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Aminoderivatives of cycloalkanespirohydantoins: synthesis and biological activity

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Abstract

3-Aminocycloalkanespiro-5-hydantoins were synthesized and their biological activity was studied. In contrast to hydantoins, these compounds failed to induce either anticonvulsive effects in the central nervous system or inhibitory effects on cholinergic contractions in the enteric nervous system. However, they exerted well pronounced, atropinsensitive, contractile effects on the guinea-pig ileum longitudinal muscle preparations. Structure–activity relationships established allow the assumption that: (i) the reduction of the ring size in the molecule of the spirohydantoins leads to an increase in the potency of the respective analogue to induce contractile effect; (ii) the introduction of $-NH_2$ in position 3 increases the ability of all the compounds studied to exert contractions; (iii) the enlargement of the ring leads to: (1) an increase of the degree of desensitization of the preparations; and (2) a decrease (except **1a**) of the potency of the analogues to exert contractile effects.

Keywords: Anticonvulsive activity; Contractile effect; Hydrazine reaction; NH-group reactivity

1. Introduction

Hydantoins and cycloalkanespirohydantoins are the subject of extensive investigations due to their biological properties [1]. The anticonvulsive activity is the strongest expressed in hydantoins. It has been found that this activity can be increased on introducing amino groups in the hydantoin ring [2]. Some cycloalkanespiro-5-hydantoins also have modest anticonvulsive effect. It has been observed that enlargement of the cycloalkane ringsize increases the effect [3]. Many derivatives of these compounds have been synthesized and studied [4], but there are no data about the influence of the amino group in the hydantoin ring on their biological activity. The objectives of the present study are: (i) the synthesis of 3-aminoderivatives of the cycloalkanespiro-5-hydantoins with 5-, 6-, 7-, 8- and 12-membered rings and (ii) the investigation of their biological activity, including the role of the amino group, both in the central and in the enteric nervous systems.

2. Results and discussion

2.1. Synthesis and characteristics of cycloalkanespiro-5-hydantoins and aminoderivatives

The cycloalkanespiro-5-hydantoins **1a**–**5a** were obtained from the corresponding cyclic ketones after the Bucherer–Lieb reaction [5,6]. Modifying a previous method [7], we transformed them into 3-aminoderivatives by means of $NH_2NH_2{\cdot}H_2O$ (Scheme 1).We ran the reaction for 1 h with 98% $NH_2NH_2{\cdot}H_2O$ and then for 3 h in refluxing with 80% $NH_2NH_2{\cdot}H_2O$ (yields over 90%). The correction of the concentration was done by adding the corresponding amount of H_2O to the reaction mixture without interrupting the heating. For obtaining 3-aminocyclododecanespiro-5-hydantoin, the

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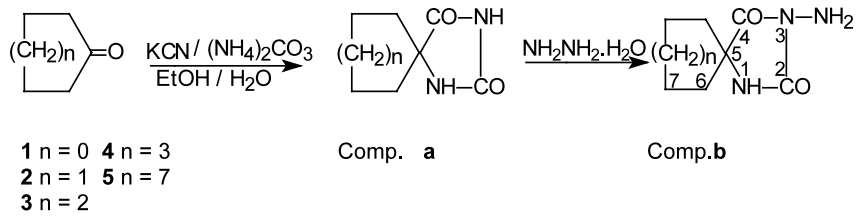
corresponding cyclododecanespiro-5-hydantoin was dissolved in ethanol at a high temperature, and $NH_2NH_2 \cdot H_2O$ was added. The duration of this reaction was 10 h, and a yield of 60% was obtained. All synthesized compounds were analyzed by means of IR-, ^1H NMR-, ^{13}C NMR- and mass-spectra. The results of the spectral analysis not previously reported showed that only the NH-group at position 3 in the hydantoine ring took part in the reaction. The increased reactivity at position 3 of the starting cycloalkanespiro-5-hydantoins is due to the presence of two carbonyl groups. As a result of their influence the signal of the N-3 proton on the ^1H-NMR spectrum appears in a weaker field (~ 10 ppm), while the signal for the N-1 proton appears at ~ 8 ppm. When aminoderivatives were obtained, the signal at ~ 10 ppm disappeared, but the one at ~ 8 ppm was preserved. This confirms the generation of a monoderivative at position 3. The two protons of the introduced $-NH_2$ group were detected as an intensive peak at 4.6–4.8 ppm. The structures were confirmed by mass-spectral analysis, a chemical ionization with NH_3 being applied. The most intense peak (100%) was $(MH)^+$ for all the compounds. The spectral data of the starting cycloalkanespiro-5-hydantoins and their aminoderivatives are presented in Table 1.

2.2. Biological activity

The 3-aminoderivatives of the cycloalkanespiro-5-hydantoins investigated (**1b**–**5b**) did not show an anticonvulsive activity using the pentylenetetrazole seizure and electroconvulsive shock seizure models. Moreover, the compounds **2b**, **3b** and **5b** induced seizures. This finding is in agreement with the decreased anticonvulsive effects of cycloalkanespiro-5-hydantoins as compared to that of hydantoins and their analogues [1]. The observed failure of 3-aminoderivatives of cycloalkanespiro-5-hydantoins, which was studied, to induce any antiseizure activity in the brain provoked further investigation on the enteric nervous system. The guinea pig ileum longitudinal muscle preparation is a classical model for investigation of exogenously applied drugs on the neurotransmitter interactions. To more fully characterize the compounds we followed their effects on both



Scheme 1. Synthesis of 3-aminoderivatives of the cycloalkanspiro-5-hydantoins.

Table 1
Physical and analytical data of the synthesized cycloalkanespiro-5-hydantoins and their aminoderivatives

Product	Yield (%)	m.p. (°C)	^1H NMR, δ (ppm)	IR (KBr) ν_{max} (cm^{-1})	MS (m/z)
1a [6] $C_7H_{10}N_2O_2$	90	205–205.5	(CD_3COCD_3) 1.84–2.11 (8H, m), 7.3 (1H, s), 10.4 (1H, s)	3215, 3074, 1788, 1737	154 $(M)^+$
2a [6] $C_8H_{12}N_2O_2$	95	219–220	(DMSO) 1.6–1.96 (10H, m), 8.2 (1H, s), 10.3 (1H, s)	3198, 3070, 1775, 1727	168 $(M)^+$
3a [6] $C_9H_{14}N_2O_2$	95	213–215	(DMSO) 1.6–2.31 (12H, m), 8.3 (1H, s), 10.4 (1H, s)	3183, 3080, 1782, 1730	182 $(M)^+$
4a [6] $C_{10}H_{16}N_2O_2$	91	240–241	(DMSO) 1.5–2.11 (14H, m), 8.2 (1H, s), 10.4 (1H, s)	3250, 3183, 1765, 1713	196 $(M)^+$
5a [3] $C_{14}H_{24}N_2O_2$	87	278–279	(DMSO) 1.29–1.98 (22H, m), 7.95 (1H, s), 10.44 (1H, s)	3183, 3063, 1772, 1728	252 $(M)^+$
1b [7] $C_7H_{11}N_3O_2$	80	140–141	($CDCl_3$) 1.7–2.3 (8H, m), 4.06 (2H, s), 6.7 (1H, s)	3320, 3200, 1788, 1738, 1612	169 $(M)^+$
2b* [7] $C_8H_{13}N_3O_2$	95	166–166.5	(DMSO) 1.2–1.6 (10H, m), 4.6 (2H, s), 8.5 (1H, s)	3320, 3200, 1775, 1732, 1610	183 $(M)^+$
3b [7] $C_9H_{15}N_3O_2$	90	162–163	($CDCl_3$) 1.5–2.18 (12H, m), 4.13 (2H, s), 7.4 (1H, s)	3320, 3200, 2500, 1770, 1730, 1610	(Cl, NH_3, 100 °C): $(MH)^+$ 198, $(MH_3)^+$ 200, $(M)^+$ 197
4b [7] $C_{10}H_{17}N_3O_2$	90	175–176	($CDCl_3$) 1.5–2.1 (14H, m), 3.9 (2H, s), 5.6 (1H, s)	3310, 3220, 1782, 1730, 1615	(Cl, NH_3, 100 °C): $(MH)^+$ 212 $(M)^+$ 211
5b $C_{14}H_{25}N_3O_2$	60	191–191.5	(DMSO) 1.17–1.8 (22H, m), 4.62 (2H, s), 7.9 (1H, s)	3300, 3266, 2857, 1773, 1727, 1660	(Cl, NH_3, 100 °C): $(MH)^+$ 268, $(MH_3)^+$ 270, $(M)^+$: 267

2b*: ^{13}C NMR δ 175.4 (C4), 156.1 (C2), 59.6 (C5), 33.6 (C6, C10), 24.6 (C8), 21.0 (C7, C9).

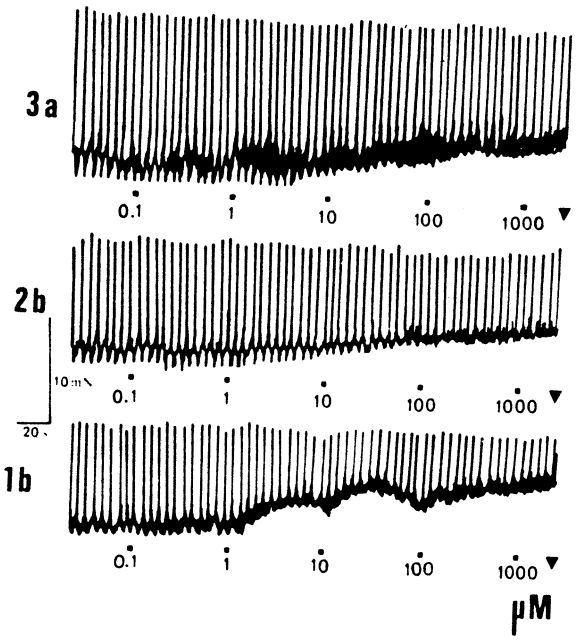


Fig. 1. Longitudinal layer of guinea-pig ileum. Tonic contractile effects of **3a**, **2b** and **1b** on the electrically-evoked (single pulses, 0.5 ms duration, 40 V, 0.1 Hz frequency) contractions; (■) application; (▼) washing.

electrically-stimulated and spontaneous mechanical activity of the isolated preparations. Electrical field stimulation (0.5 ms duration, 40 V, 0.1 Hz, frequency) elicited well-expressed, twitch-like contractions of the longitudinal layer of guinea pig ileum. The mean amplitude of the responses before treatment was 1.26 ± 0.14 g (calculated in 20 preparations isolated from eight animals). During stimulation, neither the amplitude of the contractions nor the tone of the preparations were considerably changed. Significant differences between the amplitudes of the control electrically evoked contractions before the application of the test substances were not observed. Cumulative or noncumulative addition of all compounds studied at concentrations increasing from 1 pM to 1 mM at 1–2 min intervals, did not exert any inhibitory effect on evoked contractions. Moreover, at concentration over 1 μM some compounds such as **3a**, **2b** and **1b** induced pronounced, dose dependent tonic contractile effects (Fig. 1). These results indicate that the compounds were unable to inhibit contractions, which according to parameters of the stimulation are mainly cholinergic by nature. This assumption closely corresponds with our data for absence of anticonvulsive activity of the compounds tested. However, despite the evoked release of acetylcholine during electrically-stimulated responses, the compounds reveal their ability to increase the tonic contractile activity of the preparations. The longitudinal layer of segments isolated from guinea-pig ileum showed pronounced spontaneous mechanical activity. After the equilibration period, the activity was characterized by a steady state tone and spontaneous contractions with amplitude of 0.35 ± 0.11 g (25 preparations from 10 animals). All compounds applied at concentrations of 1 pM to 1 mM caused concentration-dependent contractile effects in the spontaneous activity of the smooth muscle preparations. The effect of compound **2b** is shown at Fig. 2. The contractile effects comprised a tonic and a phasic component expressed as a total contraction. These effects were followed using cumulative or non-cumulative manners of application depending on the type of the desensitization of the preparations. Desensitization was achieved in separate experiments by applying the compounds in a non-cumulative way at a concentration of 1 μM, a contact time of 5–7 min, and without intervening washings. When intervals of 25–30 min, or 20 min, or 15 min, or

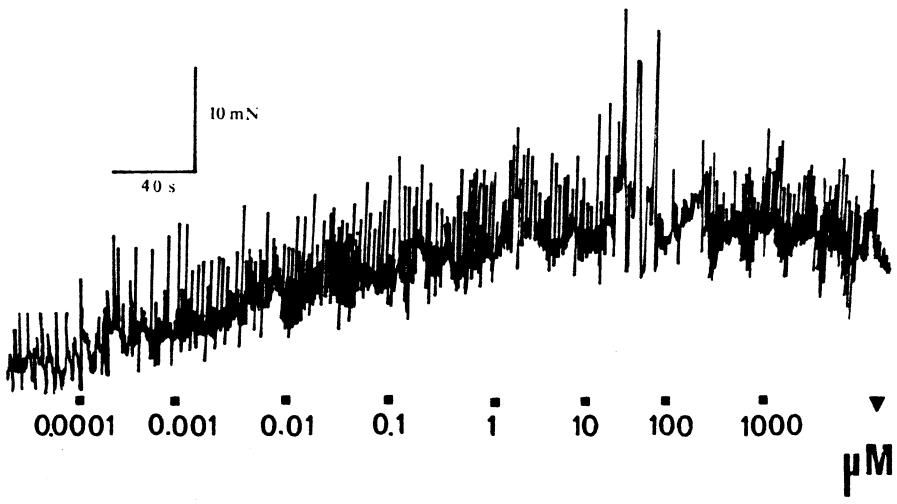
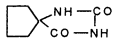
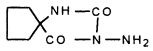
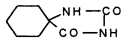
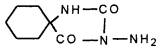
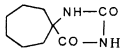
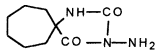


Fig. 2. Longitudinal layer of guinea-pig ileum. Dose dependent contractile effect of **2b** on the spontaneous mechanical activity; (■) application; (▼) washing.

Table 2
Longitudinal layer of guinea pig ileum. EC_{50} (μM) values of the contractile effects produced by cyclopentanespiro-5-hydantoin (**1a**), cyclohexanespiro-5-hydantoin (**2a**), cycloheptanespiro-5-hydantoin (**3a**), 3-aminocyclopentanespiro-5-hydantoin (**1b**), 3-aminocyclohexanespiro-5-hydantoin (**2b**) and 3-aminocycloheptanespiro-5-hydantoin (**3b**), on the spontaneous mechanical activity. The maximum contractile effects (g) and the type of desensitization are also presented.

Compounds	EC_{50} [μM]	Maximum contractile effect [g]	Desensitization
1a 	0.800±0.0700	0.60±0.08	-
1b 	0.010±0.0020**	1.20±0.25	-
2a 	0.010±0.0020	1.50±0.19	+
2b 	0.008±0.0015	1.00±0.25	+
3a 	0.500±0.0800	0.35±0.04	++
3b 	0.100±0.0200*	0.35±0.03	+++

The values for EC_{50} and maximum contractile effect are the mean ± SEM; the number of observations are at least 6. Asterisks indicate significant difference vs. the corresponding value for the compound without $-NH_2$ (*$p<0.05$ and **$p<0.01$). Designations for the type of the desensitization: (-), very poor or absent; (+), poor; (++), middle; and (+++), powerful.

10 min (with washings) between single doses of compounds were sufficient to prevent desensitization, it was determined, respectively, as: powerful, middle, poor and very poor or absent. Comparing the EC_{50} values of the respective concentration–response curves (Table 2) it could be assumed that: (i) the reduction of the ring size in the molecule of the spirohydantoins leads to an increase in the potency of the respective analogue to induce contractile effect; the most potent are **1b** and **2b**; and (ii) the introduction of $-NH_2$ in position 3 increases the ability of all the compounds studied to exert contractions; this effect is statistically insignificant between **2a** and **2b**, statistically significant ($P<0.05$) between **3a** and **3b**, and maximal ($P<0.01$) between **1a** and **1b**. The parameters of maximal contractile effect, expressed in absolute values (g), provide information concerning the efficacy of the compounds. All compounds (except **3a** and **3b**) induced high amplitude contractile effects. The substitution with amino group subsequently does not change at **3a**, decreases at **2a**, and increases twice the efficacy at **1a** of the respective compounds. The enlargement of the ring lead to: (1) an increase of the degree of desensitization of the preparations; and (2) a decrease (except **1a**) of the potency of the analogues to exert contractile effects. Our recent (unpublished) data showed that the acylation of the amino group of the spirohydantoins with an amino acid, or dipeptides eliminated the contractile effects of the respective compounds. The cholineblocker atropine, applied at a concentration of 3 μM, reduced or absolutely blocked the contractile effects of all compounds tested. This finding suggests that contractile effects of the spirohydantoins and their aminoderivatives on spontaneous activity of guinea-pig ileum longitudinal muscle preparations, could be due to an interaction with cholinergic neurotransmission which probably provoked the release of acetylcholine [8].

3. Experimental

Infrared spectra were recorded by the use of Perkin–Elmer FTIR-1600 spectrophotometer. For recording the NMR spectra, a Bruker WM-250 spectrometer was used at 250 and 62.9 MHz. Mass-spectra were recorded using 5890-plus mass spectrometer (Hewlett Packard). All melting points were determined using a Kofler apparatus without correction. Analytical TLC plates were purchased from E. Merck: silica gel 60F-254, aluminum backed. The plates were developed with ninhydrin, Cl_2-*o*-tolidine or UV light (254 nm). The following chromatography systems were used: $CHCl_3$/MeOH, 9:1; $CHCl_3$/MeOH/AcOH, 9:2:1; *n*-BuOH/AcOH/H_2O, 3:1:1.

3.1. Preparation of cycloalkanespiro-5-hydantoins (**1a–5a**) by Bucherer–Lieb synthesis

To a solution of cycloalkanone (290 mmol) in ethanol (225 ml) and water (200 ml) were added sodium cyanide (28.5 g, 438 mmol) and ammonium carbonate (119 g, 1.13 mol). The mixture was refluxed for 6 h with stirring. After dilution with water, the cooled mixture was acidified with concentrated hydrochloric acid. The crude cycloalkanespiro-5-hydantoin precipitated overnight upon cooling at 5 °C. Pure compound was crystallized from water as colorless crystals.

3.2. Synthesis of 3-aminocycloalkanespiro-5-hydantoins (**1b–4b**)

The corresponding cycloalkanespiro-5-hydantoin (0.03 mol) was refluxed with 10 ml (0.2 mol) of 98% $NH_2NH_2{\cdot}H_2O$ for 1 h, and with 80% $NH_2NH_2{\cdot}H_2O$ for 3.5 h (the correction of the concentration was done by adding the corresponding amount of H_2O to the reaction mixture without interrupting the heating). The reaction mixture was cooled, then poured over a small amount of crushed ice. Upon standing, the product crystallized slowly and was filtered, washed with a minimum of cold water, dried, and recrystallized from water or aqueous ethanol. Results are recorded in Table 1.

3.3. Synthesis of 3-aminocyclododecanespiro-5-hydantoin (**5b**)

5 g (0.02 mol) of the cyclododecanespiro-5-hydantoin (**5a**) were dissolved in hot ethanol (25 ml) then 10 ml (0.2 mol) 98% $NH_2NH_2{\cdot}H_2O$ were added. The optimal duration of the reaction was 10 h. The compound was obtained according to the above method as a white solid: *Anal*. Calc. for $C_{14}H_{25}N_3O_2$: C, 62.92; H, 9.36; N, 15.73. Found: C, 62.95; H, 9.30; N, 15.71%.

3.4. Biological activity on the central nervous system

Experimental seizure models (pentylenetetrazole seizures and electroconvulsive shock)

The experiments were carried out on male Wistar mice (10 mice per group), weighing 18–20 g.

3.4.1. Pentylenetetrazole seizure model

Pentylenetetrazole was injected subcutaneously in a dose of 85 mg/kg. This dose was chosen on the basis of preliminary experiments in which moderate seizures were produced in the majority of animals. The effect was evaluated by the differences between the control and the experimental group of mice in seizure intensity determined by a six point scale [9]. The animals were observed 1 h after pentylenetetrazole injection.

3.4.2. Electroconvulsive shock

An electroshock stimulation (monophase rectangular pulses with a current intensity of 50 mA, single phase duration 1 ms; stimulation frequency of 50 Hz and a trial duration of 0.2 s) was applied by means of silver corneal electrodes inducing clonic–tonic seizures. A sham electroshock was applied. The percentage of the tonic seizure was calculated.

The 3-aminocyclohexanespiro-5-hydantoin, 3-aminocycloheptanespiro-5-hydantoin, and 3-amino-cyclododecanespiro-5-hydantoin were administered i.p. at the following doses: 400 mg/kg, 600 mg/kg, 30 min before pentylenetetrazole or electroconvulsive shock.

The data of both seizure intensity and latency to first seizure were assessed by multifactor ANOVA. The data of mortality and the percentage of the tonic seizures were analyzed by Fisher's Exact Probability Two Tailed test.

3.5. Biological activity in the enteric nervous system

3.5.1. Smooth muscle preparations

Male guinea pigs (250–300 g) were used in the experiments. A segment of the ileum (excluding the portion 15 cm proximal to the ileocaecal junction) 10–15 cm long, was isolated and the intraluminal contents were removed with aerated Krebs solution containing (mM): NaCl 120, KCl 5.9, $NaHCO_3$ 15.4, NaH_2PO_4 1.2, $MgCl_2$ 2.5 and glucose 11.5. In order to retain the myenteric plexus intact, segments of the ileum approximately 2.0 cm long were cut out.

3.5.2. Spontaneous mechanical activity

The isolated segments were mounted along the axis of the longitudinal layer in 3 ml organ baths, containing Krebs solution, continuously aerated with 95% O_2 and 5% CO_2 at 36.5 °C. The spontaneous mechanical activity as well as the compound- or electrically-evoked responses of the longitudinal layer were followed under isometric conditions after standard calibration of a mechanoelectrical transducer (Experimetria Ltd., Hungary) connected to a recording device TZ 4620 (Laboratorni Pristroje, Praha) at a tension equivalent to a load of 0.5 g. There was a 60 min equilibration period before application of the compounds. Concentration–response curves were constructed for each of the compounds. The EC_{50} values, presented as means $\pm$ SEM and significance of the differences (Student's t-test at $P < 0.05$ and $P < 0.01$) were calculated using computer programs [10]. The concentration–response curves were further followed in the presence of cholinoblocker atropine (3 μM) which remained in the organ bath before retesting the compounds for 10 min.

3.5.3. Electrical stimulation

Electrical field stimulation was applied by a pair of ring wire platinum electrodes (0.45 mm thick), to

induce neurogenic contraction of the smooth muscle preparations. Stimulation with single rectangular pulses (generated by a stimulator ST-02, Experimetria MM, Hungary) of supramaximal voltage, 75 mA current, 0.5 ms duration, and 0.1 Hz frequency was applied. Since the electrically stimulated release of neurotransmitters by specific activation of the neural structures in the intestine depends on the stimulus frequency [11], we used stimulation with a relatively low frequency to provoke responses, mainly cholinergic by nature.

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