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## ORIGINAL ARTICLE

# 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 $B_4$ 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-biphenylyl-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

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thus 4-(2',4'-difluorobiphenylyl)-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 (flobufen) and 4-(2',4'-difluorobiphenylyl)-2-methylbutyric acid (deoxoflobufen, **1**) in a series of models including acute carrageenan-induced inflammation, adjuvant arthritis, in vitro inhibition of $LTB_4$ 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 $F_{254}$, Fertigplaten, Merck, Germany) in the mixture cyclohexane:chloroform: methanol:acetic acid, 60:30:5:5, v/v/v/v ($R_F$ of deoxoflobufen 0.52). The IR spectra were measured on Nexus (Thermo Nicolet) 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 : water (80:20 v/v) containing 10 mmol/l $HCOONH_4$ (spray voltage 4.00 kV, spray current 6.0 μA, capillary voltage 26.9 V, capillary temperature 199.9°C). The $^1H$, $^{13}C$, and $^{19}F$ NMR spectra of deoxoflobufen were measured on a Varian INOVA-400 instrument **(**399.90 MHz for $^1H$, 100.56 MHz for $^{13}C$, 376.25 MHz for $^{19}F$, $CDCl_3$, 30°C), and spectra of derivatives were measured on a Bruker DPX 250 spectrometer. The $^1H$ and $^{13}C$ chemical shifts are relative to tetramethylsilane, the $^{19}F$ chemical shifts are relative to $CFCl_3$.

*Deoxoflobufen (1)*

4-(2',4'-Difluorobiphenyl-4-yl)-2-methylen-4-oxobutanoic acid (Jegorov et al. 1997) (20 g), was dissolved in acetic acid (100 ml). Pd/C was added (10% Pd/C, 50% $H_2O$, 8 g), and the reaction mixture was hydrogenated at ambient pressure under vigorous stirring with a magnetic stirrer and heating at 60°C. After 5 h, Pd/C was filtered off and evaporated on a rotary vacuum evaporator. 4-(2',4'-Difluorobiphenylyl)-2-methylbutyric acid (deoxoflobufen, VÚFB 19053) was obtained by crystallisation from methanol. Yield: 17.8 g. TLC $R_F$ 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 ($CH_3OH$) 244.8 nm, $\varepsilon_M$ = 1.77 $10^4$. MS (+ESI), $[M+NH_4]^+$ m/z 308, $ms^2$ $[M+H]^+$ m/z 291 and $[R\text{-}C{\equiv}O]^+$ m/z 273, $ms^3$ (308) $C_{13}H_9F_2^+$ m/z 203 (splitting of βC- γC bond). $^1H$ NMR ($CDCl_3$, 400 MHz) 7.43 AA´BB´X, Σ *J* = 8.3, 1.8, 2 H (2, 6); 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 ddd *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); $^{13}C$ NMR ($CDCl_3$) 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); $^{19}F$ NMR (376.25 MHz, $CDCl_3$, 30°C): -114.80 dtddd (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); $J_{F,F}$ is given in bold.

*N-Benzyl-4-(2',4'-difluoro-biphenyl-4-yl)-2-methyl-butyramide (2)*

Deoxoflobufen (**1**, 5 g, 0.0172 mol) was dissolved in a mixture of dimethylformamide (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 (1.65 ml, 0.0173 mol) were added. The reaction mixture was stirred for 30 min, then cooled down to –30°C, and a solution of benzylamine (1.86 ml, 0.0170 mol) in dichloromethane (10 ml) was added. The mixture was allowed to heat to room temperature and stirred at this temperature for 2 h. The reaction mixture was extracted with aqueous sodium hydrogencarbonate (3×100 ml, 5%), water (100 ml), and hydrochloric acid (3×100 ml of 1 N). The organic layer was evaporated and a solid residue was crystallised from the mixture dichloromethane:hexane = 1:4, v/v. The crystals were separated with a suction funnel and washed with cold hexane. Yield 3.25 g (47.7%). TLC $R_F$ 0.54. Melting point 111–113°C. For $C_{24}H_{23}F_2NO$ (379.452) calculated: 75.97% C, 6.11% H, 10.01% F, 3.69% N, 4.22% O; found 75.41% C, 6.08% H, 9.57% F, 3.52% N. IR (KBr) ν(C=O) 1640 $cm^{-1}$. UV ($CH_3OH$) 245.8 nm, $\varepsilon_M$ = 1.83 $10^4$. MS (+ESI), $[M+NH_4]^+$ m/z 397, $[M+H]^+$ m/z 380, $ms^2$ (380) $[R\text{-}C{\equiv}O]^+$ m/z 273 and $C_{13}H_9F_2^+$ m/z 203 (splitting of βC- γC bond). $^1H$ NMR ($CDCl_3$) 7.23 m 8 H (2, 6, 6´, and Bn); 7.10 d *J* = 8.2, 2 H (3, 5); 6.81 m, 2 H (3´, 5´); 5.82 m, 1 H (NH); 4.35 m, 2H, ($CH_2$-Bn); 2.56 m, 2 H (γ); 2.16 m 1 H (α); 1.97 m and 1.66 m 2 H (β); 1.11 d *J* = 6.6, 3 H (α-Me); $^{13}C$ NMR ($CDCl_3$) 174.91 (C=O), 161.10 $J_{CF}$ = 248.3 and 11.5 (2´); 158.68 $J_{CF}$ = 250 and 11.5 (4´);

140.36 (4); 137.52 (ipso-Bn); 131.56 $J_{CF}$ = 1.2 (1), 130.28 $J_{CF}$ = 5.4 and 9.7 (6′), 127.86 $J_{CF}$ = 3 (2, 6); 127.69 (meta-Bn); 127.54 (3, 5); 126.78 (ortho-Bn); 126.47 (para-Bn); 124.05 (1′); 110.46 $J_{CF}$ = 4.2 and 21.2 (5′); 103.28 $J_{CF}$ = 25.4 and 27.2 (3′); 42.45 ($CH_2$ -Bn); 39.80 (α); 34.58 (β); 32.21 (γ); and 17.05 (α-Me); $^{19}$F NMR ($CDCl_3$) 112.21 (2′); 113.98 (4′).

*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 dimethylformamide (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 hours. The reaction mixture was extracted with aqueous sodium hydrogencarbonate (3 × 100 ml, 5%), water (100 ml), and hydrochloric acid (3 × 100 ml of 1 N). The organic layer was evaporated and a solid residue was crystallised from the mixture of dichloromethane : hexane = 1:4, v/v. The crystals were separated with a suction funnel and washed with cold hexane. Yield 2.3 g (35.6%). TLC $R_F$ 0.57. Melting point 131–132°C. For $C_{23}H_{27}F_2NO$ (371.974) calculated: 74.36% C, 7.33% H, 10.23% F, 3.74% N, 4.31% O; found 74.12% C, 7.27% H, 10.14% F, 3.53% N. IR (KBr) ν(C=O) 1637 cm$^{-1}$, ν(N-H) 3298 cm$^{-1}$. UV ($CH_3OH$) 245.3 nm, $\varepsilon_M$ = 1.99 · 10$^4$. MS (+ESI), $[M+NH_4]^+$ m/z 389, $[M+H]^+$ m/z 372, ms$^2$ (372) $[R\text{-}C{\equiv}O]^+$ m/z 273 and $C_{13}H_9F_2^+$ m/z 203 (splitting of βC- γC bond). $^1$H NMR ($CDCl_3$) 7.28 m 3 H (2, 6, 6′); 7.15 d *J* = 8.2, 2 H (3, 5); 6.81 m, 2 H (3′, 5′); 5.40 d, *J* = 8.5, 1 H (NH); 3.72 m, 1 H, (CH cyclohexyl); 2.56 m, 2 H (γ); 2.08 m 1 H (α); 2.00-2.00 and 1.39-0.94 multiplets (β and cyclohexyl); 1.08 d *J* = 6.6, 3 H (α-Me); $^{13}$C NMR ($CDCl_3$) 174.06 (C=O), 161.10 $J_{CF}$ = 248.9 and 12.1 (2′); 158.71 $J_{CF}$ = 250 and 12.1 (4′); 140.54 (4); 131.53 $J_{CF}$ = 1.2 (1), 130.29 $J_{CF}$ = 5.2 and 9.7 (6′), 127.85 $J_{CF}$ = 3 (2, 6); 127.57 (3, 5); 124.05 (1′); 110.45 $J_{CF}$ = 4.2 and 21.2 (5′); 103.27 $J_{CF}$ = 26.6 and 25.4 (3′); 46.98 (αC cyclohexyl); 39.93 (α); 34.69 (β); 32.26 (γ); and 17.13 (α-Me); 32.37, 32.18, 24.25, 23.91 (cyclohexyl); $^{19}$F NMR ($CDCl_3$) 112.25 (2′); 114.00 (4′).

*4-(2',4'-Difluoro-biphenyl-4-yl)-N-(2-hydroxy-ethyl)-2-methyl-butyramide (4)*

Deoxoflobufen (**1**, 4.0 g, 0.014 mol) was dissolved in a mixture of dimethylformamide (30 ml) and dichloromethane (120 ml). The reaction mixture was cooled down to –15°C, and *N*-methylmorpholine (2.3 ml, 0.021 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 hours. 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 3.4 g (74.1%). TLC $R_F$ 0.17. Melting point 108–109 °C. For $C_{19}H_{21}F_2NO_2$ (333.386) calculated: 68.45% C, 6.35% H, 11.39% F, 4.20% N, 9.59% O; found 68.25% C, 6.31% H, 10.72% F, 3.87% N. IR (KBr) ν(C=O) 1645 cm$^{-1}$, ν(N-H) and ν(O-H) 3291 cm$^{-1}$, broad. UV ($CH_3OH$) 245.5 nm, $\varepsilon_M$ = 1.86 10$^4$. MS (+ESI), $[M+H]^+$ m/z 334, ms$^2$ (334) $[M+H\text{-}H_2O]^+$ m/z 317, $[R\text{-}C{\equiv}O]^+$ m/z 273, and $C_{13}H_9F_2^+$ m/z 203 (splitting of βC- γC bond). $^1$H NMR ($CDCl_3$) 7.35 m 3 H (2, 6, 6′); 7.22 d *J* = 8.2, 2 H (3, 5); 6.88 m, 2 H (3′, 5′); 6.15 m, 1 H (NH); 3.70 t *J* = 5, 1 H, ($CH_2$ -OH); 3.40 t *J* = 5, 1 H, ($CH_2$ -NH); 3.05 broad s (OH); 2.65 m, 2 H (γ); 2.27 m 1 H (α); 2.03 m and 1.73 m (β); 1.18 d *J* = 6.9, 3 H (α-Me); $^{13}$C NMR ($CDCl_3$) 177.98 (C=O), 162.52 $J_{CF}$ = 248.9 and 12.1 (2′); 160.08 $J_{CF}$ = 250 and 12.1 (4′); 141.68 (4); 133.01 $J_{CF}$ = 1.2 (1), 131.68 $J_{CF}$ = 5.2 and 9.7 (6′), 129.30 $J_{CF}$ = 3 (2, 6); 128.94 (3, 5); 125.50 $J_{CF}$ = 13.9 and 4.2 (1′); 111.89 $J_{CF}$ = 3.6 and 21.2 (5′); 104.68 $J_{CF}$ = 26.6 and 24.8 (3′); 62.52 ($CH_2$ -OH); 42.71 ($CH_2$ -NH); 41.15 (α); 35.97 (β); 33.60 (γ); and 18.35 (α-Me); $^{19}$F NMR ($CDCl_3$) 112.32 (2′); 113.89 (4′).

*[4-(2',4'-Difluoro-biphenyl-4-yl)-2-methyl-butyrylamino]-acetic acid ethyl ester (5)*

Deoxoflobufen (**1**, 6.2 g, 0.0213 mol) was dissolved in the mixture of dimethylformamide (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 (4.2 ml, 0.032 mol) were added. The reaction mixture was stirred for 30 min at ambient temperature, then cooled down to –30°C. A solution of glycine ethyl ester (2.98 g) in dichloromethane (10 ml) was added. The reaction mixture was allowed to heat to room temperature and stirred for an additional 2 h. The reaction mixture was extracted with aqueous sodium hydrogencarbonate (3×130 ml, 5%), water (130 ml), and hydrochloric acid (3×130 ml of 1 N). The organic layer was evaporated to a solid residue and crystallised from the mixture dichloromethane : hexane = 1:4, v/v. The crystals were washed with cold hexane. Yield 2.89 g (75.07%). TLC $R_F$ 0.36. Melting point 93-95°C. For $C_{21}H_{23}F_2NO_3$ (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 ($CH_3OH$) 245.5 nm, $\varepsilon_M$ = 1.91 $10^4$. MS (+ESI), $[M+NH_4]^+$ m/z 393, $[M+H]^+$ m/z 376, $ms^2$ (376) m/z 330 splitting of an ester group, $[R\text{-}C\equiv O]^+$ m/z 273 and $C_{13}H_9F_2^+$ m/z 203 (splitting of βC- γC bond). $^1$H NMR ($CDCl_3$) 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 ($CH_2$ – Et); 4.04 m 2 H ($CH_2$ – CO); 2.68 m, 2 H (γ); 2.32 m 1 H (α); 2.07 m and 1.75 m (β); 1.29 t 3 H ($CH_3$ – Et); 1.21 d *J* = 6.9, 3 H (α-Me); $^{13}$C NMR ($CDCl_3$) 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 $J_{CF}$ = 1.2 (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 ($CH_2$ -Et); 41.67 ($CH_2$ -NH); 40.93 (α); 35.97 (β); 33.53 (γ); 18.35 (α-Me); and 14.52 ($CH_3$ – Et); $^{19}$F NMR ($CDCl_3$) 112.34 (2´); 114.02 (4´).

*Benzyl-ammonium, 4-(2',4'-Difluoro-biphenyl-4-yl)-2-methyl-butyrate (6)*

Deoxoflobufen (**1**, 2.4 g, 0.083 mol) was dissolved in the mixture acetone : ether (19.8 ml, 2:1, v/v). A solution of benzylamine (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 $R_F$ 0.43. Melting point 120–123°C. For $C_{23}H_{23}F_2NO_2$ (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 ($CH_3OH$) 245.1 nm, $\varepsilon_M$ = 1.82 $10^4$. MS (+ESI), $[M+H]^+$ m/z 398, $ms^2$ (398) m/z 270, 231, and 213. $^1$H NMR (DMSO-$d_6$) 7.51 m 1 H (6´); 7.41 dd *J* = 8.5 and 1.6, 2 H (2, 6); 7.29 m 7 H (3, 5, Bn); 7.11 m 2 H (3´, 5´); 3.77 m, 2H, ($CH_2$ -Bn); 2.68 t *J* = 7.8, 2 H (γ); 2.4 m 1 H (α); 1.97 m and 1.70 m 2 H (β); 1.14 d *J* = 6.9, 3 H (α-Me); $^{13}$C NMR (DMSO-$d_6$) 174.84 (C=O), 162.56 $J_{CF}$ = 247 and 12.1 (2´); 160.18 $J_{CF}$ = 248.3 and 12.1 (4´); 144.50 (ipso-Bn); 142.65 (4); 132.81 $J_{CF}$ = 1.2 (1), 132.48 $J_{CF}$ = 4.8 and 9.7 (6´), 129.44 $J_{CF}$ = 2.5 (2, 6); 129.27 (3, 5); 128.86 (meta-Bn); 127.54 (3, 5); 127.87 (ortho-Bn); 127.00 (para-Bn); 125.82 (1´); 112.53 $J_{CF}$ = 21.2 and 3.6 (5´); 105.00 $J_{CF}$ = 27.3 and 26 (3´); 46.40 ($CH_2$ -Bn); 35.71 (α); 39.54 (β); 33.42 (γ); and 17.67 (α-Me); $^{19}$F NMR (DMSO-$d_6$) 112.27 (2´); 113.67 (4´).

*Cyclohexylammonium, 4-(2',4'-Difluoro-biphenyl-4-yl)-2-methyl-butyrate (7)*

Deoxoflobufen (**1**, 2.0 g, 0.0069 mol) was dissolved in the mixture acetone : ether (16.8 ml, 2:1, v/v). A solution of cyclohexylamine (0.079 ml, 0.0069 mol) in the mixture acetone : ether (13.2 ml, 2:1, v/v) was added. The product precipitated within several min. The precipitate was stirred for an additional 1 h, separated and washed with cold acetone (2×6 ml). Yield 2.03 g (75.7%). TLC $R_F$ 0.45. Melting point 133-138°C. For $C_{23}H_{29}F_2NO_2$ (389.49) calculated: 70.93% C, 7.5% H, 9.76% F, 3.6% N, 8.22% O; found 70.98% C, 7.47% H, 9.78% F, 3.55% N. IR (KBr) ν(C=O) 1541 $cm^{-1}$, ν(N-H) 3431 $cm^{-1}$, broad. UV ($CH_3OH$) 246.0 nm, $\varepsilon_M$ = 1.82 $10^4$. $[M+H]^+$ m/z 390, $ms^2$ (398) m/z 270, 231, and 213. $^1$H NMR (DMSO-$d_6$) 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 ($CH_2$ cyclohexyl); 1.10 d *J* = 6.9, 3 H (α-Me); $^{13}$C NMR (DMSO-$d_6$) 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 (δC cyclohexyl); 25.15 (γC cyclohexyl); and 17.87 (α-Me); $^{19}$F NMR (DMSO-$d_6$) 112.33.4 (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 (intrapleural 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.

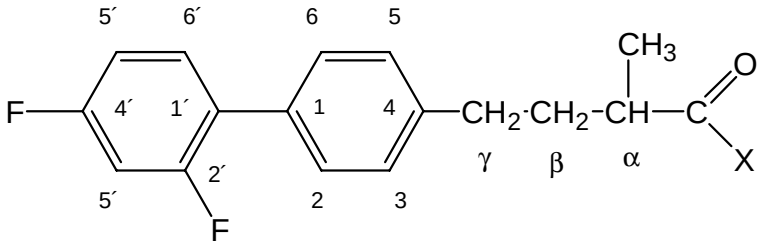
The production of leucotriene $B_4$ ($LTB_4$) was determined in rat polymorphonuclear cells from pleural exudate elicited by heat-inactivated rat serum Palmer and Salmon 1983). The cells were stimulated by the $Ca^{2+}$ ionophore A23187 (Sigma) and incubated with a solution of tested drugs. $LTB_4$ was 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'-difluorobiphelyl-4-yl) moiety. Spectral similarities are manifested - namely by very similar $^1H$, $^{13}C$, and $^{19}F$ NMR spectra - and also there is a close similarity of the conformation of (2',4'-difluorobiphelyl-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 $CH_2$ 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, $\varepsilon_M$ = $2.010^4$, methanol), *p*-transition ($^1B_{1u}$) 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 ($\varepsilon_M$ = 1.8–1.9 $10^4$, methanol).

The formation of deoxoflobufen derivatives is accompanied by a typical shift of $\nu$(C=O) vibration in deoxoflobufen (1) from 1701 $cm^{-1}$ to about 1640 and 1540 $cm^{-1}$ 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+benzylammonium or cyclohexylammonium]$^+$ 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 ion $C_{13}H_9F_2^+$ in the $ms^n$ spectra formed by the preference splitting of $\beta$C-$\gamma$C bond of deoxoflobufen.




| | | |
|---|---|---|
| **(1)** | X = OH | Deoxoflobufen |
| **(2)** | X = NH-Bn | *N*-Benzyl-4-(2',4'-difluoro-biphenyl-4-yl)-2-methyl-butyramide |
| **(3)** | X = NH-$C_6H_{11}$ | *N*-Cyclohexyl-4-(2',4'-difluoro-biphenyl-4-yl)-2-methyl-butyramide |
| **(4)** | X = NH-$(CH_2)_2$OH | 4-(2',4'-Difluoro-biphenyl-4-yl)-*N*-(2-hydroxy-ethyl)-2-methyl-butyramide |
| **(5)** | X = NH-$CH_2$-COOEt | [4-(2',4'-Difluoro-biphenyl-4-yl)-2-methyl-butyrylamino]-acetic acid ethyl ester |
| **(6)** | X = $O^-$ $N^+H_3$-Bn | Benzyl-ammonium; 4-(2',4'-difluoro-biphenyl-4-yl)-2-methyl-butyrate |
| **(7)** | X = $O^-$ $N^+H_3$-$C_6H_{11}$ | Cyclohexyl-ammonium; 4-(2',4'-difluoro-biphenyl-4-yl)-2-methyl-butyrate |

Fig. 1. **Structure of deoxoflobufen** (4-(2',4'-Difluoro-biphenyl-4-yl)-2-methyl-butyric acid) and its derivatives

Table 1. **Biological activities of deoxoflobufen derivatives**

| Compound | Paw oedema[a], % | Pleuritis[b], % | | | Ear inflammation[c], % | | $LTB_4$[d] |
|---|---|---|---|---|---|---|---|
| | | A | B | C | A | B | |
| | $69^+$ | $66^+$ | $67^+$ | $4^n$ | $6^n$ | $22^+$ | 85 |
| **(1)** | $56^+$ | 11 | 12 | 3 | 15 | 13 | 79 |
| **(2)** | $32^+$ | 15 | 11 | 4 | 12 | 0 | 76 |
| **(3)** | $29^+$ | $58^+$ | $68^+$ | $25^+$ | 16 | 3 | 86 |
| **(4)** | $46^+$ | $48^+$ | $52^+$ | 11 | $5^n$ | $33^+$ | 89 |
| **(5)** | $49^+$ | $27^+$ | $62^+$ | $48^+$ | $7^n$ | $36^+$ | 78 |
| **(6)** | $64^+$ | $26^+$ | $45^+$ | $25^+$ | $10^n$ | $43^+$ | 83 |

[a] percent of inhibition, dose 100 mg/kg; [b] percent inhibition, dose 100 mg/kg: A volume of exudate, B number of cells, C cellularity; [c] percent inhibition, dose 200 mg/kg: A weight of ear lobes, B degree of ear-lobe hyperemia; [d] 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 $LTB_4$ 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 $LTB_4$ 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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