Studies on Antiinflammatory Agents. III.[1] Synthesis and Pharmacological Properties of Metabolites of 4′-Acetyl-2′-(2,4-difluorophenoxy)methanesulfonanilide (FK3311)

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We synthesized putative metabolites of 4′-acetyl-2′-(2,4-difluorophenoxy)methanesulfonanilide (FK3311, 1), a novel antiinflammatory agent, in order to confirm their structures and also to investigate their pharmacological properties. The structures of the metabolites 2—5 were confirmed by direct comparison with the synthesized authentic compounds. We employed the following tests to assess pharmacological activities: zymosan-induced prostaglandin E_2 production for *in vitro* activity, adjuvant-induced arthritis for antiinflammatory activity, and acetic acid-induced writhing for analgesic activity. Metabolite 2 is nearly equipotent to 1 in the *in vivo* tests and metabolite 3 is an active metabolite with *in vitro* activity comparable to that of 1.

Keywords metabolite; 4′-acetyl-2′-(2,4-difluorophenoxy)methanesulfonanilide; FK3311; prostaglandin E_2; antiinflammatory activity; analgesic activity

In the preceding paper,[2] we reported 4′-acetyl-2′-(2,4-difluorophenoxy)methanesulfonanilide (FK3311, 1) as a novel type of antiinflammatory agent. The chemical structure of this compound is quite different from those of typical nonsteroidal antiinflammatory drugs such as indomethacin or aspirin and the pharmacological properties, namely well-balanced antiinflammatory, analgesic and antipyretic activities along with low ulcerogenicity, appear to be of interest. Based on these properties and the toxicological results, we selected this compound as a candidate for clinical trials in rheumatoid arthritis and osteoarthritis patients.

The metabolism of **1** in rats was studied using a ^{14}C-labelled derivative.[3] When the bile was collected by bile duct-cannulation, almost all the radioactivity (89.3%) was found in the bile as various metabolites in the 48 h following i.v. dosing. The yields of radioactivity of the four major metabolites **2**—**5** were determined to be 13.6%, 23.6%, 4.8% and 29.2%, respectively (total 71.2%). These metabolites were isolated by preparative thin layer chromatography. The chemical structures of the metabolites were proposed on the basis of proton magnetic resonance and mass spectral studies but needed to be confirmed by direct comparison with synthesized authentic samples. In this paper, we describe the synthesis of these metabolites and the results of an examination of their pharmacological activities.

Chemistry

All metabolites described in this paper were synthesized from **1** as shown in Chart 1. Metabolite **2** was prepared by direct reduction of **1** with sodium borohydride in methanol[4] in good yield. Metabolite **3**, having a hydroxyacetyl group, was obtained from a bromoacetyl derivative **6**[2] by reaction with potassium formate and subsequent hydrolysis.[5] The carbonyl group in **3** was reduced in a manner similar to that used for compound **2** to afford metabolite **4**. Metabolite **5** was also prepared by the reduction of glyoxylic acid **7**, which was prepared by the oxidation of **1** with selenium dioxide in pyridine.[6] These synthetic metabolites **2**—**5** were identical with the corresponding samples isolated from the bile of rats both in terms of spectral data (proton magnetic resonance and mass spectra) and chromatographic behavior (thin-layer

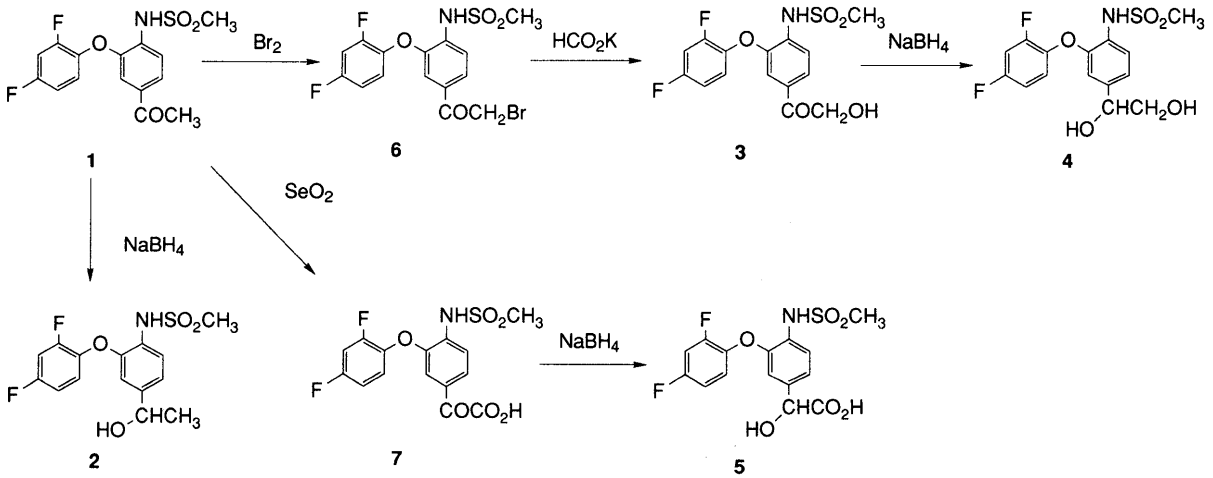

Chart 1

TABLE I. Effect of Metabolites and Reference Compounds on PGE_2 Production by Rat Neutrophils

Compound	Concentration (μg/ml)	Inhibition (%)
FK 3311 (**1**)	0.1	45[a]
2	0.32	23
3	0.1	51[a]
4	0.32	33
5	0.32	20
Indomethacin	0.01	55[b]

Significant difference from control; *a*) $p<0.05$, *b*) $p<0.01$.

TABLE II. Antiinflammatory and Analgesic Activities of **1** and Metabolites

Compound	Adjuvant arthritis % inhibition (10 mg/kg *p.o.*)		Writhing syndrome % inhibition (100 mg/kg *p.o.*)
	Injected	Uninjected	
FK 3311 (**1**)	76[a]	79[a]	81[a]
2	73[a]	63[a]	62[a]
3	28	30	26
4	2	13	31
5	2	−1	46[b]

Significant difference from control: *a*) $p<0.01$, *b*) $p<0.05$.

and high-performance liquid chromatographies).

Pharmacological Results and Discussion

The parent compound **1**, metabolites **2**—**5**, and indomethacin were assayed for *in vitro* activity to inhibit zymosan-induced prostaglandin E_2 (PGE_2) production by rat peritoneal neutrophils (Table I). It is noteworthy that metabolite **3** exhibited potent activity, comparable to that of compound **1** (51% and 45% inhibition at 0.1 μg/ml, respectively). However, the other metabolites showed little activity even at 0.32 μg/ml.

The *in vivo* antiinflammatory activity of the metabolites was assessed using adjuvant-induced arthritis in rats. The analgesic activity was investigated by using the acetic acid-induced writhing model in mice. The test samples were administered orally in both tests, and the results are summarized in Table II. Metabolite **2** exhibited fairly potent activities in both adjuvant arthritis and writhing tests in spite of its weak inhibitory activity against PGE_2 production. On the other hand, the other metabolites **3**—**5** were inactive in both tests, except that metabolite **5** showed weak activity in the writhing test. These results indicate that metabolite **2**, when administered orally, can be easily oxidized in the body to yield the parent compound **1** and thus exhibits potent *in vivo* activities. The conversion of a methylphenylcarbinol to an acetophenone in rats has been reported in the literature.[7] These results also show that metabolite **3** is an active metabolite of **1** and its poor activity in the *in vivo* tests is ascribable to its low oral absorbability.

In conclusion, we confirmed the proposed structures of the four major metabolites **2**—**5** of the novel antiinflammatory agent, FK3311 (**1**). We also elucidated that metabolite **2** is nearly equipotent to **1** in *in vivo* tests and metabolite **3** is an active metabolite with *in vitro* activity comparable to that of **1**.

Experimental

Melting points were measured on a Mitamura capillary melting-point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-408 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken with a Varian EM-390 instrument using tetramethylsilane as an internal standard. Electron impact mass spectra (EI-MS) were obtained with a Hitachi M80 mass spectrometer. Organic extracts were dried over anhydrous $MgSO_4$.

2′-(2,4-Difluorophenoxy)-4′-(1-hydroxyethyl)methanesulfonanilide (2) Sodium borohydride (0.18 g, 4.7 mmol) was added portionwise to a stirred solution of **1**[2] (1.34 g, 3.9 mmol) in methanol (25 ml) at 15 °C. The resulting mixture was stirred for 30 min at room temperature, treated with acetic acid and then evaporated under reduced pressure. The residue was dissolved in a mixture of EtOAc and water. The organic layer was washed with saturated $NaHCO_3$ solution, dried, and evaporated under reduced pressure. The residual oil was recrystallized from a mixture of EtOH and water to afford **2** (0.88 g, 65%) as crystals, mp 103—105 °C. IR (Nujol): 3438, 3100, 1615, 1585, 1500 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.37 (3H, d, J=6 Hz), 2.10 (1H, d, J=4 Hz), 3.00 (3H, s), 4.77 (1H, m), 6.77 (1H, br s), 6.80—7.30 (5H, m), 7.56 (1H, d, J=8 Hz). MS *m/z*: 343 (M^+), 328, 222. *Anal.* Calcd for $C_{15}H_{15}F_2NO_4S$: C, 52.47; H, 4.40; N, 4.08. Found: C, 52.28; H, 4.38; N, 4.05.

2′-(2,4-Difluorophenoxy)-4′-(hydroxyacetyl)methanesulfonanilide (3) Ethyl formate (3.1 g, 42 mmol) was added gradually to a solution of potassium hydroxide (2.4 g, 41 mmol) in methanol (20 ml), and the resulting mixture was refluxed for 2 h. It was then cooled to ambient temperature, and **6**[2] (11.8 g, 28 mmol) was added. The mixture was refluxed for 4 h and evaporated under reduced pressure. The residue was dissolved in a mixture of EtOAc and water and the organic layer was separated, washed with water, dried, and evaporated under reduced pressure. The oily residue was crystallized from a mixture of EtOAc and EtOH to afford **3** (6.0 g, 58%) as crystals, mp 144—145 °C. IR (Nujol): 3470, 3400, 1680, 1610, 1510 cm^{-1}. ^{1}H-NMR (DMSO-d_6+D_2O): 3.15 (3H, s), 4.68 (2H, s), 7.1—7.8 (6H, m). MS *m/z*: 357 (M^+), 326. *Anal.* Calcd for $C_{15}H_{13}F_2NO_5S$: C, 50.42; H, 3.67; N, 3.92. Found: C, 50.76; H, 3.74; N, 3.84.

2′-(2,4-Difluorophenoxy)-4′-(1,2-dihydroxyethyl)methanesulfonanilide (4) Sodium borohydride (18 mg, 0.47 mmol) was added portionwise to a stirred solution of **3** (150 mg, 0.40 mmol) in tetrahydrofuran (5 ml) and methanol (5 ml) at 15 °C. The resulting mixture was stirred for 1 h at ambient temperature, treated with acetic acid and then evaporated under reduced pressure. The residue was dissolved in a mixture of EtOAc and water. The organic layer was washed with saturated $NaHCO_3$ solution, dried, and evaporated under reduced pressure. The residual oil was recrystallized from a mixture of EtOH and water to afford **4** (130 mg, 84%) as crystals, mp 122—123 °C. IR (Nujol): 3450, 3200, 1610, 1500 cm^{-1}. ^{1}H-NMR (DMSO-d_6+D_2O): 3.02 (3H, s), 3.33 (2H, d, J=7 Hz), 4.43 (1H, t, J=7 Hz), 6.75 (1H, s), 7.0—7.6 (5H, m). MS *m/z*: 359 (M^+), 328. *Anal.* Calcd for $C_{15}H_{15}F_2NO_5S$: C, 50.14; H, 4.21; N, 3.90. Found: C, 50.48; H, 4.28; N, 3.83.

3-(2,4-Difluorophenoxy)-4-(methylsulfonylamino)phenylglyoxylic Acid (7) A mixture of **1** (3.4 g, 9.9 mmol) and selenium dioxide (1.8 g, 16.2 mmol) in pyridine (16 ml) was stirred at 100 °C for 8 h. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in an aqueous solution of sodium bicarbonate. The solution was washed with EtOAc, acidified with hydrochloric acid and then extracted with EtOAc. The extract was washed with water, dried and evaporated under reduced pressure to afford **7** (3.70 g, 100%) as an oil. IR (Nujol): 3250, 1740, 1680, 1600, 1510 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 3.17 (3H, s), 6.70—8.10 (7H, m), 8.67 (1H, s). MS *m/z*: 371 (M^+).

3-(2,4-Difluorophenoxy)-4-(methylsulfonylamino)phenylglycolic Acid (5) Sodium borohydride (1.2 g, 32 mmol) was added to an ice-cooled solution of **7** (8.2 g, 22 mmol) in tetrahydrofuran (100 ml) and methanol (20 ml). The resulting mixture was stirred at 0 °C for 1 h and at ambient temperature for 1 h. The resulting mixture was evaporated under reduced pressure, and the residue was dissolved in water. The solution was washed with EtOAc, acidified with hydrochloric acid and extracted with EtOAc. The extract was washed with water, dried, and evaporated under reduced pressure. The residue was crystallized from a mixture of EtOAc and hexane to afford **5** (6.5 g, 87%) as crystals, mp 167—168 °C. IR (Nujol): 3425, 3250, 1730, 1610, 1510 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.04 (3H, s), 4.96 (1H, s), 6.81 (1H, s), 7.0—7.6 (6H, m). MS *m/z*: 373 (M^+), 328. *Anal.* Calcd for $C_{15}H_{13}F_2NO_5S$: C, 48.26; H, 3.51; N, 3.75. Found: C, 48.23; H, 3.44; N, 3.75.

PGE_2 Production by Rat Peritoneal Neutrophils Male Sprague Dawley

rats were injected intraperitoneally with sodium casein, and 18 h later, neutrophils were collected from the peritoneal cavity. The neutrophils were incubated for 1 h at 37 °C with or without a test compound in the presence of arachidonic acid and zymosan. PGE_2 in the supernatant was determined by radioimmunoassay.

Adjuvant Arthritis and Writhing Syndrome These experiments were carried out according to the procedure described in the previous report.[2)]

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