College of Pharmacy, Yeungnam University, Kyongsan, Korea

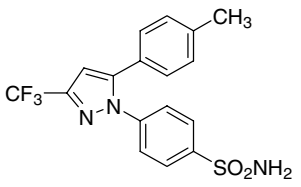
# Synthesis and biological activity of disubstituted 4,5-polymethylenepyrazoles as selective cyclooxygenase-2 inhibitors

J. W. Yu, H. H. Kim and Y. Jahng

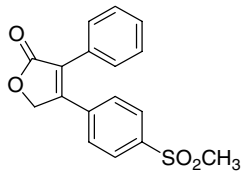
A series of 1,3- and 2,3-disubstituted 4,5-polymethylenepyrazole derivatives were prepared and their inhibitory activities on cyclooxygenase-2 were evaluated. Among the compounds prepared, 1,3-isomer, 3-cyclohexyl-1-(4-fluorophenyl) 4,5-trimethylenepyra-zole (**5be**) showed the most potent ($IC_{50} = 0.008$ μM) inhibitory activity with little selectivity (13-fold) on cyclooxygenase-2.

## 1. Introduction

Inflammation is mediated by prostaglandins (PGs), especially $PGE_2$ to cause pain, fever and swelling, and has long been a target to be controlled. Nonsteroidal antiinflammatory drugs (NSAIDs), for example aspirin, are the most widely used agents for the treatment or control of such symptoms [1, 2]. Some of these drugs have been in clinical use for over a century, but only recently was it established that these drugs reduce the pain and swelling of joints in acute or chronic inflammatory disorders by blocking the production of PGs *via* the cyclooxygenase pathway [3]. Additionally, the induction of gastrointestinal mucosal lesions, perforations, and bleeding in some patients and suppression of renal function of the patients with inflammatory conditions have been found to be due to the inhibition of PG production in the affected organs [4]. The discovery of a second, inducible isoform cyclooxygenase-2 (COX-2) that is a key enzyme in the arachidonic acid cascade and is associated with inflammation, has afforded a novel target for designing therapeutic agents that could effectively control inflammatory conditions with greatly reduced gastrointestinal and/or renal toxicity [5, 6]. Efforts in designing such a system have been concentrated on establishing selectivity on COX-2 with little or no activity on COX-1, a constitutive form which is associated with the protection of gastrointestinal tracts and the kidney. A number of classes of selective COX-2 inhibitors have emerged which include the substituted phenyl, the disubstituted five-membered (hetero)aryl ring, the (thio)phenoxy-indanone, and the disubstituted bicyclic rings as main anchors [7–16]. Among the compounds designed and synthesized as selective COX-2 inhibitors, celecoxib (**1**) [9], rofecoxib (**2**) [10], and valdecoxib (**3**) [11] have recently been launched on the market and some other candidates are under clinical trials. Structure-activity relationship studies on COX-2 inhibitors have afforded three major requirements for maximal activity and selectivity: i) a five-membered anchor, ii) two aromatic rings at adjacent positions, and iii) either $SO_2NH_2$ or $SO_2CH_3$ substitutent on the phenyl ring [12–17]. Computer-assisted docking mode studies on COX-1 and COX-2, however, suggest that one of the two aryl groups in COX-2 inhibitors could be replaced by saturated cycloalkyls [18] which are expected to interact with the Tyr385 residue, thus inactivating the enzyme. We describe here a synthesis and biological activity study of 1,3- and 2,3-disubstituted 4,5-polymethylenepyrazole derivatives in which a polymethylene bridge at the C4,C5-postion and a cyclohexyl group at the C3 position are expected to afford spatial, structural and lipophilic requirements for maximal interaction with the enzyme.




**1**

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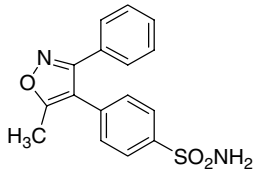



**3**

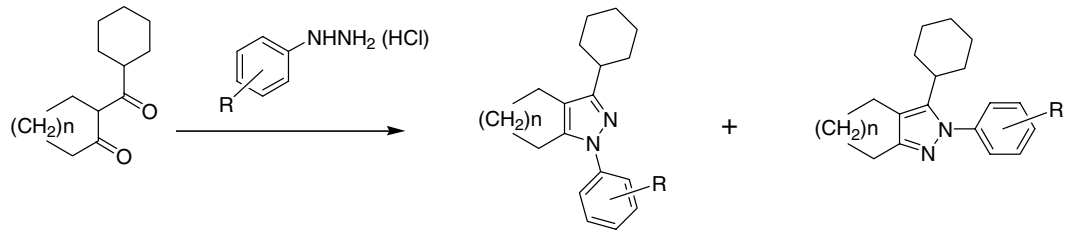
## 2. Investigations, results, and discussion

### 2.1. Chemistry

The designed compounds **5** and **6** were readily prepared in moderate yields from 2-cyclohexylcycloalkanone with (substituted)phenylhydrazines as outlined in the Scheme. The ratios of the two regioisomers ranged from 1:2.3 to 1:5.5 for **5**:**6** in most cases. Interestingly such a product distribution was not observed in the reactions of phenylhydrazine and bromophenylhydrazines where *N*2-isomers (**6aa**, **ab**, **bg**, **bh**, and **bh**) were the only products while the reactions of 4-sulfamoylphenylhydrazine hydrochloride afforded *N*1-isomer (**5ai**) as the only product, respectively. The reactions of 3- and 4-fluorophenylhydrazines with 2-cyclohexylcyclohexanone also afforded only *N*2-isomers (**6bd** and **be**). The reactions with 2-cyclohexylcyclopentanone, however, afforded two resioisomers. The reason for such distributions remained to be clarified.

Each regioisomer was readily separated by column chromatography and assigned by comparing $^1$H NMR spectra. In *N*1-isomers, the proton resonances of each phenyl ring were well-separated and assigned by COSY studies. In addition, 4–6% of NOE effect were observed between *peri*-H of the annulated cycloalkene and *ortho*-H of the *N*1-phenyl group in **5**, but not in **6**. On the other hand, in *N*2-isomers, the cycloalkyl rings are close enough to be

**Scheme**




**4** n = 1, 2
R = halogens, $OCH_3$, $SO_2NH_2$, $SO_2CH_3$, *etc*

**5**

**6**

magnetically influenced by the *N*2-phenyl rings, thus showing more complicated NMR spectra in the aliphatic region compared to those of *N*1-isomers.

The 2-cyclohexylcycloalkanones required were prepared using a previously reported method from enamines of cycloalkanones with cyclohexanecarbonyl chloride in 78–86% yields [19].

### *2.2. Biological results and discussion*

The inhibitory activities on COX-2 of the compounds prepared were evaluated by employing bone marrow mast cells from Balb/cj mice by a previously reported method [20] and are summarized in the Table. Most of the compounds prepared showed significant inhibitory activity at the μM level. Surprisingly, 1,3-isomers are more potent than the corresponding 1,2-disubstituted regioismers [21–23]. Among those, **5be** ($IC_{50} = 0.008\ \mu M$) and **5bf** ($IC_{50} = 0.010\ \mu M$) are the most potent with 12.5- and 64-fold selectivity on COX-2, respectively. Although such a result is somewhat inconsistent with the general requirement that two aryl groups are in adjacent positions for the maximal activity and selectivity, a similar pattern of activity was observed in annulated pyrazole derivatives [21]. With the increase of the ring size at the 4,5-position, activity generally decreased and so did selectivity, losing activity in most of the pentamethylene system (not described herein).

In conclusion, a series of 1,3- and 2,3-disubstituted 4,5-polymethylenepyrazoles were prepared and evaluated for their inhibitory activities as well as selectivities on COX-2. Unlike typical selective COX-2 inhibitors which have two aryl groups in a 1,2-relationship, both 1,2- and 1,3-disubstituted compounds showed potent inhibitory activity and selectivity on COX-2 with higher potencies for 1,3-disubstituted ones. Consequently, it is believed that a system with a 1,3-disubstituted compound would be a promising candidate with higher potency and selectivity on COX-2. Concurrent studies for *in vivo* activity, pharmaceutical profile study, and regiospecific synthesis of both isomers are in progress for completion in the future.

## 3. Experimental

Melting points were determined using a Fischer-Jones apparatus and are not corrected. IR spectra were obtained using a Perkin-Elmer 1330 spectrophotometer. NMR spectra were obtained using a Bruker-250 spectrometer at 250 MHz for $^1H$ NMR and 62.5 MHz for $^{13}C$ NMR and are reported as parts per million (ppm) from the internal standard tetramethylsilane (TMS). Chemicals and solvents were commercial reagent grade and used without further purification. Elemental analyses for C, H, and N were performed on a Hewlett-Packard Model 185B elemental analyzer and were within ±0.4% of the theoretical values. The starting 2-acylcycloalkanones **4** were prepared by either a previously reported method or a modification of such a method [19].

### *3.1. Compounds synthesized*

#### *3.1.1. 3-Cyclohexyl-2-phenyl-4,5-trimethylenepyrazole* (**6aa**)

A mixture of 1.94 g (10 mmol) of 2-cyclohexylcyclopentanone and 1.60 g (11 mmol) of phenylhydrazine-HCl in 20 ml of abs. EtOH was refluxed for 2 h. The reaction mixture was concentrated to give 1.92 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (4:1). The early fractions ($R_f = 0.46$) gave 1.81 g (68%) of **6aa** as pale yellow needles: m.p. 61–62 °C. IR (KBr) ν 2926, 2852, 1599, 1510, 1383, 1265, 750, 687 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.53 (dm, J = 7.6, 2 H), 7.32 (t, J = 7.6, 2 H), 7.1 (tm, J = 7.6, 1 H), 2.8 (t, J = 7.0, 2 H), 2.66–2.46 (m, 5 H), 1.93–1.88 (m, 2 H), 1.75–1.64 (m, 3 H), 1.48–1.16 (m, 5 H).

**Table: Inhibition of COX-2 and COX-1 enzyme by selected disubstituted 4,5-polymethylenepyrazoles**

| Compounds | n | R | $IC_{50}(\mu M)$[a] | | Selectivity[b] |
|---|---|---|---|---|---|
| | | | COX-2 | COX-1 | |
| **5ab** | 1 | $OCH_3$ | 0.81 | 1.79 | |
| **5ac** | 1 | 2-F | 78%@2.5 μg/ml | NT[c] | |
| **5ad** | 1 | 3-F | 65%@2.5 μg/ml | NT | |
| **5ae** | 1 | 4-F | 0.008 | 0.10 | 13 |
| **5ai** | 1 | 4-$SO_2NH_2$ | 0.010 | 0.64 | 64 |
| **5aj** | 1 | 4-$SO_2CH_3$ | 0.031 | 0.84 | 27 |
| **5ba** | 1 | H | 95%@2.5 μg/ml[d] | 0.056 | |
| **5bb** | 1 | $OCH_3$ | 0.24 | NT | |
| **5bc** | 1 | 2-F | 67%@2.5 μg/ml | NT | |
| **5bi** | 1 | 4-$SO_2NH_2$ | 0.67 | 16.41 | 25 |
| **5bj** | 1 | 4-$SO_2CH_3$ | 0.031 | 0.84 | 27 |
| **6aa** | 1 | H | 0.02 | 0.10 | 5 |
| **6ab** | 1 | $OCH_3$ | 0.79 | 2.11 | 3 |
| **6ac** | 1 | 2-F | 58%@2.5 μg/ml | NT | |
| **6ad** | 1 | 3-F | 45%@2.5 μg/ml | NT | |
| **6ae** | 1 | 4-F | 0.18 | 0.63 | 4 |
| **6aj** | 1 | 4-$SO_2CH_3$ | 0.061 | 1.84 | 27 |
| **6ba** | 2 | H | 0.02 | 0.10 | 5 |
| **6bb** | 2 | $OCH_3$ | 0.96 | 2.0 | 2 |
| **6bc** | 2 | 2-F | 4.11 | NT | |
| **6bd** | 2 | 3-F | 45%@2.5 μg/ml | NT | |
| **6be** | 2 | 4-F | 55%@2.5 μg/ml | NT | |
| **6bf** | 2 | 2-Br | 85%@2.5 μg/ml | NT | |
| **6bg** | 2 | 3-Br | 75%@2.5 μg/ml | NT | |
| **6bh** | 2 | 4-Br | 75%@2.5 μg/ml | NT | |
| **6bi** | 2 | 4-$SO_2NH_2$ | 0.014 | 0.54 | 38 |
| **6bj** | 2 | 4-$SO_2CH_3$ | 0.021 | 0.74 | 35 |
| **Celecoxib** | | | 0.019 | 2.18 | 115 |
| **NS-398** | | | 0.10 | >100 | >1000 |

a All data were the arithmetic means of triplicate determinations.
b Values are the ratios between $IC_{50}$'s of COX-1 and COX-2.
c Not tested.
d $IC_{50}$ value was not obtainable due to the death of cell

*3.1.2. 3-Cyclohexyl-1-(4-methoxyphenyl)-4,5-trimethylenepyrazole* (**5ab**) *and 3-cyclohexyl-2-(4-methoxyphenyl)-4,5-trimethylenepyrazole* (**6ab**)

The same procedure as described above for **6aa** with 1.94 g (10 mmol) of 2-cyclohexylcyclopentanone and 1.91 g (11 mmol) of 4-methoxyphenylhydrazine-HCl yielded 1.92 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (4 : 1). The early fractions ($R_f = 0.53$) gave 0.54 g (17%) of 1-(4-methoxyphenyl)-3-cyclohexyl-4,5-trimethylenepyrazole as pale yellow needles: m.p. 64–65 °C. IR (KBr) ν 2924, 2850, 1520, 1390, 1294, 1250, 1169, 1092, 1026, 822 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.49 (dm, J = 9.0, 2 H), 6.91 (dm, J = 9.0, 2 H), 3.81 (s, 3 H), 2.88 (t, J = 7.0, 2 H), 2.75–2.52 (m, 5 H), 1.99–1.95 (m, 2 H), 1.82–1.70 (s, 2 H), 1.54–1.22 (m, 5 H). The later fractions ($R_f = 0.26$) gave 1.26 g (40%) of 2-(4-methoxyphenyl)−3-cyclohexyl−4,5-tri-methylenepyrazole as pale yellow liquid: IR (KBr) ν 2927, 2852, 1587, 1518, 1452, 1381, 1250, 1032, 835 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.22 (dm, J = 8.8, 2 H), 6.87 (dm, J = 8.8, 2 H), 3.78 (s, 3 H), 2.65 (overlapped t, J = 7.0, 4 H), 2.48 (tt, J = 11.8, 3.2, 1 H), 2.34 (quintet, J = 7.1, 2 H), 1.74–1.66 (m, 5 H), 1.43–1.26 (m, 2 H), 1.21–1.12 (m, 3 H).

*3.1.3. 3-Cyclohexyl-1-(2-flurophenyl)-4,5-trimethylenepyrazole* (**5ac**) *and 3-cyclohexyl-2-(2-fluorophenyl)-4,5-trimethylenepyrazole* (**6ac**)

The same procedure as described above for **6aa** with 1.94 g (10 mmol) of 2-cyclohexylcyclopentanone and 1.78 g (11 mmol) of 2-fluorophenylhydrazine-HCl yielded 1.96 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (4 : 1). The early fractions ($R_f = 0.56$) gave 0.30 g (10%) of 1-(2-fluorophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole as a pale yellow liquid. IR (KBr) υ 2927, 2854, 1731, 1616, 1518, 1386, 1265, 1028, 814, 758 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.62 (ddd, J = 9.0, 4.7, 2.5, 2 H), 7.38–7.11 (m, 3 H), 2.77–2.59 (m, 5 H), 2.57–2.49 (m, 2 H), 2.00–1.96 (broad d, J = 11, 2 H), 1.83–1.70 (m, 4 H), 1.45–1.25 (m, 4 H). The later fractions ($R_f = 0.25$) gave 2-(2-fluorophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole as pale yellow needles (1.64 g, 54%): m.p. 62–63 °C. IR (KBr) ν 2927, 2852, 1591, 1514, 1452, 1379, 1263, 1225, 1059, 995, 761 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.50–7.35 (m, 2 H), 7.26–7.1 (m, 2 H), 2.74 (t, J = 7.2, 4 H), 2.46 (quintet, J = 7.3, 2 H), 2.33 (tm, J = 11.9, 1 H), 1.80–1.64 (m, 5 H), 1.49–1.34 (m, 2 H), 1.25–1.13 (m, 3 H).

*3.1.4. 3-Cyclohexyl-1-(3-fluorophenyl)-4,5-trimethylenepyrazole* (**5ad**) *and 3-cyclohexyl-2-(3-fluorophenyl)-4,5-trimethylenepyrazole* (**6ad**)

The same procedure as described above for **6aa** with 1.94 g (10 mmol) of 2-cyclohexylcyclopentanone and 1.78 g (11 mmol) of 3-fluorophenylhydrazine-HCl yielded 1.99 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (4 : 1). The early fractions ($R_f = 0.66$) gave 0.38 g (13%) of 1-(3-fluorophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole as a pale yellow liquid. IR (KBr) 2926, 2852, 1732, 1614, 1506, 1385, 1261, 1032, 922, 773 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.40–7.31 (m, 3 H), 6.91–6.82 (m, 1 H), 2.96 (t, J = 6.7, 2 H), 2.72–2.60 (m, 5 H), 1.99–1.62 (m, 5 H), 1.54–1.26 (m, 5 H). The later fractions ($R_f = 0.42$) gave 2-(3-fluorophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole as pale yellow needles (1.56 g, 51%): m.p. 85–86 °C. IR (KBr) ν 2929, 2854, 1729, 1610, 1502, 1452 1376, 1271, 1201, 926, 866, 783 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.45–7.35 (m, 1 H), 7.20–7.16 (m, 1 H), 7.13–7.09 (m, 1 H), 7.07–7.02 (m, 1 H), 2.72 (t, J = 7.4, 4 H), 2.64 (dt, J = 11.8, 3.4, 1 H), 2.45 (quintet, J = 7.4, 2 H), 1.84–1.69 (m, 6 H), 1.53–1.39 (m, 2 H), 1.32–1.23 (m, 2 H). $^{13}C$ NMR (62.9 MHz, $CDCl_3$) δ 163.0, 142.5, 130.5 (d, $^4J_{C-F} = 3.1$), 123.9, 121.4 (d, $^3J_{C-F} = 7.9$), 114.7 (d, $^2J_{C-F} = 21.4$), 113.4, 35.9, 32.7, 30.4, 26.7, 26.2, 24.7, 24.6.

*3.1.5. 3-Cyclohexyl-1-(4-fluorophenyl)-4,5-trimethylenepyrazole* (**5ae**) *and 3-cyclohexyl-2-(4-fluorophenyl)-4,5-trimethylenepyrazole* (**6ae**)

The same procedure as described above for **6aa** with 1.94 g (10 mmol) of 2-cyclohexylcyclopentanone and 1.78 g (11 mmol) of 4-fluorophenylhydrazine-HCl yielded 1.92 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (4 : 1). The early fractions ($R_f = 0.70$) gave 0.34 g (11%) of 1-(4-fluorophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole as pale yellow needles: m.p. 62–63 °C. IR (KBr) υ 2922, 2850 1568, 1518, 1390, 1294, 1223, 1082, 1043 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.35 (ddd, J = 9.0, 4.7, 2.1, 2 H), 7.12 (ddd, J = 8.6, 8.4, 2.2, 2 H), 2.75–2.61 (overlapped t, J = 6.9, 4 H), 2.57 (tt, J = 11.9, 3.1, 1 H), 2.42 (quintet, J = 6.9, 2 H), 1.91–1.76 (m, 5 H), 1.51–1.38 (m, 2 H), 1.20 (broad s, 3 H). The later fractions ($R_f = 0.35$) gave 1.50 g (49%) of 2-(4-fluorophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole as pale yellow needles: m.p. 86–87 °C. IR (KBr) ν 2931, 2852, 1583, 1514, 1452, 1377, 1215, 1153, 1051, 993, 833 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.54 (ddd, J = 9.0, 4.7, 2.1, 2 H), 7.07 (ddd, J = 8.6, 8.3, 2.2, 2 H), 2.90 (dd, J = 7.2, 6.6, 2 H), 2.71–2.54 (m, 5 H), 1.99–1.89 (m, 2 H), 1.82–1.70 (m, 3 H), 1.54–1.22 (m, 5 H).

*3.1.6. 2-(2-Bromophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole* (**6af**)

The same procedure as described above for **6aa** with 0.96 g (5 mmol) of 2-cyclohexylcyclopentanone and 1.23 g (5.5 mmol) of 2-bromophenylhydrazine-HCl yielded 1.22 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (4 : 1). The latter fractions ($R_f = 0.20$) gave 1.04 g (60%) of 2-(2-bromophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole as pale yellow crystals: m.p. 95–96 °C. IR (KBr) ν 2929, 2852, 1724, 1587, 1500, 1450, 1379, 1271, 1034, 757 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ−2.40 (m, 4 H), 2.24 (tt, J = 15.2, 3.4, 1 H), 1.93–1.88 (br d, 1 H), 1.70–1.64 (m, 4 H), 1.41–1.15 (m, 5 H).

*3.1.7. 2-(3-Bromophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole* (**6ag**)

The same procedure as described above for **6aa** with 0.96 g (5 mmol) of 2-cyclohexylcyclopentanone and 1.23 g (5.5 mmol) of 3-bromophenylhydrazine-HCl afforded 1.35 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (4 : 1). The early fractions ($R_f = 0.50$) gave 1.23 g (71%) of 2-(3-bromophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole as pale yellow needles: m.p. 105–107 °C. IR (KBr) ν 2927, 2843, 1589, 1493, 1466, 1425, 1373, 1053, 993, 862, 789, 690 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.62 (dd, J = 2.4, 0.7, 1 H), 7.53–7.45 (m, 1 H), 7.36–7.26 (m, 2 H), 2.74 (td, J = 7.2, 1.4, 4 H), 2.65 (tt, J = 15.2, 3.4, 1 H), 2.43 (quintet, J = 6.9, 2 H), 1.85–1.74 (m, 5 H), 1.54–1.41 (m, 2 H), 1.24 (m, 3 H).

*3.1.8. 2-(4-Bromophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole* (**6ah**)

The same procedure as described above for **6aa** with 0.96 g (5 mmol) of 2-cyclohexylcyclopentanone and 1.23 g (5.5 mmol) of 4-bromophenylhydrazine-HCl afforded 1.22 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (4 : 1). The early fractions ($R_f = 0.43$) gave 1.10 g (63%) of 1-(4-bromophenyl)-3-cyclohexyl-4,5-trimethylenepyrazole as pale yellow needles: m.p. 83.5–84 °C. IR (KBr) ν 2922, 2848, 1587, 1500, 1377, 1356, 1053, 987, 829 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.56 (dm, J = 9.0, 2 H), 7.27 (dm, J = 9.0, 2 H), 2.72 (overlapped t, J = 7.3, 4 H), 2.62 (tt, J = 11.8, 3.3, 1 H), 2.42 (quintet, J = 7.3, 2 H), 1.82–1.65 (m, 5 H), 1.52–1.38 (m, 2 H), 1.29–1.18 (m, 3 H).

*3.1.9. 3-Cyclohexyl-1-(4-sulfamoylphenyl)-4,5-trimethylenepyrazole* (**5ai**)

The same procedure as described above for **6aa** with 1.92 g (10 mmol) of 2-cyclohexylcyclopentanone and 2.46 g (11 mmol) of 4-sulfamoylphenylhydrazine-HCl afforded 2.55 g of crude material which was chromatographed on silica gel, eluting with EtOAc. The early fractions ($R_f = 0.82$) gave 2.38 g (68%) of 3-cyclohexyl-1-(4-sulfamoylphenyl)-4,5-trimethylenepyrazole as pale yellow needles: m.p. 229–231 °C. IR (KBr) ν 2931, 2850, 1595, 1454, 1333, 1171, 1026, 897. 839 $cm^{-1}$. $^1H$ NMR (250 MHz, DMSO-$d_6$) δ 7.93 (dm, J = 8.5, 2 H), 7.61 (dm, J = 8.5, 2 H), 7.46 (br. s, $NH_2$), 2.71 (t, J = 7.3, 2 H), 2.62 (t, J = 7.3, 2 H), 2.77–2.68 (m, 1 H), 2.36 (quintet, J = 7.2, 2 H), 1.81–1.70 (m, 5 H).

*3.1.10. 3-Cyclohexyl-1-(4-methanesulfonylphenyl)-4,5-trimethylenepyrazole* (**5aj**) *and 3-cyclohexyl-2-(4-methanesulfonylphenyl-)-4,5-trimethylenepyrazole* (**6aj**)

The same procedure as described above for **6aa** with 1.92 g (10 mmol) of 2-cyclohexylcarbonylcyclopentanone and 2.05g (11 mmol) of 4-methanesulfonylphenylhydrazine-HCl afforded 2.55 g of crude material which was chromatographed on silica gel, eluting with EtOAc. The early fractions ($R_f = 0.58$) gave 2.38 g (69%) of 3-cyclohexyl-1-(4-methanesulfonylphenyl)-4,5-trimethylenepyrazole as a pale yellowish semisolid. IR(KBr) ν 2930, 1600, 1455, 1335, 1170, 1025, 890, 850, 839 $cm^{-1}$. $^1H$ NMR(250 MHz, $CDCl_3$) δ 7.80 (d, J = 8.9 Hz, 2 H), 7.14 (d, J = 8.9 Hz, 2 H), 3.64 (quintet, J = 7.5 Hz, 1 H), 3.04 (s, $CH_3$), 2.46 (t, J = 7.4 Hz, 2 H), 2.34 (t, J = 7.4 Hz, 2 H), 1.88–1.79 (m, 6 H), 1.57 (quintet, 2 H), 1.34–1.21 (m, 2 H). 1.52–1.38 (m, 2 H), 1.29–1.18 (m, 2 H). The later fractions ($R_f = 0.38$) gave 0.48 g (14%) of 3-cyclohexyl-2-(4-methanesulfonylphenyl)-4,5-tetramethylenepyrazole as pale yellow needles: m.p. 108–109 °C. IR (KBr) ν 2925, 1555, 1450, 1380, 1060, 990, 830 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 8.03 (dm, J = 8.7, 2 H), 7.55 (dm, J = 8.7, 2 H), 3.65 (quintet, J = 7.5, 1 H), 3.06 (s, 3 H), 2.72–2.59 (m, 6 H), 1.86–1.80 (m, 8 H), 1.24–1.18 (m, 2 H).

*3.1.11. 3-Cyclohexyl-2-phenyl-4,5-tetramethylenepyrazole* (**6ba**)

The same procedure as described above for **6aa** with 0.25 g (1.2 mmol) of 2-cyclohexylcyclohexanone and 0.15 g (1.3 mmol) of phenylhydrazine-HCl afforded 0.32 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (5 : 1). The early fractions ($R_f = 0.41$) gave 0.20 g (60%) of 3-cyclohexyl-2-phenyl-4,5-tetramethylenepyrazole as pale yellow needles: m.p. 87–88 °C. IR (KBr) ν 2930, 2850, 1600, 1500, 1448, 1383, 1095, 991, 767, 700 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.48–7.33 (m, 5 H), 2.73 (t, J = 5.8, 2 H), 2.68 (t, J = 5.6 2 H), 2.75–2.61 (m, 1 H), 1.82–1.60 (m, 11 H), 1.29–1.19 (m, 3 H).

*3.1.12. 3-Cyclohexyl-1-(4-methoxyphenyl)-4,5-tetramethylenepyrazole* (**5bb**) *and 3-cyclohexyl-2-(4-methoxy-phenyl)-4,5-tetramethylenepyrazole* (**6bb**)

The same procedure as described above for **6aa** with 0.25 g (1.2 mmol) of 2-cyclohexylcyclohexanone and 0.23 g (1.3 mmol) of phenylhydrazine-HCl afforded 0.40 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (5 : 1). The early fractions ($R_f = 0.58$) gave 0.04 g (11%) of 3-cyclohexyl-1-(4-methoxyphenyl)-4,5-tetramethylenepyrazole as pale yellow needles: m.p. 63–64 °C. IR (KBr) ν 2930, 2850, 1520, 1443, 1250, 1170, 1030, 830 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.36 (dt, J = 8.93, 2.7, 2 H), 6.92 (dd, J = 8.96, 2.5, 2 H), 3.82 (s, 3 H), 2.72–2.55 (m, 5 H), 1.96–1.54 (m, 11 H), 1.45–1.20 (m, 3 H). The later fractions ($R_f = 0.27$) afforded 0.17 g (46%) of 3-cyclohexyl-2-(4-methoxyphenyl)-4,5-tetramethylenepyrazole as a pale yellow liquid. IR (KBr) ν 2930, 2850, 1516, 1450, 1380, 1250, 1030, 835 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.26 (dt, J = 8.91, 2.7, 2 H), 6.95 (dd, J = 8.98, 2.7, 2 H), 3.85 (s, 3 H), 2.71 (t, J = 5.9, 2 H), 2.66 (t, J = 5.8, 2 H), 2.58 (t, J = 11.6, 1 H), 1.81–1.65 (m, 11 H), 1.26–1.17 (m, 3 H).

*3.1.13. 3-Cyclohexyl-1-(2-fluorophenyl)-4,5-tetramethylenepyrazole* (**5bc**) *and 3-cyclohexyl-2-(2-fluorophenyl)-4,5-tetramethylenepyrazole* (**6bc**)

The same procedure as described above for **6aa** with 0.25 g (1.2 mmol) of 2-cyclohexylcyclohexanone and 0.21 g (1.3 mmol) of phenylhydrazine-HCl afforded 0.42 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (5 : 1). The early fractions ($R_f = 0.70$) gave 0.04 g (10%) of 3-cyclohexyl-1-(2-fluorophenyl)-4,5-tetramethylenepyrazole as pale yellow liquid. IR (KBr) ν 2928, 2850, 1614, 1515, 1390, 1050, 760 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.48–7.41 (m, 1 H) 7.36–7.13 (m, 3 H), 2.66 (t, J = 11.9, 3.5, 1 H), 2.62–2.49 (m, 2 H), 1.97–1.54 (m, 13 H), 1.44–1.24 (m, 4 H). The latter fractions ($R_f = 0.27$) afforded 0.18 (51%) of 3-cyclohexyl-2-(2-fluorophenyl)-4,5-tetramethylenepyrazole as liquid. IR (KBr) ν 2930, 2850, 1565, 1450, 1225, 990, 760 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.42–7.34 (m, 2 H), 7.24–7.15 (m, 2 H), 2.72 (t, J = 5.5, 2 H), 2.67 (t, J = 5.4, 2 H), 2.38 (t, J = 12, 1 H), 1.81–1.53 (m, 11 H), 1.28–1.11 (m, 3 H).

*3.1.14. 3-Cyclohexyl-2-(3-fluorophenyl)-4,5-tetramethylenepyrazole* (**6bd**)

The same procedure as described above for **6aa** with 0.25 g (1.2 mmol) of 2-cyclohexylcyclohexanone and 0.21 g (1.3 mmol) of phenylhydrazine-HCl afforded 0.37 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (5 : 1). The major fractions ($R_f = 0.48$) gave 0.22 g (62%) of 3-cyclohexyl-2-(3-fluorophenyl)-4,5-tetramethylenepyrazole as pale yellow needles: m.p. 82–83 °C. IR (KBr) ν 2936, 2850, 1610, 1450, 1380, 1204, 1090, 900, 800, 700 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.40 (td, J = 8.1, 6.3, 1 H), 7.15–7.04 (m, 3 H), 2.71 (t, J = 5.4, 2 H), 2.67 (t, J = 5.6, 2 H), 2.73–2.63 (overlapped 1 H), 1.81–1.60 (m, 11 H), 1.26–1.21 (m, 3 H).

*3.1.15. 3-Cyclohexyl-2-(4-fluorophenyl)-4,5-tetramethylenepyrazole* (**6be**)

The same procedure as described above for **6aa** with 0.25 g (1.2 mmol) of 2-cyclohexylcyclohexanone and 0.21 g (1.3 mmol) of phenylhydrazine-HCl afforded 0.37 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (5 : 1). The early fractions ($R_f = 0.44$) gave 0.21 g (59%) of 3-cyclohexyl-2-(4-fluorophenyl)-4,5-tetramethylenepyrazole as pale yellow needles: m.p. 111–112 °C. IR (KBr) ν 2928, 2850, 1560, 1515, 1385, 1210, 1090, 990, 840 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.32 (dd, J = 8.8, $J_{H-F}$ = 6.9, 2 H), 7.13 (dd, J = 8.6, $J_{H-F}$ = 8.6, 2 H), 2.70 (t, J = 5.4, 2 H), 2.66 (t, J = 5.4, 2 H), 2.57 (t, J = 11.6, 3.5, 1 H), 1.81–1.58 (m, 11 H), 1.25–1.18 (m, 3 H).

*3.1.16. 2-(2-Bromophenyl)-3-cyclohexyl-4,5-tetramethylenepyrazole* (**6bf**)

The same procedure as described above for **6aa** with 0.25 g (1.2 mmol) of 2-cyclohexylcyclohexanone and 0.29 g (1.3 mmol) of phenylhydrazine-HCl afforded 0.55 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (5 : 1). The early fractions ($R_f = 0.55$) gave 0.30 g (70%) of 2-(2-bromophenyl)-3-cyclohexyl-4,5-tetramethylenepyrazole as pale yellow liquid. IR (KBr) υ 2928, 2850, 1585, 1500, 1445, 1290, 1125, 760 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.65 (d, J = 7.9, 1 H), 7.37 (d, J = 4.0, 2 H), 7.30–7.22 (m, 2 H), 2.56 (br s, 2 H), 2.65 (t, J = 11.8, 3.5, 1 H), 2.39 (br s, 2 H), 2.05–1.53 (m, 11 H), 1.45–1.17 (m, 3 H).

*3.1.17. 2-(3-Bromophenyl)-3-cyclohexyl-4,5-tetramethylenepyrazole* (**6bg**)

The same procedure as described above for **6aa** with 0.25 g (1.2 mmol) of 2-cyclohexylcyclohexanone and 0.29 g (1.3 mmol) of phenylhydrazine-HCl afforded 0.56 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (5 : 1). The early fractions ($R_f = 0.53$) gave 0.27 g (62%) of 2-(2-bromophenyl)-3-cyclohexyl-4,5-tetramethylenepyrazole as pale yellow needles: m.p. 68–69 °C. IR (KBr) ν 2928, 2850, 1680, 1590, 1485, 1380, 1065, 875, 780 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.56 (s, 1 H), 7.49 (dt, J = 6.8, 1.8, 1 H), 7.30 (t, J = 7.1, 1 H), 7.29 (d, J = 7.0, 1 H) 2.70 (t, J = 5.4, 2 H), 2.66 (t, J = 5.4, 2 H), 2.72–2.56 (overlapped 1 H), 1.81–1.54 (m, 11 H), 1.21 (br s, 3 H).

*3.1.18. 2-(4-Bromophenyl)-3-cyclohexyl-4,5-tetramethylenepyrazole* (**6bh**)

The same procedure as described above for **6aa** with 0.25 g (1.2 mmol) of 2-cyclohexylcyclohexanone and 0.29 g (1.3 mmol) of phenylhydrazine-HCl afforded 0.57 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (5 : 1). The early fractions ($R_f = 0.57$) gave 0.29 g (68%) of 2-(4-bromophenyl)-3-cyclohexyl-4,5-tetramethylenepyrazole as pale yellow needles: m.p. 111–112 °C. IR (KBr) υ 2928, 2850, 1585, 1500, 1450, 1380, 1065, 990, 830 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 7.22 (dt, J = 8.7, 2.25, 2 H), 7.57 (dt, J = 8.66, 2.38, 2 H), 2.70 (t, J = 5.5, 2 H), 2.66 (t, J = 5.3, 2 H), 2.61 (t, J = 11.6, 3.7, 1 H), 1.90 (br s, 3 H), 1.81–1.63 (m, 11 H).

*3.1.19. 3-Cyclohexyl-1-(4-sulfamoylphenyl)-4,5-tetramethylenepyrazole* (**5bi**)

The same procedure as described above for **6aa** with 1.92 g (10 mmol) of 2-cyclohexylcyclopentanone and 2.46 g (11 mmol) of 4-sulfamoylphenylhydrazine-HCl afforded 2.55 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (5 : 1). The early fractions ($R_f = 0.72$) gave 2.53 g (70%) of 3-cyclohexyl-1-(4-sulfamoylphenyl)-4,5-tetramethylenepyrazole as pale yellow needles: m.p. >300 °C. IR (KBr) ν 2930, 2860, 1600, 1450, 1350, 1170, 1025, 900. 840 $cm^{-1}$. $^1H$ NMR (250 MHz, DMSO-$d_6$) δ 7.96 (dm, J = 8.5, 2 H), 7.56 (dm, J = 8.5, 2 H), 7.43 (br. s, $NH_2$), 3.53 (quintet, J = 7.5 Hz, 1 H), 2.76–2.72 (m, 4 H), 1.86–1.82 (m, 10 H), 1.24–1.21 (m, 4 H).

*3.1.20. 3-Cyclohexyl-1-(4-methanesulfonylphenyl)-4,5-tetramethylenepyrazole* (**5bj**) *and 3-cyclohexyl-2-(4-methanesulfonylphenyl)-4,5-tetramethylenepyrazole* (**6bj**)

The same procedure as described above for **6aa** with 0.25 g (1.2 mmol) of 2-cyclohexylcyclopentanone and 0.24 g (1.3 mmol) of 4-methansulfonylphenylhydrazine-HCl afforded 0.35 g of crude material which was chromatographed on silica gel, eluting with *n*-hexane : EtOAc (5 : 1). The early fractions ($R_f = 0.58$) gave 0.34 g (72%) of 3-cyclo-hexyl-1-(4-methanesulfonylphenyl)-4,5-tetramethylenepyrazole as pale yellow needles: m.p. 111–112 °C. IR (KBr) ν 2930, 2850, 1555, 1450, 1380, 1065, 990, 830 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 8.05 (dm, J = 8.7, 2 H), 7.58 (dm, J = 8.7, 2 H), 3.51 (quintet, J = 7.5 Hz, 1 H), 3.12 (s, 3 H), 2.76–2.69 (m, 4 H), 1.84–1.80 (m, 10 H), 1.26–1.20 (m, 4 H). The later fractions ($R_f = 0.23$) gave 0.06 g (13%) of 3-cyclohexyl-2-(4-methanesulfonyl-phenyl)-4,5-tetramethylenepyrazole as pale yellow needles: m.p. 128–129 °C. IR (KBr) ν 2925, 1600, 1460, 1390, 1070, 990, 830 $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$) δ 8.03 (dm, J = 8.7, 2 H), 7.55 (dm, J = 8.7, 2 H), 3.65 (quintet, J = 7.5, 1 H), 2.74–2.61 (m, 6 H), 1.86–1.80 (m, 8 H), 1.24–1.18 (m, 4 H).

### *3.2. 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 a previously described procedure [20]. For measuring inhibitory activity of the compounds on COX-2, cells suspended at a cell density of $5 \times 10^5$ cells/ml in enriched medium were preincubated with aspirin (10 μg/ml) for 2 h in order to irreversibly inactivate preexisting COX-1. After washing, BMMC were activated with KL (100 ng/ml), IL-10 (100 U/ml) and LPS (100 μg/ml) at 37 °C for 8 h in the presence or absence of the 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 a $PGD_2$ assay kit (Amersham, Buckinghamshire, 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. All data were the arithmetic mean of triplicate determinations.

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Prof. Y. Jahng
College of Pharmacy
Yeungnam University
Gyongsan 712–749
Korea