Studies on Anti-inflammatory Agents. IV.¹ Synthesis and Pharmacological Properties of 1,5-Diarylpyrazoles and Related Derivatives

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A series of novel 1,5-diarylpyrazole derivatives was synthesized and tested for anti-inflammatory and analgesic activities to develop anti-inflammatory agents with fewer side effects than existing nonsteroidal anti-inflammatory drugs. The structure-activity relationships in this series were extensively studied. Electron-withdrawing substituents such as CN and CF₃ were optimal at the 3-position of the pyrazole ring. Replacement of these substituents with bulky ones gave less active compounds. The 4-(methylsulfonyl)phenyl group seemed to be the optimal group at the 5-position of the pyrazole ring. The most potent compound was 1-[4-(fluorophenyl)-5-[4-(methylsulfonyl)phenyl]pyrazole-3-carbonitrile (19a), with oral ED₃₀ values of 0.030 and 0.47 mg/kg on adjuvant-induced arthritis and collagen-induced arthritis, respectively, and an ED₃₀ value of 7.4 mg/kg in the yeast-induced hyperalgesia (Randall-Selitto) assay. Compound 19a also showed potent inducible cyclooxygenase (COX-2)-inhibitory activity (IC₅₀ = 0.24 μM) with no COX-1 inhibition even at 100 μM.

Key words anti-inflammatory agent; 1,5-diarylpyrazole; cyclooxygenase; structure-activity relationship; synthesis

Nonsteroidal anti-inflammatory drugs (NSAIDs), represented by indomethacin and aspirin, have been demonstrated to be useful for relief of the symptoms of a number of arthritic conditions, such as rheumatoid arthritis. It has been pointed out, however, that the adverse effects of NSAIDs, namely gastrointestinal (GI) irritation and suppression of renal function, have to be ameliorated.²³ Recently, it has been shown that cyclooxygenase (COX) exists in two isoforms, termed COX-1 and COX-2.²³ It is believed that the anti-inflammatory effects of NSAIDs are mediated by inhibition of COX-1, while the side effects seem to be caused by inhibition of COX-1. A selective COX-2 inhibitor may be able to provide the desired therapeutic profile of an anti-inflammatory drug without the adverse effects commonly associated with COX-1 inhibition in the GI tract and kidney.⁴⁹

We have already reported on some methanesulfonanilide derivatives such as FK3311, which is a well-balanced anti-inflammatory, analgesic, and antipyretic agent that does not cause GI irritation.⁵⁵ Structurally distinct Dup697 (1) was also reported to be a potent anti-inflammatory drug which did not cause stomach ulcers or alter renal blood flow.⁶⁰ We were interested in the exceptionally strong inhibitory activity of 1 in the rat adjuvant-induced arthritis model. However, the 5-bromothiophene structure in 1 is biologically unstable and might have toxic effects, such as mutagenicity.⁷¹

From among the isosteric ring systems, the 3-bromo-1,5-diphenylpyrazole skeleton was found to be more stable and to have a steric conformation very similar to that of the 5-bromo-2,3-diphenylthiophene skeleton through a comparison of their frontier orbitals and three-dimensional structures by MO calculation.⁶⁸ On the basis of this finding, we designed novel 1,5-diarylpyrazole derivatives, expecting to achieve superior pharmacological and safety profiles. This paper describes the syntheses and pharmacological activities of various 1,5-diarylpyrazoles and related derivatives, and the identification of 1-[4-(fluorophenyl)-5-[4-(methylsulfonyl)phenyl]pyrazole-3-carbonitrile (19a) as the optimal compound.

Chemistry

Compounds 7, 9, 10 and 11 were synthesized via the 3-pyrazolomamine derivative 5, as shown in Chart 1. Compound 5 was prepared from 4-(methylthio)benzaldehyde 2 by Wittig reaction, pyrazoline ring formation with 4-fluorophenylhydrazine, and selective oxidation with MnO₂, according to the method reported by Appleton et al.⁶⁹ The bromo derivative 6 was obtained by diazotization of 5 and subsequent decomposition of the obtained diazonium salt in the presence of CuBr. A similar reaction using tert-BuONO and CuCl₂ gave only the reduced product 8. Oxidation of the sulfides (6, 8, 5) with peracetic acid or m-chloroperbenzoic acid (mCPBA) afforded the sulfones (7, 9, 10). Compounds 11 were prepared from 10 by treatment with the appropriate acylating agents.

Syntheses of compounds 16, 18 and 19 are outlined in

Fig. 1

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Chart 1. The 1,3-diketones 13 were prepared from the acetophenone 12 and the appropriate esters in the presence of NaH. Compounds 13 and the appropriate hydrazines were heated in EtOH to afford the desired 1,5-diarylpyrazoles 14 as the major products (80—90% yield) and 1,3-diarylpyrazoles 15 as the minor products (5—10% yield). The structural discrimination between 14a and 15a (Ar = 4-FPh, R = COOEt) was finally achieved by the derivation of 14a to 19a and the X-ray crystallographic analysis of 19a, as shown in Fig. 2. The treatment of compounds 14 with peracetic acid gave the sulfones 16. The esters 16 (R = COOEt) were hydrolyzed and treated with PCl₃ to afford the acid chlorides, which were allowed to react with the appropriate amines to give the amides 18. The nitriles 19 were obtained by dehydration of 18 (R = R' = H) with methanesulfonyl chloride and pyridine.

Compounds 20, 23, 24, 25 and 26a, d were obtained as shown in Chart 3. The nitrile 19a was treated with azide salt to give the tetrazole 20. Treatment of the acetophenone 12 with NaH and CS₂ and subsequent methylation gave the 3,3-bis(methylthio)-2-propen-1-one 21, which was treated with the hydrazine, followed by oxidation of the methylthio moieties to afford the methylsulfonyl derivative 23. Compounds 25a—d were prepared by alkylation of the amino derivative 24, which was obtained by reduction of the nitro derivative 19. The sulfone 26a was synthesized by oxidation of the sulfide 26b with sodium periodate. The methylamino derivative 26d was prepared by acidic removal of the formyl group in 27, which was
obtained according to the literature.\textsuperscript{11} Compounds 26b, c, e–h, 28 and 29 (Tables 3, 4) were synthesized following the procedure described for 19a (Chart 2).

Syntheses of the 19a-related nitrile compounds 33 and 37 are summarized in Chart 4. The imidazole 32 was synthesized by cyclization of the amidine 31 with bromopyruvate in 60% yield, following the reported synthetic route.\textsuperscript{12} The triazole 36 was obtained from 4-(methylthio)benzoic acid 34 by chlorination with SOCl\textsubscript{2}, amidation with aminomalonic acid, and cyclization with diazonium salt (via 35). The desired products 33 and 37 were prepared from 32 or 36 by hydrolysis, amidation, dehydration, and oxidation in the usual manner.

**Pharmacological 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 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—4.

From the structure-activity relationships (SARs) of 1 and the related thiope derivative, 4-(methylsulfonyl)-phenyl and 4-fluorophenyl groups seemed to play an important role in the strong anti-inflammatory activity of compound 1.\textsuperscript{13} As a first step in the SAR studies, we therefore designed a series of 3-substituted pyrazoles having 4-fluorophenyl and 4-(methylsulfonyl)phenyl groups at the 1 and 5 positions, respectively, as depicted in Table 1. The bromo derivative (7) showed very potent anti-inflammatory activity. This suggested the usefulness of the pyrazole ring as a surrogate of the thiophene ring. The sterically small unsubstituted (9) and amino (10), and electron-withdrawing trifluoromethyl (16b), carbamoyl (18a—c), cyano (19a), tetrazolyl (20) and sulfonyl (23) derivates also showed potent anti-inflammatory activities. On the other hand, bulky substituents (e.g., 11b, 18d) and less electron-attracting or less lipophilic substituents (e.g., 16c, d) gave less active compounds. Finally, the maximum anti-inflammatory and analgesic activities were achieved with the cyano derivative (19a). We chose 19a as a lead compound for further modification.

The results of the structural modification of the aryl group (Ar) at the 1 position of the pyrazole ring are summarized in Table 2. Removal of the 4-fluoro substituent in 19a resulted in some loss of the activities (19b). The 2- and 3-fluorophenyl (19c, d) and 2,4-difluorophenyl (19e) analogs showed fairly potent anti-inflammatory activities. Unfortunately, analgesic activities of these analogs were not as favorable as that of the lead compound 19a. We therefore focused our attention on the 4-substituted phenyl analogs. Replacing the 4-fluoro substituent in 19a with electron-donating moieties such as methyl, methoxy, methyliio or methylamino afforded compounds (19f—h, 25a—d) with good potency for inhibition of adjuvant arthritis. On the other hand, electron-withdrawing cyano and nitro substituents gave less active compounds (19i, j). The anti-inflammatory activity of compound 25a was inferior to that of 19a, but the discovery of a structure with excellent analgesic potency and hydrophobic character was utilized in the following study.\textsuperscript{14}

It was suggested that the 4-(methylsulfonyl)phenyl group played an essential role in the interaction between 1 and the target enzyme, COX, from the SARs of 1-related...
derivatives, as mentioned above.\textsuperscript{15} We conducted a brief modification study of the 5-aryl part, as depicted in Table 3. Only sulfone and sulfide analogs (26a, b) showed activities comparable to the parent 19a. The sulfone 26a was especially attractive in terms of its potent analgesic activity. However, 26a could be metabolically converted to the sulfone 19a, existed as a mixture of two optical isomers, and thus was not further evaluated.

Various analogs structurally related to compound 19a were synthesized and tested, as summarized in Table 4. Among the positional isomers (28, 29), compound 28, structurally very similar to 19a, showed moderate activity, which was inferior to that of 19a. The three-dimensional structures of the imidazole and triazole derivatives (33, 37) maintain a high similarity to that of the pyrazole 19a, but replacement of pyrazole with these skeletons resulted in loss of anti-inflammatory activity. Compounds 33 and 37 were also shown to be much less active than 19a in COX inhibitory assay (in vitro).\textsuperscript{16} These results may be attributable to their unfavorable charge distribution in comparison with 19a.

Based on the above evaluation, 19a (FR123826) was selected for further development. The IC\textsubscript{50} values towards both constitutive (COX-1) and inducible (COX-2) forms of human recombinant COX are compared in Table 5. Compound 19a showed COX-2-inhibitory activity comparable to that of indomethacin (IC\textsubscript{50} = 0.24 and 0.61 μM, respectively) with no COX-1 inhibition even at 100 μM. This finding demonstrates that 19a is a highly selective COX-2 inhibitor.

The in vivo data are summarized in Table 6. Compound 19a was more potent than the reference compounds (DuP697 and indomethacin) in two representative chronic arthritis models, namely adjuvant arthritis and collagen-induced arthritis (ED\textsubscript{50} = 0.030 and 0.47 mg/kg, respectively). Compound 19a also showed good analgesic activity and no ulcerogenicity, as expected from the in vitro COX-2 selectivity. These data suggest that selective COX-2 inhibitors such as 19a may represent a new generation of NSAIDs useful for the treatment of various inflammatory diseases such as rheumatoid arthritis.

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. 1H-NMR spectra were taken with a Varian EM-390 instrument using tetramethylsilane as an internal standard. Electron impact MS were obtained with a Hitachi 80 mass spectrometer. Organic extracts were dried over anhydrous MgSO\textsubscript{4}. Column chromatography was performed using Kieselgel 60 (70–230 mesh, E. Merck).

\textbf{Table 4. Analogs of Compound 19a}

<table>
<thead>
<tr>
<th>No.</th>
<th>Structure</th>
<th>Adjuvant arthritis % inhibition\textsuperscript{a}</th>
<th>Randall-Selitto relative potency\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td><img src="image" alt="Structure" /></td>
<td>81\textsuperscript{c}</td>
<td>1.12\textsuperscript{d}</td>
</tr>
<tr>
<td>29</td>
<td><img src="image" alt="Structure" /></td>
<td>36</td>
<td>1.17\textsuperscript{d}</td>
</tr>
<tr>
<td>33</td>
<td><img src="image" alt="Structure" /></td>
<td>5</td>
<td>1.05</td>
</tr>
<tr>
<td>37</td>
<td><img src="image" alt="Structure" /></td>
<td>-14</td>
<td>1.08</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Uninjected paw. \textsuperscript{b} Ratio of the pain threshold in the treated vs. control animals. \textsuperscript{c} p<0.01. \textsuperscript{d} p<0.05, significant difference from control.

\textbf{Table 5. Comparison of Compound 19a with Reference Compounds (in Vitro)}

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC\textsubscript{50}, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COX-1</td>
</tr>
<tr>
<td>19a</td>
<td>&gt;100</td>
</tr>
<tr>
<td>DuP697</td>
<td>11</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.23</td>
</tr>
</tbody>
</table>

\textsuperscript{c} Selectivity = IC\textsubscript{50}(COX-1)/IC\textsubscript{50}(COX-2).

\textbf{Table 6. Comparison of Compound 19a with Reference Compounds (in Vivo)}

<table>
<thead>
<tr>
<th>Compound</th>
<th>DuP697</th>
<th>Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>19a</td>
<td>7.4</td>
<td>1.3</td>
</tr>
</tbody>
</table>

\textsuperscript{c} In rats. \textsuperscript{b} Uninjected paw. \textsuperscript{c} The median dose for production of GI lesions. \textsuperscript{d} UD\textsubscript{50}/ED\textsubscript{50}. \textsuperscript{e} Type II collagen-induced arthritis in mice.
(2H, s), 6.6–7.5 (10H, m). MS m/z: 301 (M⁺).
1-[(4-Fluorophenyl)-5-[(4-methylthiophenyl)-3-pyrazolyl]acetamide (11a)] A mixture of 10 (0.7 g, 2.11 mmol) and AcOEt (0.22 ml, 2.33 mmol) in CH₂Cl₂ (15 ml) was stirred at room temperature for 3 h and then concentrated in vacuo. The residue was chromatographed (toluene-EtOAc, 2:1) over silica gel and the product was recrystallized from EtOH to give 11a (0.52 g, 66%) as pale brown crystals, mp 203–205°C. IR (Nujol): 3350, 1690, 1580, 1510 cm⁻¹. 1H-NMR (DMSO-d₆) δ: 2.43 (3H, s), 4.03 (2H, t), 7.0–7.2 (10H, m). MS m/z: 301 (M⁺).

2-Bromo-1-(4-fluorophenyl)-5-[(4-methylthiophenyl)-3-pyrazolyl]pyrazole (5) A solution of Na₂O₂ (0.26 g, 3.4 mmol) in H₂O (0.3 ml) was added to an ice-salt-cooled mixture of 5 (1 g, 3.34 mmol), MeCN (1 ml), concentrated H₂SO₄ (0.6 ml), and H₂O (1.6 ml). The mixture was stirred at 5°C for 20 min and added portionwise to a mixture of CuBr (645 mg, 4.54 mmol), NaBr (582 mg, 5.65 mmol), concentrated HBr (1.7 ml), and H₂O (3 ml) at 80°C. The whole was stirred at 80°C for 30 min and then evaporated with toluene. The extract was washed with H₂O, dried, and evaporated in vacuo. The residue was chromatographed (toluene) over silica gel (10 g) and the product was recrystallized from hexane-EtOH to give 6 (0.35 g, 29%), mp 98–99°C. IR (Nujol): 1600, 1510 cm⁻¹. 1H-NMR (CDCl₃) δ: 2.48 (3H, s), 6.49 (1H, s), 6.9–7.3 (8H, m). MS m/z: 364 (M⁺). Anal. Calcd for C₁₂H₁₇BrF₂N₅O₂S: C: 38.97, H: 5.29, N: 7.09. Found: C: 38.87, H: 5.49, N: 7.09.

Ethyl 4-[(4-methylthiophenyl)phenyl]-3-carboxylate (13a) A mixture of 12 (1 g, 6.02 mmol) and Na₂O₂ (60%; 288 mg, 7.2 mmol) in N,N-dimethylformamide (DMF) (7 ml) was stirred at room temperature for 30 min, then cooled to 0°C, and diethyl oxalate (0.98 ml, 7.2 mmol) was added dropwise to it. The reaction mixture was stirred at room temperature for 3h, poured into ice H₂O, and acidified with dilute HCl. The precipitates were collected and washed with H₂O to afford 13a. 1H-NMR (CDCl₃) δ: 1.29 (3H, t, J = 7.6 Hz), 2.54 (3H, s), 4.25 (2H, q, J = 7.6 Hz), 6.78 (1H, s), 7.35 (2H, d, J = 8.5 Hz), 7.91 (2H, d, J = 8.5 Hz). MS m/z: 266 (M⁺), 193 (M⁺) + Na⁺.

1-[(4-Fluorophenyl)-5-[(4-methylthiophenyl)-3-pyrazolyl]pyrazole-3-carboxylate (13a) A mixture of 12 (1 g, 6.02 mmol) and 4-fluorophenylhydrazine hydrochloride (14g, 87.1 mmol) in EtOH (180 ml) and dioxane (180 ml) was refluxed for 4h. The insoluble material was removed by filtration and the filtrate was evaporated. The residue was chromatographed (toluene-EtOAc, 2:1) over silica gel to afford 13a (0.4 g, 86%) as a pale yellow solid. 1H-NMR (DMSO-d₆) δ: 2.79 (3H, s), 7.1–7.6 (8H, m), 7.4–7.6 (2H, s). MS m/z: 398 (M⁺), 299 (M⁺) + Na⁺.

Ethyl 4-[(4-methylthiophenyl)phenyl]-3-carboxylate (13a) A mixture of 12 (1 g, 6.02 mmol) and Na₂O₂ (60%; 288 mg, 7.2 mmol) in N,N-dimethylformamide (DMF) (7 ml) was stirred at room temperature for 30 min, then cooled to 0°C, and diethyl oxalate (0.98 ml, 7.2 mmol) was added dropwise to it. The reaction mixture was stirred at room temperature for 3h, poured into ice H₂O, and acidified with dilute HCl. The precipitates were collected and washed with H₂O to afford 13a. 1H-NMR (CDCl₃) δ: 1.29 (3H, t, J = 7.6 Hz), 2.54 (3H, s), 4.25 (2H, q, J = 7.6 Hz), 6.78 (1H, s), 7.35 (2H, d, J = 8.5 Hz), 7.91 (2H, d, J = 8.5 Hz). MS m/z: 266 (M⁺), 193 (M⁺) + Na⁺.

Following the same procedure as described for compound 16a, the following compounds were obtained from the appropriate 14a.

[1-[(4-Fluorophenyl)-5-[(4-methylthiophenyl)phenyl]-3-(trifluoromethyl)pyrazole (16b)] mp 210–212°C (EtOH-EtOAc), colorless crystals. IR (Nujol): 3360, 1660, 1590, 1550, 1640, 1220, 1060, 750, 730 cm⁻¹. 1H-NMR (DMSO-d₆) δ: 3.26 (3H, s), 7.3–7.6 (7H, m), 7.96 (2H, d, J = 8Hz). MS m/z: 388 (M⁺). Anal. Calcd for C₂₃H₁₈F₂N₅O₂S: C: 42.38, H: 3.4, N: 7.15. Found: C: 42.38, H: 3.4, N: 7.15.
3.21 mmol) in toluene (16 ml) and THF (9 ml) was stirred at room temperature for 2 h. The insoluble material was removed by filtration and the filtrate was evaporated to give the acid chloride (1.37 g) as an oil.

A mixture of 25% MeNH₂ (2 ml), ice-H₂O (5 ml), and THF (10 ml) was added to the above chloride, and the whole was stirred overnight. The precipitates were collected and the filtrate was extracted with EtOAc. The extract was washed with H₂O, dried, and evaporated. The residue and the former precipitates were combined and recrystallized from EtOAc-EOH to afford 18b (1 g, 85%) as colorless crystals, mp 271–273 °C. IR (Nujol): 3400, 1600, 1605, 1550, 1510 cm⁻¹. ¹H-NMR (DMSO-d₆): δ 7.28 (3H, d, J = 5.1 Hz), 3.25 (3H, s), 1.71 (1H, s), 7.3–7.7 (6H, m), 7.91 (2H, d, J = 8.7 Hz), 8.35 (1H, q, J = 5.1 Hz). MS m/z: 373 (M⁺). Anal. Caled for C₂₆H₂₃N₂O₃S: C, 57.90; H, 4.32; N, 11.25. Found: C, 57.86; H, 4.53; N, 10.83.

Following the same procedure as described for compound 18b, the following compounds were obtained from 17a.

1-(4-Methoxyphenyl)-5-[4-(methylosulfonyl)phenyl]pyrazole-3-carboxamide (18a): mp 215–217 °C (EtOAc-EtOH). IR (Nujol): 3470, 3200, 1680, 1600, 1515 cm⁻¹. ¹H-NMR (DMSO-d₆): δ 3.25 (3H, s), 1.71 (1H, s), 7.2–7.6 (7H, m), 7.77 (1H, s), 7.91 (2H, d, J = 8.5 Hz). MS m/z: 359 (M⁺). Anal. Caled for C₂₆H₂₁N₂O₃S: C, 56.81; H, 3.93; N, 11.6. Found: C, 56.82; H, 4.00; N, 11.35.

5-[4-(Methylosulfonyl)phenyl]pyrazole-3-carboxamide (18c): mp 171–173 °C (EtOAc-EtOH), off-white crystals. IR (Nujol): 1620, 1510 cm⁻¹. ¹H-NMR (DMSO-d₆): δ 3.02 (3H, s), 3.25 (3H, s), 3.32 (3H, q), 7.08 (1H, s), 7.2–8.0 (6H, m). MS m/z: 387 (M⁺). Anal. Caled for C₂₆H₂₃N₂O₃S: C, 58.90; H, 4.68; N, 10.85. Found: C, 58.84; H, 4.66; N, 10.06.

1-(4-Fluorophenyl)-5-[4-(methylosulfonyl)phenyl]pyrazole-3-carboxamide (18d): mp 173–180 °C (EtOAc-EtOH), colorless crystals. IR (Nujol): 1615, 1515, 1500 cm⁻¹. ¹H-NMR (CDCl₃): δ 1.77–2.07 (4H, m), 3.00 (3H, s), 3.67 (2H, t, J = 6.1 Hz), 3.97 (2H, t, J = 6.1 Hz), 6.95–7.5 (7H, m), 7.78 (2H, d, J = 8.7 Hz). MS m/z: 413 (M⁺). Anal. Caled for C₂₆H₂₂F₂N₂O₃S: C, 61.02; H, 4.84; N, 10.17. Found: C, 61.17; H, 4.96; N, 10.07.


Following the same procedure as described for compound 19a, the following compounds were obtained from the appropriate 18.

5-[4-(Methylosulfonyl)phenyl]-1-phenylpyrazole-3-carbonitrile (19b): mp 175–180 °C (EtOAc). IR (Nujol): 2250, 1600, 1500 cm⁻¹. ¹H-NMR (CDCl₃): δ 3.07 (3H, s), 2.09 (2H, q, J = 6.9 Hz). MS m/z: 387 (M⁺). Anal. Caled for C₂₆H₂₂N₂O₃S: C, 58.91; H, 4.68; N, 12.87. Found: C, 58.30; H, 4.12; N, 12.67.

1-(2-Fluorophenyl)-5-[4-(methylosulfonyl)phenyl]pyrazole-3-carbonitrile (19c): mp 147–148 °C (EtOH). IR (Nujol): 2250, 1600, 1500 cm⁻¹. ¹H-NMR (CDCl₃): δ 3.07 (3H, s), 7.00 (1H, s), 7.2–8.0 (6H, m). MS m/z: 387 (M⁺). Anal. Caled for C₂₆H₂₂N₂O₃S: C, 58.91; H, 4.54; N, 12.31. Found: C, 59.00; H, 4.72; N, 12.19.


1-(4-Fluorophenyl)-5-[4-(methylosulfonyl)phenyl]pyrazole-3-carbonitrile (19e): mp 129–130 °C (EtOH). IR (Nujol): 2250, 1610, 1520 cm⁻¹. ¹H-NMR (CDCl₃): δ 3.08 (3H, s), 6.8–8.0 (8H, m). MS m/z: 359 (M⁺). Anal. Caled for C₂₆H₂₁N₂O₃S: C, 56.82; H, 3.09; N, 11.69. Found: C, 57.07; H, 3.10; N, 11.61.

1-(4-Methylphenyl)-5-[4-(methylosulfonyl)phenyl]pyrazole-3-carbonitrile (19f): mp 210–211 °C (EtOH). IR (Nujol): 2250, 1600, 1515 cm⁻¹. ¹H-NMR (DMSO-d₆): δ 2.41 (3H, s), 3.08 (3H, s), 6.96 (1H, s), 7.1–8.0 (8H, m). MS m/z: 337 (M⁺). Anal. Caled for C₂₆H₂₃N₂O₃S: 1/2H₂O: C, 63.40; H, 4.55; N, 12.32. Found: C, 63.42; H, 4.45; N, 11.98.

1-(Methylamino)phenyl]-5-[4-(methylosulfonyl)phenyl]pyrazole-3-carbonitrile (25a): A mixture of 24 (1 g, 2.96 mmol), Mel (0.42 g, 2.96 mmol), and K₂CO₃ (0.6 g, 4.35 mmol) in DMF (10 ml) was stirred
at room temperature for 1 h. The mixture was poured into H$_2$O and extracted with EtOAc. The extract was washed with H$_2$O, dried, and concentrated. The residue was chromatographed (CHCl$_3$) over silica gel to afford 2 (30.1 g, 30%) as crystals. mp 166–168°C. IR (NH$_2$): 3450, 2240, 1610, 1530 cm$^{-1}$. $^{1}$H-NMR (DMSO-d$_6$): $\delta$ 2.51 (3H, d, J = 5 Hz), 3.25 (3H, s), 6.17 (1H, q, J = 5 Hz), 6.5–8.0 (9H, m). Anal. Caled for C$_{35}$H$_{30}$N$_2$O$_7$S: C, 63.15; H, 4.58; N, 15.90. Found: C, 61.09; H, 4.57; N, 15.81.

Following the same procedure as described for compound 25a, the following compounds were obtained from 24.

1-(4-Ethylamino)phenyl)-5-(4-(methylsulfonyl)phenyl)pyrazole-3-carbonitrile (25a): mp 167–168°C (EtOAc). IR (Nujol): 3400, 2240, 1610, 1525 cm$^{-1}$. $^{1}$H-NMR (CDCl$_3$): $\delta$ 1.18 (6H, t, J = 7 Hz), 3.07 (3H, s), 3.37 (4H, q, J = 7 Hz), 6.5–8.0 (9H, m). MS m/z: 366 (M$^+$). Anal. Caled for C$_{32}$H$_{26}$N$_2$O$_7$: C, 62.88; H, 4.95; N, 15.29. Found: C, 61.82; H, 4.88; N, 15.00.

1-(4-Dimethylamino)phenyl)-5-(4-(methylsulfonyl)phenyl)pyrazole-3-carbonitrile (25b): mp 155–156°C (EtOH). IR (Nujol): 2240, 1610, 1520 cm$^{-1}$. $^{1}$H-NMR (CDCl$_3$): $\delta$ 1.18 (6H, t, J = 7 Hz), 3.07 (3H, s), 3.37 (4H, q, J = 7 Hz), 6.5–8.0 (9H, m). MS m/z: 394 (M$^+$). Anal. Caled for C$_{32}$H$_{26}$N$_2$O$_7$: C, 63.94; H, 5.62; N, 14.20. Found: C, 63.57; H, 5.45; N, 14.04.

1-(4-Dimethylamino)phenyl)-5-(4-(methylsulfonyl)phenyl)pyrazole-3-carbonitrile (25c): mp 171–172°C (EtOH). IR (Nujol): $\delta$ 1.18 (6H, t, J = 7 Hz), 3.07 (3H, s), 3.37 (4H, q, J = 7 Hz), 6.5–8.0 (9H, m). MS m/z: 366 (M$^+$). Anal. Caled for C$_{32}$H$_{26}$N$_2$O$_7$: C, 62.88; H, 4.95; N, 15.29. Found: C, 62.10; H, 4.95; N, 15.02.

1-(4-Dimethylamino)phenyl)-5-(4-(methylsulfonyl)phenyl)pyrazole-3-carbonitrile (25d): A solution of Na$_2$O (0.7 g, 3.30 mmol) in H$_2$O (5 ml) was added to an ice-cooled solution of 26 (0.6 g, 1.94 mmol) in MeOH (50 ml). The resulting solution was stirred at room temperature for 1 h and the insoluble material was filtered off. The filtrate was evaporated and the residue was dissolved in EtOAc. This solution was washed with aqueous Na$_2$SO$_4$ and H$_2$O successively, dried, and concentrated. The residue was chromatographed (CH$_2$Cl$_2$-MeOH, 50:1) over silica gel and the product was recrystallized from EtOAc to afford 26a (0.04 g, 71%) as crystals, mp 104–105°C. IR (Nujol): 2250, 1600, 1515 cm$^{-1}$. $^{1}$H-NMR (CDCl$_3$): $\delta$ 2.76 (3H, s), 6.94 (1H, s), 7.0–7.7 (8H, m). MS m/z: 525 (M$^+$). Anal. Caled for C$_{32}$H$_{28}$N$_2$O$_7$: C, 62.76; H, 3.72; N, 12.91. Found: C, 62.73; H, 3.74; N, 12.70.

1-(4-Dimethylamino)phenyl)-5-(4-(methylsulfonyl)phenyl)pyrazole-3-carbonitrile hydrochloride (26a) was chromatographed (CH$_2$Cl$_2$-MeOH, 50:1) over silica gel and the product was recrystallized from hexane-EtOH to afford 26a (0.04 g, 71%) as crystals, mp 104–105°C. IR (Nujol): 2250, 1600, 1515 cm$^{-1}$. $^{1}$H-NMR (CDCl$_3$): $\delta$ 2.76 (3H, s), 6.94 (1H, s), 7.0–7.7 (8H, m). MS m/z: 525 (M$^+$). Anal. Caled for C$_{32}$H$_{28}$N$_2$O$_7$Cl: C, 62.76; H, 3.72; N, 12.91. Found: C, 62.73; H, 3.74; N, 12.70.

1-(4-Dimethylamino)phenyl)-5-(4-(methylsulfonyl)phenyl)pyrazole-3-carbonitrile (26b): A solution of 27 (0.7 g, 2.19 mmol) and 10% HCl (3ml) in MeOH (15 ml) was stirred at 60°C for 2 h. The solvent was evaporated and the residue was washed with EtOAc to afford 26b (0.43 g, 60%), mp 189–191°C. IR (Nujol): 2650, 2450, 2250, 1510 cm$^{-1}$. $^{1}$H-NMR (DMSO-d$_6$): $\delta$ 2.73 (3H, s), 6.7–7.5 (9H, m). MS m/z: 292 (M$^+$). Anal. Caled for C$_{32}$H$_{25}$N$_2$O$_7$: C, 62.11; H, 4.29; N, 17.04. Found: C, 61.95; H, 4.31; N, 17.03.

Following the same procedure as described for 19a, the following compounds were prepared from the appropriate substituted acetophenones or acetylphenyl. 

1-(4-Fluorophenyl)-5-(4-(methylthio)phenyl)pyrazole-3-carbonitrile (26c): A solution of Na$_2$O (0.4 g, 2.06 mmol) in H$_2$O (10 ml) was added dropwise to a solution of 4-fluorotoluene (3.4 g, 20.5 mmol) and concentrated HCl (5 ml) in MeOH (15 ml) at 0°C. The mixture was stirred at 0°C for 15 min, then a solution of 35 (1.4 g, 0.71 mmol) in anhydrous MeOH (10 ml) was added and the mixture was stirred at 0°C for 30 min. After 30 min, the mixture was filtered and concentrated. The residue was recrystallized from EtOAc to afford 35 (2.2 g, 60%) as a yellow oil. $^{1}$H-NMR (DMSO-d$_6$): $\delta$ 2.53 (3H, s), 3.73 (6H, s), 3.56 (H, d, J = 8 Hz), 7.35 (2H, d, J = 8 Hz), 7.86 (2H, d, J = 8 Hz), 9.31 (1H, d, J = 8 Hz). MS m/z: 297 (M$^+$).

1-(4-Fluorophenyl)-5-(4-(methylthio)phenyl)H$_2$-1,2,4-triazole-3-carboxylate (36) A solution of NaN$_2$O (1.4 g, 20.6 mmol) in H$_2$O (10 ml) was added dropwise to a solution of 4-fluorotoluene (2.3 g, 20.5 mmol) and concentrated HCl (5 ml) in MeOH (15 ml) at 0°C. The mixture was stirred at 0°C for 15 min, then a solution of 35 (1.4 g, 0.71 mmol) in anhydrous MeOH (10 ml) was added and the mixture was stirred at 0°C for 30 min. After 30 min, the mixture was filtered and concentrated. The residue was recrystallized from EtOAc to afford 35 (2.2 g, 60%) as a yellow oil. $^{1}$H-NMR (DMSO-d$_6$): $\delta$ 2.53 (3H, s), 3.73 (6H, s), 3.56 (H, d, J = 8 Hz), 7.35 (2H, d, J = 8 Hz), 7.86 (2H, d, J = 8 Hz), 9.31 (1H, d, J = 8 Hz). MS m/z: 297 (M$^+$).
Table 7. Crystallographic Data for 19a

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Carbonitrile (37): mp 263–264°C (AcOH-H₂O). IR (Nujol): 2250, 1600, 1510 cm⁻¹. ¹H-NMR (DMSO-d₆) δ: 3.27 (3H, s), 5.40–5.81 (8H, m), MS m/z: 342 (M⁺). Anal. Calcd for C₁₅H₁₂F₁₁N₃O₇S: C, 56.14; H, 3.24; N, 16.36. Found: C, 55.80; H, 3.17; N, 16.16. X-Ray crystallographic analysis of 19a: Diffraction measurements were performed on a Rigaku AFC-SUD diffractometer using graphite-monochromated CuKα radiation (λ = 1.5418 Å). Crystallographic data are listed in Table 7.

Biological Methods. Adjuvant Arthritis and Collagen-Induced Arthritis

These experiments were carried out according to the procedures described in the previous report.¹⁵

Inflammatory Hyperalgesia Induced by Brewer’s Yeast in Rats (Randall–Selitto Assay)

Ten male Sprague Dawley rats were used per group. A suspension, 0.1mL of 5% brewer’s yeast in 0.5% methyl cellulose was injected into the right hind paw. The pain threshold was determined 3h after yeast injection, by applying pressure to the foot and reading the pressure at which the rat withdrew the foot. The drugs were given orally 2h after yeast injection. The pain threshold in the treated rats was compared with that in the control rats.

bCOX-1 and bCOX-2 Enzyme Assays (in Vitro)

CHO cells expressing either recombinant human COX-1 or COX-2 were used as the enzyme source.²⁰ COX activity was assayed as prostaglandin (PG) E₂ formation using radioimmunoassay (RIA), bCOX-1 (1 μg/10 μl) or bCOX-2 (3 μg/10 μl) was preincubated with an inhibitor in 0.1 M Tris-HCl buffer (pH 7.3) containing 2 μM heme and 5 mM L-tryptophan at 30°C for 5min, followed by a 5min incubation with arachidonic acid (10 μM) at 30°C. The enzyme reaction was stopped by the addition of 1N HCl. The PGE₂ formed was extracted with EtOAc and measured by RIA (Amersham).

Acknowledgments

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References and Notes

7) Compound 1 was metabolized to a 5-(methylsulfonoyl)thiophene derivative in rats. Compound 1 also showed mutagenicity in a chromosome aberration test conducted by Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd. (unpublished results).
8) The energy levels and the orbital distributions of HOMO and LUMO, and the torsion angles of the benzene rings of the most stable conformer of 5-bromo-3-(4-acylphenyl)-2-(4-fluorophenyl)thiophene (38) and that of 3-bromo-3-(4-acylphenyl)-1-(4-fluorophenyl)pyrazole (39) were calculated by the MNDO method: e.g., the torsion angle of the 4-acylphenyl ring was +7.7° (38) and +79.1° (39) and that of the 4-fluorophenyl ring was +77.4° (38) and +77.3° (39), respectively.
10) The production of the by-product 15 was suppressed when the reaction was conducted in AcOH.
16) COX-1 inhibition at 100 μM: 0% (33), 8% (37); COX-2 inhibition at 100 μM: 19% (33), 49% (37).
17) This product was used for the next step in the synthesis without further purification.
20) Supplied by Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd. (Tsukuba, Ibaraki).