ELSEVIER

www.elsevier.com/locate/farmac

Il Farmaco 56 (2001) 719–724

IL FARMACO

Short Communication

Synthesis of some novel 3-methyl-6-(2-substituted propanoyl/propyl)-2-benzoxazolinone derivatives and anti-nociceptive activity

Ünsal Çalış *, Nesrin Gökhan, Hakkı Erdoğan

Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06100 Ankara, Turkey

Received 2 November 2000; accepted 18 April 2001

Abstract

In this study, 12 new 3-methyl-6-(2-substituted aminopropanoyl)-2-benzoxazolinone and 3-methyl-6-(1-hydroxy-2-substituted aminopropyl)-2-benzoxazolinone derivatives have been prepared. Their structures have been elucidated by IR, ^{1}H NMR spectra and by elementary analysis. The anti-nociceptive activity of these compounds has been investigated by using a modified Koster test. It was found that most compounds are capable of inducing anti-nociception in animals.

Keywords: 2-Benzoxazolinone; Acylation; Reduction; Anti-nociceptive activity

1. Introduction

Pain is a fundamental event that is normally beneficial and works as a physiological advice for potentially tissue-damaging situations, e.g. the manifestation of inflammatory dysfunctions [1,2]. However, the very significant emotional and subjective components of human pain and the therapy of chronically debilitating pain makes the search for new peripheral analgesic agents, potent, selective and with reduced toxicity very important.

Since the first report on the hypnotic properties of 2-benzoxazolinone [3], a number of derivatives of this compound have been tested for various activities including anticonvulsant [4], analgesic [5–9], cardiotonic [10], antiulcer [11] or antibacterial, antimicrobial and antifungal effects [12–14]. In the literature it has been reported that 6-acyl-2-benzoxazolinone derivatives showed favourable analgesic activity [5]. On this basis, recently our research group has reported that some derivatives of 2-benzoxazolinone, especially 3-substituted-6-acyl-2-benzoxazolinones, presented high analgesic activity in which the activity was found to be comparable to acetyl salicylic acid (Aspirin®). Therefore these findings led us to synthesize some new 3-methyl-6-amino ketone and amino alcohol-2-benzoxazolinone derivatives and screen them for their anti-nociceptive activity. Then it was found that all compounds are capable of inducing analgesia in animals.

2. Experimental

2.1. Chemistry

All chemicals were obtained from Aldrich Chemical Co. (Steinheim, Germany). Melting points were determined with a Thomas–Hoover capillary melting point apparatus (Philadelphia, PA, USA) and are uncorrected. IR spectra (KBr) were recorded on a Perkin–Elmer 1720X FT Infrared Spectrophotometer (Beaconfield, UK), ^{1}H NMR spectra were recorded

* Correspondence and reprints.
E-mail address: sucalis@tr.net (Ü. Çalış).

0014-827X/01/$ - see front matter
PII: S0014-827X(01)01132-6

using a Bruker AC 200 MHz FT NMR spectrometer (Bruker, Karlsruhe, Germany) using tetramethylsilane as internal standard. Microanalyses were performed by the Scientific and Technical Research Council of Turkey Instrumental Analysis Laboratory (Carlo Erba, Milan, Italy). The purity of the compounds was assessed by TLC on silica gel $HF_{254+366}$ (Merck, Darmstadt, Germany).

2.1.1. 3-Methyl-2-benzoxazolinone and 6-acyl-2-benzoxazolinone were made according to the literature [5,6]

*2.1.1.1. 3-Methyl-6-(2-substituted aminopropanoyl)-2-benzoxazolinone (**I**).* 3-Methyl-6-(2-bromopropanoyl)-2-benzoxazolinone (0.01 mol) dissolved in 5 ml dimethylformamide was added dropwise to a solution of substituted amino derivatives (0.03 mol) with triethylamine (0.03 mol) in 10 ml dimethylformamide. The mixture was stirred at room temperature for 24 h, poured into crushed ice-water and the solid mass which separated out was filtered, dried and crystallized.

*2.1.1.2. 3-Methyl-6-(1-hydroxy-2-substituted aminopropyl)-2-benzoxazolinone (**II**).* 0.01 mol 6-substituted aminopropanoyl-2-benzoxazolinone was dissolved in 100 ml of methanol. To this mixture 0.012 mol/l of $NaBH_4$ was added, and stirring was continued for 4 h at reduced pressure. The residue was recrystallized from different solvents.

2.2. Pharmacology

Mice used in the present study were housed and cared for in accordance with the Hacettepe University Animal Care Unit, which applies the guidelines of National Institutes of Health (NIH) on laboratory animal welfare.

Female albino mice (Refik Saydam Hıfzısıhha Institute's Animal House, Ankara, Turkey), weighing 22 ± 2 g, were used (local breed). A minimum of six animals was used in each group. The animals were left for 2 days for acclimatization to animal room conditions and were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but free access to water was allowed.

2.2.1. Anti-nociceptive activity

This test was performed following the technique of Koster et al. [15] which is based on the property of the test compounds to antagonize the acetic acid induced pain syndrome in mice. Compounds were given orally to mice in groups of six at 100 mg/kg dose levels as a suspension in 0.5% carboxymethylcellulose. Stretching was induced 1 h later by i.p. injection of 3% solution of acetic acid at 300 mg/kg. Two control groups ($n = 6$) received carboxymethylcellulose 1 h prior to injection of acetic acid. The number of writhes induced in each mouse was counted for 5 min (between 5 and 10 min) after injection of the irritant. Anti-nociceptive activity was calculated using the formula.

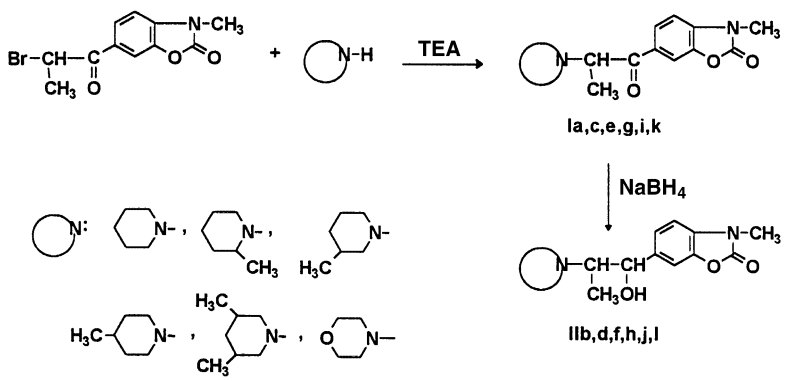
$$\text{Anti-nociceptive activity } (\%) = (n - n')/n \times 100$$

n and n' are the mean stretching number of the control and test groups, respectively. Acetyl salicylic acid was used as a reference analgesic and administered according to the test protocol.

3. Results and discussion

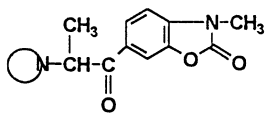
3.1. Chemistry

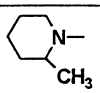
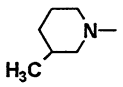
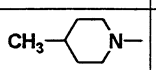
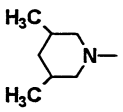
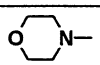
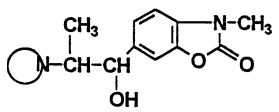
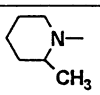
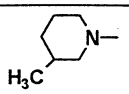
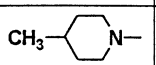
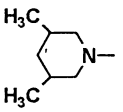
Twelve new 3-methyl-6-(2-substituted aminopropanoyl)-2-benzoxazolinone and 1-(3-methyl-2-benzoxazolinone)-2-(substituted amino-1-yl)propanol derivatives were synthesized and the structure of the synthesized compounds was proved by IR, ^{1}H NMR and elemental analysis. Benzoxazolinone was acylated in PPA with 2-bromopropanoic acid and then treated with substituted amino derivatives according to *N*-alkylation. After then the aminoalcohol derivatives were



Scheme 1.

Table 1
Some characteristics of the compounds **Ia–III**



Com. No		Crystal. solvent	M.p. °C	Yield (%)	Formula
Ia		Ethanol	142-143	54.68	$C_{16}H_{20}O_3N_2$
Ic		Ethanol	150-153	61.24	$C_{17}H_{22}O_3N_2$
Ie		Ethanol	192-193	65.54	$C_{17}H_{22}O_3N_2$
Ig		Ethanol	154-155	71.32	$C_{17}H_{22}O_3N_2$
Ii		Ethanol	158-161	68.13	$C_{18}H_{24}O_3N_2$
Ik		Methanol-Water	158-159	75.28	$C_{15}H_{18}O_4N_2$
					
IIb		Methanol-Water	122-124	46.24	$C_{16}H_{22}O_3N_2$
IId		Methanol-Water	154-156	51.25	$C_{17}H_{24}O_3N_2$
IIf		Methanol-Water	139-141	55.13	$C_{17}H_{24}O_3N_2$
IIh		Methanol	147-149	49.35	$C_{17}H_{24}O_3N_2$
IIj		Methanol	113-114	44.24	$C_{18}H_{26}O_3N_2$
III		Methanol-Water	139-141	60.21	$C_{15}H_{20}O_4N_2$

obtained by the reduction of aminoketones with $NaBH_4$ (Scheme 1).

In acylation as both the 3-nitrogen and 2-oxygen atoms are electron-donating, both the 5- and 6-posi-

tions are activated and therefore, the regioselectivity in the C-acylation of benzoxazolinone cannot be easily predicted. However, it was reported that the substitution was directed by the nitrogen atom of the benzoxazolinone ring and only the 6-acyl derivative was formed [16]. We also proved the formation of 6-acylation product with ^{1}H NMR parameters (chemical shifts) and coupling constants (J, Hz) in our previous paper [17].

All the synthesized compounds have at least one asymmetric carbon atom. However, the enantiomers were not separated. When the compounds carry alcohol moiety, these derivatives have a second asymmetric centre and so the synthesized compounds have diastereomers. It was reported that the ratio of different diastereomers (Z and E diastereomer) was dependent on the reduction method used for the carbonyl group

Table 2
IR and ^{1}H NMR spectroscopic data of the compounds **Ia**–**III**

Comp.	IR (KBr) ν (cm^{-1})	^{1}H NMR ($CDCl_3$) δ (ppm)
Ia	1660 (arom. ketone), 1760 (lactam C=O), 2920 (aliph. C–H)	1.05–1.15 (3H; d; CH–CH_3), 1.3–1.45 (6H; m; piperidine H_3, H_4, H_5), 2.35–2.55 (4H; m; piperidine H_2, H_6), 3.3–3.4 (3H; s; N–CH_3), 4.2–4.3 (1H; q; CH–CH_3), 7.3–7.4 (1H; d; 2-benzoxazolinone H_4), 7.95–8.1 (2H; m; 2-benzoxazolinone H_5, H_7)
IIb	1760 (lactam C=O), 2920 (aliph. C–H), 3400 (O–H)	0.6–0.75 (3H; d; CH–CH_3), 1.3–1.7 (6H; m; piperidine H_3, H_4, H_5), 2.3–2.7 (4H; m; piperidine H_2, H_6), 3.3–3.4 (3H; s; N–CH_3), 4.25–4.35 (1H; q; CH–CH_3), 5.0–5.1 (1H; d; CH–OH, J:14 Hz), 7.1–7.25 (2H; m; 2-benzoxazolinone H_4, H_5), 7.25–7.35 (1H; s; 2-benzoxazolinone H_7)
Ic	1670 (arom. ketone), 1750 (lactam C=O), 2920 (aliph. C–H)	1.0–1.1 (3H; d; CH_3–pip.), 1.1–1.7 (9H; m; piperidine H_3, H_4, H_5, CH–CH_3), 2.1–2.25 (1H; m; piperidine H_2), 2.4–2.6 (2H; t; piperidine H_6), 3.35–3.45 (3H; s; N–CH_3), 4.6–4.7 (1H; q; CH–CH_3), 7.3–7.4 (1H; d; 2-benzoxazolinone H_4), 7.9–8.2 (2H; m; 2-benzoxazolinone H_5, H_7)
IId	1760 (lactam C=O), 2920 (aliph. C–H), 3320 (O–H)	0.6–0.7 (3H; d; CH_3–pip.), 1.0–1.8 (9H; m; piperidine H_3, H_4, H_5, CH–CH_3), 2.05–2.15 (1H; m; piperidine H_2), 2.8–2.9 (2H; t; piperidine H_6), 3.3–3.4 (3H; s; N–CH_3), 4.2–4.35 (1H; q; CH–CH_3), 4.95–5.0 (1H; d; CH–OH, J:15 Hz), 7.15–7.25 (2H; d; 2-benzoxazolinone H_4,H_5), 7.25–7.35 (1H; m; 2-benzoxazolinone H_7)
Ie	1660 (arom. ketone), 1770 (lactam C=O), 2920 (aliph. C–H)	0.7–0.85 (3H; d; CH_3–pip.), 1.05–1.2 (3H; d; CH–CH_3), 1.3–1.8 (5H; m; piperidine H_3, H_4, H_5), 2.2–2.9 (4H; m; piperidine H_2, H_6), 3.35–3.45 (3H; s; N–CH_3), 4.2–4.3 (1H; q; CH–CH_3), 7.3–7.4 (1H; d; 2-benzoxazolinone H_4), 7.95–8.1 (2H; m; 2-benzoxazolinone H_5, H_7)
IIf	1760 (lactam C=O), 2920 (aliph. C–H), 3320 (O–H)	0.75–0.85 (3H; d; CH_3–pip.), 1.1–1.2 (3H; d; CH–CH_3), 1.2–1.7 (5H; m; piperidine H_3, H_4, H_5), 2.3–2.8 (4H; m; piperidine H_2, H_6), 3.30–3.50 (3H; s; N–CH_3), 4.25–4.35 (1H; q; CH–CH_3), 4.95–5.0 (1H; d; CH–OH, J:17 Hz), 7.1–7.2 (1H; d; 2-benzoxazolinone H_4), 7.85–8.0 (2H; m; 2-benzoxazolinone H_5, H_7)
Ig	1670 (arom. ketone), 1770 (lactam C=O), 2900 (aliph. C–H)	0.8–0.95 (3H; d; CH_3–pip.), 1.0–1.6 (8H; m; piperidine H_3, H_4, H_5, CH–CH_3), 2.0–2.9 (4H; m; piperidine H_2,H_6), 3.35–3.45 (3H; s; N–CH_3), 4.2–4.3 (1H; q; CH–CH_3), 7.3–7.4 (1H; d; 2-benzoxazolinone H_4), 7.95–8.1 (2H; m; 2-benzoxazolinone H_5, H_7)
IIh	1760 (lactam C=O), 2900 (aliph. C–H), 3440 (O–H)	0.6–0.8 (3H; d; CH_3–pip.), 0.85–1.0 (3H; d; CH–CH_3) 1.0–2.2 (5H; m; piperidine H_3, H_4, H_5), 2.4–2.8 (4H; m; piperidine H_2 H_6), 3.35–3.5 (3H; s; N–CH_3), 4.25–4.35 (1H; q; CH–CH_3), 4.95–5.05 (1H; d; CH–OH, J: 14 Hz), 7.15–7.35 (3H; m; 2-benzoxazolinone H_4,H_5,H_7)
Ii	1660 (arom. ketone), 1790 (lactam C=O), 2920 (aliph. C–H)	0.7–0.85 (6H; d; CH_3–pip.), 1.1–1.2 (6H; d; CH–CH_3), 1.5–1.8 (4H; m; piperidine H_3, H_4, H_5), 2.2–2.6 (4H; m; piperidine H_2, H_6), 3.15–3.25 (3H; s; N–CH_3), 4.3–4.5 (1H; q; CH–CH_3), 7.2–7.4 (1H; d; 2-benzoxazolinone H_4), 7.95–8.15 (2H; m; 2-benzoxazolinone H_5, H_7)
IIj	1770 (lactam C=O), 2940 (aliph. C–H), 3300 (O–H)	0.75–0.90 (6H; d; CH_3–pip.), 1.2–1.3 (3H; d; CH–CH_3), 1.4–1.8 (4H; m; piperidine H_3, H_4, H_5), 2.3–2.8 (4H; m; piperidine H_2, H_6), 3.25–3.45 (3H; s; N–CH_3), 4.4–4.5 (1H; q; CH–CH_3), 4.95–5.05 (1H; d; CH–OH, J:14 Hz), 7.2–7.4 (1H; d; 2-benzoxazolinone H_4), 7.85–8.1 (2H; m; 2-benzoxazolinone H_5, H_7)
Ik	1660 (arom. ketone), 1770 (lactam C=O), 2940 (aliph. C–H)	1.1–1.2 (3H; d; CH–CH_3), 2.4–2.6 (4H; s; morpholine H_2,H_6), 3.3–3.6 (7H; m; morfoline H_3,H_5, N–CH_3), 4.25–4.35 (1H; q; CH–CH_3), 7.3–7.4 (1H; d; 2-benzoxazolinone H_4), 8.0–8.1 (2H; m; 2-benzoxazolinone H_5, H_7)
III	1750 (lactam C=O), 2840 (aliph. C–H), 3380 (O–H)	1.2–1.4 (3H; d; CH–CH_3), 2.5–2.6 (4H; s; morpholine H_2,H_6), 3.3–3.5 (7H; m; morfoline H_3,H_5, N–CH_3), 4.15–4.35 (1H; q; CH–CH_3), 4.90–5.05 (1H; d; CH–OH, J: 15 Hz), 7.3–7.4 (1H; d; 2-benzoxazolinone H_4), 7,95–8.1 (2H; m; 2-benzoxazolinone H_5, H_7)

Table 3
Anti-nociceptive activity of the compounds **Ia**–**Il**

Comp.	Dose (mg/kg) [a]	Constriction number *	Anti-nociceptive activity% [b]
Ia	100	32 ± 1.838	55.55
IIb	100	52 ± 0.727	27.77
Ic	100	12 ± 2.561	83.33
IId	100	20 ± 1.094	72.22
Ie	100	64 ± 1.871	11.11
IIf	100	8 ± 0.9209	88.88
Ig	100	12 ± 1.986	83.33
IIh	100	26 ± 1.583	63.88
Ii	100	36 ± 0.676	50.00
IIj	100	20 ± 2.158	72.22
Ik	100	8 ± 2.331	88.88
Il	100	44 ± 1.151	38.88
Control	–	72 ± 2.345	–
ASA	100	41 ± 1.124	42.11

[a] All compounds were administered p.o.
[b] % of inhibition obtained by comparison with vehicle control group.
* $P < 0.05$.

[10]. These reduction reactions are carried out by $NaBH_4$ or Pd/C (10%) and catalytic hydrogenation. But we could not try Pd/C (10%) and catalytic hydrogenation owing to lack of equipment in our laboratory. It is also known from the published literature that when the reaction was catalyzed with $NaBH_4$, the ratio of Z/E was 15–85% and spin–spin constants of E form are 11–18 Hz. The careful analysis of the ^{1}H NMR spectra (**IIb**, **IId**, **IIf**, **IIh**, **IIj**, **Il**) allowed us to detect only the presence of one methyne hydrogen signal, which was attributed to the E diastereomer. In fact, this attribute was favoured by the previously published paper of Erdoğan and Debaert [6]. The doublets of (–CH) methyne protons of the compounds synthesized were found to be 4.95–5.10 ppm which suggests these compounds are E diastereomers.

The crystallization solvents, melting points, percentage yields, and molecular formula of the synthesized compounds are shown in Table 1. All spectral data are in accordance with the assumed structures. In the IR spectra of the compounds, the lactam and ketone stretching bands were seen at about 1790–1760 and 1670–1660 cm^{-1}. Other stretchings seen at spectra confirmed the structures (Table 2).

In the ^{1}H NMR spectrum, methylene protons at the 6-position in the benzoxazolinone were seen at 4.95–5.10 ppm. The neighbouring methyl protons to nitrogen atom in benzoxazolinone were observed at 3.3–3.6 ppm. In addition to these, morpholino protons were seen at 2.4–2.6 and 3.3–3.6 ppm, respectively. The protons belonging to the piperidine ring appeared at approximately 1.5 and 2.5 ppm. Aromatic ring protons were observed at the expected values (Table 2).

The results of microanalyses were within ± 0.4% of theoretical values.

3.2. Pharmacology

The evaluation of the anti-nociceptive profile of all benzoxazolinone derivatives was performed using the classical acetic acid-induced mice abdominal constrictions test (Modified Koster test), p.o. with acetyl salicylic acid as standard since it was easy to carry out and was sensitive to mild analgesics. The results are shown in Table 3.

In the first series of derivatives (for amino ketone derivatives) the most active derivatives (**Ic**, 83.33%, **Ig**, 83.33%, and **Ik**, 88.88%) possess 2-methyl/4-methylpiperidine and morpholine rings at the ketone moiety of the benzoxazolinone framework and presented higher activity than ASA used as standard, at the same dose. The compound possessing the piperidinyl moiety (**Ia**, 55.55%) was the third most active compound, presenting approximately the same activity of the ASA.

When the contribution of the amino alcohol moiety in benzoxazolinone to the anti-nociceptive activity was measured by comparison of the activities with amino ketone moiety, the results indicated that this molecular modification seems to be deleterious to the anti-nociceptive activity (compound **IIb**, 27.77%, **Il**, 38.88%) except for compound **IIf** (88.88%) and **IIj** (72.22%).

Finally, the anti-nociceptive activity of compounds seem to be insignificantly influenced by the different electronic or lipophilic contributions of the substituents at the benzoxazolinone framework. This may be the partially contradicting results so far obtained. But still it can be said that these derivatives show promising anti-nociceptive effect.

Acknowledgements

We thank Dr. Z. Gören, Pharmacology Department, Faculty of Medicine, Marmara University, Turkey for doing the anti-nociceptive activity.

References

[1] A. Dray, L. Urban, New pharmacological strategies for pain relief, Annu. Rev. Pharmacol. Toxicol. 36 (1996) 253–280.
[2] S.H. Ferreira, B.H. Lorenzetti, M. Devissaguet, D. Lesieur, Y. Tsouderos, S14080, a peripheral analgesic acting by release of an endogenous circulating opioid-like substance, Br. J. Pharmacol. 114 (1995) 303–308.
[3] A. Lespagnol, Mme. Lefebvre, Hypnotic effect of benzoxazolone substitutes, Bull. Soc. Chim. 12 (1945) 386–387.
[4] S. Dalkara, Ü. Çalış, R. Sunal, Synthesis and anticonvulsant activity of some new 2(3H)-benzoxazolone derivatives, J. Pharm. Belg. 43 (1988) 372–378.
[5] J.P. Bonte, D. Lesieur, C. Lespagnol, J.C Cazin, Acyl-6-benzoxazolinones, Eur. J. Med. Chem.-Chim. Ther. 9 (1974) 491–496.
[6] H. Erdoğan, M. Debaert, J.C. Cazin, Synthesis of some 2-benzoxazolinone derivatives and their analgesic properties, Arzneim. Forsch-/Drug Res. 41 (1991) 73–76.

[7] N. Gökhan, H. Erdoğan, B.C. Tel, R. Demirdamar, Analgesic and antiinflammatory activity screening of 6-acyl-3-piperidinomethyl-2-benzoxazolinone derivatives, Eur. J. Med. Chem. 31 (1996) 625–628.
[8] N. Gökhan, H. Erdoğan, T.N. Durlu, R. Demirdamar, Novel antiinflammatory analgesics: 3-(2-/4-pyridylethyl)-benzoxazolinone and oxazolo[4,5-b]pyridin-2-one derivatives, Arch. Pharmazie Pharm. Med. Chem. 332 (1999) 43–49.
[9] E. Palaska, N. Gökhan, H. Erdoğan, B.C. Tel, R. Demirdamar, Synthesis of some new pyridyl-ethylated benzoxazolinones with analgesic and antiinflammatory activities, Arzneim. Forsch-/Drug Res. 49 (1999) 754–758.
[10] M.P. Vaccher, D. Lesieur, C. Lespagnol, J.P. Bonte, J. Lamar, M. Beaughard, G. Dureng, Benzoxazolinone phenylethanolamines: adrenergic receptor antagonists, chemical and pharmacodynamic study, Farmaco Ed. Sci. 41 (4) (1986) 257–269.
[11] Y. Katsura, S. Nishino, H. Takasugi, Studies on antiulcer drugs. I. Synthesis and antiulcer activities of imidazo[1,2-*a*]pyridinyl-2-oxobenzoxazolidines-3-oxo-2*H*-1,4-benzoxazines and related compounds, Chem. Pharm. Bull. 39 (11) (1991) 2937–2943.
[12] R.S. Varma, A. Kapoor, Potential biologically active agents. Part XXI. 1-(4-Benzoxazolylmethylaminobenzoyl)morpholines and piperidines as antimicrobial agents, Indian J. Chem. 18B (2) (1979) 200–204.
[13] D.D. Erol, H. Erdoğan, N. Yuluğ, Synthesis and biological activity of 6-acyl-3-substituted-2(3*H*)-benzoxazolones, J. Pharm. Belg. 44 (5) (1989) 334–336.
[14] V. Kalcheva, Z. Mincheva, P. Andreeva, Synthesis and in vitro activity of new cephalosporin derivatives containing a benzoxazolone ring, Arzneim. Forsch. 40 (9) (1990) 1030–1034.
[15] R. Koster, M. Anderson, E.J. Debeer, Acetic acid for analgesic screening, Fed. Proc. 18 (1959) 412.
[16] H. Aichaoui, J.H. Poupaert, D. Lesieur, J.P. Henichart, Regioselectivity in the *C*-acylation of 2(3*H*)-benzoxazolones, Tetrahedron 47 (1991) 6649–6654.
[17] N. Gökhan, H. Erdoğan, T.N. Durlu, R. Demirdamar, Analgesic activity of acylated 2-benzoxazolinone derivatives, Farmaco 54 (1999) 112–115.