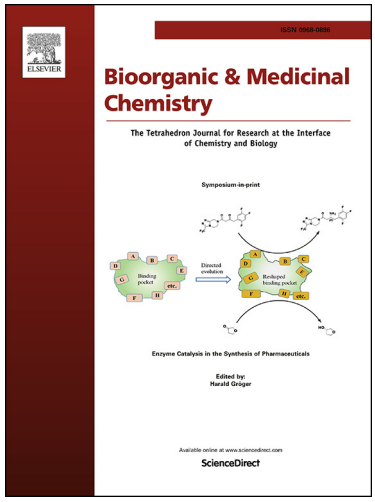
Accepted Manuscript



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PII: S0968-0896(19)30506-1
DOI: https://doi.org/10.1016/j.bmc.2019.05.041
Reference: BMC 14928

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 2 April 2019
Revised Date: 24 May 2019
Accepted Date: 27 May 2019

Please cite this article as: Sami, Y., Morita, M., Kubota, H., Hirabayashi, R., Seo, R., Nakagawa, N., Discovery of a novel orally active TRPV4 inhibitor: Part 1. Optimization from an HTS hit, *Bioorganic & Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.bmc.2019.05.041

Discovery of a novel orally active TRPV4 inhibitor: Part 1. Optimization from an HTS hit

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1. Introduction

Transient receptor potential vanilloid type 4 (TRPV4)[1] is a non-selective cation channel constitutively expressed across species and regulates a multitude of physiological mechanisms. Its first identified function dictated the early nomenclature, vanilloid receptor-related osmotically activated channel (VR-OAC)[2] or osmosensitive transient receptor potential channel 4 (OTRPC4)[2b]; When the osmolarity of the extracellular medium diverts from that of the endothelial cells on which TRPV4 channels are expressed, the cell volumes are controlled through the channel's trafficking of extracellular Ca^{2+}. Taken together with the fact that TRPV4 is expressed in organs such as the lung and the heart, evidences allow one to hypothesize that its malfunction results in pulmonary[3] and cardiovascular[3b] diseases. Indeed, it was shown by a Glaxo-Smith-Kline, Inc. group with compounds **1** and **2** (figure 1) that a TRPV4 inhibitor can improve surrogate parameters of pulmonary edema, in high pulmonary venous pressure (PVP) induced ex vivo rodent and canine models[4] or in a TRPV4 agonist-induced rodent in vivo model[4b], respectively. The results of a series of clinical studies involving yet another TRPV4 inhibitor GSK-2798745 for cardiac failure, congestive heart failure and cough will clarify the validity of this therapeutic approach in these areas.[5]

However, TRPV4 channels are now known to be expressed in a more diverse list of organs, and hence to be tasked with a wider variety of essential biological functions. One such aspect that we became intrigued with was its relationship in nociceptive perceptions as the channel is found in the peripheral nervous systems, *e.g.* sensory neurons and DRG neurons. It is believed that TRPV4 channel regulates both the inflammatory[6a] and mechanical pain[6b] in pathophysiological conditions. One example of the value of its modulator has been provided by a Shionogi group, demonstrating the effectiveness of a TRPV4 inhibitor **4** in Freund's Complete Adjuvant (FCA) induced hyperalgesia guinea pig model.[7a-c] Furthermore, another group from the same institute reported TRPV4 inhibitors such as **1** and **5** can attenuate a loss of grip strength induced by MIA injection in an acute OA pain model.[7d] Exemplified by these reports, the pharmacological implications continue to garner wide attention.

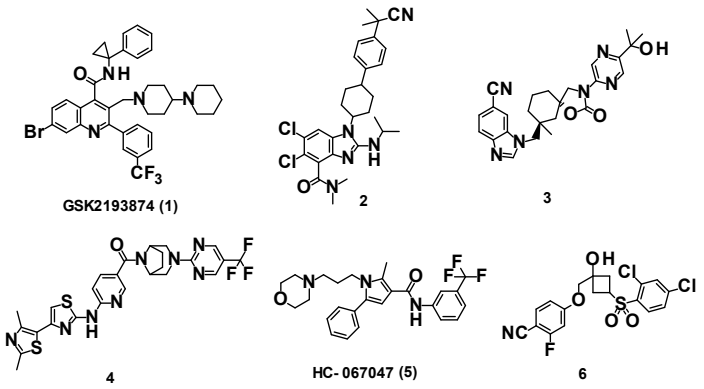


Figure 1. Selected small molecule organic compounds with TRPV4 inhibitory activities[8]

Intrigued by the pharmaceutical value a TRPV4 inhibitor would have for these reasons, we have

decided to start our own research for a TRPV4 inhibitor that can be orally administered to human patients, especially in the field of pain management. One of the several hit compounds that were found from an HTS campaign with an internal small molecule library was compound **7**. With a unique structure compared to the known TRPV4 inhibitors (figure 1, **1**-**6**[8]), its decent *in vitro* potency for an HTS hit, and also a low species variance between human and rat,[9] we have selected this compound as the starting point towards an orally active TRPV4 inhibitor with requisite safety profile.

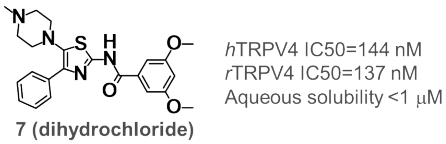
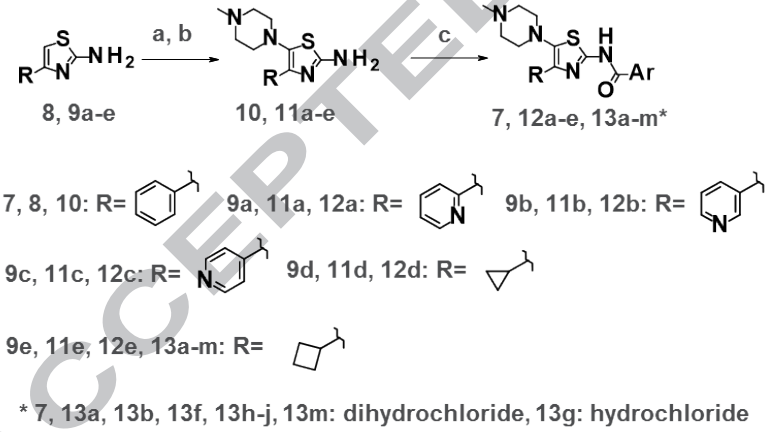


Figure 2. HTS hit compound (7) from Astellas chemical library

2. Results and discussion

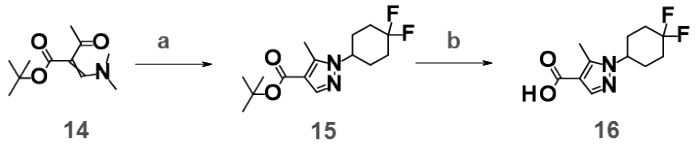
2.1. Chemistry

Compound **7** and its derivatives on table 1-3 were synthesized according to schemes 1 and 2. 1-Methyl piperazine was introduced to commercially available 2-aminothiazoles following thiazole C5 bromination. Condensation with aromatic carboxylic acids or acid chlorides gave the desired compounds.



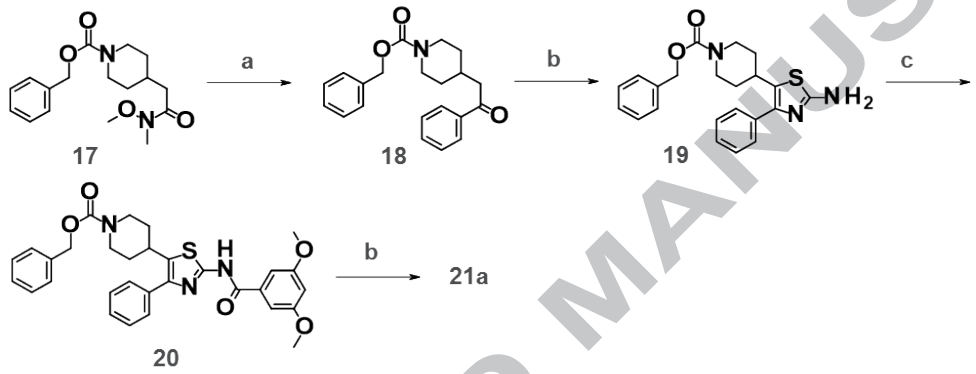
Scheme 1. Syntheses of **7**, **12a-e**, **13a-m**. Reagents and conditions: (a) NBS, NMP; (b) *N*-Methyl piperazine, NMP; (c) (i) ArCOCl, Et_3N, DCM *or* (ii) ArCOOH, HATU, DIPEA, THF, microwave irradiation; then 4M HCl (dioxane), solvents.

One aromatic carboxylic acid **16** that was used as an intermediate for **13j** in scheme 1 was synthesized as shown in scheme 2.



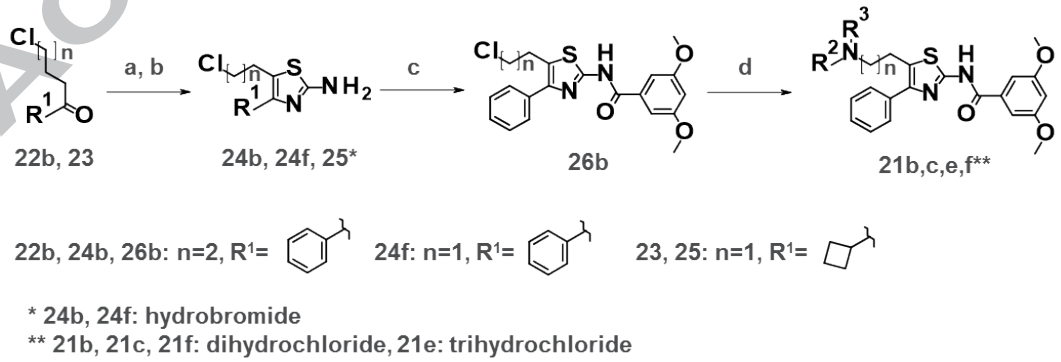
Scheme 2. Synthesis of **16**. Reagents and conditions: (a) (4,4-difluorocyclohexyl)hydrazine hydrogen chloride, K_2CO_3, EtOH, 70 °C, 1 d; (b) TFA, DCM, rt, 1 d.

Compound **21a** was synthesized as shown in scheme 3. Reaction of a commercially available Weinreb amide **17** with a Grignard reagent followed by bromination and thiazole ring formation gave aminothiazole **19**. Amide formation, and then replacement of a Cbz group with a methyl group produced the desired compound.



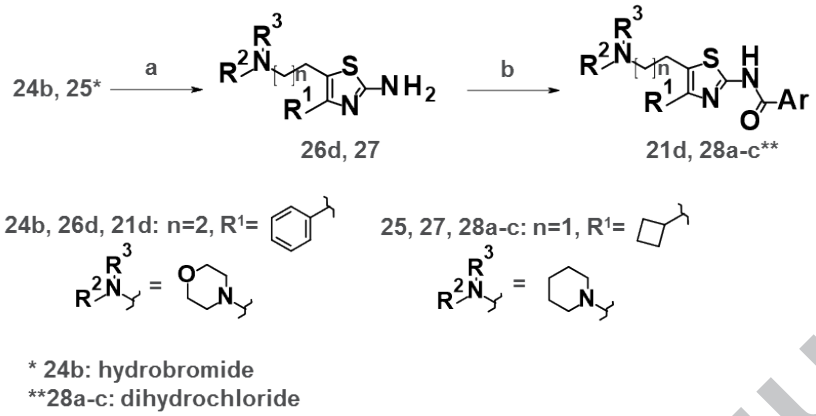
Scheme 3. Synthesis of **21a**. Reagents and conditions: (a) PhMgBr, THF, rt, 3 hr; (b) Br_2, AcOH, rt, 3 hr; thiourea, EtOH, reflux, 1 hr; (c) 3,5-$(MeO)_2$PhCOCl, Et_3N, DCE, 50 °C, 12 hr; (d) H_2 (3 atm), Pd/C, AcOH, MeOH, DMF, rt, 6 hr; 37% aq. formaldehyde, $NaBH(OAc)_3$, AcOH, DMF, rt, 5 hr.

Compounds **21b,c,e,f** were synthesized according to scheme 4. Bromination followed by condensation with thiourea afforded a 2-aminothiazole derivative from commercially available ketones. Following formation of an amide bond by condensation with 3,5-dimethoxybenzoyl chloride, the chlorine was substituted with various secondary amines to yield the desired compounds.



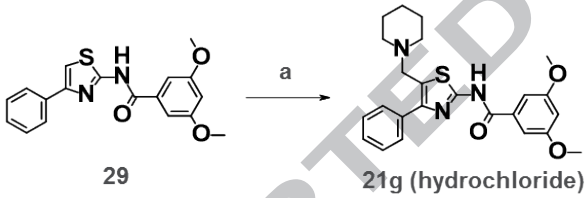
Scheme 4. Syntheses of **21b**, **21c**, **21e, 21f**. Reagents and conditions: (a) Br_2, HBr in EtOH, MeOH; (b) Thiourea, EtOH; (c) 3,5-$(MeO)_2$PhCOCl, Et_3N, DCE; (d) Amine, K_3PO_4, KI, DMF; then conc. HCl, EtOH.

Compounds **21d** and **28a-c** were synthesized as drawn in scheme 5. In these cases, intermediates **24b** and **25** were first reacted with morpholine or piperidine, and then with either 3,5-dimethoxybenzoyl chloride or commercially available carboxylic acids to yield the desired compounds.



Scheme 5. Synthesis of **21d, 28a-c**. Reagents and conditions: (a) morpholine, DMF, 60 °C, 2 d; 15 hr; *or* piperidine, DMF, 60 °C, 1 d; (b) 3,5-$(MeO)_2$PhCOCl, Et_3N, DCE; *or* ArCOOH, HATU, DIPEA, NMP; then 4M HCl (dioxane).

Compound **21g** was synthesized as drawn in scheme 6. Mannich reaction utilizing piperidine afforded the desired compound in a single step from a commercially available reagent **29**.



Scheme 6. Synthesis of **21g**. Reagents and conditions: (a) Piperidine, 36% aq. formaldehyde, AcOH, 80 °C, 18 hr; then 4M HCl (EtOAc).

2.2. Human TRPV4 inhibition and structure-activity relationship

Our goal of this research was to obtain an orally active TRPV4 inhibitor with a safety profile that permits use in common diseases, *e.g.* pain management in osteoarthritis patients. Thus, the initial aim was set on improvements of not only the *in vitro* potency, but also of factors that affect effective oral administration; namely, aqueous solubility and intrinsic metabolic clearance. Though it was initially believed that the poor solubility is primarily a result of the presence of two benzene rings (regions A, B), region C was also explored with the same objective.

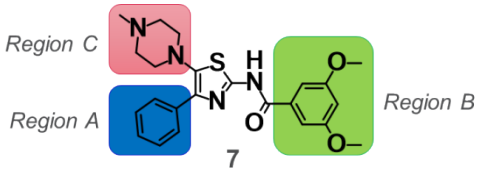
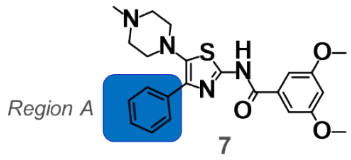


Figure 3. Regions A, B and C of compound 7.

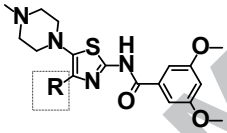
2.2.1. Region A

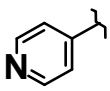


Our structure-activity relationship (SAR) investigation started with replacement of the phenyl with all three regioisomers of a pyridine (**12a-c**, table 1). Unfortunately, these analogs did not retain the *in vitro* TRPV4 antagonistic activity.

Next, we turned to a series of nonaromatic substituents (**12d-e**). It was revealed that cyclobutyl (**12e**) is a substituent that brings both comparative potency and solubility compared to **7**. It can be speculated that the protein pocket this region binds to prefers a lipophilic ligand with appropriate size. Although it was encouraging to see improvements in both aspects, we have turned our attention to region B with the hope of further optimization.

Table 1. Analogs of 7 with different substructures in region A.

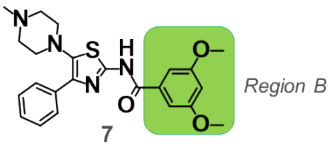


Compound No.	R	*h* TRPV4 IC50 (nM)	Aqueous Solubility* (μM)	Measured log$D_{7.4}$
7**		144	<1	>4.6
12a		1800	≥100	3.7
12b		290	2.2	4.1
12c		>10000	1.7	4.1
12d		240	15	4.0

12e		84	4.7	4.6

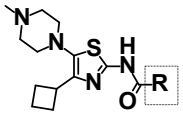
* pH6.8. ** Dihydrochloride salt.

2.2.2. Region B

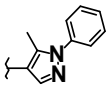
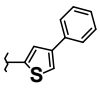
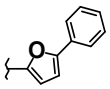
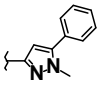


With the objective of further improving the *in vitro* potency and solubility by exploration of region B, we have investigated the SAR of various scaffolds. One such scaffold that we became intrigued with was a dimethylpyrazole (**13a**, table 2) because a much higher solubility (≥100 μM) was achieved while the ligand lipophilicity efficiency based on measured LogD values was comparable (LipE=3.0 (**13a**) *vs* 2.5 (**12e**))[10]. This proved to be a critical choice for optimizing the potency and solubility, as is discussed later in this paper. Subsequently, a benzene ring was introduced to *N*1 of this scaffold with the intention of clarifying the spatial requirement of the binding pocket. Thus, the result that this additional phenyl significantly improved the potency indicates that there is lipophilic region within the pocket. However, no other aromatic linker between the amide bond and the terminal benzene ring showed the same degree of potency (**13c-f**), which in turn means the linkage between the core and terminal structures are crucial due to either or both of electronic and steric reasons. The solubility improvement from **12e** to **13b** is discussed later.

Table 2. Analogs of 12e with different substructures in region B.



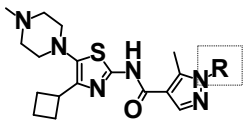
Compound No.	R	*h* TRPV4 IC_{50} (nM)	Aqueous Solubility* (μM)	Measured log$D_{7.4}$
12e		84	4.7	4.6
13a***		730	≥100	3.1

13b***		11	≥100	4.4
13c		240	NT**	NT**
13d		830	NT**	NT**
13e		3600	<1	4.9
13f***		81	<1	5.0

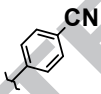
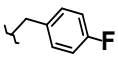
* pH6.8. ** NT is a value that has not been tested. *** Dihydrochloride salt.

Next, having observed the success with a simple phenyl ring on *N*1, further optimization was conducted. Introduction of a tertiary alkyl substituent resulted in less potency (**13g**, table 3), but the bioactivity was regained with a cyclic substituent (**13h**). While the potency was lost when a THP ring was introduced (**13i**), a difluorocyclohexyl substituent retained the potency, albeit with a loss of solubility (**13j**). Addition of a fluorine atom to the original benzene moiety improved the potency while maintaining the high solubility (**13k**), somewhat contrasting the SAR of the cyclohexane analogs (**13h, j**). Introduction of a polar CN group onto the same position (**13l**), or an additional methylene spacer between the two aromatic rings (**13m**), resulted in not only a decreased potency, but also a compromised solubility.

Table 3. Analogs of 13b with different *N*1 substituents.

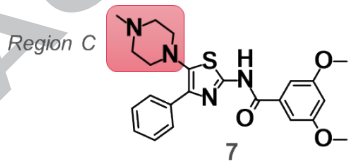


Compound No.	R	*h* TRPV4 IC_{50} (nM)	Aqueous Solubility* (μM)	Measured log$D_{7.4}$

13b**		11	≥100	4.4
13g***		78	48	4.5
13h**		11	98	5.0
13i**		880	≥100	3.1
13j**		15	7.6	4.3
13k		1.9	≥100	4.8
13l		22	<1	4.0
13m**		30	2.4	4.8

* pH6.8. ** Dihydrochloride salt. *** Hydrochloride salt.

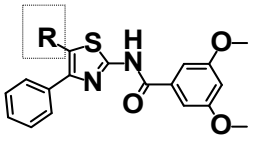
2.2.3. Region C

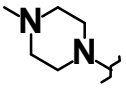
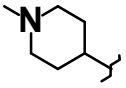
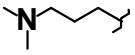


As the final part of the initial round of investigations, the SAR of region C was explored to identify a substituent with improved *in vitro* potency and physicochemical property. We were intrigued when an early exploration quickly revealed that the nitrogen directly attached to the core can be substituted to a carbon atom without loss of activity in the context of cyclic amines (**21a**, table 4). This finding also met our desire to remove one of the two amino groups bound to the thiazole, since we suspected that there is a potential risk of mutagenicity for a diaminothiazole compounds.[11,12] Interestingly, the

bioactivity was mostly retained even when the piperidine was exchanged with a linear amine (**21b**), possibly because the original piperidine ring was not forcing the ligand in an optimal conformation to bind to the target protein pocket.

Table 4. Analogs of 7 with different substructures in region C.

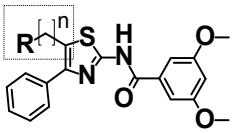


Compound No.	R	*h* TRPV4 IC_{50} (nM)	Aqueous Solubility* (μM)	Measured log$D_{7.4}$
7**		144	<1	>4.6
21a		84	≥100	3.6
21b**		86	≥100	3.5

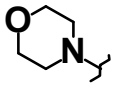
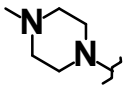
* pH6.8. ** Dihydrochloride salt.

Taking advantage of these findings, we next turned to a series of structures with linear, carbon based linkers between cyclic amines and the core. With the exception of piperazine that has an additional basic amine (**21e**, table 5), the introduced structures maintained the *in vitro* potency with a propylene linker (**21c, d**). Ethylene linker (**21f**) was found to be optimal from a survey of shorter linkers (**21f, h**).

Table 5. Analogs of 21b with different terminal amine structures.



Compound No.	n	R	*h* TRPV4 IC_{50} (nM)	Aqueous Solubility* (μM)	Measured log$D_{7.4}$

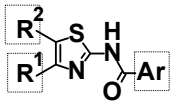
21b**	3		86	≥100	3.5
21c**	3		120	≥100	4.1
21d	3		99	<1	4.7
21e***	3		1700	≥100	4.1
21f**	2		51	84	4.5
21g****	1		310	<1	4.9

* pH6.8. ** Dihydrochloride salt. *** Trihydrochloride salt. **** Hydrochloride salt.

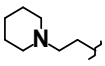
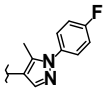
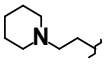
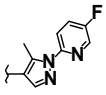
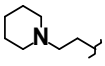
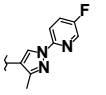
2.2.4. Combination of the obtained SAR

Based on the findings described above, we have decided to prepare one compound that contains all the optimized substituents (**28a**, table 6). While the *in vitro* potency and solubility was significantly improved compared to **7**, the human microsomal clearance value was found to be higher. In an effort to increase the metabolic stability, two pyridine analogs were prepared (**28b, c**), and **28c** was found to possess a satisfactory profile across the aspects of our interest.

Table 6. Overall profile of advanced compounds 28a-c in comparison to 7.



Compound No.	R^1	R^2	Ar	*h* TRPV4 IC_{50} (nM)	*r* TRPV4 IC_{50} (nM)	Aqueous Solubility* (□M)	Measured log$D_{7.4}$	*h* CLint (ml/min/kg)	*r* CLint (ml/min/kg)

7**	Ph			144	137	<1	>4.6	131	>1000
28a**	cBu			0.85	1.1	≥100	4.1	527	>1000
28b**	cBu			1.2	0.32	≥100	3.8	292	688
28c**	cBu			3.3	0.73	≥100	4.5	120	798

* pH6.8. ** Dihydrochloride salt.

2.3. In vivo pharmacology

After confirming the species variance in the IC_{50} values against rat and human TRPV4 is negligible, we have decided to confirm if **28c**, the most metabolically stable compound in human microsomes from table 6, is also active *in vivo*. An *in vivo* rat plasma extravasation experiment utilizing a TRPV4 activator was built to demonstrate the *in vivo* TRPV4 blockade by **28c**, because TRPV4 activators are known to elicit vascular permeability[13] which can be quantified by the leakage of Evans blue dye. Thus, **28c** was orally dosed at 3 mg/kg to a rat pretreated with a TRPV4 agonist, and was successfully shown to be orally active (88% inhibition). This finding validates our belief that this series of compounds merits further optimization efforts towards a clinical candidate after future examination of toxicological aspects.

2.4. Solubility improvements

Improvement of aqueous solubility is one of the key factors in drug discovery research.[14] According to the formulation proposed by Yalkowski and Valvani, aqueous solubility of organic compounds should be given by[15]

$$\log S_W \approx -\log P - 0.01 \times \mathrm{mp} + 1.05 \qquad (1)$$

Thus, the mechanisms underlying the solubility improvement that is achieved through modification to regions A, B and C can be assessed from the two critical aspects noted in this formula; the lipophilicity (log *P*) and the melting point (mp).

In tables 4 and 5, compounds with higher pKb values, *e.g.* **7** (5.8), **21d** (6.1) and **21g** (6.2), all have

lower solubility values than the others, such as **21a** (4.5), **21b** (4.1), **21c** (4.2), **21e** (4.5) and **21f** (4.7).[16] This is most critically a product of higher measured lipophilicity that resulted from lower basicity values for the former group compared to the latter, because the trends of solubility (log S_W) and lipophilicity (measured log$D_{7.4}$) are opposite to each other. Similar relationship is observed among **7** and **12a-c**. In this case, subtly greater exposure of the basic nitrogen to the surface might explain the lipophilicity trend, as the dihedral angle values between the pyridine and thiazole moieties in their most stable conformations in *in silico* analyses are 53 degrees for **12a** and 39, 40 degrees for **12b, 12c**.[17]

In contrast to these observations is the data obtained in tables 2 and 3. Particularly, the solubility difference between **12e** and **13b** (4.7 *vs* ≥100 μM) despite similar measured log$D_{7.4}$ values (4.6 *vs* 4.4) is interesting. The other critical component of Yalkowski's formula is the melting point (mp). Mp continues to be one of the most difficult physicochemical property to predict,[18] but it has been well established from various studies to possess correlation with the symmetry[19] and planarity[20] of the compound.[21] The structural difference between **12e** and **13b** is limited to region B, and **12e** has a C_2-symmetrical 3,5-$(MeO)_2$-phenyl that also has low dihedral angles between the thiazole-amide-benzene system. On the other hand, **13b** has an unsymmetrical pyrazole, which induces a non-planar, bent shape to the entire molecular. Therefore, it is reasonable to understand the solubility difference between the two compounds are caused by these structural factors, and it also highlights the importance of selecting the right scaffold upon which a compound is built. Further crystallographic studies are needed for a decisive analysis.

3. Conclusion

Optimization of the two benzene substituents on the thiazole ring of our HTS hit compound **7** resulted in identifications of a cBu and a pyrazole scaffold in regions A and B that led to both increased solubility and *in vitro* potency. Another structural modification that yielded similar improvements was discovered within region C in the form of a linear 2-piperidinylethyl. We believe this structure also lowered the intrinsic risk for mutagenicity. Combination of these findings yielded a small set of TRPV4 inhibitors with vastly improved potency and solubility. Comparison of the human metabolic stability revealed an interesting pattern contrary to the general trend with lipophilicity within this set of compounds. Finally, **28c** was shown in a rat *in vivo* model to be an orally active TRPV4 inhibitor. We aim to further optimize this compound and identify a clinical candidate in the near future.

4. Experimental section

4.1 Biological assays

4.1.1. *In vitro* human and rat TRPV4 inhibition assay

4.1.1-1 Ca^{2+} Influx assay

Human TRPV4 channel-expressing HEK293 cells and rat TRPV4 channel-expressing HEK293 cells

were plated at a density of 3.5×10^3 cells/well for human TRPV4 and 6.5×10^3 cells/well for rat TRPV4 into black-wall, clear bottom, Poly-D-lysine coated, 384-well plate and incubated with Dulbecco's Modified Eagle Medium alpha (DMEM) containing 10% fetal bovine serum, 50 units / mL penicillin, and 50 μg/mL streptomycin. At approximately 72 hr after cell plating, the medium was removed and the cells were loaded with 20 μL/well of Loading solution {1μM Fluo4-AM (DOJINDO, Kumamoto, Japan) diluted in assay buffer (1xHBSS containing 20mM HEPES and 1% CHAPS. In case of rat TRPV4 assay, 1.25mM $CaCl_2$ was also added)}. After approximately 1.5-h incubation at room temperature in shaded condition, 10μL/well of diluted test compounds (final, 0-10 μM), vehicle (assay buffer) or Ruthenium Red (final, 3.3 μM) as positive control TRPV4 inhibitor were added to respective wells. After approximately 30min incubation at room temperature in shaded condition, 25μl of hypotonic solution (88% H_2O in assay buffer for hTRPV4 and 100% H_2O for rat TRPV4) was added to respective wells and change in RFU (excitation wavelength: 475-495 nm, emission wavelength: 515-575 nm) from immediately before to 3 min after the addition of hypotonic solution was recorded as an indicator of change in Ca^{2+} influx. Data in an experiment was obtained in duplicate.

4.1.1-2 Data Analysis

The response to each concentration of test compound was expressed as a percentage where average values of the responses to vehicle and Ruthenium Red in each test plate were defined as 0% and 100% inhibition, respectively. IC_{50} value was calculated by Sigmoid-Emax model non-linear regression analysis using the Statistical Analysis System (SAS Institute Japan Ltd., Tokyo, Japan). IC_{50} value was defined as the test compound concentration which produces 50% inhibition.

4.1.2. *In vivo* rat TRPV4 inhibition assay

4.1.2-1 Measurement of plasma extravasation

As TRPV4 activator elicits vascular permeability[13], the effect of TRPV4 inhibitors were evaluated in a TRPV4 activator[22] induced vascular permeability test. Female Lewis rats (7 weeks of age; Charles River Laboratories Japan, Yokohama, Japan) were used. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc.

All animals were used under fed conditions. The animals' back fur was shaved under isoflurane anesthesia. After rats awoke, 0.5% methyl cellulose (MC) as a negative control or a TRPV4 inhibitor of choice was orally administered (N = 4–5). After 1 hour, 1.5% Evans blue dye (3 mL/kg) was injected via the tail vein immediately after intradermal application of the TRPV4 activator (10 μM, 10 μL) and PEG-400 (Vehicle, 10 μL) into 4 points symmetrically on the dorsal region under isoflurane anesthesia. After 10 minutes, the rats were killed by cervical dislocation. The skin area around the TRPV4 activator and Vehicle application point (8 mm in diameter) was removed, and the Evans blue dye was extracted by incubating the skin samples in 1 mL of formamide for approximately 24 hours at room temperature. The absorbance of Evans blue dye extracted in formamide from individual skin samples was measured by spectrophotometry at 620 nm using a plate

reader (SPECTRAmax PLUS 384; Molecular Devices Japan, Tokyo, Japan). The mean value of the four skin samples where Vehicle or TRPV4 activator was injected was used as the Vehicle or TRPV4 activator-induced Evans blue extravasation value.

4.1.2-2 Data analysis

The concentration of the Evans blue was calculated from a standard curve, subtracted by the value corresponding to skin samples when Vehicle was injected. Inhibitory activity (%) was calculated as follow.

Inhibitory activity (%) = {1-Value(Test compound) / Value(MC)} x 100

4.1.3. *In vitro* human and rat microsomal clearance

Pooled human and rat liver microsomes (Xenotech LLC.) were diluted in 100 mM KH_2PO_4/Na_2HPO_4 buffer (pH7.4) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA). The incubation mixtures (270 μL total volume), which contained 0.2 mg/mL of microsomal proteins, and 0.2 μM of substrates (0.093 μM in the case of compound **7**) were pre-incubated for approximately about 15 min at 37 °C. Reactions were initiated by the addition of 1 mM NADPH (30 μL). After the appropriate incubation time (0, 15, 30, and 45 min), 40 μL of incubation mixture was transferred into 80% acetonitrile containing internal standard (50 nM propranolol, 250 μL), stood at 4 °C for over 10 min, and centrifuged for 20 min at 2800 rpm. The supernatant (200 μL) was prepared and analyzed via LC-MS/MS with UPLC system (Waters) and Xevo TQ (Waters). The in vitro intrinsic clearance (CLint, vitro) was calculated using Equation 1, which is based on the time course of the residual ratio of the compounds.

$$CL_{int,\ vitro}\ (mL/min/kg) = \frac{\boldsymbol{Ke(1/min) \times MScontent(mg/kg)}}{\boldsymbol{MS\ Protein\ Conc.(mg/mL)}} \quad (2)$$

where the Ke is the disappearance rate constant.

4.2. Physicochemical property assay

4.2.1. Aqueous solubility

Solubility in Japanese Pharmacopoeia 2nd fluid for disintegration test (JP2; pH = 6.8 buffer) was evaluated by following method. 10 mM DMSO stock solution of test compounds were prepared and added into JP2. Shook by 1000 rpm at 25 °C light protected condition for 20 h. Precipitates were filtrated through PVDF membrane filter (pore size 0.22 μm, MERCK) and the compound concentration (μM) of filtrate was assayed by HPLC.

4.2.2. Measured log$D_{7.4}$

Log$D_{7.4}$ value was calculated as the natural logarithm of the compound concentration ratio between 1-octanol and Dulbecco's phosphate buffered salts (PBS) aqueous solution (pH7.4). The

concentration values within the two layers were respectively evaluated by LC-MS after 10 mM DMSO stock solution of the compound was partitioned at 23 °C.

5. Syntheses

5.1. Materials

The following abbreviations are used: NMP, *N*-methylpyrrolidone; NBS, *N*-bromosuccinimide; TFA, trifluoroacetic acid; DMF, *N*,*N*-dimethylformamide; MeOH, methanol; EtOH, ethanol; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-Oxide Hexafluorophosphate; DIPEA, diisopropylethylamine; DMSO, *N*,*N*-dimethylsulfoxide; EtOAc, ethyl acetate; IPA, isopropanol; Et_2O, diethylether; IPE, diisopropylether.

5.2. Instrumentation

1H NMR spectra were recorded on a Varian VNS-400, JEOL JNM-LA400 or JEOL JNM-AL400 spectrometer. Chemical shifts were expressed in δ values (ppm) using tetramethylsilane as the internal standard (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet, br = broad peak, and brs = broad singlet). Mass spectra (MS) were recorded on a JEOL LX-2000, Waters ZQ-2000, Waters LCT Premier mass spectrometer or Thermo Fisher Exactive Plus Orbitrap. Elemental analyses were conducted using a Yanaco JM10 (C, H, N), Elementar Vario EL III (C, H, N), Dionex ICS-3000 (S, halogene), and Dionex DX-5000 (S, halogene) and were within ±0.4% of theoretical values. All reactions were carried out using commercially available reagents and solvents without further purification.

5.3. Synthetic procedures

5.3.1. 5-(4-Methylpiperazin-1-yl)-4-phenyl-1,3-thiazol-2-amine (**10**)

To a mixture of **8** (10.0 g, 57 mmol) and NMP (100 mL) was added NBS (10.6 g, 60 mmol, 1.1 equiv.) portionwise on ice water bath, and the whole was stirred on the same bath for 1 hr, then at rt for 2 hr. To the reaction mixture was added *N*-methylpiperazine (28.5 g, 285 mmol, 5.0 equiv.) in NMP (20 mL) on an ice water bath, and the whole was stirred at 100 °C for 8 hr. To the reaction mixture was added H_2O, and the whole was stirred at room temperature for 10 minutes. The precipitate was collected by filtration, washed with H_2O and dried *in vacuo* to give the title compound (12.5 g, 45 mmol, 80%) as a pale brown powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.35 (3H, s), 2.50–2.63 (4H, m), 2.85–2.97 (4H, m), 4.80 (2H, s), 7.21–7.28 (1H, m, overlapped with CHCl3), 7.32–7.42 (2H, m), 8.04–8.14 (2H, m); MS (ESI) *m/z* 275 $[M+H]^+$.

5.3.2. 3,5-Dimethoxy-*N*-[5-(4-methylpiperazin-1-yl)-4-phenyl-1,3-thiazol-2-yl]benzamide dihydrochloride **(7)**

To a mixture of **10** (5.0 g, 18 mmol) and CH_2Cl_2 (60 mL) was added Et_3N (5.4 mL, 39 mmol, 2.1

equiv.) and 3,5-dimethoxybenzoyl chloride (7.3 g, 36 mmol, 2.0 equiv.) on an ice water bath, and the whole was stirred on the same bath for 10 minutes, then at rt for 1 d. To the reaction mixture was added EtOAc, and the organic layer was washed with H_2O and sat. brine. The organic layer was dried over anhydrous MgSO4 and concentrated *in vacuo*, and the residue was purified on a silica gel column chromatography ($CHCl_3$:MeOH). To the crude product was added EtOH, EtOAc and 4 M HCl in dioxane, and the whole was stirred at rt overnight. The precipitation was collected by filtration, washed with EtOAc and dried *in vacuo*. The obtained pale yellow powder was dissolved into H_2O, and 1 M aq. NaOH was added therein. The suspension was extracted with $CHCl_3$. The combined organic layers were concentrated *in vacuo*, and the residue was purified on a silica gel column chromatography ($CHCl_3$:MeOH) to give a pale yellow powder. To a solution of this product in MeOH and EtOAc was added 4 M HCl in dioxane, and the mixture was stirred at rt overnight. The precipitate was collected by filtration, washed with a hot mixture of EtOH and EtOAc, then dried *in vacuo* to give the title compound (2.8 g, 5.0 mmol, 28%) as a pale yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.79–2.88 (3H, m), 3.10–3.35 (6H, m), 3.42–3.53 (2H, m), 3.83 (6H, s), 6.75 (1H, t, J = 2.3Hz), 7.27–7.36 (3H, m), 7.42–7.49 (2H, m), 8.10–8.16 (2H, m), 11.07 (1H brs), 12.59 (1H, brs); MS (ESI) *m/z* 439 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{26}N_4O_3S \cdot 2H_2O \cdot 2HCl$: C, 50.46, H, 5.89, N, 10.23, S, 5.86, Cl, 12.95. Found: C, 50.59, H, 6.12, N, 10.18, S, 5.74, Cl, 12.71.

5.3.3. 5-(4-Methylpiperazin-1-yl)-4-(pyridin-2-yl)-1,3-thiazol-2-amine (**11a**)

The title compound was obtained in a similar manner to **10** using **9a** as a yellow solid in 10% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.20 (3H, s), 2.37–2.47 (4H, m), 2.81–2.92 (4H, m), 6.70 (2H, s), 7.17 (1H, ddd, J = 1.1, 4.7, 7.5 Hz), 7.74 (1H, td, J = 2.0, 7.6 Hz), 7.82–7.88 (1H, m), 8.51–8.58 (1H, m); MS (ESI) *m/z* 276 $[M+H]^+$.

5.3.4. 3,5-Dimethoxy-*N*-[5-(4-methylpiperazin-1-yl)-4-(pyridin-2-yl)-1,3-thiazol-2-yl]benzamide (**12a**)

The title compound was obtained in a similar manner to **7** using **11a** as a pale yellow solid in 43% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.24 (3H, s), 2.43–2.55 (4H, m, overlapped with DMSO), 3.00–3.08 (4H, m), 3.83 (6H, s), 6.73 (1H, t, J = 2.3 Hz), 7.26 (1H, ddd, J = 1.1, 4.8, 7.4 Hz), 7.31 (2H, d, J = 2.3 Hz), 7.80–7.87 (1H, m), 8.02–8.07 (1H, m), 8.60–8.64 (1H, m), 12.54 (1H, brs); MS (ESI) *m/z* 440 $[M+H]^+$.

5.3.5. 5-(4-Methylpiperazin-1-yl)-4-(pyridin-3-yl)-1,3-thiazol-2-amine (**11b**)

The title compound was obtained in a similar manner to **10** using **9b** as a beige solid in 47% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.23 (3H, s), 2.42–2.50 (4H, m, overlapped with DMSO), 2.73–2.80 (4H, m), 6.90 (2H, s), 7.39 (1H, ddd, J = 0.78, 4.8, 8.2 Hz), 8.33 (1H, dt, J = 1.9, 8.0 Hz), 8.40 (1H, dd, J = 1.7, 4.7 Hz), 9.17-9.21 (1H, m); MS (ESI) *m/z* 276 $[M+H]^+$.

5.3.6. 3,5-Dimethoxy-*N*-[5-(4-methylpiperazin-1-yl)-4-(pyridin-3-yl)-1,3-thiazol-2-yl]benzamide (**12b**)

The title compound was obtained in a similar manner to **7** using **11b** as a colorless solid in 10% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.25 (3H, s), 2.44–2.59 (4H, m, overlapped with DMSO), 2.90–2.96 (4H, m), 3.83 (6H, s), 6.74 (1H, t, J = 2.3 Hz), 7.30 (2H, d, J = 2.4 Hz), 7.47 (1H, ddd, J = 0.83, 4.8, 8.0 Hz), 8.42 (1H, dt, J = 2.1, 8.0 Hz), 8.48 (1H, dd, J = 1.7, 4.7 Hz), 9.26–9.30 (1H, m), 12.49 (1H, brs); MS (ESI) *m/z* 440 $[M+H]^+$; Anal. Calcd. for $C_{22}H_{25}N_5O_3S$: C, 60.12, H, 5.73, N, 15.93, S, 7.30. Found: C, 59.98, H, 5.72, N, 15.92, S, 7.30.

5.3.7. 5-(4-Methylpiperazin-1-yl)-4-(pyridin-4-yl)-1,3-thiazol-2-amine (**11c**)

The title compound was obtained in a similar manner to **10** using **9c** as a beige solid in 45% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.24 (3H, s), 2.43–2.52 (4H, m, overlapped with d-DMSO), 2.72–2.81 (4H, m), 6.91 (2H, s), 7.94–7.99 (2H, m), 8.51-8.56 (2H, m); MS (ESI) *m/z* 276 $[M+H]^+$.

5.3.8. 3,5-Dimethoxy-*N*-[5-(4-methylpiperazin-1-yl)-4-(pyridin-4-yl)-1,3-thiazol-2-yl]benzamide (**12c**)

The title compound was obtained in a similar manner to **7** using **11c** as a beige solid in 28% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.27 (3H, s), 2.47–2.60 (4H, m, overlapped with DMSO), 2.92–2.99 (4H, m), 3.83 (6H, s), 6.74 (1H, t, J = 2.3 Hz), 7.30 (2H, d, J = 2.4 Hz), 8.02–8.07 (2H, m), 8.61–8.65 (2H, m), 12.57 (1H, brs); MS (ESI) *m/z* 440 $[M+H]^+$; Anal. Calcd. for $C_{22}H_{25}N_5O_3S$: C, 60.12, H, 5.73, N, 15.93, S, 7.30. Found: 59.85, H, 5.81, N, 15.70, S, 7.23.

5.3.9. 4-Cyclopropyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-amine (**11d**)

The title compound was obtained in a similar manner to **10** using **9d** as a brown solid in 51% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.73–0.87 (4H, m), 1.95–2.05 (1H, m), 2.33 (3H, s), 2.48–2.61 (4H, m), 2.81–2.93 (4H, m), 4.63 (2H, s); MS (ESI) *m/z* 239 $[M+H]^+$.

5.3.10. *N*-[4-Cyclopropyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-3,5-dimethoxybenzamide (**12d**)

The title compound was obtained in a similar manner to **7** using **11d** as a pale yellow solid in 83% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.80–0.89 (4H, m), 2.00–2.09 (1H, m), 2.23 (3H, s), 2.40–2.55 (4H, m, overlapped with DMSO), 2.82–2.94 (4H, m), 3.81 (6H, s), 6.69 (1H, t, J = 2.3 Hz), 7.24 (2H, d, J = 2.3 Hz), 12.2 (1H, brs); MS (ESI) *m/z* 403 $[M+H]^+$; Anal. Calcd. for $C_{20}H_{26}N_4O_3S \cdot 0.5H_2O$: C, 58.37, H, 6.61, N, 13.61, S, 7.79. Found: C, 58.18, H, 6.56, N, 13.44, S, 7.72.

5.3.11. 4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-amine (**11e**)

The title compound was obtained in similar manner to **10** using **9e** as a brown powder in 62% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.80–2.10 (2H, m), 2.10–2.20 (2H, m), 2.24–2.40 (5H, m), 2.44–2.56 (4H, m), 2.70–2.85 (4H, m), 3.55–3.68 (1H, m), 4.77 (2H, s); MS (ESI) *m/z* 253 $[M+H]^+$.

5.3.12. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-3,5-dimethoxybenzamide

(**12e**)

A mixture of **11e** (400 mg, 1.6 mmol), HATU (540 mg, 1.7 mmol, 1.1 equiv.), 3,5-dimethoxybenzoic acid (350 mg, 1.9 mmol, 1.2 equiv.), DIPEA (619 mg, 4.8 mmol, 3.0 equiv.) and THF (4.0 mL) was stirred under irradiation of microwave at 160 °C for 20 minutes. To the reaction mixture was added EtOAc, and washed with sat. aq. $NaHCO_3$ and sat. brine. The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by a silica gel column chromatography ($CHCl_3$:MeOH) and recrystallization from a mixture of EtOAc and hexane to give the title compound (236 mg, 0.57 mmol, 36%) as a pale yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.76–1.87 (1H, m), 1.90–2.06 (1H, m), 2.12–2.26 (2H, m), 2.22 (3H, s), 2.28–2.40 (2H, m), 2.42–2.48 (4H, m), 2.75–2.83 (4H, m), 3.64–3.75 (1H, m), 3.82 (6H, s), 6.71 (1H, t, J = 2.3 Hz), 7.27 (2H, d, J = 2.3 Hz), 12.35 (1H, s); MS (ESI) *m/z* 417 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{28}N_4O_3S$: C, 60.55, H, 6.78, N, 13.45, S, 7.70. Found: C, 60.40, H, 6.68, N, 13.25, S, 7.67.

5.3.13. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-1,5-dimethyl-1*H*-pyrazole-4-carboxamide dihydrochloride (**13a**)

To a dioxane (2.5 mL) solution of the free form of the title compound (261 mg), which was obtained in a similar manner to **12e** using 1,5-dimethyl-1*H*-pyrazole-4-carboxylic acid, was added 4 M HCl dioxane (1.5 mL) and concentrated in vacuo. The residue was suspended in a hot mixture of IPA, EtOH and EtOAc, allowed to rt, and then the resulting precipitation was filtered to give the title compound as a colorless solid in 35% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.77–1.89 (1H, m), 1.89–2.04 (1H, m), 2.12–2.24 (2H, m), 2.24–2.40 (2H, m), 2.36 (3H, s), 2.81 (3H, d, J = 4.6 Hz), 3.01–3.13 (4H, m), 3.14–3.27 (2H, m), 3.35–3.48 (2H, m), 3.63–3.76 (1H, m), 3.81 (3H, s), 8.52 (1H, s), 11.02 (1H, brs), 11.93 (1H, brs); MS (ESI) *m/z* 375 $[M+H]^+$; Anal. Calcd. for $C_{18}H_{26}N_6OS\cdot 2HCl\cdot 0.03C_3H_8O\cdot 0.3H_2O$: C, 47.79, H, 6.39, N, 18.49, S, 7.05, Cl,15.60. Found: C, 47.78, H, 6.39, N, 18.28, S, 7.19, Cl, 15.55.

5.3.14. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide dihydrochloride (**13b**)

The title compound was obtained in a similar manner to **13a** using 5-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid as a colorless solid in 56% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.78–1.91 (1H, m), 1.91–2.05 (1H, m), 2.14–2.26 (2H, m), 2.27–2.42 (2H, m), 2.57 (3H, s), 2.81 (3H, d, J = 4.7 Hz), 3.01–3.30 (6H, m), 3.36–3.51 (2H, m), 3.72 (1H, tt, J = 8.6, 8.6 Hz), 7.46–7.65 (5H, m), 8.55 (1H, s), 11.17 (1H, brs), 12.18 (1H, brs); MS (ESI) *m/z* 437 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{28}N_6OS\cdot 2HCl\cdot 0.9H_2O$: C, 52.55, H, 6.10, N, 15.99, S, 6.10, Cl, 13.49. Found: C, 52.56, H, 6.17, N, 16.03, S, 6.08, Cl, 13.37.

5.3.15. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-4-phenylthiophene-2-carboxamide (**13c**)

The title compound was obtained in a similar manner to **12e** using 4-phenylthiophene-2-carboxylic acid in 46% yield. MS (ESI) *m/z* 439 $[M+H]^+$.

5.3.16. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-5-phenylfuran-2-carboxamide (**13d**)

The title compound was obtained in a similar manner to **12e** using 5-phenylfuran-2- carboxylic acid in 35% yield. MS (ESI) *m/z* 423 $[M+H]^+$.

5.3.17. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-1-methyl-5-phenyl-1*H*-pyrazole-3-carboxamide (**13e**)

The title compound was prepared in a similar manner to **12e** using 1-methyl-5-phenyl-1*H*-pyrazole-3-carboxylic acid as a colorless solid in 14% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.76–1.88 (1H, m), 1.89–2.03 (1H, m), 2.11–2.25 (2H, m), 2.22 (3H, s), 2.25–2.38 (2H, m), 2.40–2.54 (4H, m, overlapped with DMSO), 2.74–2.87 (4H, m), 3.65 (1H, tt, J = 8.6, 8.6 Hz), 3.96 (3H, s), 7.15 (1H, s), 7.45–7.64 (5H, m), 11.64 (1H, s); MS (ESI) *m/z* 437 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{28}N_6OS$: C, 63.28, H, 6.46, N, 19.25, S, 7.34. Found: C, 63.07, H, 6.40, N, 19.23, S, 7.24.

5.3.18. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-4-methyl-2-phenyl-1,3-oxazole-5-carboxamide dihydrochloride (**13f**)

The title compound was prepared in a similar manner to **13a** using 4-methyl-2-phenyl-1,3-oxazole-5-carboxylic acid as a pale yellow solid in 52% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.81–1.92 (1H, m), 1.92–2.06 (1H, m), 2.16–2.28 (2H, m), 2.29–2.43 (2H, m), 2.86 (3H, s), 2.95–3.07 (2H, m), 3.11–3.20 (2H, m), 3.20–3.31 (2H, m), 3.44–3.51 (2H, m), 3.54 (3H, s, overlapped with residual H_2O), 3.73 (1H, tt, J = 8.6, 8.6 Hz), 7.54–7.69 (3H, m), 8.29–8.38 (2H, m); MS (ESI) *m/z* 438 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{27}N_5O_2S \cdot 2HCl \cdot 0.3H_2O$: C, 53.55, H, 5.78, N, 13.58, S, 6.22, Cl, 13.74. Found: C, 53.65, H, 5.78, N, 13.51, S, 6.28, Cl, 13.87.

5.3.19. 1-*tert*-Butyl-*N*-[4-cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-5-methyl-1*H*-pyrazole-4-carboxamide hydrogen chloride (**13g**)

The title compound was prepared in a similar manner to **12e** using 1-*tert*-butyl-5-methyl-1*H*-pyrazole-4-carboxylic acid as a colorless solid in 33% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.61 (9H, s), 1.77–1.88 (1H, m), 1.89–2.03 (1H, m), 2.13–2.23 (2H, m), 2.26–2.39 (2H, m), 2.74 (3H, s), 2.82 (3H, d, J = 4.3Hz), 2.99–3.13 (4H, m), 3.15–3.27 (2H, m), 3.4–3.49 (2H, m), 3.70 (1H, tt, J = 8.7, 8.7Hz), 8.23 (1H, s), 10.80 (1H, brs), 11.95 (1H, s); MS (ESI) *m/z* 417 $[M+H]^+$; Anal. Calcd. for $C_{21}H_{32}N_6OS \cdot HCl \cdot 1.7H_2O$: C, 52.15, H, 7.59, N, 17.38, S, 6.63, Cl, 7.33. Found: C, 51.92, H, 7.68, N, 17.32, S, 6.63, Cl, 7.61.

5.3.20. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-1-cyclohexyl-5-methyl-1*H*-pyrazole-4-carboxamide dihydrochloride (**13h**)

The title compound was obtained in a similar manner to **13a** using 1-cyclohexyl-5-methyl-1*H*-pyrazole-4-carboxylic acid as an off white amorphous semisolid in 20% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.11–1.29 (1H, m), 1.33–1.51 (2H, m), 1.60–1.88 (8H, m), 1.89–2.02 (1H, m), 2.10–

2.24 (2H, m), 2.26–2.41 (2H, m), 2.57 (3H, s), 2.81 (3H, d, J = 4.7 Hz), 2.99–3.30 (6H, m), 3.36–3.54 (2H, m), 3.70 (1H, tt, J = 8.5, 8.5 Hz), 4.11–4.29 (1H, m), 8.32 (1H, s), 10.99 (1H, brs), 11.95 (1H, brs); MS (ESI) *m/z* 443 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{34}N_6OS \cdot 1.9HCl \cdot 1.9H_2O$: C, 50.58, H, 7.33, N, 15.39, S, 5.87, Cl, 12.33. Found: C, 50.60, H, 7.40, N, 15.27, S, 5.91, Cl, 12.19.

5.3.21. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-5-methyl-1-(oxan-4-yl)-1*H*-pyrazole-4-carboxamide dihydrochloride (**13i**)

The title compound was obtained in a similar manner to **13a** using 5-methyl-1-(oxan-4-yl)-1*H*-pyrazole-4-carboxylic acid as a pale yellow amorphous semisolid in 40% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.74–1.88 (3H, m), 1.89–2.09 (3H, m), 2.13–2.24 (2H, m), 2.27–2.39 (2H, m), 2.60 (3H, s), 2.81 (3H, d, J = 4.8Hz), 2.95–3.14 (4H, m), 3.15–3.28 (2H, m), 3.40–3.55 (4H, m), 3.64-3.77 (1H, m), 3.96 (2H, dd, J = 4.0, 11.0Hz), 8.35 (1H, s), 10.84 (1H, brs), 11.99 (1H, brs); MS (ESI) *m/z* 445 $[M+H]^+$; Anal. Calcd. for $C_{22}H_{32}N_6O_2S \cdot 1.7HCl \cdot 2H_2O$: C, 48.70, H, 7.00, N, 15.49, S, 5.91, Cl, 11.11. Found: C, 48.86, H, 7.24, N, 15.39, S, 5.76, Cl, 10.96.

5.3.22. *tert*-Butyl 1-(4,4-difluorocyclohexyl)-5-methyl-1*H*-pyrazole-4-carboxylate (**15**)

A mixture of (4,4-difluorocyclohexyl)hydrazine hydrochloride (1047 mg, 5.6 mmol), *tert*-butyl 2-[(dimethylamino)methylidene]-3-oxobutanoate (**14**) (1300 mg, 6.1 mmol, 1.1 equiv.), and K_2CO_3 (620 mg, 6.3 mmol, 1.1 equiv.) in EtOH (14 mL) was stirred at 70 °C for 1 d. To the mixture was added H_2O, and the mixture was extracted with $CHCl_3$. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column chromatography (hexane:EtOAc) to give the title compound (718 mg, 2.4 mmol, 43%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.55 (9H, s), 1.80–2.00 (4H, m), 2.25–2.40 (4H, m), 2.54 (3H, s), 4.07–4.21 (1H, m), 7.79 (1H, s); MS (ESI) *m/z* 301 $[M+H]^+$.

5.3.23. 1-(4,4-Difluorocyclohexyl)-5-methyl-1*H*-pyrazole-4-carboxylic acid (**16**)

TFA (4.5 mL, 59 mmol, 25 equiv.) was added to a solution of **15** (718 mg, 2.4 mmol) in CH_2Cl_2 (4.5 mL) at rt, and the mixture was stirred at rt for 1 d. The reaction mixture was concentrated *in vacuo*. Toluene was added to the residue, and the mixture was concentrated *in vacuo*. EtOAc was added to the residue, and then the mixture was concentrated *in vacuo* to give the title compound (570 mg, 2.3 mmol, 98%) as an off white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.86–2.20 (8H, m), 2.53 (3H, s), 4.38–4.54 (1H, m), 7.75 (1H, s), 12.17 (1H, brs); MS (ESI) *m/z* 245 $[M+H]^+$.

5.3.24. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-1-(4,4-difluorocyclohexyl)-5-methyl-1*H*-pyrazole-4-carboxamide dihydrochloride (**13j**)

The title compound was obtained in a similar manner to **13a** using **16** as a colorless solid in 11% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.76–2.25 (12H, m), 2.26–2.40 (2H, m), 2.59 (3H, s), 2.81 (3H, d, J = 4.7 Hz), 3.00–3.13 (4H, m), 3.14–3.30 (2H, m), 3.38–3.50 (2H, m), 3.70 (1H, tt, J = 8.6, 8.6 Hz), 4.44–4.59 (1H, m), 8.35 (1H, brs), 10.95 (1H, brs), 11.99 (1H, brs); MS (ESI) *m/z* 479 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{32}F_2N_6OS \cdot 2HCl \cdot 0.5H_2O$: C, 49.28, H, 6.29, N, 14.99, S, 5.72, Cl,

12.65, F, 6.78. Found: C, 49.25, H, 6.29, N, 14.98, S, 5.65, Cl, 12.18, F, 6.76.

5.3.25. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-1-(4-fluorophenyl)-5-methyl-1*H*-pyrazole-4-carboxamide (**13k**)

The title compound was obtained in a similar manner to **12e** using 1-(4-fluorophenyl)-5-methyl-1*H*-pyrazole-4-carboxylic acid as a pale brown powder in 21% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.77–1.87 (1H, m), 1.90–2.04 (1H, m), 2.12–2.25 (5H, m), 2.27–2.39 (2H, m), 2.40–2.48 (4H, m), 2.53–2.59 (3H, m), 2.76–2.82 (4H, m), 3.61–3.73 (1H, m), 7.36–7.45 (2H, m), 7.57–7.66 (2H, m), 8.53 (1H, s), 12.06 (1H, s); MS (ESI) *m/z* 455 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{27}FN_6OS$: C, 60.77, H, 5.99, N, 18.49, S, 7.05. Found: C, 60.43, H, 5.98, N, 18.13, S, 7.22.

5.3.26. 1-(4-Cyanophenyl)-*N*-[4-cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-5-methyl-1*H*-pyrazole-4-carboxamide (**13l**)

The title compound was obtained in a similar manner to **12e** using 1-(4-cyanophenyl)-5-methyl-1*H*-pyrazole-4-carboxylic acid as a beige solid in 48% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.75–1.88 (1H, m), 1.90–2.05 (1H, m), 2.12–2.40 (7H, m), 2.44–2.57 (4H, m, overlapped with DMSO), 2.64 (3H, s), 2.74–2.88 (4H, m), 3.67 (1H, tt, *J* = 8.6, 8.6 Hz), 7.78–7.86 (2H, m), 8.03–8.10 (2H, m), 8.60 (1H, s), 12.13 (1H, s); MS (ESI) *m/z* 462 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{27}N_7OS \cdot 0.07C_4H_8O_2 \cdot 0.5H_2O$: C, 61.17, H, 6.04, N, 20.57, S, 6.73. Found: C, 61.53, H, 5.88, N, 20.64, S, 6.79.

5.3.27. *N*-[4-Cyclobutyl-5-(4-methylpiperazin-1-yl)-1,3-thiazol-2-yl]-1-[(4-fluorophenyl)methyl]-5-methyl-1*H*-pyrazole-4-carboxamide dihydrochloride (**13m**)

The title compound was prepared in a similar manner to **13a** using 1-[(4-fluorophenyl)methyl]-5-methyl-1*H*-pyrazole-4-carboxylic acid as a colorless solid in 39% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.74–2.06 (2H, m), 2.12–2.25 (2H, m), 2.25–2.40 (2H, m), 2.53 (3H, s), 2.81 (3H, d, *J* = 4.7 Hz), 2.97–3.31 (6H, m), 3.34–3.51 (2H, m), 3.70 (1H, tt, *J* = 8.6, 8.6 Hz), 5.36 (2H, s), 7.13–7.31 (4H, m), 8.37 (1H, s), 10.95 (1H, brs), 12.03 (1H, brs); MS (ESI) *m/z* 469 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{29}FN_6OS \cdot 2HCl \cdot 0.8H_2O$: C, 51.85, H, 5.91, N, 15.12, S, 5.77, Cl, 12.75, F, 3.42. Found: C, 52.15, H, 5.95, N, 15.23, S, 5.89, Cl, 12.41, F, 3.48.

5.3.28. Benzyl 4-(2-oxo-2-phenylethyl)piperidine-1-carboxylate (**18**)

To the stirred solution of benzyl 4-{2-[methoxy(methyl)amino]-2-oxoethyl}piperidine-1-carboxylate (310 mg, 0.97 mmol) in THF (6.2 mL) was added PhMgBr (2.0 M in THF, 968 μL, 1.9 mmol, 2.0 equiv.). The reaction mixture was stirred at rt for 3 hr. The reaction was quenched with sat. aq. NH_4Cl, and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column chromatography to give the title compound (175 mg, 0.52 mmol, 54%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.13–1.35 (2H, m), 1.78 (2H, d, *J* = 12.9 Hz), 2.14–2.26 (1H, m), 2.77–2.92 (2H, m), 2.89 (2H, d, *J* = 6.4 Hz), 4.18 (2H, brs), 5.12 (2H, s), 7.20–7.40

(5H, m, overlapped with chloroform), 7.43–7.50 (2H, m), 7.53–7.60 (1H, m), 7.91–7.97 (2H, m); MS (APCI/ESI) *m/z* 338 $[M+H]^+$.

5.3.29. Benzyl 4-(2-amino-4-phenyl-1,3-thiazol-5-yl)piperidine-1-carboxylate (**19**)

To a stirred solution of **18** (1.2 g, 3.6 mmol) in AcOH (20 mL) was added bromine (277 μL, 5.4 mmol, 1.5 equiv.) in AcOH (4.2 mL) dropwise at rt. The reaction mixture was stirred for 3 hr at the same temperature. The solvent was evaporated off, and residue was dissolved into EtOAc and washed with sat. aq. $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. To a solution of the residue in EtOH (20 mL) was added thiourea (300 mg, 3.9 mmol, 1.1 equiv). The whole mixture was refluxed for 1 hr. After cooling to rt, the reaction mixture was concentrated *in vacuo*, and water was added. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified on a silica gel column chromatography to give the title compound (224 mg, 0.57 mmol, 16%) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.93 (2H, d, J = 12.2 Hz), 2.79 (2H, t, J = 12.2 Hz), 3.11 (1H, tt, J = 12.2, 3.7 Hz), 4.26 (2H, brs), 4.78 (2H, s), 5.14 (2H, s), 7.28–7.50 (10H, m); MS (APCI/ESI) *m/z* 394 $[M+H]^+$.

5.3.30. Benzyl 4-[2-(3,5-dimethoxybenzamido)-4-phenyl-1,3-thiazol-5-yl]piperidine-1-carboxylate (**20**)

The title compound was obtained in similar manner to **7** using **19** as a pale yellow powder in 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.66–1.83 (2H, m), 1.98 (2H, d, J = 12.8 Hz), 2.82 (2H, t, J = 12.8 Hz), 3.21 (1H, tt, J = 11.3, 3.5 Hz), 3.81 (6H, s), 4.30 (2H, brs), 5.16 (2H, s), 6.62 (1H, t, J = 2.2 Hz), 6.97 (2H, d, J = 2.2 Hz), 7.29–7.48 (10H, m), 9.85 (1H, brs); MS (APCI/ESI) *m/z* 558 $[M+H]^+$.

5.3.31. 3,5-Dimethoxy-*N*-[5-(1-methylpiperidin-4-yl)-4-phenyl-1,3-thiazol-2-yl]benzamide (**21a**)

To a mixture of **20** (185 mg, 0.33 mmol), MeOH (2.0 mL) and DMF (2.0 mL) were added 10% Pd/C (50% wet, 400 mg) and AcOH (300 μL, 5.2 mmol, 16 equiv.). The whole was stirred under 3 atm of H_2 atmosphere at rt for 6 hr. The catalyst was removed by filtration and washed with MeOH. The filtrate was concentrated *in vacuo*. To the residue was added sat. aq. $NaHCO_3$, and the precipitate was collected by filtration, washed with H_2O and dried *in vacuo*.

To a mixture of the obtained product (101 mg, 0.24 mmol) and DMF (2.0 mL) was added 37% aq. formaldehyde (100 mg, 1.2 mmol, 5.2 equiv.), AcOH (68 μL, 1.2 mmol, 5.0 equiv.) and $NaBH(OAc)_3$ (252 mg, 1.2 mmol, 5.0 equiv.) on an ice water bath, and the whole was stirred at room temperature for 5 hr. To the reaction mixture was added sat. aq. $NaHCO_3$, and the mixture was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrate *in vacuo*. The residue was purified on a silica gel column chromatography ($CHCl_3$:MeOH), and recrystallized from a mixture of EtOAc and hexane to give the title compound (57 mg, 0.13 mmol, 55%) as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.62–1.75 (2H, m), 1.85–1.95 (4H, m), 2.18 (3H, s), 2.80–3.01 (3H, m), 3.82 (6H, s), 6.73 (1H, t, J = 2.3 Hz),

7.31 (2H, d, J = 2.3 Hz), 7.35–7.42 (1H, m), 7.44–7.52 (2H, m), 7.54–7.60 (2H, m), 12.61 (1H, brs); MS (ESI) *m/z* 438 $[M+H]^+$.

5.3.32. 5-(3-Chloropropyl)-4-phenyl-1,3-thiazol-2-amine hydrogen bromide (**24b**)

To a mixture of 5-chloro-1-phenylpentan-1-one (**22b**) (700 mg, 3.6 mmol), hydrobromic acid (1 drop), and AcOH (3.5 mL) was added Br_2 (185 μL, 3.6 mmol, 1.0 equiv.) over 5 minutes, and the mixture was stirred at rt for 1.5 hr. After the mixture was concentrated *in vacuo*, EtOH (3.0 mL) and thiourea (271 mg, 3.6 mmol, 1.0 equiv.) were added, and the mixture was stirred at reflux for 5 hr. After cooling to rt, the mixture was concentrated *in vacuo*. The residue was washed with EtOAc to give the title compound (1.1 g, 3.3 mmol, 93%) as a pale brown solid. MS (ESI) *m/z* 253 $[M+H]^+$.

5.3.33. *N*-[5-(3-Chloropropyl)-4-phenyl-1,3-thiazol-2-yl]-3,5-dimethoxybenzamide (**26b**)

The title compound was obtained in a similar manner to **7** using **24b** as a pale yellow solid in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.14–2.21 (2H, m), 3.07–3.13 (2H, m), 3.59 (2H, t, J = 6.4 Hz), 3.84 (6H, s), 6.65 (1H, t, J = 2.3 Hz), 7.01 (2H, d, J = 2.3 Hz), 7.32–7.37 (1H, m), 7.40–7.45 (2H, m), 7.52–7.56 (2H, m), 9.62 (1H, brs); MS (ESI) *m/z* 417 $[M+H]^+$.

5.3.34. *N*-{5-[3-(Dimethylamino)propyl]-4-phenyl-1,3-thiazol-2-yl}-3,5-dimethoxybenzamide dihydrochloride (**21b**)

A mixture of **26b** (140 mg, 0.34 mmol), dimethylamine (106 μL, 1.0 mmol, 3.0 equiv.), K_3PO_4 (71 mg, 0.34 mmol, 1.0 equiv.), KI (56 mg, 0.34 mmol, 1.0 equiv.) in DMF (2.0 mL) was stirred at 60 °C for 1 d. The mixture was concentrated *in vacuo*. The residue was purified on a silica gel column chromatography (NH_2 silica gel, hexane:EtOAc). To a solution of the obtained free product in EtOH was added conc. HCl (60 μL), and the mixture was allowed to stand at rt for 14 hr. The precipitate was collected by filtration, washed with EtOH, dried *in vacuo* to give the title compound (96 mg, 0.19 mmol, 57%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.01–2.11 (2H, m), 2.72 (6H, d, J = 4.9 Hz), 2.96 (2H, t, J = 7.6 Hz), 3.04–3.13 (2H, m), 3.83 (6H, s), 6.75 (1H, t, J = 2.4 Hz), 7.31 (2H, d, J = 2.4 Hz), 7.37–7.42 (1H, m), 7.45–7.52 (2H, m), 7.61–7.66 (2H, m), 10.45 (1H, brs), 12.68 (1H, brs); MS (ESI) *m/z* 426 $[M+H]^+$; Anal. Calcd. for $C_{23}H_{27}N_3O_3S{\cdot}2HCl{\cdot}0.3C_2H6O{\cdot}H_2O$: C, 53.45, H, 6.23, N, 7.92, S, 6.05, Cl, 13.37. Found: C, 53.67, H, 6.20, N, 7.88, S, 6.04, Cl, 13.20.

5.3.35. 3,5-Dimethoxy-*N*-{4-phenyl-5-[3-(piperidin-1-yl)propyl]-1,3-thiazol-2-yl}benzamide dihydrochloride (**21c**)

The title compound was obtained in a similar manner to **21b** using piperidine as a colorless solid in 72% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.29–1.44 (1H, m), 1.64–1.84 (5H, m), 2.05–2.19 (2H, m), 2.75–2.89 (2H, m), 2.95 (2H, t, J = 7.6 Hz), 3.00–3.10 (2H, m), 3.39 (2H, d, J = 12 Hz), 3.83 (6H, s), 6.75 (1H, t, J = 2.4 Hz), 7.31 (2H, d, J = 2.4 Hz), 7.36–7.42 (1H, m), 7.44–7.52 (2H, m), 7.61–7.67 (2H, m), 10.33 (1H, brs), 12.68 (1H, brs); MS (ESI) *m/z* 466 $[M+H]^+$.

5.3.36. 5-[3-(Morpholin-4-yl)propyl]-4-phenyl-1,3-thiazol-2-amine (**26d**)

A mixture of 5-(3-chloropropyl)-4-phenyl-1,3-thiazol-2-amine (242 mg, 0.96 mmol), morpholine (0.25 mL, 2.9 mmol, 3.0 equiv.) and DMF (3.5 mL) was stirred at 60 °C for 1 d. To the mixture was added morpholine (0.10 mL, 1.1 mmol, 1.2 equiv.), and the mixture was stirred on the same bath for 15 hr. After allowing to rt, to the mixture were added H_2O and sat. aq. $NaHCO_3$, and the mixture was extracted with EtOAc. The combined organic layers were washed with H_2O, sat. brine, dried over anhydrous Na_2SO_4, then concentrated *in vacuo*. The residue was washed with Et_2O to give the title compound (203 mg, 0.67 mmol, 70%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.68 (2H, tt, J = 7.4, 7.4 Hz), 2.18–2.34 (6H, m), 2.75 (2H, t, J = 7.4 Hz), 3.47–3.57 (4H, m), 6.75 (2H, s), 7.25–7.30 (1H, m), 7.34–7.42 (2H, m), 7.48–7.56 (2H, m); MS (ESI) *m/z* 304 $[M+H]^+$.

5.3.37. 3,5-Dimethoxy-*N*-{5-[3-(morpholin-4-yl)propyl]-4-phenyl-1,3-thiazol-2-yl}benzamide (**21d**)

The title compound was obtained in a similar manner to **7** using **26d** as a colorless solid in 26% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.79 (2H, tt, J = 7.2, 7.2 Hz), 2.22–2.35 (6H, m), 2.94 (2H, t, J = 7.5 Hz), 3.48–3.58 (4H, m), 3.83 (6H, s), 6.73 (1H, t, J = 2.3 Hz), 7.31 (2H, d, J = 2.2 Hz), 7.34–7.40 (1H, m), 7.41–7.51 (2H, m), 7.61–7.69 (2H, m), 12.61 (1H, s); MS (ESI) *m/z* 468 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{29}N_3O_4S \cdot 0.5H_2O$: C, 63.00, H, 6.34, N, 8.82, S, 6.73. Found: C, 62.84, H, 6.12, N, 8.68, S, 6.68.

5.3.38. 3,5-Dimethoxy-*N*-{5-[3-(4-methylpiperazin-1-yl)propyl]-4-phenyl-1,3-thiazol-2-yl}benzamide trihydrochloride (**21e**)

The title compound was obtained in a similar manner to **21b** using *N*-methylpiperazine as a colorless solid in 53% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.09–2.24 (2H, m), 2.82 (3H, s), 2.93–3.04 (2H, m), 3.14–3.93 (10H, m), 3.83 (6H, s), 6.74 (1H, t, J = 2.3 Hz), 7.32 (2H, d, J = 2.2 Hz), 7.36–7.42 (1H, m), 7.44–7.52 (2H, m), 7.59–7.68 (2H, m), 11.74–12.97 (2H, m); MS (ESI) *m/z* 481 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{32}N_4O_3S \cdot 2.9HCl \cdot 2H_2O$: C, 50.17, H, 6.30, N, 9.00, S, 5.15, Cl, 16.52. Found: C, 50.13, H, 6.44, N, 8.84, S, 5.13, Cl, 16.42.

5.3.39. 3,5-Dimethoxy-*N*-{4-phenyl-5-[2-(piperidin-1-yl)ethyl]-1,3-thiazol-2-yl}benzamide dihydrochloride (**21f**)

The title compound was obtained in a similar manner to **7** and then **21b**, whereas excessive piperidine was used without any other co-solvent, using 5-(2-chloroethyl)-4-phenyl-1,3-thiazol-2-amine hydrobromide (**24f**) in 37% total yield over 2 steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.30–1.44 (1H, m), 1.65–1.85 (5H, m), 2.85–2.97 (2H, m), 3.29–3.42 (4H, m), 3.45–3.53 (2H, m), 3.83 (6H, s), 6.75 (1H, t, J = 2.3 Hz), 7.31 (2H, d, J = 2.3 Hz), 7.39–7.44 (1H, m), 7.47–7.53 (2H, m), 7.65–7.70 (2H, m), 10.15 (1H, brs), 12.75 (1H, brs); MS (ESI) *m/z* 452 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{29}N_3O_3S \cdot 1.95HCl \cdot 0.1C_2H_6O \cdot 2.3H_2O$: C, 53.22, H, 6.41, N, 7.39, S, 5.64, Cl, 12.16. Found: C, 53.14, H, 6.33, N, 7.37, S, 5.65, Cl, 12.08.

5.3.40. 3,5-Dimethoxy-*N*-{4-phenyl-5-[(piperidin-1-yl)methyl]-1,3-thiazol-2-yl}benzamide hydrogen chloride (**21g**)

A mixture of 3,5-dimethoxy-*N*-(4-phenyl-1,3-thiazol-2-yl)benzamide (**29**) (120 mg, 0.35 mmol), piperidine (73 μL, 0.74 mmol, 2.1 equiv.), 36% aq. formaldehyde (42 μL, 0.56 mmol, 1.6 equiv.) and AcOH (2.0 mL) was stirred at 80 °C for 18 hr. The reaction mixture was concentrated *in vacuo*, and sat. aq. $NaHCO_3$ was added. The mixture was extracted with $CHCl_3$. The organic layer was dried over anhydrous $MgSO_4$, then concentrated *in vacuo*. The residue was purified on a silica gel column chromatography ($CHCl_3$:MeOH). The obtained crude free product was washed with EtOH. After 4 M HCl in EtOAc (0.15 mL) was added to the suspension of the resultant solid, the mixture was concentrate *in vacuo*. The residue was recrystallized from EtOH to give the title compound (116 mg, 0.24 mmol, 69%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.21-1.42 (1H, m), 1.53–1.78 (5H, m), 2.69–2.84 (2H, m), 3.19–3.31 (2H, m), 3.83 (6H, s), 4.56 (2H, d, J = 4.7 Hz), 6.77 (1H, t, J = 2.2 Hz), 7.32 (2H, d, J = 2.3 Hz), 7.43–7.57 (3H, m), 7.60–7.67 (2H, m), 10.01 (1H, brs), 12.97 (1H, s); MS (ESI) *m/z* 438 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{27}N_3O_3S \cdot HCl \cdot 0.3H_2O$: C, 60.13, H, 6.01, N, 8.76, S, 6.69, Cl, 7.39. Found: C, 60.19, H, 5.91, N, 8.83, S, 6.64, Cl, 7.44.

5.3.41. 4-Cyclobutyl-5-[2-(piperidin-1-yl)ethyl]-1,3-thiazol-2-amine (**27**)

In a 1000ml three neck flask which was fitted with a thermometer, a dropping funnel and a reflux condenser, to a mixture of 4-chloro-1-cyclobutylbutan-1-one (**23**) (36 g, 222 mmol), 20% HBr in EtOH (9.0 mL) and MeOH (131 mL) was added bromine (11.4 mL, 222 mmol, 1.0 equiv.) on an ice-salt water bath over 5 minutes. It was stirred for 1 hr at 0 °C, then it was allowed to rt and stirred for 1.5 hr. H_2O was added and stirred for 1hr. It was diluted with H_2O, and then extracted with Et_2O. The combined organic layers were washed with 10% aq. K_2CO_3, sat. brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. To the residue, thiourea (18 g, 233 mmol, 1.1 equiv.) and EtOH (130 mL) were added, and the mixture was refluxed for 12 hr under Ar. The reaction mixture was cooled to rt, passed through a Kiriyama filter, and then was concentrated *in vacuo*. The residue was diluted with EtOAc, and the organic layer was washed with sat. aq. $NaHCO_3$. The two phases were separated, and the aq. phase was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and then concentrated *in vacuo*. The residue was purified on a silica gel column chromatography (hexane:EtOAc) to give the intermediate (**25**) (14 g, 65 mmol).

A mixture of the obtained intermediate (**25**) (5 g, 23 mmol), piperidine (9.8 g, 115 mmol, 5.0 equiv.) and DMF (60 mL) was stirred at 60 °C for 1 d. H_2O was added to the mixture, and the whole was stirred at rt for 14 hr. The precipitation was collect by filtration, and was washed with IPE to give the title compound (3.3 g, 12 mmol, 16%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.28–1.53 (6H, m), 1.68–1.95 (2H, m), 2.00–2.12 (2H, m), 2.15–2.39 (8H, m), 2.60 (2H, t, J = 7.2 Hz), 3.38 (1H, tt, J = 8.6, 8.6 Hz), 6.57 (2H, s); MS (ESI) *m/z* 266 $[M+H]^+$.

5.3.42. *N*-{4-Cyclobutyl-5-[2-(piperidin-1-yl)ethyl]-1,3-thiazol-2-yl}-1-(4-fluorophenyl)-5-methyl-

1*H*-pyrazole-4-carboxamide dihydrochloride (**28a**)

To a mixture of **27** (330 mg, 1.2 mmol), 1-(4-fluorophenyl)-5-methyl-1*H*-pyrazole-4-carboxylic acid (329 mg, 1.5 mmol, 1.2 equiv.) and NMP (6.6 mL) was added DIPEA (482 mg, 3.7 mmol, 3.0 equiv.) and HATU (567 mg, 1.5 mmol, 1.2 equiv.), and the whole was stirred at 70 °C for 1 d. The reaction was quenched with sat. aq. $NaHCO_3$, and the mixture was extracted with EtOAc. The organic layer was concentrated *in vacuo*. The resulting crude product were purified on a silica gel column chromatography. The obtained residue was dissolved in 4 M HCl dioxane, and then was concentrated *in vacuo*. The residue was recrystallized from IPA to give the title compound (297 mg, 0.55 mmol, 44%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.29–1.45 (1H, m), 1.67–1.92 (6H, m), 1.92–2.08 (1H, m), 2.16–2.30 (2H, m), 2.30–2.43 (2H, m), 2.55 (3H, s), 2.79–2.99 (2H, m), 3.04–3.29 (4H, m), 3.43–3.57 (2H, m), 3.71 (1H, tt, J = 8.6, 8.6 Hz), 7.37–7.47 (2H, m), 7.57–7.66 (2H, m), 8.56 (1H, s), 10.56 (1H, brs), 12.29 (1H, brs); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 11.59, 17.81, 19.59, 21.38, 22.23, 25.39, 28.45, 32.94, 51.68, 56.19, 61.90, 113.31, 116.11 (d, J = 23.2 Hz), 117.72, 127.62 (d, J = 9.1 Hz), 134.73 (d, J = 2.7 Hz), 139.65, 143.81, 149.93, 155.56, 160.56, 160.71, 162.51; MS (ESI) *m/z* 468 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{30}FN_5OS \cdot 2HCl \cdot 0.5H_2O$: C, 54.64, H, 6.05, N, 12.74, S, 5.83, Cl, 12.90, F, 3.46. Found: C, 54.28, H, 6.12, N, 12.46, S, 5.75, Cl, 12.82, F, 3.38.

5.3.43. *N*-{4-Cyclobutyl-5-[2-(piperidin-1-yl)ethyl]-1,3-thiazol-2-yl}-1-(5-fluoropyridin-2-yl)-5-methyl-1*H*-pyrazole-4-carboxamide dihydrochloride (**28b**)

The title compound was obtained in a similar manner to **28a** using 1-(5-fluoropyridin-2-yl)-5-methyl-1*H*-pyrazole-4-carboxylic acid as a colorless solid in 60% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.31–1.46 (1H, m), 1.67–1.90 (6H, m), 1.92–2.07 (1H, m), 2.19–2.29 (2H, m), 2.30–2.42 (2H, m), 2.81 (3H, s), 2.85–2.95 (2H, m), 3.07–3.15 (2H, m), 3.16–3.24 (2H, m), 3.42–3.59 (2H, m), 3.66–3.74 (1H, m), 7.89 (1H, dd, J = 4.1, 9.0 Hz), 8.03 (1H, td, J = 3.0, 8.5 Hz), 8.58 (1H, s), 8.60 (1H, d, J = 3.0 Hz), 10.68 (1H, brs), 12.31 (1H, brs); ; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 12.44, 17.80, 19.60, 21.38, 22.24, 25.39, 28.45, 32.95, 51.70, 56.20, 61.90, 114.46, 117.81, 119.78 (d, J = 5.5 Hz), 126.64 (d, J = 20.5 Hz), 135.65 (d, J = 26.4 Hz), 140.29, 144.47, 148.16 (d, J = 3.0 Hz), 150.02, 155.46, 157.12, 159.14, 160.61; MS (ESI) *m/z* 469 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{29}FN_6OS \cdot 2HCl \cdot 0.5H_2O \cdot 0.6C_3H_7O$: C, 52.88, H, 6.23, N, 14.34, S, 5.47, Cl, 12.10, F, 3.24. Found: C, 52.47, H, 6.34, N, 14.06, S, 5.35, Cl, 11.89, F, 3.21.

5.3.44. *N*-{4-Cyclobutyl-5-[2-(piperidin-1-yl)ethyl]-1,3-thiazol-2-yl}-1-(5-fluoropyridin-2-yl)-3-methyl-1*H*-pyrazole-4-carboxamide dihydrochloride (**28c**)

The title compound was obtained in a similar manner to **28a** using 1-(5-fluoropyridin-2-yl)-3-methyl-1*H*-pyrazole-4-carboxylic acid as a colorless solid in 44% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.31–1.46 (1H, m), 1.66–1.92 (6H, m), 1.93–2.07 (1H, m), 2.19–2.42 (4H, m), 2.83–2.97 (2H, m), 3.07–3.25 (4H, m), 3.45–3.57 (2H, m), 3.70 (1H, tt, J = 8.6, 8.6 Hz), 7.95–8.00 (2H, m), 8.55–8.59 (1H, m), 9.61 (1H, s), 10.47 (1H, brs), 12.40 (1H, brs); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 13.67, 17.84, 19.62, 21.38, 22.24, 28.46, 32.97, 51.71, 56.21, 113.75 (d, J = 5.0 Hz), 114.90,

117.75, 126.81 (d, J = 20.4 Hz), 129.59, 136.16 (d, J = 26.4 Hz), 146.44 (d, J = 2.5 Hz), 150.09, 152.70, 155.38, 156.89, 158.88, 160.35; MS (ESI) *m/z* 469 $[M+H]^+$; Anal. Calcd. for $C_{24}H_{29}FN_6OS \cdot 1.9HCl \cdot 2.1H_2O$: C, 50.07, H, 6.15, N, 14.60, S, 5.57, Cl, 11.70, F, 3.30. Found: C, 49.84, H, 6.12, N, 14.44, S, 5.59, Cl, 11.76, F, 3.31.

6. Acknowledgements

The corresponding author would like to thank all the staff members of Astellas Research Technologies Co., Ltd. for generating the data that supported this research. Special gratitude is due towards Dr. Susumu Watanuki, Mr. Hiroyuki Kaizawa, Dr. Makoto Oku and Dr. Ayako Moritomo for their invaluable advices on the manuscript preparation.

7. Declarations of interest

The authors declare no conflict of interest.

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Graphical Abstract