Turk J Chem
35 (2011) , 121 – 130.

doi:10.3906/kim-1004-584

Synthesis and evaluation of the analgesic activity of some new isoxazolo[4,5-d]pyridazin-4(5*H*)-one derivatives

Oya ÜNSAL TAN[1,*], Keriman ÖZADALI[1], Özgür YEŞİLYURT[2], Hakan KAYIR[2], Tayfun UZBAY[2] and Ayla BALKAN[1]

[1] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, Ankara-TURKEY*

e-mail: oyaunsal@hacettepe.edu.tr

[2] *Psychopharmacology Research Unit, Department of Medical Pharmacology, Gülhane Military Medical Academy, Ankara-TURKEY*

Received 27.04.2010

Some isoxazolo[4,5-d]pyridazin4(5*H*)-one derivatives were synthesized and tested for their analgesic activity. The analgesic activities of the compounds were determined by hot-plate and acetic acid writhing tests using morphine and diclofenac as references. Compounds **4a**, **4f**, **4g**, and **4i** (25 mg/kg) showed analgesic profiles similar to that of morphine (5 mg/kg).

Key Words: Isoxazolo[4,5-d]pyridazin-4(5*H*)-one, hot-plate test, writhing test, analgesic effect

Introduction

3(2H)-Pyridazinones are heterocyclic systems that have been widely studied due to their different biological activities.[1−7] Condensed 3(2H)-pyridazinones have become interesting structures for medicinal chemists.[8,9] However, the biological properties of isoxazolo[4,5-d]pyridazin-4(5*H*)-ones, which are condensed 3(2H)-pyridazinone derivatives, are not well known even though they were described by Erichomovitch and Desimoni in the 1960s.[10,11]

One of the most extensive studies on biological activities of isoxazolopyridazinones was done by Giovannoni and co-workers.[12] They evaluated the analgesic activities of a series of isoxazolo[3,4-d]- and isoxazolo[4,5-d]pyridazinone derivatives. They reported that 5-{[4-(3-chlorophenyl)piperazine-1-yl]propyl}-3-methyl-7-phenylisoxazolo[4,5-d]pyridazin-4(5*H*)-one (Figure 1) showed higher analgesic activity than morphine. This interest-

*Corresponding author

ing result prompted us to synthesize and screen the analgesic activity of a series of isoxazolo[4,5-d]pyridazine-4(5*H*)-ones.

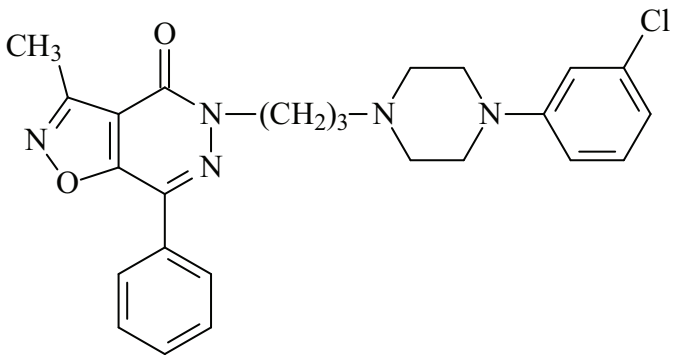

Figure 1. Structure of 5-{[4-(3-chlorophenyl)-piperazine-1-yl]propyl}-3-methyl-7-phenylisoxazolo[4,5-d]pyridazin-4(5*H*)-one.

Experimental

Chemistry

All chemicals used in this study were supplied by Aldrich (Steinheim, Germany). Melting points were determined by a Thomas Hoover capillary melting point apparatus (Philadelphia, PA, USA) and are uncorrected. IR spectra (KBr disc) were recorded on a Bruker Vector 22 IR (Beaconsfield, UK). The ^{1}H-NMR spectra (DMSO-d$_6$) were recorded on a Varian Mercury 400 FT NMR spectrophotometer using TMS as an internal reference (chemical shift represented in δ ppm). The ESI-MS spectra were measured on a Micromass ZQ-4000 single quadruple mass spectrometer.

The purity of the compounds was controlled by thin layer chromatography (Merck, silicagel, HF$_{254+366}$, type 60, 0.25 mm, Darmstadt, Germany). The elemental analyses (C, H, N) were performed using a Leco CHNS 932 (Leco Coop, St. Joseph, MI, USA) analyzer by the Scientific and Technological Research Council of Turkey Instrumental Analysis Laboratories (Ankara, Turkey). The elemental analysis results were within 0.4% of theoretical values.

Methyl 2-methyl-4-oxo-4,5-dihydrofuran-3-carboxylate 1

This compound was prepared according to the literature method.[13]

General procedure for the preparation of methyl 5-substituted benzylidene-2-methyl-4-oxo-4,5-dihydrofuran-3-carboxylates 2a-i

1 (3.25 mmol) and an equimolar amount of appropriate benzaldehyde were dissolved in 25 mL of anhydrous benzene. p-Toluensulfonic acid (0.08 g) was added as a catalytic reagent. The mixture was then heated with a Dean-Stark separator for 2 h. Benzene was evaporated and residue recrystallized from an ethyl acetate and n-hexane mixture (1:1).

Methyl 5-benzylidene-2-methyl-4-oxo-4,5-dihydrofuran-3-carboxylate 2a

Yield 64%. Mp 118-9 °C. IR (KBr); 1707 (C=O, ester), 1655 (C=O, ring). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.75 (3H, s, -CH_3), 3.76 (3H, s, -OCH_3), 6.90 (1H, s, -CH=), 7.5-7.52 (3H, m, phenyl-H_3, H_4, H_5), 7.92-7.95 (2H, m, phenyl-H_2, H_6). ESI-MS (m/z); 267 $[M+Na]^+$ (100%), 245 $[M+H]^+$.

Methyl 5-(2-chlorobenzylidene)-2-methyl-4-oxo-4,5-dihydrofuran-3-carboxylate 2b

Yield 73%. Mp 131-2 °C. IR: 1711 (C=O, ester), 1654 (C=O, ring). ESI-MS (m/z); 303 $[M+Na+2]^+$, 301 $[M+Na]^+$ (100%), 279 $[M+H]^+$, 247, 102, 73.

Methyl 5-(4-chlorobenzylidene)-2-methyl-4-oxo-4,5-dihydrofuran-3-carboxylate 2c

Yield 55%. Mp 148-9 °C. IR: 1714 (C=O, ester), 1646 (C=O, ring), ESI-MS (m/z); 303 $[M+Na+2]^+$, 301 $[M+Na]^+$ (100%), 279 $[M+H]^+$, 247, 102, 73.

Methyl 5-(2-methylbenzylidene)-2-methyl-4-oxo-4,5-dihydrofuran-3-carboxylate 2d

Yield 21%. Mp 146 °C (dec.). IR: 1703 (C=O, ester), 1646 (C=O, ring), ESI-MS (m/z); 281 $[M+Na]^+$ (100%), 259 $[M+H]^+$, 188, 160, 102, 73.

Methyl 5-(4-methylbenzylidene)-2-methyl-4-oxo-4,5-dihydrofuran-3-carboxylate 2e

Yield 31%. Mp 149-50 °C. IR: 1708 (C=O, ester), 1649 (C=O, ring), ESI-MS (m/z); 281 $[M+Na]^+$ (100%), 259 $[M+H]^+$, 188, 102, 73.

Methyl 5-(2-nitrobenzylidene)-2-methyl-4-oxo-4,5-dihydrofuran-3-carboxylate 2f

Yield 60%. Mp 122-3 °C. IR: 1714 (C=O, ester), 1653 (C=O, ring), 1522, 1343 (N=O), ESI-MS (m/z); 312 $[M+Na]^+$ (100%), 290 $[M+H]^+$, 243, 217, 188, 160, 102, 73.

Methyl 5-(4-nitrobenzylidene)-2-methyl-4-oxo-4,5-dihydrofuran-3-carboxylate 2g

Yield 62%. Mp 202 °C (dec.). IR: 1706 (C=O, ester), 1655 (C=O, ring), 1512, 1341 (N=O), ESI-MS (m/z); 312 $[M+Na]^+$ (100%), 290 $[M+H]^+$, 243, 217, 188, 160, 102, 73.

Methyl 5-(2-methoxybenzylidene)-2-methyl-4-oxo-4,5-dihydrofuran-3-carboxylate 2h

Yield 70%. Mp 161 °C (dec.). IR: 1704 (C=O, ester), 1659 (C=O, ring), ESI-MS (m/z); 297 $[M+Na]^+$ (100%), 275 $[M+H]^+$, 102, 73.

Methyl 5-(4-methoxybenzylidene)-2-methyl-4-oxo-4,5-dihydrofuran-3-carboxylate 2i

Yield 52%. Mp 153 °C (dec.). IR: 1712 (C=O, ester), 1658 (C=O, ring). ESI-MS (m/z); 297 $[M+Na]^+$ (100%), 275 $[M+H]^+$, 102, 73.

General procedure for the preparation of methyl 5-(1-hydroxy-2-(substituted phenyl)vinyl)-3-methylisoxazole-4-carboxylates 3a-i

A mixture of appropriate **2** (0.01 mol), sodium acetate (0.012 mol), hydroxylamine hydrochloride (0.011 mol), water (10 mL), and ethanol (50 mL) was refluxed for 2 h. After cooling, yellow crystals were collected and recrystallized from ethanol to give pure compound.

Methyl 5-(1-hydroxy-2-phenylvinyl)-3-methylisoxazole-4-carboxylate 3a

Yield 66%. Mp 135-6 °C. IR (KBr); 1672 (C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.40 (3H, s, -CH_3), 3.85 (3H, s, -OCH_3), 6.27 (1H, s, -CH=), 7.33-7.39 (3H, m, phenyl-H_3, H_4, H_5), 7.72-7.82 (2H, m, phenyl-H_2, H_6), 10.53 (1H, s, -OH). ESI-MS (m/z); 282 $[M+Na]^+$ (100%), 260 $[M+H]^+$, 102.

Methyl 5-(1-hydroxy-2-(2-chlorophenyl)vinyl)-3-methylisoxazole-4-carboxylate 3b

Yield 36%. Mp 131 °C. IR: 1676 (C=O), ESI-MS (m/z); 318 $[M+Na+2]^+$, 316 $[M+Na]^+$ (100%), 294 $[M+H]^+$, 262, 130, 102, 82, 73.

Methyl 5-(1-hydroxy-2-(4-chlorophenyl)vinyl)-3-methylisoxazole-4-carboxylate 3c

Yield 39%. Mp 150-1 °C IR: 1677 (C=O), ESI-MS (m/z); 318 $[M+Na+2]^+$, 316 $[M+Na]^+$ (100%), 294 $[M+H]^+$, 262, 130, 102, 82, 73.

Methyl 5-(1-hydroxy-2-(2-methylhenyl)vinyl)-3-methylisoxazole-4-carboxylate 3d

The compound could not be isolated. The oily residue was directly used for the synthesis of compound **4d**.

Methyl 5-(1-hydroxy-2-(4-methylhenyl)vinyl)-3-methylisoxazole-4-carboxylate 3e

Yield 58%. Mp 162-3 °C IR: 1668 (C=O), ESI-MS (m/z); 296 $[M+Na]^+$ (100%), 274 $[M+H]^+$, 214, 102, 73.

Methyl 5-(1-hydroxy-2-(2-nitrophenyl)vinyl)-3-methylisoxazole-4-carboxylate 3f

Yield 60%. Mp 110-1 °C IR: 1677 (C=O), 1523, 1332 (N=O), ESI-MS (m/z); 327 $[M+Na]^+$ (100%), 305 $[M+H]^+$, 273, 122, 102, 82, 73.

Methyl 5-(1-hydroxy-2-(4-nitrophenyl)vinyl)-3-methylisoxazole-4-carboxylate 3g

Yield 63%. Mp 181 °C IR: 1674 (C=O), 1507, 1328 (N=O), ESI-MS (m/z); 327 $[M+Na]^+$ (100%), 305 $[M+H]^+$, 273, 163, 122, 102, 82, 73.

Methyl 5-(1-hydroxy-2-(2-methoxyphenyl)vinyl)-3-methylisoxazole-4-carboxylate 3h

Yield 49%. Mp 133 °C IR: 1674 (C=O), ESI-MS (m/z); 312 $[M+Na]^+$ (100%), 290 $[M+H]^+$, 273, 82.

Methyl 5-(1-hydroxy-2-(4-methoxyphenyl)vinyl)-3-methylisoxazole-4-carboxylate 3i

Yield 65%. Mp 156 °C IR: 1673 (C=O), ESI-MS (m/z); 312 $[M+Na]^+$ (100%), 290 $[M+H]^+$, 273, 102, 82.

General procedure for the preparation of 7-(substituted benzyl)-3-methylisoxazolo[4,5-d]pyridazin-4(5*H*)-ones 4a-i

The mixture of appropriate **3** (0.005 mol), hydrazine hydrate (0.01 mol), and ethanol (10 ml) was refluxed for 2 h. After cooling, the white precipitate was collected and recrystallized from ethanol to give pure compound.

7-Benzyl-3-methylisoxazolo[4,5-d]pyridazin-4(5*H*)-one (4a)

Yield 91%. Mp 182 °C (179 °C).[14] IR (KBr); 3176-2955 (CON-H), 1682 (C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz); δ 2.53 (3H, s, -CH_3), 4.20 (2H, s, -CH_2-), 7.23-7.34 (5H, m, phenyl), 13.07 (1H, br, NH). ESI-MS (m/z); 264 $[M+Na]^+$ (100%), 242 $[M+H]^+$, 104, 82.

7-(2-Chlorobenzyl)-3-methylisoxazolo[4,5-d]pyridazin-4(5*H*)-one (4b)

Yield 44%. Mp 206 °C. IR: 1701 (C=O), 1562 (C=N), ^{1}H-NMR: 2.55 (3H, s, -CH_3), 4.34 (2H, s, -CH_2-), 7.31-7.48 (4H, m, ar.), 13.04 (1H, br, NH). ESI-MS (m/z); 300 $[M+Na+2]^+$, 298 $[M+Na]^+$ (100%), 276 $[M+H]^+$, 240, 225, 125, 82.

7-(4-Chlorobenzyl)-3-methylisoxazolo[4,5-d]pyridazin-4(5*H*)-one (4c)

Yield 39%. Mp 206 °C (202 °C).[14] IR: 1689 (C=O), 1567 (C=N), ^{1}H-NMR: 2.54 (3H, s, CH_3), 4.22 (2H, s, -CH_2-), 7.34 (2H, d, ar., H_2, H_6, J: 8.4 Hz), 7.39 (2H, d, ar., H_3, H_5, J: 8.4 Hz), 13.10 (1H, br, NH). ESI-MS (m/z); 300 $[M+Na+2]^+$, 298 $[M+Na]^+$ (100%), 276 $[M+H]^+$, 240, 225, 125, 82.

7-(2-Methylbenzyl)-3-methylisoxazolo[4,5-d]pyridazin-4(5*H*)-one (4d)

Mp 191-2 °C. IR: 1688 (C=O), 1559 (C=N), ^{1}H-NMR: 2,28 (3H, s, -ar.-CH_3), 2.52 (3H, s, CH_3), 4.19 (2H, s, -CH_2-), 7.13-7.19 (4H, m, ar.), 13.05 (1H, br, NH), ESI-MS (m/z); 278 $[M+Na]^+$ (100%), 256 $[M+H]^+$, 151, 136, 102, 82, 73.

7-(4-Methylbenzyl)-3-methylisoxazolo[4,5-d]pyridazin-4(5*H*)-one (4e)

Yield 51%. Mp 202-3 °C. IR: 1680 (C=O), 1565 (C=N), ^{1}H-NMR: 2.22 (3H, s, -ar.-CH_3), 2.50 (3H, s, CH_3), 4.11 (2H, s, -CH_2-), 7.09 (2H, d, ar., J: 8.4 Hz), 7.15 (2H, d, ar., J: 8.4 Hz), 13.02 (1H, br, NH). ESI-MS (m/z); 278 $[M+Na]^+$ (100%), 256 $[M+H]^+$, 240, 225, 212, 82.

7-(2-Nitrobenzyl)-3-methylisoxazolo[4,5-d]pyridazin-4(5*H*)-one (4f)

Yield 35%. Mp 190-1 °C. IR: 1686 (C=O), 1579 (C=N), 1523, 1344 (N=O), ^{1}H-NMR: 2.57 (3H, s, CH_3), 4.60 (2H, s, -CH_2-), 7.57-7.65 (2H, m, ar., H_4,H_6), 7.75 (1H, t, ar., H_5), 8.10 (1H, d, ar., H_3), 12.95 (1H, br, NH). ESI-MS (m/z); 309 $[M+Na]^+$ (100%), 287 $[M+H]^+$, 240, 102, 73.

7-(4-Nitrobenzyl)-3-methylisoxazolo[4,5-d]pyridazin-4(5*H*)-one (4g)

Yield 31%. Mp>250 °C. IR: 1691 (C=O), 1571 (C=N), 1514, 1349 (N=O), ^{1}H-NMR: 2.55 (3H, s, CH_3), 4.40 (2H, s, -CH_2-), 7.61 (2H, d, ar., H_2, H_6, J: 8.8 Hz), 8.20 (2H, d, ar., H_3, H_5, J: 8.8 Hz), 13.13 (1H, br, NH). ESI-MS (m/z): 309 $[M+Na]^+$ (100%), 287 $[M+H]^+$, 102, 73.

7-(2-Methoxybenzyl)-3-methylisoxazolo[4,5-d]pyridazin-4(5*H*)-one (4h)

Yield 61%. Mp 186 °C. IR: 1688 (C=O), 1559 (C=N), ^{1}H-NMR: 2.52 (3H, s, CH_3), 3.79 (3H, s, OCH_3), 4.19 (2H, s, -CH_2-), 7.13-7.19 (4H, m, ar.), 13.05 (1H, br, NH), ESI-MS (m/z): 294 $[M+Na]^+$ (100%), 272 $[M+H]^+$, 225, 116, 82.

7-(4-Methoxybenzyl)-3-methylisoxazolo[4,5-d]pyridazin-4(5*H*)-one (4i)

Yield 70%. Mp 179 °C (176 °C).[14] IR: 1693 (C=O), 1565 (C=N), ^{1}H-NMR: 2.50 (3H, s, CH_3), 3.77 (3H, s, OCH_3), 4.11 (2H, s, -CH_2-), 7.09 (2H, d, ar.,H_3, H_5, J: 8.0 Hz), 7.15 (2H, d, ar., H_2, H_6, J: 8.0 Hz), 13.02 (1H, br, NH). ESI-MS (m/z): 294 $[M+Na]^+$ (100%), 272 $[M+H]^+$, 240, 225, 82.

Pharmacology

Animals: Adult male albino Swiss-Webster mice (25-30 g) were used. They were housed in a quiet and temperature and humidity controlled room (22 ± 2 °C and 60 ± 5%, respectively) in which a 12-h (light/dark) cycle was maintained (0700-1900 light). All experiments were performed at the same time of the day and in the light period (0900-1130).

All procedures in the present study are in accordance with the Guide for the Care and Use of Laboratory Animals as adapted by the National Institutes of Health (Washington, DC, USA, 1996). Local ethical committee approval was also obtained (March 21 2005; No: 05/22). All efforts were made to minimize animal suffering to reduce the number of animals used. Each animal was used only once and the animals were killed immediately after the termination of tests.

Evaluation of the analgesic effect

Analgesia was assessed by using acetic acid-induced writhing test and hot-plate test in mice. Each group consisted of 6-8 mice.

Writhing test: Writhing syndrome was elicited by ip injection of 0.3% acetic acid in a volume of 10 mL/kg. The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection. Injections of compounds and vehicle (0.5% CMC Na) to mice were done 60 min before testing. The degree of analgesia was expressed as the percentage decrease in the number of writhes and calculated according to the formula: % inhibitions of writhes= [(Control – Test)/Control] × 100 where control is the number of writhes in CMC Na-treated animals and test is the number of writhes in compound-treated animals.[15]

Hot-Plate test: The hot-plate test (52.5 ± 0.4 °C) (MAYCOM-9601) was used to assess paw withdrawal latency to a thermal pain stimulus. Each mouse was placed individually on a hot plate and the reaction time was measured starting from time the mouse was placed on the plate until the mouse either demonstrated hind

paw licking or jumping. We used the cut-off latency time as 90 s to prevent tissue damage. The percentage of the maximum possible effect (MPE%) was calculated for each animal using the following formula Eq. (1):

$$\%\text{MPE} = \frac{\left(\begin{smallmatrix}\text{Test}\\ \text{latency}\end{smallmatrix}\right) - \left(\begin{smallmatrix}\text{Pre-drug}\\ \text{latency}\end{smallmatrix}\right)}{(\text{Cut-off}) - \left(\begin{smallmatrix}\text{Pre-drug}\\ \text{latency}\end{smallmatrix}\right)}$$

where predrug latency is time elapsed from when the mouse was placed on the plate until the mouse either demonstrated hind paw licking or jumping before injection of any drugs and test latency is time elapsed during the same procedure after drug injection in the same mouse.[16]

Drugs: We used **4a-i**, morphine HCl, and diclofenac. Morphine HCl, diclofenac, and the compounds were suspended in 0.5% CMC Na (vehicle) and injected into the mice intraperitoneally. We used the doses of compounds according to the locomotor activity test results that did not inhibit locomotor activity (data not shown).

Statistical analysis: Results were expressed as mean ± S.E.M. The analgesic effects of the compound for each observation time were evaluated by one way analysis of variance (a one-way ANOVA) followed by Dunnet's test for post-hoc comparisons. The level of significance was set at the $P < 0.05$ level.

Results and discussion

Chemistry

The route used to synthesize 7-substituted benzyl-3-methylisoxazolo[4,5-d]pyridazin-4(5*H*)-ones **4a-i** is outlined in Figure 2. Some of these compounds (**4a**, **4c**, and **4i**) were synthesized by Chantegrel and co-workers[14] earlier. In our synthetic procedure, to use methyl acetoacetate instead of ethyl acetoacetate was the only exception.[13] We observed that the yield of **1** increased using methyl acetoacetate. The structures of our target compounds were confirmed using IR, ^{1}H-NMR, mass spectra, and elemental analysis (except **4a**, **4c**, and **4i**) data.

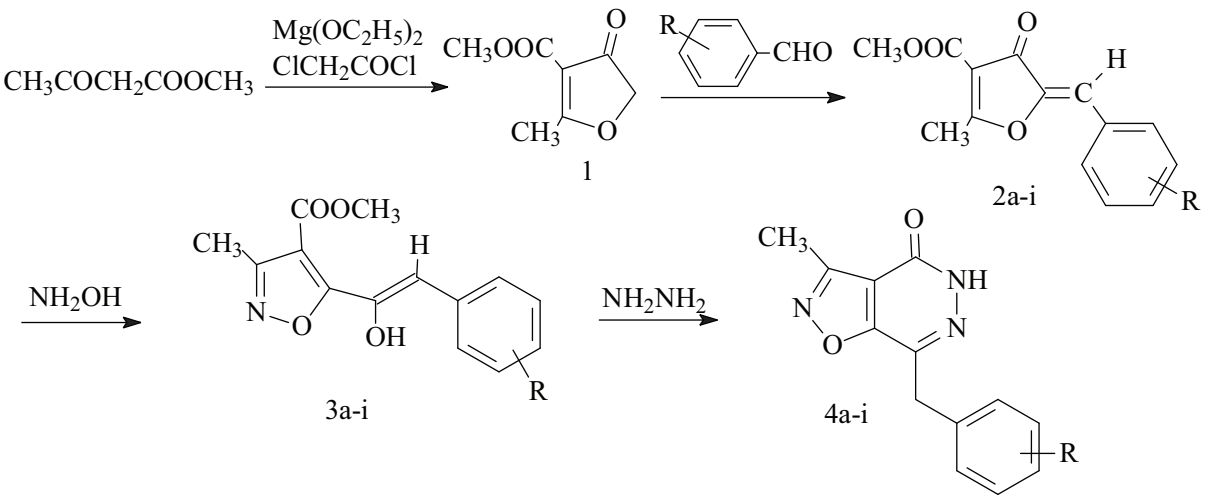

Figure 2. Synthetic route of **4a-i**.

Analgesic activity

The analgesic activities of the synthesized compounds in this study were measured using hot-plate and acetic acid induced writhing tests in mice.

Generally it is well known that opiates have analgesic effects in both writhing and hot-plate tests but non-steroidal antiinflammatory drugs (NSAIDs) have analgesic activity only in the writhing test. For this reason we evaluated the analgesic effect of morphine in both tests but we evaluated diclofenac only in the writhing test.

In the writhing test, it was observed that all compounds tested showed at least equal analgesic activity to diclofenac. However, these compounds had less activity in comparison with mice that received morphine 5 mg/kg. Compounds **4h** and **4i** (25 mg/kg), which had electron donating substituents on the phenyl ring, were the most potent compounds. In addition, **4a**, which had no substituent on the phenyl ring, showed moderate analgesic activity (Figure 3).

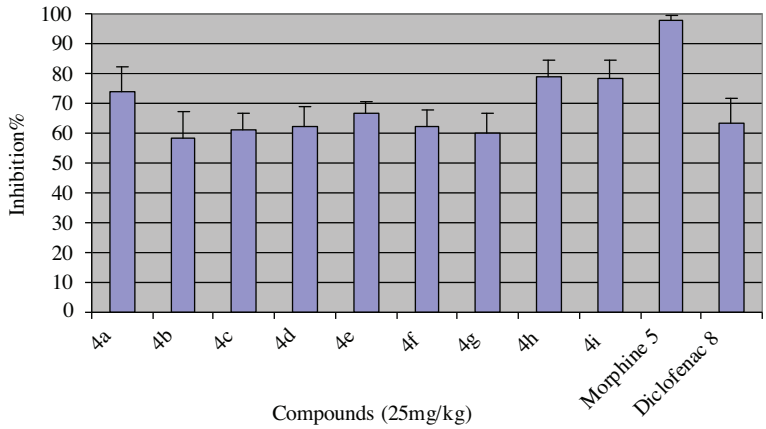

Figure 3. Analgesic effects of compounds **4a-i** in acetic acid-induced writhing test.

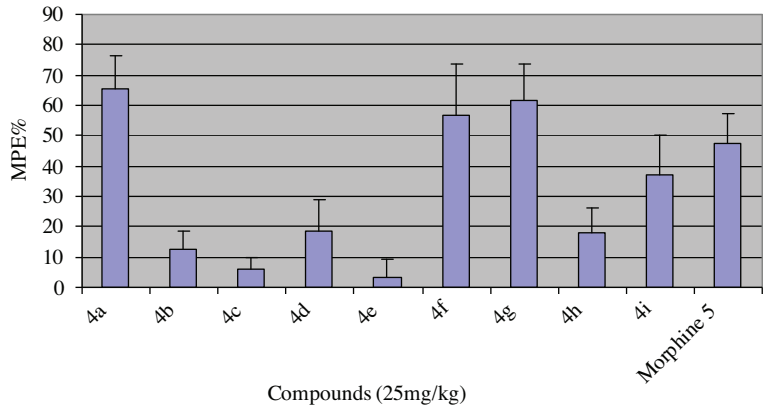

Figure 4. Analgesic effects of compounds **4a-i** in hot-plate test.

The results of the hot-plate test of all compounds are shown in the Table. Morphine increased paw withdrawal latency 47.47%. It was observed that **4a**, **4f**, **4g**, and **4i** (65.38%, 56.67%, 61.56%, and 36.82%,

respectively) had an analgesic effect similar to that of morphine. Among them **4a** was the most effective compound in the series. In addition to these results **4b-e** and **4h** did not demonstrate a significant effect (Figure 4).

Table. Effects of the compounds in acetic acid induced abdominal writhing and hot-plate tests.

Compounds	R	Doses (mg/kg) (mg/kg)	Inhibition on writhing test ± SEM	% MPE on Hot-plate test ±
Control	-	-	0.1 ± 8.93	1.11 ± 6.49
Morphine	-	5	97.97 ± 1.67*	47.47 ± 9.56*
Diclofenac	-	8	63.25 ± 8.54*#	-
4a	H	25	73.86 ± 8.29*#	65.38 ± 10.88*
4b	2-Cl	25	58.33 ± 8.92*#	12.33 ± 6.01
4c	4-Cl	25	61.36 ± 5.42*#	6.25 ± 3.81
4d	2-CH_3	25	62.5 ± 6.13*#	18.70 ± 10.22
4e	4-CH_3	25	66.66 ± 3.62*#	3.46 ± 6.00
4f	2-NO_2	25	62.12 ± 5.75*#	56.67 ± 16.75*
4g	4-NO_2	25	60.22 ± 6.36*#	61.56 ± 12.10*
4h	2-OCH_3	25	78.78 ± 5.46*#	17.99 ± 8.28
4i	4-OCH_3	25	78.40 ± 5.85*#	36.82 ± 13.18*

*P $<$ 0.05 significantly different from control. # P $<$ 0.05 significantly different from morphine. n = 6-8.

Conclusion

In the present study, 9 (6 of them are new compounds) isoxazolo[4,5-d]pyridazin-4(5H)-one derivatives were synthesized in order to evaluate their analgesic activities. According to the results we can conclude the following: (i) All compounds have an analgesic effect in the acetic acid-induced writhing test. (ii) The compounds (**4h** and **4i**) that have a methoxy substituent on the phenyl ring are the most potent compounds. (iii) Substitution of highly electron donating groups such as OH or NH_2 may be useful to increase activity in the writhing test. (iv) In the hot-plate test, the compounds carrying NO_2 groups (**4f** and **4g**) are the most potent ones among the substituted derivatives. (v) When the results of the 2 tests are considered together, it can be suggested that **4a**, **4f**, **4g**, and **4i** have analgesic profiles similar to that of morphine in acetic acid-induced writhing and hot-plate tests. However, **4b-4e** and **4h** showed an analgesic effect only in the writhing test, which means they do not have the same analgesic profile as morphine and the analgesic profiles of these compounds appear to be more similar to those of NSAIDs. (vi) Among the compounds, **4a** showed remarkable activity in all tests.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the Scientific Research Fund of Hacettepe University though Project 0501301001. The authors would also like to thank Mr. Selami Alan for his technical assistance during the study.

References

1. Curran, W. V.; Ross, A. *J. Med. Chem.* **1974**, *17*, 273-281.
2. Takaya, M.; Sato, M.; Terashima, K.; Tanizawa, H. *J. Med. Chem.* **1979**, *22*, 53-58.
3. Rubat, C.; Courdert, P.; Albuisson, E.; Bastide, J.; Couquelet, J.; Tronche, P. *J. Pharm. Sci.* **1992**, *81*, 1084-1089.
4. Dal Piaz, V.; Ciciani, G.; Giovannoni, M. *Il Farmaco*, **1997**, *52*, 173-178.
5. Balkan, A.; Özkanlı, F.; Ünsal, O.; Gören, Z.; Terzioğlu, B. *FABAD J. Pharm. Sci.* **2003**, *28*, 19-25.
6. Ünsal, O.; Balkan, A.; Aypak, C.; Terzioğlu, B.; Gören, Z. *FABAD J. Pharm. Sci.* **2004**, *29*, 185-194.
7. Betti, L.; Botta, M.; Corelli, F.; Floridi, M.; Fossa, P.; Giannaccini, G.; Manetti, F.; Strappaghetti, G.; Corsano, S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 437-440.
8. Strappaghetti, G.; Brodi, C.; Giannaccini, G.; Betti, L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2575-2579.
9. Pakulska, W.; Malinowski, Z.; Szczesniak, A. K.; Czarnecka, E.; Epsztajn, *J. Arch. Pharm. Chem. Life Sci.* **2009**, *342*, 41-47.
10. Erichomovitch, L.; Chubb, F. *Can. J. Chem.* **1966**, *44*, 2095-2100.
11. Desimoni, G.; Finzi, P. *Tetrahedron* **1967**, *23*, 681-685.
12. Giovannoni, M. P.; Vergelli, C.; Gherlardini, C.; Galeotti, N.; Bartolini, A.; Dal Piaz, V. *J. Med. Chem.* **2003**, *46*, 1055-1059.
13. Ünsal-Tan, O.; Özden, K.; Rauk, A.; Balkan, A. *Eur. J. Med. Chem.* **2010**, *45*, 2345-2352.
14. Chantegrel, B.; Deshayes, C.; Pujol, B. *J. Heterocycl. Chem.* **1990**, *27*, 927-34.
15. Balkan, A.; Gören, Z.; Urgun, H.; Çalış, Ü.; Çakar, A. N.; Atilla, P.; Uzbay, İ. T. *Arzneim.-Forsch./Drug Res.* **2002**, *52*, 462-467.
16. Uzbay, İ. T.; Çınar, M. G.; Aytemir, M.; Tuğlular, İ. *Life Sciences* **1999**, *64*, 1313-1319.