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Novel potent and selective Ca^{2+} release-activated Ca^{2+} (CRAC) channel inhibitors. Part 3: Synthesis and CRAC channel inhibitory activity of 4′-[(trifluoromethyl)pyrazol-1-yl]carboxanilides

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ABSTRACT

From a series of 4'-[(trifluoromethyl)pyrazol-1-yl]carboxanilides derived from 4-methyl-4'-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide, one inhibited thapsigargin-induced Ca^{2+} influx in Jurkat T cells (IC_{50} = 77 nM) and exhibited high selectivity for the CRAC channel over the VOC channel (index: >130). Another acted as an inhibitor for both T lymphocyte activation-induced diseases and ovalbumin-induced airway eosinophilia in rats (ED_{50} = 1.3 mg/kg) *p.o.*

1. Introduction

It is well-established that intracellular Ca^{2+} plays an important role in various cellular functions, and that its concentration is regulated by Ca^{2+} influx through Ca^{2+} channels on the cell membrane. Ca^{2+} channels, which are located in the nervous, endocrine, cardiovascular, and skeletal systems and are modulated by membrane potential, are called voltage-operated Ca^{2+} (VOC) channels. These channels are classified into L, N, P, Q, R, and T subtypes.[1] Excessive Ca^{2+} influx through the VOC channels causes hypertension and brain dysfunction.

In contrast, Ca^{2+} channels on inflammatory cells, including lymphocytes, mast cells, and neutrophils, can be activated regardless of their membrane potential. This type of Ca^{2+} channel has been reported to act in the crisis and exacerbation of inflammation and autoimmune diseases.[2] In the T cells, it has been reported that the early stages of activation consist of pre- and post-Ca^{2+} events.[3] The stimulation of T cell receptors induces pre-Ca^{2+} events, including the generation of IP_3, followed by the release of Ca^{2+} from the endoplasmic reticulum (ER).[4] In post-Ca^{2+} events, depletion of Ca^{2+} in the ER induces the activation of Ca^{2+} release-activated Ca^{2+} (CRAC) channels, and capacitative Ca^{2+} influx through the CRAC channel sustains high intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$).[5] This prolonged high $[Ca^{2+}]_i$ activates cytosolic signal transduction to produce lipid mediators (e.g., LTD_4), cytokines [e.g., interleukin-2 (IL-2)], and matrix metalloproteinases, which participate in the pathogenesis of inflammation and autoimmune diseases.

These facts suggest that CRAC channel inhibitors can be useful for the treatment of diseases caused by the activation of inflammatory cells without side effects observed in steroids.[6] Since VOC channel inhibitors would cause adverse events in the nervous and cardiovascular systems, it may be necessary for CRAC channel inhibitors to exhibit sufficient selectivity over VOC channels if they are to be used as anti-inflammatory drugs. In the last decade, SK&F 96365 (**1**),[7] econazole (**2**),[8] and L-651582 (**3**)[9] have been reported to inhibit the CRAC channel (Fig. 1); however, their potency and selectivity over VOC channels was not sufficient for use as anti-inflammatory drugs.

Our effort to discover novel CRAC channel inhibitors led to the discovery of a series of aryl-3-trifluoromethylpyrazoles, including compounds **4–6** (Fig. 2), that showed potent and selective CRAC channel inhibitory activity.[10] Among these, 4-methyl-4'-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide (**6**)[10,11] was found to have inhibitory activity (IC_{50}: 0.15 μM, selectivity index: 31) selective for the CRAC channel over the VOC channel. Although similar carboxanilide derivatives, including

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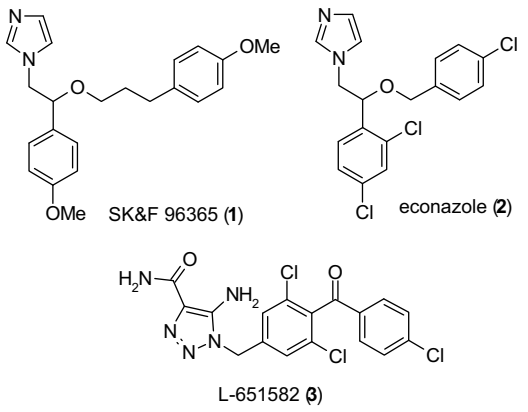


Figure 1. Structures of SK&F 96365 (**1**), econazole (**2**), L-651582 (**3**).

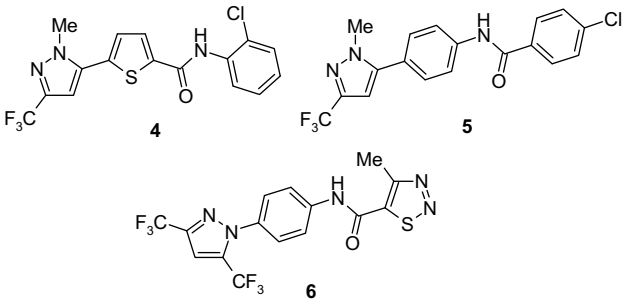
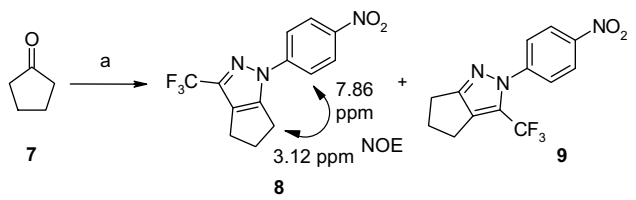


Figure. 2. Pyrazole derivatives **4**–**6** identified as CRAC channel inhibitors.

compound 6, were recently reported to inhibit the transcriptional activity of nuclear factor in activated T cells,[12] inhibition of CRAC channels was not observed. In addition, compound 6 inhibited concanavalin A-induced liver injury and trinitrochlorobenzene (TNCB)-induced ear swelling in mice with ED_{50} values of 0.61 and 1.1 mg/kg *p.o.*, respectively.[10] We believe that compound **6** should be considered a lead compound in the discovery of potent and selective CRAC channel inhibitors, and designed some carboxanilides substituting 3,5-bis(trifluoromethyl)-1*H*-pyrazole and 4-methyl-1,2,3-thiadiazole moieties as other pyrazoles and heteroaryl groups, respectively. In this paper, we describe the synthesis and the structure–activity relationships (SARs) of 4'-[(trifluoromethyl)pyrazol-1-yl]carboxanilide derivatives as novel CRAC channel inhibitors.

2. Chemistry

The routes of synthesis for intermediates **11a** and **b** are shown in Schemes 1 and 2. The cyclopentapyrazoles **8** and **9** were obtained via the trifluoroacetylation of cyclopentanone **7** with ethyl trifluoroacetate in the presence of sodium methoxide (NaOMe),



Scheme 1. Reagents: (a) i—CF_3CO_2Et, NaOMe, EtOH; ii—4-$NO_2C_6H_4NHNH_2$, concd HCl, EtOH.

followed by cyclization with 4-nitrophenylhydrazine (Scheme 1). The structure of compound **8** was determined by observing the nuclear Overhauser effect (NOE) between the protons of the methylene group (3.12 ppm) and the benzene (7.86 ppm, Scheme 1). The hydrogenation of compound **8** and 5-methyl-1-(4-nitrophenyl)-3-(trifluoromethyl)pyrazole (**10**)[13] yielded the anilines **11a** and **b**, respectively (Scheme 2).

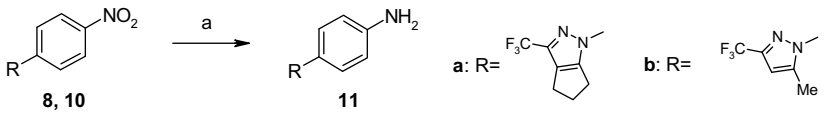
The desired 4'-[(trifluoromethyl)pyrazol-1-yl]carboxanilides (**13**) were prepared from the anilines (**11**) via the methods depicted in Schemes 3–7. The anilines (**11**) were converted into compounds **13a**–**z** via treatment with the carboxylic acids (**12**) in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide·HCl (EDC HCl) (Method A, Scheme 3) or EDC HCl and 1-hydroxybenzotriazole hydrate (HOBT) (Method B). The acid chlorides were prepared from the corresponding carboxylic acids (**12**) via treatment with oxalyl chloride and catalytic amounts of dimethylformamide (DMF), after which they were coupled with the anilines (**11**) in the presence of triethylamine (Et_3N) to yield the desired anilides (**13**) (Method C). The coupling reaction of the corresponding acids (**12**) and anilines (**11**) afforded the anilides (**13**) in the presence of *O*-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU) and Et_3N (Method D). The imidazole-4-carboxanilide (**14**) and 5-aminonicotinanilide (**15**) derivatives were obtained by deprotecting the trityl and *t*-butoxycarbonyl groups in compounds **13y**[14] and **13z**,[15] respectively (Schemes 4 and 5). The 4-chloronicotinanilide derivative (**13n**) was reacted with methanol (MeOH) under acidic conditions to yield the 4-methoxy derivative (**16**), and amination of compound 13n in liquid ammonia yielded the 4-amino derivative (**17**) (Scheme 6). Acidic treatment of compounds **13n** and **13p** yielded the 4-hydroxy (**18**, Scheme 6) and 2-hydroxy (**19**, Scheme 7) derivatives, respectively.

3. Results and discussion

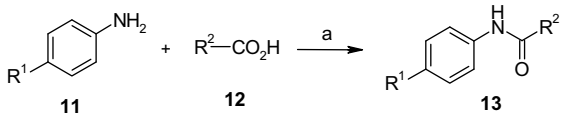
The synthesized compounds were evaluated for their inhibition of Ca^{2+} influx through the CRAC and VOC channels on Jurkat T cells[16] and PC12-h5 cells,[17] respectively. The results are summarized in Table 1.

We first investigated the effect of substituents in the pyrazole moiety of compound **6** on CRAC channel inhibitory activity and selectivity over VOC channels (Table 1). Removal of the 5-trifluoromethyl group (**13a**) dramatically reduced CRAC channel inhibitory activity. In contrast, the 5-methyl derivative **13b** was equipotent to compound 6, which suggested that the trifluoromethyl and methyl groups would be interchangeable at this position. These results indicated that the substituents at the 5-position, neighboring the benzene ring, might be essential for CRAC channel inhibition. Introduction of the larger tolyl group (**13c**) reduced both the activity and selectivity of the compounds. The cyclopentapyrazole derivative (**13d**) showed less potent activity and lower selectivity than compound **6**. This indicated that the trifluoromethyl or small alkyl groups at the 5-position on the pyrazole ring were favorable for inhibiting CRAC channels, and that bulky groups may interfere with interactions with target molecules. In contrast, compounds **13e–g**, which were substituted with electron-withdrawing groups for the 5-trifluoromethyl group, exhibited potent and selective CRAC channel inhibitory activity. In particular, the chloro (**13e**) and bromo (**13f**) derivatives potently inhibited the CRAC channel with IC_{50} values of 0.082 and 0.093 μM, respectively.

To investigate the SARs in detail, we attempted to calculate the torsion angles between the pyrazole and benzene rings for compounds **6**, **13a–g** using the MOPAC/AM1 program, and then compared these values with the IC_{50} values of the CRAC channel (Table 2). The torsion angle of the inactive compound (**13a**) was 30 degrees. In contrast, the substituents at the 5-position in com-



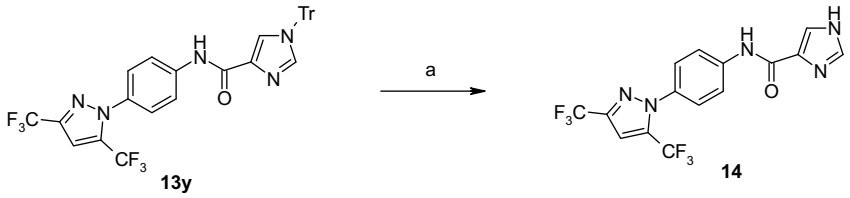
Scheme 2. Reagents: (a) H_2, Pd–C, EtOH.



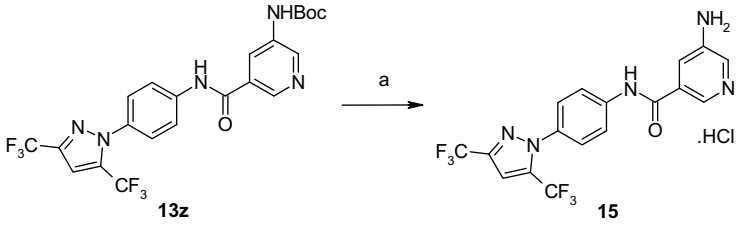
Scheme 3. Reagents: (a) Method A: EDC·HCl, THF; Method B: EDC·HCl, HOBT, DMF; Method C: $(COCl)_2$, cat. DMF, CH_2Cl_2, and then Et_3N, CH_2Cl_2; Method D: HBTU, Et_3N, DMF.

torsion between the pyrazole and benzene rings is necessary for CRAC channel inhibition.

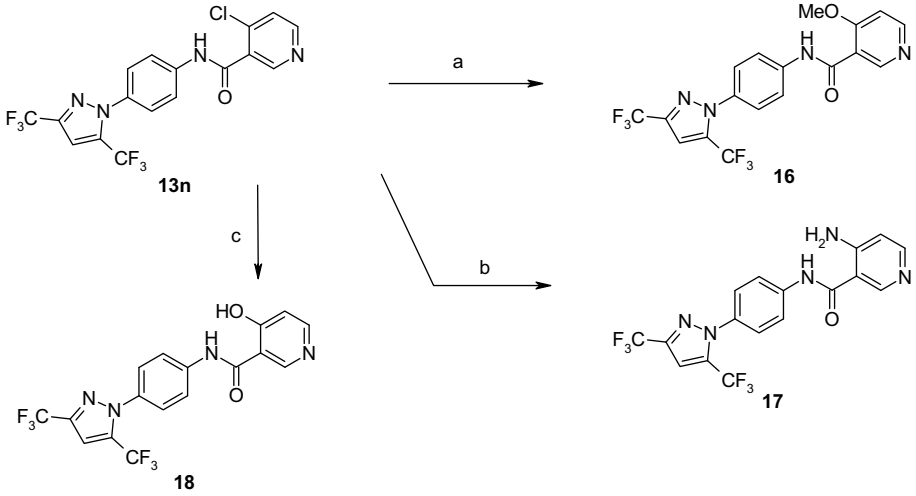
Second, we investigated the effect of substituting various heteroaryl groups for the 4-methyl-1,2,3-thiadiazole moiety of compound **6** (Table 3). In the series of compounds possessing 5-membered heteroaryl rings (**13h**, **13i**, **14**), the thiazolyl derivatives (**13h**, **13i**) were less active than compound **6**, and the imidazolyl group (**14**) dramatically decreased the activity. Although the 2-pyr-



Scheme 4. Reagents: (a) concd HCl, acetone.



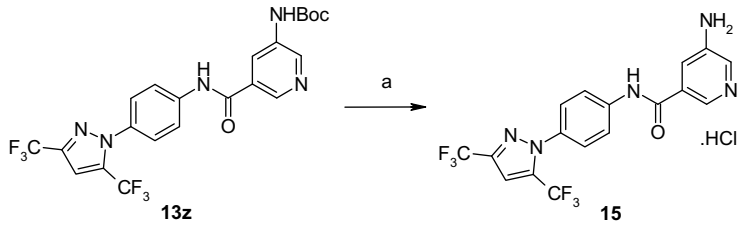
Scheme 5. Reagents: (a) CF_3CO_2H, CH_2Cl_2, and then HCl, AcOEt.



Scheme 6. Reagents: (a) HCl, AcOEt, MeOH; (b) liq. NH_3; (c) aq HCl, dioxane.

pounds 6, **13b–g**, which showed CRAC channel inhibitory activity with IC_{50} values less than 10^{-6} M, generated torsion angles greater than 40 degrees. The results indicated that these substituents may be sterically repulsed by the phenyl group, and that the resultant

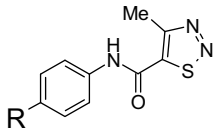
idyl derivative **13j** showed no inhibitory activity against the CRAC channel, substitution of the 3- and 4-pyridyl groups (**13k**, **13l**) for the thiadiazole group retained the activity and improved selectivity for the CRAC channel over the VOC channel. Compound **13k**, in

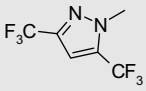
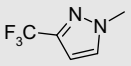
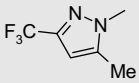
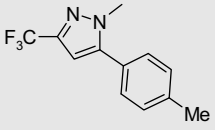
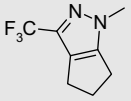
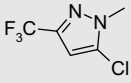
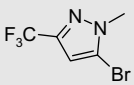
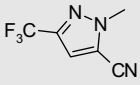


Scheme 7. Reagents: (a) HCl, AcOEt.

particular, showed almost no VOC channel inhibitory activity, which meant that CRAC channel selectivity was high (index: >40). Given this, we speculated that the amide moiety and the pyridine ring of compound **13j** would settle into a co-planar conformation through hydrogen bonding between the amide proton and the nitrogen atom of the pyridine ring, and that this conformation may be unfavorable for CRAC channel inhibition. This speculation is supported by previous results.[10]

Table 1
Biological Properties of the 4-methyl-1,2,3-thiadiazole-5-carboxanilides



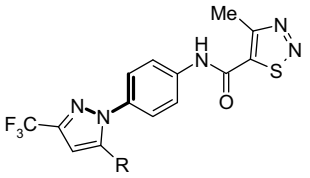
Compound	R	IC_{50} (μM)		CRAC channel selectivity[c]
		CRAC[a]	VOC[b]	
6		0.15	4.7	31
13a		40%[d]	NT[e]	—
13b		0.22	46%[d]	>45
13c		0.72	2.5	3.5
13d		0.40	3.5	8.8
13e		0.082	2.9	35
13f		0.092	2.6	28
13g		0.22	45%[d]	>45

[a] The inhibition of Ca^{2+} influx through the CRAC channels on Jurkat T-cells. See Section 5.
[b] The inhibition of Ca^{2+} influx through the VOC channels on PC12-h5 cells. See Section 5.
[c] IC_{50} to VOC channel/IC_{50} to CRAC channel.
[d] % inhibition at 10 μM.
[e] Not tested.

Next, we examined the effect of substituents in the 3-pyridyl group of compound **13k**. In the series of compounds possessing substituents at the 4-position (**13m–o**, **16–18**), the methyl (**13m**), chloro (**13n**), and methoxy (**16**) derivatives exhibited potent and selective CRAC channel inhibitory activity. The trifluoromethyl derivative (**13o**) was almost as potent as compound **13k**; however, its selectivity for the CRAC channel was not sufficient. Substitution with the amino group (**17**) resulted in decreased activity, and the hydroxy derivative (**18**) exhibited no CRAC channel inhibitory activity. Similar substituent effects were observed in the series of compounds substituted at the 2-position (**13p**, **13q**, **19**) in the 3-pyridyl group. The chloro (**13p**) and methoxy (**13q**) derivatives exhibited potent CRAC channel inhibitory activity. In contrast, a more than 10-fold decrease in activity was observed for the hydroxy derivative **19**. We speculated that hydrogen bonding would exist between the carbonyl groups of the amide moiety and the protons of the hydroxy or amino groups in compounds **17–19**, and that the resultant conformations may cause a loss of CRAC channel inhibitory activity. This speculation is supported by the fact that the 5-amino derivative (**15**) was equipotent to compound **13k**. Among the 5-substituted nicotinanilides (**13r-u**, **15**), the 5-fluoro derivative **13s** (IC_{50} = 0.077 μM) was a 3-fold more potent CRAC channel inhibitor than compound **13k**. This compound also showed no inhibitory activity against the VOC channel at 10 μM, which resulted in excellent selectivity for the CRAC channel (index: >130). The chloro (**13t**) and bromo (**13u**) groups also improved CRAC channel inhibitory activity and selectivity. These results implied that the introduction of halogens at the 5-position would result in potent and selective CRAC channel inhibitory activity. This activity was maintained in compounds **13r** and **13v** after introduction of a methyl group at the 5- and 6-positions, respectively. In compounds with further substitutions, the 4,6-dimethyl derivative (**13w**) maintained activity, but compound **13x**, which possessed two methyl groups at the 2- and 4-positions, was less potent than the other methylated derivatives.

Selected compounds with potent and selective CRAC channel inhibitory activity were evaluated for their ability to inhibit phytohemagglutinin (PHA)-induced IL-2 production in Jurkat T cells[7] (Table 4). Compound **6** showed potency with an IC_{50} value of 17 nM. The methyl (**13b**), chloro (**13e**), and bromo (**13f**) derivatives also demonstrated potent inhibitory activity against IL-2 production; in particular, the 5-bromo-3-trifluoromethylpyrazole derivative (**13f**) exhibited highly potent activity with an IC_{50} value of 5.9 nM. In the series of compounds possessing pyridine rings, the 4-pyridyl derivative (13l) was as potent as compound 6; however, the 3-pyridyl derivative **13k** was 5-fold less potent than compound **6**. In the 3-pyridyl group, substituents such as halogens (**13n**, **13s–u**) and methyl groups (**13w**) improved the activity, which resulted in compounds as potent as compound **6**. In contrast, the introduction of an amino group (**15**) decreased in the activity. It is known that IL-2 plays an important role in the activation, cell cycle progression, and proliferation of T cells; thus, these results imply that the function of T lymphocytes would be inhibited by CRAC channel inhibitors in vitro.

Table 2
Torsion angle and CRAC channel inhibitory activity of the 4-methyl-1,2,3-thiadiazole-5-carboxanilides

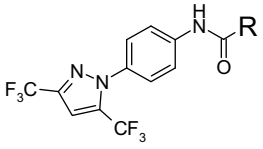


Compound	CRAC IC_{50} (μM)[a]	Torsion angle (deg.)[b]
6	0.15	55
13a	40%[d]	30
13b	0.22	43
13c	0.72	46
13d	0.40	60
13e	0.082	52
13f	0.092	57
13g	0.22	42

[a] See footnote *a* in Table 1.
[b] Torsion angle between the N–N bond in the pyrazole and the C–C bond in the benzene (bold in scheme).

These compounds were also tested in a delayed-type hypersensitivity (DTH) model[19] (Table 3). Substitution of the methyl (**13b**), chloro (**13e**), and bromo (**13f**) groups for the trifluoromethyl (**6**) group at the 5-position in the pyrazole moiety tended to reduce activity in vivo. However, several nicotinanilide derivatives were found to inhibit TNCB-induced ear swelling in sensitized mice effectively at a dose of 30 mg/kg *p.o.* Selected compounds (**13k**, **13w**, **15**) were further evaluated for their inhibitory activity against ovalbumin (OA)-induced airway eosinophilia in rats.[20] Among the compounds tested, **13w** was found to be the most potent inhibitor, with an ED_{50} value of 1.3 mg/kg *p.o.* Airway eosinophilia is widely studied as a disease model of asthma, and compounds **6** and **13w**, which are expected to exhibit potent inhibitory activity in this model, could become candidates for novel anti-asthmatic agents.[21]

Table 3
Biological Properties of the 4′-[3,5-bis(trifluoromethyl)pyrazol-1-yl]carboxanilides



Compound.	R	IC_{50} (μM)		CRAC channel selectivity[c]
		CRAC[a]	VOC[b]	
6	4-Methyl-1,2,3-thiadiazol-5-yl	0.15	4.7	31
13h	Thiazol-5-yl	0.96	10	10
13i	Thiazol-2-yl	0.46	37%[d]	>22
14	1*H*-imidazol-4-yl	29%[d]	NT[e]	—
13j	Pyridin-2-yl	14%[d]	NT[e]	—
13k	Pyridin-3-yl	0.25	30%[d]	>40
13l	Pyridin-4-yl	0.24	10	42
13m	4-Methylpyridin-3-yl	0.29	6.3	20
13n	4-Chloropyridin-3-yl	0.16	5.1	32
13o	4-Trifluoromethylpyridin-3-yl	0.43	2.0	5
16	4-Methoxypyridin-3-yl	0.23	4.8	21
17	4-Aminopyridin-3-yl	0.78	2.4	3.1
18	4-Hydroxypyridin-3-yl	21%[d]	NT[e]	—
13p	2-Chloropyridin-3-yl	0.24	4.5	19
13q	2-Methoxypyridin-3-yl	0.12	2.9	24
19	2-Hydroxypyridin-3-yl	3.3	NT[e]	—
13r	5-Methylpyridin-3-yl	0.21	3.1	15
13s	5-Fluoropyridin-3-yl	0.077	27%[d]	>130
13t	5-Chloropyridin-3-yl	0.11	31%[d]	>91
13u	5-Bromopyridin-3-yl	0.15	29%[d]	>67
15	5-Aminopyridin-3-yl	0.27	14%[d]	>37
13v	6-Methylpyridin-3-yl	0.22	27%[d]	>45
13w	4,6-Dimethylpyridin-3-yl	0.29	41%[d]	>34
13x	2,4-Dimethylpyridin-3-yl	1.6	NT[e]	—

[a–e] See the corresponding footnotes in Table 1.

4. Conclusion

Novel 4'-[(trifluoromethyl)pyrazol-1-yl]carboxanilides derived from compound **6** were designed and evaluated for their CRAC and VOC channel inhibitory activity. SARs on the pyrazole ring indicated that substituents would be required at the 5-position in order to inhibit the CRAC channel, and that the introduction of electron-withdrawing groups at this position (**13e–g**) would increase the activity. It was found that the steric repulsion between the pyrazole and benzene rings caused by introducing these substituents at the 5-position would be suitable for showing potent CRAC channel inhibitory activity. On the other hand, replacement of the 4-methyl-1,2,3-thiadiazole moiety of compound **6** with the 3- and 4-pyridyl groups (**13k**, **13l**) resulted in the inhibition of a potent and selective CRAC channel. In particular, the 5-halogenated nicotinanilides (**13s–u**) showed favorable potency and selectivity. Potent and highly selective CRAC channel inhibitors were also found to inhibit PHA-induced IL-2 production with IC_{50} values at the level of 10^{-8} to 10^{-9} M. In addition, some compounds were orally available and effective inhibitors in DTH models associated with T cell activation. Moreover, compound **13w** inhibited antigen-induced airway eosinophilia in rats as potently as compound **6**. We expect these CRAC channel inhibitors to become a new class of anti-inflammatory and immunosuppressive agents.

5. Experimental

Melting points were determined without correction using a Yanagimoto MP-3 melting point apparatus. ^{1}H NMR spectra were recorded on a JEOL JNM-LA300, a JEOL JNM-EX400, or a JEOL JNM-A500 spectrometer, and chemical shifts were measured in ppm using tetramethylsilane as an internal standard. The signal pattern abbreviations are as follows: s: singlet, br s: broad singlet, d: doublet, t: triplet, dd: double doublet, dt: double triplet, and m: multiplet. FAB-MS were recorded on a JEOL JMS-DX300, a JEOL JMS-DX2000, a HP 5970-MSD, a Fisons TRIO-1000, or a Finnigan mat TSQ 700 mass spectrometer. The elemental analyses were performed with a Yanako MT-5 microanalyzer and a Yokogawa IC-7000S ion chromatographic analyzer. The drying of organic solutions during workup was done over anhydrous $MgSO_4$. Column chromatography was performed with Wakogel C-200 or Merck silica gel 60.

5.1. 1-(4-Nitrophenyl)-3-(trifluoromethyl)-1,4,5,6-tetrahydrocyclopenta[*c*]pyrazole (8) and 2-(4-nitrophenyl)-3-(trifluoromethyl)-2,4,5,6-tetrahydrocyclopenta[*c*]pyrazole (9)

Ethyl trifluoroacetate (4.26 g, 30.0 mmol) was added to a mixture of NaOMe (1.78 g, 33.0 mmol) in Et_2O (30 mL) dropwise below 30 °C, then cyclopentanone (7, 2.52 g, 30.0 mmol) was added dropwise. The mixture was stirred for 24 h at room temperature, and then 1 M HCl was added. This mixture was extracted with AcOEt, washed with H_2O and brine, and then dried and concentrated *in vacuo*. The residue (4.71 g) was added to a mixture of 34-nitrophenylhydrazine (4.59 g, 30.0 mmol), concentrated HCl

Table 4
Biological Properties of Selected Compounds

Compound	IC_{50} (μM) CRAC[a]	IC_{50} (nM) IL-2[b]	DTH[c] % inhibition at 30 mg/kg *p.o.*	Eosinophilia[d] ED_{50} (mg/kg *p.o.*)
6	0.15	17	ca.100[e]	2.4
13b	0.22	18	24	NT[f]
13e	0.082	15	42	NT[f]
13f	0.092	5.9	8.3	NT[f]
13k	0.25	93	62	4.7
13l	0.24	16	25	NT[f]
13n	0.16	18	-18	NT[f]
13s	0.077	18	44	NT[f]
13t	0.11	34	42	NT[f]
13u	0.15	24	17	NT[f]
15	0.27	160	59	4.4
13v	0.22	94	46	NT[f]
13w	0.29	33	64	1.3

[a] See footnote *c* in Table 1.
[b] Inhibition of PHA-induced IL-2 production in Jurkat T-cells. See Section 5.
[c] Inhibition of TNCB-induced contact hypersensitivity in mice. See Section 5.
[d] The inhibition of OA-induced airway eosinophilia in rats. See Section 5.
[e] ED_{50} = 1.1 mg/kg *p.o.*
[f] Not tested.

(30 mL) and EtOH (30 mL). This mixture was heated at 80 °C for 4 h, and then H_2O was added and extracted with AcOEt, washed with 1 M HCl, brine, dried, and concentrated in vacuo. The residue was purified via column chromatography (hexane/AcOEt 19:1–9:1) to yield **8** (1.65 g, 19%) and **9** (178 mg, 2%) as light yellow needle crystals.

5.1.1. 1-(4-Nitrophenyl)-3-(trifluoromethyl)-1,4,5,6-tetrahydrocyclopenta[*c*]pyrazole (8)

^{1}H NMR ($CDCl_3$) δ *2.60–2.85* (4H, m), 3.12 (2H, t, *J* = 6.9 Hz), 7.86 (2H, d, *J* = 9.2 Hz), 8.33 (2H, d, *J* = 9.2 Hz); GC–MS *m/z* 297 (M^+).

5.1.2. 2-(4-Nitrophenyl)-3-(trifluoromethyl)-2,4,5,6-tetrahydrocyclopenta[*c*]pyrazole (9)

^{1}H NMR ($CDCl_3$) δ 2.46-2.57 (2H, m), 2.80-2.92 (4H, m), 7.70 (2H, d, *J* = 9.0 Hz), 8.34 (2H, d, *J* = 9.0 Hz); GC-MS *m/z* 297 (M^+).

5.1.3. 4-[3-(Trifluoromethyl)-5,6-dihydrocyclopenta[*c*]pyrazol-1(4*H*)-yl]aniline (11a)

1-(4-Nitrophenyl)-3-(trifluoromethyl)-1,4,5,6-tetrahydrocyclopenta[*c*]pyrazole (**8**, 1.00 g, 3.36 mmol) was hydrogenated at room temperature for 1 h in the presence of 10% Pd–C in EtOH (10 mL) and THF (10 mL). After the catalyst was removed via filtration, the filtrate was concentrated *in vacuo* and recrystallized from AcOEt/hexane to yield **11a** (583 mg, 65%) as a gray powder: ^{1}H NMR ($CDCl_3$) δ *2.57–2.69* (2H, m), 2.76 (2H, t, *J* = 7.1 Hz), 2.93 (2H, t, *J* = 7.1 Hz), 3.75 (2H, br s), 6.71 (2H, d, *J* = 8.8 Hz), 7.38 (2H, d, *J* = 8.8 Hz); FAB-MS *m/z* 268 [$(M+H)^+$].

The compound described below was prepared similarly.

5.1.4. 4-[5-Methyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]aniline (11b)

^{1}H NMR (DMSO-d_6) δ 2.24 (3H, s), 5.47 (2H, s), 6.59-6.68 (3H, m), 7.10–7.15 (2H, m); FAB-MS *m/z* 242 [$(M+H)^+$].

5.1.5. 4′-[3,5-Bis(trifluoromethyl)-1*H*-pyrazol-1-yl]thiazole-5-carboxanilide (13h)

A mixture of thiazole-5-carboxylic acid (75 mg, 0.581 mmol), 4-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]aniline (**11c**, 171 mg, 0.579 mmol), EDC HCl (117 mg, 0.610 mmol), and THF (3 ml) were stirred together for 5 h at room temperature, and then H_2O (10 ml) was added. This mixture was extracted with AcOEt and washed with 1 M HCl, saturated aqueous $NaHCO_3$, and brine. The extract was dried and concentrated in vacuo, and the residue was recrystallized from AcOEt/hexane to yield **13h** (77 mg, 33%) as a pale brown powder. mp 194–196 °C; ^{1}H NMR (DMSO-d_6) δ 7.64 (2H, d, *J* = 9.0 Hz), 7.83 (1H, s), 7.94 (2H, d, *J* = 9.0 Hz), 8.74 (1H, s), 9.35 (1H, s), 10.76 (1H, s); FAB-MS *m/z* 407 [$(M+H)^+$]. Anal. calcd for $C_{15}H_8F_6N_4OS$: C, 44.34; H, 1.98; N, 13.79; F, 28.05; S, 7.89. Found: C, 44.18; H, 2.05; N, 13.77; F, 27.89; S, 7.94.

The compounds described below were prepared using the same method. Among the corresponding amines, 4-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]aniline[18] was prepared using the method reported previously.

5.1.6. 4-Methyl-4′-(3-trifluoromethyl-1*H*-pyrazol-1-yl)-1,2,3-thiadiazole-5-carboxanilide (13a)

(66%): mp 158–161 °C (AcOEt-Hexane); ^{1}H NMR (DMSO-d_6) δ 2.84 (3H, s), 7.05 (1H, d, *J* = 2.5 Hz), 7.84-7.94 (4H, m), 8.70 (1H, dd, *J* = 2.5, 1.0 Hz), 10.92 (1H, s); FAB-MS *m/z* 354 [$(M+H)^+$]. Anal. calcd for $C_{14}H_{10}F_3N_5OS$: C, 47.59; H, 2.85; N, 19.82; F, 16.13; S, 9.08. Found: C, 47.43; H, 3.03; N, 19.96; F, 16.20; S, 9.09.

5.1.7. 4-Methyl-4′-(5-methyl-3-trifluoromethyl-1*H*-pyrazol-1-yl)-1,2,3-thiadiazole-5-carboxanilide (13b)

(76%): mp 75–76 °C (EtOH-Et_2O); ^{1}H NMR (DMSO-d_6) δ 2.35 (3H, s), 2.84 (3H, s), 6.76 (1H, s), 7.60 (2H, d, *J* = 8.8 Hz), 7.88 (2H, d, *J* = 8.8 Hz), 10.97 (1H, s); FAB-MS *m/z* 368 [$(M+H)^+$]. Anal. calcd for $C_{15}H_{12}F_3N_5OS{\cdot}0.3C_2H_6O{\cdot}0.5H_2O$: C, 48.02; H, 3.82; N, 17.95; F, 14.61; S, 8.22. Found: C, 47.97; H, 3.84; N, 17.61; F, 14.26; S, 7.95.

5.1.8. 4-Methyl-4′-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide (13c)

(77%): ^{1}H NMR (DMSO-d_6) δ 2.31 (3H, s), 2.82 (3H, s), 7.13 (1H, s), 7.16–7.22 (4H, m), 7.37 (2H, d, *J* = 8.8 Hz), 7.76 (2H, d, *J* = 8.8 Hz), 10.91 (1H, s); FAB-MS *m/z* 444 [$(M+H)^+$]. Anal. calcd for $C_{21}H_{16}F_3N_5OS$: C, 56.88; H, 3.64; N, 15.79; F, 12.85; S, 7.23. Found: C, 56.72; H, 3.61; N, 15.61; F, 12.62; S, 7.06.

5.1.9. 4-Methyl-4′-(3-trifluoromethyl-1,4,5,6-tetrahydrocyclopentapyrazol-1-yl)-1,2,3-thiadiazole-5-carboxanilide (13d)

(35%): mp 155–156 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ *2.57–2.65* (2H, m), 2.72 (2H, t, *J* = 6.8 Hz), 2.83 (3H, s), 3.08 (2H, t, *J* = 7.1 Hz), 7.73 (2H, d, *J* = 9.1 Hz), 7.85 (2H, d, *J* = 9.1 Hz), 10.91 (1H, s); FAB-MS *m/z* 394 [$(M+H)^+$]. Anal. calcd for $C_{17}H_{14}F_3N_5OS$: C, 51.90; H, 3.59; N, 17.80; F, 14.49; S, 8.15. Found: C, 51.70; H, 3.51; N, 17.89; F, 14.45; S, 8.08.

5.1.10. 4′-[3,5-Bis(trifluoromethyl)-1*H*-pyrazol-1-yl]thiazole-2-carboxanilide (13i)

(49%): mp 174–175 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 7.63 (2H, d, *J* = 8.8 Hz), 7.83 (1H, s), 8.10 (2H, d, *J* = 8.8 Hz), 8.15 (1H, d, *J* = 3.2 Hz), 8.19 (1H, d, *J* = 3.2 Hz), 11.17 (1H, s); FAB-MS *m/z* 407 [$(M+H)^+$]. Anal. calcd for $C_{15}H_8F_6N_4OS$: C, 44.34; H, 1.98; N, 13.79; F, 28.05; S, 7.89. Found: C, 44.15; H, 1.85; N, 13.87; F, 27.83; S, 7.84.

5.1.11. 4′-[3,5-Bis(trifluoromethyl)-1*H*-pyrazol-1-yl]pyridine-2-carboxanilide (13j)

(55%): mp 171–173 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 7.62 (2H, d, *J* = 9.0 Hz), 7.70-7.74 (1H, m), 7.82 (1H, s), 8.10 (1H, td, *J* = 7.8, 1.5 Hz), 8.16 (2H, d, *J* = 9.0 Hz), 8.20 (1H, d, *J* = 7.8 Hz), 8.78 (1H, dd, *J* = 4.9, 1.5 Hz), 11.20 (1H, s); FAB-MS *m/z* 401 [$(M+H)^+$]. Anal. calcd for $C_{17}H_{10}F_6N_4O$: C, 51.01; H, 2.52; N, 14.00; F, 28.48. Found: C, 50.82; H, 2.50; N, 14.04; F, 28.72.

5.1.12. 4′-[3,5-Bis(trifluoromethyl)-1*H*-pyrazol-1-yl]nicotinanilide (13k)

(53%): mp 188–190 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ *7.58–7.62* (1H, m), 7.64 (2H, d, J = 9.0 Hz), 7.83 (1H, s), 8.00 (2H, d, J = 9.0 Hz), 8.33 (1H, dt, J = 7.8, 1.8 Hz), 8.80 (1H, dd, J = 4.7, 1.8 Hz), 9.14 (1H, d, J = 1.8 Hz), 10.77 (1H, s); FAB-MS *m/z* 401 [(M+H)$^+$]. Anal. calcd for $C_{17}H_{10}F_6N_4O$: C, 51.01; H, 2.52; N, 14.00; F, 28.48. Found: C, 50.91; H, 2.33; N, 14.02; F, 28.77.

5.1.13. 4'-[3,5-Bis(trifluoromethyl)-1*H*-pyrazol-1-yl]isonicotinanilide monohydrochloride (13l)

(66%): mp 170–172 °C (EtOH–MeCN); ^{1}H NMR (DMSO-d_6) δ 7.66 (2H, d, J = 9.0 Hz), 7.84 (1H, s), 8.04 (2H, d, J = 9.0 Hz), 8.19 (2H, d, J = 5.9 Hz), 8.94–9.02 (2H, m), 11.13 (1H, s); FAB-MS *m/z* 401 [(M+H)$^+$]. Anal. calcd for $C_{17}H_{10}F_6N_4O$·HCl: C, 46.75; H, 2.54; N, 12.83; Cl, 8.12; F, 26.10. Found: C, 46.71; H, 2.46; N, 12.98; Cl, 8.09; F, 26.15.

5.1.14. 5-Methyl-4'-[3,5-bis(trifluoromethyl)-1>*H*-pyrazol-1-yl] nicotinanilide (13r)

(10%): mp 178–181 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 2.42 (3H, s), 7.63 (2H, d, J = 8.8 Hz), 7.83 (1H, s), 8.00 (2H, d, J = 8.8 Hz), 8.14 (1H, s), 8.64 (1H, d, J = 1.5 Hz), 8.94 (1H, d, J = 1.5 Hz), 10.73 (1H, s); FAB-MS *m/z* 415 [(M+H)$^+$]. Anal. calcd for $C_{18}H_{12}F_6N_4O$: C, 52.18; H, 2.92; N, 13.52; F, 27.51. Found: C, 52.10; H, 2.80; N, 13.57; F, 27.66.

5.1.15. 5-Fluoro-4'-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-nicotinanilide (13s)

(49%): mp 176–178 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 7.64 (2H, d, J = 8.8 Hz), 7.82 (1H, s), 7.99 (2H, d, J = 8.8 Hz), 8.25 (1H, dt, J = 9.3, 1.9 Hz), 8.83 (1H, d, J = 3.0 Hz), 9.02 (1H, s), 10.81 (1H, s); FAB-MS *m/z* 419 [(M+H)$^+$]. Anal. calcd for $C_{17}H_9F_7N_4O$: C, 48.82; H, 2.17; N, 13.39; F, 31.80. Found: C, 48.62; H, 2.13; N, 13.43; F, 31.64.

5.1.16. 5-Chloro-4'-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-nicotinanilide (13t)

(25%): mp 175–177 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 7.64 (2H, d, J = 8.8 Hz), 7.82 (1H, s), 7.99 (2H, d, J = 8.8 Hz), 8.45 (1H, t, J = 2.2 Hz), 8.86 (1H, d, J = 2.2 Hz), 9.07 (1H, d, J = 2.2 Hz), 10.82 (1H, s); FAB-MS *m/z* 435 [(M+H)$^+$]. Anal. calcd for $C_{17}H_9ClF_6N_4O$: C, 46.97; H, 2.09; N, 12.89; Cl, 8.16; F, 26.22. Found: C, 46.96; H, 2.02; N, 12.97; Cl, 8.17; F, 26.08.

5.1.17. 4′-(5-Chloro-3-trifluoromethyl-1*H*-pyrazol-1-yl)-4-methyl-1,2,3-thiadiazole-5-carboxanilide (13e)

A mixture of 1,2,3-thiadiazole-5-carboxylic acid (166 mg, 1.15 mmol), 4-(5-chloro-3-trifluoromethyl-1*H*-pyrazol-1-yl)aniline[12] (**11d**, 250 mg, 0.956 mmol), EDC HCl (220 mg, 1.15 mmol), HOBT (155 mg, 1.15 mmol), and DMF (5 ml) was stirred overnight at room temperature, and then H_2O was added. This mixture was extracted with AcOEt and washed with brine. The extract was dried and concentrated in vacuo, and the residue was recrystallized from AcOEt–hexane to yield **13e** (282 mg, 76%) as a colorless powder. mp 148 °C; ^{1}H NMR (DMSO-d_6) δ 2.84 (3H, s), 7.30 (1H, s), 7.66 (2H, d, J = 8.8 Hz), 7.91 (2H, d, J = 8.8 Hz), 11.00 (1H, s); FAB-MS *m/z* 388 [(M+H)$^+$]. Anal. calcd for $C_{14}H_9ClF_3N_5OS$: C, 43.36; H, 2.34; N, 18.06; Cl, 9.14; F, 14.70; S, 8.27. Found: C, 43.41; H, 2.21; N, 18.30; Cl, 8.90; F, 14.72; S, 8.24.

The compounds described below were prepared following the same method. Among the corresponding amines, 4-(5-bromo-3-trifluoromethyl-1*H*-pyrazol-1-yl)aniline and 4-(5-cyano-3-trifluoromethyl-1*H*-pyrazol-1-yl)aniline were prepared using the methods reported previously.[12]

5.1.18. 4′-(5-Bromo-3-trifluoromethyl-1*H*-pyrazol-1-yl)-4-methyl-1,2,3-thiadiazole-5-carboxanilide (13f)

(73%): mp 150 °C (MeOH–H_2O); ^{1}H NMR (DMSO-d_6) δ 2.84 (3H, s), 7.32 (1H, s), 7.64 (2H, d, J = 9.0 Hz), 7.90 (2H, d, J = 9.0 Hz), 11.02 (1H, s); FAB-MS *m/z* 432, 434 [(M+H)$^+$]. Anal. calcd for $C_{14}H_9BrF_3N_5OS$: C, 38.90; H, 2.10; N, 16.20; Br, 18.49; F, 13.19; S, 7.42. Found: C, 38.81; H, 1.99; N, 16.29; Br, 18.41; F, 13.11; S, 7.38.

5.1.19. 4′-(5-Cyano-3-trifluoromethyl-1*H*-pyrazol-1-yl)-4-methyl-1,2,3-thiadiazole-5-carboxanilide (13g)

(60%): mp 215–216 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 2.84 (3H, s), 7.83 (2H, d, J = 8.8 Hz), 7.95 (2H, d, J = 8.8 Hz), 8.08 (1H, s), 11.06 (1H, s); FAB-MS *m/z* 379 [(M+H)$^+$]. Anal. calcd for $C_{15}H_9F_3N_6OS$: C, 47.62; H, 2.40; N, 22.21; F, 15.06; S, 8.48. Found: C, 47.60; H, 2.37; N, 22.43; F, 15.06; S, 8.51.

5.1.20. 4-Chloro-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-nicotinanilide (13n)

A mixture of 4-chloronicotinic acid (564 mg, 3.58 mmol), oxalyl chloride (0.26 ml, 2.98 mmol), and DMF (5 μl) in dichloromethane (6 ml) was stirred for 3 h at room temperature and concentrated in vacuo. The residue was added to dichloromethane (6 ml), 4-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]aniline (**11c**[12], 590 mg, 2.00 mmol), and Et_3N (0.42 ml, 3.01 mmol) at 0 °C, and this mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with AcOEt and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried and concentrated *in vacuo*. The residue was recrystallized from EtOH–H_2O to yield **13n** (470 mg, 54%) as a pink powder. mp 170–171 °C; ^{1}H NMR (DMSO-d_6) δ 7.64 (2H, d, J = 8.8 Hz), 7.74 (1H, d, J = 5.6 Hz), 7.83 (1H, s), 7.93 (2H, d, J = 8.8 Hz), 8.68 (1H, d, J = 5.6 Hz), 8.85 (1H, s), 11.06 (1H, s); FAB-MS *m/z* 435 [(M+H)$^+$]. HRMS *m/z* calcd for $C_{17}H_{10}ClF_6N_4O$ [M+H]$^+$: 435.0447. Found: 435.0440.

The compounds described below were prepared following the same method.

5.1.21. 4-Trifluoromethyl-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]nicotinanilide (13o)

(91%): mp 179–180 °C (EtOH–H_2O); ^{1}H NMR (DMSO-d_6) δ 7.65 (2H, d, J = 8.8 Hz), 7.83 (1H, s), 7.90 (2H, d, J = 8.8 Hz), 7.94 (1H, d, J = 5.1 Hz), 9.02 (1H, d, J = 5.1 Hz), 9.10 (1H, s), 11.14 (1H, s); FAB-MS *m/z* 469 [(M+H)$^+$]. Anal. calcd for $C_{18}H_9F_9N_4O$: C, 46.17; H, 1.94; N, 11.96; F, 36.51. Found: C, 46.14; H, 1.64; N, 12.21; F, 36.57.

5.1.22. 2-Methoxy-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]nicotinanilide (13q)

(44%): mp 128 °C (hexane); ^{1}H NMR (DMSO-d_6) δ 4.00 (3H, s), 7.14–7.20 (1H, m), 7.61 (2H, d, J = 8.6 Hz), 7.82 (1H, s), 7.94 (2 H, d, J = 8.6 Hz), 8.07 (1H, d, J = 7.3 Hz), 8.36 (1H, d, J = 4.8 Hz), 10.56 (1H, s); FAB-MS *m/z* 431 [(M+H)$^+$]. Anal. calcd for $C_{18}H_{12}F_6N_4O_2$: C, 50.24; H, 2.81; N, 13.02; F, 26.49. Found: C, 50.25; H, 2.97; N, 13.06; F, 26.33.

5.1.23. 6-Methyl-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]nicotinanilide monohydrochloride (13v)

(27%): mp 215–216 °C (EtOH–H_2O); ^{1}H NMR (DMSO-d_6) δ 2.74 (3H, s), 7.65 (2H, d, J = 9.0 Hz), 7.84 (1H, s), 7.82-7.86 (1H, m), 8.05 (2H, d, J = 9.0 Hz), 8.70 (1H, dd, J = 8.3, 1.7 Hz), 9.30 (1H, d, J = 1.7 Hz), 11.13 (1H, s); FAB-MS *m/z* 415 [(M+H)$^+$]. Anal. calcd for $C_{18}H_{12}F_6N_4O$·HCl: C, 47.96; H, 2.91; N, 12.43; Cl, 7.87; F, 25.29. Found: C, 48.01; H, 2.87; N, 12.43; Cl, 7.67; F, 25.02.

5.1.24. 4,6-Dimethyl-4′-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]nicotinanilide (13w)

(9%): mp 194-196 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 2.41 (3H, s), 2.50 (3H, s), 7.25 (1H, s), 7.61 (2H, d, J = 8.8 Hz), 7.82 (1H, s), 7.95 (2H, d, J = 8.8 Hz), 8.59 (1H, s), 10.76 (1H, s); FAB-MS m/z 429 [(M+H)$^+$]. Anal. calcd for $C_{19}H_{14}F_6N_4O$: C, 53.28; H, 3.29; N, 13.08; F, 26.61. Found: C, 53.16; H, 3.19; N, 13.22; F, 26.71.

5.1.25. 2,4-Dimethyl-4′-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]nicotinanilide monohydrochloride (13x)

(35%): mp 190 °C (decomposed) (EtOH–AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s), 2.71 (3H, s), 7.66 (2H, d, J = 8.8 Hz), 7.81 (1H, d, J = 5.9 Hz), 7.84 (1H, s), 7.96 (2H, d, J = 8.8 Hz), 8.72 (1H, d, J = 5.9 Hz), 11.42 (1H, s); FAB-MS m/z 429 [(M+H)$^+$]. Anal. calcd for $C_{19}H_{14}F_6N_4O$·HCl: C, 49.10; H, 3.25; N, 12.05; Cl, 7.63; F, 24.53. Found: C, 49.03; H, 3.22; N, 12.10; Cl, 7.65; F, 24.43.

5.1.26. 4-Methyl-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-nicotinanilide monohydrochloride (13m)

Et_3N (1.90 ml, 13.6 mmol) and HBTU (2.46 g, 6.49 mmol) were added to a chilled solution of 4-methylnicotinic acid monohydrochloride (1.04 g, 5.99 mmol) in DMF (15 ml), and the mixture was stirred for 3 h at room temperature. The mixture was then added to 4-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]aniline (**11c**, 1.47 g, 4.99 mmol) and stirred for 6 days at room temperature, after which H_2O was added. This mixture was extracted with AcOEt and washed with H_2O, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried and concentrated in vacuo. The residue was purified via column chromatography ($CHCl_3$/MeOH 99: 1), and added AcOEt (30 ml) and 4 M HCl–AcOEt (3 ml) to yield the crude hydrochloride, and the crude product was recrystallized from EtOH to yield **13m** (735 mg, 33%) as a colorless powder. mp 181 °C (decomposed); ^{1}H NMR (DMSO-d_6) δ 2.62 (3H, s), 7.65 (2H, d, J = 8.8 Hz), 7.83 (1H, s), 7.85 (1H, d, J = 5.9 Hz), 7.98 (2H, d, J = 8.8 Hz), 8.81 (1H, d, J = 5.9 Hz), 9.04 (1H, s), 11.24 (1H, s); FAB-MS m/z 415 [(M+H)$^+$]. Anal. calcd for $C_{18}H_{12}F_6N_4O$·HCl: C, 47.96; H, 2.91; N, 12.43; F, 25.29; Cl, 7.87. Found: C, 47.74; H, 2.74; N, 12.42; F, 25.18; Cl, 7.86.

The compounds described below were prepared following the same method.

5.1.27. 2-Chloro-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]nicotinanilide (13p)

(55%): mp 154–157 °C (EtOH–H_2O); ^{1}H NMR (DMSO-d_6) δ 7.60 (1H, dd, J = 7.8, 4.8 Hz), 7.64 (2H, d, J = 9.0 Hz), 7.83 (1H, s), 7.92 (2H, d, J = 9.0 Hz), 8.15 (1H, dd, J = 7.8, 2.0 Hz), 8.57 (1H, dd, J = 4.8, 2.0 Hz), 11.02 (1H, s); FAB-MS m/z 435 [(M+H)$^+$]. Anal. calcd for $C_{17}H_9ClF_6N_4O$: C, 46.97; H, 2.09; N, 12.89; Cl, 8.16; F, 26.22. Found: C, 46.98; H, 2.02; N, 12.97; Cl, 8.16; F, 26.01.

5.1.28. 5-Bromo-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]nicotinanilide (13u)

(48%): mp 169–170 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 7.64 (2H, d, J = 9.0 Hz), 7.83 (1H, s), 7.98 (2H, d, J = 9.0 Hz), 8.58 (1H, t, J = 2.0 Hz), 8.94 (1H, d, J = 2.0 Hz), 9.09 (1H, d, J = 2.0 Hz), 10.82 (1H, s); FAB-MS m/z 479, 481 [(M+H)$^+$]. Anal. calcd for $C_{17}H_9BrF_6N_4O$: C, 42.61; H, 1.89; N, 11.69; Br, 16.68; F, 23.79. Found: C, 42.63; H, 1.75; N, 11.73; Br, 16.76; F, 23.55.

5.1.29. 4′-[3,5-Bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-1*H*-imidazole-4-carboxanilide (14)

A mixture of 4'-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-1-trityl-1*H*-imidazole-4-carboxanilide (**13y**, 180 mg, 0.285 mmol), concentrated HCl (0.1 ml) and acetone (3 ml) was stirred for 17 h at room temperature, and then concentrated in vacuo. The residue was purified via column chromatography (hexane/AcOEt 1:1–2:3) and recrystallized from AcOEt–hexane to yield 14 (35 mg, 13%) as a colorless powder. mp 200 °C (decomposed); ^{1}H NMR (DMSO-d_6) δ 7.55 (2H, d, J = 9.0 Hz), 7.80 (1H, s), 7.86 (1H, s), 7.87 (1H, s), 8.07 (2H, d, J = 9.0 Hz), 10.23 (1H, s), 12.64-12.76 (1H, br s); FAB-MS m/z 390 [(M+H)$^+$]. Anal. calcd for $C_{15}H_9F_6N_5O$·0.25H_2O: C, 45.75; H, 2.43; N, 17.79; F, 28.95. Found: C, 45.84; H, 2.34; N, 17.96; F, 28.75.

5.1.30. 5-Amino-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]nicotinanilide monohydrochloride dihydrate (15)

A mixture of *t*-butyl 5-{4-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]phenylcarbamoyl}pyridin-3-ylcarbamate (**13z**, 147 mg, 0.285 mmol), trifluoroacetic acid (2 ml) and CH_2Cl_2 (2 ml) was stirred for 2 h at room temperature, and then concentrated in vacuo. This mixture was diluted with AcOEt and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried and concentrated in vacuo. The residue was added to AcOEt (10 ml) and 4 M HCl–AcOEt (1 ml) to yield the crude hydrochloride, and the crude product was recrystallized from EtOH–AcOEt to yield **15** (116 mg, 83%) as colorless needle crystals. mp 260 °C (sublimated); ^{1}H NMR (DMSO-d_6) δ 7.64 (2H, d, J = 8.8 Hz), 7.82 (1H, s), 7.97 (1H, s), 8.00 (2H, d, J = 8.8 Hz), 8.18 (1H, s), 8.58 (1H, s), 11.02 (1H, s); FAB-MS m/z 416 [(M+H)$^+$]. Anal. calcd for $C_{17}H_{11}F_6N_5O$·HCl·2H_2O: C, 41.86; H, 3.31; N, 14.36; Cl, 7.27; F, 23.37. Found: C, 41.82; H, 3.02; N, 14.47; Cl, 7.09; F, 23.42.

5.1.31. 4-Methoxy-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]nicotinanilide monohydrochloride (16)

A mixture of 4-chloro-4'-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-nicotinanilide (**13n**, 100 mg, 0.230 mmol), 4 M HCl–AcOEt (0.115 ml, 0.460 mmol) and MeOH (2 ml) was stirred for 5 h at room temperature, and then concentrated in vacuo, and the residue was recrystallized from MeOH–Et_2O to yield **16** (70 mg, 65%) as a colorless powder. mp 272–275 °C; ^{1}H NMR (DMSO-d_6) δ 4.13 (3H, s), 7.64 (2H, d, J = 8.8 Hz), 7.67 (1H, d, J = 6.9 Hz), 7.82 (1H, s), 7.93 (2H, d, J = 8.8 Hz), 8.86 (1H, d, J = 6.9 Hz), 8.95 (1H, s), 10.91 (1H, s); FAB-MS m/z 431 [(M+H)$^+$]. Anal. calcd for $C_{18}H_{12}F_6N_4O_2$·HCl: C, 46.32; H, 2.81; N, 12.00; Cl, 7.60; F, 24.42. Found: C, 45.97; H, 2.66; N, 11.94; Cl, 7.57; F, 24.15.

5.1.32. 4-Amino-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]nicotinanilide (17)

A mixture of 4-chloro-4'-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-nicotinanilide (**13n**, 450 mg, 1.04 mmol) and liquid ammonia (10 ml) was stirred overnight at 80 °C in a sealed tube, and then concentrated in vacuo. The residue was purified via column chromatography ($CHCl_3$/MeOH 50:1–20:1) and recrystallized from AcOEt–hexane to yield 17 (72 mg, 17%) as a pale yellow powder. mp 241 °C (decomposed); ^{1}H NMR (DMSO-d_6) δ 6.69 (1H, d, J = 5.6 Hz), 7.09 (2H, s), 7.59 (2H, d, J = 8.8 Hz), 7.82 (1H, s), 7.94 (2H, d, J = 8.8 Hz), 8.09 (1H, d, J = 5.6 Hz), 8.68 (1H, s), 10.49 (1H, s); FAB-MS m/z 416 [(M+H)$^+$]. Anal. calcd for $C_{17}H_{11}F_6N_5O$·0.25H_2O: C, 48.64; H, 2.76; N, 16.68; F, 27.15. Found: C, 48.85; H, 2.54; N, 16.77; F, 27.42.

5.1.33. 4-Hydroxy-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]nicotinanilide (18)

A mixture of 4-chloro-4'-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-nicotinanilide (13n, 350 mg, 0.806 mmol), 1 M HCl (4 ml), and dioxane (4 ml) was stirred overnight at 50 °C, and then 1 M NaOH was added. This mixture was extracted with AcOEt and washed with brine. The organic layer was dried and concentrated *in vacuo*. The residue was recrystallized from AcOEt–hexane to yield 18 (110 mg, 33%) as a colorless powder. mp >300 °C; ^{1}H NMR (DMSO-d_6) δ *6.53–6.57* (1H, m), 7.60 (2H, d, J = 8.8 Hz), 7.81

(1H, s), 7.89 (2H, d, J = 8.8 Hz), 7.89–7.93 (1H, m), 8.60-8.63 (1H, m), 12.25-12.55 (1H, br s), 13.21 (1H, s); FAB-MS m/z 417 [$(M+H)^+$]. Anal. calcd for $C_{17}H_{10}F_6N_4O_2 \cdot 0.25H_2O$: C, 48.52; H, 2.52; N, 13.31; F, 27.09. Found: C, 48.51; H, 2.28; N, 13.36; F, 27.20.

The compounds described below were prepared following the same method.

5.1.34. 2-Hydroxy-4'-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]nicotinanilide (19)

(5%): mp >300 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 6.61 (1H, t, J = 6.7 Hz), 7.61 (2H, d, J = 9.0 Hz), 7.82 (1H, s), 7.86 (1H, dd, J = 6.7, 2.4 Hz), 7.91 (2H, d, J = 9.0 Hz), 8.50 (1H, dd, J = 6.7, 2.4 Hz), 12.48 (1H, s), 12.82 (1H, s); FAB-MS m/z 417 [$(M+H)^+$]. Anal. calcd for $C_{17}H_{10}F_6N_4O_2$: C, 49.05; H, 2.42; N, 13.46; F, 27.38. Found: C, 49.07; H, 2.38; N, 13.51; F, 27.45.

5.1.35. Fura-2 loading and population intracellular calcium measurements

Cells were suspended in HEPES buffered solution (pH 7.4) of the following composition (mM). NaCl: 137, KCl: 5.8, $MgCl_2$: 1, $CaCl_2$: 2.5, glucose: 5, and HEPES: 10. The cells were loaded with 1 μM Fura-2 /AM at room temperature for 45 min followed by successive washes to remove unincorporated dye, and resuspended in HEPES buffered solution. Cell suspensions were studied in a 96-well black plate. Fluorescence measurements for the determination of intracellular calcium concentration were made using a fluorescence microplate reader (Fluostar, SLT Labinstruments GmbH, Austria) with excitation wavelengths of 340 nm and 380 nm and an emission fluorescence detection wavelength of 500 nm. The cell-free fluorescence of each compound was then measured, after which it was deducted from the cell fluorescence value. The final intracellular calcium concentration in each well was calculated from the fluorescence ratio using the standard equation. Wells treated with 25 μM ionomycin were used to obtain the R_{max} value, while the R_{min} value was obtained from wells treated with 25 μM ionomycin/50 mM EGTA.

5.1.36. CRAC channel inhibition assay

CRAC channel inhibition was evaluated in Jurkat cells (2×10^6 cells/ml). Fura-2 loaded Jurkat cells were stimulated with 1 μM thapsigargin for 30 min, and the intracellular calcium concentration was measured at the endpoint (30 min). The CRAC channel inhibition IC_{50} value was calculated for each compound from the percent inhibition of the thapsigargin-induced calcium influx in Jurkat cells.

5.1.37. VOC channel inhibition assay

VOC channel inhibition was evaluated in murine neuroblastoma, PC12-h5 cells (1×10^6 cells/ml). Fura-2 loaded PC12-h5 cells were stimulated with 50 mM KCl for 20 min, and then the intracellular calcium concentration was measured at the endpoint of 20 min. The IC_{50} values for the VOC channel inhibition of each compounds were calculated from the percent inhibition of KCl-induced calcium influx in PC12 cells.

5.1.38. IL-2 production assay

Jurkat T lymphocytes (5×10^6 cells/ml) were placed in a 96-well microplate and incubated with PHA (20 μg/ml) for 20 h, after which the supernatant was collected. The IL-2 concentration in each supernatant was measured using a human IL-2 ELISA system (DuoSeT™, Genzyme).

5.1.39. TNCB-induced contact hypersensitivity in mice

On day 0, 100 μL of 7% TNCB solution was applied to the abdominal region (fur removed) of anesthetized five-week-old male CD-1 mice. Seven days after TNCB sensitization, the thickness of the ear was measured, and 10 μL of 0.25% TNCB was applied to both the inside and outside of the ear pinnas. Only the solvent was applied to negative control mice. Ear thickness was measured 24 h after the TNCB challenge using a dial thickness gauge, and the change due to swelling were calculated from the pre-exposure value. The compounds were administered orally 1 h before exposure to TNCB, and 0.5% MC was administered orally to the negative control and control animals.

5.1.40. Antigen-induced airway eosinophilia in rats

Four-week-old female BN rats were sensitized via intraperitoneal injections of OA (1 mg) and $Al(OH)_3$ (20 mg) once daily for three consecutive days. Three weeks after sensitization, the rats were exposed to an aerosol of 1% (w/v) OA solution for 15 min. Test compounds were administered orally 1 h before the start of antigen exposure. Bronchoalveolar lavage (BAL) was performed 24 h after antigen exposure. The number of white blood cells in the BAL fluid from each animal was determined using a blood cell counter. A cell smear was prepared, and then observed microscopically to identify and classify the cells as eosinophils, neutrophils, or monocytes according to their morphological characteristics. The number of eosinophils in each animal's BAL fluid was then calculated.

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