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5-Nitrobenzylidene Derivatives or 3-Benzoyl-2-thiohydantoin and Their Pharmacological Properties

5-Nitrobenzylidenowe pochodne 3-benzoilo-2-tiohydantoiny oraz ich właściwości farmakologiczne

INTRODUCTION

Derivatives of 2-thiohydantoin substituted in 3 and 5 positions form a considerable group of physiologically active substances that show anticonvulsant [2—4, 6—7] and hypoglycemic [2, 3, 9] activity.

In present modification only nitro group was introduced in one of three isomeric positions of benzylidene system. It allowed to evaluate the effect of nitro substituent and its influence on pharmacological properties of compounds 1—18. Both benzoylthiohydantoic acids described in a present paper (19—20) together with those obtained earlier (21—24) [2, 3] were also included in the screening studies.

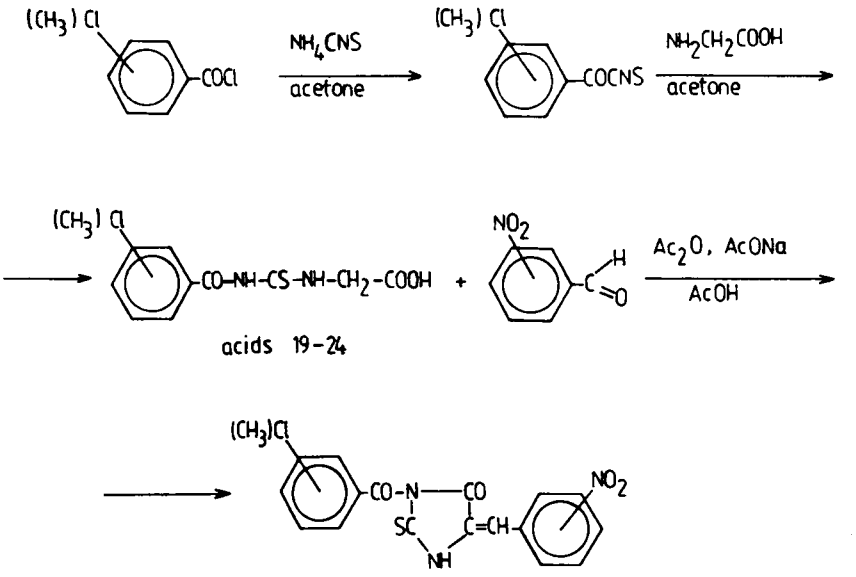
The new derivatives were prepared by condensation of ω-benzoylthiohydantoic acids with nitroaldehydes in glacial acetic acid medium [3]. The attempts at receiving the free 3-benzoyl-2--thiohydantoin by cyclization of acids in a presence of hydrochloric acid have failed. In this reaction only 2-thiohydantoin was obtained instead of expected 3-benzoyl-2-thiohydantoin.

Reactions leading to preparation of acids 19—24 and benzylidene derivatives of 3-benzoyl-2--thiohydantoins 1—18 are present in Scheme 1.

The structures of compounds 1—18 and 19—20 were confirmed by spectral data. The IR spectra of all compounds 1—20 were investigated while the ^{1}H NMR spectra were performed only for compounds 10—12 and 19—20. The data showed in the experimental part are in agreement with those described previously [2, 3].

The data of mass spectra were examined for compounds 7—12. The main fragments of their molecules were analogous to those indicated for 3-o-chloro- and 3-o-methylbenzoyl-5-benzylidene--2-thiohydantoins [3].

Scheme 1



compounds 1—18

CHEMISTRY

Melting points were uncorrected. The IR spectra were performed in a form of nujol suspensions in a range of 4000—400 cm^{-1} using Zeiss IR-10 apparatus. The mass spectra were measured in a range of m/e 1—400 using a 9000 S LKB gas chromatograph-mass spectrophotometer at ionization energy 70 eV. The ^1H NMR spectra were recorded by Tesla BS 487 C (80 Hz) apparatus (the solvent DMS O-d_6 containing below 4 ppm of water and TMS as internal standard). Measurement were carried out at room temperature.

Preparation of ω-benzoylthiohydantoic acids

ω-m-Chlorobenzoylthiohydantoic acid 19 was synthesized by refluxing of equivalent amounts of ammonium thiocyanate, glycine and m-chlorobenzoyl chloride in anhydrous acetone for 2 hrs. After filtration of precipitated ammonium chloride, the excess of acetone was distilled off and residue was crystallized from water. Yield 60%, colourless needles, mp. 164—5°C.

Analysis:

For: $C_{10}H_9ClN_2O_3S$ (272.56) — calc.: 44.06% C, 3.30% H, 10.27% N;
found: 44.38% C, 3.19% H, 10.38% N.

IR (cm^{-1}): 3110 NH; 1700 C=O; 3330 OH; ^1H NMR (ppm): 11.76 COOH (s, 1H); 11.22 NH (s, 1H); 4.41—4.55 CH_2 (d, 2H).

ω-m-Methylbenzoylthiohydantoic acid 20 was obtained from m-methylbenzoyl chloride, ammonium thiocyanate and glycine similarly as described above. Yield 50%, colorless needles, mp. 181—2°C.

Analysis:

For: $C_{11}H_{12}N_2O_3S$ (252.16) — calc.: 52.39% C, 4.75% H, 11.10% N;
found: 52.38% C, 4.74% H, 11.18% N.

IR (cm^{-1}): 3180 NH; 1730 and 1680 C=O; 3320 OH; ^1H NMR (ppm): 11.58 COOH (s, 1H); 11.31 NH (s, 1H); 4.47—4.56 CH_2 (d, 2H); 2.52 CH_3 (s, 3H).

General procedure for preparation of compounds 1—24

0.05 mole of corresponding ω-benzoylthiohydantoic acid and 0.05 mole of appropriate nitroaldehyde in 150 ml of glacial acetic acid, with addition of 10 g of anhydrous sodium acetate and 10 ml of acetic anhydride, were refluxed for 1.5 hrs. Towards the end of the reaction, the color of the mixture turned to reddish brown. The reaction mixture was then poured into 500 ml of cold water, stirred and filtered after cooling. Crude precipitates were crystallized from 40—80% aqueous solutions of acetic and dried at 90°C. Most compounds synthesized were precipitated in a form of strongly contaminated oils which subsequently solidified at room temperature. Their yields were calculated for products obtained after first crystallization.

Compounds 1—24 are very soluble in acetone, pyridine and chloroform, easy soluble in alcohols and acetic acid and insoluble in water, ethers and conc. sodium hydroxide.

Analytical results and other characteristics of the compounds 1—18 are summarized in Table 1.

IR (cm^{-1}): 3250—3300 NH; 1670—1705 C=O (single band), exception: compound 7, 1680 and 1720 (two bands); 1610—1630 C=C;

^1H NMR (ppm): compound 10: 2.45 CH_3 (s, 3H); 7.13 CH (s, 1H); 10.07 NH (s, 1H); 7.43—7.73 $C_6H_4CH_3$ (m, 4H); 8.21—8.55 $C_6H_4NO_2$ (m, 4H); compound 11: 2.44 CH_3 (s, 3H); 7.15 CH (s, 1H); 10.06 NH (s, 1H); 7.33—7.80 $C_6H_4CH_3$ (m, 4H); 8.17—9.03 $C_6H_4NO_2$ (m, 4H); compound 12: 2.40 CH_3 (s, 3H); 7.27 CH (s, 1H); 10.10 NH (s, 1H); 7.32—7.70 $C_6H_4CH_3$ (m, 4H); 8.03—8.62 $C_6H_4NO_2$ (m, 4H);

PHARMACOLOGY

All compounds were preliminarily tested to determine their LD_{50} values, anticonvulsant and hypoglycemic activity.

Table 1. 5-Nitrobenzylidene derivatives of 3-chloro-

No.	R_1	R_2	Formula m. wt.	Yeld %	m.p. °C
1	p-Cl	4'-NO_2	$C_{17}H_{10}ClN_3O_4S$	85.7	>300
2	p-Cl	3'-NO_2	387.80	78.3	215-6
3	p-Cl	2'-NO_2		53.8	235-6
4	p-CH_3	4'-NO_2	$C_{18}H_{13}N_3O_4S$	89.2	>300
5	p-CH_3	3'-NO_2	367.38	64.8	238-9
6	p-CH_3	2'-NO_2		67.5	234-5
7	o-Cl	4'-NO_2	$C_{17}H_{10}ClN_3O_4S$	86.8	218-9
8	o-Cl	3'-NO_2	387.80	71.7	231-2
9	o-Cl	2'-NO_2		68.6	224-5
10	o-CH_3	4'-NO_2	$C_{18}H_{13}N_3O_4S$	87.9	250-1
11	o-CH_3	3'-NO_2	367.38	73.8	172-3
12	o-CH_3	2'-NO_2		64.7	197-8
13	m-Cl	4'-NO_2	$C_{17}H_{10}ClN_3O_4S$	89.9	>300
14	m-Cl	3'-NO_2	387.80	78.6	233-4
15	m-Cl	2'-NO_2		65.7	208-9
16	m-CH_3	4'-NO_2	$C_{18}H_{13}N_3O_4S$	58.7	266-7
17	m-CH_3	3'-NO_2	367.38	78.9	182-3
18	m-CH_3	2'-NO_2		58.4	183-4

Materials and methods

Experiments were performed on Albino Swiss mice of either sexes (17—28 g) and male Wistar rats (160—240 g).

Phenytoin (Pharm. Works „Polfa", Warszawa) was administered ip as suspension in 3% Tween 80, tolbutamide (Diabetol, Pharm. Works „Polfa", Starogard), glucose (*Glucosum pulvis*, Pharm. Works „Polfa" Kraków) and pentetrazole (*Cardiazolum*, Lublin, Druggist's Council, Lab. of Reagents „Cefarm" No. ser. 020277) were injected as aqueous solutions.

Investigated compounds and acids were given i.p. in 3% Tween 80 in a volume of 10 ml/kg. Controls received the same volume of the solvent.

Acute toxicity. LD_{50} were estimated by the method of Litchfield and Wilcoxon [5]. Female, Albino Swiss mice (10—15 in group) were observed for 3 hrs and then 24 hrs after administration of various doses of investigated compounds the survivors were counted.

If doses of 3 or 2.5 g/kg did not cause a lethal outcome of mice, these doses were accepted as LD_{50} ($LD_{min.}$) and the partial doses applied in tests were calculated in relation to them.

and 3-methylbenzoyl-2-thiohydantoins (1—18)

Analyses					
Calculated %			Found %		
C	H	N	C	H	N
52.65	2.59	10.83	52.33	2.53	10.89
52.65	2.59	10.83	52.73	2.57	10.84
52.65	2.59	10.83	52.71	2.54	10.59
58.84	3.56	11.43	58.83	3.57	11.45
58.84	3.56	11.43	58.32	3.58	11.45
58.84	3.56	11.43	57.82	3.55	11.32
52.65	2.59	10.83	52.42	2.55	11.00
52.65	2.59	10.83	52.48	2.53	10.80
52.65	2.59	10.83	52.44	2.59	10.59
58.84	3.56	11.43	58.84	3.56	11.22
58.84	3.56	11.43	58.77	3.50	11.26
58.84	3.56	11.43	58.84	3.50	11.27
52.65	2.59	10.83	52.58	2.53	10.81
52.65	2.59	10.83	52.38	2.52	10.82
52.65	2.59	10.83	52.47	2.52	10.84
58.84	3.56	11.43	58.80	3.49	11.31
58.84	3.56	11.43	58.55	3.50	11.28
58.84	3.56	11.43	58.61	3.49	11.29

Anticonvulsant action. Maximum electroshock (MES) and penetrazole (PTZ) tests were used. The MES test was carried out according to Swinyard [8]. Convulsions were produced by alternating current (60 Hz, 50 mA, 0.2 s) using corneal electrodes. The anticonvulsive activity was evaluated by noting a number of mice protected from hind limb extension. Compounds examined and acids were given 1 hr before MES in doses of 2/5 LD_{50}. Each experimental group consisted of 10 mice.

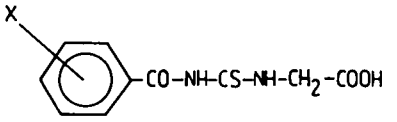
In PTZ test a dose of 115 mg/kg of penetrazole for compounds 19—24 and 120 mg/kg for compounds 1 – 18 was administered. These doses involved a general clonic convulsions in 100% and tonic convulsions in 90% of mice examined in groups consisted of 10 animals. Compounds tested were given in doses of 2/5, 1/5, 1/10 and 1/20 LD_{50} and acids in a dose of 2/5 LD_{50} half hour before penetrazole application.

Hypoglycemic action. In rats hyperglycemia was evoked by oral administration of 8 g/kg of glucose in aqueous solution, in a volume of 20 ml/kg. The blood was sampled from the tail tip three times: before glucose administration (0 min) and 90 and 150 min afterwards. Tolbutamide (10, 50 and 100 mg/kg s.c.) and the investigated compounds in dose of 2/5 LD_{50} were given 30 min after glucose administration. Each experimental group consisted of 3 rats. Blood glucose was determined in the samples using the Hyvarinen and Nikkilä [1] method.

RESULTS AND DISCUSSION

The acute toxicity of compounds 19—24 ranges between 450—1120 mg/kg (Table 2). Examined ω-benzoylthiohydantoic acids applied in doses of 2/5 LD_{50} exert no protective activity in MES and PTZ tests.

Table 2. Acute toxicity of benzylothiohydantoic acids 19—24



Compound No.	x	LD_{50} i.p. mg/kg
19	m-Cl	800.0 (740.7—864.0)
20	m-CH_3	1000.0 (833.3—1800.0)
21	p-Cl	1120.0 (1047.0—1198.4)
22	p-CH_3	760.0 (619.2—942.4)
23	o-Cl	450.0 (354.3—571.5)
24	o-CH_3	1100.0 (924.3—1309.0)

In hypoglycemic test these compounds show better activity. All preparations distinctly depress the level of glucose in blood of rats after 90 min and increase or maintain further activity during 150 min after their application (Table 3).

The arrangement of acids in relation to their decreasing activity an the chemical structure gives the following sequence:

Substituents:	m-CH_3	p-CH_3	o-CH_3	m-Cl	p-Cl	o-Cl
Acids:	24	20	22	23	19	21

(after 150 min)

This dependence indicates that more active group form the methylbenzoylthiohydantoic acids than corresponding chlorobenzoylthiohydantoic acids and allows to notice the characteristic arrangement of methyl- and chloro-substituents, expressed in a sequence of meta-, para- and ortho- with the dominating influence of meta-substitution on hypoglycemic properties of compounds 19—24.

New 5-nitrobenzylidene derivatives of 3-benzoyl-2-thiohydantoin (1—18) are substances of low toxicity with LD_{50} values above 3000 mg/kg i.p.

Their anticonvulsant properties are very weak. Similarly to acids examined, compounds 1—18 are also completely inactive in MES test. None of them

Table 3. Hypoglycemic properties of compounds 1—24

Compound* No.	Levels of glucose mg % in blood samples before 0' and after 90' 150' administration 8 g/kg of glucose p.o.			% Increment of glucose in blood evaluated to 0' time	
	Time (min) 0'	90'	150'	90'	150'
1	121.8	155.1	157.3	27.3	29.1
2	62.4	132.6	116.8	112.5	87.1
3	77.9	195.0	213.9	150.3	174.5
4	51.3	129.2	124.7	151.8	143.0
5	45.2	137.1	161.8	203.3	257.9
6	50.1	139.7	132.7	176.6	162.7
7	65.0	188.8	146.2	187.3	124.9
8	69.4	141.7	149.0	104.1	114.6
9	84.0	147.8	127.8	79.9	51.6
10	73.5	138.1	131.5	87.8	78.9
11	68.3	178.9	196.5	161.9	187.7
12	61.1	223.3	184.8	265.4	202.4
13	76.5	195.6	179.9	155.6	135.1
14	83.3	188.8	178.2	126.6	113.5
15	82.9	185.1	150.9	123.2	82.0
16	63.6	177.6	155.5	179.2	144.7
17	66.1	153.6	129.0	132.3	95.1
18	60.5	115.0	97.4	90.0	60.9
19	97.5	165.4	168.3	69.6	72.6
20	110.9	199.2	138.5	79.6	24.9
21	92.6	176.4	169.6	90.5	83.2
22	119.5	188.4	155.0	57.7	29.7
23	86.7	159.8	162.6	84.4	87.5
24	90.8	144.6	146.2	59.3	61.0
As a control served Tolbutamide used s.c. in doses:					
10 mg/kg	70.2	104.4	130.8	48.7	86.3
50 mg/kg	64.5	154.9	120.4	140.1	86.6
100 mg/kg	68.7	67.1	68.1	−2.3	−0.5
Solvent H_2O+ +3% Tween 80					
10 mg/kg	86.2	151.4	174.0	75.6	101.8

* Compounds were injected i.p. in a dose of 2/5 LD_{50} 30 min after glucose administration.

ensures the protection of mice against convulsions induced by alternating current.

In PTZ examination only several derivatives of 3-m-methyl- and 3-m--chlorobenzoyl-2-thiohydantoin exert activity of no account protecting 10—30% of mice. The remaining preparates occur to be inactive at doses of pentetrazole used in the investigations.

Results of hypoglicemic test presented in Table 3 indicate that most derivatives are hypoglycemically active substances, although their activity is weaker in comparison to that of thiohydantoin acids and the tolbutamide that was used as comparative drug during examination.

Relationship between hypoglycemic activity and the chemical structure of compounds 1—18 is difficult to appoint. In general, evaluation of both series of chloro- and methylbenzoyl-2-thiohydantoin derivatives shows that the more active group are chloro-substituted derivatives. The influence of three possible isomeric localizations of nitro-substituent in the benzylidene system on activity of compounds has rather slight contribution. In prevalent number of substances introduction of the nitro group mainly in ortho- or para-position of the benzylidene gives conspicuous hypoglycemic effects. The meta-nitrosubstituted compounds are less effective and several of them are active even hyperglycemically, e.g. compounds 5, 8 or 11.

Finding out the relation between the chemical structure of compounds investigated and their activity required further studies.

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STRESZCZENIE

Przeprowadzono syntezę 24 nowych pochodnych 3-benzylo-2-thiohydantoiny. Wykazano, że odznaczają się one małą toksycznością, słabymi właściwościami przeciwdrgawkowymi w teście PTZ, brakiem działania ochronnego w teście MES (związki 1—18) oraz słabo zaznaczonym działaniem hipoglikemicznym. Niektóre z meta-nitropodstawnych związków wykazują działanie hiperglikemiczne (związki 5, 8, 11).