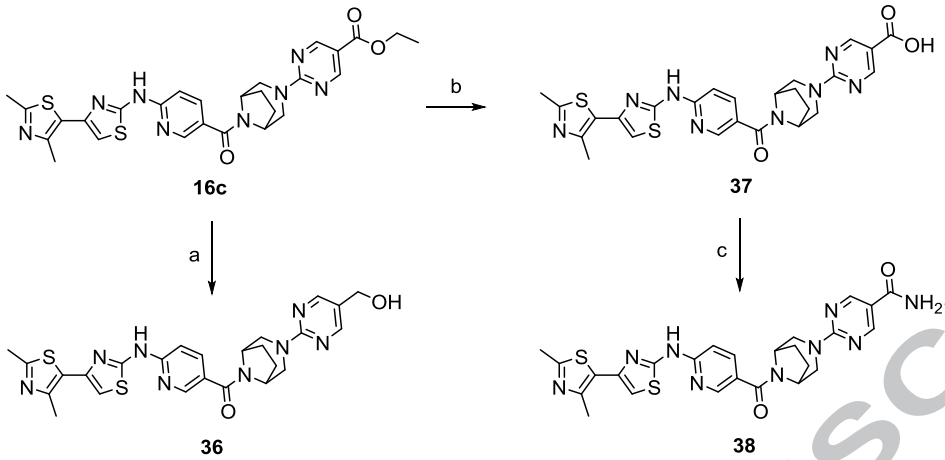
17, **25a**: $R_1 = R_2 = R_3 = R_4 = H$
17, **25b**: R_1 = (S) CH_3, $R_2 = R_3 = R_4 = H$
17, **25c**: R_1 = (R) CH_3, $R_2 = R_3 = R_4 = H$
17, **25d**: $R_1 = CF_3$, $R_2 = R_3 = R_4 = H$
17, **25e**: $R_1 = CH_2OH$, $R_2 = R_3 = R_4 = H$
17, **25f**: $R_1 = R_2 = H$, R_3 = (R) CH_3, $R_4 = H$
17, **25g**: $R_1 = R_2 = H$, R_3 = (S) CH_3, $R_4 = H$

17, **25h**: $R_1 = R_2 = H$, $R_3 = R_4$= 

17, **25i**: $R_1 = R_2$ =  , $R_3 = R_4 = H$

17, **25j**: $R_1 = R_2$ =  , $R_3 = R_4 = H$

Scheme 6. Synthesis of the compound **26a-i** and **27-35**. Reagents and conditions: (a) 4N HCl in 1,4-dioxane, 0°C~r.t., 2h; (b) 2-chloro-pyrimidine or 3-chloro-pyridazine or 2-chloro-pyridine, K_2CO_3, NMP, 90°C, 3h.



Scheme 7. Synthesis of the compound **36-38.** Reagents and conditions: (a) $LiBH_4$, THF, MeOH, r.t., 2.5h; (b) 2N NaOH, THF, EtOH, r.t.~60°C, 4h; (c) COMU, NH_4Cl, Et_3N, DMF, r.t., 24h.

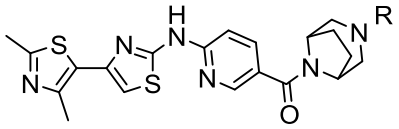
2.2. In vitro pharmacology. The synthesized TRPV4 ligands were evaluated in vitro for antagonism as measured by inhibition of activation by 4αPDD or osmotic stimuli. The assays were conducted using a fluorometric imaging plate reader (FLIPR) with human, guinea pig or rat TRPV4 stably expressed in recombinant Chinese hamster ovary (CHO) K1 cells. The results were summarized in Table 1-4 as following, which included some previously reported antagonist values for comparing the results. Table 1 showed human TRPV4 antagonist potencies against 4αPDD as TRPV4 selective agonist and human hypotonicity IC_{50} values as using osmotic stimuli. The human TRPV4 antagonist potency against 4αPDD of compound **27** resulted in nearly 2-fold improvement compared with compound **16a** and 3,5-pyrimidine analog **16b** resulted in nearly 3-fold loss of potency compared with **27**, which indicated the improvement of the potency to add the nitrogen at 2-position on phenyl ring. The pyridine **27**, pyridazine **28** and pyrazine **29** showed almost same TRPV4 antagonist potency against 4αPDD, which indicated no influence of the improvement of the potency based on the nitrogen position of **27**. Additional 6-position nitrogen substituted on 2-pyridine ring as in the case of **16d** resulted in a significant improvement of human TRPV4 antagonist potency against 4αPDD. Moreover, the hypotonic potency showed almost same trend of 4αPDD potency except for **29**. Most potent analog **16d** against 4αPDD showed best TRPV4

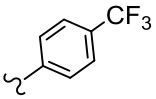
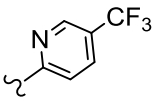
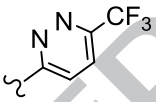
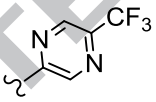
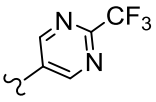
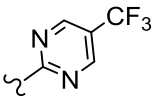
hypotonic potency and **16b** which indicated weaker potency against 4αPDD than **16d** showed slightly loss in hypotonic potency. There are some exceptions in the correlation between 4αPDD and hypotonicity IC_{50} values, but it could be predicted by the result of the 4αPDD as using the activation in human TRPV4 antagonist potency. The influence of the substitution at 4-position on 2,6-pyrimidine ring was given in Table 2. The compounds which have halogen, methanesulfonyl, cyano and trifluoromethyl group showed more potent for human TRPV4 and resulted in a slightly improvement of non-substituted analog **30**. The alcohol analog **36** retained good potency, but amide analog **38** resulted in 8-fold loss in potency. Furthermore, the carboxylic acid analog **37** showed significant loss in potency. These result suggested that the region of near the 4-position is not to be tolerated in improvement of the human TRPV4 potency. Next, the SAR and result of human, guinea pig and rat of the piperazine ring was shown in Table 3. The (S)-methyl isomer at R_1 position **26b** resulted in 10-fold improvement in human TRPV4 potency compared with non-substituted piperazine analog **26a**. On the other hand, the (R)-methyl isomer at R_1 position **26c** showed slightly improvement compared with **26a**, which suggested that the potency could be depended on the conformation on this region. The compound **26d** having enantio-mixture trifluoromethyl group at R_1 position resulted in nearly 20-fold improvement in human TRPV4 potency. Comparison with the

result of the **26b** suggested that it could be important for improvement of the TRPV4 potency to increase the lipophilicity. There is a support information that the compound **26e** including alcohol, hydrophilic group at R_1 position showed 10-fold loss in human TRPV4 potency. The regio-substitution of methyl group at R_3 position showed slightly differences their potencies, as in **26f** and **26g**. The compounds including bridge structure at R_1 and R_2 showed more potent than compound **26h** including bridge structure at R_3 and R_4 position, which indicated that the steric hindrance near the R_1 and R_2 region could be important to improve the human TRPV4 potency. There is no support data which influence is important or not, but these data suggested that it was very important for keeping the good potency to maintain high lipophilicity and/or steric hindrance at R_1 and R_2 region. The guinea pig TRPV4 antagonist potencies showed almost same result with human, which indicated that there are about 1-4 fold species differences between them. On the other hand, there are big species differences between human and rat, which indicated the chemo type dependence in Table 3. Fortunately, more human TRPV4 potent compound **26d**, **26i** and **16d** showed single nano-molar IC_{50} values for rat TRPV4 in spite of about 20-30 fold species differences. The left part SAR was shown in Table 4. The 1,3-dimethyl-5-substituted pyrazole analog **18** and the compound including alcohol group at 2-position at thiazole ring, as in **19**, showed 6-fold loss in

potency compared with **16d**. The potency of 1,3-dimethyl-4-substituted pyrazole analog **20** showed as same TRPV4 IC_{50} value as **16d**. On the other hand, 3-substituted-1,2,4-thiadiazol-5-amine analogs, as in **21** and **22**, resulted in a significant loss in TRPV4 potency compared with corresponding thiazole analogs **20** and **16d**, respectively. The degree of the species differences between human and rat showed almost same result in Table 3.

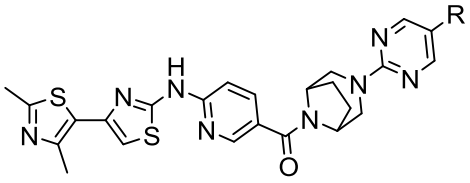
Table 1. Relationship between human 4αPDD and hypotonicity as agonist with trifluoromethyl benzene and hetero aromatic ring on the right part.



compd.	R	h_4αPDD[a,c] IC_{50} (nM)	h_hypotonicity[b,c] IC_{50} (nM)
16a		3.9	-
27		2.2	1.4
28		2.2	1.3
29		2.6	0.71
16b		6.3	23
16d		0.22	0.4

[a]Concentration of 4αPDD was 1000nM, determined as ED_{70} for human TRPV4. [b]Hypotonic solution(218 mOsm), equivalent to ED_{70} of 4αPDD. [c]The IC_{50} value of HC-067047 with using the 4αPDD and /or hypotonicity as the agonist was 57nM and /or 29nM for human TRPV4 as a control of the assay respectively.

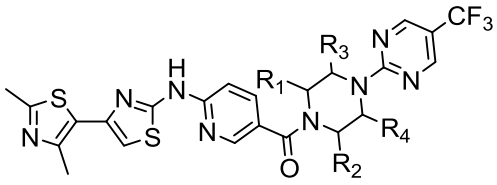
Table 2. SAR with human 4αPDD and microsomal stability of pyrimidine at 4-position

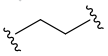
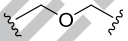
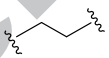


compd.	R	h_4αPDD[a] IC_{50} (nM)	h_microsomal stability (%)[b]	LogD[c]
30	H	0.5	8.5	3.30
31	F	0.31	38.4	3.51
32	Cl	0.3	76.4	3.97
33	Br	0.21	72.8	4.05
34	SO_2Me	0.39	52.7	2.83
35	CN	0.18	37.7	3.18
36	CH_2OH	0.65	61.4	2.70
37	COOH	170	86.2	1.46
38	$CONH_2$	4.2	51.8	2.31
16d	CF_3	0.22	94.8	4.25

[a]Concentration of 4αPDD was 1000nM, determined as ED_{70} for human TRPV4.
[b]percent of compound remaining after 30 min incubation with human liver microsomes (0.5 mg protein/mL) at 37°C with or without NADPH generating system. [c]LogD was calculated value by Pipeline pilot version 9.5.0.831.

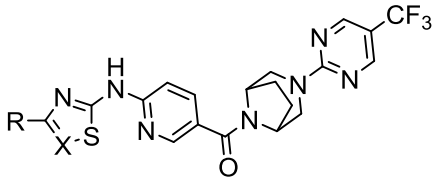
Table 3. SAR of the piperidine ring and each species TRPV4 antagonist potencies against 4αPDD as agonist.

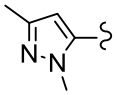
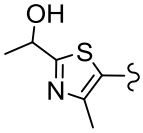
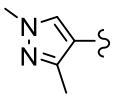
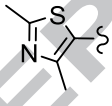


compd.	R_1, R_2	R_3, R_4	4αPDD[a, b]			h_microsomal stability (%)[c]	LogD[d]
			h_IC_{50} (nM)	g_IC_{50} (nM)	r_IC_{50} (nM)		
26a	H, H	H, H	6.8	-	-	77.5	3.71
26b	(S)Me, H	H, H	0.62	1.3	22	69.5	4.09
26c	(R)Me, H	H, H	2.4	2.1	62	76.3	4.09
26d	CF_3	H, H	0.29	1.1	8.2	88.4	4.75
26e	CH_2OH, H	H, H	64	-	-	82.1	3.20
26f	H, H	(R)Me, H	0.99	-	-	75.1	3.69
26g	H, H	(S)Me, H	2	1.8	47	64.1	3.69
26h	H, H		0.97	1.2	50	57.4	5.01
26i		H, H	0.19	0.73	4.4	79.8	3.47
16d		H, H	0.22	0.9	8.6	94.8	4.25

[a]Concentrations of 4αPDD were 1000nM, 250nM, 300nM for humans, guinea pigs, and rats, respectively, determined as ED_{70} for each TRPV4. [b]The IC_{50} value of HC-067047 was 180nM for rat TRPV4 as a control of the assay. [c]percent of compound remaining after 30 min incubation with human liver microsomes (0.5 mg protein/mL) at 37°C with or without NADPH generating system. [d]LogD was calculated value by Pipeline pilot version 9.5.0.831.

Table 4. SAR of the azole on the left part and each species TRPV4 antagonist potencies against 4αPDD as agonist.



compd.	R	X	4αPDD[a] h_IC$_{50}$ (nM)	4αPDD[a] g_IC$_{50}$ (nM)	4αPDD[a] r_IC$_{50}$ (nM)	h_microsomal stability (%)[b]	LogD[c]
18		C	1.3	1.3	39	99.7	4.16
19		C	1.8	1.4	38	79.9	4.06
20		C	0.87	0.93	-	89	3.87
21		N	6.2	4	180	96.7	3.43
22		N	3.7	2.6	-	85.2	3.80
16d		C	0.22	0.9	8.6	94.8	4.25

[a]Concentrations of 4αPDD were 1000nM, 250nM, 300nM for humans, guinea pigs, and rats, respectively, determined as ED$_{70}$ for each TRPV4. [b]percent of compound remaining after 30 min incubation with human liver microsomes (0.5 mg protein/mL) at 37°C with or without NADPH generating. [c]LogD was calculated value by Pipeline pilot version 9.5.0.831.

2.3. Human microsomal stability. According to our expectation of future oral drug, the human microsomal stability data and corresponding calculated LogD as the reference were put on the table 2-4. This chemo type showed good human microsomal stability in spite of high lipophilic derivatives, but it was depended on the 4-position substitutions on pyridine or substitutions on piperazine ring. In Table 2, non-substituted pyrimidine analog **30** showed poor microsomal stability due to the position to be easily metabolize at 4-position on pyrimidine ring, generally. The florine substituted at 4-position analog **31** showed better microsomal stability than **30**, which indicated the support data. The chlorine, bromine and trifluoromethyl substituted analogs **32**, **33** and **16d** showed more stable respectively. Contrary to our expectations, the methane sulfonyl, cyano and amide substituted analog, as in **34**, **35** and **38**, resulted in poor metabolic stability in spite of lower calculated LogD. The compounds including hydrophilic group at 4-position on pyrimidine showed poor or moderate microsomal stability. In Table 3, there seems to be no influence the substitutions at R_1 and R_2 on piperazine ring. On the other hand, the R_3 and R_4 substituted compound showed slightly decreased in human microsomal stability, as in **26h**, compared with corresponding analogs, as in **16d**, which indicated one possibility that higher LogD showed lower human microsomal stability. However, the compound including alcohol group at 2-

position at thiazole ring, as in **19**, decreased the human microsomal stability compared with **16d** in spite of lower lipophilicity than **16d**. As the result, microsomal stability of these derivatives did not depend on the lipophilicity as general theory.

2.4. In vivo pharmacology and selectivity for TRPV1. The analgesic effect of compound **16d** and **26i** was evaluated via subcutaneous administration in FCA induced mechanical hyperalgesia model in guinea pig and its effect was compared with ibuprofen as the reference compound in Table 5. The analgesic effect of compound **16d** has already been confirmed via oral administration and its result was reported in our previous communication[32]. In this paper, we put the analgesic effect of compound **16d** as the comparison data with **26i** via subcutaneous administration. Both compounds **16d** and **26i** showed dose-dependent reversal of mechanical hyperalgesia with 3mg/kg, 10mg/kg and 30mg/kg and its effect showed significant effect compared with ibuprofen 10mg/kg and 30mg/kg. Furthermore, the 3mg/kg dose of compound **26i** showed better efficacy than corresponding dose of **16d**, which might be possible to give a target efficacy with lower plasma exposure level than **16d**. In Table 6, the analgesic effect via subcutaneous administration in FCA induced mechanical hyperalgesia model in rat of compound **26i** was illustrated, which was plateaued in a dose of 10mg/kg and 100mg/kg due to the solubility issue or ceiling effect. However, improvement of rat potency has led us to confirm the analgesic effect in rat FCA induced mechanical hyperalgesia model in spite of species differences between human, guinea pig and rat. In Table 7, the selectivity data for human TRPV1 of compound **16d** and **26i** was indicated and its

potency was compared to those of reference BCTC as known TRPV1 selective antagonist. As the result, both compounds did not show the human TRPV1 antagonist potency.

Table 5. In vivo efficacy comparison data of ibuprofen, **16d** and **26i** in guinea pig FCA induced mechanical hyperalgesia model at 3h.

dose (s.c.)	analgesic effect (%reversal)		
	ibuprofen[a]	**16d**	**26i**
vehicle (DMAA+PG 1:1)	-2.7±10.1	-2.4±2.9	-1.7±1.2
3 mg/kg	-	4.0±2.4	21.4±4.8
10 mg/kg	10.1±5.4	20.5±2.7	29.0±2.6
30 mg/kg	13.6±4.1	30.9±2.6	34.7±3.6

[a]comparison.

Table 6. In vivo efficacy of **26i** in rat FCA induced mechanical hyperalgesia model.

dose (s.c.)	analgesic effect (%reversal)	
	1h	3h
vehicle (0.5%MC)	0±28.9	0±16.9
10 mg/kg	31.6±14.0	45.6±13.7
100 mg/kg	39.7±11.8	47.3±7.4

Table 7. Comarison data of **16d** and **26i** about hTRPV4 antagonist potency against 4αPDD as agonist and selectivity for hTRPV1 antagonist potency of activation by capsaicin.

compd.	h_TRPV4	h_TRPV1
	4αPDD IC_{50} (nM)	CAP IC_{50} (nM)[c]
16d	0.22	>10000
26i	0.19	>10000
BCTC[a]	n.t.[b]	1.83

[a]N-(4-tertiarybutylphenyl)-4-(3-cholorphyridin-2-yl)tetrahydropyrazine-1(2H)-carboxyamide as selective TRPV1 ligand. [b]no try. [c]The IC_{50} values of TRPV1 was determined against 100nM capsaicin in human TRPV1 CHO cells.

3. Conclusion

In summary, a novel series of (6-aminopyridin-3-yl)(4-(pyridin-2-yl)piperazin-1-yl) methanone derivatives were synthesized and evaluated as TRPV4 antagonists. Optimization of these substitutions led to improvement of TRPV4 antagonist potency in several species such as human, guinea pig and rat, which still remain some species differences between human and rat. The SAR analysis indicated that 2,6-pyrimidine at the nitrogen atom on bicyclic piperazine in right part based on the 2,4-dimethyl thiazole resulted in a significant potency. Among these derivatives, compound **26i** showed excellent metabolic stability and TRPV4 antagonism (human_IC_{50}=0.19nM, guinea pig_IC_{50}=0.73nM, rat_IC_{50}=4.4nM) as using selective TRPV4 agonist 4αPDD and indicated dose-dependent analgesic effect in FCA induced mechanical hyperalgesia model in guinea pig and rat. Despite the excellent in vivo efficacy of compound **26i**, there are still some studies, such as non-rodent PK profiles and safety pharmacology, to proceed clinical phase. In future, we believe that compound **26i** or other related compounds could be a potential utility in the management of pain.

4. Experimental section

4.1. General chemistry. All reagents and solvents were commercially available and

used without further purification. All flash chromatographic separations were performed on an automated purification system using Yamazen prepacked silica gel columns. ^{1}H NMR spectra were recorded on Bruker 400 MHz. Chemical shifts are reported in parts per million σ (ppm) and tetramethylsilane was used as internal standard for spectra obtained in DMSO-d_6 or $CDCl_3$. All J values were given in Hz. Splitting pattern designed as follows; s = singlet, d = doublet, dd = double doublets, dt = double triplet, t = triplet, q = quartet, m = multiplet, br = broad peak. Analytical LC/MS was performed on Shim-pack XR-ODS (2.2μM, 50mm x 3.0mm, a linear gradient from 10% to 100% B over 3 min and then 100% B for 0.5min (A = H_2O + 0.1% formic acid, B = MeCN + 0.1% formic acid), flow rate 3.0 mL/min) using a Shimadzu system. High resolution mass spectra (HRMS) were recorded on a Thermo Fisher Scientific LTQ Orbitrap using electrospray positive ionization.

4.1.1. 2',4'-Dimethyl-[4,5'-bithiazol]-2-amine (2a). To a solution of compound **1** (40g, 258mmol) in THF (480mL) was added Et_3N (39.3mL, 283mmol) and TMSOTf (51.3mL, 283mmol) dropwise at 0°C over 1h. The mixture was stirred at same temperature for 1h. To a reaction mixture was added NBS (50.5g, 283mmol) at 0°C over 10min. The mixture was stirred at same temperature for 20min. After quenching with water, the resulting mixture was extracted with EtOAc. The combined organic

extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. To a solution of crude material in EtOH (600mL) was added thiourea (19.61g, 258mmol) at room temperature. The mixture was refluxed for 1h. After cooling to room temperature, EtOH was concentrated until about half volume. To a resulting mixture was added sat. $NaHCO_3$ solution (500mL) and H_2O (300mL) at room temperature. The mixture was stirred at same temperature for 30min. The resulting solid was filtered and rinsed with H_2O. The residue was purified by crystallization (EtOAc/hexane = 1/5) to afford **2** (49.9g, 91.7%) as a pale brown solid. ^{1}H-NMR (DMSO-d_6) δ 2.45 (s, 3H), 2.55 (s, 3H), 6.63 (s, 1H), 7.15 (s, 2H). MS-ESI (*m/z*) = 212 $[M+H]^+$.

4.1.2. 4-(1,3-Dimethyl-1H-pyrazol-5-yl)thiazol-2-amine (2b). The title compound was prepared in a similar method to **2a** in 84.0% yield (376mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 2.12 (s, 3H), 3.92 (s, 3H), 6.22 (s, 1H), 6.80 (s, 1H), 7.12 (s, 2H). MS-ESI (*m/z*) = 195 $[M+H]^+$.

4.1.3. 1-(2-Amino-4'-methyl-[4,5'-bithiazol]-2'-yl)ethan-1-ol (2c). The title compound was prepared in a similar method to **2a** in 58.3% yield (4070mg). Yellow solid. ^{1}H-NMR ($CDCl_3$) δ 1.67 (d, *J* = 6.5 Hz, 3H), 2.13 (s, 3H), 2.58 (s, 3H), 5.00 (s,

2H), 6.08 (q, *J* = 6.6 Hz, 1H), 6.54 (s, 1H). MS-ESI (*m/z*) = 284 $[M+H]^+$.

4.1.4. 4-(1,3-Dimethyl-1H-pyrazol-4-yl)thiazol-2-amine (2d). The title compound was prepared in a similar method to **2a** in 32.9% yield (231mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 2.29 (s, 3H), 3.74 (s, 3H), 6.37 (s, 1H), 6.93 (s, 2H), 7.73 (s, 1H). MS-ESI (*m/z*) = 195 $[M+H]^+$.

4.1.5. Ethyl 6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinate (3a). A mixture of **2a** (5000mg, 23.66mmol), Ethyl-6-chloronicotinate (4392mg, 23.66mmol), $Pd(dba)_2$ (1361mg, 2.366mmol), xantphos (2054mg, 3.55mmol) and Na_2CO_3 (3010mg, 28.4mmol) in 1,4-dioxane (50ml) was refluxed for 16h. After cooling to room temperature, the reaction mixture was diluted with water. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by crystalization (EtOAc/diethyl ether = 1:1) to afford **3a** (4010mg, 47.0%) as a pale brown solid. ^{1}H-NMR (DMSO-d_6) δ 1.33 (t, *J* = 7.1 Hz, 3H), 2.51 (d, *J* = 9.7 Hz, 6H), 2.60 (s, 3H), 4.31 (q, *J* = 7.1 Hz, 2H), 7.17 (t, *J* = 11.7 Hz, 2H), 8.16 (dd, *J* = 8.7, 2.0 Hz, 1H), 8.86 (d, *J* = 2.0 Hz, 1H), 11.98 (s, 1H). MS-ESI (*m/z*) = 361 $[M+H]^+$.

4.1.6. Ethyl 6-((4-(1,3-dimethyl-1H-pyrazol-5-yl)thiazol-2-yl)amino)nicotinate

(3b). The title compound was prepared in a similar method to **3a** in 98.0% yield (8660mg). Pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ 1.40 (t, *J* = 7.2 Hz, 3H), 2.28 (s, 3H), 4.01 (s, 3H), 4.39 (q, *J* = 7.2 Hz, 2H), 6.30 (s, 1H), 6.61 (d, *J* = 8.7 Hz, 1H), 6.97 (s, 1H), 8.11 (dd, *J* = 8.7, 2.3 Hz, 1H), 9.02 (d, *J* = 2.0 Hz, 1H), 9.66 (s, 1H). MS-ESI (*m/z*) = 344 $[M+H]^+$.

4.1.7. Ethyl 6-((2'-(1-acetoxyethyl)-4'-methyl-[4,5'-bithiazol]-2-yl)amino)nicotinate (3c). The title compound was prepared in a similar method to **3a** in 74.7% yield (1140mg). Pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ 1.41 (t, *J* = 7.2 Hz, 3H), 1.68 (d, *J* = 6.5 Hz, 3H), 2.12 (d, *J* = 8.9 Hz, 3H), 2.63 (s, 3H), 4.41 (td, *J* = 13.7, 7.3 Hz, 2H), 6.09 (q, *J* = 6.6 Hz, 1H), 6.77 (d, *J* = 8.7 Hz, 1H), 6.91 (s, 1H), 8.19 (d, *J* = 8.7 Hz, 1H), 8.83 (s, 1H), 9.04 (s, 1H). MS-ESI (*m/z*) = 433 $[M+H]^+$.

4.1.8. Ethyl 6-((4-(1,3-dimethyl-1H-pyrazol-4-yl)thiazol-2-yl)amino)nicotinate (3d). The title compound was prepared in a similar method to **3a** in 98.4% yield (1740mg). Orange solid. ^{1}H-NMR ($CDCl_3$) δ 1.40 (t, *J* = 7.2 Hz, 3H), 2.48 (s, 3H), 3.83 (s, 3H), 4.38 (q, *J* = 7.1 Hz, 2H), 6.74 (d, *J* = 7.5 Hz, 2H), 7.65 (s, 1H), 8.13 (dd, *J* = 8.5, 2.0 Hz, 1H), 9.02 (s, 1H), 9.25 (s, 1H). MS-ESI (*m/z*) = 344 $[M+H]^+$.

4.1.9. 6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinic acid (4a). To a

solution of **3a** (4010 mg, 11.12 mmol) in ethanol (80 ml) was added 2N NaOH (16.69 ml, 33.4 mmol) solution at room temperature. The mixture was stirred at 80°C for 5h. After concentration, the residue was neutralized with 2N HCl solution at 0°C to afford the white precipitation. The resulting solid was collected by filtration to afford **4a** (3600mg, 97.4%) as the colorless solid. ^{1}H-NMR (DMSO-d_6) δ 2.53 (s, 3H), 2.60 (s, 3H), 7.10 (d, *J* = 8.7 Hz, 1H), 8.14 (dd, *J* = 8.7, 2.1 Hz, 1H), 8.81 (d, *J* = 1.9 Hz, 1H), 11.84 (s, 1H). MS-ESI (*m/z*) = 333 $[M+H]^+$.

4.1.10. 6-((4-(1,3-Dimethyl-1H-pyrazol-5-yl)thiazol-2-yl)amino)nicotinic acid (4b). The title compound was prepared in a similar method to **4a** in 99.8% yield (7940mg). Pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ 2.17 (s, 3H), 4.01 (s, 1H), 6.37 (s, 1H), 7.16 (d, *J* = 8.5 Hz, 1H), 7.37 (s, 1H), 8.16 (d, *J* = 8.5 Hz, 1H), 8.84 (s, 1H). MS-ESI (*m/z*) = 316 $[M+H]^+$.

4.1.11. 6-((2'-(1-Hydroxyethyl)-4'-methyl-[4,5'-bithiazol]-2-yl)amino)nicotinic acid (4c). The title compound was prepared in a similar method to **4a** in 100.0% yield (955mg). Pale yellow solid. ^{1}H-NMR (DMSO-d_6) δ 1.45 (d, *J* = 6.5 Hz, 3H), 2.53 (s, 3H), 4.89 (q, *J* = 6.5 Hz, 1H), 7.14 (d, *J* = 8.7 Hz, 1H), 7.19 (s, 1H), 8.14 (dd, *J* = 8.8, 2.3 Hz, 1H), 8.83 (d, *J* = 2.0 Hz, 1H), 11.87 (s, 1H). MS-ESI (*m/z*) = 363 $[M+H]^+$.

4.1.12. 6-((4-(1,3-Dimethyl-1H-pyrazol-4-yl)thiazol-2-yl)amino)nicotinic acid (4d).

The title compound was prepared in a similar method to **4a** in 98.5% yield (1600mg). Pale brown solid. ^{1}H-NMR (DMSO-d_6) δ 2.37 (s, 3H), 3.79 (s, 3H), 6.92 (s, 1H), 7.16 (d, J = 8.8 Hz, 1H), 7.84 (s, 1H), 8.13 (dd, J = 8.5, 2.3 Hz, 1H), 8.82 (d, J = 2.0 Hz, 1H), 11.74 (s, 1H). MS-ESI (m/z) = 316 $[M+H]^+$.

4.1.13. 3-Bromo-1,2,4-thiadiazol-5-amine (6). To a solution of **5** (5000mg, 25.1mmol) in ethanol (15ml) was added 28% NH_3 solution (3.39ml, 50.1mmol) at room temperature. The mixture was stirred at 70°C for 3h. After cooling to room temperature, sat.$NaHCO_3$ solution was poured into the residue to afford the precipitation. The resulting solid was collected by filtration and washed with water to afford **6** (3.08g, 68.0%) as the white solid. ^{1}H-NMR (DMSO-d_6) δ 8.38 (2H, s). MS-ESI (m/z) = 180 $[M+H]^+$.

4.1.14. tert-Butyl (3-bromo-1,2,4-thiadiazol-5-yl)carbamate (7). To a solution of **6** (3080mg, 17.11mmol) in THF (60ml) was added DMAP (105mg, 0.86mmol) and $(Boc)_2O$ (4480, 20.53mmol) at room temperature. The mixture was stirred at 50°C for 1h. After concentration, the residue was purified by flash column chromatography (EtOAc/hexane=1/5) to afford **7** (3955mg, 82.5%) as the white solid. ^{1}H-NMR ($CDCl_3$)

δ 1.62 (s, 9H), 9.10 (br, 1H). MS-ESI (*m/z*) = 280 $[M+H]^+$.

4.1.15. tert-Butyl (3-(1,3-dimethyl-1H-pyrazol-4-yl)-1,2,4-thiadiazol-5-yl)carbamate (8a). To a solution of **7** (2000mg, 7.14mmol) in 1,4-dioxane (20ml) and H_2O (2ml) was added 1,3-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (1903mg, 8.57 mmol), CsF (2169mg, 14.28mmol) and A-TAPHOS (506mg, 0.714mmol) at room temperature. The mixture was stirred at 90°C overnight. After cooling to room temperature, the reaction mixture was diluted with water. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (silica gel: EtOAc/hexane=1/5) to afford **8a** (1170mg, 55.5%) as the pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 1.53 (s, 9H), 2.58 (s, 3H), 3.87 (s, 3H), 7.85 (s, 1H), 8.82 (s, 1H). MS-ESI (*m/z*) = 296 $[M+H]^+$.

4.1.16. tert-Butyl (3-(2,4-dimethylthiazol-5-yl)-1,2,4-thiadiazol-5-yl)carbamate (8b). The title compound was prepared in a similar method to **8a** in 100.0% yield (1923mg). Orange oil. ^{1}H-NMR ($CDCl_3$) δ: 1.54 (s, 9H), 2.68 (s, 3H), 2.77 (s, 3H), 8.84 (br, 1H). MS-ESI (*m/z*) = 313 $[M+H]^+$.

4.1.17. 3-(1,3-Dimethyl-1H-pyrazol-4-yl)-1,2,4-thiadiazol-5-amine (9a). The mixture

of **8a** (1258mg, 4.26mmol) and 4N HCl in EtOAc solution (10ml) was stirred at room temperature overnight. After concentration, sat.$NaHCO_3$ solution was poured into the residue to afford the white precipitation. The resulting solid was collected by filtration to afford **9a** (741mg, 89.0%) as the white solid. ^{1}H-NMR (DMSO-d_6) δ 2.40 (s, 3H), 3.78 (s, 3H), 7.85 (s, 2H), 7.95 (s, 1H). MS-ESI (*m/z*) = 196 $[M+H]^+$.

4.1.18. 3-(2,4-Dimethylthiazol-5-yl)-1,2,4-thiadiazol-5-amine (9b). The title compound was prepared in a similar method to **9a** in 75.8% yield (476mg). white solid. ^{1} H-NMR (DMSO-d_6) δ 2.59 (3H, s), 2.65 (3H, s), 8.08 (2H, s). MS-ESI (*m/z*) = 213 $[M+H]^+$.

4.1.19. 6-((3-(1,3-Dimethyl-1H-pyrazol-4-yl)-1,2,4-thiadiazol-5-yl)amino)nicotinic acid (11a). A mixture of **9a** (390mg, 2mmol), ethyl-6-chloronicotinate (389mg, 2.1mmol), $Pd_2(dba)_3$ (55mg, 0.6mmol), xantphos (69.3mg, 0.12mmol) and K_3PO_4 (636mg, 3.0mmol) in 1,4-dioxane (0.5ml) was heated at 130°C for 2h under microwave irradiation. After cooling to room temperature, the reaction mixture was diluted with water. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by crystalization with EtOAc to afford **10a**

(530mg, 77.0%) as a pale brown solid. MS-ESI (*m/z*) = 345 $[M+H]^+$. To a solution of **10a** (530 mg, 1.53 mmol) in ethanol (5 ml) was added 2N NaOH (1.53 ml, 3.06 mmol) solution at room temperature. The mixture was stirred at 70°C for 8h. After concentration, the residue was neutralized with 2N HCl solution at 0°C to afford the brown precipitation. The resulting solid was collected by filtration to afford **11a** (438mg, 90.5%) as the brown solid. ^{1}H-NMR (DMSO-d_6) δ 2.50 (s, 3H), 3.83 (s, 3H), 7.23 (d, *J* = 8.5 Hz, 1H), 8.26 (d, *J* = 8.5 Hz, 1H), 8.93 (s, 1H), 12.53 (s, 1H). MS-ESI (*m/z*) = 317 $[M+H]^+$.

4.1.20. 6-((3-(2,4-Dimethylthiazol-5-yl)-1,2,4-thiadiazol-5-yl)amino)nicotinic acid (11b). The title compound was prepared in a similar method to **11a** in 97.0% yield (389mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 2.63 (s, 3H), 2.74 (s, 3H), 7.23 (d, *J* = 8.7 Hz, 1H), 8.27 (dd, *J* = 8.7, 2.1 Hz, 1H), 8.93 (d, *J* = 2.0 Hz, 1H), 12.72 (s, 1H), 13.15 (br, 1H). MS-ESI (*m/z*) = 334 $[M+H]^+$.

4.1.21. tert-Butyl 3-(4-(trifluoromethyl)phenyl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (13a). A mixture of **12** (100mg, 0.471mmol), 1-bromo-4-(trifluoromethyl)benzene (117mg, 0.518mmol), $Pd(dba)_2$ (13.54mg, 0.024mmol), RuPhos (21.98, 0.024mmol) and NaO*t*-Pen (130mg, 1.178mmol) in 1,4-dioxane (1.0ml)

was heated at 100°C for 2h under microwave irradiation. After cooling to room temperature, the reaction mixture was diluted with water. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (silica gel: EtOAc/hexane=1/10) to afford **13a** (157mg, 94.0%) as the pale brown solid. ^{1}H-NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.81 (d, *J* = 7.0 Hz, 2H), 1.98 (t, *J* = 4.5 Hz, 2H), 3.06 (s, 2H), 3.47 (d, *J* = 11.3 Hz, 2H), 4.39 (s, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 7.47 (d, *J* = 8.3 Hz, 2H). MS-ESI (*m/z*) = 357 $[M+H]^+$.

4.1.22. tert-Butyl 3-(2-(trifluoromethyl)pyrimidin-5-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (13b). The title compound was prepared in a similar method to **13a** in 96.0% yield (162mg). White solid. ^{1}H-NMR ($CDCl_3$) δ 1.49 (s, 9H), 1.81 (d, *J* = 7.3 Hz, 2H), 2.06 (t, *J* = 4.3 Hz, 2H), 3.20 (s, 2H), 3.50 (d, *J* = 11.3 Hz, 2H), 4.46 (s, 2H), 8.34 (s, 2H). MS-ESI (*m/z*) = 359 $[M+H]^+$.

4.1.23. tert-Butyl 3-(5-(ethoxycarbonyl)pyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (13c). To a solution of **12** (1000mg, 4.71mmol) in NMP (9.4ml) was added ethyl 2-chloropyrimidine-5-carboxylate (967mg, 5.18mmol) and diisopropyl ethylamine (913mg, 7.07mmol) at room temperature. The

reaction mixture was stirred at 80°C for 5h. The reaction mixture was diluted with water to afford the white precipitation. The resulting solid was collected by filtration to afford **13c** (1627mg, 95.3%) as the white solid. ^{1}H-NMR ($CDCl_3$) δ 1.37 (t, *J* = 7.2 Hz, 3H), 1.49 (s, 9H), 1.65 (dd, *J* = 14.0, 6.6 Hz, 2H), 1.92 (t, *J* = 4.6 Hz, 2H), 3.21 (br, 2H), 4.34 (q, *J* = 7.2 Hz, 2H), 4.35 (br, 2H) 4.56 (d, *J* = 12.8 Hz, 2H), 8.84 (d, *J* = 5.8 Hz, 2H). MS-ESI (*m/z*) = 363 $[M+H]^+$.

4.1.24. tert-Butyl 3-(5-(trifluoromethyl)pyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (13d). The title compound was prepared in a similar method to **13c** in 84.8% yield (6800mg). White solid. ^{1}H-NMR ($CDCl_3$) δ 1.49 (s, 9H), 1.66 (d, *J* = 7.3 Hz, 2H), 1.92 (s, 2H), 3.19 (s, 2H), 4.33 (s, 2H), 4.49 (d, *J* = 13.1 Hz, 2H), 8.48 (s, 2H). MS-ESI (*m/z*) = 359 $[M+H]^+$.

4.1.25. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-(4-(trifluoromethyl)phenyl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (16a). The mixture of **13a** (151mg, 0.423mmol) and 4N HCl in 1,4-dioxane solution (1.38ml) was stirred at room temperature overnight. After concentration, the residue was crystalized with ether to afford the white precipitation. The resulting solid was collected by filtration to afford **14a** (121mg, 98.0%) as the white solid. MS-ESI (*m/z*) = 257 $[M+H]^+$.

To a solution of **4a** (116mg, 0.35mmol) in DMF (2.3ml) was added **14a** (120mg, 0.42mmol), COMU (225mg, 0.525mmol) and Et_3N (177mg, 1.75mmol) at room temperature. The reaction mixture was stirred at same temperature for 1.5h. The reaction mixture was diluted with sat.$NaHCO_3$ solution to afford the pale yellow precipitation. The resulting solid was collected by filtration to **16a** (86.5mg, 43.3%) as the pale yellow solid. ^{1}H-NMR (DMSO-d_6) δ ^{1}H-NMR (DMSO-d_6) δ 1.79 (d, *J* = 7.8 Hz, 2H), 1.94 (s, 2H), 2.53 (s, 3H), 2.60 (s, 3H), 3.06 (d, *J* = 11.5 Hz, 2H), 3.71 (d, *J* = 10.0 Hz, 2H), 4.22-5.00 (br, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 7.14 (d, *J* = 11.0 Hz, 2H), 7.51 (d, *J* = 8.5 Hz, 2H), 7.91 (d, *J* = 8.3 Hz, 1H), 8.52 (s, 1H), 11.80 (s, 1H). MS-ESI (*m/z*) = 571 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{26}F_3N_6OS_2$, 571.1556; found, 571.1552.

4.1.26. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-(2-(trifluoromethyl)pyrimidin-5-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (16b). The title compound was prepared in a similar method to **16a** in 87.3% yield (186mg). Pale yellow solid. ^{1}H-NMR (DMSO-d_6) δ 1.83 (d, *J* = 8.0 Hz, 2H), 1.97 (s, 2H), 2.53 (s, 3H), 2.60 (s, 3H), 3.22 (d, *J* = 11.3 Hz, 2H), 3.83 (d, *J* = 9.5 Hz, 2H), 4.50 (s, 2H), 7.15 (d, *J* = 9.3 Hz, 2H), 7.92 (d, *J* = 8.5 Hz, 1H), 8.55 (d, *J* = 7.5 Hz, 3H). MS-ESI (*m/z*) = 573 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{24}F_3N_8OS_2$,

573.1461; found, 573.1462.

4.1.27. Ethyl 2-(8-(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyrimidine-5-carboxylate (16c). The title compound was prepared in a similar method to **16a** in 99.8% yield (2488mg). Pale red solid. ^{1}H-NMR (DMSO-d_6) δ 1.29 (t, *J* = 7.1 Hz, 3H), 1.58 (d, *J* = 7.5 Hz, 2H), 1.88 (t, *J* = 4.3 Hz, 2H), 2.58 (s, 3H), 4.28 (q, *J* = 7.1 Hz, 2H), 4.59 (d, *J* = 12.4 Hz, 4H), 6.94 (s, 2H), 7.73 (s, 1H), 8.46 (s, 1H), 8.81 (s, 2H). MS-ESI (*m/z*) = 577 $[M+H]^+$.

4.1.28. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-(5-(trifluoromethyl)pyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (16d). The title compound was prepared in a similar method to **16a** in 73.1% yield (151mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.60 (d, *J* = 8.0 Hz, 2H), 1.90 (s, 2H), 2.52 (s, 3H), 2.59 (s, 3H), 3.28 (d, *J* = 12.8 Hz, 2H), 4.08-5.01 (m, 4H), 7.11-7.21 (m, 2H), 7.91 (d, *J* = 8.3Hz, 1H), 8.53 (s, 1H), 8.73 (s, 2H), 11.78 (s, 1H). MS-ESI (*m/z*) = 573 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{24}F_3N_8OS_2$, 573.1461; found, 573.1462.

4.1.29. (6-((4-(1,3-Dimethyl-1H-pyrazol-5-yl)thiazol-2-yl)amino)pyridin-3-yl)(3-(5-(trifluoromethyl)pyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (18).

The title compound was prepared in a similar method to **16a** in 88.9% yield (198mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.61 (d, J = 8.0 Hz, 2H), 1.91 (s, 2H), 2.16 (s, 3H), 3.29 (d, J = 12.8 Hz, 2H), 4.01 (s, 3H), 4.54 (d, J = 13.1 Hz, 4H), 6.35 (s, 1H), 7.17 (d, J = 8.8 Hz, 1H), 7.32 (s, 1H), 7.92 (d, J = 8.5 Hz, 1H), 8.54 (s, 1H), 8.74 (s, 2H), 11.75 (s, 1H). MS-ESI (*m/z*) = 556 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{25}F_3N_9OS$, 556.1849; found, 556.1849.

4.1.30. (6-((2'-(1-Hydroxyethyl)-4'-methyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-(5-(trifluoromethyl)pyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (19). The title compound was prepared in a similar method to **16a** in 77.6% yield (172mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.45 (d, J = 6.3 Hz, 3H), 1.61 (d, J = 7.5 Hz, 2H), 1.90 (s, 2H), 2.54 (s, 3H), 3.29 (d, J = 12.5 Hz, 2H), 4.54 (d, J = 12.0 Hz, 4H), 4.88 (t, J = 6.0 Hz, 1H), 6.05 (d, J = 4.8 Hz, 1H), 7.16 (d, J = 7.3 Hz, 2H), 7.92 (d, J = 8.8 Hz, 1H), 8.54 (s, 1H), 8.74 (s, 2H). MS-ESI (*m/z*) = 603 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{26}F_3N_8O_2S_2$, 603.1567; found, 603.1566.

4.1.31. (6-((4-(1,3-Dimethyl-1H-pyrazol-4-yl)thiazol-2-yl)amino)pyridin-3-yl)(3-(5-(trifluoromethyl)pyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (20). The title compound was prepared in a similar method to **16a** in 48.3% yield (153mg).

Pale red solid. ^{1}H-NMR (DMSO-d_6) δ 1.61 (d, J = 8.0 Hz, 2H), 1.91 (s, 2H), 2.35 (d, J = 17.6 Hz, 3H), 3.29 (d, J = 14.1 Hz, 2H), 3.79 (s, 3H), 4.54 (d, J = 12.8 Hz, 4H), 6.89 (s, 1H), 7.17 (d, J = 8.5 Hz, 1H), 7.84 (s, 1H), 7.90 (t, J = 4.1 Hz, 1H), 8.52 (s, 1H), 8.74 (s, 2H), 11.59 (s, 1H). MS-ESI (*m/z*) = 556 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{25}F_3N_9OS$, 556.1849; found, 556.1846.

4.1.32. (6-((3-(1,3-Dimethyl-1H-pyrazol-4-yl)-1,2,4-thiadiazol-5-yl)amino)pyridin-3-yl)(3-(5-(trifluoromethyl)pyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (21). The title compound was prepared in a similar method to **16a** in 72.0% yield (190mg). White solid. ^{1}H-NMR ($CDCl_3$) δ 1.80 (d, J = 8.0 Hz, 2H), 2.01-2.03 (m, 2H), 2.63 (s, 3H), 3.32 (s, 2H), 3.88 (s, 3H), 4.66 (d, J = 13.2 Hz, 4H), 6.91 (d, J = 8.4 Hz, 1H), 7.90-7.93 (m, 2H), 8.52 (s, 2H), 8.67 (s, 1H), 9.21 (s, 1H). MS-ESI (*m/z*) = 557 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{24}F_3N_{10}OS$, 557.1802; found, 557.1802.

4.1.33. (6-((3-(2,4-Dimethylthiazol-5-yl)-1,2,4-thiadiazol-5-yl)amino)pyridin-3-yl)(3-(5-(trifluoromethyl)pyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (22). The title compound was prepared in a similar method to **16a** in 88.8% yield (107mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.61 (d, J = 8.8 Hz, 2H),

1.92 (s, 2H), 2.63 (s, 3H), 2.75 (s, 3H), 3.28 (s, 2H), 4.51 (s, 4H), 7.24 (d, *J* = 8.8 Hz, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 8.65 (s, 1H), 8.74 (s, 2H), 12.63 (s, 1H). MS-ESI (*m/z*) = 574 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{23}F_3N_9OS_2$, 574.1414; found, 574.1409.

4.1.34. tert-Butyl 4-(6-chloronicotinoyl)-3-(trifluoromethyl)piperazine-1-carboxylate (24). To a solution of commercially available **23** (5050mg, 19.86mmol) in CH_2Cl_2 (50ml) and THF (50ml) was added diisopropyl ethylamine (3850mg, 29.8mmol) and 6-chloronicotinoyl chloride (3850mg, 21.85mmol) at 0°C. The reaction mixture was stirred at room temperature for 30min. The reaction mixture was diluted with water. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography (silica gel: EtOAc/hexane=2/3) to afford **24** (7260mg, 92.8%) as the pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ 1.47 (s, 9H), 1.58 (s, 1H), 2.77 (s, 1H), 3.20 (s, 1H), 3.54 (s, 1H), 4.04-4.21 (m, 1H), 4.57 (s, 1H), 5.28 (s, 1H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 8.47 (s, 1H).MS-ESI (*m/z*) = 394 $[M+H]^+$.

4.1.35. tert-Butyl 4-(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-

yl)amino)nicotinoyl)piperazine-1-carboxylate (17a). The title compound was prepared in a similar method described for the preparation of the compound **16a**, employing commercially available tert-butyl piperazine-1-carboxylate (520 mg, 2.79mmol) and **4a** (774mg, 2.33mmol) to afford **17a** in 92.5% yield (1079mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.42 (s, 9H), 2.53 (s, 3H), 2.60 (s, 3H), 3.40 (s, 4H), 3.51 (s, 4H), 7.12 (t, *J* = 7.2 Hz, 2H), 7.79 (d, *J* = 8.0 Hz, 1H), 8.40 (d, *J* = 1.5 Hz, 1H), 11.76 (s, 1H). MS-ESI (*m/z*) = 501 $[M+H]^+$.

4.1.36. tert-Butyl (S)-4-(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-3-methylpiperazine-1-carboxylate (17b). The title compound was prepared in a similar method described for the preparation of the compound **16a**, employing commercially available tert-butyl (S)-3-methylpiperazine-1-carboxylate (300 mg, 1.50mmol) and **4a** (498mg, 1.50mmol) to afford **17b** in 92.3% yield (712mg). Yellow solid. ^{1}H-NMR ($CDCl_3$) δ 1.28 (d, *J* = 6.7 Hz, 3H), 1.48 (s, 9H), 1.75 (s, 2H), 2.61 (s, 3H), 2.65 (s, 3H), 3.25 (d, *J* = 4.5 Hz, 2H), 3.69 (d, *J* = 4.4 Hz, 2H), 3.92-3.94 (m, 3H), 6.73 (d, *J* = 8.4 Hz, 1H), 6.85 (s, 1H), 7.63 (d, *J* = 7.7 Hz, 1H), 8.42 (s, 1H), 9.57 (s, 1H). MS-ESI (*m/z*) = 515 $[M+H]^+$.

4.1.37. tert-Butyl (R)-4-(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-3-

methylpiperazine-1-carboxylate (17c). The title compound was prepared in a similar method described for the preparation of the compound **16a**, employing commercially available tert-butyl (R)-3-methylpiperazine-1-carboxylate (300 mg, 1.50mmol) and **4a** (498mg, 1.50mmol) to afford **17c** in 82.9% yield (639mg). Yellow solid. ^{1}H-NMR ($CDCl_3$) δ 1.28 (d, *J* = 6.7 Hz, 3H), 1.48 (s, 9H), 2.61 (s, 3H), 2.66 (s, 3H), 2.78 (s, 1H), 2.96-3.11 (m, 3H), 3.92-3.95 (m, 3H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.84 (s, 1H), 7.29 (dd, *J* = 11.4, 5.1 Hz, 1H), 7.67 (dd, *J* = 17.9, 8.5 Hz, 1H), 8.43 (s, 1H), 8.63 (d, *J* = 4.4 Hz, 1H). MS-ESI (*m/z*) = 515 $[M+H]^+$.

4.1.38. tert-Butyl 4-(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-3-(trifluoromethyl)piperazine-1-carboxylate (17d). The title compound was prepared in a similar method described for the preparation of the compound **3a**, employing **24** (5001mg, 12.72mmol) and **2a** (2560mg, 12.12mmol) to afford **17d** in 77.4% yield (5330mg). Pale yellow solid. ^{1}H-NMR (DMSO-d_6) δ 2.53 (s, 3H), 2.60 (s, 3H), 2.92 (s, 1H), 3.73 (s, 1H), 3.95 (s, 1H), 4.33 (s, 1H), 5.32 (s, 1H), 7.15 (t, *J* = 7.5 Hz, 2H), 7.82 (d, *J* = 8.4 Hz, 1H), 8.44 (s, 1H), 11.83 (s, 1H). MS-ESI (*m/z*) = 569 $[M+H]^+$.

4.1.39. tert-Butyl 4-(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-3-(hydroxymethyl)piperazine-1-carboxylate (17e). The title compound was prepared in

a similar method described for the preparation of the compound **16a**, employing commercially available tert-butyl 3-(hydroxymethyl)piperazine-1-carboxylate (300mg, 1.39mmol) and **4a** (461mg, 1.39mmol) to afford **17e** in 77.5% yield (571mg). Brown oil. ^{1}H-NMR ($CDCl_3$) δ 1.48 (s, 9H), 2.61 (s, 3H), 2.66 (s, 3H), 2.83-2.86 (m, 3H), 3.01-3.11 (m, 1H), 3.95 (br, 1H), 4.23-4.26 (m, 2H), 6.79-6.85 (m, 2H), 7.30 (d, *J* = 6.7 Hz, 2H), 7.68 (t, *J* = 7.6 Hz, 1H), 8.63 (s, 2H). MS-ESI (*m/z*) = 531 $[M+H]^+$.

4.1.40. tert-Butyl (R)-4-(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-2-methylpiperazine-1-carboxylate (17f). The title compound was prepared in a similar method described for the preparation of the compound **16a**, employing commercially available tert-butyl (R)-2-methylpiperazine-1-carboxylate (300 mg, 1.50mmol) and **4a** (498mg, 1.50mmol) to afford **17f** in 45.4% yield (350mg). Pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ 1.16 (s, 2H), 1.48 (s, 9H), 2.61 (s, 3H), 2.66 (s, 3H), 3.11-3.17 (m, 2H), 3.24 (t, *J* = 4.3 Hz, 1H), 3.62-3.70 (m, 2H), 3.92 (d, *J* = 11.0 Hz, 1H), 4.33 (s, 1H), 6.80 (d, *J* = 8.5 Hz, 1H), 6.84 (s, 1H), 7.70 (d, *J* = 8.5 Hz, 1H), 8.46 (s, 1H), 8.91 (s, 1H). MS-ESI (*m/z*) = 515 $[M+H]^+$.

4.1.41. tert-Butyl (S)-4-(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-2-methylpiperazine-1-carboxylate (17g). The title compound was prepared in a similar

method described for the preparation of the compound **16a**, employing commercially available tert-butyl (S)-2-methylpiperazine-1-carboxylate (300 mg, 1.50mmol) and **4a** (498mg, 1.50mmol) to afford **17g** in 83.9% yield (647mg). Pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ 1.17 (s, 3H), 1.46 (s, 9H), 2.61 (s, 3H), 2.66 (s, 3H), 3.11 (br, 3H), 3.24 (t, *J* = 4.8 Hz, 1H), 3.69 (t, *J* = 4.8 Hz, 1H), 3.90 (s, 1H), 4.33 (s, 1H), 6.78 (d, *J* = 8.5 Hz, 1H), 6.84 (s, 1H), 7.29 (dd, *J* = 7.5, 5.8 Hz, 1H), 7.69 (t, *J* = 4.3 Hz, 1H), 9.10 (s, 1H). MS-ESI (*m/z*) = 515 $[M+H]^+$.

4.1.42. tert-Butyl 3-(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (17h). The title compound was prepared in a similar method described for the preparation of the compound **16a**, employing commercially available tert-butyl 3,8-diazabicyclo[3.2.1]octane-8-carboxylate (201mg, 0.948mmol) and **4a** (300mg, 0.903mmol) to afford **17h** in 51.8% yield (246mg). White solid. ^{1}H-NMR ($CDCl_3$) δ 1.49 (s, 9H), 1.82 (s, 1H), 1.95 (t, *J* = 7.4 Hz, 2H), 2.60 (s, 3H), 2.67 (s, 3H), 3.12-3.48 (m, 3H), 4.27 (br, 4H), 6.84 (t, *J* = 4.3 Hz, 2H), 7.68 (dd, *J* = 8.5, 2.3 Hz, 1H), 8.44 (d, *J* = 1.8 Hz, 1H), 8.95 (s, 1H). MS-ESI (*m/z*) = 527 $[M+H]^+$.

4.1.43. tert-Butyl 9-(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-3-oxa-7,9-diazabicyclo[3.3.1]nonane-7-carboxylate (17i). The title compound was

prepared in a similar method described for the preparation of the compound **16a**, employing commercially available tert-butyl 3-oxa-7,9-diazabicyclo[3.3.1]nonane-7-carboxylate (165mg, 0.722mmol) and **4a** (200mg, 0.602mmol) to afford **17i** in 100.0% yield (326mg). Pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ 1.48 (s, 9H), 2.61 (s, 3H), 2.67 (s, 3H), 3.17-3.36 (m, 2H), 3.93-4.34 (m, 8H), 6.80-6.85 (m, 2H), 7.74 (dd, *J* = 8.5, 2.0 Hz, 1H), 8.46 (t, *J* = 7.2 Hz, 1H), 8.84 (s, 1H). MS-ESI (*m/z*) = 543 $[M+H]^+$.

4.1.44. tert-Butyl 8-(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-3,8-diazabicyclo[3.2.1]octane-3-carboxylate (17j). The title compound was prepared in a similar method described for the preparation of the compound **16a**, employing commercially available tert-butyl 3,8-diazabicyclo[3.2.1]octane-3-carboxylate (2108mg, 9.93mmol) and **4a** (3000mg, 9.03mmol) to afford **17j** in 83.5% yield (3971mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.41 (s, 9H), 1.61 (t, *J* = 6.9 Hz, 2H), 1.86 (s, 2H), 2.53 (s, 3H), 2.60 (s, 3H), 3.01-3.15 (m, 2H), 3.78-3.81 (m, 2H), 4.24-4.59 (m, 2H), 7.13 (t, *J* = 9.2 Hz, 2H), 7.87 (d, *J* = 7.9 Hz, 1H), 7.95 (s, 1H), 8.48 (s, 1H). MS-ESI (*m/z*) = 527 $[M+H]^+$.

4.1.45. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(piperazin-1-yl)methanone (25a). To a solution of **17a** (130mg, 0.259mmol) in MeOH (0.6ml) was

added 4N HCl in 1,4-dioxane solution (0.65ml, 2.59mmol) at 0°C. The reaction mixture was stirred at room temperature for 2h. The solvent was concentrated to afford **25a** (113mg, 100.0%) as the white solid. ^{1}H-NMR (DMSO-d_6) δ 2.53 (s, 3H), 2.62 (s, 3H), 3.16 (s, 4H), 3.74 (s, 4H), 7.15 (t, *J* = 10.9 Hz, 2H), 7.84 (d, *J* = 8.5 Hz, 1H), 8.46 (s, 1H), 9.21 (s, 2H), 11.79 (s, 1H). MS-ESI (*m/z*) = 401 $[M+H]^+$.

4.1.46. (S)-(6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(2-methylpiperazin-1-yl)methanone (25b). The title compound was prepared in a similar method to **25a** in 100.0% yield (663mg). Pale yellow solid. ^{1}H-NMR (DMSO-d_6) δ 1.34 (d, *J* = 6.8 Hz, 3H), 2.54 (s, 3H), 2.65 (s, 3H), 3.06 (s, 2H), 3.18-3.23 (m, 3H), 4.01 (s, 1H), 4.50 (s, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 7.22 (s, 1H), 7.82 (d, *J* = 8.3 Hz, 1H), 8.43 (s, 1H), 9.05 (s, 1H), 9.53 (d, *J* = 10.3 Hz, 1H), 11.80 (s, 1H). MS-ESI (*m/z*) = 415 $[M+H]^+$.

4.1.47. (R)-(6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(2-methylpiperazin-1-yl)methanone (25c). The title compound was prepared in a similar method to **25a** in 100.0% yield (605mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.34 (d, *J* = 6.8 Hz, 3H), 2.53 (s, 3H), 2.61 (s, 3H), 3.04-3.07 (m, 2H), 3.19-3.22 (m, 3H), 4.02 (s, 1H), 4.54 (s, 1H), 7.15 (t, *J* = 8.7 Hz, 2H), 7.82 (d, *J* = 9.4 Hz, 1H), 8.44 (s, 1H), 8.79

(s, 1H), 9.29 (s, 1H), 11.79 (s, 1H). MS-ESI (*m/z*) = 415 $[M+H]^+$.

4.1.48. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(2-(trifluoromethyl)piperazin-1-yl)methanone (25d). The title compound was prepared in a similar method to **25a** in 76.6% yield (2210mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 2.43 (s, 1H), 2.53 (s, 3H), 2.60 (s, 3H), 2.88 (s, 2H), 3.26 (s, 2H), 3.57 (s, 1H), 5.14 (s, 1H), 7.14 (t, *J* = 6.8 Hz, 2H), 7.79 (d, *J* = 8.0 Hz, 1H), 8.40 (s, 1H), 11.81 (s, 1H). MS-ESI (*m/z*) = 469 $[M+H]^+$.

4.1.49. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(2-(hydroxymethyl)piperazin-1-yl)methanone (25e). The title compound was prepared in a similar method to **25a** in 100.0% yield (305mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.59 (s, 2H), 2.55 (s, 3H), 2.66 (s, 3H), 3.01-3.27 (m, 3H), 3.56 (s, 2H), 3.99-4.62 (m, 2H), 7.13 (d, *J* = 8.5 Hz, 1H), 7.22 (s, 1H), 7.84 (d, *J* = 8.5 Hz, 1H), 8.47 (s, 1H), 8.98 (s, 1H), 9.62 (d, *J* = 11.0 Hz, 1H), 11.80 (s, 1H). MS-ESI (*m/z*) = 431 $[M+H]^+$.

4.1.50. (R)-(6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-methylpiperazin-1-yl)methanone (25f). The title compound was prepared in a similar method to **25a** in 99.9% yield (298mg). Yellow solid. ^{1}H-NMR (DMSO-d_6) δ 1.24 (s, 3H), 2.54 (s, 3H), 2.66 (s, 3H), 3.06-3.07 (m, 2H), 3.39-3.42 (m, 3H), 4.10-4.13 (m,

2H), 7.13 (d, *J* = 8.8 Hz, 1H), 7.22 (s, 1H), 7.83 (dd, *J* = 8.7, 2.1 Hz, 1H), 8.45 (d, *J* = 1.8 Hz, 1H), 9.25 (s, 1H), 9.47 (s, 1H), 11.81 (s, 1H). MS-ESI (*m/z*) = 415 $[M+H]^+$.

4.1.51. (S)-(6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-methylpiperazin-1-yl)methanone (25g). The title compound was prepared in a similar method to **25a** in 99.9% yield (284mg). Yellow solid. ^{1}H-NMR (DMSO-d_6) δ 1.24 (s, 3H), 2.55 (s, 3H), 2.66 (s, 3H), 3.06-3.08 (m, 1H), 3.34-3.37 (m, 1H), 4.06-4.08 (m, 5H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.22 (s, 1H), 7.84 (d, *J* = 8.5 Hz, 1H), 8.31 (s, 1H), 8.46 (s, 1H), 9.26 (s, 1H), 9.48 (s, 1H), 11.82 (s, 1H). MS-ESI (*m/z*) = 415 $[M+H]^+$.

4.1.52. (3,8-Diazabicyclo[3.2.1]octan-3-yl)(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)methanone (25h). The title compound was prepared in a similar method to **25a** in 100.0% yield (211mg). White solid. ^{1}H-NMR ($CDCl_3$) δ 1.77 (s, 2H), 1.93 (s, 2H), 2.54 (s, 3H), 2.63 (s, 3H), 4.02 (s, 3H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.19 (s, 1H), 7.83 (dd, *J* = 8.8, 2.3 Hz, 1H), 8.44 (d, *J* = 1.8 Hz, 1H), 9.32 (t, *J* = 23.7 Hz, 2H). MS-ESI (*m/z*) = 427 $[M+H]^+$.

4.1.53. (3-Oxa-7,9-diazabicyclo[3.3.1]nonan-9-yl)(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)methanone (25i). The title compound was prepared in a similar method to **25a** in 99.3% yield (270mg). White solid. ^{1}H-NMR (DMSO-d_6) δ

2.59 (s, 3H), 3.38-3.50 (m, 7H), 4.01-4.05 (m, 3H), 4.54 (br, 1H), 7.13 (t, *J* = 7.4 Hz, 2H), 7.84 (d, *J* = 8.5 Hz, 1H), 8.44 (d, *J* = 25.8 Hz, 2H), 9.34 (s, 1H), 11.73 (s, 1H). MS-ESI (*m/z*) = 443 $[M+H]^+$.

4.1.54. (3,8-Diazabicyclo[3.2.1]octan-8-yl)(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)methanone (25j). The title compound was prepared in a similar method to **25a** in 100.0% yield (492mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 2.05-2.08 (m, 4H), 2.58 (br, 3H), 2.72 (s, 3H), 3.18-3.20 (m, 4H), 4.51-4.53 (br, 2H), 7.17 (d, *J* = 8.5 Hz, 1H), 7.29 (s, 1H), 7.93 (dd, *J* = 8.5, 2.3 Hz, 1H), 8.54 (d, *J* = 1.8 Hz, 1H), 9.46 (s, 1H), 9.92 (d, *J* = 10.8 Hz, 1H), 11.88 (s, 1H). MS-ESI (*m/z*) = 427 $[M+H]^+$.

4.1.55. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(4-(5-(trifluoromethyl)pyrimidin-2-yl)piperazin-1-yl)methanone (26a). To a solution of **25a** (200mg, 0.458mmol) in NMP (4ml) was added 2-chloro-5-(trifluoromethyl)pyrimidine (100ng, 0.549mmol) and K_2CO_3 (189mg, 1.374mmol) at room temperature. The reaction mixture was heated at 90°C for 3h. After cooling to room temperature, the reaction mixture was diluted with water. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was

purified by flash column chromatography (silica gel: $CHCl_3$/MeOH=95/5) to afford **26a** (136mg, 54.4%) as the pale yellow solid. ^{1}H-NMR (DMSO-d_6) δ 2.53 (s, 3H), 2.60 (s, 3H), 3.65 (s, 4H), 3.93 (s, 4H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.84 (d, *J* = 8.5 Hz, 1H), 8.45 (s, 1H), 8.75 (s, 2H), 11.77 (s, 1H). MS-ESI (*m/z*) = 547 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{23}H_{22}F_3N_8OS_2$, 547.1305; found, 547.1303.

4.1.56. (S)-(6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(2-methyl-4-(5-(trifluoromethyl)pyrimidin-2-yl)piperazin-1-yl)methanone (26b). The title compound was prepared in a similar method to **26a** in 66.3% yield (622mg). Brown solid. ^{1}H-NMR ($CDCl_3$) δ 1.28 (d, *J* = 6.8 Hz, 3H), 2.61 (s, 3H), 2.66 (s, 3H), 3.10 (t, *J* = 11.2 Hz, 1H), 3.28 (d, *J* = 11.9 Hz, 1H), 4.16 (s, 1H), 4.64 (s, 1H), 4.72 (d, *J* = 13.4 Hz, 2H), 4.84 (d, *J* = 12.5 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 6.85 (s, 1H), 7.69 (d, *J* = 8.5 Hz, 1H), 8.47 (s, 1H), 8.51 (s, 2H), 9.41 (s, 1H). MS-ESI (*m/z*) = 561 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{24}F_3N_8OS_2$, 561.1461; found, 561.1463.

4.1.57. (R)-(6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(2-methyl-4-(5-(trifluoromethyl)pyrimidin-2-yl)piperazin-1-yl)methanone (26c). The title compound was prepared in a similar method to **26a** in 61.2% yield (342mg). Brown solid. ^{1}H-NMR ($CDCl_3$) δ 1.28 (d, *J* = 6.8 Hz, 3H), 2.64 (d, *J* = 15.2 Hz, 3H), 3.09 (t, *J*

= 11.3 Hz, 1H), 3.28 (d, *J* = 12.2 Hz, 1H), 3.39 (t, *J* = 7.1 Hz, 2H), 4.15 (s, 1H), 4.64 (s, 1H), 4.72 (d, *J* = 13.3 Hz, 1H), 4.84 (d, *J* = 12.8 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 6.86 (s, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 8.47 (s, 1H), 8.51 (s, 2H), 9.64 (s, 1H). MS-ESI (*m/z*) = 561 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{24}F_3N_8OS_2$, 561.1461; found, 561.1466.

4.1.58. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(2-(trifluoromethyl)-4-(5-(trifluoromethyl)pyrimidin-2-yl)piperazin-1-yl)methanone (26d). The title compound was prepared in a similar method to **26a** in 95.3% yield (150mg). Pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ 2.62 (s, 3H), 2.67 (s, 3H), 3.04-3.07 (m, 1H), 3.40 (d, *J* = 10.8 Hz, 1H), 3.68 (s, 1H), 3.91 (s, 1H), 4.90 (s, 1H), 5.39-5.46 (m, 2H), 6.79 (d, *J* = 8.5 Hz, 1H), 6.87 (s, 1H), 7.73 (d, *J* = 8.5 Hz, 1H), 8.53 (d, *J* = 8.5 Hz, 3H), 9.24 (s, 1H). MS-ESI (*m/z*) = 615 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{21}F_6N_8OS_2$, 615.1178; found, 615.1180.

4.1.59. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(2-(hydroxymethyl)-4-(5-(trifluoromethyl)pyrimidin-2-yl)piperazin-1-yl)methanone (26e). The title compound was prepared in a similar method to **26a** in 37.8% yield (43mg). Pale yellow solid. ^{1}H-NMR (DMSO-d_6) δ 1.86-1.93 (m, 1H), 2.17 (t, *J* = 8.3

Hz, 1H), 2.52 (s, 3H), 2.59 (s, 3H), 3.13 (s, 2H), 3.51 (s, 1H), 4.72 (s, 2H), 4.93 (s, 1H), 7.13 (d, *J* = 9.5 Hz, 2H), 7.84 (d, *J* = 8.5 Hz, 1H), 8.46 (s, 1H), 8.73 (s, 2H), 11.74 (s, 1H). MS-ESI (*m/z*) = 577 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{24}F_3N_8O_2S_2$, 577.1410; found, 577.1414.

4.1.60. (R)-(6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-methyl-4-(5-(trifluoromethyl)pyrimidin-2-yl)piperazin-1-yl)methanone (26f). The title compound was prepared in a similar method to **26a** in 42.2% yield (136mg). Brown solid. ^{1}H-NMR ($CDCl_3$) δ 1.24 (s, 3H), 2.61 (s, 3H), 2.66 (s, 3H), 3.18 (s, 1H), 3.36-3.39 (m, 2H), 3.64-3.67 (m, 2H), 4.68 (d, *J* = 12.8 Hz, 1H), 5.05 (s, 1H), 6.79 (d, *J* = 8.4 Hz, 1H), 6.86 (s, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 8.51 (s, 1H), 8.52 (s, 2H), 9.23 (s, 1H). MS-ESI (*m/z*) = 561 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{24}F_3N_8OS_2$, 561.1461; found, 561.1464.

4.1.61. (S)-(6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-methyl-4-(5-(trifluoromethyl)pyrimidin-2-yl)piperazin-1-yl)methanone (26g). The title compound was prepared in a similar method to **26a** in 63.6% yield (183mg). Brown solid. ^{1}H-NMR ($CDCl_3$) δ 1.24 (s, 3H), 2.61 (s, 3H), 2.66 (s, 3H), 3.19 (s, 1H), 3.34-3.36 (m, 2H), 3.64-3.66 (m, 2H), 4.68 (d, *J* = 12.2 Hz, 1H), 5.05 (s, 1H), 6.76 (d, *J* = 8.5

Hz, 1H), 6.86 (s, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 8.51 (s, 1H), 8.52 (s, 2H), 9.38 (s, 1H). MS-ESI (*m/z*) = 561 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{24}F_3N_8OS_2$, 561.1466; found, 561.1466.

4.1.62. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(8-(5-(trifluoromethyl)pyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)methanone (26h). The title compound was prepared in a similar method to **26a** in 87.6% yield (130mg). Pale brown solid. ^{1}H-NMR ($CDCl_3$) δ 1.77-2.06 (m, 4H), 2.61 (s, 3H), 2.67 (s, 3H), 3.16-3.19 (br, 1H), 3.59-3.62 (br, 2H), 4.64-4.88 (br, 3H), 6.82 (t, *J* = 8.6 Hz, 2H), 7.68 (dd, *J* = 8.5, 2.1 Hz, 1H), 8.48 (t, *J* = 16.4 Hz, 3H), 8.90 (s, 1H). MS-ESI (*m/z*) = 573 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{24}F_3N_8OS_2$, 573.1461; found, 573.1461.

4.1.63. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(7-(5-(trifluoromethyl)pyrimidin-2-yl)-3-oxa-7,9-diazabicyclo[3.3.1]nonan-9-yl)methanone (26i). The title compound was prepared in a similar method to **26a** in 69.2% yield (68mg). White solid. ^{1}H-NMR ($CDCl_3$) δ 2.62 (s, 3H), 2.67 (s, 3H), 3.40-3.47 (m, 2H), 3.90-4.01 (m, 5H), 4.68 (s, 1H), 5.10-5.14 (m, 2H), 6.84 (t, *J* = 9.3 Hz, 2H), 7.77 (d, *J* = 8.5 Hz, 1H), 8.51 (d, *J* = 8.0 Hz, 3H), 9.14 (s, 1H). MS-ESI (*m/z*) =

589 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{24}F_3N_8O_2S_2$, 589.1410; found, 589.1410.

4.1.64. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-(5-(trifluoromethyl)pyridin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (27).

The title compound was prepared in a similar method described for the preparation of the compound **26a**, employing commercially available 2-fluoro-5-(trifluoromethyl)pyridine (40mg, 0.24mmol) and **25j** (80mg, 0.16mmol) to afford **27** in 44.8% yield (92mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.68 (d, *J* = 7.8 Hz, 2H), 1.91 (s, 2H), 2.53 (s, 3H), 2.60 (s, 3H), 3.19 (d, *J* = 12.3 Hz, 2H), 4.31-4.59 (m, 3H), 6.90 (d, *J* = 9.0 Hz, 1H), 7.15 (d, *J* = 9.3 Hz, 2H), 7.84 (d, *J* = 9.0 Hz, 1H), 7.92 (d, *J* = 8.5 Hz, 1H), 8.43 (s, 1H), 8.53 (s, 1H), 11.80 (s, 1H). MS-ESI (*m/z*) = 572 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{25}F_3N_7OS_2$, 572.1509; found, 572.1515.

4.1.65. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-(6-(trifluoromethyl)pyridazin-3-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (28).

The title compound was prepared in a similar method described for the preparation of the compound **26a**, employing commercially available 3-chloro-6-(trifluoromethyl)pyridazine (47mg, 0.26mmol) and **25j** (100mg, 0.23mmol) to afford **28**

in 44.8% yield (60mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.73 (d, J = 7.8 Hz, 2H), 1.95 (s, 2H), 2.53 (s, 3H), 2.60 (s, 3H), 3.29 (s, 2H), 4.27 (d, J = 11.5 Hz, 2H), 4.53-4.56 (m, 2H), 7.15 (d, J = 5.8 Hz, 2H), 7.35 (d, J = 9.8 Hz, 1H), 7.85 (d, J = 9.5 Hz, 1H), 7.93 (d, J = 8.5 Hz, 1H), 8.55 (s, 1H), 11.80 (s, 1H). MS-ESI (*m/z*) = 573 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{24}F_3N_8OS_2$, 573.1461; found, 573.1456.

4.1.66.

1.1.1. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-(5-(trifluoromethyl)pyrazin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (29).

The title compound was prepared in a similar method described for the preparation of the compound **26a**, employing commercially available 2-fluoro-5-(trifluoromethyl)pyrazine (40mg, 0.24mmol) and **25j** (80mg, 0.16mmol) to afford **29** in 46.9% yield (43mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.71 (d, J = 7.8 Hz, 2H), 1.94 (s, 2H), 2.53 (s, 3H), 2.60 (s, 3H), 3.29 (s, 2H), 4.24 (d, J = 11.8 Hz, 2H), 4.44-4.75 (m, 2H), 7.15 (d, J = 8.5 Hz, 2H), 7.92 (d, J = 8.8 Hz, 1H), 8.38 (s, 1H), 8.53 (d, J = 6.0 Hz, 2H), 11.81 (s, 1H). MS-ESI (*m/z*) = 573 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{24}F_3N_8OS_2$, 573.1461; found, 573.1460.

4.1.67. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-(pyrimidin-2-

yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (30). The title compound was prepared in a similar method described for the preparation of the compound **26a**, employing commercially available 2-chloropyrimidine (30mg, 0.26mmol) and **25j** (100mg, 0.23mmol) to afford **30** in 58.7% yield (70mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.62 (d, *J* = 7.3 Hz, 2H), 1.88 (s, 2H), 2.52 (s, 3H), 2.59 (s, 3H), 3.15 (d, *J* = 12.8 Hz, 2H), 4.49-4.64 (m, 4H), 6.68 (d, *J* = 4.3 Hz, 1H), 7.14 (d, *J* = 8.3 Hz, 2H), 7.90 (d, *J* = 9.0 Hz, 1H), 8.37 (d, *J* = 4.8 Hz, 2H), 8.51 (s, 1H), 11.76 (s, 1H). MS-ESI (*m/z*) = 505 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{25}N_8OS_2$, 505.1587; found, 505.1580.

4.1.68. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-(5-fluoropyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (31). The title compound was prepared in a similar method described for the preparation of the compound **26a**, employing commercially available 2-chloro-5-fluoropyrimidine (43mg, 0.32mmol) and **25j** (200mg, 0.40mmol) to afford **31** in 31.5% yield (66mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.63 (d, *J* = 7.3 Hz, 2H), 1.89 (s, 2H), 2.53 (s, 3H), 2.60 (s, 3H), 3.17 (d, *J* = 12.8 Hz, 2H), 4.32 (d, *J* = 9.6 Hz, 2H), 4.51-4.92 (br, 2H), 6.68 (d, *J* = 4.3 Hz, 1H), 7.14 (d, *J* = 8.3 Hz, 2H), 7.90 (d, *J* = 9.0 Hz, 1H), 8.49 (d, *J* = 4.8 Hz, 3H), 11.78 (s, 1H). MS-ESI (*m/z*) = 523 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for

$C_{24}H_{24}FN_8OS_2$, 523.1493; found, 523.1495.

4.1.69. (3-(5-Chloropyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)methanone (32). The title compound was prepared in a similar method described for the preparation of the compound **26a**, employing commercially available 2,5-dichloropyrimidine (51mg, 0.34mmol) and **25j** (150mg, 0.32mmol) to afford **32** in 67.6% yield (118mg). White solid. ^{1}H-NMR ($CDCl_3$) δ 1.80 (d, *J* = 7.9 Hz, 2H), 1.99-2.03 (m, 3H), 2.61 (s, 4H), 2.67 (s, 4H), 2.85 (d, *J* = 5.1 Hz, 1H), 3.24-3.40 (m, 2H), 4.47 (d, *J* = 12.8 Hz, 2H), 4.90-4.92 (m, 1H), 6.85 (t, *J* = 4.1 Hz, 2H), 7.84 (dd, *J* = 8.5, 2.0 Hz, 1H), 8.25 (s, 2H), 8.59 (d, *J* = 1.5 Hz, 1H), 8.74 (s, 1H).MS-ESI (*m/z*) = 539 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{24}ClN_8OS$, 539.1198; found, 539.1198.

4.1.70. (3-(5-Bromopyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)(6-((2',4'-dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)methanone (33). The title compound was prepared in a similar method described for the preparation of the compound **26a**, employing commercially available 5-bromo-2-chloropyrimidine (66mg, 0.34mmol) and **25j** (150mg, 0.32mmol) to afford **33** in 51.8% yield (98mg). White solid. ^{1}H-NMR ($CDCl_3$) δ 1.79 (d, *J* = 7.8 Hz, 2H), 1.98-1.99 (m, 2H), 2.61 (s, 3H), 2.66

(s, 3H), 3.23-3.26 (m, 2H), 4.46 (d, *J* = 12.8 Hz, 3H), 4.90-4.93 (m, 1H), 6.83 (t, *J* = 9.3 Hz, 2H), 7.82 (dd, *J* = 8.5, 2.0 Hz, 1H), 8.31 (s, 2H), 8.58 (d, *J* = 1.6 Hz, 1H), 9.00 (s, 1H). MS-ESI (*m/z*) = 583 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{24}H_{24}BrN_8OS_2$, 583.0692; found, 583.0696.

4.1.71. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-(5-(methylsulfonyl)pyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (34). The title compound was prepared in a similar method described for the preparation of the compound **26a**, employing commercially available 2-chloro-5-(methylsulfonyl)pyrimidine (92mg, 0.48mmol) and **25j** (200mg, 0.43mmol) to afford **34** in 77.9% yield (196mg). Pale brown solid. ^{1}H-NMR ($CDCl_3$) δ 1.77 (d, *J* = 8.0 Hz, 2H), 2.03-2.05 (m, 2H), 2.61 (s, 3H), 2.66 (s, 3H), 3.09 (s, 3H), 3.35-3.39 (m, 2H), 4.70-4.73 (m, 4H), 6.83 (t, *J* = 11.0 Hz, 2H), 7.82 (dd, *J* = 8.5, 2.1 Hz, 1H), 8.59 (d, *J* = 2.0 Hz, 1H), 8.73 (s, 2H), 9.20 (s, 1H). MS-ESI (*m/z*) = 583 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{27}N_8O_3S_3$, 583.1363; found, 583.1359.

4.1.72. 2-(8-(6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyrimidine-5-carbonitrile (35). The title compound was prepared in a similar method described for the preparation of the compound **26a**,

employing commercially available 2-chloropyrimidine-5-carbonitrile (67mg, 0.48mmol) and **25j** (300mg, 0.60mmol) to afford **35** in 67.9% yield (216mg). Pale yellow solid. ^{1}H-NMR (DMSO-d_6) δ 1.58 (d, J = 7.9 Hz, 2H), 1.91 (s, 2H), 2.53 (s, 3H), 2.60 (s, 3H), 3.29-3.32 (m, 5H), 4.53-4.56 (m, 4H), 7.15 (d, J = 9.5 Hz, 2H), 7.92 (d, J = 8.5 Hz, 1H), 8.54 (s, 1H), 8.80 (s, 2H), 11.80 (s, 1H). MS-ESI (m/z) = 530 $[M+H]^+$. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{25}H_{24}N_9OS_2$, 530.1540; found, 530.1543.

4.1.73. (6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)pyridin-3-yl)(3-(5-(hydroxymethyl)pyrimidin-2-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)methanone (36).

To a suspension of **16c** (150mg, 0.26mmol) in THF (3ml) and MeOH (0.3ml) was added $LiBH_4$ (17mg, 0.78mmol) at room temperature. The reaction mixture was stirred at same temperature for 3h. The reaction mixture was diluted with water. The aqueous layer was separated and then extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (silica gel: EtOAc/hexane=3/7) to afford **36** (26mg, 18.5%) as the white solid. ^{1}H-NMR (DMSO-d_6) δ 1.04 (d, J = 6.0 Hz, 1H), 1.61 (d, J = 7.8 Hz, 2H), 1.88 (s, 2H), 2.53 (s, 3H), 2.60 (s, 3H), 3.16 (d, J = 12.8 Hz, 2H), 4.34 (s, 2H), 4.44 (d, J = 12.0 Hz, 2H), 4.71-5.08 (m, 2H), 7.15 (d, J = 8.5 Hz, 2H), 7.91 (d, J = 8.3 Hz, 1H), 8.32 (s, 2H), 8.52 (s, 1H), 11.78 (s, 1H). MS-ESI (m/z) =

535 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{27}N_8O_2S_2$, 535.1693; found, 535.1693.

4.1.74. 2-(8-(6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyrimidine-5-carboxylic acid (37). To a suspension of **16c** (1500mg, 2.6mmol) in THF (15ml) and EtOH (15ml) was added 2N NaOH solution (2.86ml, 5.2mmol) at room temperature. The reaction mixture was stirred at 60°C for 4h. After cooling to room temperature, the reaction mixture was diluted with water. The aquious layer was neutralized with 2N HCl to afford the pale brown precipitation. The resulting solid was collected by filtration to afford **37** (904mg, 63.3%) as the white solid. ^{1}H-NMR (DMSO-d_6) δ 1.60 (d, *J* = 7.9 Hz, 2H), 1.91 (s, 2H), 2.53 (s, 3H), 2.60 (s, 3H), 3.27 (s, 2H), 4.41-4.53 (m, 4H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.92 (dd, *J* = 8.7, 2.1 Hz, 1H), 8.53 (d, *J* = 2.0 Hz, 1H), 8.79 (s, 2H), 11.79 (s, 1H), 12.87-12.90 (br, 1H). MS-ESI (*m/z*) = 549 $[M+H]^+$. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{25}N_8O_3S_2$, 549.1486; found, 549.1487.

4.1.75. 2-(8-(6-((2',4'-Dimethyl-[4,5'-bithiazol]-2-yl)amino)nicotinoyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyrimidine-5-carboxamide (38). The title compound was prepared in a similar method described for the preparation of the compound **16a**,

employing commercially available ammonium chloride (88mg, 1.64mmol) and **37** (150mg, 0.273mmol) to afford **38** in 22.8% yield (34mg). White solid. ^{1}H-NMR (DMSO-d_6) δ 1.60 (d, J = 7.8 Hz, 2H), 1.90 (d, J = 5.9 Hz, 2H), 2.53 (s, 3H), 2.60 (s, 3H), 3.25 (d, J = 11.9 Hz, 2H), 4.53-4.56 (m, 4H), 7.15 (d, J = 8.4 Hz, 2H), 7.31 (s, 1H), 7.87-7.93 (m, 2H), 8.53 (d, J = 1.9 Hz, 1H), 8.80 (s, 2H), 11.79 (s, 1H). MS-ESI (m/z) = 548 $[M+H]^+$. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{25}H_{26}N_9O_2S_2$, 548.1645; found, 548.1644.

4.2. TRPV4 fuctional assay. The TRPV4 functional assays were performed using CHO-K1 cells stably expressing human, guinea pig or rat TRPV4. The TRPV4-CHO cells were cultured in 384-well plates at densities of 4 x 10^3 cells/well in the culture medium (high glucose DMEM with GlutaMAX, 3%FBS, 25mM HEPES, Penicillin/Streptomycin) at 37°C, 5 % CO_2 overnight. The cells were washed with assay buffer (Hanks, 20 mmol/L HEPES, 2.5 mmol/L probenecid, pH7.4), and incubated with loading buffer（3 μmol/L Fluo 4-AM, 0.03 % Pluronic F-127 in assay buffer）for 1 h at 37°C, 5 % CO_2. After washing with assay buffer, the cells were incubated with the compounds for 5 minutes and stimulated with 4αPDD (final 1 μM) or hypotonic solution (final 218mOsm). The fluorescent intensity was measured with FLIPR TETRA system (Ex 470-495,Em 515-575 nm) for 5 minutes, and the IC_{50} was determined.

4.3. Human microsomal stability. The human hepatic microsomes were prepared by commercially available pooled human hepatic microsomes. The microsomal stability of test compounds in human liver microsomes was determined at one concentration (0.5μM). A reaction was performed at 37˚C for 0 minutes or 30 minutes in the presence of 1 mmol/L NADPH in 0.2 mL of a buffer (50 mmol/L Tris-HCl pH 7.4, 150 mmol/L potassium chloride, 10 mmol/L magnesium chloride) containing 0.5 mg protein/mL of human liver microsomes. Incubations were terminated by addition of 2-fold volume of organic solvent (MeCN/MeOH = 1:1). The test compound in the supernatant was quantified by LC/MS/MS, and a remaining amount of the test compound after the reaction was calculated, letting a compound amount at 0 minute reaction time to be 100%.

4.4. Freund's Complete Adjuvant (FCA) induced mechanical hyperalgesia test fot compound (26i)[33]. Male Sprague−Dawley rats (6 weeks, 180−280g) or male Hartley guinea pigs (4 weeks, 300-400g) were used. FCA induced mechanical hyperalgesia was measured by using an analgesymeter (Ugo Basile, Italy). The 50 % FCA solution (50 μL for rat, 40 μL for guinea pig) were injected into the left hind paw. Withdrawal latency was measured before FCA injection to obtain a naïve value (naïve). The next day after FCA injection, the withdrawal latency was measured before

(predose) or 3 h after the compound administration (postdose). The analgesic effects were indicated as the % of reversal which calculated as following formula; % of reversal = (postdose - predose)/(postdose – naïve) × 100. The 0.5 w/v% methylcellulose aqueous solution was used as vehicle. Statistical analysis was performed by Windows SAS program. The statistical tests (p value) were performed at a 2-sided 0.05 level.

Notes

The authors declare no competing financial interest.

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Graphical abstract