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SYNTHESIS AND BIOLOGICAL ACTIVITY OF S-CONTAINING BETULIN DERIVATIVES

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S-Containing derivatives of the natural triterpenoid betulin were synthesized. It has been found that the 30-thio- and 30-sulfinylbenzimidazole derivatives of betulin exhibit anti-inflammatory activity comparable to that of sodium diclofenac.

Key words: triterpenoids, betulin, sulfides, sulfoxides, anti-inflammatory activity.

Research on the chemistry and biology of pentacyclic lupane-type triterpenoids, betulin (**1**) and its derivatives, has recently vigorously developed. A literature review dedicated to this topic has been published [1].

It has been observed that betulinic and betulonic acids and their numerous N-containing derivatives (nicotinates, amides, imines, urethanes, etc.) possess distinct anti-inflammatory, antitumor, immunomodulating, and anti-HIV activities [2-7]. However, S-containing compounds, which could be prepared by the reaction of alkali-metal thiolates with the corresponding halo- or sulfoxyl-derivatives (mesylates, tosylates) of betulin, betulinic acid, and lupeol with subsequent oxidation of the resulting sulfides to the sulfoxides, were not investigated during a study of the properties of modified lupane-type triterpenoids. We note that the literature contains only a single example of the preparation of several 30-thio derivatives of betulinic acid amide with ω-undecylamine, which are potential HIV-1 inhibitors [8].

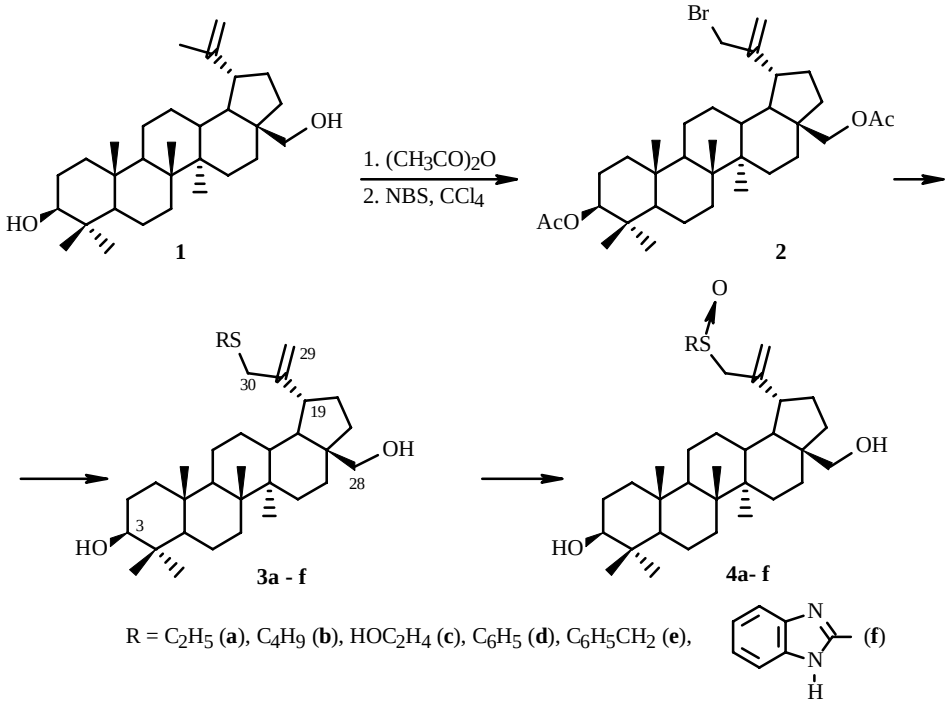

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TABLE 1. Anti-Inflammatory Activity of Betulin (**1**) and Its Derivatives (**3f**, **4f**)

Compound	Growth of pododema relative to initial values	Inhibition of pododema relative to control
	%	
Control 1	54.68±2.80	-
1	52.09±3.84	4.7
3f	30.28±4.81*	44.6
4f	40.64±4.30*	25.7
Control 2	71.34±8.14	-
Sodium diclofenac	36.74±6.50*	48.50

*$p < 0.05$ relative to the appropriate control.

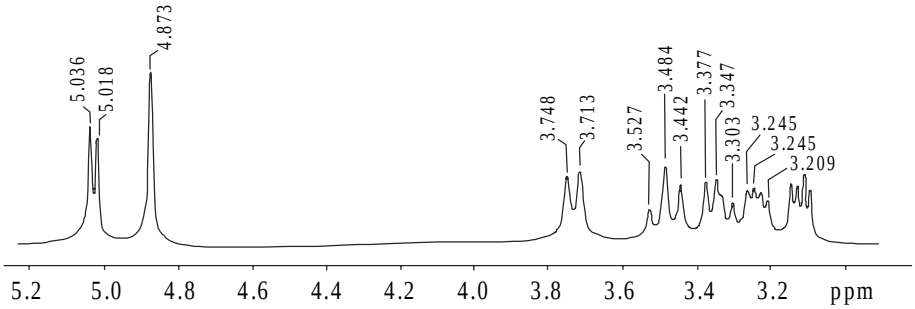

Fig. 1. A portion of the PMR spectrum (2.0-5.2 ppm) of **4d**.

Herein we report the synthesis and preliminary evaluation of the biological activity of certain 30-alkylthio-, 30-arylthio-, 30-alkylsulfinyl-, and 30-arylsulfinyl-derivatives of betulin.

The starting material for the S-containing derivatives of betulin **3a-f** was the known 30-bromo-3β,28-diacetoxylup-20(29)-ene (**2**), which was prepared by the literature method [9]. Compound **2** was thiylated using sodium ethyl-, *n*-butyl-, 2′-hydroxyethyl-, phenyl-, benzyl-, or benzimidazolyl-thiolates to form 30-thiosubstituted lup-20(29)-en-3β,28-diols **3a-f**, the yields of which were from 50 to 70%. The resulting sulfides **3a-f** were oxidized by H_2O_2 (30%) in acetone:acetic acid to the corresponding sulfoxides **4a-f** (yield 50-65%).

IR spectra of the sulfoxides contained bands charactistic of the IR spectrum of betulin at 1034, 3400 (OH) and 880 cm^{-1} (=CH_2) and an additional absorption band for the sulfoxide (1100 cm^{-1}). The formation of sulfoxides was confirmed by PMR spectra, from which it follows that the resulting sulfinyl group has the most significant effect on the C-30 protons. Thus, whereas the signal for H_2-30 protons in the PMR spectra of sulfides **3a-f** appears as a singlet at 2.97-4.00 ppm, those of the analogous protons in the spectra of sulfoxides **4a-f** corresponding to them appear as two doublets at 3.27-3.94 ppm owing to deshielding by the sulfinyl. For this same reason, signals corresponding to protons of the C-29 exomethyl shift to weak field.

It should be noted that certain characteristic signals in the spectrum of the phenyl sulfoxide **4d** (Fig. 1) are doubled. This may indicate that rotation of the substituents around the CH_2–S bond is hindered or that two diasteroisomers formed. The lack of changes in the nature of the PMR spectra recorded at temperatures from 30 to 50°C argue in favor of the second hypothesis. Use of double resonance and differences in the integrated intensities enabled signals for the C-28 and C-30 protons for each of the isomers of **4d** to be correctly assigned. According to the relative integrated intensities of the signals at 5.02 and 5.04 ppm (each corresponds to exomethyl proton Ha-29 of one of the isomers), the ratio of isomers is 1.00:0.82.

Isomers also formed for the sulfoxides with butyl (**4b**, 1.00:0.79) and benzyl (**4e**, 1.00:0.92) substituents. It can be assumed that the observed difference in the chemical shifts is due to the presence of bulky substituents on the asymmetric S atom of enantiomeric sulfoxides. In fact, PMR spectra of an artificial mixture of sulfoxide and (*S*)-α-methoxyphenylacetic acid as a chiral reagent [10] showed a significant increase in the differences of the chemical shifts of nonequivalent protons of the diastereomers.

The study produced S-containing triterpenoids **3a-f** and **4a-f**, of which **3f** and **4f** exhibited distinct anti-inflammatory activity. The activity of **3f** was almost two times greater than that of the corresponding sulfoxide **4f** in a comparative experiment and similar to the anti-inflammatory activity of sodium diclofenac (Table 1). The results of a toxicity study showed that the LD_{50} values of **1**, **3f**, and **4f** were >3000 mg/kg.

EXPERIMENTAL

IR spectra were recorded on a UR-20 instrument in vaseline oil. UV spectra were obtained on a SF-46 spectrophotometer in EtOH solutions. PMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solutions on a Varian Mercury Plus 300 spectrometer (working frequency 300 MHz, HMDS internal standard). Melting points were measured on a Kofler stage. Specific optical rotations were recorded in $CHCl_3$ or EtOH solutions on a Perkin—Elmer Model 341 polarimeter at 589 nm.

Column chromatography (CC) was performed using SiO_2 (Merck, 60-200 μm) with a compound:sorbent ratio ≈ 1:20 and hexane:ethylacetate (10-50% of the latter) eluent. TLC used Sorbfil (Russia) plates and development by spraying with phosphomolybdic acid solution (20%) in EtOH with subsequent heating at 100-120°C for 2-3 min. Anhydrous solvents were prepared by standard methods [11]. Betulin (**1**) was isolated from birch bark by the literature method [12] and crystallized from propan-2-ol, mp 254-256°C, $[\alpha]_D^{22}$ +20.1° (*c* 2.0, Py) {lit. [13] mp 256-257°C, $[\alpha]_D^{25}$ +19.0° (*c* 2.0, Py)}.

30-Bromo-3*β*,28-di-O-acetyllup-20(29)-ene (2) was prepared by the literature method [9], R_f 0.4 (hexane:ethylacetate 5:1), mp 183-185°C, $[\alpha]_D^{22}$ +8.0° (*c* 1.8, $CHCl_3$) {lit. [9] mp 185°C, $[\alpha]_D^{25}$ +7.4° (*c* 0.49, $CHCl_3$)}.

IR spectrum (ν, cm^{-1}): 880 (=CH_2), 1720 (CH_3COO).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.77, 0.92, 0.97 (3 × 3H, 3s, $3CH_3$), 0.78 (6H, s, $2CH_3$), 1.96, 1.99 (2 × 3H, 2s, $2CH_3COO$), 2.37 (1H, td, J = 10.8, 5.1, H-19), 3.91 (2H, s, H_2-30), 3.78, 4.19 (2H, 2d, J = 11.10, H_2-28), 4.40 (1H, dd, J = 9.8, 5.4, H-3), 4.96, 5.07 (2H, 2s, =CH_2-29).

Synthesis of Betulin Sulfides (3a-f). A solution of NaOMe in MeOH was prepared by adding metallic Na (1 g, 43.5 mmol) to anhydrous MeOH (5 mL), treated with the appropriate mercaptan (1.7 mmol), and stirred at room temperature for 3 h. Solvent was evaporated. The resulting sodium salt of the mercaptan was treated with a mixture of 3*β*,28-diacetoxy-30-bromolup-20(29)-ene (1.7 mmol) in anhydrous DMSO (16 mL), stirred at room temperature for 6 h, and diluted with water. The resulting precipitate was filtered off, washed with water, HCl solution (5%), and water again until the pH value of the rinsings was neutral. The resulting mixture of crystalline products was separated by CC over SiO_2. Crystallization from EtOH produced betuline sulfides **3a-f** as fine white crystals.

30-(Ethylthio)lup-20(29)-en-3*β*,28-diol (3a), yield 0.56 g (56%), R_f 0.4 ($CHCl_3$:ethylacetate 10:1), mp 190-193°C, $[\alpha]_D^{18}$ -0.2° (*c* 4.7, EtOH).

UV spectrum (EtOH, λ_{max}, nm, log ε): 220 (1.604).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.69, 0.75, 0.90, 0.92, 0.95 (5 × 3H, 5s, $5CH_3$), 1.33 (3H, t, J = 7.2, $C\underline{H}_3CH_2S$–), 2.29 (1H, td, J = 11.1, 5.1, H-19), 2.38 (2H, q, J = 7.2, $CH_3C\underline{H}_2S$–), 3.07 (2H, s, H_2-30), 3.12 (1H, dd, J = 10.8, 5.1, H-3), 3.24, 3.73 (2H, 2d, J = 11.1, H_2-28), 4.76, 4.83 (2H, 2s, =CH_2-29).

30-(Butylthio)lup-20(29)-en-3*β*,28-diol (3b), yield 0.6 g (69%), R_f 0.5 ($CHCl_3$:ethylacetate 10:1), mp 144-146°C, $[\alpha]_D^{18}$ -12.5° (*c* 3.0, EtOH).

UV spectrum (EtOH, λ_{max}, nm, log ε): 220 (0.764).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.69, 0.76, 0.90, 0.93, 0.96 (5 × 3H, 5s, $5CH_3$), 1.19 (3H, t, J = 7.2, $C\underline{H}_3(CH_2)_2CH_2S$–), 2.30 (1H, td, J = 10.1, 5.4, H-19), 2.35 (2H, t, J = 7.2, $CH_3(CH_2)_2C\underline{H}_2S$–), 3.06 (2H, s, H_2-30), 3.12 (1H, dd, J = 10.7, 5.1, H-3), 3.26, 3.73 (2H, 2d, J = 10.8, H_2-28), 4.76, 4.83 (2H, 2s, =CH_2-29).

30-(2-Hydroxyethylthio)lup-20(29)-en-3*β*,28-diol (3c), yield 0.5 g (57%), R_f 0.35 (ethylacetate:hexane:CH_2Cl_2 5:2:3), mp 182-183°C, $[\alpha]_D^{18}$ -30.3° (*c* 2.7, EtOH).

UV spectrum (EtOH, λ_{max}, nm, log ε): 240 (2.828).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.70, 0.76, 0.91, 0.93, 0.96 (5 × 3H, 5s, $5CH_3$), 2.30 (1H, td, J = 10.9, 5.4, H-19), 2.59 (2H, t, J = 6.0, $HOCH_2C\underline{H}_2S$), 3.08 (2H, s, H_2-30), 3.12 (1H, dd, J = 10.5, 5.1, H-3), 3.26, 3.73 (2H, 2d, J = 10.5, H_2-28), 3.66 (2H, t, J = 6.0, $HOC\underline{H}_2CH_2S$), 4.79, 4.87 (2H, 2s, =CH_2-29).

30-(Phenylthio)lup-20(29)-en-3*β*,28-diol (3d), yield 0.56 g (56%), R_f 0.3 ($CHCl_3$:ethylacetate 10:1), mp 168-170°C, $[\alpha]_D^{18}$ -25.5° (*c* 2.9, EtOH).

UV spectrum (EtOH, λ_{max}, nm, log ε): 230, 250 (2.657, 3.338).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.69, 0.76, 0.95 (3 × 3H, 3s, $3CH_3$), 0.90 (6H, s, $2CH_3$), 2.34 (1H, td, J = 10.9, 5.4, H-19), 3.12 (1H, dd, J = 10.7, 5.1, H-3), 3.24, 3.73 (2H, 2d, J = 10.5, H_2-28), 3.51 (2H, s, H_2-30), 4.84, 4.89 (2H, 2s, =CH_2-29), 7.25 (5H, m, arom.).

30-(Benzylthio)lup-20(29)-en-3*β*,28-diol (3e), yield 0.30 g (45%), R_f 0.5 ($CHCl_3$:MeOH 20:1), mp 135-137°C, $[\alpha]_D^{18}$ -14.9° (*c* 1.9, EtOH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.69, 0.75, 0.95 (3 × 3H, 3s, $3CH_3$), 0.90 (6H, s, $2CH_3$), 2.27 (1H, td, J = 11.0, 5.7, H-19), 2.97 (2H, s, H_2-30), 3.12 (1H, dd, J = 10.1, 5.1, H-3), 3.22, 3.71 (2H, 2d, J = 10.8, CH_2-28), 3.56 (2H, s, S–C$\underline{H}_2$–Ph), 4.78, 4.86 (2H, 2s, =CH_2-29), 7.24 (5H, m, arom).

30-(Benzimidazolylthio)lup-20(29)-en-3,28-diol (3f), yield 0.55 g (50%), R_f 0.3 ($CHCl_3$:MeOH 20:1), mp 174-176°C, $[\alpha]_D^{22}$ -38.4° (*c* 3.6, EtOH).

UV spectrum (EtOH, λ_{max}, nm, log ε): 230, 280 (2.931, 2.809).

PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.65, 0.75, 0.87, 0.92, 0.96 (5 × 3H, 5s, $5CH_3$), 2.32 (1H, m, H-19), 2.97 (1H, m, H-3), 3.04, 3.49 (2H, 2d, J = 10.8, H_2-28), 4.00 (2H, s, H_2-30), 4.93, 5.02 (2H, 2s, =CH_2-29), 7.12, 7.43 (4H, 2m, arom).

Synthesis of Betulin Sulfoxides 4a-f. Betulin sulfide (**3a-f**, 0.5 mmol) was dissolved in acetone:acetic acid (20 mL, 9:1), heated to 45-50°C, treated with H_2O_2 (0.5 mL, 30%), and stirred on a magnetic stirrer at room temperature for 2 h. The course of the reaction was followed using TLC. The reaction mixture was extracted with ethylacetate (3 × 20 mL). The extract was washed with aqueous $NaHCO_3$ (10%) until the washings were neutral and dried over Na_2SO_4. The solvent was evaporated. The solid was separated over a column of SiO_2 with elution of each sulfoxide by an individually selected mixture. Crystallization from EtOH produced betulin sulfoxides **4a-f** as fine white crystals.

30-(Ethylsulfinyl)lup-20(29)-en-3*β*,28-diol (4a), yield 0.2 g (55%), R_f 0.3 ($CHCl_3$:ethylacetate 10:1), mp 170-172°C, $[\alpha]_D^{21}$ -15.4° (*c* 3.2, EtOH).

UV spectrum (EtOH, λ_{max}, nm, log ε): 220 (1.288).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.70, 0.76, 0.90, 0.92, 0.95 (5 × 3H, 5s, $5CH_3$), 1.34 (3H, t, J = 7.2, C$\underline{H}_3$$CH_2$S–), 2.42 (1H, td, J = 10.2, 5.1, H-19), 2.96 (2H, q, J = 7.2, CH_3C$\underline{H}_2$S–), 3.12 (1H, dd, J = 10.8, 5.4, H-3), 3.25, 3.71 (2H, 2d, J = 11.1, H_2-28), 3.58, 3.67 (2H, 2d, J = 13.8, H_2-30), 5.10, 5.20 (2H, 2s, =CH_2-29).

30-(Butylsulfinyl)lup-20(29)-en-3*β*,28-diol (4b), yield 0.3 g (58%), R_f 0.25 ($CHCl_3$:ethylacetate 10:1), mp 171-173°C, $[\alpha]_D^{18}$ -11.4° (*c* 3.3, EtOH).

UV spectrum (EtOH, λ_{max}, nm, log ε): 220 (0.324).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.69, 0.76, 0.90, 0.92, 0.95 (5 × 3H, 5s, $5CH_3$), 1.19 [3H, t, J = 7.2, C$\underline{H}_3$$(CH_2)_2CH_2$S], 2.30 (1H, m, H-19), 2.64 [2H, t, J = 7.5, $CH_3(CH_2)_2$C$\underline{H}_2$S], 3.12 (1H, dd, J = 10.5, 5.1, H-3), 3.23, 3.72 (2H, 2d, J = 10.8, H_2-28), 3.30, 3.39 (2H, 2d, J = 12.9, H_2-30), 4.93 (0.44H, s, =CHa-29, isomer A), 4.95 (0.56H, s, =CHa-29, isomer B), 5.03 (1H, s, =CHb-29).

30-(Phenylsulfinyl)lup-20(29)-en-3*β*,28-diol (4d), yield 0.25 g (50%), R_f 0.4 ($CHCl_3$:MeOH 20:1), mp 127-129°C, $[\alpha]_D^{18}$ -2.7° (*c* 2.9, EtOH).

UV spectrum (EtOH, λ_{max}, nm, log ε): 230 (2.689).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.69, 0.75, 0.95 (3 × 3H, 3s, $3CH_3$), 0.90 (6H, s, $2CH_3$), 2.32 (1H, m, H-19), 3.12 (1H, dd, J = 11.3, 5.1, H-3), 3.22 (0.45H, d, J = 10.2, H-28a, isomer A), 3.24 (0.55H, d, J = 11.1, H-28a, isomer B), 3.32, 3.50 (0.9H, 2d, J = 13.2, H_2-30, isomer A), 3.33, 3.40 (1.1H, 2d, J = 13.2, H_2-30, isomer B), 3.73 (1H, d, J = 10.8, Hb-28), 4.87 (1H, s, =CHa-29), 5.02 (0.45H, s, =CHb-29, isomer A), 5.04 (0.55H, s, =CHb-29, isomer B), 7.46, 7.59 (5H, 2m, arom).

30-(Benzylsulfinyl)lup-20(29)-en-3*β*,28-diol (4e), yield 0.15 g (50%), R_f 0.3 ($CHCl_3$:MeOH 20:1), mp 116-118°C, $[\alpha]_D^{21}$ -12.7° (*c* 2.6, EtOH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.69, 0.74, 0.90, 0.93 (4 × 3H, 4s, $4CH_3$), 0.82 (3.12H, s, CH_3, isomer B), 0.84 (2.88H, s, CH_3, isomer A), 2.28 (1H, td, J = 11.1, 5.6, H-19), 3.12 (1H, dd, J = 10.1, 4.8, H-3), 3.17, 3.68 (2H, 2d, J = 10.8, H_2-28), 3.27, 3.36 (2H, 2d, J = 13.2, H_2-30), 3.96 (2H, s, S–C$\underline{H}_2$–Ph), 4.98, 5.04 (2H, 2d, =CH_2-29), 7.24-7.33 (5H, m, arom).

30-(Benzimidazolylsulfinyl)lup-20(29)-en-3,28-diol (4f), yield 0.3 g (65%), R_f 0.25 ($CHCl_3$:MeOH 20:1), mp 170-172°C, $[\alpha]_D^{22}$ -12.4° (*c* 5.1, EtOH).

UV spectrum (EtOH, λ_{max}, nm, log ε): 260 (3.199).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.69, 0.74, 0.88, 0.90, 0.92 (5 × 3H, 5s, $5CH_3$), 2.33 (1H, m, H-19), 3.12 (1H, m, H-3), 3.21, 3.70 (2H, 2d, J = 10.5, H_2-28), 3.94 (2H, s, H_2-30), 4.93, 5.02 (2H, 2d, =CH_2-29), 7.12, 7.44 (4H, 2m, arom).

Acute toxicity of 1, 3f, and 4f was studied by the rapid method of Prozorovskii et al. [14] on mongrel white mice of both sexes (18-20 g) by i.p. injection.

Anti-inflammatory activity of 1, 3f, and 4f was studied on mongrel white rats of both sexes (180-220 g) using the carrageenan adema model [15] induced by subplantar injection of aqueous carrageenan (0.1 mL, 1%). Each group contained six animals. Compounds **1**, **3f**, and **4f** were injected i.p. at doses of 50 mg/kg in starchy mucus 1 h before injection of the phlogogenic agent. The volume of the paw was measured oncometrically before the experiment and 4 h after injection of carrageenan. The control was sodium diclofenac at a dose of 8 mg/kg. The growth of the adema in the inflamed paw relative to the initial volume and the inhibition of adema relative to the control (for **1**, **3f**, and **4f**, control 1; for sodium diclofenac, control 2) were calculated. Statistical processing used the Student *t*-criterion [16]. The effect was considered reliable for $p < 0.05$.

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