Synthesis and activity of 4-(2',4'-difluorobiphenylyl)-2-methylbutyric acid (deoxoflobufen) and its derivatives

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Summary
The series of 4-(2',4'-difluorobiphenylyl)-2-methylbutyric acid (deoxoflobufen, 1) and its four amides and two salts were prepared and tested for anti-inflammatory activity in rats and mice, using as models carrageenan-induced paw oedema, pleuritis, and arachidonic acid-induced ear inflammation, and on leucotriene B4 production in cells.

Keywords: deoxoflobufen – derivatives – salts – anti-inflammatory activity

INTRODUCTION

Since the introduction of modern non-steroidal anti-inflammatory drugs (NSAIDs) with ibuprofen and indomethacin, numerous compounds have been synthesised and tested for the treatment of rheumatic diseases. Since long term therapy with NSAIDs is occasionally associated with serious side effects, e.g., large intestinal ulcers, bleeding, perforation, relapse of classic inflammatory bowel disease, or complications of diverticular disease (fistula and perforation) (Szabo et al. 1989, Bjarnason et al. 1993), the development of novel compounds with improved efficacy and less toxic effect is constantly desirable. Possible targets of this development are compounds with improved influence on the biosynthesis of various mediators of inflammation and the inhibition of enzymes of arachidonic acid cascade, and modulators of the activity of immune systems.

In the framework of basic screening for new anti-inflammatory and anti-arthritic drugs the series of aryloxoalkanoic acids was synthesised and their structure was further optimised based on the QSAR analysis (Kuchař et al. 1988). In order to hinder the aromatic moiety from the metabolic hydroxylation and elimination, a new fluorine-containing representative of ω-biphenyl-ω-oxoalkanoic acids 4-(2',4'-difluorobiphenyl-4-yl)-2-methyl)-4-oxobutanoic acid (flobufen) was developed (Kuchař et al. 1988, Jegorov et al. 1995, Panajotova et al. 1997, Kuchař et al. 1997).

Further modification of the structure has indicated recently that the reductive elimination of a keto group in 4-(2',4'-difluorobiphenyl-4-yl)-2-methyl)-4-oxobutanoic acid (flobufen) providing
thus 4-(2’;4’-difluorobiphenyl)-2-methylbutyric acid (deoxoflobufen, 1) contributed further to the improvement of the immunomodulating effect with apparently reduced toxicity (Bulej et al. 2005). This study was dedicated to the comparison of 4-(2’;4’-difluorobiphenyl-4-yl)-2-methyl-4-oxobutanoic acid (flohuben) and 4-(2’;4’-difluorobiphenyl)-2-methylbutyric acid (deoxoflobfen, 1) in a series of models including acute carrageenan-induced inflammation, adjuvant arthritis, in vitro inhibition of LTB4 formation, graft-versus-host reaction (GVHR), the production of specific antibodies against ovalbumin, peritoneal exudate formation induced by thioglycollate, and phagocytosis of thioglycollate-stimulated mouse peritoneal macrophages (Bulej et al. 2005). In this work we describe in detail the synthesis, characterisation and biological activity of deoxoflobufen and some of its derivatives.

MATERIAL AND METHODS

Synthetic part
The melting points were determined on a Kofler block. TLC of compounds was measured using a silica gel (Kieselgel 60 FlSi, Fertigplatten, Merck, Germany) in the mixture cyclohexane:chloroform:methanol:acetic acid, 60:30:5:5, v/v/v/v (Rf of deoxoflobufen 0.52). The IR spectra were measured on a Nexus (Thermo Nicolet) instrument in KBr pellets. UV spectra were measured on a Varian DMS 300 instrument at the concentration 5×10^{-5} mol/l in methanol. Positive ion ESI MS spectra were measured on a Finnigan LCQ instrument in methanol:acetic acid (100 ml), and hydrochloric acid (3×100 ml of 1 N). The reaction mixture was allowed to heat to room temperature and stirred for 30 min, then cooled down to –30°C, and spectra of derivatives were measured. The IR spectra were measured on a Bruker DPX 250 spectrometer. The 1H, 13C, and 19F NMR spectra were measured on a Varian INOVA-400 instrument (399.90 MHz for 19F, 376.25 MHz for 13C, 100.56 MHz for 1H, 399.90 MHz for 1H, 376.25 MHz for 19F, 141.14 (1); 132.74 (4); 131.22 (6’), 128.93 (2, 6); 128.61 (3, 5); 125.22 (1’); 111.47 (5’); 103.31 (3’); 38.80 (α); 35.02 (β); 33.07 (γ); and 16.93 (α-Me); 19F NMR (376.25 MHz, CDCl3, 30°C): -114.80 dddd (1.1, 1.8, 8.7, 10.5, 7.9, 2-F), and -113.13 dddd (8.9, 8.0, 7.9, 6.5, 4-F; JF-JF = 4.7) in the form of free acid and benzammonium salt (Bulej et al. 2004a, 2004b). IR (KBr) ν(C=O) 1740 cm⁻¹, MS (+ESI), [M+H]⁺ m/z 203 (m/z 203) and C 13H9F2O+N⁺ m/z 273, [M+H]+ m/z 197 and [M+H]+ (308) C8H10F2O⁺ m/z 203 (splitting of βC-γC bond). H NMR (CDCl3, 400 MHz) 7.43 AA’BB’X, Σ J = 8.3, 1.8, 2 H (2, 2: 7.39 ddd J = 8.7, 8.7, 6.5, 1 H (6’); 7.27 AA’BB’X, Σ J = 8.3, 2 H (3, 5); 6.94 dddd J = 8.7, 8.0, 2.6, 1.1, 1 H (5’); 6.90 dddd J = 10.5, 8.9, 2.6, 1 H (3’); 2.72 m 2 H (γ); 2.55 ddq J = 7.0, 7.0, 7.0, 1 H (α); 2.09 and 1.79 m 2 H (β); 1.26 ddq J = 7.0, 7.0, 7.0, 3 H (α-Me); 13C NMR (CDCl3, 100 MHz) 182.47 (C=O), 166.22 (2’); 159.74 (4’); 141.14 (1); 132.74 (4); 131.22 (6’), 128.93 (2, 6); 128.61 (3, 5); 125.22 (1’), 111.47 (5’); 103.31 (3’); 38.80 (α); 35.02 (β); 33.07 (γ); and 16.93 (α-Me); 19F NMR (376.25 MHz, CDCl3, 30°C) -114.80 dddd (1.1, 1.8, 8.7, 10.5, 7.9, 2-F), and -113.13 dddd (8.9, 8.0, 7.9, 6.5, 4-F; JF-JF = 4.7).

Deoxoflobufen (1)
4-(2’;4’-Difluorobiphenyl-4-yl)-2-methylbutyric acid (deoxoflobfen, VÚFB 19053) was obtained by crystallisation from methanol. Yield: 17.8 g. TLC Rf 0.52. Deoxoflobufen was characterised by spectral methods and X-ray crystal structure determination in the form of free acid and benzylammonium salt (Bulej et al. 2004a, 2004b). IR (KBr) ν(C=O) 1701 cm⁻¹, UV (CH3OH) 244.8 nm, νs = 1.77 10⁻³, MS (+ESI), [M+NH4]⁺ m/z 308, m/2 291 and [R-C=O]⁺ m/z 273, ms 3 (308) C8H10F2O+N⁺ m/z 203 (splitting of βC-γC bond). H NMR (CDCl3, 400 MHz) 7.43 AA’BB’X, Σ J = 8.3, 1.8, 2 H (2, 2: 7.39 ddd J = 8.7, 8.7, 6.5, 1 H (6’); 7.27 AA’BB’X, Σ J = 8.3, 2 H (3, 5); 6.94 dddd J = 8.7, 8.0, 2.6, 1.1, 1 H (5’); 6.90 dddd J = 10.5, 8.9, 2.6, 1 H (3’); 2.72 m 2 H (γ); 2.55 ddq J = 7.0, 7.0, 7.0, 1 H (α); 2.09 and 1.79 m 2 H (β); 1.26 ddq J = 7.0, 7.0, 7.0, 3 H (α-Me); 13C NMR (CDCl3, 100 MHz) 182.47 (C=O), 166.22 (2’); 159.74 (4’); 141.14 (1); 132.74 (4); 131.22 (6’), 128.93 (2, 6); 128.61 (3, 5); 125.22 (1’), 111.47 (5’); 103.31 (3’); 38.80 (α); 35.02 (β); 33.07 (γ); and 16.93 (α-Me); 19F NMR (376.25 MHz, CDCl3, 30°C) -114.80 dddd (1.1, 1.8, 8.7, 10.5, 7.9, 2-F), and -113.13 dddd (8.9, 8.0, 7.9, 6.5, 4-F; JF-JF = 4.7).
N-Cyclohexyl-4-(2',4'-difluoro-biphenyl-4-yl)-2-methyl-butyramide (3)

Deoxoflobufen (1, 5 g, 0.0172 mol) was dissolved in a mixture of dimethylfomamide (35 ml) and dichloromethane (150 ml). The solution was cooled down to –15°C, N-methylmorpholine (2.85 ml, 0.026 mol) and ethyl chloroformate (3.38 ml, 0.026 mol) were added. The reaction mixture was stirred for 30 min, then cooled down to –30°C and a solution of cyclohexylamine (1.97 ml, 0.0172 mol) in dichloromethane (10 ml) was added. The mixture was allowed to heat to room temperature and stirred at this temperature for an additional 2 h.

The reaction mixture was extracted with aqueous sodium hydrogencarbonate (3 × 100 ml, 5%), water (80 ml), and hydrochloric acid (3 × 80 ml of 1 N). The organic layer was evaporated to a solid residue and crystallised from the mixture of dichloromethane : hexane = 1:4, v/v. Crystals were washed with cold hexane. Yield 2.3 g (35.6%). TLC Rf 0.57. Melting point 93-95°C. For C21H23F2NO3 (375.422) calculated: 67.18% C, 6.18% H, 10.12% F, 3.73% N. IR (KBr) ν(C=O) 1645 cm−1, ν(N-H) and ν(O-H) 3291 cm−1.

{4-(2',4'-Difluoro-biphenyl-4-yl)-2-methyl-butyramino]-acetic acid ethyl ester (5)

Deoxoflobufen (1, 6.2 g, 0.0213 mol) was dissolved in the mixture of dimethylfomamide (45 ml) and dichloromethane (190 ml). The solution was cooled down to –15°C, of 1-ethylpiperidine (10.3 ml, 0.0746 mol) and isobutyl chloroformate (2.7 ml) were added. The reaction mixture was stirred for 30 minutes at the ambient temperature and then cooled down to –30°C. Ethanolamine (0.9 ml, 0.914 mol) was added and the temperature was allowed to rise to ambient temperature and stirred for an additional 2 h.

The reaction mixture was extracted with aqueous sodium hydrogencarbonate (3 × 80 ml, 5%), water (80 ml), and hydrochloric acid (3 × 80 ml of 1 N). The organic layer was evaporated to a solid residue and crystallised from the mixture of dichloromethane : hexane = 1:4, v/v. Crystals were washed with cold hexane. Yield 2.89 g (75.07%). TLC Rf 0.36. Melting point 93-95°C. For C21H23F2NO3 (375.422) calculated: 67.18% C, 6.18% H, 10.12% F, 3.73%
N, 12.78% O; found 67.05% C, 6.14% H, 10.14% F, 3.59% N. IR (KBr) ν(C=O) 1540 cm⁻¹. UV (CH₃OH) 245.5 nm, ϵ₂₃ = 1.91 10³. MS (+ESI), [M+NH₄]⁺ m/z 393, [M+H]⁺ m/z 376, m/z 376 m/z 330 splitting of an ester group, [R-C=O]⁻ m/z 273 and C₂H₄F₄⁻ m/z 203 (splitting of β-C bonds). ¹H NMR (CDCl₃) 7.38 m 3 H (2, 6, 6'); 7.26 d J = 8.5, 2 H (3, 5'); 6.90 m, 2 H (3', 5'); 6.02 m, 1 H (NH); 4.22 q 2 H (CH₂ – Et); 4.04 m 2 H (CH₂ – CO₂); 2.68 m, 2 H (γ); 2.32 m 1 H (α); 2.07 m and 1.75 m (β); and 1.29 t 3 H (CH₃ – Et); 1.21 d J = 6.9, 3 H (α-Me); ¹³C NMR (CDCl₃) 176.67 (C=O); 170.48 (C=O side chain); 162.51 J CF = 248.9 and 11.5 (2'); 160.11 J CF = 250 and 12.1 (4'); 141.77 (4'); 132.98 J CF = 12 (1); 131.70 J CF = 9.7 and 4.8 (6'); 129.00 (2, 6); 128.94 (3, 5); 125.68 (1'); 111.86 J CF = 21.2 and 4.2 (5'); 104.68 J CF = 27.3 and 25.4 (3'); 61.88 (CH₂ – Et); 41.67 (CH₂ – NH); 40.93 (α); 35.97 (β); 33.53 (γ); 18.35 (α-Me); and 14.52 (CH₃ – Et); ¹⁹F NMR (CDCl₃) 112.34 (2'); 114.02 (4').

Benzylammonium, 4-(2',4'-difluorobiphenyl-4-yl)-2-methylbutyrate (6)
Deoxylsobufen (I, 2.4 g, 0.083 mol) was dissolved in the mixture acetone : ether (19.8 ml, 2:1, v/v). A solution of benzyamine (0.9 ml, 0.0082 mol) in the mixture acetone : ether (16.2 ml, 2:1, v/v) was added. The reaction mixture was stirred for 1 h and then chilled in the refrigerator to 4°C. The crystals were separated and washed with cold acetone (2×9 ml). Yield 2.03 g (64.05%). TLC Rf 0.43. Melting point 120-123°C. For C₈H₁₈F₄NO₂ (383.442) calculated: 72.05% C, 6.05% H, 9.91% F, 3.65% N, 8.34% O; found 72.71% C, 6.38% H, 9.68% F, 3.39% N. X-ray crystal structure determination (Bulej et al 2004b). IR (KBr) ν(C=O) 1540 cm⁻¹, ν(N-H) 3434 cm⁻¹, broad. UV (CH₃OH) 245.1 nm, ϵ₂₃ = 1.82 10³. MS (+ESI), [M+NH₄]⁺ m/z 398, m/z 376 (398) m/z 270, 231, and 213. ¹H NMR (DMSO-d₆) 7.51 m 1 H (6); 7.41 dd J = 8.5 and 1.6, 2 H (2, 6); 7.28 d J = 8.5, 2 H (3, 5); 7.11 m, 2 H (3', 5'); 2.68 t J = 7.8 2 H (γ); 2.68 m, 1 H (CH cyclohexyl); 2.36 m, 1 H (α); 1.97 m, 1 H (β) 1.83-1.47 and 1.34-1.00 multiplets (CH₂ cyclohexyl); 1.10 d J = 6.9, 3 H (α-Me); ¹³C NMR (DMSO-d₆) 178.23 (C=O), 162.51 J CF = 247 and 12.1 (2'); 160.16 J CF = 248.3 and 12.1 (4'); 142.87 (4'); 132.71 J CF = 1.2 (1); 132.45 J CF = 9.7 and 5.5 (6); 129.36 J CF = 3 (2, 6); 129.24 (3, 5); 125.90 J CF = 13.9 and 4.2 (1'); 112.49 J CF = 21.2 and 3.6 (5'); 104.95 J CF = 27.3 and 26 (3'); 50.47 (αC cyclohexyl); 40.02 (α); 36.03 (βC cyclohexyl); 35.96 (β); 33.55 (γ); 26.18 (8C cyclohexyl); 25.15 (γC cyclohexyl); and 17.87 (α-Me); ¹⁹F NMR (DMSO-d₆) 112.33 (2'); 113.68 (4').

Biological Assays
The inhibition of carrageenan-induced rat paw oedema was evaluated by the method described by Winter (Winter et al. 1962); the experimental conditions are described in our previous works (Panajotova et al. 1997, Bulej et al. 2005). The effect was expressed in per cent of oedema inhibition compared with an untreated control.

The inhibition of experimental pleuritis was evaluated by the method described by Hidaka (Hidaka et al. 1986) in a group of Wistar Han female rats pre-treated with 0.5% carrageenan in saline (intraperitoneal injection) The tested compounds were suspended with gum arabic and applied orally in a single dose 1 h before the application of carrageenan. The volume of the exudate from the pleural cavity was compared with that of untreated animals; the total cell number and cellularity (determined by Sysmex cell counter) were also compared.

Arachidonic acid-induced ear inflammation in mice was produced by the method described by Opas (Opas et al. 1985); the ear-lobe inflammation was induced by application of 20 µl arachidonic acid solution in acetone. The compound was given orally 16 h before oedema induction. The degree of ear-lobe hyperemia and the weight of ear lobes were evaluated 1 h after the application of arachidonic acid. The results were expressed as
percent of inhibition in comparison with untreated control.

The production of leucotriene B\textsubscript{4} (LTB\textsubscript{4}) was determined in rat polymorphonuclear cells from pleural exudate elicited by heat-inactivated rat serum Palmer and Salmon 1983). The cells were stimulated by the Ca\textsuperscript{2+} ionophore A23187 (Sigma) and incubated with a solution of tested drugs. LTB\textsubscript{4} was determined in supernatants using commercial RIA kit (Amersham). All results are summarised in Table 1.

RESULTS AND DISCUSSION

The series of 4-(2',4'-difluorobiphenylyl)-2-methylbutyric acid (deoxoflobufen, 1) and its four amides (2-5), and two salts (6, 7) were prepared, starting directly from (4-(2',4'-Difluorobiphenyl-4-yl)-2-methylen-4-oxobutanoic acid) (Jegorov et al. 1997), Fig. 1.

From the chemical point of view, novel derivatives share a number of spectroscopic properties of 4-(2',4'-difluorobiphenyl-4-yl)-2-methyl-4-oxobutanoic acid (flobufen) facilitated by the presence of common 4-(2',4'-difluorobiphenyl-4-yl) moiety. Spectral similarities are manifested - namely by very similar \(\text{^1H, ^13C, and ^19F NMR spectra - and also there is a close similarity of the conformation of (2',4'-difluorobiphenyl-4-yl) group in the solid state (Bulej et al 2004a, 2004b).}

The main spectral differences associated with the reduction of a C=O group in 4-(2',4'-difluorobiphenyl-4-yl)-2-methyl)-4-oxobutanoic acid (flobufen) to a CH\textsubscript{2} group in deoxoflobufen can be observed in the UV spectra. Whereas flobufen starts to absorb at about 320 nm with a maximum at 272 nm (\(\alpha\)-transition, \(\epsilon\text{M} = 2.010^4\), methanol), \(\beta\)-transition (\(1B1u\)) lies about 215 nm and continues to the cut of wavelength, derivatives of deoxoflobufen start to absorb at about 300 nm, with a shoulder at about 280 nm and a maximum at 245 nm (\(\epsilon\text{M} = 1.8–1.910^4\), methanol).

The formation of deoxoflobufen derivatives is accompanied by a typical shift of \(v(C=O)\) vibration in deoxoflobufen (1) from 1701 cm\textsuperscript{-1} to about 1640 and 1540 cm\textsuperscript{-1} in its amides (2-5) and salts (6, 7), respectively. Positive ion ESI spectra were found useful particularly with respect to the possibility of detection of [deoxoflobufen\textsuperscript{+}benzylammonium or cyclohexylammonium]\textsuperscript{+} ions in the case of deoxoflobufen salts even in the presence of ammonium formate in the solution. Deoxoflobufen and its derivatives share also a common fragment \(C_{13}H_{9}F_{2}\textsuperscript{+}\) in the ms\textsuperscript{n} spectra formed by the preference splitting of \(\beta\text{-C-\gammaC bond of deoxoflobufen.}\)

![Fig. 1. Structure of deoxoflobufen (4-(2',4'-Difluoro-biphenyl-4-yl)-2-methyl-butyric acid) and its derivatives](image-url)
Table 1. Biological activities of deoxoflobufen derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>Paw oedema&lt;sup&gt;a&lt;/sup&gt;, %</th>
<th>Pleuritis&lt;sup&gt;b&lt;/sup&gt;, %</th>
<th>Ear inflammation&lt;sup&gt;c&lt;/sup&gt;, %</th>
<th>LTB&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
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<td>(1)</td>
<td>69&lt;sup&gt;+&lt;/sup&gt;</td>
<td>66&lt;sup&gt;+&lt;/sup&gt;</td>
<td>67&lt;sup&gt;+&lt;/sup&gt;</td>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>(2)</td>
<td>56&lt;sup&gt;+&lt;/sup&gt;</td>
<td>11</td>
<td>12</td>
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<td>(3)</td>
<td>32&lt;sup&gt;+&lt;/sup&gt;</td>
<td>15</td>
<td>11</td>
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<td>(4)</td>
<td>29&lt;sup&gt;+&lt;/sup&gt;</td>
<td>58&lt;sup&gt;+&lt;/sup&gt;</td>
<td>68&lt;sup&gt;+&lt;/sup&gt;</td>
<td>25&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>(6)</td>
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<td>27&lt;sup&gt;+&lt;/sup&gt;</td>
<td>62&lt;sup&gt;+&lt;/sup&gt;</td>
<td>48&lt;sup&gt;+&lt;/sup&gt;</td>
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<sup>a</sup> percent of inhibition, dose 100 mg/kg; <sup>b</sup> percent inhibition, dose 100 mg/kg: A volume of exudate, B number of cells, C cellularity; <sup>c</sup> percent inhibition, dose 200 mg/kg: A weight of ear lobes, B degree of ear-lobe hyperemia; <sup>d</sup> percent inhibition at the concentration of 30 µg/ml + statistically significant data, n statistically insignificant data, for denomination of individual compounds see Fig. 1

Deoxoflobufen exhibited very pronounced anti-inflammatory effects in the models of acute inflammation and inhibited LTB<sub>4</sub> production, Table 1. The test derivatives did not show better pharmacodynamic profile in comparison with deoxoflobufen, but still had interesting anti-inflammatory properties. All compounds inhibited significantly carrageenan-induced paw oedema and lowered LTB<sub>4</sub> production. The derivatives 4, 5, 6 and 7 decreased exudate formation in the pleural cavity and the compounds 5, 6 and 7 markedly reduced hyperemia development in the model of arachidonic acid-induced ear inflammation. The anti-inflammatory activity of highly lipophilic amides 2 and 3 in the model of experimental pleuritis is markedly reduced. Their poor bioavailability in the pleural cavity could be a plausible explanation.

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