Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 5370–5383
Bioorganic & Medicinal Chemistry

Novel potent and selective calcium-release-activated calcium (CRAC) channel inhibitors. Part 2: Synthesis and inhibitory activity of aryl-3-trifluoromethylpyrazoles☆

Yasuhiro Yonetoku,* Hirokazu Kubota, Yoshinori Okamoto, Jun Ishikawa, Makoto Takeuchi, Mitsuaki Ohta and Shin-ichi Tsukamoto

Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan

Received 16 February 2006; revised 20 March 2006; accepted 21 March 2006
Available online 17 April 2006

Abstract—To identify potent and selective calcium-release-activated calcium (CRAC) channel inhibitors, we examined the structure–activity relationships of the pyrazole and thiophene moieties in compound **4**. Compound **25b** was found to exhibit highly potent and selective inhibitory activity for CRAC channels and further modifications of the pyrazole and benzoyl moieties of compound **25b** produced compound **29**. These compounds were potent inhibitors of IL-2 production in vitro and also acted as inhibitors in pharmacological models of diseases resulting from T-lymphocyte activation, after oral administration.

1. Introduction

Ca^{2+} is known to regulate many physiological activities by controlling various cellular functions, and the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is precisely regulated by plasmalemmal Ca^{2+} channels. These channels can be divided into two broad categories depending on their activation mechanisms. Ca^{2+} channels modulated by membrane potential are called voltage-operated calcium (VOC) channels, and have been well studied with respect to their distribution and cellular functions.[2] VOC channel inhibitors have been used for the treatment of hypertension and brain dysfunction. In contrast, inflammatory cells have Ca^{2+} channels that are regulated by signal transduction pathways activated by other receptors.[3a–d]

In activated T-cells, the stimulation of cell surface receptors results in a transient increase in $[Ca^{2+}]_i$ as a result of Ca^{2+} efflux from inositol triphosphate-sensitive Ca^{2+} stores, and subsequently a sustained Ca^{2+} influx occurs through the calcium-release-activated calcium (CRAC) channels.[3e–o] This prolonged high $[Ca^{2+}]_i$ has been shown to activate cytosolic signal transduction, inducing the production of lipid mediators (e.g., LTD_4), cytokines (e.g., interleukin-2 (IL-2)), and matrix metalloproteases, which are known to be involved in the pathogenic processes in inflammation and autoimmune diseases. Thus, CRAC channel inhibitors may be useful for the treatment of diseases, such as asthma and rheumatoid arthritis, in which inflammatory cells are activated.[4] Since inhibition of VOC channels is known to affect the nervous and cardiovascular systems, compounds that inhibit CRAC channels will need to be sufficiently selective in their action, when compared to VOC channels, to be therapeutically useful and avoid serious side effects.

Recently, several compounds, including SK&F 96365 (**1**),[5] econazole (**2**),[6] and L-651582 (**3**,[7] Fig. 1), have been reported to inhibit CRAC channels. However, their potency and selectivity have not been adequate for them to be candidates for anti-inflammatory drugs. Screens of our chemical library for potent and selective CRAC channel inhibitors identified 4′-chloro-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxanilide (**4**) as a lead compound. Subsequent modification of the 4-chlorophenyl moiety of compound **4** produced 2′-chloro-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-thiophene-2-carboxanilide (**5**) which was a highly potent

Keywords: Anti-inflammatory; Channel inhibitors; Ca^{2+} release-activated Ca^{2+} channel; CRAC channel; Interleukin-2; X-ray diffraction studies.
☆ See Ref. 1.
* Corresponding author. Tel.: +81 029 847 8611; fax: +81 029 847 8313; e-mail: yasuhiro.yonetoku@jp.astellas.com

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.03.039

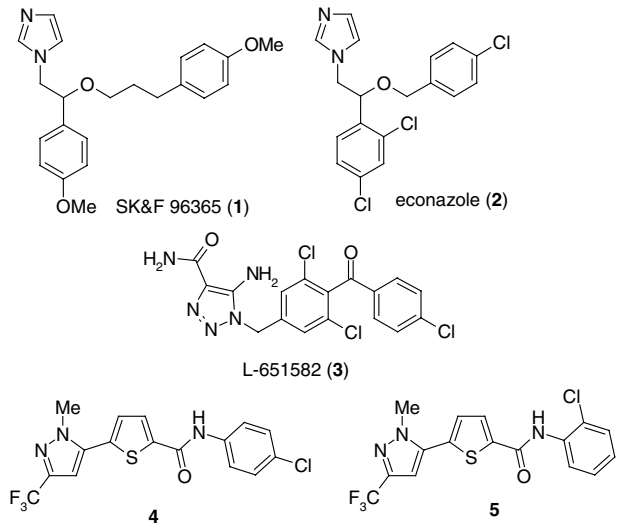
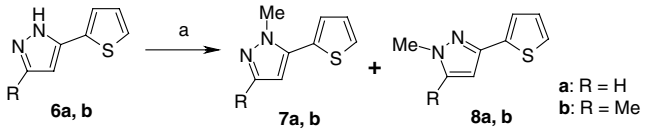


Figure 1. Structures of SK&F 96365 (**1**), econazole (**2**), L-651582 (**3**), and compounds **4** and **5**.

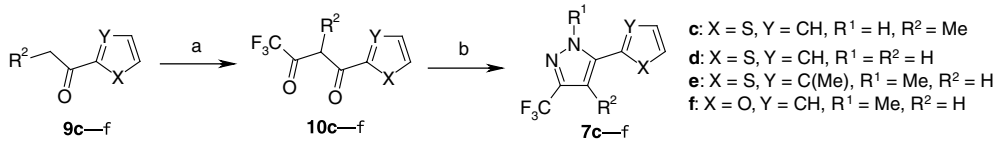
and selective CRAC channel inhibitor with an IC_{50} value of 0.050 μM and a selectivity index of more than 200 (Fig. 1).[1,8] In an attempt to further examine the details of the structure–activity relationships (SARs) of compound **4**, we substituted its thiophene and (1-methyl-3-trifluoromethyl)pyrazole groups, and in this paper, we describe the preparation of a series of aryl-3-trifluoromethylpyrazoles and the SARs for these moieties.



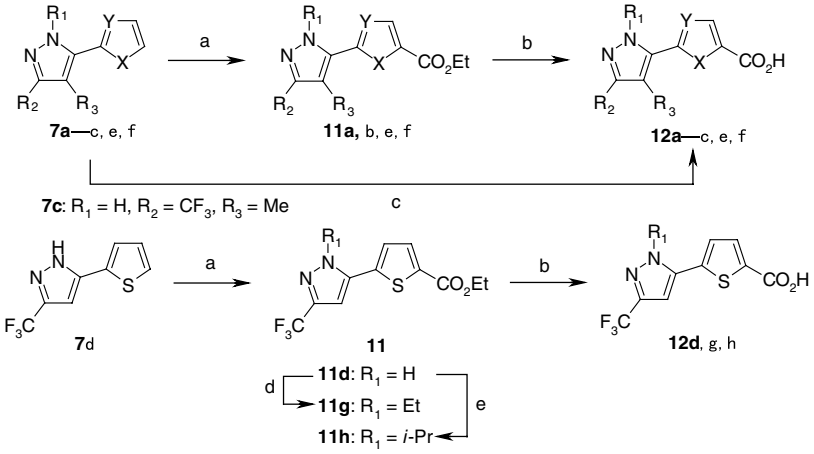
Scheme 1. Reagents: (a) MeI, K_2CO_3, DMF.

2. Chemistry

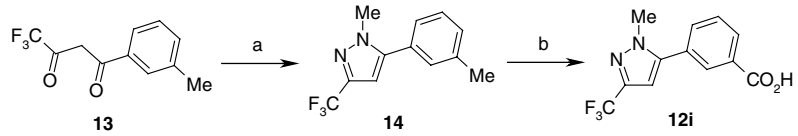
Synthetic routes for the arylcarboxylic acid intermediates **12a–i** are shown in Schemes 1–4. Pyrazoles **6a** and **6b** were subjected to N-methylation with methyl iodide to give the 1-methylpyrazoles **7a** and **7b**, respectively, which were accompanied by the corresponding regioisomers (Scheme 1). Other 3-trifluoromethylpyrazoles **7c–f** were obtained by trifluoroacetylation of the ketone derivatives **9c–f** with ethyl trifluoroacetate or trifluoroacetic anhydride in the presence of bases, followed by cyclization of the diones **10c–f** with hydrazines (Scheme 2). This cyclization proceeded regioselectively when methylhydrazine was used and gave the corresponding pyrazoles **7e** and **7f**. Compounds **7a–c**, **7e**, and **7f** were treated with *n*-butyllithium in tetrahydrofuran at −78 °C, and treatment of the resultant lithium salts with ethyl chloroformate gave the esters **11a–c**, **11e**, and **11f** (Scheme 3). Lithiation of compound **7d** proceeded at 0 °C, and the resultant dianion was converted into the ester **11d** by treatment with ethyl chloroformate and then aqueous $NaHCO_3$. Compound **11d** was alkylated with ethyl iodide and isopropyl iodide to give the 1-alkylpyrazoles **11g** and **11h**, respectively. The esters **11a**, **11b**, and **11d–h** obtained in this way were



Scheme 2. Reagents: (a) with R^2 = Me: LDA, THF, then $(CF_3CO)_2O$, with R^2 = H: CF_3CO_2Et, NaOMe, MeOH; (b) with R^1 = Me: $MeNHNH_2$, AcOH, EtOH, with R^1 = H: $NH_2NH_2{\cdot}HCl$, EtOH.



Scheme 3. Reagents and condition: (a) with R_1 = Me: *n*-BuLi, THF, −78 °C, then $ClCO_2Et$, with R_1 = H: (i) *n*-BuLi, THF, 0 °C, then $ClCO_2Et$, (ii) $NaHCO_3$(aq), EtOH; (b) NaOH(aq), EtOH; (c) i—*n*-BuLi, THF, 0 °C, then $ClCO_2Et$, ii—NaOH(aq), EtOH; (d) EtI, K_2CO_3, DMF; (e) *i*-PrI, K_2CO_3, DMF.



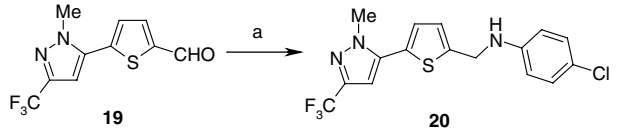
Scheme 4. Reagents: (a) $MeNHNH_2$, EtOH; (b) $KMnO_4$, H_2O.

hydrolyzed with aqueous NaOH to form the acids **12a**, **12b**, and **12d**–**h**. Lithiation of compound **7c** and coupling with ethyl chloroformate, followed by hydrolysis, directly gave the corresponding acid **12c**. The benzoic acid **12i** was obtained by regioselective cyclization of the dione **13**[9] with methylhydrazine, followed by oxidation of the resultant pyrazole **14** with $KMnO_4$ (Scheme 4).

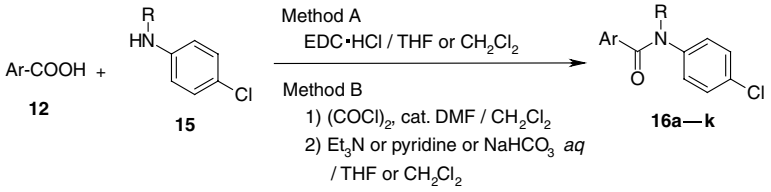
As shown in Scheme 5, compound **12** was mixed with the corresponding aniline **15** in the presence of 1-ethyl-3-dimethylaminopropyl carbodiimide hydrochloride (EDC · HCl) to produce the desired carboxanilide **16** (Method A). Compound **16** was also synthesized by treating compound **12** with $(COCl)_2$, followed by coupling with the corresponding aniline **15** in the presence of triethylamine, pyridine, or aqueous $NaHCO_3$ (Method B). The 1,3-dimethyl- and 1-benzylpyrazole derivatives **17c** and **17d** were obtained by alkylation of compounds **16c** and **16d**, respectively, in the presence of K_2CO_3 (Scheme 6). The structure of compound **17c** was determined as illustrated in Scheme 6, using the nuclear Overhauser effect (NOE), observed between the protons of the *N*-methyl group (3.91 ppm) and the thiophene (7.50 ppm). In the same way, the NOE between protons of the benzyl group (5.53 ppm) and the thiophene (7.01 ppm) in compound **17d** corroborated the structure of this compound shown in Scheme 6.

As shown in Scheme 7, the anilinomethyl derivative **20** was obtained by reductive amination of the aldehyde **19** with 4-chloroaniline in the presence of sodium triacetoxyborohydride ($NaBH(OAc)_3$). The reversed amide **22** was prepared by Curtius rearrangement of the acid **12j**, followed by coupling of the resultant amine **21** with 4-chlorobenzoyl chloride (Scheme 8). As shown in Scheme 9, the acetophenones **23a**–**c**[10] were treated with ethyl trifluoroacetate and then methylhydrazine to form the desired pyrazoles **25a**–**c**.

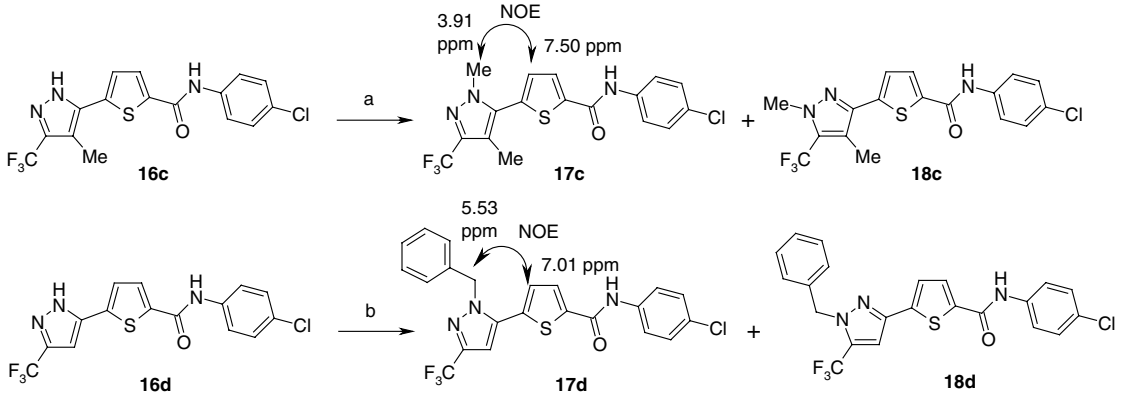
The bis(trifluoromethyl)pyrazole derivatives **27** and **28** were prepared by coupling the aniline **26**[11] with



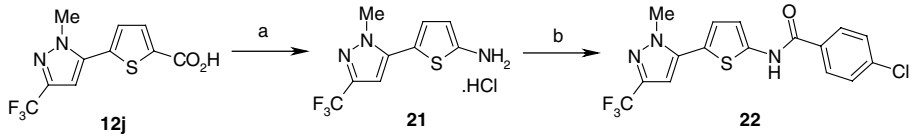
Scheme 7. Reagents: (a) 4-ClPhNH_2, $NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$.



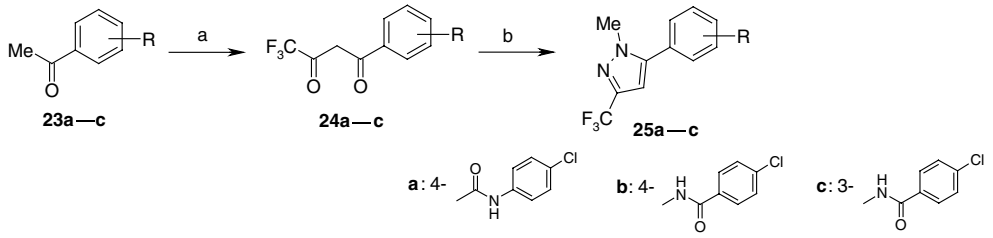
Scheme 5.



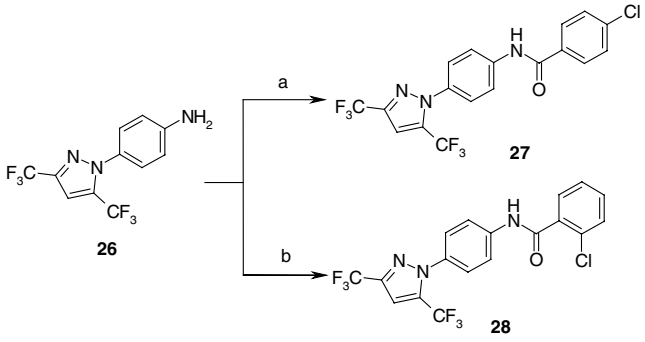
Scheme 6. Reagents: (a) MeI, K_2CO_3, DMF; (b) BnBr, K_2CO_3, DMF.



Scheme 8. Reagents: (a) i—DPPA, Et_3N, toluene, then *t*-BuOH, ii—CF_3CO_2H, CH_2Cl_2; (b) 4-ClC_6H_4COCl, $NaHCO_3$(aq), $ClCH_2CH_2Cl$.



Scheme 9. Reagents: (a) CF_3CO_2Et, NaOMe, DMI; (b) $MeNHNH_2$, AcOH, EtOH.



Scheme 10. Reagents: (a) 4-ClPhCOCl, Et_3N, THF; (b) 2-$ClC_6H_4CO_2H$, EDC.HCl, THF.

4-chlorobenzoyl chloride and 2-chlorobenzoic acid, respectively (Scheme 10).

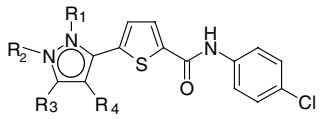
3. Results and discussion

Compounds **16**, **17**, **20**, **22**, **25**, and **27**–**29** were evaluated for their ability to inhibit Ca^{2+} influx through CRAC and VOC channels on Jurkat T-cells[12] and PC12-h5 cells,[13] respectively. The results are summarized in Tables 1–3.

We first examined the effect of substitutions in the pyrazole moiety of compound **4** on CRAC channel inhibitory activity and selectivity over VOC channels (Table 1). Since substitution of the 3-trifluoromethyl group with hydrogen (**16a**) or a methyl group (**16b**) resulted in loss of activity, the trifluoromethyl group seemed to be essential to the inhibition of CRAC channels. Removal of the 1-methyl group (**16d**) also dramatically reduced the inhibitory activity, and the 1-methylpyrazol-3-yl derivative **16j** showed no CRAC channel inhibitory activity. From these results, we hypothesized that the methyl group on the nitrogen neighboring the thiophene ring might be essential for CRAC channel inhibition. We proposed that the methyl group may cause steric repulsion of the thienyl group, and that the resultant torsion between the pyrazole and thiophene rings would result in inhibitory activity for CRAC channels. Methyl and ethyl groups (**16g**) were found to be interchangeable at this site, however, the introduction of larger moieties, such as isopropyl (**16h**) and benzyl (**17d**) groups, tended to reduce both the activity and selectivity of the compounds. These facts indicated that small alkyl groups at the 1-position on the pyrazole ring were favorable for inhibiting CRAC channels, and that bulky groups may interfere with interactions with the target molecules. The 1,4-dimethyl derivative **17c** had a reduced inhibitory activity and we speculated that this change at the 4-position may have been unfavorable for CRAC channel inhibition because it changed the torsional angle between the pyrazole and thiophene rings.

Second, we examined the effect of the substitutions in the thiophene and amide moieties of compound **4** on inhibitory activity and selectivity (Table 2). The fact that the methyl derivative **16e** was about 7-fold less potent than compound **4** led us to the view that a methyl group introduced on the thiophene ring would also alter the torsional angle between the pyrazole and thiophene rings. The amine derivative **20** was 35-fold less potent than compound **4**, and N-methylation (**16k**) also resulted in a reduction in its activity. These results implied that both the carbonyl and the N–H groups were necessary for high potency. Since the reversed amide derivative **22** showed weak activity, we speculated that the distance of the carbonyl and the N–H groups from the pyrazole moiety may influence CRAC channel inhibitory activity. Substitution of the thiophene ring by the furan (**16f**) or by benzenes (**16i**, **25a**) also resulted in loss of activity. We speculated that substitutions in the center of the compounds would have considerable effects on the whole conformation of the molecule and such conformational change caused by introducing furan and 1,3-phenylene may be incompatible with inhibition of CRAC channels. On the other hand, the 1,4-phenylene group in compound **25a** would alter the distance between the amide groups on the pyrazole moiety compared to compound **4**, and this may also be unfavor-

Table 1. Physical and biological properties of 4′-chloro-5-(substituted-pyrazolyl)thiophene-2-carboxanilides



Compound	R_1	R_2	R_3	R_4	Formula[a]	Method	Yield (%)	Mp (°C)	Solvent[b]	IC_{50} (μM)		CRAC channel selectivity[e]
										CRAC[c]	VOC[d]	
4	Me	—	CF_3	H	$C_{16}H_{11}ClF_3N_3OS$	—	—	—	—	0.13	0.75	5.8
16a	Me	—	H	H	$C_{15}H_{12}ClN_3OS$	A	54	206—208	D–A	19%[f]	NT	—
16b	Me	—	Me	H	$C_{16}H_{14}ClN_3OS$	B	6.6	198—200	A–B	31%[f]	NT	—
16d	H	—	CF_3	H	$C_{15}H_9ClF_3N_3OS \cdot 0.1C_4H_8O_2$	A	23	246—247	A–B	0%[f]	NT	—
16j	—	Me	CF_3	H	$C_{16}H_{11}ClF_3N_3OS$	B	60	241—242	A	8%[f]	NT	—
16g	Et	—	CF_3	H	$C_{17}H_{13}ClF_3N_3OS$	A	59	177	A–B	0.25	2.0	8.0
16h	*i*-Pr	—	CF_3	H	$C_{18}H_{15}ClF_3N_3OS$	A	36	205—210	A–B	0.56	1.9	3.4
17d	Bn	—	CF_3	H	$C_{22}H_{15}ClF_3N_3OS$	—	3.5	oil	—	3.4	1.8	0.53
17c	Me	—	CF_3	Me	$C_{17}H_{13}ClF_3N_3OS$	—	35	192—196	A–B	0.37	1.5	4.1

[a] Elemental analyses were within ±0.4% of calculated values, unless otherwise noted.
[b] Recrystallization solvents: A, AcOEt; B, hexane; C, EtOH; D, MeOH.
[c] Inhibition of Ca^{2+} influx through CRAC channels on Jurkat T-cells. See Section 5.
[d] Inhibition of Ca^{2+} influx through VOC channels on PC12-h5 cells. See Section 5.
[e] IC_{50} to VOC channel/IC_{50} to CRAC channel.
[f] % inhibition at 10 μM.

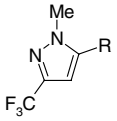
able for inhibitory activity. In the series of phenylene derivatives possessing the reversed amide (**25c**, **25b**), the 1,3-phenylene group (**25c**) reduced the activity. Surprisingly, the 1,4-phenylene derivative **25b** was a more potent CRAC channel inhibitor than compound **4** with an IC_{50} value of 0.085 μM. Additionally, this compound showed high selectivity for CRAC channels with an index of more than 31, due to a reduced inhibitory activity for VOC channels. We identified the positions of the carbonyl and the N–H groups in compound **25b** as important for a high inhibitory activity for CRAC channels.

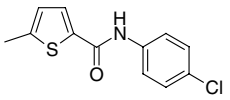
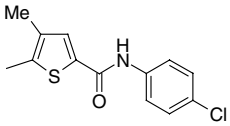
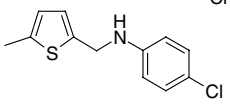
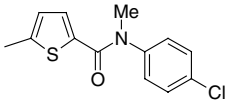
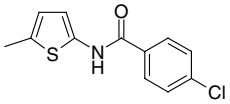
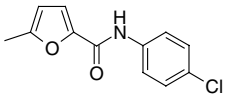
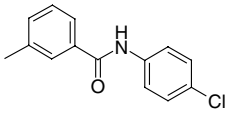
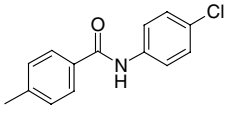
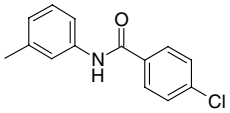
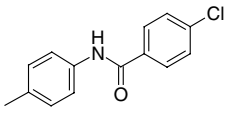
To investigate the SARs of the arylpyrazole series, in detail we carried out single-crystal X-ray diffraction studies of compounds **5**, **25c**, and **25b** (Fig. 2). This showed that the pharmacologically active compounds **5** and **25b** had an 'extended' conformation, in contrast to the 'bended' conformation of the inactive compound **25c**. Additionally, we found that the distance between the carbon of the trifluoromethyl group and the amide proton in compounds **5** and **25b** was similar: 9.71 and 9.44 Å, respectively. In contrast, the corresponding distances in the inactive compound **25c** were found to be much shorter (7.68 Å). We therefore concluded that the overall conformation of the compounds and the distance between the trifluoromethyl group and the amide proton are important determinants of the inhibitory activity of the compounds.

The SARs observed in the series of compounds possessing a (1-alkyl-3-trifluoromethyl)pyrazole moiety are summarized below. The degree of torsional angle between the pyrazole and thiophene rings should influence the CRAC channel inhibitory activity. The 1-alkyl and 3-trifluoromethyl groups were necessary for CRAC channel inhibition, and we suggested that the hydrophobic groups in the pyrazole moiety were also favorable for inhibitory activity. In addition, the amide group was essential for the inhibition of CRAC channels, and compounds possessing this group at a suitable distance from the trifluoromethyl moiety seemed to retain potent activity, even in the series of reversed-type amide derivatives such as compound **25b**.

These results led us to design a new type of molecular skeleton. We hypothesized that the presence of highly hydrophobic groups such as bis(trifluoromethyl)pyrazole might be favorable for potent CRAC channel inhibitory activity and therefore evaluated the pharmacological properties of some derivatives of this type (Table 3). In the series of compounds possessing chloro groups (**27**, **28**), the 4-chloro group (**27**) reduced activity. In contrast, the 2-chloro derivative **28** inhibited CRAC channel activity with an IC_{50} value of the order of 10^{-7} M. These results resembled the observations that compound **5**, bearing the 2-chlorophenyl group, was a more potent and selective CRAC channel inhibitor than the 4-chlorophenyl derivative **4**.[1]. This suggested that the substitution at the 2-position on the phenyl group would also favour high CRAC channel inhibitory activity in the case of bis(trifluoromethyl)pyrazole derivatives. Based on these SARs, we subsequently investigated the known 4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-arylcarboxanilide derivatives with substitutions neighboring the amide bond. As a result, we identified 4-methyl-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide (**29**) as a CRAC channel inhibitor that was more selective than compound **4**, with an IC_{50} value of 0.15 μM and a selectivity index of 31 compared to VOC channels. Although similar carboxanilide derivatives, including compound **29**, were recently reported to inhibit transcriptional activity in activated T-cells, with nuclear factors being the target for these immunosuppressants,[14] their inhibitory activity against CRAC channels has not been

Table 2. Physical and biological properties of 1-methyl-5-substituted-3-trifluoromethyl-1*H*-pyrazoles



Compound	R	Formula[a]	Method	Yield (%)	Mp (°C)	Solvent[b]	IC_{50} (μM)		CRAC channel selectivity[e]
							CRAC[c]	VOC[d]	
4		$C_{16}H_{11}ClF_3N_3OS$	—	—	—	—	0.13	0.75	5.8
16e		$C_{17}H_{13}ClF_3N_3OS$	A	51	162—164	A–B	0.96	1.5	1.6
20		$C_{16}H_{13}ClF_3N_3S$	—	86	92—94	—	4.5	3.3	0.73
16k		$C_{17}H_{13}ClF_3N_3OS$	B	58	136—137	A–B	5.1	3.9	0.76
22		$C_{16}H_{11}ClF_3N_3OS$	—	53	166—168	A–B	1.6	2.3	1.4
16f		$C_{16}H_{11}ClF_3N_3O_2$	A	46	183—185	A–B	8.6	6.2	0.72
16i		$C_{18}H_{13}ClF_3N_3O$	A	66	190—192	A–B	14%[f]	NT	
25a		$C_{18}H_{13}ClF_3N_3O$	—	37	155—160	A–B	36%[f]	NT	
25c		$C_{18}H_{13}ClF_3N_3O$	—	17	150—153	A–B	1.1	2.0	1.8
25b		$C_{18}H_{13}ClF_3N_3O$	—	33	187—188	A–B	0.085	2.6	31

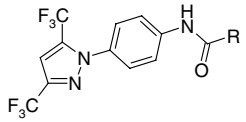
[a] Elemental analyses were within ±0.4% of calculated values, unless otherwise noted.
[b] Recrystallization solvents: A, AcOEt; B, hexane; C, EtOH; D, MeOH.
[c] Inhibition of Ca^{2+} influx through CRAC channels on Jurkat T-cells. See Section 5.
[d] Inhibition of Ca^{2+} influx through VOC channels on PC12-h5 cells. See Section 5.
[e] IC_{50} to VOC channel/IC_{50} to CRAC channel.
[f] % inhibition at 10 μM.

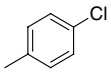
described. We consider these compounds will provide useful leads for the discovery of other potent and selective CRAC channel inhibitors.

As shown in Figure 2, single-crystal X-ray diffraction studies of compounds **29** revealed that the amide proton was located 9.24 Å from the farther trifluoromethyl group on the pyrazole ring, which was similar to the distance measured in active compounds such as compounds **5** and **25b** (9.71 and 9.44 Å, respectively).

Selected compounds (**16g**, **25b**, **28**, and **29**) were evaluated for their ability to inhibit phytohemagglutinin (PHA)-induced IL-2 production in Jurkat T-cells[5]

Table 3. Physical and biological properties of 4′-[3,5-bis(trifluoromethyl)pyrazol-1-yl]carboxanilides



Compound	R	Formula[a]	Yield (%)	Mp (°C)	Solvent[b]	IC_{50} (μM)		CRAC channel selectivity[e]
						CRAC[c]	VOC[d]	
4	—	$C_{16}H_{11}ClF_3N_3OS$	—	—	—	0.13	0.75	5.8
27		$C_{18}H_{10}ClF_6N_3O$	48	196—197	A–B	41%[f]	NT	—
28		$C_{18}H_{10}ClF_6N_3O$	21	183—185	A–B	0.63	24%[f]	>16
29		$C_{15}H_9F_6N_5OS$	—	—	—	0.15	4.7	31

[a] Elemental analyses were within ±0.4% of calculated values, unless otherwise noted.
[b] Recrystallization solvents: A, AcOEt; B, hexane; C, EtOH; D, H_2O.
[c] See the corresponding footnotes to Table 1.
[d] Inhibition of Ca^{2+} influx through VOC channels on PC12-h5 cells. See Section 5.
[e] IC_{50} to VOC channel/IC_{50} to CRAC channel.
[f] % inhibition at 10 μM.

(Table 4). These compounds did inhibit IL-2 production, indicating that CRAC channel inhibitors can inhibit T-cell function. In particular, the 4′-[3,5-bis(trifluoromethyl)pyrazol-1-yl]carboxanilide derivatives **28** and **29** potently inhibited IL-2 production with IC_{50} values of 0.013 and 0.017 μM, respectively, which were about 35 times higher than that for compound **4**. We believe that the new type of molecular skeleton, the 4′-[3,5-bis(trifluoromethyl)pyrazol-1-yl]carboxanilide moiety, will be very useful for discovering potent inhibitors of IL-2 production derived from CRAC channel activation.

Compounds **25b** and **29** were also evaluated for their inhibitory activity in the concanavalin A (Con A)-induced hepatitis model in mice[15] (Table 5). Compound **5** was already known to act as a moderately effective inhibitor of liver injury at a dose of 30 mg/kg *p.o.*[1] In contrast, compounds **25b** and **29** showed potent inhibitory activities, with ED_{50} values of 4.2 and 0.61 mg/kg *p.o.*, respectively. Compound **29** was also tested in a delayed-type hypersensitivity (DTH) model,[16] and was found to inhibit trinitrochlorobenzene (TNCB)-induced ear swelling in sensitized mice with an ED_{50} value of 1.1 mg/kg *p.o.* These results demonstrated that compounds **25b** and **29** were orally available and effective inhibitors in these pharmacological models, associated with T-cell activation. We suggest that the 4′-pyrazolyl carboxanilides may exhibit potent activities in vivo.

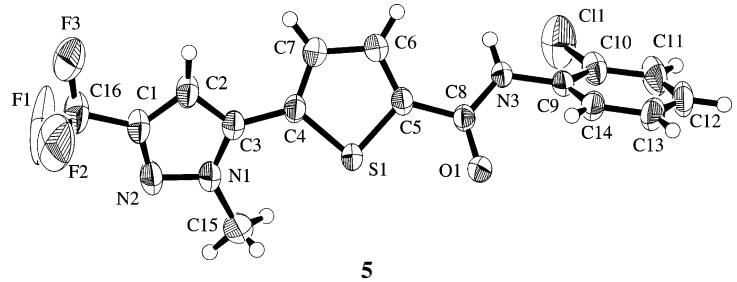
4. Conclusion

We designed novel aryl-3-trifluoromethylpyrazole derivatives from compound **4** and evaluated their ability to inhibit CRAC and VOC channels. SAR showed that the 1-methyl group on the pyrazole ring is essential to the inhibition of CRAC channels, as a result of its effect on the torsional angle between the pyrazole and thiophene rings. We found that the distance between the trifluoromethyl group and the amide proton also influenced the activity. We identified compound **25b** as a potent and highly selective CRAC channel inhibitor, with an IC_{50} value for CRAC channels of 0.085 μM and a selectivity index of more than 31. We also designed a new type of CRAC channel inhibitor, 4′-[3,5-bis(trifluoromethyl)pyrazol-1-yl]carboxanilides, utilizing the SARs obtained from the series of compounds with a (1-alkyl-3-trifluoromethyl)pyrazole moiety, and as a result found that compound **29** showed selective CRAC channel inhibitory activity with an IC_{50} value of 0.15 μM and a selectivity index of 31. In addition, compounds **25b** and **29** were found to inhibit the function of T-lymphocytes both in vitro and in vivo. These compounds have the potential to provide a new class of orally active, anti-inflammatory agents.

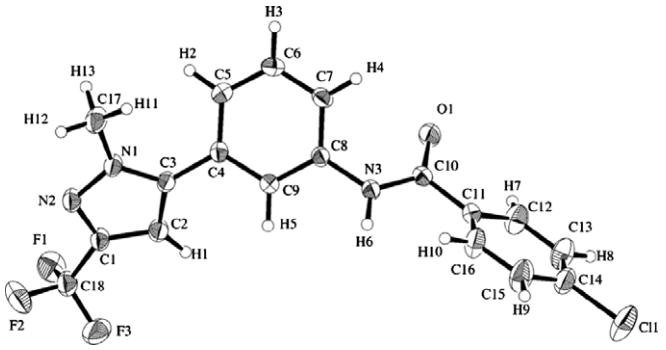
5. Experimental

5.1. Chemistry

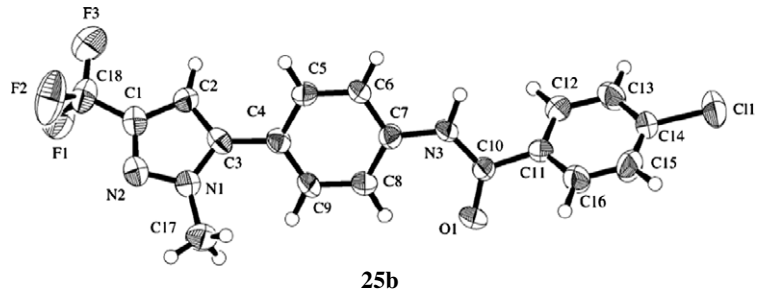
Melting points were determined with a Yanagimoto MP-3 melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a JEOL JNM-90, JEOL JNM-LA300, JEOL JNM-EX400, or JEOL JNM-A500 spectrometer and were referenced to an internal standard, tetramethylsilane. The abbreviations used for the signal patterns are as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; dd, double doublet;



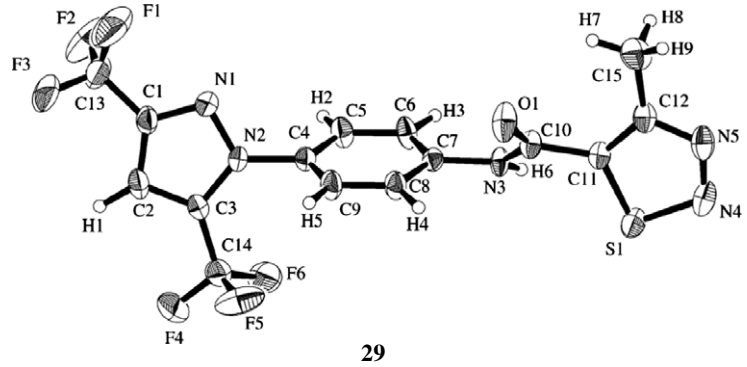
5



25c



25b



29

Figure 2. ORTEP view with the labeling scheme for compounds **5**, **25c**, **25b**, and **29**.

Table 4. Biological properties of selected compounds in vitro

Compound	IC_{50} (μM)	
	CRAC[a]	IL-2[b]
4	0.13	0.53
16g	0.25	0.26
25b	0.085	0.21
28	0.63	0.013
29	0.15	0.017

[a] Inhibition of Ca^{2+} influx through CRAC channels on Jurkat T-cells. See Section 5.

[b] Inhibition of PHA-induced IL-2 production in Jurkat T-cells. See Section 5.

Table 5. Effects of compounds **5**, **25b**, and **29** in vivo

Compound	Hepatitis[a]		DTH[b]
	ED_{50} (mg/kg *p.o.*)	% inhibition at 30 mg/kg *p.o.*	ED_{50} (mg/kg *p.o.*)
5		46	
25b	4.2		
29	0.61		1.1

[a] Inhibition of Con A-induced liver injury in mice. See Section 5.

[b] Inhibition of TNCB-induced contact hypersensitivity in mice. See Section 5.

dt, double triplet; tt, triple triplet; m, multiplet. Mass spectra were recorded on a JEOL JMS-DX300, JEOL JMS-DX2000, HP 5970-MSD, Fisons TRIO-1000 or Finnigan mat TSQ 700 mass spectrometer, and the ionization method was chosen from EI and FAB. 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). Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values. 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)-1*H*-pyrazole (7a) and 1-methyl-3-(2-thienyl)-1*H*-pyrazole (8a)

A mixture of 3-(2-thienyl)-1*H*-pyrazole (**6a**, 1.58 g, 10.5 mmol), K_2CO_3 (2.18 g, 15.8 mmol), and MeI (0.98 mL, 16 mmol) in DMF (20 mL) was stirred for 24 h at room temperature. The mixture was added H_2O and extracted with AcOEt, washed with H_2O, brine, dried, and concentrated in vacuo. The residue was purified by column chromatography (hexane/AcOEt = 8:1–5:1) to give **7a** (344 mg, 20%) as a pale yellow oil and **8a** (758 mg, 44%) as a pale yellow oil.

5.2.1. 1-Methyl-5-(2-thienyl)-1*H*-pyrazole (7a). ^{1}H NMR ($CDCl_3$) δ 3.98 (3H, s), 6.39 (1H, d, J = 2.2 Hz), 7.11 (1H, dd, J = 5.4, 3.7 Hz), 7.16 (1H, dd, J = 3.7, 1.6 Hz), 7.39 (1H, dd, J = 5.4, 1.6 Hz), 7.47 (1H, d, J = 2.2 Hz); FAB-MS m/z 165 [$(M+H)^+$].

5.2.2. 1-Methyl-3-(2-thienyl)-1*H*-pyrazole (8a). ^{1}H NMR ($CDCl_3$) δ 3.91 (3H, s), 6.43 (1H, d, J = 2.1 Hz), 7.03 (1H, dd, J = 4.8, 3.8 Hz), 7.21 (1H, dd, J = 4.8, 1.1 Hz), 7.29 (1H, dd, J = 3.8, 1.1 Hz), 7.32 (1H, d, J = 2.1 Hz); FAB-MS m/z 165 [$(M+H)^+$].

The following compound was similarly prepared.

5.3. A mixture of 1,3-dimethyl-5-(2-thienyl)-1*H*-pyrazole (7b) and 1,5-dimethyl-3-(2-thienyl)-1*H*-pyrazole (8b)

^{1}H NMR ($CDCl_3$) δ 2.28 (3H, s), 3.79, 3.90 (total 3H, s), 6.18, 6.23 (total 1H, s), 7.00–7.38 (total 3H, m); FAB-MS m/z 179 [$(M+H)^+$].

5.4. 4-Methyl-3-(2-thienyl)-5-trifluoromethyl-1*H*-pyrazole (7c)

A 1.6-M solution of *n*-BuLi (21.0 mL, 33.6 mmol) in hexane was added to a mixture of diisopropylamine (3.34 g, 33 mmol) in THF (25 mL) at −40 °C, then the whole was cooled at −70 °C and 2-propionylthiophene (**9c**, 4.21 g, 30.0 mmol) was added dropwise below −60 °C. The mixture was stirred for 1.5 h at the same temperature and poured dropwise to a mixture of trifluoroacetic anhydride (31.5 g, 150 mmol) in THF (30 mL) at −60 °C. The mixture was stirred for 1 h and then aqueous NH_4Cl was added. The whole was extracted with AcOEt, washed with saturated aqueous $NaHCO_3$ and brine, then dried and concentrated in vacuo. The residue was purified by column chromatography (hexane/AcOEt = 16:1–8:1) to give a pale yellow solid (**10c**, 1.01 g). This diketone (569 mg, 2.41 mmol) was added to a mixture of hydrazine hydrochloride (181 mg, 2.64 mmol) and EtOH (5 mL), and the whole was heated at 50 °C for 2 h. Aqueous $NaHCO_3$ was added to the mixture and extracted with AcOEt, washed with brine, dried, and concentrated in vacuo to give **7c** (448 mg, 11%) as a brown solid: ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, d, J = 1.0 Hz), 7.16 (1H, dd, J = 5.1, 3.7 Hz), 7.25 (1H, dd, J = 3.5, 1.0 Hz), 7.45 (1H, dd, J = 4.9, 1.0 Hz), 10.80 (1H, br s); FAB-MS m/z 233 [$(M+H)^+$].

5.5. 5-(2-Thienyl)-3-trifluoromethyl-1*H*-pyrazole (7d)

NaOMe (17.3 g, 319 mmol) in MeOH (100 mL) was added to a solution of 2-acetylthiophene (**9d**, 31.0 g, 246 mmol) in MeOH (150 mL), and the whole was stirred for 1 h at room temperature. It was cooled on ice-bath, then added ethyl trifluoroacetate (41.9 g, 295 mmol), and the whole was heated to reflux for 24 h. H_2O was added to the mixture and extracted with AcOEt, washed with brine, dried, and concentrated in vacuo to give a brown oil (**10d**). This crude compound was added to a mixture of hydrazine hydrate (13.1 mL, 271 mmol), AcOH (25 mL), and EtOH (250 mL), and the whole was heated to reflux for 13 h. 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 crystallized from AcOEt/hexane to give **7d** (11.1 g, 21%) as a pale brown powder: ^{1}H NMR ($CDCl_3$) δ 6.72 (1H, s), 7.10–7.14 (1H, m), 7.31 (1H, dd, J = 3.8, 1.1 Hz), 7.38–7.42 (1H, m), 10.66 (1H, br s); FAB-MS m/z 219 [$(M+H)^+$].

The following compounds were prepared following the same methods with the modification that methylhydrazine and AcOH were used instead of hydrazine hydrochloride.

5.6. 1-Methyl-5-(3-methyl-2-thienyl)-3-trifluoromethyl-1*H*-pyrazole (7e)

^{1}H NMR ($CDCl_3$) δ 2.18 (3H, s), 3.83 (3H, s), 6.56 (1H, s), 6.98 (1H, d, J = 4.8 Hz), 7.40 (1H, d, J = 5.2 Hz); FAB-MS m/z 247 [$(M+H)^+$].

5.7. 5-(2-Furyl)-1-methyl-3-trifluoromethyl-1*H*-pyrazole (7f)

^{1}H NMR ($CDCl_3$) δ 4.09 (3H, s), 6.54 (1H, dd, J = 3.8, 2.0 Hz), 6.61–6.64 (1H, m), 6.71 (1H, s), 7.54 (1H, dd, J = 1.8, 0.9 Hz); FAB-MS m/z 217 [$(M+H)^+$].

5.8. Ethyl 5-(1-methyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylate (11a)

A 1.6 M solution of *n*-BuLi (1.23 mL, 1.9 mmol) in hexane was added dropwise to a solution of 1-methyl-5-(2-thienyl)-1*H*-pyrazole (**7a**, 281 mg, 1.71 mmol) in THF (6 mL) at −78 °C. The mixture was stirred for 1 h at

−50 °C and then ethyl chloroformate (0.33 mL, 3.42 mmol) was added at the same temperature. The mixture was stirred for 3 h at −70 °C, then H_2O was added. The whole was extracted with AcOEt, washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography (hexane/AcOEt = 5:1) to give **11a** (338 mg, 84%) as a pale yellow solid: ^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, J = 7.1 Hz), 4.03 (3H, s), 4.38 (2H, q, J = 7.2 Hz), 6.47 (1H, d, J = 2.1 Hz), 7.15 (1H, d, J = 3.9 Hz), 7.49 (1H, d, J = 2.4 Hz), 7.78 (1H, d, J = 3.9 Hz); FAB-MS m/z 237 [(M+H)$^+$].

The following compounds were prepared using similar methods.

5.9. Ethyl 5-(1,3-dimethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylate (11b) [a mixture with ethyl 5-(1,5-dimethyl-1*H*-pyrazol-3-yl)thiophene-2-carboxylate]

^{1}H NMR ($CDCl_3$) δ 1.37, 1.39 (total 3H, t, J = 7.4 Hz), 3.80, 3.94 (total 3H, s), 4.35, 4.37 (total 2H, q, J = 7.2 Hz), 6.25, 6.27 (total 1H, s), 7.11, 7.21 (total 1H, d, J = 3.7 Hz), 7.71, 7.76 (total 1H, d, J = 3.9 Hz); GC-MS m/z 250 (M$^+$).

5.10. Ethyl 3-methyl-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylate (11e)

^{1}H NMR ($CDCl_3$) δ 1.39 (3H, t, J = 7.1 Hz), 2.19 (3H, s), 3.86 (3H, s), 4.37 (2H, q, J = 7.2 Hz), 6.60 (1H, s), 7.66 (1H, s); FAB-MS m/z 319 [(M+H)$^+$].

5.11. Ethyl 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)furan-2-carboxylate (11f)

^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, J = 7.1 Hz), 4.17 (3H, s), 4.40 (2H, q, J = 7.1 Hz), 6.72 (1H, d, J = 3.6 Hz), 6.85 (1H, s), 7.26 (1H, d, J = 3.9 Hz); FAB-MS m/z 289 [(M+H)$^+$].

5.12. Ethyl 5-(5-trifluoromethyl-1*H*-pyrazol-3-yl)thiophene-2-carboxylate (11d)

A 1.6-M solution of *n*-BuLi (30.0 mL, 48.0 mmol) in hexane was added dropwise to a solution of 5-(2-thienyl)-3-trifluoromethyl-1*H*-pyrazole (**7d**, 5.02 g, 23.0 mmol) in THF (50 mL) below −60 °C. The mixture was stirred for 1 h at 0 °C, then ethyl chloroformate (4.62 mL, 48.3 mmol) was added below −60 °C. The mixture was stirred for 1 h at −78 °C, then H_2O was added. The whole was extracted with AcOEt, washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography (hexane/AcOEt = 9:1–6:1) to give a pale yellow solid (4.67 g). This diester was added to a mixture of $NaHCO_3$ (1.08 g, 12.9 mmol), H_2O (20 mL), EtOH (50 mL), and dioxane (30 mL), and the whole was stirred for 1 day at room temperature. The mixture was concentrated in vacuo, and the residue was extracted with AcOEt, washed with H_2O, brine, dried, and concentrated in vacuo to give **11d** (3.48 g, 52%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, J = 7.1 Hz), 4.39 (2H, q, J = 7.1 Hz), 6.79 (1H, s), 7.28 (1H, d, J = 3.6 Hz), 7.76 (1H, d, J = 4.2 Hz), 11.46 (1H, br s); FAB-MS m/z 291 [(M+H)$^+$].

5.13. Ethyl 5-(1-ethyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylate (11g)

The title compound was prepared from **11d** by a procedure similar to that described for **7a** and **8a** (33% from **11d**): ^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, J = 7.2 Hz), 1.50 (3H, t, J = 7.2 Hz), 4.35 (2H, q, J = 7.2 Hz), 4.39 (2H, q, J = 7.2 Hz), 6.67 (1H, s), 7.17 (1H, d, J = 3.9 Hz), 7.80 (1H, d, J = 3.9 Hz); FAB-MS m/z 319 [(M+H)$^+$].

The following compounds were prepared using similar methods.

5.14. Ethyl 5-(1-isopropyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylate (11h)

^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, J = 7.1 Hz), 1.53 (6H, d, J = 6.8 Hz), 4.39 (2H, q, J = 7.0 Hz), 4.77 (1H, sept, J = 6.6 Hz), 6.62 (1H, s), 7.14 (1H, d, J = 3.9 Hz), 7.80 (1H, d, J = 3.5 Hz); FAB-MS m/z 333 [(M+H)$^+$].

5.15. 5-(1-Methyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylic acid (12a)

A mixture of ethyl 5-(1-methyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylate (**11a**, 310 mg, 1.31 mmol), 5 M NaOH (1 mL) and EtOH (5 mL) was stirred for 19 h at room temperature. The mixture was added 1 M HCl (5 mL) and H_2O, then filtered and recrystallized from EtOH to give **12a** (175 mg, 64%) as a colorless powder: ^{1}H NMR (DMSO-d_6) δ 3.98 (3H, s), 6.63 (1H, d, J = 1.8 Hz), 7.46 (1H, d, J = 3.9 Hz), 7.49 (1H, d, J = 1.8 Hz), 7.77 (1H, d, J = 3.6 Hz), 13.29 (1H, s); FAB-MS m/z 209 [(M+H)$^+$].

The following compounds were prepared using similar methods.

5.16. 5-(1,3-Dimethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylic acid (12b) [a mixture with 5-(1,5-dimethyl-1*H*-pyrazol-3-yl)thiophene-2-carboxylic acid]

^{1}H NMR (DMSO-d_6) δ 2.16, 2.27 (total 3H, s), 3.75, 3.89 (total 3H, s), 6.40, 6.49 (total 1H, s), 7.33, 7.40 (total 1H, d, J = 3.9 Hz), 7.64, 7.74 (total 1H, d, J = 3.9 Hz), 13.12 (1H, br s); FAB-MS m/z 223 [(M+H)$^+$].

5.17. 5-(5-Trifluoromethyl-1*H*-pyrazol-3-yl)thiophene-2-carboxylic acid (12d)

^{1}H NMR (DMSO-d_6) δ 7.23 (1H, s), 7.60 (1H, d, J = 4.0 Hz), 7.76 (1H, d, J = 4.0 Hz); FAB-MS m/z 263 [(M+H)$^+$].

5.18. 3-Methyl-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylic acid (12e)

^{1}H NMR (DMSO-d_6) δ 2.16 (3H, s), 3.85 (3H, s), 7.03 (1H, s), 7.70 (1H, s), 13.36 (1H, s); FAB-MS m/z 289 [(M+H)$^+$].

5.19. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)furan-2-carboxylic acid (12f)

^{1}H NMR ($CDCl_3$) δ 4.20 (3H, s), 6.77 (1H, d, J = 3.9 Hz), 6.89 (1H, s), 7.41 (1H, d, J = 3.6 Hz); FAB-MS m/z 261 [$(M+H)^+$].

5.20. 5-(1-Ethyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylic acid (12g)

^{1}H NMR (DMSO-d_6) δ 1.39 (3H, t, J = 7.2 Hz), 4.36 (2H, q, J = 7.2 Hz), 7.15 (1H, s), 7.53 (1H, d, J = 3.9 Hz), 7.80 (1H, d, J = 3.9 Hz), 13.46 (1H, br s); FAB-MS m/z 291 [$(M+H)^+$].

5.21. 5-(1-Isopropyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylic acid (12h)

^{1}H NMR (DMSO-d_6) δ 1.44 (6H, d, J = 6.9 Hz), 4.83 (1H, sept, J = 6.5 Hz), 7.08 (1H, s), 7.48 (1H, d, J = 3.9 Hz), 7.80 (1H, d, J = 3.4 Hz); FAB-MS m/z 305 [$(M+H)^+$].

5.22. 5-(4-Methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl)-thiophene-2-carboxylic acid (12c)

The title compound was prepared from **7c** by a procedure similar to that described for **11d**, using 1-M NaOH instead of aqueous $NaHCO_3$: ^{1}H NMR (DMSO-d_6) δ 2.28 (3H, s), 7.53 (1H, d, J = 3.9 Hz), 7.81 (1H, d, J = 3.9 Hz), 13.35 (1H, s), 14.11 (1H, s); FAB-MS m/z 275 [$(M-H)^-$].

5.23. 1-Methyl-5-(3-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazole (14)

Methylhydrazine (1.89 g, 41.0 mmol) was added to an ice-cooled mixture of 4,4,4-trifluoro-1-(3-methylphenyl)butane-1,3-dione (**13**, 8.98 g, 37.3 mmol), AcOH (12 mL), and EtOH (70 mL), and the whole was stirred for 0.5 h at room temperature, then heated to reflux for 1 h. 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/toluene = 4:1) to give **14** (2.28 g, 25%) as a pale yellow oil: ^{1}H NMR (DMSO-d_6) δ 2.38 (3H, s), 3.92 (3H, s), 6.87 (1H, s), 7.28–7.46 (4H, m); FAB-MS m/z 241 [$(M+H)^+$].

5.24. 3-[1-Methyl-3-(trifluoromethyl)-1*H*-pyrazol-5-yl]benzoic acid (12i)

A mixture of 1-methyl-5-(3-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazole (**14**, 961 mg, 4.00 mmol) and $KMnO_4$ (632 mg, 4.00 mmol) in H_2O (30 mL) was heated to reflux for 12 h. Further $KMnO_4$ (3.16 g, 20.0 mmol) was added and the whole was heated to reflux for 9 h. The mixture was filtered on Celite and washed with 1 M NaOH and H_2O, then the filtrate was added Et_2O and extracted with H_2O. The aqueous layer was acidified with 1 M HCl and extracted with AcOEt, washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography ($CHCl_3$/MeOH = 10:1) and recrystallized from i-Pr_2O to give **12i** (90 mg, 8.3%) as a colorless solid ^{1}H NMR (DMSO-d_6) δ 3.94 (3H, s), 7.01 (1H, s), 7.68 (1H, t, J = 7.7 Hz), 7.85–7.91 (1H, m), 8.03–8.11 (2H, m), 13.24 (1H, s); FAB-MS m/z 271 [$(M+H)^+$].

5.25. 4′-Chloro-5-(1-methyl-1*H*-pyrazol-5-yl)thiophene-2-carboxanilide (16a)

A mixture of 5-(1-methyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylic acid (**12a**, 149 mg, 0.716 mmol), 4-chloroaniline (110 mg, 0.859 mmol), and EDC·HCl (206 mg, 1.07 mmol) in THF (5 mL) was stirred for 5 h at room temperature. H_2O was added to the whole and extracted with AcOEt, washed with saturated aqueous $NaHCO_3$, brine, dried, and concentrated in vacuo. The residue was recrystallized from MeOH–AcOEt to give **16a** (124 mg, 54%) as a colorless powder: ^{1}H NMR ($CDCl_3$) δ 4.01 (3H, s), 6.64 (1H, d, J = 1.9 Hz), 7.43 (2H, d, J = 8.8 Hz), 7.50 (1H, d, J = 2.0 Hz), 7.52 (1H, d, J = 4.4 Hz), 7.77 (2H, d, J = 8.8 Hz), 8.07 (1H, d, J = 3.9 Hz), 10.43 (1H, s); FAB-MS m/z 318 [$(M+H)^+$].

The following compounds were prepared using similar methods.

5.26. 4′-Chloro-5-(4-methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl)thiophene-2-carboxanilide (16c)

^{1}H NMR (DMSO-d_6) δ 2.30 (3H, s), 7.44 (2H, d, J = 8.8 Hz), 7.57 (1H, d, J = 3.9 Hz), 7.78 (2H, d, J = 8.8 Hz), 8.09 (1H, d, J = 3.9 Hz), 10.46 (1H, s), 14.09 (1H, br s); FAB-MS m/z 386 [$(M+H)^+$].

5.27. 4′-Chloro-5-(3-trifluoromethyl-1*H*-pyrazol-5-yl)-thiophene-2-carboxanilide (16d)

^{1}H NMR (DMSO-d_6) δ 7.20 (1H, s), 7.43 (2H, d, J = 8.8 Hz), 7.64 (1H, d, J = 3.9 Hz), 7.77 (2H, d, J = 8.7 Hz), 8.04 (1H, d, J = 3.9 Hz), 10.45 (1H, s), 14.34 (1 H, s); FAB-MS m/z 372 [$(M+H)^+$].

5.28. 4′-Chloro-4-methyl-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxanilide (16e)

^{1}H NMR (DMSO-d_6) δ 2.21 (3H, s), 3.87 (3H, s), 7.03 (1H, s), 7.43 (2H, d, J = 8.8 Hz), 7.77 (2H, d, J = 8.8 Hz), 7.98 (1H, s), 10.45 (1H, s); FAB-MS m/z 400 [$(M+H)^+$].

5.29. 4′-Chloro-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)furan-2-carboxanilide (16f)

^{1}H NMR ($CDCl_3$) δ 4.13 (3H, s), 6.78 (1H, d, J = 4.0 Hz), 6.89 (1H, s), 7.35 (2H, d, J = 8.8 Hz), 7.37 (1H, d, J = 3.9 Hz), 7.62 (2H, d, J = 8.7 Hz), 7.96 (1H, s); FAB-MS m/z 370 [$(M+H)^+$].

5.30. 4′-Chloro-5-(1-ethyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxanilide (16g)

^{1}H NMR ($CDCl_3$) δ 1.51 (3H, t, J = 7.3 Hz), 4.36 (2H, q, J = 7.3 Hz), 6.69 (1H, s), 7.21 (1H, d, J = 3.9 Hz),

7.36 (2H, d, J = 8.7 Hz), 7.58 (2H, d, J = 8.8 Hz), 7.61 (1H, d, J = 3.9 Hz), 7.66 (1H, s); FAB-MS m/z 400 [(M+H)$^+$].

5.31. 4′-Chloro-5-(1-isopropyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxanilide (16h)

^{1}H NMR ($CDCl_3$) δ 1.54 (6H, d, J = 6.8 Hz), 4.78 (1H, sept, J = 6.6 Hz), 6.64 (1H, s), 7.17 (1H, d, J = 3.9 Hz), 7.35 (2H, d, J = 8.8 Hz), 7.58 (2H, d, J = 8.8 Hz), 7.61 (1H, d, J = 3.4 Hz), 7.67 (1H, s); FAB-MS m/z 414 [(M+H)$^+$].

5.32. 4′-Chloro-3-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)benzanilide (16i)

^{1}H NMR (DMSO-d_6) δ 4.21 (3H, s), 7.03 (1H, s), 7.43 (2H, d, J = 8.8 Hz), 7.71(1H, t, J = 7.8 Hz), 7.82 (2H, d, J = 8.8 Hz), 7.83–7.87 (1H, m), 8.04–8.08 (1H, m), 8.14 (1H, t, J = 1.5 Hz), 10.43 (1H, s); FAB-MS m/z 380 [(M+H)$^+$].

5.33. 4′-Chloro-5-(1,3-dimethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxanilide (16b)

A mixture of 5-(1,3-dimethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylic acid (**12b**, 202 mg, 0.909 mmol, accompanied with regioisomer), oxalyl chloride (127 μL, 1.45 mmol), and DMF (1 drop) in THF (5 mL) was stirred for 2 h at room temperature. The mixture was concentrated in vacuo, and the residue in THF (3 mL) was added dropwise to an ice-cooled mixture of 4-chloroaniline (139 mg, 1.09 mmol), Et_3N (190 μL, 1.36 mmol), and THF (2 mL). The whole was stirred for 20 h at room temperature then H_2O was added and extracted with AcOEt, washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography (toluene/AcOEt = 9:1–4:1) and recrystallized from AcOEt–hexane to give **16b** (20 mg, 6.6%) as a colorless powder: ^{1}H NMR (DMSO-d_6) δ 2.17 (3H, s), 3.91 (3H, s), 6.41 (1H, s), 7.43 (2H, d, J = 8.8 Hz), 7.47 (1H, d, J = 3.9 Hz), 7.77 (2H, d, J = 8.8 Hz), 8.05 (1H, d, J = 3.9 Hz), 10.41 (1H, s); FAB-MS m/z 332 [(M+H)$^+$].

The following compounds were prepared according to this method, substituting aqueous $NaHCO_3$ or pyridine for Et_3N as appropriate.

5.34. 4′-Chloro-5-(1-methyl-5-trifluoromethyl-1*H*-pyrazol-3-yl)thiophene-2-carboxanilide (16j)

^{1}H NMR (DMSO-d_6) δ 4.01 (3H, s), 7.42 (2H, d, J = 9.2 Hz), 7.46 (1H, s), 7.63 (1H, d, J = 3.7 Hz), 7.77 (1H, d, J = 9.1 Hz), 7.99 (1H, d, J = 4.3 Hz), 10.37 (1H, s); FAB-MS m/z 386 [(M+H)$^+$].

5.35. 4′-Chloro-*N*-methyl-5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxanilide (16k)

^{1}H NMR (DMSO-d_6) δ 3.35 (3H, s), 3.96 (3H, s), 6.55 (1H, d, J = 3.9 Hz), 7.03 (1H, s), 7.31 (1H, d, J = 3.9 Hz), 7.46 (2H, d, J = 8.7 Hz), 7.55 (2H, d, J = 8.3 Hz); FAB-MS m/z 400 [(M+H)$^+$].

5.36. 4′-Chloro-5-(1,5-dimethyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxanilide (17c)

The title compound was prepared from **19e** by a procedure similar to that described for **7a** and **8a** (35% from **16c**): ^{1}H NMR (DMSO-d_6) δ 2.15 (3H, s), 3.91 (3H, s), 7.44 (2H, d, J = 9.2 Hz), 7.50 (1H, d, J = 4.3 Hz), 7.77 (2H, d, J = 9.1 Hz), 8.14 (1H, d, J = 3.7 Hz), 10.50 (1H, s); FAB-MS m/z 400 [(M+H)$^+$].

The following compounds were prepared using similar methods.

5.37. 5-(1-Benzyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-4′-chlorothiophene-2-carboxanilide (17d) (3.5% from 16d)

^{1}H NMR ($CDCl_3$) δ 5.53 (2H, s), 6.76 (1H, s), 7.01 (1H, d, J = 3.9 Hz), 7.05–7.09 (2H, m), 7.30–7.36 (5H, m), 7.51 (1H, d, J = 3.9 Hz), 7.55 (2H, d, J = 9.3 Hz), 7.60 (1H, s); FAB-MS m/z 462 [(M+H)$^+$].

5.38. 5-{5-[(4-Chloroanilino)methyl]-2-thienyl}-1-methyl-3-trifluoromethyl-1*H*-pyrazole (20)

A mixture of 4-chloroaniline (65 mg, 0.512 mmol), 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxaldehyde (**19**, 127 mg, 0.488 mmol), $NaBH(OAc)_3$ (259 mg, 1.22 mmol), and AcOH (0.1 mL) in $ClCH_2CH_2Cl$ (2 mL) was stirred for 2.5 h at room temperature. Saturated aqueous $NaHCO_3$ was added and extracted with AcOEt. The extract was washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography (hexane/AcOEt = 5:1) to give **20** (156 mg, 86%) as a pale yellow solid: ^{1}H NMR ($CDCl_3$) δ 3.99 (3H, s), 4.18 (1H, br s), 4.53 (2H, d, J = 4.9 Hz), 6.59 (1H, s), 6.61 (2H, d, J = 8.7 Hz), 7.02 (1H, d, J = 3.9 Hz), 7.06 (1H, d, J = 3.9 Hz), 7.14 (2H, d, J = 8.8 Hz); FAB-MS m/z 372 [(M+H)$^+$].

5.39. 5-(1-Methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophen-2-ylamine monohydrochloride (21)

A mixture of 5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)thiophene-2-carboxylic acid (**12j**, 905 mg, 3.28 mmol), diphenylphosphoryl azide (741 μL, 3.44 mmol), and Et_3N (479 μL, 3.44 mmol) in toluene (20 mL) was stirred at 50 °C for 30 min. It was cooled at room temperature, then *t*-BuOH (486 mg, 6.55 mmol) in toluene (1 mL) was added and the whole was stirred at 80 °C for 5 h. H_2O was added to the mixture, extracted with AcOEt, washed with brine, dried, and concentrated in vacuo. The residue was purified by column chromatography (hexane/AcOEt = 6:1–4:1) to give *t*-butyl [5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-thiophen-2-yl]-carbamate (223 mg) as a pale yellow solid. This carbamate was added to a mixture of trifluoroacetic acid (5 mL) and CH_2Cl_2 (5 mL), and the whole was stirred for 2 days at room temperature. It was concentrated in vacuo, and the residue was added to

saturated aqueous $NaHCO_3$. It was extracted with AcOEt, washed with brine, dried, and concentrated in vacuo. AcOEt (50 mL) and 4 M HCl–dioxane (1 mL) were added to the residue, then the whole was concentrated in vacuo. The residue was crystallized from AcOEt–Et_2O to give **21** (154 mg, 17%) as a pale yellow powder: ^{1}H NMR (DMSO-d_6) δ 3.96 (3H, s), 6.29 (1H, d, J = 3.9 Hz), 6.77 (1H, s), 7.36 (1H, d, J = 3.9 Hz), 6.80–7.40 (3H, br); GC-MS m/z 247 (M^+).

5.40. 4-Chloro-*N*-[5-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)-2-thienyl]benzamide (22)

The title compound was prepared from **21** by a procedure similar to that described for **16b**: ^{1}H NMR (DMSO-d_6) δ 4.03 (3H, s), 6.93 (1H, s), 7.01 (1H, d, J = 4.4 Hz), 7.33 (1H, d, J = 3.9 Hz), 7.67 (2H, d, J = 8.3 Hz), 8.05 (2H, d, J = 8.8 Hz), 11.92 (1H, s); FAB-MS m/z 386 [$(M+H)^+$].

5.41. 4′-Chloro-4-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)benzanilide (25a)

The title compound was prepared from **23a** by a procedure similar to that described for **7d**: ^{1}H NMR (DMSO-d_6) δ 3.99 (3H, s), 7.04 (1H, s), 7.43 (2H, d, J = 8.8 Hz), 7.80 (2H, d, J = 8.3 Hz), 7.85 (2H, d, J = 8.8 Hz), 8.10 (2H, d, J = 8.3 Hz), 10.50 (1H, s); FAB-MS m/z 380 [$(M+H)^+$].

The following compounds were prepared using similar methods.

5.42. 4-Chloro-4′-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)benzanilide (25b)

^{1}H NMR ($CDCl_3$) δ 3.93 (3H, s), 6.56 (1H, s), 7.43 (2H, d, J = 8.8 Hz), 7.49 (2H, d, J = 8.4 Hz), 7.77 (2H, d, J = 8.8 Hz), 7.84 (2H, d, J = 8.0 Hz), 7.91 (1H, s); FAB-MS m/z 380 [$(M+H)^+$].

5.43. 4-Chloro-3′-(1-methyl-3-trifluoromethyl-1*H*-pyrazol-5-yl)benzanilide (25c)

^{1}H NMR ($CDCl_3$) δ 3.93 (3H, s), 6.56 (1H, s), 7.20 (1H, d, J = 7.4 Hz), 7.42–7.50 (3H, m), 7.59 (1H, d, J = 7.8 Hz), 7.80 (2H, d, J = 8.3 Hz), 7.87 (1H, s), 8.11 (1H, br s); FAB-MS m/z 380 [$(M+H)^+$].

5.44. 4-Chloro-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]benzanilide (27)

The title compound was prepared from **26** by a procedure similar to that described for **16b**: ^{1}H NMR (DMSO-d_6) δ 7.62 (2H, d, J = 8.7 Hz), 7.65 (2H, d, J = 8.8 Hz), 7.82 (1H, s), 7.97–8.03 (4H, m), 10.64 (1H, s); FAB-MS m/z 434 [$(M+H)^+$].

5.45. 2-Chloro-4′-[3,5-bis(trifluoromethyl)-1*H*-pyrazol-1-yl]benzanilide (28)

The title compound was prepared from **26** by a procedure similar to that described for **16a**: ^{1}H NMR (DMSO-d_6) δ 7.49 (1H, td, J = 7.3, 1.0 Hz), 7.54 (1H, td, J = 7.6, 1.9 Hz), 7.58–7.67 (4H, m), 7.82 (1H, s), 7.93 (2H, d, J = 9.3 Hz), 10.88 (1H, s); FAB-MS m/z 434 [$(M+H)^+$].

5.46. 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 GmbH, Austria) with excitation wavelength of each 340 nm 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 concentration in each well was 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.

5.47. 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 compound were calculated from percent inhibition of thapsigargin-induced calcium influx in Jurkat cells.

5.48. 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 compound were calculated from percent inhibition of KCl-induced calcium influx in PC12 cells.

5.49. 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).

5.50. 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.

5.51. TNCB-induced contact hypersensitivity in mice

Five-weak-old male CD-1 mice were treated with 100 μL of a 7% TNCB solution in the abdominal region after cutting their abdominal hair under anesthesia at day 0. Seven days after TNCB sensitization, the thickness of ear was measured, and 10 μL of 0.25% TNCB was applied both inside and outside of ear pinnas. Only a solvent was applied for negative control mice. Ear thickness was measured using a dial thickness gauge 24 h after the TNCB challenge, and changes in swelling were calculated from the value pre-exposure. Compound **29** was administered orally 1 h before exposure to TNCB, and 0.5% MC was administered orally in negative control and control animals.

Acknowledgments

The authors are grateful to the staff of the Division of Analytical Science Laboratories for the elemental analysis and spectral measurements.

References and notes

1. Yonetoku, Y.; Kubota, H.; Okamoto, Y.; Toyoshima, A.; Funatsu, M.; Ishikawa, J.; Takeuchi, M.; Ohta, M.; Tsukamoto, S. *Bioorg. Med. Chem.*, in press, doi:10.1016/j.bmc.2006.03.024.
2. Mori, Y.; Mikala, G.; Varadi, G.; Kobayashi, T.; Koch, S.; Wakamori, M.; Schwartz, A. *Jpn. J. Pharmacol.* **1996**, *72*, 83.
3. (a) Hoth, M.; Penner, R. *Nature* **1992**, *355*, 353; (b) Lewis, R. S.; Cahalan, M. D. *Annu. Rev. Immunol* **1995**, *13*, 623; (c) Clementi, E.; Meldolesi, J. *Cell Calcium* **1996**, *19*, 269; (d) Geiszt, M.; Kapus, A.; Nemet, K.; Farkas, L.; Ligeti, E. *J. Biol. Chem.* **1997**, *272*, 26471; (e) Clapham, D. E. *Cell* **1995**, *80*, 259; (f) Petersen, O. H.; Gerasimenko, O. V.; Gerasimenko, J. V.; Mogami, H.; Tepikin, A. V. *Cell Calcium* **1998**, *23*, 87; (g) Berridge, M. J.; Irvine, R. F. *Nature* **1989**, *341*, 197; (h) Putney, J. W., Jr. *Cell Calcium* **1990**, *11*, 611; (i) Randriamampita, C.; Tsien, R. Y. *Nature* **1993**, *364*, 809; (j) Fasolato, C.; Innocenti, B.; Pozzan, T. *Trends Pharmacol. Sci.* **1994**, *15*, 77; (k) Berridge, J. M. *Biochem. J.* **1995**, *312*, 1; (l) Hoth, M. *Pflügers Arch.* **1995**, *430*, 315; (m) Fanger, C. M.; Hoth, M.; Crabtree, G. R.; Lewis, R. S. *J. Cell Biol.* **1995**, *131*, 655; (n) Zweifach, A.; Lewis, R. S. *J. Biol. Chem.* **1995**, *270*, 14445; (o) Zweifach, A.; Lewis, R. S. *J. Gen. Physiol.* **1996**, *107*, 597.
4. (a) Slaughter, R. S.; Garcia, M. L.; Kaczorowski, G. J. *Curr. Pharm. Des.* **1996**, *2*, 610; (b) Cahalan, M. D.; Chandy, K. G. *Curr. Opin. Biotechnol.* **1997**, *8*, 749.
5. Chung, S. C.; McDonald, T. V.; Gardner, P. *Br. J. Pharmacol.* **1994**, *113*, 861.
6. Christian, E. P.; Spence, K. T.; Togo, J. A.; Dargis, P. G.; Warawa, E. *Br. J. Pharmacol.* **1996**, *119*, 647.
7. Felder, C. C.; Ma, A. L.; Liotta, L. A.; Kohn, E. C. *J. Pharm. Exp. Ther.* **1991**, *257*, 967.
8. Kubota, H.; Yonetoku, Y.; Sugasawa, K.; Funatsu, M.; Kawazoe, S.; Toyoshima, A.; Okamoto, Y.; Ishikawa, J.; Takeuchi, M.; WO 9919303; *Chem. Abstr.* **1999**, *130*, 311815.
9. Das, M.; Livingstone, S. E. *Aust. J. Chem.* **1974**, *27*, 2109.
10. Nugent, R. A. WO 9203451; *Chem. Abstr.* **1992**, *119*, 28357.
11. Neubauer, H. J.; Kuenast, C.; Hofmeister, P. *Chem. Abstr.* **1989**, *111*, 174087, DE3732541.
12. (a) Ref. 7; (b) Timmerman, L. A.; Clipstone, N. A.; Ho, S. N.; Northrop, J. P.; Crabtree, G. R. *Nature* **1996**, *383*, 837.
13. Virgilio, F. D.; Milani, D.; Leon, A.; Meldolesi, J.; Pozzan, T. *J. Biol. Chem.* **1987**, *262*, 9189.
14. Djuric, S. W.; BaMaung, N. Y.; Basha, A.; Liu, H.; Luly, J. R.; Madar, D. J.; Sciotti, R. J.; Tu, N. P.; Wagenaar, F. L.; Wiedeman, P. E.; Zhou, X.; Ballaron, S.; Bauch, J.; Chen, Y.-W.; Chiou, X. G.; Fey, T.; Gauvin, D.; Gubbins, E.; Hsieh, G. C.; Marsh, K. C.; Mollison, K. W.; Pong, M.; Shaughnessy, T. K.; Sheets, M. P.; Smith, M.; Trevillyan, J. M.; Warrior, U.; Wegner, C. D.; Carter, G. W. *J. Med. Chem.* **2000**, *43*, 2975.
15. Tiegs, G.; Hentschel, J.; Wendel, A. *J. Clin. Invest.* **1992**, *90*, 196.
16. Ishikawa, J.; Ohga, K.; Yoshino, T.; Takezawa, R.; Ichikawa, A.; Kubota, H.; Yamada, T. *J. Immunol.* **2003**, *170*, 4441.