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Synthesis and Biological Activity of Annulated Pyrazoles as Selective COX-2 Inhibitors. I.

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A series of disubstituted 4,5-polymethylenepyrazoles were synthesized and evaluated their inhibitory activities against COX-2. Some compounds showed strong (0.3 nM) inhibitory activity on COX-2 and were found somewhat selective (up to 16) on COX-2 over COX-1.

Key words : 4,5-Polymethylenepyrazoles, COX-1, COX-2 inhibitor, Anti-inflammatory

INTRODUCTION

Prostaglandins, found in many tissues, not only play an important role to elicit a variety of beneficial responses such as maintaining the gastrointestinal integrity or the renal blood flux but also contribute to inflammation. The nonsteroidal antiinflammatory drugs have been used for treating inflammation, pain and fever by control prostagladin-level in the inflammatory site (Insel, 1996; Lombardino, 1985). Their major activity was exhibited by inhibiting cyclooxygenase (COX, also known as prostaglandin endoperoxide H-synthase or PGHS: EC 1.14.99.1) which is the key enzyme in the biosynthetic sequence of prostaglandins from arachidonic acid. Recently, two isoforms of the COX have been found: the first COX-1 is constitutively expressed in a large variety of cells and is responsible in large part of the basal endogenous release of prostaglandins while the second COX-2 is rapidly induced in cells by agents such as endotoxins and cytokines and is responsible of the production of prostaglandins in response to proinflammatory stimuli (Xie *et al*, 1992, and Mitchell *et al*, 1993). Most of the side effects of the non-steroidal anti-inflammatory agents in gastrointestinal tracts and renal function were found to be caused by over suppression of prostaglandins in stomach and kidney by inhibiting COX-1. The selective or specific inhibitors of COX-2, thus, can be a new vista to control inflammation with reduced side effects (Vane, 1994). Some of the selective COX-2 inhibitors which are either in market or in clinical trials are shown in Fig. 1 (Chang and Jahng, 1998, Graul, A. *et al*, 1997).

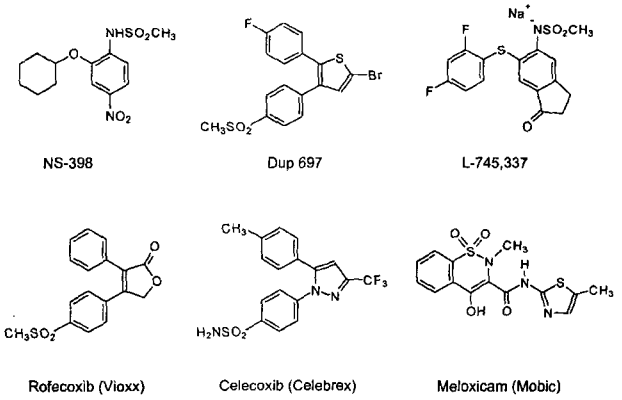
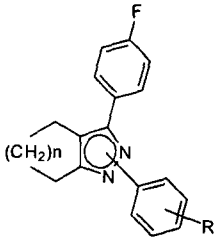


Fig. 1. Representative COX-2 inhibitors

Recent approval of the celecoxib and meloxicam



n = 1, 2.

R = halogens, OCH_3, SO_2NH_2.

Fig. 2. Disubstituted 4,5-polymethylenepyrazoles

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for clinical use spurred us to design and synthesize new candidates. We herein described design and synthesis as well as biological activity of disubstituted 4,5-polymethylenepyrazoles as potential anti-inflammatory agents.

MATERIALS AND METHODS

Experimental

Melting points were determined using a Fischer-Jones melting points apparatus and were not corrected. Infrared (IR) spectra were obtained using a Perkin-Elmer 1330 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker-250 spectrometer 250 MHz for ^{1}H NMR and 62.5 MHz for ^{13}C NMR and were reported as parts per million (ppm) from the internal standard tetramethylsilane (TMS). Chemicals and solvents were commercial reagents grade and used without further purification. Elemental analyses were taken on a Hewlett-Packard Model 185B elemental analyzer. The starting 2-acylcycloalkanones **1** (Szmuszkovicz and Skaletzky, 1967) were prepared by either previously reported method or modification of such a method.

General synthetic method for 1,3- and 2,3-diaryl-4,5-polymethylenepyrazole

To a solution of 2-acylcycloalkanone in dry methanol (30 mL) was slowly added 1.1-1.2 equivalent of (substituted)phenylhydrazine hydrate or its HCl salt. The resulting mixture was stirred for 12 h and concentrated to remove water formed by forming azeotrope with methanol. The resulting solid was either recrystallized from CH_2Cl_2: petroleum ether (1 : 3) or chromatographed on silica gel to afford two isomeric diaryl-4,5-polymethylenepyrazoles.

1- And 2- phenyl-3-(4-fluorophenyl)- 4,5 -trimethylenepyrazole (2aa/3aa)

The crude product from 2.0 g (9.7 mmol) of 2-(4-fluorobenzoyl)cyclopentanone and 2 mL of 98% phenylhydrazine hydrate was chromatographed on silica gel eluting with *n*-hexane: CH_2Cl_2 (3 : 7). The early fractions (Rf=0.69) afforded **2aa** as white needles (1.70 g, 63%): mp 148-153°C IR (KBr) υ 3055, 2951, 2854, 1597, 1506, 1363, 1219, 839, 754 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (dd, *J*=8.8, 5.5 Hz, 2H), 7.61 (dm, *J* =7.6 Hz, 2H), 7.34 (tm, J=8.0 Hz, 2H), 7.14 (tm, J=8.0 Hz, 1H), 7.00 (dd, *J*=8.8, 8.8 Hz, 2H), 2.93 (t, 2H, *J*=7.2 Hz), 2.79 (t, *J*=6.9 Hz, 2H), 2.59 (quintet, *J*=7.2 Hz, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 162.8 (d, $^1J_{C\text{-}F}$=246.5 Hz), 150.4, 145.3, 140.8, 130.4 (d, $^4J_{C\text{-}F}$=3.1 Hz), 129.7, 128.0 (d, $^3J_{C\text{-}F}$=8.1 Hz), 127.1, 125.9, 119.4, 115.9 (d, $^2J_{C\text{-}F}$=21.6 Hz), 31.4, 26.9, 24.6. Anal. Calcd. for $C_{18}H_{15}N_2F$, C: 77.68, H : 5.43, N : 10.06. Found C : 77.71, H : 5.43, N : 10.09. The latter fractions (Rf=0.19) afforded **3aa** as white needles (0.43 g, 16%): mp 77-79°C IR (KBr) υ 3068, 2953, 2850, 1595, 1510, 1219, 1157, 978, 833, 770 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.25 (m, 3H), 7.10 (dd, *J*=8.8, 5.5 Hz, 2H), 6.98 (dd, *J*=8.8, 8.8 Hz, 2H), 2.76 (t, *J*=7.2 Hz, 2H), 2.69 (t, *J*=6.9 Hz, 2H), 2.41 (quintet, *J*=7.2 Hz, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 162.7 (d, $^1J_{C\text{-}F}$=248 Hz), 162.7, 141.1, 135.6, 130.6 (d, $^4J_{C\text{-}F}$= 5.0 Hz), 129.3, 127.5 (d, $^3J_{C\text{-}F}$=8.5 Hz), 127.3, 127.0, 125.5, 115.9 (d, $^2J_{C\text{-}F}$=21.7 Hz), 30.3, 25.2, 24.1. Anal. Cald. for $C_{18}H_{15}N_2F$, C : 77.68, H : 5.43, N : 10.06. Found C: 77.71, H: 5.43, N : 10.08.

1-And 2-(4-methoxyphenyl)-3-(4-fluorophenyl)-4,5-trimethylenepyrazole (2ab/3ab)

The crude product from 1.0 g (4.9 mmol) of 2-(4-fluorobenzoyl)cyclopentanone and 0.9 g (4.9 mmol) of 4-methoxyphenylhydrazine HCl was chromatographed on silica gel eluting with *n*-hexane: CH_2Cl_2 (3:7). The early fractions (Rf=0.76) afforded **2ab** as white needles (0.18 g, 22%): mp 112-114°C. IR (KBr) υ 3035, 2943, 2846, 1518, 1250, 1225, 1028, 856, 833. cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (dd, *J*=8.8, 5.5 Hz, 2H), 7.59 (dm, 2H, *J*=9.1 Hz), 7.08 (dd, *J*=8.8, 8.8 Hz, 2H), 6.95 (dm, *J*=9.1 Hz, 2H), 3.83 (s, 3H), 2.97 (t, *J*=7.1 Hz, 2H), 2.88 (t, *J*=7.0 Hz, 2H), 2.68 (quintet, *J*=7.3 H, 2Hz). ^{13}C NMR (75.5 MHz, $CDCl_3$) 162.7 (d, $^1J_{C\text{-}F}$=246.5 Hz), 158.0, 150.2, 134.5, 130.4 (d, $^4J_{C\text{-}F}$=3.1 Hz), 127.9 (d, $^3J_{C\text{-}F}$=8.0 Hz), 126.5, 121.2, 114.8, 115.8 (d, $^2J_{C\text{-}F}$=21.6 Hz), 55.9, 31.5, 26.5, 24.7. Anal. Calcd. for $C_{19}H_{17}N_2OF$, C : 74.01, H: 5.56, N: 9.08. Found C : 74.01, H : 5.58, N : 9.11. The latter fractions (Rf=0.49) afforded **3ab** as white needles (0.29 g, 34%): mp 92-95°C. IR (KBr) υ 2960, 2854, 1730, 1518, 1252, 1217, 1036, 839, 723 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 (dm, *J*=8.9 Hz, 2H), 7.13 (dm, *J*=8.8 Hz, 2H), 6.94 (dd, *J*=8.8, 8.9 Hz, 2H), 6.82 (dm, *J* =8.8 Hz, 2H), 3.76 (s, 3H), 2.80 (t, *J*=7.3 Hz, 2H), 2.74 (t, *J*=7.0 Hz, 2H), 2.46 (quintet, *J*=7.3 Hz, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 162.4 (d, $^1J_{C\text{-}F}$=248 Hz), 162.2, 158.9, 135.6, 134.5, 130.6 (d, $^3J_{C\text{-}F}$=8.1 Hz), 127.5 (d, $^4J_{C\text{-}F}$=3.4 Hz), 126.9, 126.4, 115.9 (d, $^2J_{C\text{-}F}$=21.7 Hz), 55.8, 30.3, 25.2, 24.2. Anal. Calcd. for $C_{19}H_{17}N_2OF$, C: 74.01, H: 5.56, N: 9.08. Found C: 74.00, H: 5.53, N: 9.10.

1- And 2-(2-fluorophenyl)-3-(4-fluorophenyl)-4,5-trimethylenepyrazole (2ac/3ac)

The crude product from 1.0 g (4.9 mmol) of 2-(4-fluorobenzoyl) cyclopentanone and 0.8 g (4.9 mmol) of 2-fluorophenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: CH_2Cl_2 (3 : 7). The early fractions (Rf=0.60) afforded **2ac** as a yellow liquid (0.12 g, 10%). IR (KBr) υ 2924, 1734, 1614, 1514, 1464, 1223, 1153, 1070, 835, 750 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.74 (dd, *J*=8.7, 5.4 Hz, 2H), 7.65 (td, *J*=7.8, 2.2 Hz, 1H),

7.26-7.10 (m, 3H), 7.02 (dd, J=8.7, 8.7 2H), 2.86 (t, J=6.8 Hz, 2H), 2.77 (t, J=6.7 Hz, 2H), 2.59 (quintet, J 6.6 Hz, 2H). Anal. Calcd. for $C_{18}H_{14}N_2F_2$, C: 73.71, H: 4.81, N: 9.55. Found C: 73.70, H: 4.81, N: 9.56. The latter fractions (Rf=0.27) afforded **3ac** as pale yellow needles (0.83 g, 57%): mp 101-103°C. IR (KBr) υ 3043, 2962, 1597, 1514, 1464, 1225, 1165, 976, 852, 762 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.37 (td, J=7.6, 1.8 Hz, 1H), 7.29-7.20 (m, 1H), 7.15-6.96 (m, 4H), 6.88 (dd, J= 8.7, 8.7 2H), 2.77 (t, J=7.3 Hz, 2H), 2.72 (t, J=7.1 Hz, 2H), 2.42 (quintet, J=7.0 Hz, 2H). Anal. Calcd. for $C_{18}H_{14}N_2F_2$, C: 73.71, H: 4.81, N: 9.55. Found C: 73.69, H: 4.82, N: 9.54.

1- And 2-(3-fluorophenyl)-3-(4-fluorophenyl)-4,5-trimethylenepyrazole (2ad/3ad)

The crude product from 1.0 g (9.7 mmol) of 2-(4-fluorobenzoyl)cyclopentanone and 0.8 g (4.9 mmol) of 3-fluorophenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: CH_2Cl_2 (3:7). The early fractions (Rf=0.82) afforded **2ad** as yellow needles (0.12 g, 8%): mp 110-111°C. IR (KBr) υ 3076, 2941, 2864, 1737, 1601, 1269, 1120, 851, 760 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.76 (dd, J=8.8, 5.4 Hz, 2H), 7.45-7.27 (m, 3H), 7.04 (dd, J=8.8, 8.8 2H), 7.00-6.83 (m, 1H), 3.00 (t, J=7.0 Hz, 2H), 2.83 (t, J =6.7 Hz, 2H), 2.65 (quintet, J=6.7 Hz, 2H). Anal. Calcd. for $C_{18}H_{14}N_2F_2$, C: 73.71, H: 4.81, N: 9.55. Found C: 73.72, H: 4.80, N: 9.53. The latter fractions (Rf=0.40) afforded **3ad** as pale yellow liquid (0.79 g, 55%). IR (KBr) υ 3068, 2958, 2854, 1610, 1516, 1450, 1228, 839, 781 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.21-7.15 (m, 1H), 7.10 (dd, J=8.7, 5.3 Hz, 2H), 6.99-9.87 (m, 5H), 2.75 (t, J=7.3 Hz, 2H), 2.68 (t, J=7.0 Hz, 2H), 2.41(quintet, J=7.0 Hz, 2H). Anal. Calcd. for $C_{18}H_{14}N_2F_2$, C: 73.71, H: 4.781, N: 9.55. Found C:73.73, H: 4.82, N: 9.56.

1- And 2-(4-fluorophenyl)-3-(4-fluorophenyl)-4,5-trimethylenepyrazole (2ae/3ae)

The crude product from 1.0 g (9.7 mmol) of 2-(4-fluorobenzoyl)cyclopentanone and 0.8 g (4.9 mmol) of 4-fluorophenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: CH_2Cl_2 (3:7). The early fractions (Rf=0.78) afforded **2ae** as white needles (0.15g, 11%): mp 169-170°C. IR (KBr) υ 2922, 2856, 1560, 1514, 1448, 1217, 1090, 833, 777 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 7.82 (dd, J=8.8, 5.5 Hz, 2H), 7.66 (dd, J=8.8, 5.5 Hz, 2H), 7.10 (overlapped dd, J=8.8, 8.8 Hz, 4H), 3.01 (t, J=7.1 Hz, 2H), 2.90 (t, J=7.0 Hz, 2H), 2.70 (quintet, J=7.3 Hz, 2H). Anal. Calcd. for $C_{18}H_{14}N_2F_2$, C: 73.71, H: 4.81, N: 9.55. Found C: 73.73, H: 4.82, N: 9.54. The latter fractions (Rf=0.19) afforded **3ae** as pale yellow needles (0.82 g, 56%): mp 61-63°C. IR (KBr) υ 2947, 2845, 1726, 1597, 1508, 1225, 972, 833, 730 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 7.26 (dd, J=8.8, 5.5 Hz, 2H), 7.15 (dd, J=8.8, 5.5 Hz, 2H), 7.04-6.97 (m, 4H), 2.83 (t, J=7.3 Hz, 2H), 2.76 (t, J=7.0 Hz, 2H), 2.49 (quintet, J=7.3 Hz, 2H). Anal. Calcd. for $C_{18}H_{14}N_2F_2$, C: 73.71, H: 4.81, N: 9.55. Found C: 73.69, H: 4.79, N: 9.54.

2-(2-Bromophenyl)-3-(4-fluorophenyl)-4,5-trimethylenepyrazole (3af)

The crude product from 1.0 g (9.7 mmol) of 2-(4-fluorobenzoyl)cyclopentanone and 1.1 g (4.9 mmol) of 2-bromophenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: CH_2Cl_2 (3:7). The eluents (Rf=0.16) afforded only **3af** as pale yellow needles (1.2 g, 66%): mp 110-112°C. IR (KBr) υ 2940, 2850, 1593, 1512, 1490, 1432, 1361, 1228, 1155, 1093, 1033, 973, 833, 771 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, J=7.5 Hz, 1H), 7.38-7.34 (m, 2H), 7.28-7.23 (m, 1H), 7.11 (dd, J=8.8, 5.4 Hz, 2H), 6.92 (dd, J=8.8, Hz, 2H), 2.86 (overlapped t, J=6.4 Hz, 2H), 2.83 (t, J=6.4 Hz, 2H), 2.52 (quintet, J=6.4 Hz, 2H). Anal. Calcd. for $C_{18}H_{14}N_2BrF$, C: 60.52, H: 3.95, N: 7.84. Found C: 60.51, H: 3.93, N: 7.85.

2-(3-Bromophenyl)-3-(4-fluorophenyl)-4,5-trimethylenepyrazole (3ag)

The crude product from 1.0 g (9.7 mmol) of 2-(4-fluorobenzoyl)cyclopentanone and 1.1 g (4.9 mmol) of 3-bromophenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: CH_2Cl_2 (3:7). The eluents (Rf=0.31) afforded only **3ag** as pale yellow needles (1.23 g, 70%): mp 105-106°C. IR (KBr) υ 2962, 1587, 1511, 1423, 1452, 1219, 1160, 856, 779 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 7.56 (dd, J=1.6, 1.5, 1H), 7.38 (ddd, J=7.64, 1.5, 1.3 Hz, 1H), 7.19-7.14 (m, 3H), 7.10 (dd, 3.5, 1.2 Hz, 1H), 7.09-6.99 (m, 3H), 2.83 (t, J=6.0 Hz, 2H), 2.76 (t, J=6.1 Hz, 2H), 2.49 (quintet, J=6.1 Hz, 2H). Anal. Calcd. for $C_{18}H_{14}N_2BrF$, C: 60.52, H: 3.95, N: 7.84. Found C: 60.53, H: 3.92, N: 7.86.

2-(4-Bromophenyl)-3-(4-fluorophenyl)-4,5-trimethylenepyrazole (3ah)

The crude product from 1.0 g (9.7 mmol) of 2-(4-fluorobenzoyl)cyclopentanone and 1.1 g (4.9 mmol) of 3-bromophenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: CH_2Cl_2 (3:7). The eluents (Rf=0.49) afforded only **3ah** as pale yellow needles (1.25 g, 72%): mp 121-123°C. IR (KBr) υ 3070, 2940, 2846, 1589, 1498, 1448, 1223, 1155, 1064, 1007, 974, 825, 735 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 7.44 (ddd, J=8.6, 2.0, 1.8, 2H), 7.19-7.13 (m, 4H), 7.06-6.99 (m 2H), 2.83 (t, J=7.0 Hz, 2H), 2.76 (t, J=7.5 Hz, 2H), 2.49 (quintet, J=7.5 Hz, 2H). Anal. Calcd. for $C_{18}H_{14}N_2BrF$, C: 60.52, H: 3.95,

N: 7.84. Found C: 60.52, H: 3.94, N: 7.85.

3-(4-Fluorophenyl)-1-(4-Sulfamoylphenyl)-4,5-trimethylenepyrazole (2ai)

The crude product from 1.0 g (9.7 mmol) of 2-(4-fluorobenzoyl)cyclopentanone and 0.9 g (4.9 mmol) of 4-sulfamoylphenylhydrazine HCl salt was chromatographed on silica gel eluting with EtOAc. The eluents (Rf= 0.48) afforded only **2ai** as pale yellow needles (1.2 g, 66%): mp 214-215°C. IR (KBr) υ 3292, 3074, 2926, 1595, 1516, 1344, 1159, 1095, 843cm^{-1}. ^{1}H NMR (250 MHz, CD_3CN) 7.70 (d, J=8.8, 2.2 Hz, 2H), 7.31 (dt, J=8.8, 2.2 Hz, 2H), 7.19 (dd, J=8.9, 5.4 Hz, 2H), 7.04 (dd, J=8.9, 8.9 Hz, 2H), 5.64 (br. s, NH_2), 2.70 (t, J=7.4 Hz, 3H), 2.66 (t, J=7.3 Hz, 2H), 2.39 (quintet, J=7.0 Hz, 2H). Anal. Calcd. for $C_{18}H_{16}N_3FO_2S$, C: 60.49, H: 4.51, N: 11.76. Found C: 60.52, H: 4.53, N: 11.78.

3-(4-Fluorophenyl)-1-phenyl-4,5-tetramethylenepyrazole and 3-(4-fluorophenyl)-2-phenyl-4,5-tetramethylenepyrazole (2ba/3ba)

The crude product from 1.92 g (9.7 mmol) of 2-(4-fluorobenzoyl)cyclohexanone and 2 mL of 98% phenylhydrazinehydrate was chromatographed on silica gel eluting with *n*-hexane: CH_2Cl_2 (3:7). The early fractions (Rf=0.78) afforded **2ba** as white needles (0.19g, 8%): mp 108-109°C. IR (KBr) υ 2952, 2839, 1597, 1502, 1363, 1221, 1155, 962, 836, 756, 690 cm^{-1}. ^{1}H NMR(300 MHz, $CDCl_3$) δ 7.74 (dd, J=8.8, 5.5 Hz, 2H), 7.63 (dm, J=7.6 Hz, 2H), 7.32 (tm, J=8.0 Hz, 2H), 7.15 (tm, J=8.0 Hz, 1H), 7.00 (dd, J=8.8, 8.8 Hz, 2H), 2.81(t, J 5.0 Hz, 2H), 2.57(t, J=5.4 Hz 2H), 1.94-1.86(m, 2H), 1.83-1.75(m, 2H). Anal. Calcd. for $C_{19}H_{17}N_2F$, C: 78.06, H: 5.86, N: 9.58. Found C: 78.08, H: 5.84, N: 9.57. The latter fractions (Rf= 0.16) afforded **3ba** as pale yellow needles: mp 117-118°C. IR(KBr) υ 3059, 2926, 2839, 1597, 1502, 1363, 1221, 839, 758 cm^{-1}. ^{1}H NMR(250 MHz, $CDCl_3$) δ 7.35-7.24(m 5H), 7.18(dd, J=8.7, 5.4 Hz, 2H), 7.04(dm, J =8.7 Hz, 2H), 2.84(t, J=6.1 Hz, 2H), 2.60(t, J=6.2 Hz, 2H), 1.97-1.88(m, 2H), 1.86-1.77(m, 2H). Anal. Calcd. for $C_{19}H_{17}N_2F$, C: 78.06, H: 5.86, N: 9.58. Found C: 78.09, H: 5.84, N: 9.57.

3-(4-Fluorophenyl)-1-(4-methoxyphenyl)-4,5-tetramethylenepyrazole and 3-(4-fluorophenyl)-2-(4-methoxyphenyl)-4,5-tetramethylenepyrazole (2bb/3bb)

The crude product from 1.92 g (8.7 mmol) of 2-(4-fluorobenzoyl)cyclohexanone and 1.8 g (9.8 mmol) of 4-methoxyphenylhydrazine HCl salt was chromatographed on silica gel eluting with *n*-hexane: EtOAc (4:1). The early fractions (Rf=0.71) afforded **2bb** as white needles (0.73 g, 26%): mp 110-113°C. IR (KBr) υ 2933, 2854, 1729, 1518, 1443, 1217, 1099, 833, 727 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (dd, J=8.8, 5.5 Hz, 2H), 7.37 (dm, J=9.1 Hz, 2H), 7.03 (dd, J=8.8, 8.8 Hz, 2H), 6.90 (dm, 2H, J=9.1 Hz), 3.78 (s, 3H), 2.70 (t, J=7.1 Hz, 2H), 2.62 (t, J=7.0 Hz, 2H), 1.77 (br. s, 4H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 162.5 (d, $^1J_{C\text{-}F}$=246.2 Hz), 159.0, 148.1, 140.3, 133.6, 130.8 (d, $^4J_{C\text{-}F}$=3.1 Hz), 129.9 (d, $^3J_{C\text{-}F}$=7.9 Hz), 125.5, 115.7 (d, $^2J_{C\text{-}F}$=21.4 Hz), 115.1, 114.6, 56.0, 23.9, 23.5, 23.1, 22.9. Anal. Calcd. for $C_{20}H_{19}N_2OF$, C: 74.51, H: 5.94, N: 8.69. Found C: 74.53, H: 5.91, N: 8.68. The latter fractions (Rf=0.50) afforded **3bb** as pale yellow needles (0.86 g, 31%): mp 55-56°C. IR (KBr) υ 2933, 2046, 1601, 1516, 1443, 1248, 1155, 1026, 833, 727 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19-7.10 (m, 2H), 6.94 (dd, J=8.8 Hz, 8.9 Hz, 2H), 6.75 (dm, J=8.8 Hz, 2H), 3.73 (s, 3H), 2.73 (t, J=7.3 Hz, 2H), 2.50 (t, J=7.0 Hz, 2H), 1.85-1.79 (m, 2H), 1.78-1.68 (m, 2H). Anal. Calcd. for $C_{20}H_{19}N_2OF$, C: 74.51, H: 5.94, N: 8.69. Found C: 74.54, H: 5.92, N: 8.67.

1- And 2-(2-fluorophenyl)-3-(4-fluorophenyl)-4,5-tetramethylenepyrazole (2bc/3bc)

The crude product from 1.92 g (8.7 mmol) of 2-(4-fluorobenzoyl)cyclohexanone and 1.6 g (9.8 mmol) of 2-fluorophenylhydrazineHCl was chromatographed on silica eluting with *n*-hexane: EtOAc (4:1). The early fractions (Rf=0.75) afforded **2bc** as white needles (0.30 g, 11%) as pale yellow liquid. IR(KBr) 3076, 2941, 2864, 1736, 1601, 1269, 1120, 850, 760 cm^{-1}. ^{1}H NMR(250 MHz, $CDCl_3$) δ 7.79-7.67 (m, 2H) 7.28-7.22(m, 2H), 7.56(dd, J=8.9, 5.4 Hz, 1H), 7.19(t, J= 8.5 Hz, 1H), 7.16-7.09(m, 2H), 7.02(dd, J=8.8 Hz, 1H), 6.92-6.84(m, 3H), 2.70(s, 1H), 2.50(s, 1H), 2.41(t, J=6.2 Hz, 2H), 1.88-1.72(m, 4H). Anal. Calcd. for $C_{19}H_{16}N_2F_2$, C: 73.53, H: 5.20, N: 9.03. Found C: 73.56, H: 5.19, N: 9.05. The latter fractions (Rf= 0.53) afforded **3bc** as pale yellow liquid (1.46 g, 54%). IR (KBr) 3059, 2926, 2858, 1610, 1512, 1360, 1215, 867, 781 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.35 (td, J =7.6, 1.8 Hz, 1H), 7.26-7.18 (m, 1H), 7.13-7.03 (m, 3H), 6.99-6.86 (m, 3H), 2.73 (t, J=6.2 Hz, 2H), 2.53 (t, J=6.2 Hz, 2H), 1.88-1.78 (m, 2H), 1.77-1.68 (m, 2H). Anal. Calcd. for $C_{19}H_{16}N_2F_2$, C: 73.53, H: 5.20, N: 9.03. Found C: 73.56, H: 5.17, N: 9.06.

1- And 2-(3-fluorophenyl)-3-(4-fluorophenyl)-4,5-tetramethylenepyrazole (2bd/3bd)

The crude product from 1.92 g (8.7 mmol) of 2-(4-fluorobenzoyl)cyclohexanone and 1.6 g (9.8 mmol) of 3-fluorophenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: EtOAc (4 : 1). The early fractions (Rf=0.75) afforded **2bd** as pale yellow liquid (0.28 g, 10%). IR (KBr) u 3076, 2941, 2864, 1736, 1601, 1269, 1120, 851, 760 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.76 (dd, J=8.1, 5.7 Hz, 2H), 7.56 (dd, J=8.3, 5.6 Hz, 2H), 6.95-6.84 (m, 4H), 2.41 (t, J= 5.9 Hz, 4H), 1.87-1.70

(m, 4H). Anal. Calcd. for $C_{19}H_{16}N_2F_2$, C: 73.53, H: 5.20, N: 9.03. Found C: 73.55, H: 5.17, N: 9.04. The latter factions (Rf=0.83) afforded **3bd** as white needles (1.40 g, 50%): mp 112-113°C. IR (KBr) υ 3059, 2926, 2858, 1610, 1512, 1400, 1215, 847, 781 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.15(t, *J*=8.2 Hz, 1H), 7.10(dd, *J*=8.9, 5.3 Hz, 2H), 7.00(dd, *J*=8.8, 8.8 Hz, 2H), 7.00 (overlapped s, 1H), 6.90-6.80 (m, 2H), 2.72 (t, *J*=6.3 Hz, 2H), 2.48 (t, *J*=6.2 Hz, 2H), 1.85-1.77 (m, 2H), 1.75-1.68 (m, 2H). Anal. Calcd. for $C_{19}H_{16}N_2F_2$, C: 73.53, H: 5.20, N: 9.03. Found C: 73.55, H: 5.19, N: 9.04.

1-And 2-(4-fluorophenyl)-3-(4-fluorophenyl)-4,5-tetramethylenepyrazole (2be/3be)

The crude product from 1.92 g (8.7 mmol) of 2-(4-fluorobenzoyl)cyclohexanone and 1.6 g (9.8 mmol) of 4-fluorophenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: EtOAc (4:1). The early fractions (Rf=0.73) afforded **2bc** as pale yellow needles (0.44 g, 16%). IR (KBr) υ 2943, 2846, 1512, 1325, 1217, 1111, 995, 839, 729 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.13 (dd, *J*=9.0, 5.0 Hz, 2H), 7.07 (dd, *J*=9.3, 5.4 Hz, 2H), 6.95 (dd, *J*=9.2, 9.2 Hz, 2H), 6.91 (dd, *J*=8.7, 8.7 Hz, 2H), 2.73 (t, *J*=6.3 Hz, 2H), 2.50 (t, *J*=6.1 Hz, 2H), 1.87-1.79 (m, 2H), 1.73-1.66 (m, 2H). Anal. Calcd. for $C_{19}H_{16}N_2F_2$, C: 73.53, H: 5.20, N: 9.03. Found C: 73.55, H: 5.18, N: 9.06. The latter fractions (Rf=0.70) afforded **3be** as white needles (1.40 g, 50%): mp 136-139°C. IR (KBr) υ 3055, 2935, 2854, 1512, 1369, 1217, 1155, 852, 820 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.25.-7.12 (m, 4H), 7.08-6.96 (m, 4H), 2.82 (t, *J*=6.2 Hz, 2H), 2.59 (t, *J*=6.0 Hz, 2H), 1.97-1.87 (m, 2H), 1.85-1.79 (m, 2H). Anal. Calcd. for $C_{19}H_{16}N_2F_2$, C: 73.53, H: 5.20, N: 9.03. Found C: 73.56, H: 5.17, N: 9.05.

2-(2-Bromophenyl)-3-(4-fluorophenyl)-4,5-tetramethylenepyrazole (3bf)

The crude product from 0.96 g (4.4 mmol) of 2-(4-fluorobenzoyl)cyclohexanone and 1.1 g (4.9 mmol) of 2-bromophenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: EtOAc (4:1). The eluents (Rf=0.49) afforded only **3bf** as pale yellow liquid (1.05 g, 64%). IR (KBr) υ 3062, 2933, 2854, 1591, 1512, 1489, 1228, 839, 748 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.49 (dd, *J*=7.3 Hz, 1H), 7.28-7.22 (m, 2H), 7.16-7.09 (td, *J*=7.3, 1.8 Hz, 1H), 7.04 (dd, *J*=8.7, 5.4 Hz, 2H), 6.86 (dd, *J*=8.7, 8.7 Hz, 2H), 2.72 (t, *J*=6.0 Hz, 3H), 2.54 (t, *J*=6.0 Hz, 2H), 1.87-1.78 (m, 2H), 1.74-1.69 (m, 2H). Anal. Calcd. for $C_{19}H_{16}N_2BrF$, C: 61.47, H: 4.35, N: 7.55. Found C: 61.44, H: 4.33, N: 7.53.

2-(3-Bromophenyl)-3-(4-fluorophenyl)-4,5-tetramethylenepyrazole (3bg)

The crude product from 0.96 g (4.4 mmol) of 2-(4-fluorobenzoyl)cyclohexanone and 1.1 g (4.9 mmol) of 3-bromophenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: EtOAc (4:1). The eluents (Rf=0.76) afforded only **3bg** as pale yellow needles (1.00 g, 61%): mp 164-165°C. IR (KBr) υ 3057, 2924, 2856, 1734, 1589, 1483, 1219, 849, 779 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.35 (d, *J*=7.5 Hz, 1H), 7.15 (dd, *J*=8.7, 5.4 Hz, 2H), 710-7.01 (m, 4H), 2.80 (t, *J*=6.0 Hz, 3H), 2.55 (t, *J*=6.0 Hz, 2H), 1.92-1.85 (m, 2H), 1.82-1.78 (m, 2H). Anal. Calcd. for $C_{19}H_{16}N_2BFr$, C: 61.47, H: 4.35, N: 7.55. Found C: 61.45, H: 4.32, N: 7.54.

2-(4-Bromophenyl)-3-(4-fluorophenyl)-4,5-tetramethylenepyrazole (3bh)

The crude product from 0.96 g (4.4 mmol) of 2-(4-fluorobenzoyl)cyclohexanone and 1.1 g (4.9 mmol) of 4-bromophenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: EtOAc (4:1). The eluents (Rf=0.76) afforded only **3bh** as pale yellow needles (1.05 g, 64%): mp 134-136°C. IR (KBr) υ 3095, 2918, 2856, 1587, 1490, 1223, 974, 831 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.43 (dd, *J*=8.8, 2.8 Hz, 2H), 7.22-7.02 (m, 6H), 2.82 (t, *J*=6.0 Hz, 3H), 2.57 (t, *J*=6.0 Hz, 2H), 1.97-1.87 (m, 2H), 1.84-1.69 (m, 2H). Anal. Calcd. for $C_{19}H_{16}N_2BrF$, C: 61.47, H: 4.35, N: 7.55. Found C: 61.45, H: 4.32, N: 7.55.

3 - (4-Fluorophenyl) -1-(4-sulfamoylphenyl)-4,5-tetramethylenepyrazole (2bi)

The crude product from 1.92 g (8.7 mmol) of 2-(4-fluorobenzoyl)cyclohexanone and 1.8 g (9.8 mmol) of 4-sulfamoylphenylhydrazineHCl was chromatographed on silica gel eluting with *n*-hexane: EtOAc (4:1). The eluents (Rf=0.70) only afforded **2bi** as pale yellow needles (2.0 g, 62%): mp 198-200°C. IR (KBr) υ 3304, 3070, 2939, 1595, 1514, 1342, 1159, 843, 723 cm^{-1}. ^1H NMR (250 MHz, CD_3CN) 7.68 (dm, *J*=8.8 Hz, 2H), 7.27 (dm, *J*=8.8 Hz, 2H), 7.18 (dd, *J*=8.7, 5.4 Hz, 2H), 7.05 (dm, *J*=8.7 Hz, 2H), 5.52 (br. s, NH_2), 2.70 (t, *J*=6.0 Hz, 3H), 2.43 (t, *J*=6.0 Hz, 2H), 1.93-1.78 (m, 2H), 1.74-1.59 (m, 2H). Anal. Calcd. for $C_{19}H_{18}N_3O_2FS$, C: 61.44, H: 4.88, N: 11.31. Found C: 61.43, H: 4.86, N: 11.32.

Preparation and activation of bone marrow-derived mast cells (BMMC)

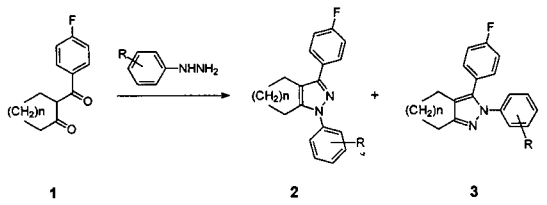
Bone marrow cells from male *Balb/cJ* mice were cultured for up to 10 weeks in 50% enriched medium (RPMI 1640 containing 2 mM L-glutamine, 0.1 mM nonessential amino acids, antibiotics and 10% fetal calf serum) and 50% WEHI-3 cell conditioned medium as a source of IL-3. After 3 weeks, over 98% of the cells were found to be BMMC checked by the previously described procedure (Murakami, *et al*, 1994, 1995). For

measuring inhibitory activity of the compounds on COX-2, cells suspended at a cell density of 5×10^5 cells/mL in enriched medium were preincubated with aspirin (10/mL) for 2 h in order to irreversibly inactivate pre-existing COX-1. After washing, BMMC were activated with KL (100 ng/mL), IL-10 (100 U/mL) and LPS (10 μg /mL) at 37°C for 8 h in the presence or absence of compound previously dissolved in DMSO. For measuring COX-1 activity, cells without aspirin pretreatment were incubated at 37°C for 2 h with activators. All reactions were stopped by centrifugation at 120 g at 4°C for 5 min. The supernatant was stored at -80°C for COX-1 or COX-2-dependent PGD_2 analysis. Concentrations of PGD_2 in the supernatant were measured using PGD_2 assay kit (Amersham, Buckin-hamshire, UK). Under the conditions employed, COX-1 and COX-2-dependent phases of PGD_2 generation reached 1.5 ng and 6 ng/ 10^6 cells, respectively (Moon, *et al*, 1998). All data were the arithmetic mean of triplicate determinations.

RESULTS AND DISCUSSION

Chemistry and properties

Reactions of 2-acylcycloalkanone with (substituted)-phenylhydrazine hydrate or its HCl salt afforded N1-isomers (**2**) and N2-isomers (**3**) in a ratio of 1:6 to 4:1, respectively. Interestingly, such a product distribution was not observed in the reactions of bromophenyl-hydrazines where N2-isomers (**3af**, **ag**, **ah**, **bf**, **bg**, and **bh**) were the only products while the reactions of 4-sulfamoyl-phenylhydrazineHCl afforded N1-isomers (**2ai**, and **2bi**) as an only product, respectively.



1 **2** **3**

aa n = 1, R = H; **ab** n = 1, R = OCH_3; **ac** n = 1, R = 2-F; **ad** n = 1, R = 3-F; **ae** n = 1, R = 4-F; **af** n = 1, R = 2-Br; **ag** n = 1, R = 3-Br; **ah** n = 1, R = 4-Br; **ai** n = 1, R = SO_2NH_2; **ba** n = 2, R = H, **bb** n = 2, R = OCH_3; **bc** n = 1, R = 2-F; **bd** n = 2, R = 3-F; **be** n = 2, R = 4-F; **bf** n = 2, R = 2-Br; **bg** n = 2, R = 3-Br; **bh** n = 2, R = 4-Br; **bi** n = 2, R = SO_2NH_2

Scheme 1. Synthesis of designed compounds **2** and **3**

Each regioisomer was readily separated by column chromatography and assigned by NMR. In N1-isomers, the proton resonances of each phenyl ring were well-separated and assigned by COSY experiment. In addition, 4.7% of NOE effect was observed between *peri*-H (H6) of the annulated cyclopentene ring and *ortho*-H of the N1-phenyl group in **2aa**, but not in **3aa**. Similar NOE effects (4-6%) were observed in N1-isomers between the two corresponding H's (Kim and Jahng, 1999). On the other hand, two phenyl rings in N2-isomers are close enough to magnetically influence each other thus showing overlapped proton resonances. One of the *ortho*-H's of the each phenyl ring also points toward the shielding region of the neighboring phenyl ring (Kim, 1999), are thus upfield-shifted about 0.5-1.0 ppm compared to those of N1-isomers.

Table I. Inhibitory activity of disubstituted 4,5-polymethylenepyrazoles on COX-2

Compds	COX-2 inhibition(%)[a]	IC_{50} on COX-2 (μM)	Selectivity[c]	Compds	COX-2 inhibition(%)[a]	IC_{50} on COX-2 (μM)	Selectivity[c]
2aa	100	0.27	0.3	2ba	71.3	0.45	
2ab	83.3			2bb	90.0[b]		
2ac	67.1			2bc	62.3		
2ad	47.4			2bd	72.4		
2ae	89.0[b]			2be	68.4		
2af	72.4			2bf	75.8		
3aa	100	0.0003	16.2	3ba	82.8	0.05	5.7
3ab	100[b]	0.07	3.0	3bb	84.0[b]		
3ac	77.0			3bc	84.9		
3ad	83.3			3bd	83.9		
3ae	90.0[b]			3be	81.6		
3af	78.0[b]			3bf	82.2		
3ag	77.0[b]			3bg	77.0		
3ah	76.0[b]			NS-398		3.8	> 26

[a]Data were taken at the concentration of 1.0 μg/mL. [b]Data were taken at the concentration of 2.5 μg/mL. [c]The ratio of IC_{50} on COX-1 and that of COX-2

Biological properties

The inhibitory activity of the diaryl polymethylenepyrazoles on COX-2 was evaluated *in vitro* using cell lines that selectively produced one or the other enzyme, which are summarized in Table I. In general, 2,3-diaryl isomers have stronger inhibitory activity against COX-2 than the corresponding 1,3-isomers. It is worthy to note that 1,3-diaryl-4,5-polymethylenepyrazoles retained significant inhibitory activity against COX-2 (Jahng, 1999). Such an activity is somewhat surprising compared to the fact that most of the reported strong and selective COX-2 inhibitors possess 1,2-diaryl sub-stituents (Chang and Jahng, 1998, Giannangeli, et *al*, 1998). Further studies are required to clarify such a structure-activity relationship. The inhibitory activity decreases with the increase of the annulated ring size. The attempts to increase the ring size further were, thus, not pursued.

In conclusion, 2,3-and 1,3-diaryl-4,5-polymethylenepyrazoles showed significant inhibitory activity against COX-2 and were somewhat selective on COX-2 over COX-1. Regioselective synthesis of each isomer along with studies on variations of the substituents and substitution positions are in progress which will be due in the future.

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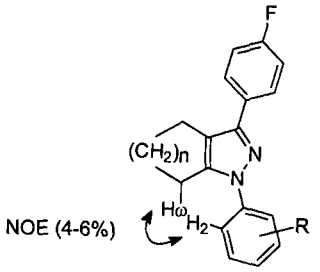
Kim, H. H., Jahng, Y. Unpublished results. The NOE's of the Hω of the annulated carbocycle and H2 of the N1-phenyl groups in compounds **2** were measured.



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