Synthesis and antimicrobial activity of new 3-substituted cyclohexanespiro-5-hydantoin derivatives

Marin Marinov, Iliana Kostova, Emilia Naydenova, Rumyana Prodanova, Petja Marinova, Plamen Penchev, Neyko Stoyanov

Synthesis and antimicrobial activity of new 3-substituted cyclohexanespiro-5-hydantoin derivatives: *The article presents a synthesis of new 3-substituted cyclohexanespiro-5-hydantoin derivatives. The target compounds were prepared by an interaction of 3-aminocyclohexanespiro-5-hydantoin with Indometacin, Nalidixic acid, 2-Thiopheneacetic acid and Myristic acid. The antimicrobial activity of the products obtained was determined against Gram-positive bacteria Staphylococcus aureus and Bacillus subtilis, Gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa and Salmonella abony, the yeasts Candida albicans and Saccharomyces cerevisiae and the molds Penicillium chrysogenum, Aspergillus niger and Fusarium moniliforme.*

Key words: *3-substituted cyclohexanespiro-5-hydantoin derivatives, antimicrobial activity*

INTRODUCTION

The study of 3-amino derivatives of spirohydantoins is mainly due to their biological effect. Different 3-aminocycloalkanespiro-5-hidantoins were synthesized and their biological activity was studied in the previous investigation of ours. It was found that 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, atropine-sensitive, contractile effects on the guinea-pig ileum longitudinal muscle preparations [1]. Recently, we have examined the cytotoxic effect of 3-amino-9'-fluorenespiro-5-hydantoin on the retinoblastoma cell line WERI-Rb-1 and its antibacterial activity against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria and the yeasts *Candida albicans*. The results obtained from these tests revealed that this compound could not serve as potential anticancer agent, but it showed pronounced antimicrobial activity against the bacteria *Escherichia coli* and no activity towards *Staphylococcus aureus* and *Candida albicans* [2].

The current work is a continuation of our investigations for the preparation of new biologically active derivatives of spirohydantoins. The interaction of 3-aminocyclohexanespiro-5-hydantoin with other biologically active substances, which have an application in the medical practice [3, 4], such as: Indometacin, Nalidixic acid, 2-Thiopheneacetic acid and Myristic acid was studied for this purpose. An additional contribution of the present study was the new information received about the antimicrobial effect of the synthesized compounds against various Gram-positive and Gram-negative bacteria, yeasts and molds.

EXPERIMENTAL

Synthetic compounds

All used chemicals were purchased from Merck and Sigma-Aldrich. The melting points were determined by a SMP-10 digital melting point apparatus. The purity of the compounds was checked by thin layer chromatography on Kieselgel 60 F_{254}, 0.2 mm Merck plates, eluent system (vol. ratio): ethyl acetate : petroleum ether = 1 : 2. The elemental analysis data were obtained with an automatic analyzer Carlo Erba 1106. The IR spectra were registered in KBr pellets on a Bruker FT-IR VERTEX 70 Spectrometer from 4000 cm^{-1} to 400 cm^{-1} at resolution 2 cm^{-1} with 25 scans. The Attenuated Total Reflection FTIR (ATR) spectra were registered on the same instrument by ATR accessory MIRacle™ with a one-reflection ZnSe element (Pike) and the stirred crystals were pressed by an anvil to the reflection element; the spectra were from 4500 cm^{-1} to 600 cm^{-1} at resolution 2 cm^{-1} with 16 scans. The Raman spectra (the stirred crystals placed in

aluminium disc) were measured on a RAM II (Bruker Optics) with a focused laser beam of 200 mW power of Nd:YAG laser (1064 nm) from 4000 cm^{-1} to 51 cm^{-1} at resolution 2 cm^{-1} with 25 scans. The initial cyclohexanespiro-5-hydantoin (**I**, Scheme 1) was synthesized *via* the Bucherer-Lieb method [5].

Synthesis of 3-aminocyclohexanespiro-5-hydantoin (II, Scheme 1)

A suspension of 5.00 g (0.027 mol) of cyclohexanespiro-5-hydantoin (I, Scheme 1) and 15 ml of concentrated hydrazine hydrate was refluxed for 5 h. After cooling to the room temperature, the mixture was poured onto a small quantity of crushed ice. The colorless obtained product (II) was filtered off and recrystallized from ethanol.

Synthesis of amides IV, VI, VIII and X (Schemes 2-5)

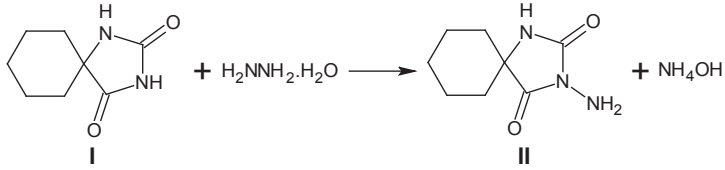
1.83 g (0.01 mol) of 3-aminocyclohexanespiro-5-hydantoin (II) and 0.01 mol of the corresponding biologically active substance (Indometacin, III, Scheme 2; Nalidixic acid, V, Scheme 3; 2-Thiopheneacetic acid, VII, Scheme 4 and Myristic acid, IX, Scheme 5) were dissolved in 50 ml of tetrahydrofuran with stirring at room temperature. *N,N'*-dicyclohexylcarbodiimide (DCC, 2.06 g, 0.01 mol) was added to the reaction mixture and the latter was left overnight. After this interaction, the *N,N'*-dicyclohexylcarbamide formed was filtered off and 1 ml of glacial acetic acid was added to the filtrate for removing of the unreacted reagent. After filtration, the solvent was evaporated to dryness and the amides obtained (IV, VI, VIII and X) were recrystallized from ethanol.

Antimicrobial assay

The microbial cultures were purchased from National Bank of Industrial Microorganisms and Cell Cultures (NBIMCC), Sofia. The antimicrobial effect of the synthesized compounds IV, VI, VIII and X against Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633, Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NTCC 6017, the yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763 and the molds *Penicillium chrysogenum*, *Aspergillus niger* and *Fusarium moniliforme* was studied. The technique used for this investigation was the agar well diffusion method. [6]. The used agar media were Soy-casein agar (Scharlau) for the tests with bacteria and Saburo-decstrose agar (Scharlau) for yeast and molds. The wells were filled with solutions (50 µl) of the compounds synthesized and, following a 30-min stay at room temperature, the Petri dishes were placed in a thermostat at 37 °C for 24 h for bacteria, 28 °C for 24 h for yeast and 72 h for molds. After cultivation, the diameter of inhibition growth zones around wells was measured in mm, and the results obtained were evaluated as follows: up to 15 mm - the microbial culture is week sensitive, 15-20 mm - it is sensitive and over 25 mm - it is very sensitive to the given synthetic compound at tested concentration. The concentrations (mg/ml) of compounds IV, VI, VIII and X in ethylene glycol were as follows: 0.5, 1, 2 and 5. The pure ethylene glycol (50 µl) was used as a control. The data on antimicrobial activity are arithmetic average of three measurements.

RESULTS AND DISCUSSION

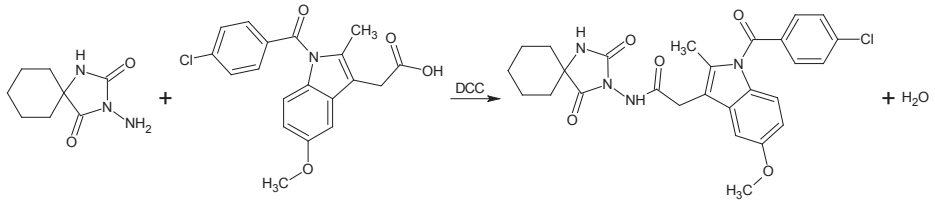
The 3-aminocyclohexanespiro-5-hydantoin (II) was synthesized by an interaction of cyclohexanespiro-5-hydantoin (I) with concentrated hydrazine hydrate (Scheme 1) following a modificated method (see the Experimental part) of the prior techniques [1, 2, 7, 8]. The product II was obtained with 98 % yield and its physicochemical parameters and spectral data were identical with the previously published results [1, 7].



I **II**

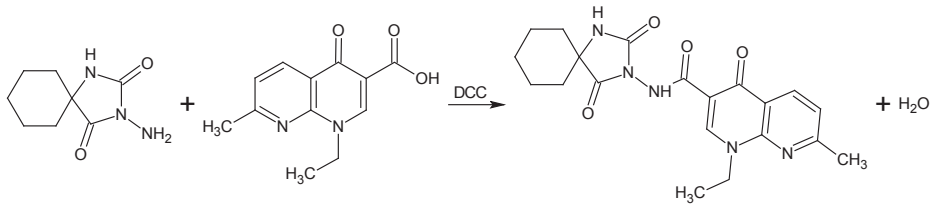
Scheme 1

The compound II was subjected to an interaction with Indometacin (III, Scheme 2), Nalidixic acid (V, Scheme 3), 2-Thiopheneacetic acid (VII, Scheme 4) and Myristic acid (IX, Scheme 5). The reactions were carried out in accordance with the DCC-method [9], and as result of this technique the corresponding amides (IV, VI, VIII and X) were obtained.



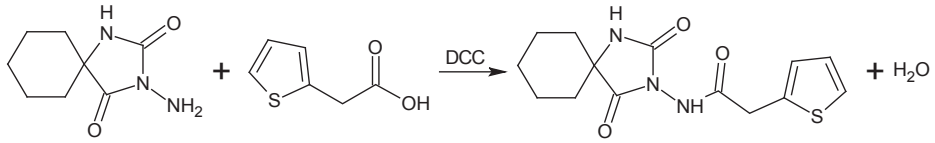
II **III** **IV**

Scheme 2



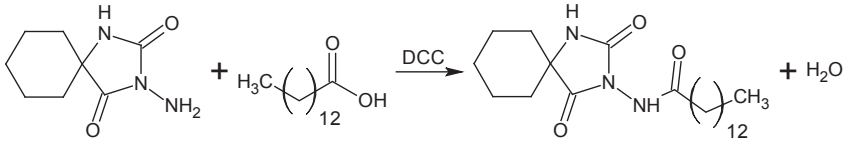
II **V** **VI**

Scheme 3



II **VII** **VIII**

Scheme 4



II **IX** **X**

Scheme 5

The preparation of compounds IV, VI, VIII and X were carried out at ambient temperature in a medium of tetrahydrofuran. The syntheses were carried out also by using other suitable solvents, such as ethyl acetate, dioxane and acetonitrile, but the resulting yields of the products were lower in comparison with the use of tetrahydrofuran. The synthesized compounds (IV, VI, VIII and X) were colorless to pale yellow crystalline substances. Their physicochemical characteristics and spectral data are listed in Tables 1 and 2 respectively.

Table 1

№	Molecular formula	Yield, %	M. p., °C	R_f	Elemental analysis, %									
					Calculated					Found				
					C	H	N	S	Cl	C	H	N	S	Cl
IV	$C_{27}H_{27}ClN_4O_5$ 522.98	89	234-5	0.57	62.01	5.20	10.71		6.78	61.84	5.11	10.59		6.61
VI	$C_{20}H_{23}N_5O_4$ 397.43	96	206-7	0.49	60.44	5.83	17.62			60.27	5.73	17.55		
VIII	$C_{14}H_{17}N_3O_3S$ 307.37	78	147-8	0.62	54.71	5.57	13.67	10.43		54.56	5.41	13.43	10.16	
X	$C_{22}H_{39}N_3O_3$ 393.56	66	82-3	0.66	67.14	9.99	10.68			66.95	9.86	10.54		

Table 2

№	IR (ν_{max}, cm^{-1})							
	ν_{NH}	$\nu_{CH\ (arom.)}$	$\nu_{CH\ (aliph.)}$	$\nu_{C=O}$	$\nu_{C=O\ (amide)}$	$\nu_{CC\ (arom.)}$	ν_{CN}	$\nu_{2\text{-thioph. core}}$
IV	3247	3007	2915	1789, 1738	1685	1596	1368	
VI	3296		2948	1775, 1763, 1704	1701	1599	1355	
VIII	3287		2944	1763, 1738, 1715	1678	1588	1376	816
X	3338		2949	1765, 1736, 1712	1683		1379	
	ATR (ν_{max}, cm^{-1})							
	ν_{NH}	$\nu_{CH\ (arom.)}$	$\nu_{CH\ (aliph.)}$	$\nu_{C=O}$	$\nu_{C=O\ (amide)}$	$\nu_{CC\ (arom.)}$	ν_{CN}	$\nu_{2\text{-thioph. core}}$
IV	3253	3001	2926	1800, 1735	1681	1599	1372	
VI	3300		2956	1785, 1767, 1707	1701	1601	1358	
VIII	3291		2948	1767, 1743, 1716	1683	1592	1379	825
X	3343		2951	1773, 1740, 1719	1679		1381	
	Raman (ν_{max}, cm^{-1})							
IV	3068, 3002, 2928, 2852, 1782, 1738, 1680, 1590, 1577, 1447, 1393, 1350, 1222, 1182, 1124, 1066, 830, 736, 663							
VI	3077, 3042, 2986, 2928, 1759, 1714, 1614, 1561, 1468, 1444, 1382, 1329, 1296, 1274, 1198, 1093, 969, 877, 781, 725, 708, 557, 539, 486, 430, 314							
VIII	2966, 2919, 2898, 2864, 2849, 2833, 2716, 1758, 1672, 1456, 1361, 1290, 1257, 1188, 1117, 1053, 1018, 912, 877, 835, 801, 766, 481, 365							
X	2956, 2925, 2901, 2881, 2860, 2848, 2726, 1767, 1680, 1468, 1372, 1296, 1267, 1195, 1127, 1064, 1028, 910, 892, 856, 807, 787, 457, 344							

The antimicrobial activity of the products obtained was determined against Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633, Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NTCC 6017, the yeasts *Candida albicans* ATCC 10231 and

Saccharomyces cerevisiae ATCC 9763 and the molds *Penicillium chrysogenum*, *Aspergillus niger* and *Fusarium moniliforme*. The results obtained from these analyses are listed in Table 3.

Table 3

Microorganism	Inhibition zone (mm)															
	Concentration of compound IV (mg/ml)				Concentration of compound VI (mg/ml)				Concentration of compound VIII (mg/ml)				Concentration of compound X (mg/ml)			
	0.5	1	2	5	0.5	1	2	5	0.5	1	2	5	0.5	1	2	5
Staphylococcus aureus ATCC 6538	-	-	-	-	-	13.3	16.9	20.8	-	-	-	-	-	-	-	-
Bacillus subtilis ATCC 6633	-	-	-	-	-	18.9	19.7	26.8	-	-	-	-	-	-	-	-
Escherichia coli ATCC 8739	-	-	-	-	-	15.4	17.3	23.1	-	-	-	13.1	-	-	-	13.4
Pseudomonas aeruginosa ATCC 9027	-	-	-	-	-	13.7	16.0	20.2	-	-	-	14.6	-	-	-	14.3
Salmonella abony NTCC 6017	-	-	-	-	-	13.7	14.4	20.0	-	-	-	12.7	-	-	-	10.3

* - No zone of inhibition

An interesting fact is that 3-aminocyclohexanespiro-5-hydantoin (II), Indometacin (III), 2-Thiopheneacetic acid (VII) and Myristic acid (IX) do not possess antimicrobial activity. The Nalidixic acid (V), which is used in the treatment of urinary tract infections, exhibits an expressed bacteriostatic effect against Gram-positive bacteria.

The amides presented in this study (IV, VI, VIII and X) showed no activity against the tested yeasts and molds. The results listed in Table 3 indicated the presence of a weak antibacterial activity of VIII and X, and the absence of such action of IV. In contrast, compound VI (in a concentration above 1 mg/ml) exhibited a pronounced antimicrobial activity against the tested Gram-positive and Gram-negative bacteria.

CONCLUSIONS

The synthesis of new 3-substituted cyclohexanespiro-5-hydantoin derivatives (IV, VI, VIII and X), based on the interaction of 3-aminocyclohexanespiro-5-hydantoin (II) with Indometacin (III), Nalidixic acid (V), 2-Thiopheneacetic acid (VII) and Myristic acid (IX) was presented. The antimicrobial tests of the products obtained showed a very important result about compound VI, which exhibited a pronounced antimicrobial activity against the tested Gram-positive and Gram-negative bacteria. This fact shows the antibacterial potential of VI and motivates the further research in this direction.

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About the authors:

Marin Marinov, Assoc. Prof., PhD, Faculty of Plant Protection and Agroecology, Department of General Chemistry, Agricultural University – Plovdiv, e-mail: m_n_marinov@abv.bg.

Iliana Kostova, Chief Assist. Prof., PhD, Department of Biotechnologies and Food technologies, “Angel Kanchev” University of Ruse, Razgrad Branch, e-mail: ikostova@uni-ruse.bg.

Emilia Naydenova, Prof., PhD, Department of Organic Chemistry, University of Chemical Technology and Metallurgy, e-mail: emilia@uctm.edu.

Rumyana Prodanova, Assist. Prof., Faculty of Plant Protection and Agroecology, Department of General Chemistry, Agricultural University – Plovdiv, e-mail: ru_prodanovakam@abv.bg.

Petja Marinova, Chief Assist. Prof., PhD, Faculty of Chemistry, Department of General and Inorganic Chemistry with Methodology of Chemistry Education, Plovdiv University “Paisii Hilendarski”, e-mail: marinova@uni-plovdiv.bg.

Plamen Penchev, Assoc. Prof., PhD, Faculty of Chemistry, Department of Analytical Chemistry and Computer Chemistry, Plovdiv University “Paisii Hilendarski”, e-mail: plamen@uni-plovdiv.bg.

Neyko Stoyanov, Prof., PhD, Department of Chemistry and Chemical Technologies, “Angel Kanchev” University of Ruse, Razgrad Branch, e-mail: nstoianov@uni-ruse.bg.

This paper has been reviewed