4-NITRO-3-TRIFLUOROMETHYLPHENYLAMIDES OF 2-(DIALKOXYPHOSPHORYL) ISOBUTRYIC ACID — A NOVEL CLASS OF NONSTEROIDAL ANTIANDROGENS

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An important achievement of experimental endocrinology in the last two decades was the discovery of nonsteroidal antiandrogens among substituted carboxanilides [1-3]. Some of these have a pronounced ability to complete with androgens by binding to receptor proteins in target organs, and are already used (flutamide, niftolid) in clinical practice to treat carcinoma of the prostate and hirsutism [4, 5].

In the present work we present data on the synthesis and antiandrogenic activity of 4-nitro-3-trifluoromethylphenylamido 2-(dialkoxyphosphoryl) isobutyric acids, in order to discover among them new, effective antiandrogenic compounds and to establish the dependence of antiandrogenic activity on chemical structure.

Starting compound for the synthesis of the test substances was 2-hydroxy-2-methyl-4-nitro-3-trifluoromethylpropionylanilide (I) [6]. Treatment of this with butyllithium followed by dialkylchlorophosphates gives 4-nitro-3-triflurophenylamides of 2-(dialkoxyphosphoryloxy)isobutyric acids (II-IV), where alkyl = C_2H_5 (II), C_3H_7 (III), or C_5H_9 (IV).

Amides II-IV (Table 1) are viscous liquids that don't distill under vacuum, are stable on storage in the refrigerator, easily soluble in most organic solvents and oils, and insoluble in water. They are hydrolyzed by mineral acids and bases to starting compound I.

Fig. 1. Ventral prostate glands of castrated rats. a) Testosterone propionate; b) testosterone propionate + compound III. Stained with hematoxylin-eosin, × 50.

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TABLE 1. Physicochemical Characteristics of Amides II-IV

Compound	Alk	Yield, %	Empirical formula
II	C_2H_5	75	$C_{15}H_{20}F_3N_2O_7P$
III	n-C_3H_7	80	$C_{17}H_{24}F_3N_2O_7P$
IV	n-C_5H_{11}	77	$C_{21}H_{32}F_3N_2O_7P$

TABLE 2. Comparison of Antiandrogenic Activity of Niftolid and 4-Nitro-3-trifluoromethylphenylamides of 2-(Dialkoxyphosphoryloxy)isobutyric Acid

Conditions of experiments	n	Mass of organs, mg per 100 g body		
		ventral lobe of prostate gland	seminal vesicles	levator ani muscle
Control, TP	48	35,5±1,9	29,6±1,6	40,9±1,8
Niftolid + TP	23	14,7±1,0	9,5±0,5	32,0±2,7
Compound II + TP	7	14,4±1,9	9,7±0,7	22,1±1,6**
Compound III + TP	20	10,2±1,2*	9,0±0,3	18,4±0,6***
Compound IV + TP	8	16,7±1,7	9,2±0,5	19,8±0,8
Mineral oil	7	17,8±1,1	10,8±0,4	17,0±1,7

Notes. Significance of differences: one asterisk, $p < 0.05$; two, $p < 0.01$; and three, $p < 0.001$ compared to the control group "niftolid + TP". For all experimental groups, $p < 0.001$ compared with the control.

The structure of amides II-IV was confirmed by elemental analysis data and IR spectroscopy. In the IR spectra of these compounds, there are intense absorption bands in the regions 1600-1640 cm^{-1} (C=O), 1239 cm^{-1} (P=O), 1080, 1050 (C–O in P–O–C), and 3280-3300 cm^{-1} (N–H).

EXPERIMENTAL (CHEMICAL)

IR spectra were recorded in a UR-20 instrument (Germany) in CCl_4 solution. Data from elemental analysis agreed with calculated values.

4-Nitro-3-trifluoromethylphenylamides of 2-(Dialkoxyphosphoryloxy)isobutyric Acid (II-IV). To a solution of 0.02 mole of compound I in 60 ml tetrahydrofuran cooled to −90°C was added, in an argon atmosphere, 0.025 mole of butyllithium in 10 ml absolute hexane and 5 ml hexamethyl phosphotriamide. The reaction mixture was stirred for 10 min and 0.02 mole dialkylchlorophosphate in 10 ml of tetrahydrofuran was added. This was stirred 2 h at −85°C, and then the temperature was raised over 12 h to 20-25°C. The solvent was removed under vacuum with a water aspirator pump. To the residue was added 60 ml of a saturated solution of sodium bicarbonate; this was extracted with methylene chloride, which was washed with water and dried with sodium sulfate. The solvent was evaporated, and the residue was dissolved in 30 ml ether and precipitated with hexane. The product, a light-yellow oil, was dried at 40-50°C under vacuum with an oil pump to constant weight.

EXPERIMENTAL (BIOLOGICAL)

The antiandrogenic activity of the synthesized compounds was studied in castrated, sexually immature male Wistar rats weighing 50-60 g. Control animals were given testosterone propionate (TP) in the form of a 0.05% solution in oil at a dose of 200 μg/kg per day, or oil, subcutaneously for a period of 7 days. Experimental animals received in addition 10 mg/kg of niftolid (control antiandrogen) or an equivalent amount of one of the test compounds – 15.52 of compound I, 16.53 of II, and 18.57 mg/kg of compound III.

Niftolid and the test preparations were first dissolved in benzyl benzoate, then in mineral oil at a ratio of 1:10 and also injected subcutaneously.

Twenty-four hours after administering the last dose of the preparations, the animals were decapitated. The ventral portion of the prostate gland (ventral prostate), seminal vesicles, and levator ani muscle were isolated and weighed. The mass of these organs was expressed as mg per 100 g body weight (relative weight).

The ventral prostate was studied histologically by staining with hematoxylin-eosin.

The results of the studies (Table 2) showed that all 3 compounds have high antiandrogenic activity. Under their influence, complete inhibition of the stimulatory effect of testosterone on the morphofunctional state of the test organs was seen: weight of the ventral prostate and seminal vesicles was decreased by 2.0-2.9 fold, and that of the levator ani muscle by 1.8-2.2 fold.

It is noteworthy that among the compounds studied, II and IV possess antiandrogenic activity comparable to that of niftolid; the effect of compound III on the ventral prostate and levator ani exceeded that of niftolid by 1.4-fold. This compound also caused a much greater decrease in the weight of the ventral prostate compared with castrated rats receiving oil. The latter is evidence that the antiandrogens cause an effective blockade both of exogenous androgens and those of endogenous, adrenal origin.

Histological studies showed that after a 7-day course of TP administration, clear signs of stimulation of the ventral prostate were seen: the acini had an irregular shape, and the epithelium was tall and cylindrical (Fig. 1a). with simultaneous administration of 2-(dipropoxyphosphoryloxy)isobutyric acid 4-nitro-3-trifluoromethylphenylamide to these animals, suppression of the androgenic response of the prostate gland was noted, with decreased diameter of the terminal sections and reduction in the height of the epithelial cells (Fig. 1b), i.e., changes took place that were similar to those seen with administration of niftolid to androgenized animals [1, 7].

Thus, the results obtained demonstrate that 4-nitro-3-trifluoromethylphenylamides of 2-(dialkoxyphosphoryloxy)isobutyric acid represent a new class of compounds with antiandrogenic activity. The antiandrogenic effect of one of these – 2-(dipropoxyphosphoryloxy)-isobutyric acid 4-nitro-3-trifluoromethylphenylamide – exceeds that of niftolid, which demonstrates its potential for use as a new antiandrogenic drug.

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