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Analgesic activity of acylated 2-benzoxazolinone derivatives

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Abstract

Ten new benzoxazolinone derivatives having a disubstituted benzoyl group at the six position of the ring were synthesized by reacting 2-benzoxazolinone with aromatic carboxylic acids in the presence of polyphosphoric acid. The structure of the compounds were elucidated by IR, ^{1}H NMR, MS and elemental analysis. Analgesic activity was evaluated by a modified Koster test. Seven compounds showed analgesic activities higher than that of acetylsalicylic acid.

Keywords: 2-Benzoxazolinone; Acylation; Analgesic activity

1. Introduction

Since the first report on the hypnotic properties of 2-benzoxazolinone [1,2], a number of derivatives have been tested for various activities including anticonvulsant [3], antipyretic, analgesic [4–10], cardiotonic [11,12], antiulcer [13], antineoplastic [14] or antibacterial, antimicrobial and antifungal [15–20] effects. 6-Methoxybenzoxazolinone is a natural occurring compound that acts as an agonist of melatonin and also serves as an environmental stimulus for reproductive activities [21,22]. This role emphasizes the potential of the benzoxazolinone structure. However, it is interesting to note that the benzoxazolinone ring could be considered as a cyclic bioisoster of pyrocatechol. This analogy was confirmed by the biological study of the compounds including a benzoxazolinone ring linked to phenylethylamine chains and these compounds were accepted as potent agonists or antagonists of dopamine or norepinephrine [23–25].

Numerous similar works are currently in progress to obtain a better understanding of the subject and it is clear that choosing a useful starting substance such as the acyl derivatives of benzoxazolinone is the key step of the medicinal chemistry.

In particular, 6-acylbenzoxazolinones exhibit analgesic properties much greater than those of the parent heterocycle and are indeed the latest development in the field of central nervous system drugs. Electrophilic substitution, such as chlorination, sulfonation and nitration was achieved using classical reagents but acylation yielding 6-acyl derivatives was found to require particular conditions. Acylation of benzoxazolinone was accomplished earlier by two methods in the literature, in which either DMF–$AlCl_3$ and acid chloride [26] or polyphosphoric and carboxylic acids [4] were used. It was reported that both methods gave the same product (6-acylbenzoxazolinone) at very similar yields [27].

The aim of the present work is to synthesize derivatives carrying benzoyl moieties at 6 position of the benzoxazolinone (Scheme 1) and to examine their analgesic activities.

2. Experimental

2.1. Chemistry

All chemicals and solvents used in the present study were purchased from Merck AG and Aldrich Chemicals. Melting points were determined using a Thomas Hoover capillary melting point apparatus (Philadelphia, USA) and were uncorrected. IR spectra were recorded

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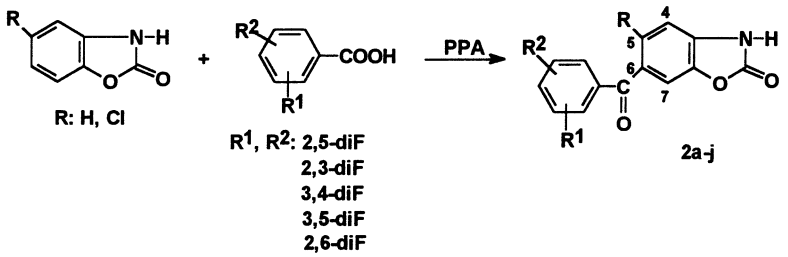

Scheme 1. Synthesis of 6-acyl-2-benzoxazolinone.

Table 1
Analytical data for the synthesized compounds

Comp.	R	R^1/R^2	Crystallization solvents	M.p. (°C)	Yield (%)	Formula	Analysis
2a	H	2,5-F	Ethanol–water	161–163	58	$C_{14}H_7O_3NF_2$	C, N, H
2b	Cl	2,5-F	Acetonitrile	192–194	62	$C_{14}H_6O_3NClF_2$	C, N, H
2c	H	2,3-F	Acetonitrile–water	176–177	57	$C_{14}H_7O_3NF_2$	C, N, H
2d	Cl	2,3-F	Acetonitrile–water	151–154	67	$C_{14}H_6O_3NClF_2$	C, N, H
2e	H	3,4-F	Ethyl acetate–*n*-hexane	170–172	51	$C_{14}H_7O_3NF_2$	C, N, H
2f	Cl	3,4-F	Ethyl acetate–*n*-hexane	175–177	64	$C_{14}H_6O_3NClF_2$	C, N, H
2g	H	3,5-F	Ethanol–water	182–184	72	$C_{14}H_7O_3NF_2$	C, N, H
2h	Cl	3,5-F	Ethanol	153–156	59	$C_{14}H_6O_3NClF_2$	C, N, H
2i	H	2,6-F	Acetonitrile–water	181–183	68	$C_{14}H_7O_3NF_2$	C, N, H
2j	Cl	2,6-F	Acetonitrile–water	139–141	74	$C_{14}H_6O_3NClF_2$	C, N, H

Table 2
Spectral data of 6-acyl-benzoxazolinone derivatives

Comp.	IR (cm^{-1})			^1H NMR (ppm)		
	Aromatic ketone C=O	Lactam C=O	N–H	2-Benzoxazolinone H^4	2-Benzoxazolinone $H^{5,7}$	Aromatic ring (benzene)
2a	1657	1747	3264	6.81–6.83 (s, 1H, $J = 8.24$ Hz)	7.63–7.69 (m, 2H)	7.13–7.26 (m, 3H)
2b	1618	1777	3293	6.87–6.89 (s, 1H, $J = 8.24$ Hz)	7.14–7.43 (m, 4H)	7.14–7.43 (m, 4H)
2c	1620	1757	3118	6.86–6.88 (s, 1H, $J = 8.24$ Hz)	7.57–7.66 (m, 2H)	7.08–7.32 (m, 3H)
2d	1623	1778	3295	6.84–6.86 (s, 1H, $J = 8.24$ Hz)	7.05–7.44 (m, 4H)	7.05–7.44 (m, 4H)
2e	1650	1789	3186	6.81–6.83 (s, 1H, $J = 8.24$ Hz)	7.63–7.70 (m, 2H)	7.13–7.26 (m, 3H)
2f	1615	1771	5155	6.90–6.92 (s, 1H, $J = 8.24$ Hz)	7.16–7.60 (m, 4H)	7.16–7.60 (m, 4H)
2g	1621	1810	3108	6.91–6.93 (s, 1H, $J = 8.24$ Hz)	7.55–7.61 (m, 2H)	7.10–7.30 (m, 3H)
2h	1622	1779	3161	6.82–6.83 (s, 1H, $J = 8.24$ Hz)	7.05–7.58 (m, 4H)	7.05–7.58 (m, 4H)
2I	1623	1771	3258	6.84–6.86 (s, 1H, $J = 8.24$ Hz)	7.64–7.40 (m, 2H)	6.95–7.11 (m, 3H)
2j	1621	1792	3197	6.82–6.84 (s, 1H, $J = 8.24$ Hz)	7.14–7.56 (m, 4H)	7.14–7.56 (m, 4H)

on a Perkin–Elmer 457 IR spectrophotometer (Überlingen, Germany). ^1H NMR spectra were recorded on a Bruker AC 400 MHz spectrometer (Karlsruhe, Germany) using dimethylsulfoxide-d_6 with chemical shifts reported as δ (ppm) with TMS as internal standard. Mass spectra were taken on a VG Analytical 70-250S or HP II Plus 5890 mass spectrometer with electron ionization (EI).

Microanalyses were performed in the laboratory of the Turkish Scientific and Technical Research Council (Ankara, Turkey). The purity of the compounds was assessed by TLC on silica gel $HF_{254+366}$ (E. Merck, Darmstadt, Germany).

2.1.1. 6-Acyl-2-benzoxazolinone

6-Acyl-2-benzoxazolinone was obtained by reacting 2-benzoxazolinone and/or 5-chloro-2-benzoxazolinone (chlorzoxazone) with the appropriate carboxylic acid in polyphosphoric acid (PPA) as reported by Pilli et al. [7].

2.2. Pharmacology

Locally-bred, female albino mice weighing 22 ± 2 g were used. The animals were transferred to the laboratory at least 2 days before the experiments to acclimatize them to their new environment. They were placed

in groups of six in glass cages, and fed with food and water ad libitum.

2.2.1. Analgesic activity [28]

Analgesic activity of the compounds was examined by employing a modified Koster test. Each compound was dispersed in carboxymethyl cellulose and then a 100 mg/kg dose was administered orally to a group of six mice. Exactly 1 h later, a 3% solution of acetic acid was injected intraperitoneally at a dose of 300 mg/kg and 5 min after the injections stretches were observed in the IR spectra for a further 10 min. Pure carboxymethyl cellulose was given to the control group ($n = 6$). As a standard analgesic, acetylsalicylic acid (ASA) was used. The analgesic activity was calculated using the following formula:

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

where n and n' indicate the average number of the stretches for the control and test groups, respectively.

3. Results

The molecular formulae, melting points, % yields and crystallization solvents used in this study are listed in Table 1. The compounds were obtained by reacting 2-benzoxazolinone or 5-chloro-2-benzoxazolinone with disubstituted fluorobenzoic acid derivatives in PPA (Scheme 1). The structure of the compounds were elucidated by IR, ^{1}H NMR, MS and microanalyses and all spectral data were in accordance with the assigned structure (Table 2). The analgesic activity of the compounds was screened by means of the modified Koster test (Table 3).

Table 3
Analgesic activities of 6-acyl-2-benzoxazolinone derivatives

Comp.[a]	R	R^1/R^2	% Analgesic activity[b]
2a	H	2,5-F	76.07
2b	Cl	2,5-F	64.26
2c	H	2,3-F	38.22
2d	Cl	2,3-F	78.63
2e	H	3,4-F	19.86
2f	Cl	3,4-F	63.40
2g	H	3,5-F	70.25
2h	Cl	3,5-F	–*
2i	H	2,6-F	53.26
2j	Cl	2,6-F	68.37
Aspirin	–	–	45.24

[a] 100 mg/kg p.o.
[b] Results are expressed as their mean values.
* No activity, $n = 6$, $P < 0.01$.

4. Discussion

Due to the fact that both the 3-nitrogen and 2-oxygen atoms were electron-donating, their 5th- and 6th-positions were activated and therefore, the regioselectivity in the C-acylation of benzoxazolones could not be easily predicted. The existence of 5-acylation products was reported earlier [29], but this report was not substantiated by any spectroscopic data. It was found that the spectral region corresponding to the aromatic protons extended over a wider range of chemical shifts (7.11–7.92 ppm) in the 6-series, and the 4- and 6-protons, which were in the vicinity of the carbonyl group, were well separated in the 5-series but overlapping occurred in the 6-series.

In order to clarify this situation, we reproduced the acylation in PPA (which unambiguously leads to 6-acyl derivatives) and also discovered that the isolated materials definitively belonged to the 6-acyl series.

In the IR spectra, absorption bands were detected at ca. 3200 cm^{-1}, thus indicating the presence of a N–H group. The lactam and ketone C=O stretching bands were seen at ca. 1810–1747 and 1657–1618 cm^{-1}, respectively. Other observed stretches confirmed the structures of these compounds.

As was reported earlier, the ^{1}H NMR spectral region extended over a wider range of chemical shifts (6.81–7.70) and so H^5 and H^7 protons (7.05–7.70), which were in close proximity to the carbonyl group, overlapped in the acylated products. The 2-benzoxazolinone H^4 proton was observed at ca. 6.81–6.89 ppm ($J = 8.2$ Hz). Other aromatic protons were seen as expected chemical shift and integral values.

The mass spectroscopic fragmentation of the 6-difluorobenzoyl-(5-chloro)-2-benzoxazolinone ring was studied under electron ionization. Molecular ion peaks were found to appear for 2-benzoxazolinone derivatives. Base peaks resulted from a loss of a difluorophenyl radical and gave a peak at m/z 162. A second cleavage occurred between the carbonyl and 2-benzoxazolinone ring. As a result, the remaining radical gave a peak at m/z 113. Molecular ion peaks were not found to appear for 5-chloro-2-benzoxazolinone (chlorzoxazone). Base peaks were seen at m/z 169, resulting from the loss of a difluorobenzoyl radical. Intense peaks were also seen as a result of CO_2 and 2CO loss from the molecular ions.

The results of microanalyses were within $\pm 0.4\%$ of theoretical values.

It was reported that the introduction of an acyl group into the 6th position of the benzoxazolinone ring caused an increase in activity [4]. These results led us to screen the compounds for analgesic activity. As seen in Table 3 (with the exception of **2c**, **2e** and **2h**), all the compounds showed analgesic activity greater than that of ASA ($P < 0.01$). In addition, substituting a chlorine atom into the 5th position of a benzoxazolinone also improved the analgesic activity of the compounds mentioned herein.

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