Studies on Anti-inflammatory Agents. V.[1] Synthesis and Pharmacological Properties of 3-(Difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl]pyrazole and Related Compounds

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A series of novel 1,5-diphenylpyrazole derivatives bearing hydrophilic substituents was prepared. The anti-inflammatory and analgesic activities of these compounds were evaluated by using the adjuvant arthritis and Randall–Selitto assays in rats, and the structure–activity relationships were studied. The optimal compound was 3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl]pyrazole (10) with oral ED_{50} values of 0.31 and 2.6 mg/kg on adjuvant-induced arthritis and carrageenin-induced foot edema, respectively. Compound 10 showed analgesic activities not only toward inflamed paw but also toward normal paw (ED_{30} = 0.55 and 1.8 mg/kg, respectively) in the Randall–Selitto assay, and moreover, 10 was effective in the tail-pinch assay (ED_{50} = 21 mg/kg) similarly to morphine. The asymmetric synthesis and pharmacological properties of the enantiomers of 10 are also reported.

Key words anti-inflammatory agent; analgesic activity; 1,5-diphenylpyrazole; structure–activity relationship; morphine

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the main therapeutic agents for the treatment of the symptoms of arthritis. The common mechanism of action of this broad class of drugs is believed to be the inhibition of cyclooxygenase (COX), which is a key enzyme for the conversion of arachidonic acid into prostaglandins (PGs). The clinical benefits are reduction of pain and swelling associated with arthritis. The major drawbacks of these NSAIDs are severe mechanism-based side effects, including gastrointestinal ulceration.[2] Additionally, there continues to be a great need for new agents to lessen the sensation of pain, especially chronic pain, which is presently undertreated. The search for new analgesic agents, devoid of the side effects typical of morphine-like opioid agonists (such as respiratory depression, constipation and physical dependence), has attracted considerable attention in recent years.[3]

We have reported some 1,5-diarylpyrazole derivatives, such as FR123826 (**1**), as potent anti-inflammatory agents with selective COX-2 inhibition.[1,4,5] The structurally similar COX-2 inhibitor SC-58125 (**4**) has also been found by Isakson *et al.*[6] In the previous report,[1] we pointed out that electron-withdrawing substituents such as CN and CF_3 at the 3-position and the 4-(methylsulfonyl)-phenyl group at the 5-position of the pyrazole ring were optimal in a series of **1**-related derivatives. We further suggested that replacement of the fluoro substituent in **1** with an electron-donating moiety such as methoxy or methylamino also afforded compounds (*e.g.*, **2**, **3**) with good anti-inflammatory and/or analgesic activities. The hydrophilic character of these compounds, as well as their good activity, encouraged us to search for novel compounds with improved analgesic potency and physicochemical properties compared to **1** and **4**. In this paper, we describe the syntheses and pharmacological properties of a series of **2** and **3**-related 1,5-diphenylpyrazoles and the identification of 3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl]pyrazole (**10**) as the optimal compound.

Chemistry

Syntheses of 1-(4-alkoxyphenyl)-5-phenylpyrazoles **6**, **7**, **9** and **10** are summarized in Chart 1. The 1,3-diketones **5**, which were prepared from 4′-(methylthio)acetophenone and the appropriate esters, were treated with 4-methoxyphenylhydrazine in AcOH to afford the 1,5-diphenylpyrazoles **6**. The sulfoxide and sulfones (**7**, **10**) were obtained by oxidation of the sulfides **6** with *m*-chloroperbenzoic acid (*m*CPBA) or peracetic acid. Treatment of the methoxy derivative **7b** with BBr_3 afforded the hydroxy derivative **8**, which was alkylated with EtI and NaH to give the ethoxy derivative **9**.

1-[4-(Methylamino)phenyl]-5-[4-(methylsulfonyl)phenyl]pyrazoles **16** were synthesized as shown in Chart 2. Syntheses of **11** and **12** were performed similarly to **6** or **7**. Reduction of the nitro derivatives with iron powder and NH_4Cl gave the amino derivatives **13**.[7] The methylamino derivatives **16** were prepared from **13** by a three-step

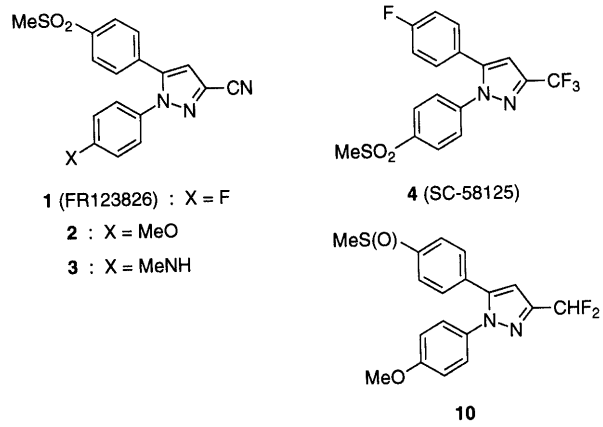

Fig. 1. Structures

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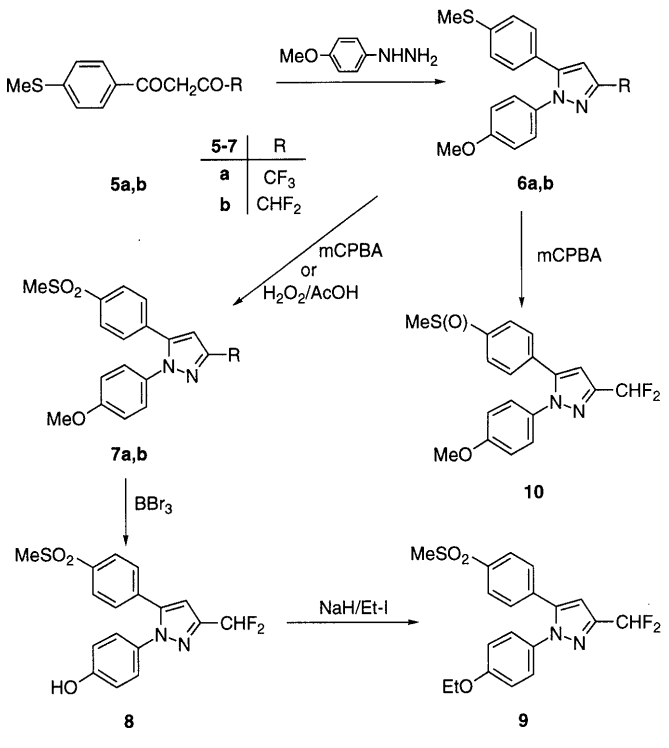


Chart 1

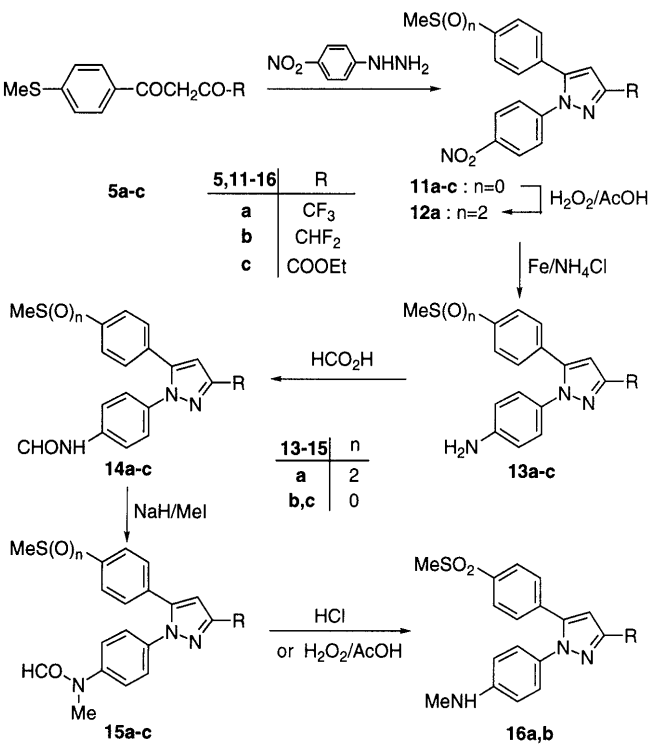


Chart 2

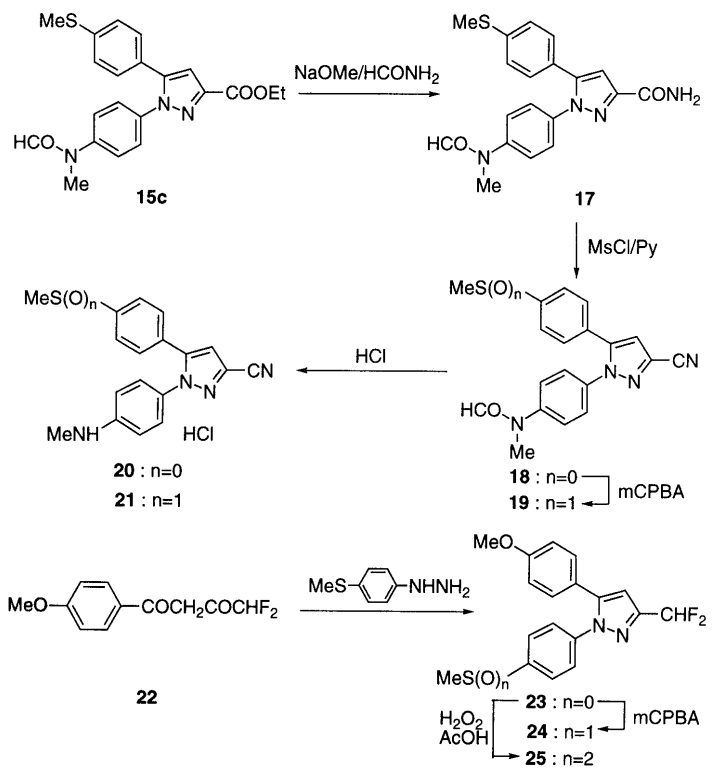


Chart 3

procedure of protection with a formyl group, methylation, and deprotection *via* **14** and **15** (81% yield from **13a** to **16a**). Oxidation and deprotection took place in one pot, when **15b** ($R=CHF_2$, $n=0$) was treated with peracetic acid (37% yield). Compound **15c** was used as an intermediate for the synthesis of the cyano derivatives **20** and **21** (Chart 3).

As depicted in Chart 3, the ester **15c** was heated with sodium methoxide and formamide, affording the amide **17**. The nitrile **18** was prepared by dehydration of **17** with methanesulfonyl chloride and pyridine. Deprotection of **18** with hydrochloric acid gave the desired sulfide **20**, and oxidation of **18** with *m*CPBA and subsequent deprotection afforded the sulfoxide **21**. The positional isomers (**23**—**25**) of **6b**, **10** and **7b** were synthesized from **22** and 4-(methylthio)phenylhydrazine similarly to **6**, **10** or **7**.

In order to study the effect of chirality,[8] we planned

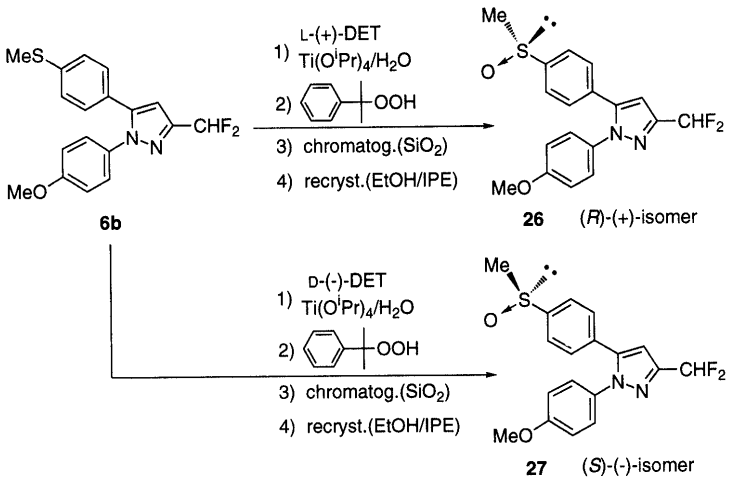


Chart 4

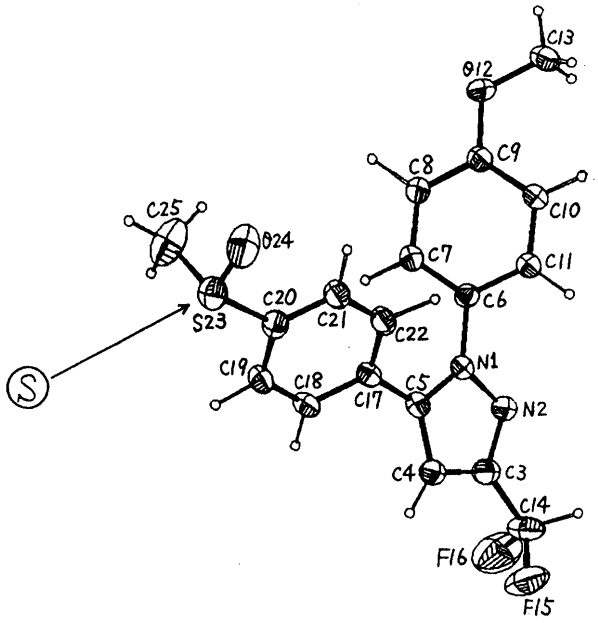


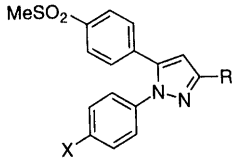
Fig. 2. An ORTEP Diagram of the X-Ray Crystal Structure of **27**

the synthesis of the optically pure enantiomers of compound **10**. Among several possible methods including asymmetric oxidation of sulfide,[9] utilization of a chiral auxiliary[10] or optical resolution, Kagan's method[9a] seemed to be the most promising. The (*R*)-(+)-isomer **26** was synthesized from the sulfide **6b** using L-(+)-diethyl tartrate (DET),[11] titanium (IV) isopropoxide, H_2O and cumene hydroperoxide as shown in Chart 4. On the other hand, D-(−)-DET was used for the preparation of the (*S*)-(−)-isomer **27**. Recrystallization from a mixture of EtOH and diisopropyl ether (IPE) afforded the optically pure isomers **26** and **27** (99.1% ee and 99.8% ee, respectively). The absolute configuration of **27** was confirmed to be *S*, a result consistent with the literature expectation,[9a] by X-ray crystallographic analysis as depicted in Fig. 2.

Results and Discussion

The compounds synthesized in this study were first tested for anti-inflammatory and analgesic activities through oral administration. The chronic anti-inflammatory activity

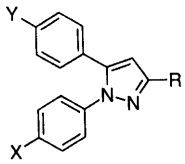
Table 1. Pharmacological Activities of 1,5-Diphenylpyrazoles



No.	X	R	Adjuvant arthritis % inhibition[a] (3.2 mg/kg *p.o.*)	Randall–Selitto relative potency[b] (10 mg/kg *p.o.*)
2[e]	MeO	CN	80[c]	1.05
7a	MeO	CF_3	93[c,g]	NT
7b	MeO	CHF_2	99[c]	1.47[c]
3[e]	MeNH	CN	68[c]	1.27[c]
16a[f]	MeNH	CF_3	81[c]	1.15[d]
16b	MeNH	CHF_2	77[c]	1.09
9	EtO	CHF_2	85[c]	1.11

a) Uninjected paw. *b*) Ratio of the pain threshold in the treated *vs.* control animals. *c*) $p<0.01$, *d*) $p<0.05$, significant difference from control. *e*) Ref. 1. *f*) HCl salt. *g*) 6/10 rats died. NT: Not tested.

Table 2. Comparison of the Sulfide, Sulfoxide and Sulfone Analogues



No.	X	Y	R	Adjuvant arthritis % inhibition[a] (3.2 mg/kg *p.o.*)	Randall-Selitto ED_{30} (mg/kg *p.o.*)
6b	MeO	MeS	CHF_2	94[c]	4.7
10	MeO	MeS(O)	CHF_2	86[c,d]	0.55
7b	MeO	$MeSO_2$	CHF_2	99[c,e]	3.0
20[b]	MeNH	MeS	CN	64[c]	>10
21[b]	MeNH	MeS(O)	CN	74[c,f]	11
3	MeNH	$MeSO_2$	CN	68[c]	37
23	MeS	MeO	CHF_2	80[c]	9.3
24	MeS(O)	MeO	CHF_2	76[c]	2.9
25	$MeSO_2$	MeO	CHF_2	88[c]	3.1

a) Uninjected paw. *b*) HCl salt. *c*) $p<0.01$, significant difference from control. ED_{50} (mg/kg): *d*) 0.31, *e*) 0.40, *f*) 0.30.

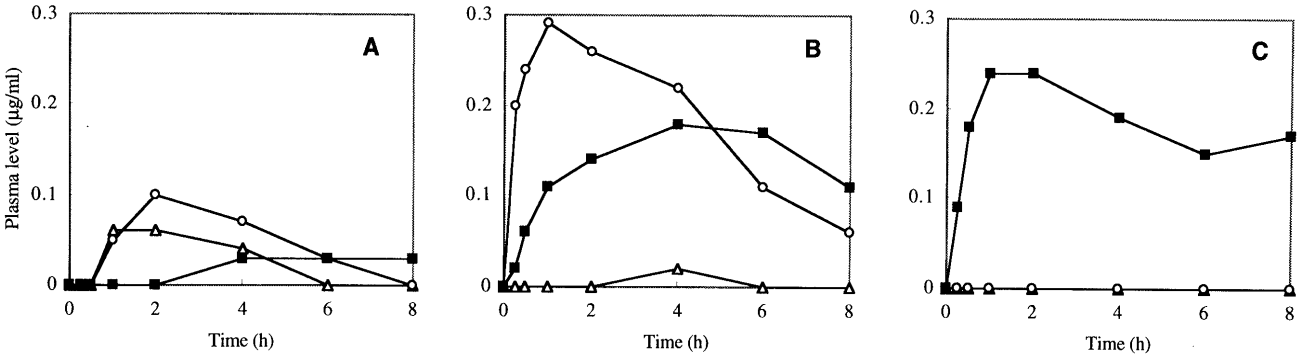


Fig. 3. Time Course of Plasma Concentrations of **10** and Related Compounds after Oral Administration of 10 mg/kg of **6b** (A), **10** (B) or **7b** (C) in Rats

Each point represents the mean level ($n=4$) of **6b** (△), **10** (○) or **7b** (■).

Table 3. Comparison of Compound **10** with Reference Compounds

	10	Indomethacin	Morphine
Adjuvant arthritis[a]			
ED_{50} (mg/kg *p.o.*)[b]	0.31	0.26	NT
UD_{50} (mg/kg *p.o.*)[c]	>3.2	0.069	NT
Safety index[d]	>10	0.27	
Carrageenin-induced foot edema[a]			
ED_{50} (mg/kg *p.o.*)	2.6	4.7	>100
Randall-Selitto, ED_{30} (mg/kg *p.o.*)[a]			
Normal paw[e]	1.8	>10	0.22
Inflamed paw[f]	0.55	3.3	0.11
Tail-pinch assay[g]			
ED_{50} (mg/kg *p.o.*)	21	>320	11

a) In rats. *b*) Uninjected paw. *c*) The median dose for production of GI lesions. *d*) UD_{50}/ED_{50}. *e*) Uninjected left hind paw. *f*) Yeast-injected right hind paw. *g*) Modified Haffner's method in mice (ref. 20). NT: Not tested.

Table 4. Compound **10** and Its Optical Isomers

	10 (±)	**26** (*R*)-(+)	**27** (*S*)-(−)
Carrageenin-induced foot edema[a]			
ED_{50} (mg/kg *p.o.*)	2.3	2.4	2.3
Randall-Selitto, ED_{30} (mg/kg *p.o.*)[a]			
Normal paw[b]	1.4	2.5	3.1
Inflamed paw[c]	0.31	0.66	0.47
Tail-pinch assay[d]			
ED_{50} (mg/kg *p.o.*)	17	17	16
PGE_2 production[e]			
IC_{50} (μM)	0.24	0.10	1.7

a) In rats. *b*) Uninjected left hind paw. *c*) Yeast-injected right hind paw. *d*) Modified Haffner's method in mice. *e*) Rat neutrophils (*in vitro*).

was assessed in terms of inhibition of adjuvant arthritis in rats. The analgesic activity against inflammation-related pain was evaluated as relative potency in the yeast-induced hyperalgesia (Randall–Selitto) assay in rats. The test results are summarized in Tables 1 and 2.

As a first step in the chemical modification of **2** and **3**, we designed a series of 3-(di- or trifluoromethyl)pyrazoles (**7a,b**, **16a,b**), because a number of diaryl heterocycles bearing polyfluoroalkyl moieties had been shown to have potent anti-inflammatory activities.[1,6,12] As shown in Table 1, all tested compounds significantly suppressed adjuvant arthritis at 3.2 mg/kg. However, **7a** showed an indication of severe toxicity (6/10 rats died in the adjuvant arthritis experiment) and both **16a** and **16b** were inferior to **3** in the Randall–Selitto assay. Fortunately, the maximum anti-inflammatory and analgesic activities were achieved with the difluoromethyl derivative **7b**. Replacement of the methoxy group in **7b** with an ethoxy moiety failed to improve the activities (compound **9**). The anti-inflammatory and analgesic activities seemed to vary inversely with the size of the alkoxy substituents. Consequently, **7b** and **3** were selected as lead compounds for further modification.

The pharmacological properties of the sulfide, sulfoxide and sulfone analogues of **7b**, its positional isomer (**25**) and **3** are compared in Table 2. The three redox analogues (sulfides, sulfoxides, sulfones) were similarly active toward adjuvant arthritis, on the other hand, the sulfoxides tended to exhibit the most potent analgesic activities in the Randall–Selitto assay. Among the potent sulfoxide analogues, compound **10** seemed to be the most promising, having exceptionally strong analgesic activity (ED_{30} = 0.55 mg/kg). The positional isomer **24** was slightly inferior to **10**. As an alternative type of lead compound, we were also interested in **21**, which had comparable activities to and more hydrophilic character than the parent compound **1**. To investigate the differences in the analgesic activities of **6b**, **10** and **7b**, we performed pharmacokinetic studies of these compounds in rats, as shown in Fig. 3. The fact that **10** had the strongest analgesic activity might be explained by the following results: 1) the bioavailability of the sulfide **6b** (7.4%) was inferior to that of the sulfoxide **10** or the sulfone **7b** (37% and 24%, respectively), giving relatively low plasma levels of **10** and **7b** (Fig. 3A), 2) **10** exhibited both NSAID-like (*i.e.*, inhibition of PG synthesis) and opioid-like activities as described later, while **7b** seemed to have only NSAID-like activity,[13] 3) the administration of **10** resulted in high plasma levels of both **10** and **7b** (Fig. 3B), on the other hand, the administration of **7b** resulted in high plasma level of only **7b**, with no detectable amount of **10** (Fig. 3C).

Based on the above evaluation, **10** (FR140423) was chosen for further development. It was equipotent to indomethacin, a representative NSAID, in both chronic and acute inflammation models, namely adjuvant arthritis and carrageenin-induced foot edema (ED_{50} = 0.31 and 2.6 mg/kg, respectively), with no indication of ulcerogenicity (Table 3). Surprisingly, **10** showed potent an-

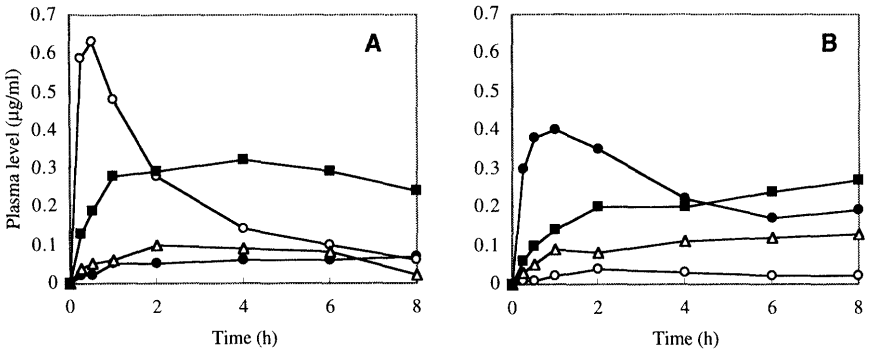


Fig. 4. Time Course of Plasma Concentrations of Enantiomers of **10** and Related Compounds after Oral Administration of 10 mg/kg of **26** (A) or **27** (B) in Rats

Each point represents the mean level ($n=4$) of **6b** (△), **26** (○), **27** (●) or **7b** (■).

algesic activities not only toward the inflamed paw, but also toward the normal paw in the Randall–Selitto assay, and moreover, **10** was effective in the tail-pinch assay. Morphine, a potent narcotic analgesic, exhibited similar analgesic activities, but indomethacin was devoid of these analgesic activities, as shown in Table 3. Naloxone, an opioid antagonist, suppressed the analgesic activities of both **10** and morphine in the tail-pinch assay.[14] However, **10** did not bind to any of the opioid receptors (μ, δ, κ) even at 10 μM.[15] Further mechanistic studies are in progress.

A comparison of the pharmacological properties of the racemate **10** and its two optical isomers **26** and **27** is presented in Table 4. These three compounds showed almost the same activities in the three *in vivo* assays, while the (*R*)-(+)-isomer **26** was more than ten times stronger than the (*S*)-(−)-isomer **27** in the *in vitro* PGE_2 production assay. Pharmacokinetic studies have indicated rapid *in vivo* conversion of **26** and **27** into a mixture of **7b**, **6b**, **26** and **27** in rats (Fig. 4). This probably explains the discrepancy between the *in vitro* and *in vivo* pharmacological results.

In conclusion, aiming at discovering compounds with better analgesic potency and physicochemical properties than **1**, we found the novel analgesic and anti-inflammatory agent **10**. Compound **10** showed exceptionally strong analgesic activity, and the solubility of **10** in water was 42 μg/ml compared to <1 μg/ml for **1**.[16] Pharmacological studies have suggested that **10** possesses not only NSAID-like peripherally acting activities, but also opioid-like centrally acting analgesic activities. Details of the pharmacological properties and mechanistic studies of **10** will be published elsewhere.

Experimental

Melting points were measured on a Mitamura capillary melting-point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IR-408 spectrophotometer. ^{1}H-NMR spectra were taken with a Varian EM-390 (90 MHz) or a Bruker AC-200 (200 MHz) instrument using tetramethylsilane as an internal standard. Electron impact (EI)-MS were obtained with a Hitachi M80 mass spectrometer. Atmospheric pressure chemical ionization (CI)-MS were obtained with a Hitachi M-1000 LC/QMS spectrometer. Optical rotations were recorded on a DIP-360 (Nihon Bunkoh Co., Ltd.) polarimeter. Solvents used for anhydrous reactions were dried over molecular sieves 3A. Organic extracts were dried over anhydrous $MgSO_4$. Column chromatography was performed using Kieselgel 60 (70—230 mesh, E. Merck).

1-(4-Methoxyphenyl)-5-[4-(methylthio)phenyl]-3-(trifluoromethyl)pyrazole (6a) A mixture of **5a** ($R=CF_3$)[4] (1.3 g, 4.96 mmol) and 4-methoxyphenylhydrazine hydrochloride (0.96 g, 5.52 mmol) in AcOH (15 ml) was refluxed for 1 h. EtOAc and H_2O were added and the organic layer was separated, washed with H_2O, dried, and evaporated. The residue was chromatographed (hexane–EtOAc, 3 : 1) over silica gel and the product was recrystallized from EtOH to give **6a** (1.19 g, 66%) as pale yellow crystals, mp 98—100 °C. IR (Nujol): 1605, 1520, 1500 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.48 (3H, s), 3.82 (3H, s), 6.71 (1H, s), 6.8—7.3 (8H, m). EI-MS *m/z*: 364 (M^+). *Anal.* Calcd for $C_{18}H_{15}F_3N_2OS$: C, 59.34; H, 4.12; N, 7.69. Found: C, 59.14; H, 3.98; N, 7.65.

3-(Difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylthio)phenyl]-pyrazole (**6b**): Compound **6b** was obtained from **5b**[4] similarly to **6a**: mp 100—101 °C (EtOH), colorless crystals. IR (Nujol): 1610, 1520, 1500 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.48 (3H, s), 3.82 (3H, s), 6.5—7.3 (10H, m). EI-MS *m/z*: 346 (M^+). *Anal.* Calcd for $C_{18}H_{16}F_2N_2OS$: C, 62.41; H, 4.66; N, 8.09. Found: C, 62.40; H, 4.56; N, 8.04.

1-(4-Methoxyphenyl)-5-[4-(methylsulfonyl)phenyl]-3-(trifluoromethyl)-pyrazole (7a) A mixture of **6a** (2.0 g, 5.49 mmol) and *m*CPBA (3.0 g, 13.9 mmol) in CH_2Cl_2 (50 ml) was stirred at room temperature for 3 h. The mixture was washed with aqueous $NaHCO_3$, dried, and evaporated. The residue was recrystallized from EtOH to afford **7a** (1.8 g, 83%) as colorless crystals, mp 155—156 °C. IR (Nujol): 1600, 1500 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.25 (3H, s), 3.80 (3H, s), 7.03 (2H, d, $J=8$ Hz), 7.2—7.6 (5H, m), 7.93 (2H, d, $J=8$ Hz). EI-MS *m/z*: 396 (M^+). *Anal.* Calcd for $C_{18}H_{15}F_3N_2O_3S$: C, 54.55; H, 3.79; N, 7.07. Found: C, 54.28; H, 3.71; N, 7.04.

3-(Difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfonyl)phenyl]-pyrazole (7b) A mixture of **6b** (2 g, 5.29 mmol), 30% H_2O_2 (2 ml, 17.6 mmol) and H_2SO_4 (2 drops) in AcOH (5 ml) was stirred at 60 °C for 1 h. The solvent was evaporated and the residue was recrystallized from EtOAc to give **7b** (0.86 g, 39%) as crystals, mp 135 °C. IR (Nujol): 1605, 1520 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.07 (3H,s), 3.84 (3H, s), 6.1—8.0 (10H, m). EI-MS *m/z*: 378(M^+), 346. *Anal.* Calcd for $C_{18}H_{16}F_2N_2O_3S$: C, 57.14; H, 4.26; N, 7.40. Found: C, 56.99; H, 4.02; N, 7.31.

3-(Difluoromethyl)-1-(4-hydroxyphenyl)-5-[4-(methylsulfonyl)phenyl]-pyrazole (8) BBr_3 (1 M in CH_2Cl_2; 30 ml) was added dropwise to an ice-cooled solution of **7b** (3.3 g, 8.73 mmol) in CH_2Cl_2 (70 ml). The mixture was stirred at 5 °C for 2 h, poured into ice-H_2O, and extracted with EtOAc. The extract was washed with dilute HCl, dried, and evaporated to afford **8** (3.1 g, 97%) as an off-white powder, mp 170—180 °C.[17] IR (Nujol): 1595, 1500 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.25 (3H, s), 6.7—7.6 (8H, m), 7.92 (2H, d, $J=8$ Hz), 9.93 (1H, s). EI-MS *m/z*: 364 (M^+).

3-(Difluoromethyl)-1-(4-ethoxyphenyl)-5-[4-(methylsulfonyl)phenyl]-pyrazole (9) NaH (60%; 0.12 g, 3 mmol) was added portionwise to a solution of **8** (1 g, 2.75 mmol) in *N*,*N*-dimethylformamide (DMF) (15 ml) at 5 °C. The mixture was stirred at room temperature for 30 min, then EtI (0.84 g, 5.38 mmol) was added dropwise at 5 °C. The whole was stirred at room temperature for 4 h and poured into a mixture of ice and dilute HCl. The precipitates were collected and chromatographed (EtOAc–toluene, 1 : 4) over silica gel to give **9** (0.25 g, 23%) as an off-white powder, mp 105—107 °C. IR (Nujol): 1605, 1520, 1500 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (3H, t, $J=7$ Hz), 3.07 (3H, s), 4.05 (2H, q, $J=7$ Hz), 6.5—7.5 (8H, m), 7.89 (2H, d, $J=8$ Hz). EI-MS *m/z*: 392 (M^+). *Anal.* Calcd for $C_{19}H_{18}F_2N_2O_3S$: C, 58.16; H, 4.59; N, 7.14. Found: C, 58.50; H, 4.60; N, 6.84.

3-(Difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl]-pyrazole (10) A mixture of **6b** (1.0 g, 2.88 mmol) and *m*CPBA (0.56 g, 2.59 mmol) in CH_2Cl_2 (23 ml) was stirred at room temperature for 1 h, then washed with aqueous $NaHCO_3$, dried, and evaporated. The residue was recrystallized from EtOH to give **10** (0.82 g, 78%) as colorless crystals, mp 123—124 °C. IR (Nujol): 1615, 1590, 1520 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.74 (3H, s), 3.83 (3H, s), 6.5—7.7 (10H, m). EI-MS *m/z*: 362(M^+), 347. *Anal.* Calcd for $C_{18}H_{16}F_2N_2O_2S \cdot 1/10H_2O$: C, 59.36; H, 4.48; N, 7.69. Found: C, 59.16; H, 4.28; N, 7.64.

5-[4-(Methylthio)phenyl]-1-(4-nitrophenyl)-3-(trifluoromethyl)pyrazole (**11a**): Compound **11a** was obtained from **5a** similarly to **6a**: mp 163—164 °C (EtOH), pale brown solid.[17] IR (Nujol): 1600, 1525 cm^{-1}.

5-[4-(Methylsulfonyl)phenyl]-1-(4-nitrophenyl)-3-(trifluoromethyl)-pyrazole (**12a**): Compound **12a** was obtained from **11a** similarly to **7b**: mp 163—164 °C (EtOH).[17] IR (Nujol): 1600, 1535 cm^{-1}. EI-MS *m/z*: 411 (M^+).

1-(4-Aminophenyl)-5-[4-(methylsulfonyl)phenyl]-3-(trifluoromethyl)-pyrazole (13a) A mixture of **12a** (1.12 g, 2.72 mmol), Fe powder (1.0 g, 17.9 mmol) and NH_4Cl (0.1 g, 1.87 mmol) in EtOH (20 ml) and H_2O (10 ml) was refluxed for 1 h. The mixture was filtered and the filtrate was evaporated. The residue was washed with H_2O to afford **13a** (1.0 g, 96%), mp 250—251 °C.[17] IR (Nujol): 3500, 3400, 1640, 1600, 1520, 1500 cm^{-1}. EI-MS *m/z*: 381 (M^+).

1-[4-(Formylamino)phenyl]-5-[4-(methylsulfonyl)phenyl]-3-(trifluoromethyl)pyrazole (14a) A mixture of **13a** (1.0 g, 2.62 mmol) and formic acid (5 ml) was refluxed for 1 h. The mixture was poured into H_2O (70 ml) and the precipitates were collected and washed with H_2O to give **14a** (1 g, 94%), mp 163—166 °C.[17] IR(Nujol): 3270, 1680, 1610, 1520 cm^{-1}. EI-MS *m/z*: 409 (M^+).

1-[4-(*N*-Methylformylamino)phenyl]-5-[4-(methylsulfonyl)phenyl]-3-(trifluoromethyl)pyrazole (15a) Compound **14a** (1 g, 2.44 mmol) was treated with NaH (60%; 107 mg, 2.67 mmol) in DMF (4 ml) at 5 °C for 30 min. A solution of MeI (0.71 g, 5 mmol) in DMF (1 ml) was added dropwise and the mixture was stirred at 5 °C for 1 h. The mixture was poured into dilute HCl and extracted with $CHCl_3$. The extract was washed with H_2O, dried, and evaporated. The residue was crystallized from IPE to afford **15a** (0.95 g, 92%), mp 118—120 °C.[17] IR (Nujol): 1660, 1610, 1520, 1500 cm^{-1}. EI-MS *m/z*: 423 (M^+).

1-[4-(Methylamino)phenyl]-5-[4-(methylsulfonyl)phenyl]-3-(trifluoromethyl)pyrazole hydrochloride (16a) A mixture of **15a** (0.95 g, 2.24 mmol) and 10% aqueous HCl (3 ml) in MeOH (15 ml) was stirred at 60 °C for 2 h. The solvent was evaporated and the residue was crystallized from EtOAc to afford **16a** (0.91 g, 94%), mp 200—202 °C. IR (Nujol): 2725, 2600, 2450, 1600, 1520, 1500 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.75 (3H, s), 3.26 (3H, s), 6.8—8.0 (9H, m), 8.42 (2H, s). EI-MS *m/z*: 395 (M^+). *Anal.* Calcd for $C_{18}H_{16}F_3N_3O_2S \cdot HCl$: C, 50.06; H, 3.97; N, 9.73. Found: C, 50.42; H, 4.03; N, 9.77.

3-(Difluoromethyl)-1-[4-(methylamino)phenyl]-5-[4-(methylsulfonyl)phenyl]pyrazole (**16b**): Compound **16b** was prepared from **15b** (R = CHF_2, $n=0$)[4] similarly to **7b**: mp 175—176 °C (EtOH). IR (Nujol): 3430, 1615, 1540 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.72 (3H, s), 3.07 (3H, s), 3.97 (1H, s), 6.5—8.1 (10H, m). EI-MS *m/z*: 377 (M^+). *Anal.* Calcd for $C_{18}H_{17}F_2N_3O_2S \cdot 1/8EtOH$: C, 57.21; H, 4.67; N, 10.97. Found: C, 57.13; H, 4.53; N, 10.58.

1-[4-(*N*-Methylformylamino)phenyl]-5-[4-(methylthio)phenyl]pyrazole-3-carboxamide (17) A mixture of **15c** (4.3 g, 10.9 mmol)[4] and NaOMe (1.8 g, 33.3 mmol) in $HCONH_2$ (20 ml) was stirred at 100 °C for 1 h. The solvent was evaporated and the residue was washed with H_2O to afford **17** (3.5 g, 91%), mp 183—189 °C.[17] IR (Nujol): 3350, 3200, 2650, 1670, 1655, 1605, 1520 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.47 (3H, s), 3.23 (3H, s), 6.9—7.7 (11H, m), 8.65 (1H, s). EI-MS *m/z*: 366 (M^+).

1-[4-(*N*-Methylformylamino)phenyl]-5-[4-(methylthio)phenyl]pyrazole-3-carbonitrile (18) A mixture of **17** (3.5 g, 9.42 mmol) and methanesulfonyl chloride (6.5 g, 56.7 mmol) in pyridine (40 ml) was stirred at 60 °C for 1.5 h. The solvent was evaporated, and H_2O and EtOAc were added to the residue. The organic layer was separated, washed with dilute HCl, dried, and evaporated. The residue was recrystallized from EtOH to give **18** (2.2 g, 66%), mp 132—134 °C.[17] IR (Nujol): 2250, 1670, 1600, 1515 cm^{-1}. EI-MS *m/z*: 348 (M^+).

1-[4-(*N*-Methylformylamino)phenyl]-5-[4-(methylsulfinyl)phenyl]-pyrazole-3-carbonitrile (**19**): Compound **19** was prepared from **18** similarly to **10**: oil.[17] IR (Film): 2250, 1680, 1610, 1515 cm^{-1}.

1-[4-(Methylamino)phenyl]-5-[4-(methylthio)phenyl]pyrazole-3-carbonitrile hydrochloride (**20**): Compound **20** was obtained from **18** similarly to **16a**: mp 113—120 °C (EtOH). IR (Nujol): 3400, 2650, 2450, 2250, 1600, 1515 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.46 (3H, s), 2.74 (3H, s), 6.57 (2H, s), 6.5—7.4 (9H, m). EI-MS *m/z*: 320 (M^+). *Anal.* Calcd for $C_{18}H_{16}N_4S \cdot HCl$: C, 60.58; H, 4.80; N, 15.70. Found: C, 60.39; H, 4.83; N, 15.53.

1-[4-(Methylamino)phenyl]-5-[4-(methylsulfinyl)phenyl]pyrazole-3-carbonitrile hydrochloride (**21**): Compound **21** was obtained from **19** similarly to **16a**: mp 175—177 °C (dec.) (EtOH). IR (Nujol): 2630, 2450, 2250, 1600, 1515 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.74 (3H, s), 2.76 (3H, s), 6.53 (2H, s), 6.7—7.8 (9H, m). EI-MS *m/z*: 336 (M^+). *Anal.* Calcd for $C_{18}H_{16}N_4OS \cdot HCl \cdot 1/6H_2O$: C, 57.52; H, 4.65; N, 14.91. Found: C, 57.92; H, 4.63; N, 14.37.

3-(Difluoromethyl)-5-(4-methoxyphenyl)-1-[4-(methylthio)phenyl]-pyrazole (**23**): Compound **23** was obtained from **22** similarly to **6a**: mp 90—92 °C (EtOH), light pink crystals. IR (Nujol): 1610, 1545, 1495 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.49 (3H, s), 3.81 (3H, s), 6.4—7.3 (10H, m). CI-MS *m/z*: 347 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{16}F_2N_2OS$: C, 62.42; H, 4.66; N, 8.09. Found: C, 62.75; H, 4.66; N, 7.99.

3-(Difluoromethyl)-5-(4-methoxyphenyl)-1-[4-(methylsulfinyl)phenyl]pyrazole (**24**): Compound **24** was prepared from **23** similarly to **10**: mp 123—124 °C (EtOH), light yellow crystals. IR (Nujol): 1610, 1550, 1500 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.74 (3H, s), 3.83 (3H, s), 6.4—7.7 (10H, m). CI-MS *m/z*: 363 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{16}F_2N_2O_2S$: C, 59.66; H, 4.45; N, 7.72. Found: C, 59.50; H, 4.50; N, 7.65.

3-(Difluoromethyl)-5-(4-methoxyphenyl)-1-[4-(methylsulfonyl)phenyl]pyrazole (**25**): Compound **25** was prepared from **23** similarly to **7b**: mp 118—120 °C (iso-PrOH), colorless crystals. IR (Nujol): 1615, 1595, 1500 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.07 (3H, s), 3.84 (3H, s), 6.4—8.0 (10H, m). CI-MS *m/z*: 379 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{16}F_2N_2O_3S$: C, 57.13; H, 4.26; N, 7.40. Found: C, 56.83; H, 4.22; N, 7.28.

(*R*)-(+)-3-(Difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)-phenyl]pyrazole (26) L-(+)-DET (3.71 g, 18 mmol) and dry CH_2Cl_2 (75 ml) were added to a three-necked, round-bottomed 500 ml flask under N_2. Then titanium(IV) isopropoxide (2.7 ml, 9 mmol) was added *via* a syringe. The mixture was stirred at room temperature for 5 min, and H_2O (162 μl, 9 mmol) was added. The solution was vigorously stirred for 25 min, by which time the solution was clear and slightly yellow. To this solution was added **6b** (6.24 g, 18 mmol), and the whole was chilled to −30 °C and stirred at −30 to −20 °C for 40 min. Then cumene hydroperoxide (3.0 ml, 18 mmol) was added dropwise. After an additional 10 min, the reaction vessel was sealed and allowed to stand in the freezer (−23 °C) for 18 h.

H_2O (3.03 ml) was added to the reaction vessel, and the solution was stirred vigorously at room temperature for 90 min. The reaction mixture was filtered through Celite and the Celite pad was washed with CH_2Cl_2 (110 ml). Then 2 N NaOH (48 ml) and brine (24 ml) were added to the filtrate and the mixture was stirred vigorously for 1 h. The organic layer was separated, dried, and concentrated *in vacuo*. The crude oil (9.5 g) was chromatographed (CH_2Cl_2–MeOH, 40:1) over silica gel (450 g). The product obtained was recrystallized from EtOH–IPE (1:4) to afford **26** (0.71 g, 11%), mp 113—114 °C. $[\alpha]_D^{25}$ +69.4° ($c=1.00$, $CHCl_3$). The IR and ^{1}H-NMR spectra were identical with those of **10**. CI-MS *m/z*: 363 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{16}F_2N_2O_2S \cdot 1/10H_2O$: C, 59.36; H, 4.48; N, 7.69. Found: C, 59.26; H, 4.39; N, 7.62.

(*S*)-(−)-3-(Difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl]pyrazole (**27**): Compound **27** was obtained similarly to **26**, except that D-(−)-DET was used: mp 112—114 °C, yield 17%. $[\alpha]_D^{25}$ −70.9° ($c=1.02$, $CHCl_3$). The IR and ^{1}H-NMR spectra were identical with those of **10**. CI-MS *m/z*: 363 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{16}F_2N_2O_2S \cdot 1/10H_2O$: C, 59.36; H, 4.48; N, 7.69. Found: C, 59.21; H, 4.29; N, 7.67.

X-Ray Crystallographic Analysis of Compound 27 Diffraction measurements were performed on a Rigaku AFC-5R diffractometer using graphite-monochromated CuKα radiation ($\lambda=1.54178$ Å). Crystallographic data are listed in Table 5.

Biological Methods. Adjuvant Arthritis, Randall–Selitto Assay, and PGE_2 Production by Rat Peritoneal Neutrophils These experiments were carried out according to the procedures described in the previous reports.[1,18,19]

Carrageenin-Induced Foot Edema in Rats Five male Sprague-Dawley rats were used per group. Rats were fasted for 18—24 h prior to the test. The rats were dosed orally (5 ml/kg) with drugs suspended in vehicle (0.5% methyl cellulose) or with vehicle alone. One hour later a subplantar injection of 0.1 ml of a 1% solution of carrageenin in sterile saline was

Table 5. Crystallographic Data for Compound **27**

Formula	$C_{18}H_{16}F_2N_2O_2S$
Molecular weight	362.40
Crystal dimensions (mm)	$0.25 \times 0.10 \times 0.05$
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Lattice parameters: a (Å)	13.385 (3)
b (Å)	17.378 (4)
c (Å)	7.389 (2)
V (Å^3)	1718.7 (6)
Z	4
D_{calc} (g/cm^3)	1.400
Total reflections	1726
R	0.051

administered. The volume of the injected foot was measured with a displacement plethysmometer. Three hours after the injection of carrageenin, the volume of the foot was again measured. The average foot swelling in a group of drug-treated animals was compared with that in a group of placebo-treated animals, and the % inhibition of edema was determined.

Tail-Pinch Assay The analgesic effect of drugs in mice was analyzed by the modified Haffner's tail-pinch method as reported by Takagi *et al.*[20]

Pharmacokinetic Methods. Preparation of Plasma Sample for HPLC Analysis Drugs (10 mg/5 ml/kg) suspended in 0.5% methyl cellulose were orally administered to rats, which had been fasted overnight. After a designated time period, the blood was collected and centrifuged at 2700 rpm for 5 min. Plasma (0.1 ml) was taken and added to MeOH (0.2 ml) containing the internal standard, 2-(4-fluorophenyl)-3-[4-(methylsulfonyl)phenyl]-5-(trifluoromethyl)thiophene.[21] The mixture was stirred for 10 min and centrifuged at 2700 rpm for 5 min. The supernatant was used for HPLC analysis.

HPLC Analysis for 6b, 7b and 10 HPLC was performed using a system consisting of a Waters Model 481 (264 nm), a Waters Model 510 pump, a Waters Automated Controller, a Waters WISP 710B, and a Waters Data Module 730. The column was TSK-Gel 120T 5 μm (0.4×15 cm, Tosoh). The flow rate was 1 ml/min. Solvents A and B were 0.3% KH_2PO_4 in H_2O–MeCN (7 : 3) and 0.3% KH_2PO_4 in H_2O–MeCN (3 : 7), respectively. The mobile phase started from A/B = 66/34 and went to B over 20 min. Retention times of **6b**, **7b** and **10** were 16.8, 11.9 and 8.2 min, respectively.

HPLC Analysis for 6b, 7b, 26 and 27 HPLC was carried out using a system consisting of a Waters Model 484 (264 nm), a Waters Model M-6000A, a Waters WISP 710B, and a Hewlett Packard HP-3396. The column was CHIRALPAK AD (0.46×25 cm, Daicel) and the flow rate was 1 ml/min. The mobile phase was hexane–EtOH–Et_2NH (1600 : 400 : 1). Retention times of **6b**, **7b**, **26** and **27** were 8.5, 36.8, 29.4 and 18.5 min, respectively.

Acknowledgments The authors are grateful to Dr. T. Fujii and his colleagues in the pharmacological division for biological assays, and Dr. K. Sakane in the New Drug Research Laboratories for useful suggestions during the preparation of this paper. Thanks are also due to the staff members of the Analytical Research Laboratories for X-ray crystallographic analysis, elemental analyses and spectral measurements.

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14) Compound **10** (80 mg/kg *p.o.*) and morphine (40 mg/kg *p.o.*) were inactive when naloxone (0.2 mg/kg s.c.) was administered simultaneously in the tail-pinch assay (T. Ochi *et al.*, unpublished results).
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