Journal of Enzyme Inhibition and Medicinal Chemistry, 2010; 25(1): 74–79

informa
healthcare

RESEARCH ARTICLE

Synthesis of some imidazolyl-thioacetyl-pyrazolinone derivatives and their antinociceptive and anticancer activities

Seref Demirayak[1], Ismail Kayagil[1], Leyla Yurttas[1], and Rana Aslan[2]

[1]Department of Pharmaceutical Chemistry, and [2]Department of Pharmacology, Faculty of Pharmacy, University of Anadolu, Eskisehir, Turkey

Abstract
In this study, some 4-(1,5-diarylimidazol-2-yl)thioacetyl-1-phenyl-2,3-dimethyl-3-pyrazoline-5-one derivatives were prepared by reacting 4-(2-chloroacetyl)-1-phenyl-2,3-dimethyl-3-pyrazoline-5-one and 2-mercapto-1, 5-diarylimidazole derivatives. The antinociceptive and anticancer activities of the compounds obtained were investigated. It was observed that some of the compounds, **2a**, **2d**, **2g**, and **2j**, showed remarkable antinociceptive activity, and one of the compounds, **2i**, showed weak anticancer activity.

Keywords: *1,5-diarylimidazole-2-thione; 3-pyrazoline-5-one; antinociceptive activity; anticancer activity*

Introduction

The antinociceptive and antiinflammatory activities of 1,2-diaryl heterocyclics by cyclooxygenase-2 (COX-2) inhibition have been known since the 1970s[1–4]. Since then, extensive research in this area has been carried out, and numerous compounds have been synthesized in a search for COX-2 inhibitory, antinociceptive and antiinflammatory activities. Two of these compounds, namely celecoxib **I** and rofecoxib **II** (Figure 1), have been employed clinically. However, these drugs have been found to have serious side effects on the heart.

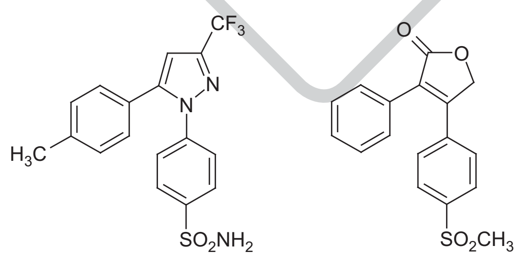


Figure 1. Structures of celecoxib **I** and rofecoxib **II**.

The heterocyclic residue of these compounds may be five- or six-membered, such as furan, thiophene, pyrrole, oxazole, thiazole, imidazole, pyrazole, pyridine, pyrimidine, etc.[5–20]. The pyrazolones, especially antipyrine (1-aryl-2, 3-dimethyl-3-pyrazoline-4-one) derivatives, are well known for their antinociceptive and antipyretic activities and have been used widely in the clinic[21]. In light of the above findings, it appears that both 1,2-diarylheterocyclic and antipyrine residues are two important active pharmacophoric structures for antinociceptive activity. Besides, it has been well documented that 1,2-diaryl heterocyclic compounds have cytotoxic activity as a result of COX-2 inhibition[1,22–26].

In the present study, we have aimed to incorporate 1, 5-diarylimidazole and antipyrine residues in a single molecule and investigate antinociceptive and anticancer activities of the resultant compounds.

Experimental

Chemistry

Melting points were determined by using an Electrothermal 9100 digital melting point apparatus and

Address for Correspondence: Seref Demirayak, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Anadolu, 26470, Eskisehir, Turkey. Fax: +90 222 3350750. E-mail: sdemiray@anadolu.edu.tr

(Received 25 November 2008; revised 03 March 2009; accepted 01 April 2009)

ISSN 1475-6366 print/ISSN 1475-6374 online © 2010 Informa UK Ltd
DOI: 10.3109/14756360903016751

were uncorrected. Spectroscopic data were recorded on the following instruments: FTIR (Fourier transform infrared), Shimadzu 8400S spectrophotometer; ^{1}H-NMR (nuclear magnetic resonance), Bruker 500 NMR spectrometer. Analyses for C, H, and N were within 0.4% of the theoretical values.

4-(2-Chloroacetyl-1-phenyl-2,3-dimethyl-3-pyrazoline-5--one[27] and 2-mercapto-1,5-diarylimidazoles[28] were prepared according to the literature methods. Some characteristics of the compounds are given in Table 1.

General methods for preparation of 4-(1, 5-diarylimidazol-2-yl)thioacetyl-1-phenyl-2, 3-dimethyl-3-pyrazoline-5-one derivatives

A mixture of 4-(2-chloroacetyl-1-phenyl-2,3-dimethyl-3-pyrazoline-5-one (5 mmol, 1.32 g), the appropriate 2-mercapto-1,5-diarylimidazole derivative (5.5 mmol), and K_2CO_3 (6 mmol, 0.83 g) in acetone was refluxed for 8 h. The excess acetone was evaporated. The residue was washed with water and recrystallized from ethanol.

2a IR (KBr) ν_{max} (cm^{-1}): 1639 (C=O), 1593–1506 (C=C), ^{1}H-NMR (500 MHz) (DMSO-d$_6$) (ppm): 2.58 (3H, s, CH$_3$), 3.35 (3H, s, CH$_3$), 4.48 (2H, s, CH$_2$), 7.06 (2H, d, *J*: 7.84 Hz, Ar-H), 7.18–7.27 (3H, m, Ar-H), 7.28–7.30 (3H, m, Ar-H), 7.36 (2H, d, *J*: 7.86 Hz, Ar-H), 7.48–7.51 (4H, m, Ar-H), 7.54–7.58 (2H, m, Ar-H).

2b IR (KBr) ν_{max} (cm^{-1}): 1651, 1637 (C=O), 1590–1506 (C=C), ^{1}H-NMR (500 MHz) (DMSO-d$_6$) (ppm): 2.49 (3H, s, CH$_3$), 2.58 (3H, s, CH$_3$), 3.34 (3H, s, CH$_3$), 4.49 (2H, s, CH$_2$), 7.08 (2H, d, *J*: 8.01 Hz, Ar-H), 7.13–7.25 (3H, m, Ar-H), 7.27–7.30 (2H, m, Ar-H), 7.36 (2H, d, *J*: 8.57 Hz, Ar-H), 7.47–7.53 (4H, m, Ar-H), 7.55–7.58 (2H, m, Ar-H).

2c IR (KBr) ν_{max} (cm^{-1}): 1647 (C=O), 1621–1520 (C=C), ^{1}H-NMR (500 MHz) (DMSO-d$_6$) (ppm): 2.62 (3H, s, CH$_3$), 3.34 (3H, s, CH$_3$), 3.69 (3H, s, OCH$_3$), 4.45 (2H, s, CH$_2$), 6.80 (2H, d, *J*: 7.71 Hz, Ar-H), 6.99 (2H, d, *J*: 7.65 Hz, Ar-H), 7.17 (1H, s, Ar-H), 7.26–7.28 (2H, m, Ar-H), 7.36 (2H, d, *J*: 7.97 Hz, Ar-H), 7.40–7.50 (4H, m, Ar-H), 7.55–7.58 (2H, m, Ar-H).

2d IR (KBr) ν_{max} (cm^{-1}): 1655, 1638 (C=O), 1593–1498 (C=C), ^{1}H-NMR (500 MHz) (DMSO-d$_6$) (ppm): 2.58 (3H, s, CH$_3$), 3.36 (3H, s, CH$_3$), 4.48 (2H, s, CH$_2$), 7.09–7.10 (4H, m, Ar-H), 7.26 (1H, s, Ar-H), 7.28–7.30 (2H, m, Ar-H), 7.36 (2H, d, *J*: 7.87 Hz, Ar-H), 7.48–7.52 (4H, m, Ar-H), 7.55–7.58 (2H, m, Ar-H).

2e IR (KBr) ν_{max} (cm^{-1}): 1635 (C=O), 1589–1514 (C=C), ^{1}H-NMR (500 MHz) (DMSO-d$_6$) (ppm): 2.36 (3H, s, CH$_3$), 2.58 (3H, s, CH$_3$), 3.34 (3H, s, CH$_3$), 4.47 (2H, s, CH$_2$), 7.08 (2H, d, *J*: 7.82 Hz, Ar-H), 7.16 (2H, d, *J*: 8.06 Hz, Ar-H), 7.19–7.26 (4H, m, Ar-H), 7.29 (2H, d, *J*: 8.00 Hz, Ar-H), 7.36 (2H, d, *J*: 7.96 Hz, Ar-H), 7.45–7.50 (1H, m, Ar-H), 7.55–7.58 (2H, m, Ar-H).

2f IR (KBr) ν_{max} (cm^{-1}): 1642 (C=O), 1605–1505 (C=C), ^{1}H-NMR (500 MHz) (DMSO-d$_6$) (ppm): 2.37 (3H, s, CH$_3$), 2.49 (3H, s, CH$_3$), 2.58 (3H, s, CH$_3$), 3.34 (3H, s, CH$_3$), 4.47 (2H, s, CH$_2$), 7.08 (2H, d, *J*: 8.32 Hz, Ar-H), 7.16 (2H, d, *J*: 8.72 Hz, Ar-H), 7.18–7.23 (2H, m, Ar-H), 7.26 (1H, s, Ar-H), 7.29 (2H, d, *J*: 8.13 Hz, Ar-H), 7.36 (2H, d, *J*: 7.78 Hz, Ar-H), 7.48–7.50 (1H, m, Ar-H), 7.54–7.58 (2H, m, Ar-H).

2g IR (KBr) ν_{max} (cm^{-1}): 1638 (C=O), 1611–1508 (C=C), 1340, ^{1}H-NMR (500 MHz) (DMSO-d$_6$) (ppm): 2.37 (3H, s, CH$_3$), 2.58 (3H, s, CH$_3$), 3.34 (3H, s, CH$_3$), 3. 69 (3H, s, OCH$_3$), 4.44 (2H, s, CH$_2$), 6.81 (2H, d, *J*: 8.02 Hz, Ar-H), 7.09 (2H, d, *J*: 8.80 Hz, Ar-H), 7.15 (2H, d, *J*: 7.92 Hz, Ar-H), 7.24 (1H, s, Ar-H), 7.28 (2H, d, *J*: 8.26 Hz, Ar-H), 7.36 (2H, d, *J*: 8.17 Hz, Ar-H), 7.47–7.51 (3H, m, Ar-H), 7.55–7.58 (2H, m, Ar-H).

2h IR (KBr) ν_{max} (cm^{-1}): 1646, 1631 (C=O), 1596–1498 (C=C), 1340, ^{1}H-NMR(500 MHz) (DMSO-d$_6$) (ppm): 2.36 (3H, s, CH$_3$), 2.58 (3H, s, CH$_3$), 3.34 (3H, s, CH$_3$), 4.47 (2H, s, CH$_2$), 7.10–7.12 (4H, m, Ar-H), 7.16 (2H, d, *J*: 8.15 Hz, Ar-H), 7.24 (1H, s, Ar-H), 7.29 (2H, d, *J*: 8.20 Hz, Ar-H), 7.36 (2H, d, *J*: 7.42 Hz, Ar-H), 7.47–7.50 (1H, m, Ar-H), 7.55–7.58 (2H, m, Ar-H).

2i IR (KBr) ν_{max} (cm^{-1}): 1636 (C=O), 1589–1514 (C=C), ^{1}H-NMR (500 MHz) (DMSO-d$_6$) (ppm): 2.58 (3H, s, CH$_3$), 3.35 (3H, s, CH$_3$), 3.80 (3H, s, OCH$_3$), 4.47 (2H, s, CH$_2$), 7.02 (2H, d, *J*: 8.67 Hz, Ar-H), 7.09 (2H, d, *J*: 7.85 Hz, Ar-H), 7.18–7.24 (5H, m, Ar-H), 7.26 (1H, s, Ar-H), 7.36 (2H, d, *J*: 8.07 Hz, Ar-H), 7.47–7.50 (1H, m, Ar-H), 7.55–7.58 (2H, m, Ar-H).

2j IR (KBr) ν_{max} (cm^{-1}): 1638 (C=O), 1595–1502 (C=C), 1340, ^{1}H-NMR (500 MHz) (DMSO-d$_6$) (ppm): 2.49 (3H, s, CH$_3$), 2.58 (3H, s, CH$_3$), 3.34 (3H, s, CH$_3$), 3.80 (3H, s, OCH$_3$), 4.47 (2H, s, CH$_2$), 7.03 (2H, d, *J*: 8.84 Hz, Ar-H), 7.09 (2H, d, *J*: 8.33 Hz, Ar-H), 7.21 (2H, d, *J*: 8.79 Hz, Ar-H), 7.25 (1H, s, Ar-H), 7.36 (2H, d, *J*: 7.37 Hz, Ar-H), 7.47–7.50 (1H, m, Ar-H), 7.55–7.58 (2H, m, Ar-H).

Table 1. Some characteristics of the compounds.

Compound	-R	-R′	M.p. (°C)	Yield (%)	Mol. formula/anal. (C, H, N, S)
2a	-H	-H	124–5	82	$C_{28}H_{24}N_4O_2S$
2b	-H	-CH$_3$	117–8	78	$C_{29}H_{26}N_4O_2S$
2c	-H	-OCH$_3$	115–7	75	$C_{29}H_{26}N_4O_3S$
2d	-H	-F	175–6	86	$C_{28}H_{23}FN_4O_2S$
2e	-CH$_3$	-H	146–7	85	$C_{29}H_{26}N_4O_2S$
2f	-CH$_3$	-CH$_3$	138–9	82	$C_{30}H_{28}N_4O_2S$
2g	-CH$_3$	-OCH$_3$	175–7	80	$C_{30}H_{28}N_4O_3S$
2h	-CH$_3$	-F	180–1	85	$C_{29}H_{25}FN_4O_2S$
2i	-OCH$_3$	-H	168–9	83	$C_{29}H_{26}N_4O_3S$
2j	-OCH$_3$	-CH$_3$	161–2	83	$C_{30}H_{28}N_4O_3S$
2k	-OCH$_3$	-OCH$_3$	140–1	78	$C_{30}H_{28}N_4O_4S$
2l	-OCH$_3$	-F	160–2	82	$C_{29}H_{25}FN_4O_3S$

2k IR (KBr) ν_{max} (cm^{-1}): 1658, 1641 (C=O), 1598–1512 (C=C), ^{1}H-NMR (500 MHz) (DMSO-d_6) (ppm): 2.58 (3H, s, CH_3), 3.35 (3H, s, CH_3), 3.69 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 4.46 (2H, s, CH_2), 6.70–6.87 (4H, m, Ar-H), 7.03 (2H, d, *J*: 8.80 Hz, Ar-H), 7.15 (1H, s, Ar-H), 7.19 (2H, d, *J*: 8.77 Hz, Ar-H), 7.35 (2H, d, *J*: 7.62 Hz, Ar-H), 7.48–7.51 (1H, m, Ar-H), 7.55–7.58 (2H, m, Ar-H).

2l IR (KBr) ν_{max} (cm^{-1}): 1632 (C=O), 1589–1498 (C=C), ^{1}H-NMR (500 MHz) (DMSO-d_6) (ppm): 2.58 (3H, s, CH_3), 3.35 (3H, s, CH3), 3.80 (3H, s, OCH_3), 4.46 (2H, s, CH_2), 7.02 (2H, d, *J*: 8.86 Hz, Ar-H), 7.10–7.12 (4H, m, Ar-H), 7.15 (1H, s, Ar-H), 7.21 (2H, d, *J*: 8.82 Hz, Ar-H), 7.23 (1H, s, Ar-H), 7.35 (2H, d, *J*: 8.54 Hz, Ar-H), 7.48–7.51 (1H, m, Ar-H), 7.55–7.58 (2H, m, Ar-H).

Pharmacology

Animals

All the animals were housed in cages with free access to food and water. They were placed in a quiet and temperature–humidity controlled room (22 ± 2°C and 60 ± 5%, respectively) in which a 12:12 light–dark cycle was maintained. Mice (25–30 g) of either sex were used in the experiments. The animals were divided into 13 groups. Seven or eight animals were used in each study group. The mice were allowed 1–2 h to adjust to the laboratory conditions. All compounds were given intraperitoneally (i.p.) at 100 mg/kg doses. The control animals received 0.1 mL dimethylsulfoxide (DMSO) i.p. Morphine sulfate (5 mg/kg) and dipyrone (100 mg/kg) were used as the reference antinociceptive agents. The study was approved by the Local Ethics Committee of Osmangazi University, Medical School, Eskisehir, Turkey.

Tail clip test

A pressure-standardized artery clip was applied 3–4 cm from the tip of the tail for evaluation of the response to noxious pressure. Turning toward or biting at the clip within 15 s of artery clip placement was the threshold used in this test[29].

Hot-plate test

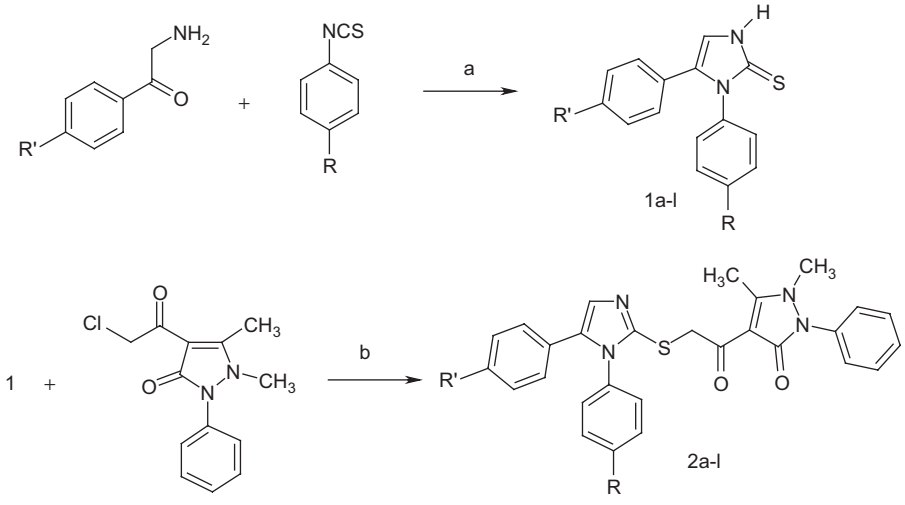
The test was based on that described by Eddy and Leimbach. A transparent glass cylinder (16 cm high, 16 cm diameter) was used to keep the mouse on the heated surface of the plate. The temperature of the hot-plate was set to 55 ± 0.5°C by using a thermoregulated water-circulating pump. The time of latency was defined as the time period between the zero point when the animal was placed on the hot-plate surface and the time when the animal licked its paw or jumped off to avoid thermal pain (cutoff time 30 s)[30–32].

Abdominal constriction test

This test was performed by the i.p. injection of 0.6% acetic acid (60 mg/kg). The number of stretching movements (arching of back, development of tension in the abdominal muscles, elongation of the body, and extension of the forelimbs) was observed. Stretching movements commenced 5 min after acetic acid injection. These contractions were counted and recorded for 10 min. Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions[33].

Anticancer activity test

The cytotoxic and/or growth inhibitory effects of the compounds were evaluated *in vitro* against approximately 66 human tumor cell lines derived from nine neoplastic diseases, namely: leukemia (L), non-small cell lung cancer (NSCLC), colon cancer (CC), central nervous system cancer (CNSC), melanoma (M), ovarian cancer (OC), renal cancer (RC), prostate cancer (PC), and breast cancer (BC). The evaluation of anticancer activity was performed at the National Cancer Institute (NCI) of Bethesda, USA, following the *in vitro* screening program, which is based upon the use of multiple panels of 66 human tumor cell lines against which our compounds were tested at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. A 48 h continuous drug exposure



Scheme 1. Synthesis of compounds **1a–l** and **2a–l**. Reagents and conditions: (a) pyridine, heating at reflux; (b) K_2CO_3, acetone.

protocol was followed and a sulforhodamine B (SRB) protein assay was used to estimate cell viability of growth[34].

Results and discussion

Chemistry

The syntheses of the title 4-(1,5-diarylimidazol-2-yl) thioacetyl-1-phenyl-2,3-dimethyl-3-pyrazoline-5-one derivatives **2a**–**l** were accomplished in accordance with the sequence of reaction depicted in Scheme 1. The starting materials, 1,5-diaryl-2-mercaptoimidazoles **1a**–**l**, were prepared by reacting the appropriate 2-amino-4′-substituted acetophenones and 4-substituted phenylisothiocyanates in pyridine according to the method described in the literature[28]. To obtain the final products **2a**–**l**, 4-(2-chloroacetyl-1-phenyl-2,3-dimethyl-3-pyrazoline-5-one was reacted with suitable imidazole derivatives **1a**–**l** under Williamson ether synthesis conditions. The structures of the obtained compounds were elucidated using spectral data. In the IR spectra, the charactesistic amide and ketone carbonyl functions were observed in the 1672– 1652 cm^{-1} region separately or as a single band[35]. The NMR spectra of the compounds **2a**–**l** exhibited singlets resulting from resonances of the thioacetyl-1-phenyl-2,3-dimethyl-3-pyrazoline-5-one residue assigned to C-CH_3 protons at 2.58 ppm, to N-CH_3 protons at 3.34–3.36 ppm, and to S-CH_2-CO protons at 4.45–4.49 ppm, respectively. The other common proton groups existing in all compounds, imidazole-C_4-H protons, were obtained as singlets at 7.26–7.29 ppm for some of the compounds. For the others, the mentioned protons were taking part in multiplets because of overlaping with aromatic protons.

Antinociceptive activity

Antinociceptive activities of the compounds were determined by using the tail clip test, hot-plate test, and abdominal constriction test. Both the tail clip and hot-plate tests were used to evaluate central antinociceptive activity and the abdominal constriction test was used to assess peripheral antinociceptive activity. The findings are shown in Table 2 and all data were compared to control groups. The results are given as a percentage of the maximal possible effect (%MPE ± SEM), which is defined by following equation:

$$\%\text{MPE} = [(\text{postdrug latency}) - (\text{predrug latency}) / (\text{cutoff time}) - (\text{predrug latency})] \times 100$$

Statistical analyses were carried out using Student's *t*-test.

Compounds **2a**, **2d**, and **2g** exhibited antinociceptive activity in the tail clip test. **2a** and **2d** showed greater antinociceptive activities than the reference compounds. The other compounds did not display any significant antinociceptive activity in this test.

In the hot-plate test, only compound **2d** evoked antinociceptive activity when compared with the control group or dipyrone. On the other hand, the other compounds did not exhibit any antinociceptive activity when compared with control or reference compounds.

In the abdominal constriction test, although all the compounds exhibited significant antinociceptive activities, compounds **2d** and **2j** especially were found to produce the most antinociceptive activity at the dose tested. Compound **2d** was antinociceptive when compared with dipyrone ($p \leq 0.05$), and at 100 mg/kg it produced antinociceptive activity equivalent to that of morphine. **2a** was the only compound not to show antinociceptive activity in the abdominal constriction test.

Thus, in the present study, it was found that compound **2d** was the most active molecule in all antinociceptive tests. Therefore, this compound was thought to produce both

Table 3. Anticancer activity of some compounds as growth percent against selected cell lines.

Compound	NCI-H460	MCF7	SF-268
2c	88	76	97
2f	94	97	101
2h	98	98	99
2i	23	37	83
2j	111	141	118
2l	88	88	96

Table 2. Antinociceptive activity of the compounds.

Compound	Tail clip test (%MPE)	Hot-plate test (%MPE)	AcOH test stretching number[a]
Control	8.41 ± 2.76	14.03 ± 3.20	19.16 ± 2.72
2a	65.73 ± 10.17***	10.67 ± 3.99	11.66 ± 3.60
2b	2.55 ± 0.81	3.98 ± 3.26	5.66 ± 2.40*
2d	77.56 ± 14.24**	53.53 ± 9.99**,+	0.50 ± 0.22***,+
2e	17.90 ± 10.01	10.75 ± 4.09	2.83 ± 1.22***
2g	36.55 ± 7.04$	22.20 ± 6.72	4.33 ± 1.52**
2h	2.85 ± 1.92	9.17 ± 4.93	2.50 ± 0.99**
2i	18.41 ± 6.49	22.50 ± 3.84	1.50 ± 0.84**
2j	0.13 ± 1.65	3.47 ± 4.10	0.83 ± 0.40***
2k	3.70 ± 3.61	13.58 ± 3.81	7.66 ± 2.59**
2l	0.19 ± 2.46	3.91 ± 4.51	2.66 ± 1.02**
Dipyrone	54.61 ± 1.92**	27.16 ± 1.11*	7 ± 1.96**
Morphine	59.46 ± 10.12**	35.13 ± 2.87***	0.33 ± 0.21**

Note. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; MPE, maximum possible effect; all values are given as mean ± SEM. +$p \leq 0.05$ as compared with dipyrone group; $$p \leq 0.05$ as compared with morphine group.

[a]Incidence of abdominal constriction.

Table 4. Mean $\log_{10}$ GI_{50} values of compound **2i** and standard compounds.

Compound	L	NSCLC	CC	CNSC	M	OC	RC	PC	BC	MG-MID
2i	−4.74	−4.36	−4.36	−4.30	−4.36	−4.30	−4.30	−4.72	−4.35	−4.39
A	−5.48	−5.17	−5.11	−5.12	−5.08	−5.18	−4.99	−4.49	−4.79	−5.09
B	−6.39	−6.20	−6.14	−6.18	−6.08	−6.45	−6.17	−6.41	−6.05	−6.20

Note. A, melphalan; B, cisplatin.

central and peripheral antinociception, while **2a** induced only a central antinociceptive effect. Overall, our results confirm that these compounds have a generally peripheral antinociceptive effect.

The compound **2d** bears a fluoro group on one of the aryl residues. The other active molecules **2g** and **2j** bear a methyl and a methoxy on each of the other aryl residues while **2a** is nonsubstituted. Although it is known that the mentioned substituents are important for the antinociceptive activity of 1,2-diarylheterocyclic compounds[1], it may not be possible to put forward an idea about the contribution of the substituent to the activity.

Anticancer activity

The compounds selected by NCI and their preliminary anticancer test results as growth percent values obtained against NSCLC (non-small cell lung cancer), BC (breast cancer), and CNSC (central nervous system cancer) cells are given in Table 3. These cells were NCI-H460, MCF7, and SF-268, respectively. Compound **2i** showed remarkable inhibition values for the cells NCI-H460 and MCF7, but the other compounds were found to be inactive. Compound **2i** was accepted for a further screening test. In this step, the selected compound was evaluated *in vitro* against 66 human tumor cell lines derived from nine neoplastic diseases. The detailed test results are given in Table 4.

According to the test method, it is stated that compounds having growth percent values greater than –4 are considered as inactive. It can be seen that for compound **2i**, $\log_{10}$ GI_{50} (logarithm of concentration that causes 50% growth inhibition) values are smaller than –4. Therefore, we may conclude that the compound provides a notable activity level. Melphalan and cisplatin (*cis*-diaminodichloroplatinum), two commonly used chemotherapeutic agents, were used as standard compounds. When the mean-graph midpoint (MG-MID) values of the compounds melphalan and cisplatin, i.e. –5.09 and –6.20 respectively, are considered, it is observed that compound **2i** provides an acceptable activity level (MG-MID –4.39).

Acknowledgements

The authors present their thanks to NCI (USA) and Anadolu University BIBAM (Turkey) AAQfor Anticancer test results and NMR spectral data respectively.

***Declaration of interest*:** The authors report no conflicts of interest.

References

1. Pariet M, Ryn JV. COX-2 Inhibitors. Berlin: Birkhauser Verlag, 2004:15–40, 227–43.
2. Talley JJ. Selective inhibitors of cyclooxygenase-2. *Prog Med Chem* 1999;36: 201–34.
3. Leval X, Delarge J, Somers F, Tullio P, Henrotin Y, Pirotte B, et al. Recent advances in inducible cyclooxygenase (COX-2) inhibition. *Curr Med Chem* 2000;7:1041–62.
4. Dannhardt G, Kiefer W. Cyclooxygenase inhibitors—current status and future prospects. *Eur J Med Chem* 2001;36:109–26.
5. Penning TD, Talley JJ, Bertenshaw SR, Carter JS, Collins PW, Docter S, et al. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-5-(4-metylphenyl)-3-(trifluorometyl)-1h-pyrazol-1-yl-benzenesulfonamide (sc-58635, celecoxib). *J Med Chem* 1997;40:1347–65.
6. Khanna IK, Weier RM, Yu Y, Collins PW, Miyashiro JM, Koboldt CM, et al. 1,2-Diarylpyrroles as potent and selective inhibitors of cyclooxygenase-2. *J Med Chem* 1997;40:1619–33.
7. Talley JJ, Brown DL, Carter JS, Graneto MJ, Koboldt CM, Masferrer JL, et al. 4-[5-Methyl-3-phenylisoxazol-4-yl]-benzenesulfonamide, valdecoxib: a potent and selective inhibitor of COX-2. *J Med Chem* 2000;43:775–7.
8. Habeeb AG, Rao PNP, Knaus EE. Design and syntheses of diarylisoxazoles: novel inhibitors of cyclooxygenase-2 (COX-2) with analgesic-antiinflammatory activity. *Drug Dev Res* 2000;51:273–86.
9. Almansa C, Arriba AF, Cavalcanti FL, Gomez LA, Miralles A, Merios M, et al. Synthesis and SAR of a new series of COX-2-selective inhibitors: pyrazolo[1,5-a]pyrimidines. *J Med Chem* 2001;44:350–61.
10. Hashimoto H, Imamura K, Haruta J, Wakitani K. 4-(4-Cycloalkyl/aryl-oxazol-5-yl)benzenesulfonamides as selective by introduction of a fluorine atom and identification of a potent, highly selective, an orally active COX-2 inhibitor jte-522. *J Med Chem* 2002;45:1511–17.
11. Laufer SA, Wagner GK. From imidazoles to pyrimidines: new inhibitors of cytokine release. *J Med Chem* 2002;45:2733–40.
12. Liu H, Huang X, Shen J, Luo X, Li M, Xiong B, et al. Inhibitory mode of 1,5-diarylpyrazole derivatives against cyclooxygenase-2 and cyclooxygenase-1: molecular docking and 3D QSAR analyses. *J Med Chem* 2002;45:4816–27.
13. Almansa C, Alfon J, Arriba A, Cavalcanti FL, Escamilla I, Gomez LA, et al. Synthesis and structure-activity relationship of a new series of COX-2 selective inhibitors: 1,5-diarylimidazoles. *J Med Chem* 2003; 46:3463–75.
14. Rao PNP, Amini M, Li H, Habeeb AG, Knaus EE. Design, synthesis, and biological evaluation of 6-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones: a novel class of diarylheterocyclic selective cyclooxygenase-2-inhibitors. *J Med Chem* 2003;46:4872–82.
15. Hu W, Guo Z, Chu F, Bai A, Yi X, Cheng G, et al. Synthesis and biological evaluation of substituted 2-sulfonyl-phenyl-3-phenyl-indoles: a new series of selective COX-2 inhibitors. *Bioorg Med Chem* 2003;11:1153–60.
16. Singh SK, Vobbalareddy S, Shivaramakrishna S, Krishnamraju A, Rajjak SA, Casturi SR, et al. Methanesulfonamide group at position-4 of the c-5-phenyl ring of 1,5-diarylpyrazole affords as a potent class of cyclooxygenase-2 (COX-2) inhibitors. *Bioorg Med Chem Lett* 2004;14:1683–8.
17. Tuyen TN, Sin K, Kim HP, Park H. Synthesis and antiinflammatory activity of 1,5-diarylimidazoles. *Arch Pharm Res* 2005;28:1013–18.
18. Mozziconacci J, Arnoult E, Bernard P, Do QT, Marot C, Morin-Allory L. Optimization and validation of a docking-scoring protocol; application to virtual screening for COX-2 inhibitors. *J Med Chem* 2005;48:1055–68.
19. Laufer SA, Zimmermann W, Ruff KJ. Tetrasubstituted imidazole inhibitors of cytokine release: probing substituents in the n-1 position. *J Med Chem* 2004;47:6311–25.
20. Navidpour L, Shadnia H, Shafaroodi H, Amini M, Dehpour AR, Shafiee A. Design, synthesis and biological evaluation of substituted 2-alkylthio-1,5-diarylimidazoles as selective COX-2 inhibitors. *Bioorg Med Chem* 2007;15:1976–82.

21. Borne RF. Nonsteroidal antiinflammatory drugs. In: Foye WO, Lemke TL, Williams DA, eds. *Principles of Medicinal Chemistry,* 4th ed. Philadelphia, PA: Williams & Wilkins, 1995:535–80.
22. Hida T, Kozaki K, Muramatsu H, Masuda A, Shimizu S, Mitsudomi T, et al. Cyclooxygenase-2 inhibitor induces apoptosis and enhances cytotoxicity of various anticancer agents in non-small cell lung cancer cell lines. *Clin Cancer Res* 2000;6:2006–11.
23. Petersen C, Baumann M, Petersen S. New targets for the modulation of radiation response-selective inhibition of the enzyme cyclooxygenase 2. *Curr Med Chem Anticancer Agents* 2003; 3:354–9.
24. Gust R, Busch S, Keilitz R, Schmidt K, Rauch M. Investigations on the influence of halide substituents on the estrogen receptor interaction of 2,4,5-tris(4-hydroxyphenyl)imidazoles. *Arch Pharm Pharm Med Chem* 2003;336:456–65.
25. Ye F, Wu J, Dunn T, Yi J, Tong X, Zhang D. Inhibition of clclooxygenase-2 activity in head and neck cancer cells by genistein. *Cancer Lett* 2004;211:39–46.
26. Johnsen JI, Lindskog M, Ponthan F, Pettersen I, Elfman L, Orrego A. Cyclooxygenase-2 is expressed in neuroblastoma, and nonsteroidal anti-inflammatory drugs induce apoptosis and inhibit tumor growth in vivo. *Cancer Res* 2004;64:7210–15.
27. Kaufmann HP, Huang S, Buckmann HJ. Über antipyril-ketone. *Berichte* 1942;75B:1214.
28. Korohoda MJ, Bojarska AB. Methylation of 4-imidazoline-2-thiones. *J Prakt Chem* 1991;333:355–60.
29. Biancchi C, Franceschini J. Experimental observations on Haffners method for testing analgesic drugs. *Br J Pharmacol* 1954;9:280–4.
30. Eddy NB, Leimbach D. Synthetic analgesic (II). *Dithienylbutenyl- and dithienylbutylamines. J Pharmacol Exp Ther* 1953;107:385–93.
31. Noble F, Smadja C, Roques BP. Role of endogenous cholecystokinin in the facilitation of mu-mediated antinociception by delta opioid agonists. *J Pharmacol Exp Ther* 1994;271:1127–34.
32. Bastos GNT, Santos ARS, Ferreira VMM, Costa AMR, Bispo CI, Silveira AJA, et al. Antinociceptive effect of aqueous extract obtained from roots of physalis angulata L. *on mice. J Ethnopharmacol* 2006;103:241–5.
33. Koster R, Anderson M, Beer EJ. Acetic acid for analgesic screening. *Fed Proc* 1959;18:412.
34. Boyd MR. Status of the NCI preclinical antitumor drug discovery screen. *Princ Pract Oncol* 1989;3:2–11.
35. Gürsoy A, Demirayak S, Capan G, Erol K, Vural K. Synthesis and preliminary evaluation of new 5-pyrazolinone derivatives as analgesic agents. *Eur J Med Chem* 2000;35:359–64.