Turk J Chem
31 (2007) , 677 – 687.

Synthesis of the Amide Derivatives of 3-[1-(3-Pyridazinyl)-5-phenyl-1H-pyrazole-3-yl]propanoic Acids as Potential Analgesic Compounds

Erden BANOĞLU[1*], Murat ŞÜKÜROĞLU[1], Burcu ÇALIŞKAN ERGÜN[1], Sultan NACAK BAYTAŞ[1], Eda AYPAR[2], Mustafa ARK[2]

[1] *Division of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Etiler, Ankara-TURKEY*

e-mail: banoglu@gazi.edu.tr

[2] *Division of Pharmaceutical Sciences, Department of Pharmacology, Faculty of Pharmacy, Gazi University, 06330 Etiler, Ankara-TURKEY*

Received 18.06.2007

A series of structurally diverse amide derivatives of 3-[1-(3-pyridazinyl)-5-phenyl-1H-pyrazole-3-yl]propanoic acids were prepared and tested for their in vivo analgesic activity using an acetic acid induced writhing test. All the test compounds displayed approximately equipotent analgesic activity to aspirin. The results showed that the analgesic activity of **5a**, **5f**, **5n**, and **5o** is significantly higher than that of **5d**.

Key Words: Pyridazine, pyrazole, analgesic, writhing.

Introduction

Although a number of nonsteroidal anti-inflammatory drugs (NSAIDs) are available for the treatment of pain syndromes, their chronic use for treatment of pain concomitant with inflammation limits their therapeutic use since they cause gastrointestinal and renal side effects.[1,2] Therefore, the main trend nowadays in pain therapy focuses on improved nonsteroidal analgesics that are effective as an analgesic but devoid of the side effects inherent to traditional NSAIDs.

In recent years, the dual inhibition of cyclooxygenase and 5-lypoxygenase enzymes for treatment of inflammation and pain has been introduced as a novel therapeutic target, and one of the first examples of dual acting analgesic and anti-inflammatory molecules was tepoxalin, a diarylpyrazole derivative,[3] as seen in Figure 1.

*Corresponding author

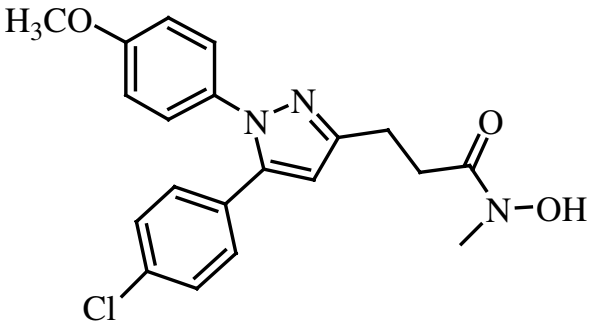


Figure 1. Tepoxalin

In addition, many studies also focused on pyridazine derivatives for developing potent and safer NSAIDs without gastric side effects,[4−6]. Among these compounds, 4-ethoxy-2-methyl-5-morpholino-3(2H)-pyridazinone (emorfazone) is currently being marketed in Japan as an analgesic and anti-inflammatory drug.[7] Dogruer et al. subsequently synthesized [6-(4-methoxyphenyl)-3(2H)-pyridazinone-2-yl]acetamide and propanamide derivatives and reported that these compounds showed potential analgesic activity.[8] Moreover, some studies for developing COX-2 inhibitors have concentrated on the preparation of the amide derivatives of currently used NSAIDs such as indomethacin[9] and meclofenamic acid[10] and found that neutralization of these NSAIDs by preparing amide derivatives resulted in compounds that selectively inhibited COX-2 but not COX-1.

These findings stimulated us to search for new compounds with a 1,5-diaryl substitution pattern about a central pyrazole ring. Based on the information that pyridazine derivatives bear potent analgesic and anti-inflammatory activities,[11−14] we chose the pyridazine ring as one of the aryl substituents about the central pyrazole ring. We also aimed to explore the presence of the propanamide side chain that is linked to the 3 position of the pyrazole ring to determine the contribution of this structural feature to the analgesic activity. Therefore, we synthesized amide derivatives of 3-[1-(3-pyridazinyl)-5-phenyl-1H-pyrazole-3-yl]propanoic acids (Figure 2) and hereby present the initial results of in vivo analgesic activity screening of the title amide derivatives.

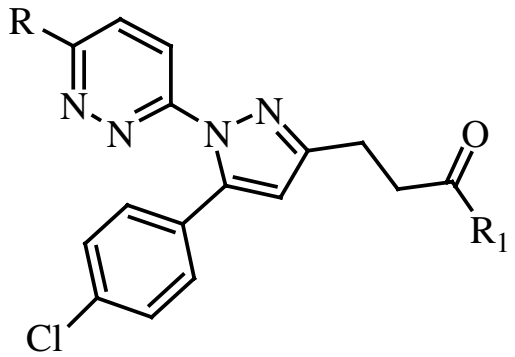


Figure 2. General structure of the synthesized compounds.

Experimental

Chemistry

All chemicals and solvents used were of reagent grade (Merck or Aldrich), and were used without further purification. Thin layer chromatographic (TLC) analyses were performed on pre-coated aluminum plates (silicagel 60 F254, Merck). TLC spots were visualized with UV light. Melting points were measured on an Electrothermal 9200 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded in KBr pellets on a Bruker 1000 FTIR spectrometer in the range 4000-400 cm^{-1}. Elemental analyses, and NMR and mass spectra were performed in the Central Laboratory of the Faculty of Pharmacy, Ankara University. NMR spectra were measured with a Varian Mercury 400 FT-NMR spectrometer in DMSO-d_6. All chemical shifts were reported as δ (ppm) values. Mass spectra were recorded on a VG Waters Micromass ZQ by the ESI (+) method. Elementary analyses were performed on a Leco CHNS 932 analyzer and satisfactory results ±0.4% of calculated values (C, H, N) were obtained. Compounds **1**-**3** were prepared according to the previously reported procedure.[15]

3-(1-(6-Methoxypyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl)propanoic acid (4)

Compound **3** (4.72 g, 13 mmol) was dissolved in anhydrous methanol, and sodium methoxide (2.1 g, 39 mmol) was added. The reaction mixture was refluxed for 5 h and 50 mL of water was added; it was then neutralized with 1 N HCl, and filtered to give **4** (3.81 g, 71%). ^{1}H-NMR (400 MHz, DMSO-d_6)δ 12.1 (s, 1H), 7.94 (d, 1H, J = 9.2 Hz), 7.48 (d, 1H, J = 9.2 Hz), 7.41 (d, 2H, J = 8.8 Hz), 7.27 (d, 1H, J = 8.4 Hz), 6.6 (s, 1H), 3.98 (s, 3H), 2.88 (t, 2H, J = 7.2 Hz), 2.65 (t, 2H, J = 7.2 Hz). Anal. ($C_{17}H_{15}ClN_4O_3$): C, H, N calc. 56.91, 4.21, 15.62 found 56.74, 4.35, 15.63.

General procedure for the synthesis of amide derivatives (5a-p)

To the solution of acid derivatives (1.3 mmol) were added triethylamine (4 mmol) and ethyl chloroformate (1.5 mmol), followed by stirring at 0 °C for 30 min. After addition of the appropriate amine derivatives (1.6 mmol), the mixture was stirred for an additional 1 h at 0 °C. Then, the reaction mixture was warmed to room temperature, and kept stirring overnight. After the solvent was evaporated under reduce pressure, acetone was added, and filtered. To precipitate the product, 20 mL of water was added to the residue. The precipitate was filtered, dried, washed with boiling ether, dried, and crystallized from the appropriate solvent.

N-(4-chlorophenyl)-3-(1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl)propanamide 5a

Yield 35%. FT-IR (KBr) cm^{-1} 1672. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 10.17 (s, 1H), 8.12 (d, 2H, J = 6.4 Hz), 7.63 (d, 2H, J = 8.8 Hz), 7.44 (d, 2H, J = 8.0 Hz), 7.34 (m, 4H), 6.67 (s, 1H), 3.00 (t, 2H), 2.79 (t, 2H). Anal. ($C_{22}H_{16}Cl_3N_5O$): C, H, N calc. 55.89, 3.41, 14.81 found 55.74, 3.53, 14.68.

N-octyl-3-(1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl)propanamide 5b

Yield 69.8%. FT-IR (KBr) cm^{-1} 1648. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 8.14 (d, 1H, J = 8.8 Hz), 8.09 (d, 1H, J = 8.8 Hz), 7.86 (t, 1H, J = 5.6 Hz), 7.42 (d, 2H, J = 8.4 Hz), 7.32 (d, 2H, J = 8.8 Hz), 6.59 (s, 1H),

3.01 (q, 2H, J = 6.4 Hz), 2.88 (t, 2H, J = 7.6 Hz), 1.26 (m, 14H), 0.82 (t, 3H, J = 7.2 Hz). ESI-MS 474 $[M+H]^+$, 496 $[M+Na]^+$. Anal. ($C_{24}H_{29}Cl_2N_5O$): C, H, N calc. 60.76, 6.16, 14.76 found 60.33, 6.09, 14.52

N-butyl-3-(1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl)propanamide 5c

Yield 52.5%. FT-IR (KBr) cm^{-1} 1633. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 8.14 (d, 1H, J = 9.2 Hz), 8.09 (d, 1H, J = 8.8 Hz), 7.86 (t, 1H, J = 5.6 Hz), 7.43 (d, 2H, J = 8.4 Hz), 7.32 (d, 2H, J = 8.8 Hz), 6.60 (s, 1H), 3.03 (q, 2H, J = 6.4 Hz), 2.88 (t, 2H, J = 7.6 Hz), 1.32 (m, 3H), 1.20 (m, 3H), 0.78 (t, 3H, J = 7.2 Hz). ESI-MS 418 $[M+H]^+$, 440 $[M+Na]^+$. Anal. ($C_{20}H_{21}Cl_2N_5O$): C, H, N calc. 57.42, 5.06, 16.74 found 57.37, 5.01, 16.66.

N-phenethyl-3-(1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl)propanamide 5d

Yield 49%. FT-IR (KBr) cm^{-1} 1637. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 8.14 (d, 1H, J = 9.2 Hz), 8.08 (d, 1H, J = 8.8 Hz), 8.01 (t, 1H, J = 5.6 Hz), 7.43 (d, 2H, J = 8.4 Hz), 7.32 (d, 2H, J = 8.8 Hz), 7.23 (t, 2H, J = 8.4, 6.4 Hz), 7.16 (d, 3H, J = 6.8 Hz), 6.60 (s, 1H), 3.26 (m, 2H), 2.88 (t, 2H, J = 7.6 Hz), 2.67 (t, 2H, J = 7.6, 6.8 Hz), 2.48 (m, 2H). ESI-MS 466 $[M+H]^+$, 488 $[M+Na]^+$. Anal. ($C_{24}H_{21}Cl_2N_5O$): C, H, N calc. 61.81, 4.54, 15.02 found 61.79, 4.39, 15.04.

4-{3-[1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl]propanoyl}morpholine 5e

Yield 40%. FT-IR (KBr) cm^{-1} 1651. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 8.15 (d, 1H, J = 9.6 Hz), 8.09 (d, 1H, J = 8.8 Hz), 7.42 (d, 2H, J = 8.0 Hz), 7.32 (d, 2H, J = 8.8 Hz), 6.68 (s, 1H), 3.48 (m, 8H), 2.90 (t, 2H, J = 8.0, 6.8 Hz), 2.75 (t, 2H, J = 8.0, 6.8 Hz). ESI-MS 432 $[M+H]^+$, 454 $[M+Na]^+$. Anal. ($C_{20}H_{19}Cl_2N_5O_2$): C, H, N calc. 55.57, 4.43, 16.20 found 55.41, 4.41, 15.99.

4-{3-[1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl]propanoyl}piperidine 5f

Yield 37%. FT-IR (KBr) cm^{-1} 1634. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 8.15 (d, 1H, J = 9.6 Hz), 8.08 (d, 1H, J = 8.8 Hz), 7.43 (d, 2H, J = 8.4 Hz), 7.32 (d, 2H, J = 8.8 Hz), 6.68 (s, 1H), 3.42 (m, 4H), 2.88 (t, 2H, J =8.0, 7.2 Hz), 2.73 (t, 2H, J = 8.0, 7.2 Hz), 1.47 (m, 6H). ESI-MS 430 $[M+H]^+$, 452 $[M+Na]^+$. Anal. ($C_{21}H_{21}Cl_2N_5O$): C, H, N calc. 58.61, 4.92, 16.27 found 58.69, 4.79, 16.12.

1-{3-[1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl]propanoyl}-4-[(4-fluorophenyl)]piperazine 5g

Yield 76%. FT-IR (KBr) cm^{-1} 1636. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 8.13 (d, 1H, J = 9.2 Hz), 8.03 (d, 1H, J = 9.6 Hz), 7.40 (d, 2H, J = 8.8 Hz), 7.30 (d, 2H, J = 8.4 Hz), 7.03 (t, 2H, J = 9.2, 8.8 Hz), 6.92 (m, 2H), 6.68 (s, 1H), 3.59 (m, 4H), 3.05 (m, 4H), 2.90 (t, 2H, J = 7.6, 6.8 Hz), 2.79 (t, 2H, J = 8.0, 6.4 Hz). Anal. ($C_{26}H_{23}Cl_2FN_6O$): C, H, N calc. 59.44, 4.41, 16.00 found 59.40, 4.47, 15.74.

1-{3-[1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl]propanoyl}-4-[(3-chlorophenyl)]piperazine 5h

Yield 40.8%. FT-IR (KBr) cm^{-1} 1642. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 8.12 (d, 1H, J = 9.2 Hz), 8.03 (d, 1H, J = 9.2 Hz), 7.39 (d, 2H, J = 8.0 Hz), 7.30 (d, 2H, J = 8.4 Hz), 7.18 (t, 2H, J = 8.0 Hz), 6.86 (bd, 1H, J = 8.4 Hz), 6.77 (bd, 1H, J = 7.6 Hz), 6.67 (s, 1H), 3.58 (m, 4H), 3.13 (m, 4H), 2.91 (t, 2H, J = 8.0,

6.4 Hz), 2.79 (t, 2H, J = 7.6, 6.8 Hz). Anal. ($C_{26}H_{23}Cl_3N_6O$): C, H, N calc. 57.63, 4.28, 15.51 found 57.65, 4.35, 15.37.

1-{3-[1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl]propanoyl}-4-[(2-fluorophenyl)]piperazine 5i

Yield 71%. FT-IR (KBr) cm^{-1} 1628. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 8.14 (d, 1H, J = 8.8 Hz), 8.05 (d, 1H, J = 8.8 Hz), 7.40 (d, 2H, J = 8.8 Hz), 7.31 (d, 2H, J = 8.0 Hz), 7.10 (m, 2H), 6.97 (m, 2H), 6.68 (s, 1H), 3.60 (m, 2H), 2.95 (m, 6H), 2.80 (t, 2H, J = 7.6, 6.8 Hz), 2.68 (t, 2H, J = 8.0, 6.4 Hz). Anal. ($C_{26}H_{23}Cl_2FN_6O$. H_2O): C, H, N calc. 57.47, 4.64, 15.47 found 57.25, 4.65, 14.99.

1-{3-[1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl]propanoyl}-4-[(2-pyridyl)] piperazine 5j

Yield 35%. FT-IR (KBr) cm^{-1} 1639. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 8.13 (d, 1H, J = 8.0 Hz), 8.09 (bd, 1H, J = 4.4 Hz), 8.04 (d, 1H, J = 8.4 Hz), 7.51 (t, 1H, J = 8.8, 7.6 Hz), 7.39 (d, 2H, J = 8.0 Hz), 7.31 (d, 2H, J = 7.6 Hz), 6.80 (d, 1H, J = 8.4 Hz), 6.68 (s, 1H), 6.63 (t, 1H, J = 6.4, 5.6 Hz), 3.49 (m, 8H), 2.91 (t, 2H, J = 7.6, 6.8 Hz), 2.80 (t, 2H, J = 8.0, 6.4 Hz). Anal. ($C_{25}H_{23}Cl_2N_7O$): C, H, N calc. 59.06, 4.56, 19.29 found 58.80, 4.53, 18.95.

1-{3-[1-(6-chloropyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl]propanoyl}-4-{[(3-trifluoromethyl)phenyl]}piperazine 5k

Yield 30%. FT-IR (KBr) cm^{-1} 1639. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 8.13 (d, 1H, J = 8.8 Hz), 8.03 (d, 1H, J = 9.2 Hz), 7.40 (m, 3H), 7.30 (d, 2H, J = 8.0 Hz), 7.20 (bd, 1H, J = 8.4 Hz), 7.15 (s, 1H), 7.06 (d, 1H, J = 7.6 Hz), 6.68 (s, 1H), 3.61 (m, 4H), 3.19 (m, 4H), 2.91 (t, 2H, J = 7.6, 6.8 Hz), 2.80 (t, 2H, J = 8.0, 6.8 Hz). Anal. ($C_{27}H_{23}Cl_2F_3N_6O$): C, H, N calc. 56.36, 4.03, 14.61 found 56.38, 4.03, 14.63.

1-{3-[1-(6-methoxypyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl]propanoyl}-4-{[(3-trifluoromethyl)phenyl]}piperazine 5l

Yield 59.2%. FT-IR (KBr) cm^{-1} 1640. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 7.81 (d, 1H, J = 9.2 Hz), 7.27 (m, 4H), 7.05 (m, 5H), 6.50 (s, 1H), 3.84 (s, 3H), 3.49 (m, 4H), 3.01 (m, 4H), 2.78 (t, 2H, J = 8.0 Hz), 2.67 (t, 2H, J = 7.6 Hz). Anal. ($C_{28}H_{26}ClF_3N_6O_2$): C, H, N calc. 58.90, 4.59, 14.72 found 58.77, 4.52, 14.81.

1-{3-[1-(6-methoxypyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl]propanoyl}-4-[(4-fluoro) phenyl]piperazine 5m

Yield 63.7%. FT-IR (KBr) cm^{-1} 1638. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 7.80 (d, 1H, J = 8.8 Hz), 7.27 (m, 3H), 7.12 (d, 2H, J = 8.4 Hz), 6.91 (t, 2H, J = 9.2, 8.4 Hz), 6.80 (m, 2H), 6.50 (s, 1H), 3.84 (s, 3H), 3.47 (m, 4H), 2.89 (m, 4H), 2.77 (t, 2H, J = 7.6 Hz), 2.66 (t, 2H, J = 8.0 Hz). Anal. ($C_{27}H_{26}ClFN_6O_2$): C, H, N calc. 62.25, 5.03, 16.13 found 62.12, 4.91, 16.14.

1-{3-[1-(6-methoxypyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl]propanoyl}-4-[(3-chloro) phenyl]piperazine 5n

Yield 58.4%. FT-IR (KBr) cm^{-1} 1638. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 7.82 (d, 1H, J = 9.2 Hz), 7.27 (dd, 3H, J = 9.2, 8.4 Hz), 7.12 (d, 2H, J = 8.8 Hz), 7.07 (t, 1H, J = 8.4 Hz), 6.76 (m, 2H), 6.66 (d, 1H, J = 8.0 Hz), 6.50 (s, 1H), 3.84 (s, 3H), 3.47 (m, 4H), 3.01 (m, 4H), 2.77 (t, 2H, J = 8.0 Hz), 2.66 (t, 2H, J = 7.6 Hz). Anal. ($C_{27}H_{26}Cl_2N_6O_2$): C, H, N calc. 60.34, 4.88, 15.64 found 60.03, 4.60, 15.65.

1-{3-[1-(6-methoxypyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl]propanoyl}-4-phenylpiperazine 5o

Yield 70%. FT-IR (KBr) cm^{-1} 1647. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 7.94 (d, 1H, J = 9.2 Hz), 7.38 (m, 3H), 7.23 (m, 4H), 6.91 (d, 2H, J = 8.0 Hz), 6.78 (t, 1H, J = 8.8, 7.2 Hz), 6.62 (s, 1H), 3.96 (s, 3H), 3.60 (m, 4H), 3.07 (m, 4H), 2.90 (t, 2H, J = 8.0 Hz), 2.78 (t, 2H, J = 7.6 Hz). Anal. ($C_{27}H_{27}ClN_6O_2$): C, H, N calc. 64.47, 5.41, 16.71 found 64.37, 5.24, 16.61.

N-butyl-3-(1-(6-methoxypyridazin-3-yl)-5-(4-chlorophenyl)-1H-pyrazol-3-yl)propanamide 5p

Yield 68.3%. FT-IR (KBr) cm^{-1} 1634. ^{1}H-NMR (400 MHz, DMSO-d_6)δ 7.94 (d, 1H, J= 8.8 Hz), 7.86 (t, 1H, J= 5.6 Hz), 7.42 (m, 3H), 7.26 (d, 2H, J= 8.4 Hz), 6.55 (s, 1H), 3.97 (s, 3H), 3.03 (q, 2H, J = 5.6 Hz), 2.87 (t, 2H, J = 8.0, 7.2 Hz), 1.33 (m, 3H), 1.22 (m, 3H), 0.79 (t, 3H, J= 7.6, 7.2 Hz). Anal. ($C_{21}H_{24}ClN_5O_2$): C, H, N calc. 60.94, 5.84, 16.92 found 60.56, 5.72, 16.92.

Pharmacology

Materials

Male Swiss albino mice weighing 18-25 g from the animal breeding Laboratories of the Refik Saydam Hıfzısıhha Institute in Ankara, Turkey, were used for all experiments. The mice were kept in groups of 6 in a temperature-controlled room and synchronized by 12:12 light-dark cycle, maintained on a standard pellet diet with water given ad libitum and left for at least 3 days for acclimatization before the experimental sessions. For each of the compounds tested, for reference (aspirin), and for controls, a group of animals comprising n = 5-9, n = 13, and n = 11 mice were used, respectively. All experiments were carried out according to the suggested ethical guidelines for the care of laboratory animals approved by the Ethics Committee of Gazi University.

Preparation of test samples for bioassay

Test samples (100 mg/kg), suspended in 0.5% carboxymethyl cellulose (0.5% CMC in distilled water), were given orally to the animals. Control animals received the same experimental handling as the test groups with the exception that the drug treatment was replaced with an appropriate volume of the dosing vehicle (0.5% CMC, 10 mL/kg). Acetyl salicylic acid (100 mg/kg) in 0.5% CMC was used as the reference drug.

Acetic Acid Induced Writhing Test[16]

The mice were intraperitoneally injected with 0.8 % (v/v) acetic acid solution in distilled water (0.1 mL/10 g bodyweight) 30 min after drug administration. The mice were housed individually for observation after acetic acid injection, and the total number of abdominal contractions (writhing movements) was counted for 15 min. The analgesic activity was expressed as the percentage change compared to writhing controls.

% analgesic activity was determined by the following formula:

$$\% \text{ Analgesic Activity} = 100 - \left(\frac{\text{drug}}{\text{control}} \times 100\right)$$

The values of % antinociception were shown as normalized by arcsin transformation:[17]

$$\arcsin \sqrt{\%\text{analgesic activity}/100}$$

Statistical Analysis Data were presented as means ± SEM. One-way ANOVA followed by post-hoc Student-Newman-Keuls was used for statistical analysis. $P < 0.05$ was considered significant.

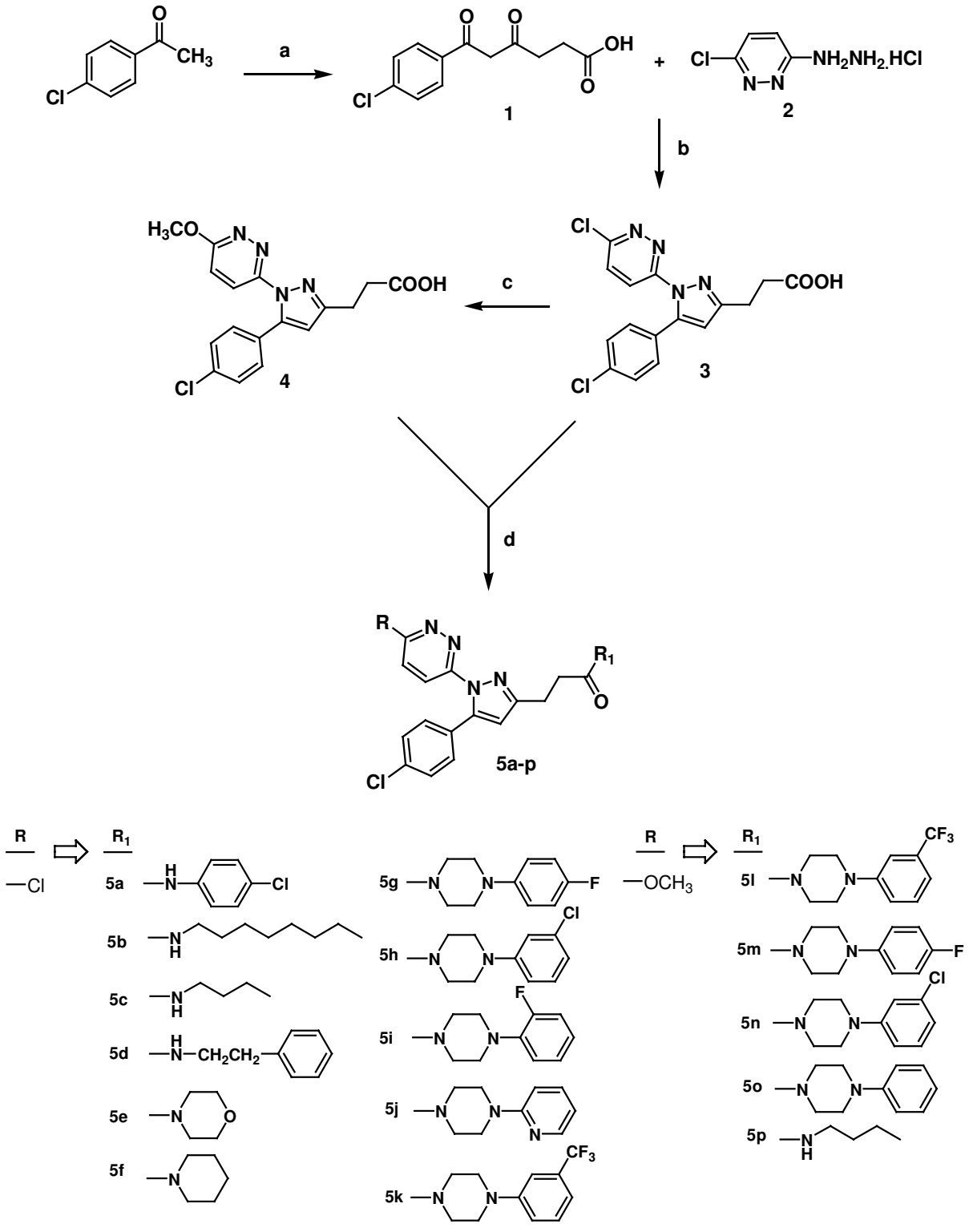
Results and Discussion

Chemistry

The synthetic route for the synthesis of the title compounds is shown in Figure 3. Accordingly, condensation of readily available p-chloroacetophenone with succinic anhydride provided an efficient route to 6-(4-chlorophenyl)-4,6-dioxohexanoic acid (**1**). This condensation was carried out in the presence of an alkoxide base in a polar aprotic solvent at an initial temperature of 0 °C, and then heating to a temperature of 50 °C under anhydrous conditions. Subsequently, the 1,5-diaryl-3-substituted pyrazole derivative **3** was generated by carrying out the condensation in the presence of the hydrochloride salt of 3-chloro-6-hydrazinopyridazine (**2**) in refluxing methanol. This carboxylic acid derivative was then refluxed in methanol in the presence of sodium methoxide to obtain methoxypyridazine derivative **4**. By treatment of **3** or **4** with appropriate amines in the presence of triethylamine and ethyl chloroformate, which was used as the carboxylate activator, the resulting amide derivatives **5** were prepared in moderate to good yield (30%-76%).

Pharmacology

Initial screening of the analgesic activity of the free carboxylic acid **3** and synthesized amide derivatives **5a-p** was assessed in mice by using an acetic acid induced writhing test.[16]



a) i. t-BuOK, DMF, 0 °C, ii. succinic anhydride, DMF, 0-55 °C, iii. H^+; b) NEt_3, MeOH, reflux; c) CH_3ONa, reflux; d) i. ClCOOEt, NEt_3, DCM, ii. appropriate amine derivative

Figure 3. Synthetic pathways of the amide derivatives of 3-[1-(3-pyridazinyl)-5-phenyl-1H-pyrazole-3-yl]propanoic acids.

Table. Analgesic activity of the synthesized compounds.

Compound #	Analgesic activity (%)
3	36.78 ± 2.7 (n = 5)
5a	63.73 ± 5.4*(n = 9)
5b	50.34 ± 5.7 (n = 5)
5c	47.85 ± 4.3 (n = 6)
5d	28.61 ± 6.9 (n = 9)
5e	43.84 ± 4.7 (n = 6)
5f	59.32 ± 6.7* (n = 6)
5g	44.48 ± 6.2 (n = 6)
5h	51.81 ± 3.8 (n = 6)
5i	38.04 ± 4.8 (n = 8)
5j	51.43 ± 5.4 (n = 6)
5k	53.53 ± 3.4 (n = 6)
5l	43.12 ± 4.4 (n = 8)
5m	44.86 ± 10.5 (n = 6)
5n	63.21 ± 5.5* (n = 9)
5o	60.83 ± 10.1* (n = 6)
5p	43.11 ± 8 (n = 6)
Aspirin	51.54 ± 5.5 (n = 13)

*statistically significant difference between **5d** and related compounds (**5d** vs. **5o**, **5d** vs. **5f** ($P < 0.05$); **5d** vs. **5n**, **5d** vs. **5a** ($P < 0.01$).

As seen in the Table, compounds having 4-chlorophenyl **5a**, 4-pyridyl **5f**, 4-(3-chlorophenyl)piperazine **5h**, and 4-(3-trifluoromethylphenyl)piperazine **5k** in the amide portion where the pyridazine is substituted with chlorine atom at the 6 position, and compounds having 4-(3-chlorophenyl)piperazine **5n**, 4-phenylpiperazine **5o** in the amide portion in 6-methoxypyridazine derivatives showed activity higher than aspirin, which served as the reference drug in the assays, although these results did not show statistical significance. Statistical results indicate that only compound **5d** showed a significant difference regarding analgesic activity when compared with derivatives **5a, 5f, 5n**, and **5o**. Meanwhile, all other amide derivatives shown in the Table resulted in activity approximately equipotent to aspirin at the same oral dose of 100 mg/kg. These results correlated well with our previous studies[18] indicating that amidation of free carboxylic acid contributes to analgesic activity.

Analgesic activity of secondary amide derivatives **5a-d, p** seems to be sensitive to electronic effects of the substituent in the amide portion since while the aromatic amide derivative **5a** showed potent analgesic activity the derivatives possessing alkyl amide groups **5b, c, p** had diminished analgesic activity although it did not reach statistical significance. This is also supported by the compound in which the incorporation of ethylene linker between the amide nitrogen and a terminal phenyl ring also caused a detrimental effect on analgesic activity, as illustrated by 2-phenethyl amide analog **5d**. Among the tertiary amide derivatives, the 3-chlorophenylpiperazine **5n** and phenylpiperazine **5o** derivatives in which the pyridazine ring is substituted with a methoxy group showed higher but not significantly different analgesic activity than aspirin.

Introduction of a chloro substituent at the 6 position of the pyridazine ring **5h** had a reducing effect on analgesic activity when compared to **5n**. In addition, tertiary amide derivative including piperidinyl side chain **5f** resulted in higher analgesic activity, while the replacement of piperidin **5f** with morpholine **5e** led to poor analgesic activity.

Some recent studies for developing safer analgesic and anti-inflammatory drugs have concentrated on the preparation of the amide derivatives of well-established NSAID templates with free carboxylic acid such as indometacin[9] and meclofenamic acid.[10] In these studies, it was observed that neutralization of the NSAIDs accomplished by preparing amide derivatives resulted in compounds with good analgesic and anti-inflammatory activity and with no gastric side effects in animal models. Based on this approach, in our previous work dealing with [6-(5-methyl-3-phenylpyrazole-1-yl)-3(2*H*)-pyridazinone-2-yl]acetamides,[18] we found that certain amide derivatives including 4-fluorophenylpiperazine, 4-phenylpiperazine, 4-(2-pyridyl)piperazine, 4-methoxyphenyl, and the $N-$octyl in the amide portion showed superior analgesic activity compared to the reference compound aspirin used in the assays. Dogruer et al. also reported that, in the case of [6-(4-methoxyphenyl)-3(2*H*)-pyridazinone-2-yl]acetamide and propanamide derivatives, the highest analgesic activity was observed with 4-fluorophenylpiperazine derivative in the amide portion of the compounds.[8] Other published research from different laboratories also indicated that the presence of substituted phenylpiperazine moiety at the amide side chain of the pyridazinone ring had a positive influence on their analgesic activity.[11,14,19–21]

Thus, this preliminary study of amidation of 3-(1-(6-chloro/methoxy-pyridazine-3-yl)-5-(4-chlorophenyl)-1H-pyrazole-3-yl)propanoic acids bearing the 1,5-diarylpyrazole template in the structure, which is well established for analgesic and anti-inflammatory activity,[22,23] indicates that the preparation of certain amide derivatives of free carboxylic acid derivatives might be important for good analgesic activity. Therefore, our initial screening results demonstrate that the presence of certain arylpiperazine and aromatic amine substituents in the amide portion might contribute to their analgesic activity. These types of compounds along with our previously published results[18] might lead to further studies for developing better candidates with potent analgesic and anti-inflammatory activities. The preparation of distinct amide derivatives based on this initial screening study is currently under investigation in our laboratory.

References

1. D. Clinch, A.K. Banerjee, G. Ostick and D.W. Levy, **J.R. Coll. Physicians Lond. 17**, 228-30 (1983).
2. P. Brooks, P. Emery, J.F. Evans, H. Fenner, C.J. Hawkey, C. Patrono, J. Smolen, F. Breedveld, R. Day, M. Dougados, E.W. Ehrich, J. Gijon-Banos, T.K. Kvien, M.H. Van Rijswijk, T. Warner and H. Zeidler, **Rheumatology**, **38**, 779-88 (1999).
3. S. Leone, A. Ottani, and A. Bertolini, **Curr. Topics Med. Chem.**, **7**, 265-75 (2007).
4. V. Dal Piaz, M.P. Giovannoni, G. Ciciani, D. Barlocco, G. Giardina, G. Petrone and G.D. Clarke, **Eur. J. Med. Chem. 31**, 65-70 (1996).
5. P. Coudert, C. Rubat, F. Rohet, F. Leal, J. Fialip and J. Couquelet, **Pharm. Pharmacol. Commun. 6**, 387-96 (2000).
6. V.K. Chintakunta, V. Akella, M.S. Vedula, P.K. Mamnoor, P. Mishra, S.R. Casturi, A. Vangoori and R. Rajagopalan, **Eur. J. Med. Chem., 37**, 339-47 (2002).

7. M. Takaya, M. Sato, K. Terashima and H. Tanizawa, **J. Med. Chem. 22**, 53-58 (1979).
8. D.S. Dogruer, M.F. Sahin, S. Ünlü and I. Shigeru, **Arch. Pharm. Pharm. Med. Chem., 333**, 79-86 (2000).
9. A.S. Kalgutkar, A.B. Marnett, B.C. Crews, R.P. Remel and L.J. Marnett, **J. Med. Chem. 43**, 2860-70 (2000).
10. A.S. Kalgutkar, S.W. Rowlinson, B.C. Crews and L.J. Marnett, **Bioorg. Med. Chem. Lett. 12**, 521-24 (2002).
11. S. Moreau, P. Coudert, C. Rubat, E. Albuisson and J. Couquelet, **Arzneim.-Forsch./Drug Res. 46**, 800-05 (1996).
12. B. Okçelik, S. Ünlü, E. Banoglu, E. Küpeli, E. Yesilada and M.F. Sahin, **Arch. Pharm. Pharm. Med. Chem. 336**, 406-12 (2003).
13. S. Pieretti, V. Dal Piaz, R. Matucci, M.P. Giovannoni and A. Galli, **Life Sci. 65**, 1381-94 (1999).
14. F. Rohet, C. Rubat, P. Coudert, E. Albuisson and J. Couquelet, **Chem. Pharm. Bull. 44**, 980-86 (1996).
15. M.P. Wachter, 1993, U.S. Patent 5, 242, 940; A. Abdel-Magid, 2002, U.S. Patent 6, 384, 233; W.V. Murray, 1998, E.P. 0293221.
16. R. Koster, M. Anderson and E.J. Debeer, **Fed. Proc**. **18**, 412-18 (1959).
17. S. Altug, O. Uludag, B. Tunctan, I. Cakici, H. Zengil and N. Abacioglu, **Chronobiol**. **Int. 23**, 583-591 (2006).
18. E. Banoglu, Ç. Akoglu, S. Unlu, E. Küpeli, E. Yesilada and M.F. Sahin, **Arch. Pharm. Pharm. Med. Chem. 337**, 7-14 (2004).
19. C. Rubat, P. Coudert, P. Tronche, J. Bastide, P. Bastide and A.M. Privat, **Chem. Pharm. Bull**. **37**, 2832-35 (1989).
20. C. Rubat, P. Coudert, E. Albuisson, J. Bastide, J. Couquelet and P. Tronche, **J. Pharm. Sci**. **81**, 1084-87 (1992).
21. M. Gokce, D. Dogruer and M.F. Sahin, **Farmaco 56**, 233-37 (2001).
22. K. Tsuji, K. Nakamura, N. Konishi, T. Tojo, T. Ochi, H. Senoh and M. Matsuo, **Chem. Pharm. Bull. 45**, 987-99 (1997).
23. J.J. Talley, S.R. Bertenshaw, J.S. Carter, P.W. Collins, S. Docter, M.J. Graneto, P.C. Isakson, L.F. Lee, J.W. Malecha, J.M. Miyashiro, T.D. Penning, R.S. Rogers, D.J. Rogier, S.S. Yu, G.D. Anderson, E.G. Burton, J.N. Cogburn, S.A. Gregory, C.M. Koboldt, W.E. Perkins, K. Seibert, A.W. Veehuizen and Y.Y. Zhang, **Act. Chim. Therap**. **25**, 123-134 (1999).