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Novel potent and selective calcium-release-activated calcium (CRAC) channel inhibitors. Part 1: Synthesis and inhibitory activity of 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamides

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Abstract—We synthesized and evaluated a series of 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamides to identify potent inhibitors of calcium-release-activated calcium (CRAC) channels with greater selectivity than voltage-operated calcium (VOC) channels. These efforts resulted in identification of compounds **22** and **24**. The former exhibits highly potent and selective CRAC channel inhibitory activity, and the latter inhibited phytohemagglutinin-induced interleukin-2 production by Jurkat T lymphocytes and concanavalin A-induced hepatitis in mice.

1. Introduction

Ca^{2+} channels in the plasma membrane are known to regulate Ca^{2+} influx, and as a result they regulate a variety of cellular functions. Voltage-operated calcium (VOC) channels, located in the nervous, endocrine, cardiovascular, and skeletal systems, are known to be modulated by the membrane potential, and are classified into L, N, P, Q, R, and T subtypes.[1] Some of these molecules have been well characterized, and their inhibitors, such as nifedipine, have been used for the treatment of hypertension[1b] and brain dysfunction.[1c] In contrast, Ca^{2+} channels in inflammatory cells, such as lymphocytes, mast cells and neutrophils, are activated regardless of their membrane potential[2a] and are known as store-operated Ca^{2+} channels. They have been shown to play important roles in the pathogenesis and exacerbation of inflammatory and autoimmune diseases.[2b–e]

The early stages in T lymphocyte activation can be divided into events pre- and post-intracellular Ca^{2+} release.[3] Before Ca^{2+} release, the stimulation of cell surface receptors induces the generation of IP_3, followed by the release of Ca^{2+} from the endoplasmic reticulum (ER).[4] After the depletion of Ca^{2+} from ER stores, calcium-release-activated calcium (CRAC) channels are activated and capacitative Ca^{2+} influx through these channels sustains a high intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$).[5] This prolonged high $[Ca^{2+}]_i$ is crucial for cytosolic signal transduction which induces the production of lipid mediators (e.g., LTD_4), cytokines (e.g., interleukin-2 (IL-2)), and matrix metalloproteinases, which are known to play important roles in the pathogenesis of inflammation and autoimmune diseases.

These facts suggested that CRAC channel inhibitors would be useful for the treatment of diseases, such as asthma and rheumatoid arthritis, caused by the activation of inflammatory cells.[6] However, inhibitors which affect both CRAC and VOC channels are predicted to show undesirable side-effects in the nervous and cardiovascular systems. Therefore, it may be necessary for CRAC channel inhibitors to exhibit sufficient selectivity over VOC channels to be useful as anti-inflammatory drugs.

Keywords: Anti-inflammatory; Channel inhibitors; Ca^{2+}-release-activated Ca^{2+} channel; CRAC channel; Interleukin-2; Quantitative structure–activity relationship.
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In the last decade, several compounds including SK&F 96365 (**1**),[7] LOE 908 (**2**),[8] and L-651582 (**3**,[9] Fig. 1) have been reported to inhibit CRAC channels. However, their potency and selectivity are unlikely to be adequate for their use as anti-inflammatory drugs. To find potent and selective CRAC channel inhibitors, we screened our chemical library and identified *N*-(4-chlorophenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (**4**,[10] Fig. 1) as a lead compound. We have studied the structure–activity relationships (SARs) of this compound and in this paper, we describe the synthesis of a series of 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamides, and the SARs of their 4-chlorophenyl moiety.

2. Chemistry

The 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamides were prepared as shown in Schemes 1–3. In the presence of sodium methoxide (NaOMe) and ethyl trifluoroacetate, 2-acetylthiophene **5** was converted to the dione **6**,[11] and regioselective cyclization of compound **6** with methylhydrazine produced the pyrazole **7** (Scheme 1). The structure of compound **7** was determined as illustrated in Scheme1, using the nuclear Overhauser effect (NOE), observed between the protons of the *N*-methyl group (4.01 ppm) and the thiophene (7.21 ppm). Lithiation of compound **7** with *n*-butyllithium (*n*-BuLi) in tetrahydrofuran (THF), followed by treatment with ethyl chloroformate, gave the ester **8**, which was hydrolyzed with aqueous NaOH to form the acid **9**. Compound **9** was treated with oxalyl chloride and the resultant acyl chloride **10** was allowed to react with amines to form 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamides (**11**–**35**). The 4-hydroxyphenyl (**36**) and the 1-*tert*-butoxycarbonylpiperidin-4-yl (**37**) derivatives were prepared by the reaction of compound **9** with the corresponding amines[12] in the presence of 1-ethyl-3-(3-

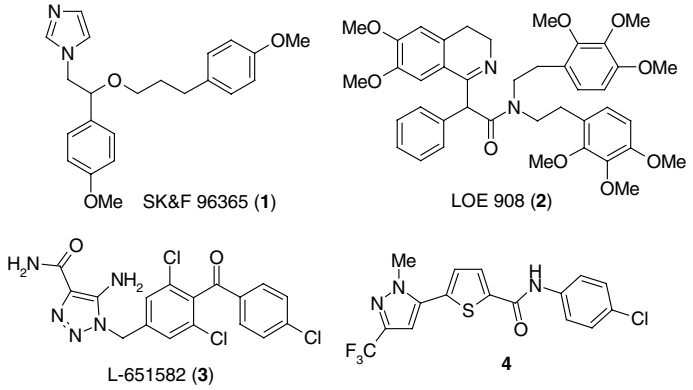
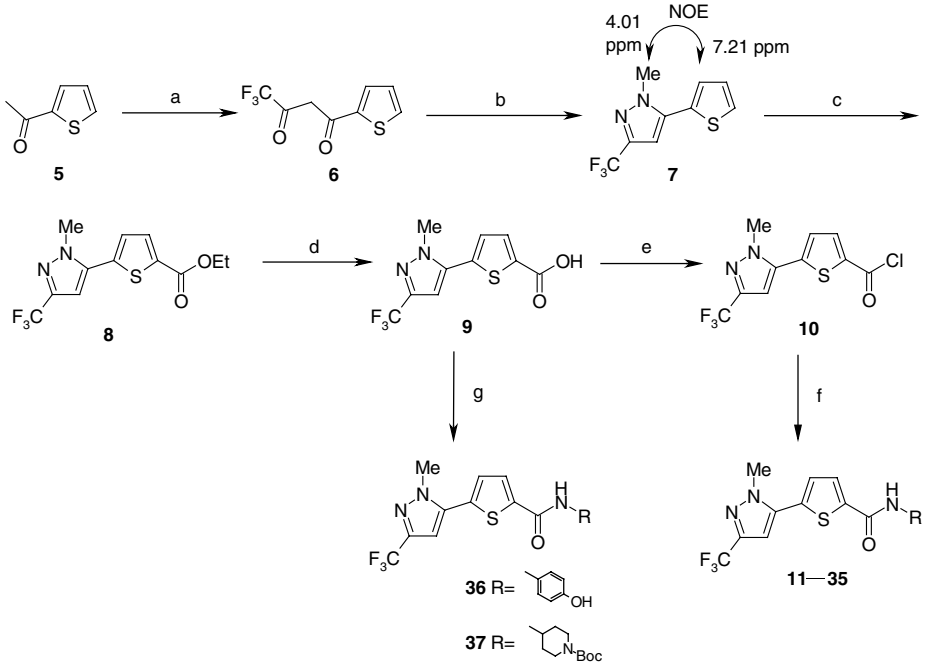
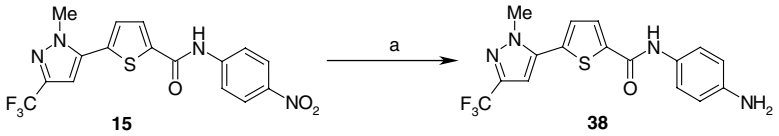


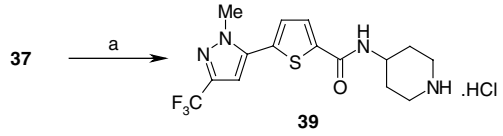
Figure 1. Structures of SK&F 96365 (**1**), LOE 908 (**2**), L-651582 (**3**), and compound **4**.



Scheme 1. Reagents: (a) CF_3CO_2Et, NaOMe, MeOH; (b) $MeNHNH_2$, AcOH–EtOH; (c) *n*-BuLi, THF, then $ClCO_2Et$; (d) 1 M NaOH, EtOH; (e) $(COCl)_2$, cat. DMF, THF; (f) R-NH_2, base; (g) R-NH_2, EDC · HCl, THF.

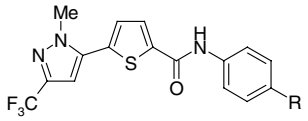


Scheme 2. Reagents: (a) Zn, NH_4Cl, EtOH–H_2O.



Scheme 3. Reagents: (a) 4 M HCl (g), AcOEt.

Table 1. Biological properties of 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(4-substituted-phenyl)-2-thiophenecarboxamides



Compound	R	IC_{50} (μM)		CRAC channel selectivity index[c]
		CRAC[a]	VOC[b]	
4	Cl	0.13	0.75	5.8
11	F	0.14	3.6	26
12	Br	0.27	2.6	10
13	CO_2Et	46%[d]	NT[e]	—
14	CN	0.56	1.5	2.7
15	NO_2	1.4	4.0	2.9
16	H	0.062	1.9	31
17	Me	0.37	2.4	6.5
18	*i*Pr	0.92	1.2	1.3
36	OH	0.24	4.3	18
19	OMe	0.44	24%[f]	>23[g]
38	NH_2	4.1	69%[f]	—
20	NMe_2	8%[f]	NT	—

[a] Inhibition of Ca^{2+} influx through CRAC channels on Jurkat T lymphocytes. See experimental section.
[b] Inhibition of Ca^{2+} influx through VOC channels on PC12-h5 cells. See experimental section.
[c] IC_{50} for VOC channels/IC_{50} for CRAC channels.
[d] % inhibition at 0.3 μM.
[e] Not tested.
[f] % inhibition at 10 μM.
[g] IC_{50} value for VOC channels was taken as >10 μM.

dimethylaminopropyl)carbodiimide hydrochloride (EDC · HCl), as shown in Scheme 1. Reduction of the 4-nitrophenyl derivative **15** with zinc powder gave the 4-aminophenyl derivative **38** (Scheme 2), and the piperidin-4-yl derivative **39** was obtained from compound **37** by treatment with HCl (Scheme 1).

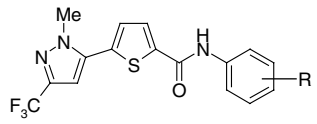
3. Results and discussion

The compounds synthesized as described above were evaluated for their ability to inhibit Ca^{2+} influx through CRAC channels on Jurkat T lymphocytes[13] and VOC channels on PC12-h5 cells.[14] The results are summarized in Tables 1–3.

We first examined the effects of substitutions at the 4-position in the phenyl group of compound **4** on the inhibitory activity and selectivity (Table 1). Substituting fluoro (**11**) and bromo (**12**) groups for the chloro group did not change the compounds' inhibitory effects on CRAC channels, but reduced their inhibitory activity against VOC channels. Substituting ethoxycarbonyl (**13**), cyano (**14**), and nitro (**15**) groups reduced the inhibitory effect on CRAC channels, suggesting that substituting groups that were highly electron-withdrawing at the 4-position would not favor increased CRAC channel inhibitory activity. In contrast, removal of the chloro group significantly increased CRAC channel inhibitory activity, with compound **16** showing an IC_{50} value of 0.062 μM, and in addition exhibiting high selectivity for CRAC channels with an index of over 31. The methyl derivative **17** was almost equipotent to compound **4**, whereas the isopropyl derivative **18** was a less effective inhibitor. From these results, we speculated that the ability to inhibit CRAC channels was sensitive to the bulkiness of the group at the 4-position. Hydroxy (**36**) and methoxy (**19**) groups, which maintained the potency, improved the selectivity for CRAC channels, with indices of approximately 20. In contrast, substituting nitrogen-containing groups such as amino (**38**) and dimethylamino (**20**) groups at this position dramatically decreased the inhibitory activity.

To investigate the SARs in detail, we performed a quantitative analysis using various parameters for side groups, such as π,[15] molecular refractivity,[16] Swein

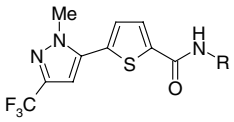
Table 2. Biological properties of 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(substituted-phenyl)-2-thiophenecarboxamides



Compound	R	IC_{50} (μM)		CRAC channel selectivity index[c]
		CRAC[a]	VOC[b]	
4	4-Cl	0.13	0.75	5.8
21	3-Cl	1.1	2.2	2.0
22	2-Cl	0.050	0%[d]	>200[e]
23	2-F	0.090	4.0	44
24	2-Me	0.30	10	33
25	2,4-diCl	0.14	18%[d]	>71[e]

[a–c] See the corresponding footnotes to Table 1.
[d] % inhibition at 10 μM.
[e] IC_{50} value for VOC channels was taken as >10 μM.

Table 3. Biological properties of 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-substituted-2-thiophenecarboxamides



Compound	R	IC_{50} (μM)		CRAC channel selectivity index[c]
		CRAC[a]	VOC[b]	
4	4-Chlorophenyl	0.13	0.75	5.8
26	Thiophen-2-yl	0.10	1.2	12
27	Thiophen-3-yl	0.61	2.3	3.8
28	1-Methylpyrrol-2-yl	0.58	10	17
29	Pyridin-3-yl	0.99	47%[d]	>10[e]
30	Pyridin-4-yl	0.42	4.1	10
31	Thiazol-2-yl	35%[f]	NT[g]	—
32	1,2,4-Triazol-4-yl	6%[d]	NT[g]	—
33	Tetrazol-5-yl	12%[d]	NT[g]	—
34	Ethyl	1.7	17%[d]	>6[e]
35	Cyclohexyl	0.36	4.3	12
39	Piperidin-4-yl	10%[d]	NT[g]	—

[a–e] See the corresponding footnotes to Table 1.
[f] % inhibition at 1 μM.
[g] Not tested.

and Lupton F/R,[17] σ_{para},[18] Taft E_s,[19] Verloop L,[20] molecular volume, molecular surface area, and Verloop B5.[20] For the series of compounds shown in Table 1, the σ_{para} values of the group substituted at the 4-position in the phenyl group showed the best correlation ($r = 0.81$) with the ability to inhibit CRAC channels. These results are shown in Figure 2 and described by the following parabolic equation:

$$pIC_{50} = -2.04(\pm 0.44)\sigma^2_{para} + 6.74 \text{ (Eq. 1, } n = 13,$$
$$r = 0.81,\ s = 0.37,\ F = 21.46).$$

These results showed that both highly electron-withdrawing and highly electron-donating groups tended to dramatically reduce the inhibitory effect on CRAC channels. On the other hand, compounds containing groups with σ_{para} values of around 0 would be expected to act as highly potent inhibitors.

In the series of compounds with chloro groups in the phenyl group (**4**, **21**, and **22**), a 3-chloro group (**21**) decreased both the activity and the selectivity (Table 2). In contrast, the 2-chloro derivative (**22**), with an

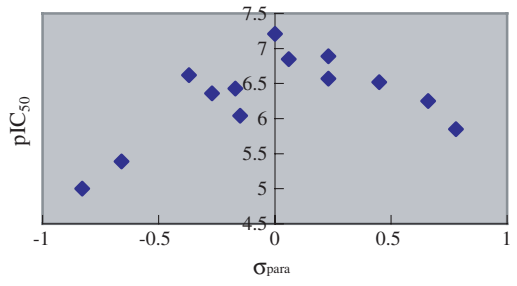


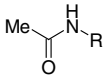
Figure 2. Correlation between Hammett constant (σ_{para}) and pIC_{50}. The pIC_{50} values of compounds **13** and **20** are plotted as 6.5 and 5.0, respectively.

IC_{50} value of 0.050 μM, was 3-fold more potent than compound **4** as a CRAC channel inhibitor. Surprisingly, this compound showed no inhibition of VOC channels at 10 μM, and an excellent selectivity for CRAC channels with an index of more than 200. Other substitutions at the 2-position (**23**–**25**) also improved the selectivity of the compounds for CRAC channels, implying that substitutions at this position would generally be expected to reduce inhibitory effects on VOC channels and produce CRAC channel-selective compounds.

Next, we investigated the effects of substituting heteroaromatic and aliphatic groups for the 4-chlorophenyl moiety of compound **4**. As shown in Table 3, in the series of compounds containing heteroaromatics, the ability of the compound to inhibit CRAC channels was unaffected by a 2-thienyl group (**26**), but was reduced 5-fold in the 3-thienyl derivative **27**. The 1-methylpyrrolyl (**28**) and pyridyl (**29**, **30**) derivatives were also less potent than compound **4**, and the introduction of thiazolyl (**31**), 1,2,4-triazolyl (**32**), and tetrazolyl (**33**) groups resulted in dramatic reductions in the inhibition of CRAC channels. Among the compounds with aliphatic groups (**34**, **35**, and **39**), the cyclohexyl derivative **35** was found to show the most potent CRAC channel inhibitory activity and was almost as potent as compound **4**. These results implied that aromatic groups were not essential features of CRAC channel inhibitors.

To investigate the SARs of this series in more detail, we attempted to calculate logP ($c\log P$) values for the acetamides **40**–**50** using the ACD/log*P* program.[21] The $c\log P$ values obtained were compared with the CRAC channel inhibitory activity of the corresponding 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamides (**4**, **26**–**30**, **32**–**35**, **39**, respectively), and the results are shown in Table 4. Among the acetamides bearing aromatic groups (**40**–**47**), the 4-chlorophenyl (**40**) and

Table 4. *c*log *P* values of acetamides **40**–**50** and CRAC channel inhibitory activity of corresponding 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamides



Compound	R	*c*log *P*[a]	Compound	CRAC IC_{50}[b] (μM)
40	4-Chlorophenyl	2.05	**4**	0.13
41	Thiophen-2-yl	1.43	**26**	0.10
49	Cyclohexyl	1.01	**35**	0.36
42	Thiophen-3-yl	0.76	**27**	0.61
43	1-Methylpyrrol-2-yl	0.74	**28**	0.58
45	Pyridin-4-yl	0.59	**30**	0.42
44	Pyridin-3-yl	0.26	**29**	0.99
48	Ethyl	−0.52	**34**	1.7
50	Piperidin-4-yl	−0.85	**39**	10%[c]
46	1,2,4-Triazol-4-yl	−1.43	**32**	6%[c]
47	Tetrazol-5-yl	−1.47	**33**	12%[c]

[a] ACD/log *P* values.
[b] See the footnote a of Table 1.
[c] % inhibition at 10 μM.

thiophen-2-yl (**41**) derivatives gave *c*log *P* values greater than 1, and the corresponding compounds **4** and **26** were potent inhibitors of CRAC channels. In contrast, 1,2,4-triazol-4-yl (**46**) and tetrazol-5-yl (**47**) groups produced hydrophilic acetamides and the corresponding thiophene-2-carboxamides **32** and **33** failed to inhibit CRAC channels. From these results, we hypothesized that a hydrophobic acetamide moiety would be an essential feature of potent inhibitors of CRAC channels. This speculation was supported by the fact that compounds **27**–**30**, whose corresponding acetamides possessed *c*log *P* values of 0.2–0.8, showed moderate activity with IC_{50} values of 0.4–1.0 μM. In addition, the *c*log *P* value of the cyclohexyl derivative (**49**) was the highest in the *N*-aliphatic acetamide series **48**–**50**, and the *N*-cyclohexylthiophene-2-carboxamide **35** was the most potent CRAC channel inhibitor of compounds **34**, **35**, and **39** (Table 4).

A selection of compounds which showed potent and selective inhibitory activity for CRAC channels (**11**, **12**, **16**, **17**, **22**–**26**, **28**, **29**, **35**, and **36**) was tested for their ability to inhibit phytohemagglutinin (PHA)-induced IL-2 production in Jurkat T lymphocytes[7] (Table 5). Compound **4** inhibited IL-2 production with an IC_{50} value of 0.53 μM. Substitutions at the 2-position of the phenyl group (**22**–**25**) increased the inhibitory activity, with compounds **23** and **25** in particular inhibiting IL-2 production with IC_{50} values of 0.023 and 0.026 μM, respectively. The 1-methylpyrrolyl derivative **28** was also a more potent inhibitor than compound **4** in this assay, despite having shown a 4-fold lower potency as a CRAC channel inhibitor. These results suggested that substitutions adjacent to the amide bond would be likely to increase the inhibitory effect of compounds on IL-2 production. The cyclohexyl derivative **35** inhibited IL-2 production with an IC_{50} value of 0.076 μM, which showed that hydrophobic groups in this moiety may be crucial for inhibitory activity against both IL-2 production and CRAC channels. Some compounds which were potent inhibitors of both CRAC channel and IL-2 production (**16**, **22**–**25**, **35**) were further tested for their ability to inhibit concanavalin A (Con A)-induced hepatitis in mice (Table 6).[22] Among the compounds tested,

Table 5. Biological properties of 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamides in vitro

Compound	IC_{50} (μM)	
	CRAC[a]	IL-2[b]
4	0.13	0.53
11	0.14	0.75
12	0.27	1.8
16	0.062	0.85
17	0.37	0.54
36	0.24	1.5
22	0.050	0.14
23	0.09	0.023
24	0.30	0.053
25	0.14	0.026
26	0.10	0.70
28	0.58	0.20
29	0.99	1.4
35	0.36	0.076

[a] See the footnote a of Table 1.
[b] Inhibition of PHA-induced IL-2 production in Jurkat T lymphocytes. See experimental section.

Table 6. Effects of selected compounds on Con A-induced hepatitis in mice

Compound	In vivo hepatitis[a]	
	ED_{50} (mg/kg *p.o.*)	% inhibition at 30 mg/kg *p.o.*
16		48
22		46
23		32
24	12.7	67
25		0
35		9

[a] Inhibitory activities against Con A-induced liver injury in mice. See experimental section.

24 was found to be the most potent inhibitor with an ED_{50} value of 12.7 mg/kg *p.o.*

4. Conclusion

We designed and synthesized novel 5-(1-methyl-3-trifluoromethyl-*1H*-pyrazol-5-yl)-2-thiophenecarboxamides based on compound **4** and evaluated their ability to inhibit CRAC and VOC channels. SAR study showed a relationship between the ability of compounds to inhibit CRAC channels and the σ_{para} values of the groups substituted at the 4-position on the phenyl ring, with values of around 0 being most favorable for a high potency. In addition, substitutions at the 2-position resulted in an improvement of the selectivity of the CRAC channel inhibitors and hydrophobic moieties such as chlorophenyl and cyclohexyl groups were shown to be essential to potent CRAC channel inhibitory activity. We identified compound **22** as the most promising novel CRAC channel inhibitor, with an IC_{50} value of 0.050 μM and a selectivity index of more than 200. Additionally, some other compounds including compound **24** were found to inhibit PHA-induced IL-2 production in vitro with IC_{50} values of the order of 10^{-8} M, and Con A-induced hepatitis in vivo, after oral administration. This study demonstrated that T lymphocyte function can be inhibited by CRAC channel inhibitors both in vitro and in vivo. These compounds have potential uses as a new class of orally active anti-inflammatory agents.

5. Experimental

5.1. Chemistry

Melting points were determined with a Yanaco MP-500D melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-EX90, JEOL JNM-LA300, JEOL JNM-EX400 or JEOL JNM-A500 spectrometer and were referenced to an internal standard, tetramethylsilane. The abbreviations used for the signal patterns are as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; dd, double doublet; dt, double triplet; tt, triple triplet; m, multiplet. Mass spectra were recorded on a Hitachi M-80 or JEOL JMS-DX300 mass spectrometer, and the ionization method was chosen from EI and FAB. High-resolution mass spectra (HRMS) were recorded on VG ZAB-VSE mass spectrometers. The elemental analyses were performed with a Yanako MT-5 microanalyzer (C, H, and N) and a Yokogawa IC-7000S ion chromatographic analyzer (halogens and S). Drying of organic solutions during workup was done over anhydrous $MgSO_4$. Preparative column chromatography was performed with Wakogel C-200 or Merck silica gel 60.

5.2. 1-Methyl-5-(2-thienyl)-3-trifluoromethyl-1*H*-pyrazole (7)

NaOMe (10.3 g, 191 mmol) was added to a solution of 2-acetylthiophene (**5**, 18.5 g, 147 mmol) in MeOH (150 mL), and the whole mixture was stirred for 20 min at room temperature. It was cooled on ice-bath, then added ethyl trifluoroacetate (25.0 g, 176 mmol), and the whole mixture was heated to reflux for 19 h. H_2O (300 mL) was added to the mixture and extracted with AcOEt, washed with brine, dried, and concentrated in vacuo to give a pale brown solid (**6**, 34.7 g). This crude compound (**6**, 20.0 g) was added to a mixture of methylhydrazine (4.56 g, 99.0 mmol), AcOH (20 mL), and EtOH (200 mL), and the whole mixture was heated to reflux for 30 min. The mixture was concentrated in vacuo, and saturated aqueous $NaHCO_3$ was added to the residue and extracted with AcOEt, washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography (hexane/AcOEt, 20:1–10:1) to give **7** (11.8 g, 60%): ^{1}H NMR ($CDCl_3$) δ 4.01 (3H, s), 6.64 (1H, s), 7.15 (1H, dd, J = 5.2, 3.7 Hz), 7.21 (1H, dd, J = 3.7, 1.3 Hz), 7.47 (1H, dd, J = 5.2, 1.3 Hz); FAB-MS m/z 233 [$(M+H)^+$].

5.3. Ethyl 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxylate (8)

A 1.6 M solution of *n*-BuLi (33.0 mL, 52.8 mmol) in hexane was added dropwise to a solution of 1-methyl-5-(2-thienyl)-3-trifluoromethyl-1*H*-pyrazole (**7**, 11.2 g, 48.2 mmol) in THF (150 mL) at −70 °C. The mixture was stirred for 1.5 h at −70 °C and then ethyl chloroformate (10.5 g, 96.8 mmol) was added at the same temperature. The mixture was stirred for 15 min and then aqueous NH_4Cl was added. The whole mixture was extracted with AcOEt, washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography (hexane/AcOEt, 7:1) to give **8** (9.58 g, 65%) as a pale yellow solid: ^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, J = 7.3 Hz), 4.05 (3H, s), 4.39 (2H, q, J = 7.0 Hz), 6.71 (1H, s), 7.20 (1H, d, J = 4.5 Hz), 7.80 (1H, d, J = 3.5 Hz); FAB-MS m/z 305 [$(M+H)^+$].

5.4. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxylic acid (9)

A mixture of ethyl 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxylate (**8**, 3.07 g, 10.1 mmol), 1 M NaOH (15 mL), and EtOH (30 mL) was stirred for 4 h at room temperature. One molar HCl (18 mL) was added to the mixture and extracted with AcOEt, washed with brine and dried, and then concentrated in vacuo to give **9** (2.79 g, 100%) as a pale brown solid: ^{1}H NMR (DMSO-d_6) δ 4.06 (3H, s), 7.18 (1H, s), 7.59 (1H, d, J = 3.9 Hz), 7.80 (1H, d, J = 3.9 Hz), 13.44 (1H, br s); FAB-MS m/z 275 [$(M-H)^-$].

5.5. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarbonylchloride (10)

A mixture of 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxylic acid (**9**, 2.21 g, 8.00 mmol), oxalyl chloride (2.03 g, 16.0 mmol), and DMF (3 drops) in $ClCH_2CH_2Cl$ (30 mL) was stirred for 90 min at room temperature. The mixture was concentrated in vacuo to give **10** (2.23 g, 95%) as a brown solid: ^{1}H NMR

($CDCl_3$) δ 4.09 (3H, s), 6.78 (1H, s), 7.30 (1H, d, J = 4.4 Hz), 8.00 (1H, d, J = 3.9 Hz); FAB-MS m/z 291 [$(M+H)^+$].

5.6. *N*-(4-Fluorophenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (11)

To a mixture of 4-fluoroaniline (93 mg, 0.84 mmol), saturated aqueous $NaHCO_3$ (3 mL), and CH_2Cl_2 (2 mL), 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarbonylchloride (**10**, 195 mg, 0.662 mmol) was added and the whole mixture was stirred for overnight at room temperature. H_2O was added to the mixture and extracted with AcOEt, washed with brine and dried, and then concentrated in vacuo. The residue was recrystallized from AcOEt to give **11** (64 mg, 26%) as a colorless powder: mp 189–192 °C; ^1H NMR ($CDCl_3$) δ 4.07 (3H, s), 6.72 (1H, s), 7.08 (2H, t, J = 8.6 Hz), 7.23 (1H, d, J = 3.9 Hz), 7.55–7.61 (2H, m), 7.62 (1H, d, J = 3.9 Hz), 7.69 (1H, br s); FAB-MS m/z 370 [$(M+H)^+$]. Anal. Calcd for $C_{16}H_{11}F_4N_3OS$: C, 52.03; H, 3.00; N, 11.38; F, 20.58; S, 8.68. Found: C, 51.98; H, 2.84; N, 11.43; F, 20.75; S, 8.72.

The following compounds were prepared following the same method. Among the corresponding amines, 2-amino-1-methylpyrrole was prepared by the methods reported previously.[23]

5.7. *N*-(4-Bromophenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (12)

Yield 11%; mp 191–192 °C (AcOEt); ^1H NMR ($CDCl_3$) δ 4.07 (3H, s), 6.73 (1H, s), 7.24 (1H, d, J = 3.9 Hz), 7.47–7.55 (4H, m), 7.62 (1H, d, J = 3.9 Hz), 7.67 (1H, br s); FAB-MS m/z 430, 432 [$(M+H)^+$]. Anal. Calcd for $C_{16}H_{11}BrF_3N_3OS$: C, 44.67; H, 2.58; N, 9.77; Br, 18.57; F, 13.25; S, 7.45. Found: C, 44.56; H, 2.75; N, 9.80; Br, 18.62; F, 13.30; S, 7.36.

5.8. *N*-(4-Cyanophenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (14)

Yield 55%; mp 211–214 °C (AcOEt–hexane); ^1H NMR (DMSO-d_6) δ 4.09 (3H, s), 7.20 (1H, s), 7.67 (1H, d, J = 3.9 Hz), 7.85 (2H, d, J = 8.8 Hz), 7.95 (2H, d, J = 9.3 Hz), 8.15 (1H, d, J = 3.9 Hz), 10.72 (1H, s); FAB-MS m/z 377 [$(M+H)^+$]. Anal. Calcd for $C_{17}H_{11}F_3N_4OS \cdot 0.15H_2O$: C, 53.87; H, 3.00; N, 14.78; F, 15.04; S, 8.46. Found: C, 53.49; H, 2.85; N, 14.53; F, 15.41; S, 8.46.

5.9. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(4-nitrophenyl)-2-thiophenecarboxamide (15)

Yield 24%; mp 183–184 °C (AcOEt–hexane); ^1H NMR (DMSO-d_6) δ 4.10 (3H, s), 7.20 (1H, s), 7.68 (1H, d, J = 3.9 Hz), 8.03 (2H, d, J = 9.3 Hz), 8.18 (1H, d, J = 4.4 Hz), 8.29 (2H, d, J = 9.2 Hz), 10.88 (1H, s); FAB-MS m/z 397 [$(M+H)^+$]. HRMS m/z Calcd for $C_{16}H_{11}F_3N_4O_3S$ [$(M+H)^+$]: 397.0582. Found: 397.0601.

5.10. *N*-(4-Isopropylphenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (18)

Yield 71%; mp 163–164 °C (AcOEt–hexane); ^1H NMR (DMSO-d_6) δ 1.21 (6H, d, J = 6.8 Hz), 2.83–2.92 (1H, m), 4.08 (3H, s), 7.17 (1H, s), 7.24 (2H, d, J = 8.3 Hz), 7.63 (1H, d, J = 3.9 Hz), 7.63 (2H, d, J = 8.8 Hz), 8.09 (1H, d, J = 3.9 Hz), 10.30 (1H, s); FAB-MS m/z 394 [$(M+H)^+$]. Anal. Calcd for $C_{19}H_{18}F_3N_3OS$: C, 58.00, H, 4.61; N, 10.68; F, 14.49; S, 8.15. Found: C, 58.17; H, 4.59; N, 10.68; F, 14.48; S, 8.16.

5.11. *N*-(4-Dimethylaminophenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (20)

Yield 60%; mp 202–203 °C (EtOH–AcOEt); ^1H NMR (DMSO-d_6) δ 2.88 (6H, s), 4.08 (3H, s), 6.74 (2H, d, J = 9.2 Hz), 7.15 (1H, s), 7.52 (2H, d, J = 9.3 Hz), 7.60 (2H, d, J = 4.4 Hz), 8.04 (1H, d, J = 4.4 Hz), 10.13 (1H, s); FAB-MS m/z 394 (M^+). Anal. Calcd for $C_{18}H_{17}F_3N_4OS$: C, 54.81, H, 4.34; N, 14.20; F, 14.45; S, 8.13. Found: C, 54.73; H, 4.43; N, 14.19; F, 14.34; S, 7.95.

5.12. *N*-(3-Chlorophenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (21)

Yield 39%; mp 127–129 °C (AcOEt–hexane); ^1H NMR (DMSO-d_6) δ 4.09 (3H, s), 7.17–7.21 (2H, m), 7.41 (1H, t, J = 8.1 Hz), 7.65 (1H, d, J = 3.9 Hz), 7.66–7.70 (1H, m), 7.91 (1H, t, J = 2.0 Hz), 8.11 (1H, d, J = 3.9 Hz), 10.51 (1H, s); FAB-MS m/z 386 [$(M+H)^+$]. Anal. Calcd for $C_{16}H_{11}ClF_3N_3OS$: C, 49.81; H, 2.87; N, 10.89; Cl, 9.19; F, 14.77; S, 8.31. Found: C, 49.74; H, 2.73; N, 10.94; Cl, 9.11; F, 14.73; S, 8.29.

5.13. *N*-(1-Methyl-1*H*-pyrrol-2-yl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (28)

Yield 36%; mp 158–159 °C (AcOEt–hexane); ^1H NMR (DMSO-d_6) δ 3.44 (3H, s), 4.07 (3H, s), 5.92 (1H, br s), 5.98 (1H, t, J = 3.2 Hz), 6.69 (1H, s), 7.16 (1H, s), 7.62 (1H, d, J = 3.9 Hz), 8.03 (1H, d, J = 3.9 Hz), 10.12 (1H, s); FAB-MS m/z 355 [$(M+H)^+$]. Anal. Calcd for $C_{15}H_{13}F_3N_4OS$: C, 50.84; H, 3.70; N, 15.81; F, 16.08; S, 9.05. Found: C, 50.90; H, 3.66; N, 15.77; F, 16.07; S, 9.01.

5.14. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(pyridin-4-yl)-2-thiophenecarboxamide monohydrochloride (30)

Yield 24%; mp 247 °C (AcOEt–hexane); ^1H NMR (DMSO-d_6) δ 4.11 (3H, s), 7.25 (1H, s), 7.73 (1H, d, J = 4.3 Hz), 8.39 (2H, d, J = 5.9 Hz), 8.53 (1H, d, J = 3.9 Hz), 8.76 (2H, d, J = 6.4 Hz), 11.93 (1H, s); FAB-MS m/z 353 [$(M+H)^+$]. Anal. Calcd for $C_{15}H_{11}F_3N_4OS \cdot HCl$: C, 46.34; H, 3.11; N, 14.41; Cl, 9.12; F, 14.66; S, 8.25. Found: C, 46.19; H, 2.99; N, 14.39; Cl, 9.07; F, 14.72; S, 8.13.

5.15. *N*-Cyclohexyl-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (35)

Yield 68%; mp 140–141 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 1.10–1.20 (1H, m), 1.24–1.36 (4H, m), 1.58–1.66 (1H, m), 1.69–1.87 (4H, m), 3.67–3.77 (1H, m), 4.05 (3H, s), 7.10 (1H, s), 7.53 (1H, d, J = 3.9 Hz), 7.87 (1H, d, J = 3.9 Hz), 8.38 (1H, d, J = 7.8 Hz); FAB-MS m/z 358 [(M+H)$^+$]. Anal. Calcd for $C_{16}H_{18}F_3N_3OS$: C, 53.77; H, 5.08; N, 11.76; F, 15.95; S, 8.97. Found: C, 53.47; H, 4.94; N, 11.68; F, 16.25; S, 8.97.

The following compounds were prepared according to this method, substituting Et_3N for aqueous $NaHCO_3$. Among the corresponding amines, 2- and 3-aminothiophene were prepared by the methods reported previously.[24]

5.16. *N*-(4-Methylphenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (17)

Yield 61%; mp 167–169 °C (AcOEt–hexane); ^{1}H NMR ($CDCl_3$) δ 2.35 (3H, s), 4.06 (3H, s), 6.72 (1H, s), 7.19 (2H, d, J = 8.3 Hz), 7.22 (1H, d, J = 3.9 Hz), 7.49 (2H, d, J = 8.3 Hz), 7.60 (1H, d, J = 3.9 Hz), 7.65 (1H, br s); FAB-MS m/z 366 [(M+H)$^+$]. Anal. Calcd for $C_{17}H_{14}F_3N_3OS \cdot 0.25H_2O$: C, 55.20; H, 3.95; N, 11.36; F, 15.41; S, 8.67. Found: C, 55.15; H, 3.77; N, 11.43; F, 15.50; S, 8.87.

5.17. *N*-(2-Fluorophenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (23)

Yield 26%; mp 147–148 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 4.09 (3H, s), 7.18 (1H, s), 7.22–7.35 (3H, m), 7.58–7.62 (1H, m), 7.64 (1H, d, J = 3.9 Hz), 8.10 (1H, d, J = 3.9 Hz), 10.33 (1H, s); FAB-MS m/z 370 [(M+H)$^+$]. Anal. Calcd for $C_{16}H_{11}F_4N_3OS$: C, 52.03; H, 3.00; N, 11.38; F, 20.58; S, 8.68. Found: C, 51.81; H, 2.92; N, 11.41; F, 20.55; S, 8.69.

5.18. *N*-(2-Methylphenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (24)

Yield 50%; mp 159–161 °C (AcOEt–hexane); ^{1}H NMR ($CDCl_3$) δ 2.35 (3H, s), 4.06 (3H, s), 6.72 (1H, s), 7.13–7.18 (1H, m), 7.22 (1H, d, J = 3.9 Hz), 7.23–7.30 (2H, m), 7.58 (1H, s), 7.61 (1H, d, J = 3.9 Hz), 7.86 (1H, d, J = 7.8 Hz); FAB-MS m/z 366 [(M+H)$^+$]. Anal. Calcd for $C_{17}H_{14}F_3N_3OS$: C, 55.88; H, 3.86; N, 11.50; F, 15.60; S, 8.78. Found: C, 55.77; H, 3.79; N, 11.64; F, 15.56; S, 8.77.

5.19. *N*-(2,4-Dichlorophenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (25)

Yield 25%; mp 181–183 °C (AcOEt–hexane); ^{1}H NMR ($CDCl_3$) δ 4.08 (3H, s), 6.74 (1H, s), 7.26 (1H, d, J = 3.9 Hz), 7.32 (1H, dd, J = 9.1, 2.2 Hz), 7.45 (1H, d, J = 2.4 Hz), 7.66 (1H, d, J = 3.9 Hz), 8.25 (1H, s), 8.45 (1H, d, J = 8.8 Hz); FAB-MS m/z 420 [(M+H)$^+$]. Anal. Calcd for $C_{16}H_{10}Cl_2F_3N_3OS$: C, 45.73; H, 2.40; N, 10.00; Cl, 16.87; F, 13.56; S, 7.63. Found: C, 45.65; H, 2.38; N, 9.99; Cl, 16.77; F, 13.40; S, 7.59.

5.20. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(thiophen-2-yl)-2-thiophenecarboxamide (26)

Yield 15%; mp 180–185 °C (Et_2O–hexane); ^{1}H NMR ($CDCl_3$) δ 4.07 (3H, s), 6.73 (1H, s), 6.83 (1H, dd, J = 3.7, 1.2 Hz), 6.92 (1H, dd, J = 5.7, 3.7 Hz), 6.97–7.00 (1H, m), 7.24 (1H, d, J = 3.9 Hz), 7.65 (1H, d, J = 3.9 Hz), 8.37 (1H, br s); FAB-MS m/z 358 [(M+H)$^+$]. Anal. Calcd for $C_{14}H_{10}F_3N_3OS_2 \cdot 0.25H_2O$: C, 46.47; H, 2.92; N, 11.61; F, 15.75; S, 17.72. Found: C, 46.37; H, 2.71; N, 11.44; F, 15.65; S, 17.72.

5.21. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(thiophen-3-yl)-2-thiophenecarboxamide (27)

Yield 62%; mp 166–168 °C (AcOEt–hexane); ^{1}H NMR ($CDCl_3$) δ 4.07 (3H, s), 6.72 (1H, s), 7.13 (1H, dd, J = 4.9, 1.5 Hz), 7.23 (1H, d, J = 3.9 Hz), 7.31 (1H, dd, J = 4.7, 3.2 Hz), 7.60 (1H, d, J = 3.9 Hz), 7.68 (1H, dd, J = 3.0, 1.4 Hz), 7.97 (1H, s); FAB-MS m/z 358 [(M+H)$^+$]. Anal. Calcd for $C_{14}H_{10}F_3N_3OS_2$: C, 47.05; H, 2.82; N, 11.76; F, 15.95; S, 17.95. Found: C, 46.91; H, 2.83; N, 11.68; F, 16.24; S, 17.91.

5.22. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(pyridin-3-yl)-2-thiophenecarboxamide (29)

Yield 62%; mp 186–187 °C (AcOEt–hexane); ^{1}H NMR ($CDCl_3$) δ 4.07 (3H, s), 6.73 (1H, s), 7.25 (1H, d, J = 3.9 Hz), 7.35 (1H, dd, J = 8.3, 3.8 Hz), 7.68 (1H, d, J = 3.9 Hz), 7.95 (1H, s), 8.24–8.28 (1H, m), 8.42 (1H, dd, J = 4.7, 1.2 Hz), 8.68 (1H, d, J = 2.5 Hz); FAB-MS m/z 353 [(M+H)$^+$]. Anal. Calcd for $C_{15}H_{11}F_3N_4OS$: C, 51.13; H, 3.15; N, 15.90; F, 16.18; S, 9.10. Found: C, 51.03; H, 3.14; N, 15.84; F, 15.89; S, 9.04.

5.23. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(1*H*-tetrazol-5-yl)-2-thiophenecarboxamide (33)

Yield 72%; mp 285–287 °C (acetone); ^{1}H NMR (DMSO-d_6) δ 4.09 (3H, s), 7.22 (1H, s), 7.69 (1H, d, J = 3.9 Hz), 8.32 (1H, d, J = 4.4 Hz), 12.67 (1H, br s); FAB-MS m/z 344 [(M+H)$^+$]. Anal. Calcd for $C_{11}H_8F_3N_7OS \cdot 0.2H_2O$: C, 38.09; H, 2.44; N, 28.26; F, 16.43; S, 9.24. Found: C, 37.95; H, 2.40; N, 28.54; F, 16.78; S, 9.24.

The following compounds were prepared according to this method, substituting pyridine for aqueous $NaHCO_3$.

5.24. Ethyl 4-{[5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarbonyl]amino}benzoate (13)

Yield 45%; mp 183–185 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, t, J = 7.1 Hz), 4.09 (3H, s), 4.31 (2H, q, J = 7.2 Hz), 7.19 (1H, s), 7.66 (1H, d, J = 3.9 Hz), 7.91 (2H, d, J = 8.7 Hz), 7.99 (2H, d, J = 8.8 Hz), 8.16 (1H, d, J = 4.4 Hz), 10.65 (1H, s); FAB-MS m/z 424 [(M+H)$^+$]. Anal. Calcd for

$C_{19}H_{16}F_3N_3O_3S$: C, 53.90; H, 3.81; N, 9.92; F, 13.46; S, 7.57. Found: C, 53.89; H, 3.79; N, 9.87; F, 13.43; S, 7.48.

5.25. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-phenylthiophene-2-carboxamide (16)

Yield 86%; mp 129–130 °C; ^{1}H NMR ($CDCl_3$) δ 4.07 (3H, s), 6.72 (1H, s), 7.19 (1H, t, J = 7.6 Hz), 7.23 (1H, d, J = 3.4 Hz), 7.40 (2H, t, J = 8.1 Hz), 7.60–7.63 (3H, m), 7.66 (1H, br s); FAB-MS m/z 352 [$(M+H)^+$]. Anal. Calcd for $C_{16}H_{12}F_3N_3OS$: C, 54.70; H, 3.44; N, 11.96; F, 16.22; S, 9.13. Found: C, 54.69; H, 3.22; N, 12.21; F, 16.10; S, 9.05.

5.26. *N*-(4-Methoxyphenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (19)

Yield 49%; mp 166–167 °C (AcOEt–hexane); ^{1}H NMR (DMSO-d_6) δ 3.75 (3H, s), 4.08 (3H, s), 6.95 (2H, d, J = 9.3 Hz), 7.16 (1H, s), 7.62 (1H, d, J = 3.9 Hz), 7.62 (2H, d, J = 9.3 Hz), 8.06 (1H, d, J = 3.9 Hz), 10.27 (1H, s); FAB-MS m/z 382 [$(M+H)^+$]. Anal. Calcd for $C_{17}H_{14}F_3N_3O_2S$: C, 53.54, H, 3.70; N, 11.02; F, 14.94; S, 8.41. Found: C, 53.45; H, 3.67; N, 10.96; F, 14.88; S, 8.28.

5.27. *N*-(2-Chlorophenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (22)

Yield 41%; mp 156 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 4.08 (3H, s), 6.74 (1H, s), 7.11 (1H, td, J = 7.8, 1.5 Hz), 7.26 (1H, d, J = 3.4 Hz), 7.35 (1H, td, J = 7.8, 1.4 Hz), 7.44 (1H, dd, J = 7.8, 1.5 Hz), 7.66 (1H, d, J = 3.9 Hz), 8.32 (1H, s), 8.48 (1H, dd, J = 8.3, 1.4 Hz); FAB-MS m/z 386 [$(M+H)^+$]. Anal. Calcd for $C_{16}H_{11}ClF_3N_3OS$: C, 49.81; H, 2.87; N, 10.89; Cl, 9.19; F, 14.77; S, 8.31. Found: C, 49.79; H, 2.80; N, 10.95; Cl, 9.18; F, 14.73; S, 8.30.

5.28. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(thiazol-2-yl)-2-thiophenecarboxamide (31)

Yield 58%; mp 235–238 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 4.09 (3H,s), 7.20 (1H, s), 7.29 (1H, d, J = 3.4 Hz), 7.57 (1H, d, J = 3.9 Hz), 7.65 (1H, d, J = 3.9 Hz), 8.28 (1H, br s), 12.94 (1H, br s); FAB-MS m/z 359 [$(M+H)^+$]. Anal. Calcd for $C_{13}H_9F_3N_4OS_2$: C, 43.57; H, 2.53; N, 15.63; F, 15.90; S, 17.90. Found: C, 43.34; H, 2.48; N, 15.64; F, 16.20; S, 17.82.

5.29. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(4*H*-1,2,4-triazol-4-yl)-2-thiophenecarboxamide (32)

Yield 71%; mp 207–210 °C (AcOEt); ^{1}H NMR (DMSO-d_6) δ 4.08 (3H, s), 7.22 (1H, s), 7.69 (1H, d, J = 3.9 Hz), 7.98 (1H, d, J = 3.9 Hz), 8.83 (2H, s), 12.34 (1H, s); FAB-MS m/z 343 [$(M+H)^+$]. Anal. Calcd for $C_{12}H_9F_3N_6OS$: C, 42.11; H, 2.65; N, 24.55; F, 16.65; S, 9.37. Found: C, 41.77; H, 2.55; N, 24.20; F, 16.59; S, 9.17.

5.30. *N*-Ethyl-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (34)

To a mixture of ethylamine (70% in H_2O, 1 mL, 15.5 mmol) and THF (4 mL), 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarbonylchloride (**10**, 150 mg, 0.509 mmol) was added and the whole mixture was stirred for 2 h at room temperature. H_2O was added to the mixture and extracted with AcOEt, washed with brine and dried, and then concentrated in vacuo. The residue was recrystallized from AcOEt–hexane to give **34** (96 mg, 62%) as a colorless powder: mp 136–137 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (3H, t, J = 7.1 Hz), 3.25–3.32 (2H, m), 4.05 (3H, s), 7.11 (1H, s), 7.54 (d, J = 3.9 Hz), 7.81 (1H, d, J = 3.9 Hz), 8.65 (1H, t, J = 5.4 Hz); FAB-MS m/z 304 [$(M+H)^+$]. Anal. Calcd for $C_{12}H_{12}F_3N_3OS$: C, 47.52; H, 3.99; N, 13.85; F, 18.79; S, 10.57. Found: C, 47.45; H, 3.98; N, 13.83; F, 19.07; S, 10.46.

5.31. *N*-(4-Hydroxyphenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (36)

A mixture of 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-**2-thiophene** (with Standard) carboxylic acid (**9**, 156 mg, 0.565 mmol), 4-aminophenol (62 mg, 0.57 mmol), and EDC · HCl (114 mg, 0.595 mmol) in THF (2 mL) was stirred for 3 days at room temperature. H_2O (10 mL) was added to the mixture and extracted with AcOEt, washed with 1 M HCl, saturated aqueous $NaHCO_3$, brine and dried, and then concentrated in vacuo. The residue was purified by column chromatography (hexane/AcOEt, 2:1) and recrystallized from AcOEt–hexane to give **36** (113 mg, 54%) as a colorless powder: mp 185–186 °C; ^{1}H NMR (DMSO-d_6) δ 4.08 (3H, s), 6.76 (2H, d, J = 8.8 Hz), 7.15 (1H, s), 7.48 (2H, d, J = 9.3 Hz), 7.61 (1H, d, J = 4.4 Hz), 8.04 (1H, d, J = 3.9 Hz), 9.32 (1H, s), 10.17 (1H, s); FAB-MS m/z 368 [$(M+H)^+$]. Anal. Calcd for $C_{16}H_{12}F_3N_3O_2S$: C, 52.31, H, 3.29; N, 11.44; F, 15.52; S, 8.73. Found: C, 51.99; H, 3.37; N, 11.27; F, 15.76; S, 8.68.

The following compound was prepared using similar method.

5.32. *tert*-Butyl 4-{[5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarbonyl]amino}piperidine-1-carboxylate (37)

^{1}H NMR ($CDCl_3$) δ 1.44–1.49 (2H, m), 1.47 (9H, s), 1.98–2.08 (2H, m), 2.83–2.97 (2H, m), 4.04 (3H, s), 4.08–4.17 (3H, m), 6.02–6.08 (1H, m), 6.68 (1H, s), 7.17 (1H, d, J = 3.6 Hz), 7.50 (1H, d, J = 3.9 Hz); FAB-MS m/z 459 [$(M+H)^+$].

5.33. *N*-(4-Aminophenyl)-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thiophenecarboxamide (38)

Zinc powder (240 mg, 3.67 mmol) and NH_4Cl (196 mg, 3.66 mmol) was added to a solution of 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(4-nitrophenyl)-2-thiophenecarboxamide (**15**, 146 mg, 0.368 mmol) in EtOH–H_2O (2:1, 6 mL) at 5 °C. The mixture was stirred for 20 min at room temperature and filtered on Celite. H_2O was added to the filtrate and extracted with AcOEt, washed with 0.1 M HCl, saturated aqueous $NaHCO_3$, brine and dried, and then concentrated in vacuo. The residue was recrystallized from AcOEt–hexane to give

38 (64 mg, 47%) as a yellow powder: mp 181–183 °C; ^{1}H NMR (DMSO-d_6) δ 4.07 (3H, s), 4.99 (2H, s), 6.56 (2H, d, J = 8.8 Hz), 7.14 (1H, s), 7.32 (2H, d, J = 8.8 Hz), 7.59 (1H, d, J = 3.9 Hz), 8.01 (1H, d, J = 3.9 Hz), 10.02 (1H, s); FAB-MS m/z 367 [(M+H)$^+$]. Anal. Calcd for $C_{16}H_{13}F_3N_4OS$: C, 52.45, H, 3.58; N, 15.29; F, 15.56; S, 8.75. Found: C, 52.65; H, 3.47; N, 15.04; F, 15.35; S, 8.60.

5.34. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-*N*-(4-piperidyl)-2-thiophenecarboxamide monohydrochloride (39)

A mixture of *tert*-butyl 4-{[5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thenoyl]amino}piperidine-1-carboxylate (**37**, 65 mg, 0.142 mmol) and 4 M HCl–AcOEt (1 mL) in AcOEt (1 mL) was stirred for 4 h at room temperature. The mixture was concentrated in vacuo, and the residue was recrystallized from EtOH to give **39** (34 mg, 61%) as a colorless powder: mp 265–266 °C; ^{1}H NMR (DMSO-d_6) δ 1.73–1.85 (2H, m), 1.93–2.01 (2H, m), 2.96–3.06 (2H, m), 3.28–3.32 (2H, m), 4.00–4.08 (1H, m), 4.05 (3H, s), 7.12 (1H, s), 7.56 (1H, d, J = 3.9 Hz), 7.97 (1H, d, J = 3.9 Hz), 8.74 (1H, d, J = 7.4 Hz), 8.78–8.90 (1H, br s); FAB-MS m/z 359 [(M+H)$^+$]. Anal. Calcd for $C_{15}H_{17}F_3N_4OS \cdot HCl$: C, 45.63; H, 4.59; N, 14.19; Cl, 8.98; F, 14.43; S, 8.12. Found: C, 45.32; H, 4.76; N, 14.20; Cl, 8.89; F, 14.63; S, 8.10.

5.35. QSAR analysis

Linear and multiple regression analyses were carried out using TSAR 3.0 (Oxford Molecular Group). One or two out of the following parameters (π, molecular refractivity, Swein and Lupton F/R, σ_{para} (σ^2_{para}), E_s, Verloop L, molecular volume, molecular surface area, and Verloop B5) were applied to the analyses. The following statistical parameters were determined for each regression equation: the number of points: n; the correlation coefficient: r; the significance of the regression model: F; the standard error: s.

5.36. Fura-2 loading and population intracellular calcium measurements

Cells were suspended in Hepes buffered solution (pH 7.4) of the following composition: NaCl: 137 mM, KCl: 5.8 mM, $MgCl_2$: 1 mM, $CaCl_2$: 2.5 mM, glucose: 5 mM, and Hepes: 10 mM. 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 in fluorescence microplate reader (Fluostar, SLT Labinstruments Ges.m.b.H, Austria) with excitation wavelength of each 340 and 380 nm at emission fluorescence detection of 500 nm. Then, self-fluorescence of each compound was measured in a cell-free way and was deducted from cell way fluorescence. Final intracellular calcium concentrations in each well were calculated from the fluorescence ratio, using the standard equation. The R_{max} value was obtained from 25 μM ionomycin-treated wells. The R_{min} value was obtained from 25 μM ionomycin/50 mM EGTA-treated wells.

5.37. 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 measured intracellular calcium concentration at the endpoint of 30 min. IC_{50} values on CRAC channel inhibition of each compounds were calculated from percent inhibition of thapsigargin-induced calcium influx in Jurkat cells.

5.38. 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 measured intracellular calcium concentration at the endpoint of 20 min. IC_{50} values on VOC channel inhibition of each compounds were calculated from percent inhibition of KCl-induced calcium influx in PC12 cells.

5.39. 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 and supernatant was collected from these cells. IL-2 concentration in each supernatant was measured by human IL-2 ELISA system (DuoSeT™, Genzyme).

5.40. Con A-induced hepatitis

Con A (20 mg/kg) was intravenously injected to female Balb/c mice in a volume of 10 mL/kg. Blood samples were obtained 24 h after Con A injection, and serum GOT and GPT levels were measured. For oral dosage groups, compounds were suspended with 0.5% MC and administered orally in a volume of 10 mL/kg 1 h before Con A injection. Cyclosporin A (CsA) was dissolved in physiological saline and subcutaneously administered at 50 mg/kg in a volume of 10 mL/kg 2 h before Con A injection.

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