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SYNTHESIS AND ANTIBACTERIAL ACTIVITY STUDIES OF NEW 2-*N*-SUBSTITUTED 2,5-DIHYDROFURANS

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New 2-*N*-substituted 2,5-dihydrofurans were successfully synthesized by the convenient and efficient method, which was based on the reaction of functionalized 2-imino-2,5-dihydrofurans with benzohydrazide in glacial acetic acid. Synthesized compounds exhibited moderate to defined antibacterial activities against Gram-positive (*Staphylococcus aureus* – 209p and 1) and Gram-negative (*Shigella Flexneri 6858, Esherichia coli 0–55*) bacteria compared to furazolidone.

Keywords: 2-imino-2,5-dihydrofurans, benzohydrazide, 2-*N*-substituted 2,5-dihydrofurans, synthesis, antibacterial activity.

Introduction. The unsaturated γ-iminolactone structure is related to unsaturated γ-lactone fragment which constitutes a part of many natural molecules, in particular L-ascorbic, penicillic and tetronic acids, protoanemonin and cardenolides (heart glycosides). Functionally substituted derivatives of unsaturated γ-lactones constitute an important class of many natural and synthetic products [1–4]. They possess a wide range of biological activity (antibacterial, antibiotic, antifungal, anti-inflammatory) and their numerous derivatives have been applied in different areas of medicine [5–11]. The biological activity of unsaturated γ-lactones is due to the presence of an unsaturated C=C bond or aromatic substituent [12, 13]. Unsaturated lactones conjugated to a double bond are also plant growth stimulators [14, 15].

In continuation of our current studies on the chemistry of 2-imino--2,5-dihydrofurans [16], we described the synthesis of new 2-*N*-substituted 2,5-dihydrofurans **3**, **a**–**g**, and the study of the antibacterial activity of the starting **1**, **a**–**g** and synthesized **3**, **a**–**g** compounds.

Results and Discussion. New 2-*N*-substituted 2,5-dihydrofurans **3**, **a**–**g**, were prepared from the reaction of 2-imino-2,5-dihydrofurans **1**, **a**–**g** with benzohydrazide **2** by using the recently reported method [17]. The synthesis of the desired 2-*N*-substituted iminodihydrofurans **3**, **a**–**g** was finally achieved by adding an equivalent amount of the benzohydrazide **2** to a warm solution of 2-imino-2,5-

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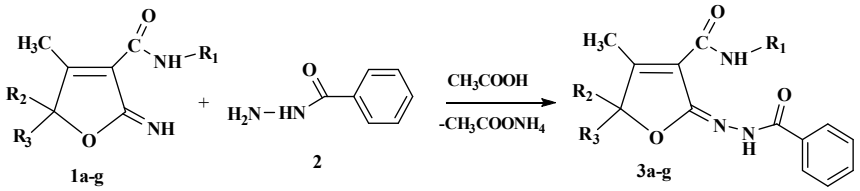
-dihydrofurans **1**, **a**–**g** in glacial acetic acid. After stirring the reaction mixture for 2 *h*, products were precipitated from the solution and subsequently isolated by filtration.

The structures of the obtained compounds were determined from their spectroscopic data. Compounds **1b** and **3b** have asymmetric carbon atom. The specific rotation was measured for these compounds. They are optically inactive compounds.



a. R_1=H, R_2=R_3= CH_3,
b. R_1=H, R_2=CH_3, R_3=C_2H_5,
c. R_1= H, R_2,R_3=(-CH_2-)$_4$,
d. R_1=H, R_2,R_3=(-CH_2-)$_5$,
e. R_1= R_2=R_3= CH_3,
f. R_1=CH_3, R_2,R_3=(-CH_2-)$_5$,
g. R_1=$CH_2C_6H_5$, R_2=R_3= CH_3,

The antibacterial activities of the starting **1**, **a**–**g** and obtained **3**, **a**–**g** compounds were evaluated against Gram-positive (*Staphylococcus aureus* – 209p and 1) and Gram-negative (*Shigella Flexneri* 6858*, Escherichia coli* 0–55) bacteria by the agar diffusion technique [18]. The antibacterial activities of compounds **1**, **a**–**g** and **3**, **a**–**g** were compared with standard drug furazolidone [19]. Furazolidone was chosen as a positive control, since it is a synthetic chemical that has antibacterial activity against both gram-positive and gram-negative microorganisms. The drug is currently used in the clinic. In addition, the furan ring is present in the structure of furazolidone.

*Antibacterial activities of **1**, **a**–**g** and **3**, **a**–**g** in comparison to furazolidone*

Compounds	*Staphylococcus aureus*		*Shigella Flexneri*	*Escherichia coli*
	209p	1	6858	0–55
	Zone of inhibition, *mm*			
1a	11.0 ± 0.1	11.0 ± 0.1	12.0 ± 0.1	11.0 ± 0.1
1b	12.0 ± 0.1	14.0 ± 0.1	12.0 ± 0.1	14.0 ± 0.1
1c	10.0 ± 0.1	10.0 ± 0.1	11.0 ± 0.1	11.0 ± 0.1
1d	10.0 ± 0.1	11.0 ± 0.1	10.0 ± 0.1	10.0 ± 0.1
1e	12.0 ± 0.1	10.0 ± 0.2	11.0 ± 0.1	12.0± 0.1
1f	11.0 ± 0.1	12.0 ± 0.1	12.0 ± 0.1	10.0 ± 0.1
1g	10.0 ± 0.1	10.0 ± 0.1	10.0 ± 0.1	10.0 ± 0.1
3a	16.3 ± 0.6	20.8 ± 0.9	16.3 ± 0.9	17.0 ± 1.4
3b	10.0 ± 0	10.0 ± 0	13.0 ± 1.0	13.3 ± 0.6
3c	12.6 ± 0.6	10.0 ± 1.0	13.3 ± 0.6	13.0 ± 1.0
3d	17.3 ± 0.9	17.6 ± 1.3	16.0 ± 1.0	20.3 ± 0.9
3e	12.2 ± 0.9	13.8 ± 1.3	11.0 ± 1.0	13.3 ± 0.9
3f	10.8 ± 0.9	10.2 ± 1.3	10.3 ± 1.0	11.1 ± 0.9
3g	19.4 ± 0.8	17.6 ± 1.6	17.1 ± 1.1	17.8 ± 0.7
Furazolidone	25.0 ± 1.0	24.0 ± 1.2	24.0 ± 1.0	24.0 ± 1.0

The antibacterial activities of **1**, **a**–**g** and **3**, **a**–**g** are shown in Table. In comparison to furazolidone, compounds **1**, **a**–**g** exhibited weak activities towards the growth of both Gram-positive and Gram-negative bacteria. Replacing the imino group to benzoylhydrazono group in compounds **3**, **a**–**g** enhanced the antibacterial activity. Compounds **3**, **a**–**g** exhibited moderate to defined activities.

Probably, the antibacterial activity of the compounds **3**, **a**–**g** can be explained by their ability to interact with bacterial proteins, as well as with certain enzymes containing sulfhydryl groups and which are of great importance for the normal life of microorganisms, which are known in unsaturated γ-lactone series [20, 21]. It is assumed that the bactericidal action of unsaturated γ-iminolactones is associated with the ability of the iminolactone double bond to attach the SH groups present in bacterial proteins, inhibiting the development of bacteria.

Experimental Part.

Chemical Part. The ^{1}H and ^{13}C NMR spectra were recorded at 300 *MHz* and 75 *MHz* on a Varian Mercury-300 *MHz* ("Varian", USA) spectrometer in the mixture of solvents DMSO-d_6+CCl_4 (1: 3), using tetramethylsilane as internal standard. IR spectra were recorded on Avatar 330FT-IR ("Thermo Nicolet", USA) spectrometer, using attenuated total reflectance (ATR) method. The reaction progress and the purity of the obtained substances were checked by using TLC method on UV-254 plates ("Silufol", Czech Republic) with acetone/benzene mixture (1:2) as an eluent, visualization was with iodine vapors. All melting points were measured with an Electrothermal 9100 apparatus. Specific optical rotation was decided on a Polartronic H532 polarimeter ("Schmidt+Haensch GmbH & Co.", Berlin, Germany).

Compounds **1b**, **1c** were synthesized by the known procedure [16].

5-Ethyl-2-imino-4,5-dimethyl-2,5-dihydrofuran-3-carboxamide ***(1b)***. Yield 83%; white solid; m.p. 141–142°*C* (from ethanol), $[\alpha]_D^{20}$ 0 (c 0.5, DMSO). IR (KBr), *v, cm*$^{-1}$: 3300 (NH_2), 3140 (NH), 1683 (C=O), 1642 (C=N), 1625 (C=C). ^{1}H NMR spectrum, *δ, ppm*: 0.95 t (*J* = 8.5 *Hz*, 3H, $CH_2C\underline{H}_3$), 1.40 s (3H, CH_3), 1.79 dq (*J* = 14.5, 7.4 *Hz*, 1H, $C\underline{H}_2CH_3$), 1.92 dq (*J* = 14.5, 7.4 *Hz*, 1H, $C\underline{H}_2CH_3$), 2.34 s (3H, CH_3), 7.20 s (1H, =NH), 7.16 br.s (1H) and 8.84 br.s (1H, $N\underline{H}_2$). ^{13}C NMR spectrum, *δ*, *ppm*: 6.88 (CH_3), 11.88 (CH_3), 23.06 (CH_3), 29.50 (CH_2), 89.68 (C_5), 120.16 (C_3), 162.09 (C_2), 166.68 (C_4), 169.45 (C=O).

2-Imino-4-methyl-5,5-tetramethylene-2,5-dihydrofuran-3-carboxamide ***(1c)***. Yield 75%; white solid; m.p. 193–195°*C*(from ethanol). IR (KBr), *v, cm*$^{-1}$: 3300 (NH_2), 3140 (NH), 1680 (C=O), 1640 (C=N), 1620 (C=C). ^{1}H NMR spectrum, *δ*, *ppm*: 1.42 m (2H) and 1.58–1.85 m (6H, $(CH_2)_4$), 2.33 s (3H, CH_3), 7.23 s (1H, =NH), 7.15 br.s (1H) and 8.86 br.s (1H, $N\underline{H}_2$). ^{13}C NMR spectrum, *δ*, *ppm*: 22.10 (CH_2), 22.22 (CH_2), 25.09 (CH_2), 26.95 (CH_2), 35.91 (CH_3), 86.29 (C_5), 116.76 (C_3), 162.10 (C_2), 166.37 (C_4), 173.27 (C=O).

General Procedure for the Preparation of compounds 3, a–g. To a well-stirred warm (40–50°*C*) solution of compound **1**, **a**–**g** (10 *mmol*) in 10 *mL* glacial acetic acid was added an equivalent amount of benzohydrazide **2**. The reaction mixture was stirred at room temperature for 2 *h*. The products, which precipitated in the course of the reaction, were filtered, washed with water, and recrystallized.

2-(2-Benzoylhydrazono)-4,5,5-trimethyl-2,5-dihydrofuran-3-carboxamide ***(3a)***. Yield 91%; white solid; m.p. 221–222°*C*. IR (KBr), *v, cm*$^{-1}$: 3394 (NH_2), 3294 (NH), 1722 (C=O), 1683 (C=O), 1642 (C=N), 1625 (C=C), 1500–1600 (C=C_{arom}). ^{1}H NMR spectrum, *δ*, *ppm*: 1.45 s (6H, $2CH_3$), 2.37 s (3H, CH_3), 7.44–7.52 m (3H, C_6H_5), 7.80–7.84 m (2H, C_6H_5), 7.40 br.s (1H) and 8.34 br.s (1H, $N\underline{H}_2$), 9.98 s (1H, NH). ^{13}C NMR spectrum, *δ*, *ppm*: 11.78 (CH_3), 20.44 (CH_3), 24.37 (CH_3), 91.38 (C_5), 117.93 (C_3), 127.15 ($2C_{arom.}$), 127.74 ($2C_{arom.}$), 130.57 ($C_{arom.}$), 133.45 ($C_{arom.}$), 161.44 (C_2), 161.93 (C_4), 167.73 (C=O), 171.12 (C=O).

*2-(2-Benzoylhydrazono)-5-ethyl-4,5-dimethyl-2,5-dihydrofuran-3-carboxamide (**3b**)*. Yield 89%; white solid; m.p. 170–171ºC, $[\alpha]_D^{20}$ 0 (c 0.5, DMSO). IR (KBr), *v, cm*$^{-1}$: 3394 (NH_2), 3294 (NH), 1722 (C=O), 1683 (C=O), 1642 (C=N), 1625 (C=C), 1500–1600 (C=$C_{arom.}$). ^{1}HNMR spectrum, *δ, ppm*: 0.95 t (3H, *J* = 8.5 *Hz*, $CH_2C\underline{H}_3$), 1.45 s (3H, CH_3), 1.79 dq (*J* = 14.5, 7.4 *Hz*, 1H, $C\underline{H}_2CH_3$), 1.92 dq (*J* = 14.5, 7.4 *Hz*, 1H, $C\underline{H}_2CH_3$), 2.37 s (3H, CH_3), 7.45–7.52 m (3H, C_6H_5), 7.79–7.84 m (2H, C_6H_5), 7.40 br.s (2H) and 8.34 br.s (2H, $N\underline{H}_2$), 9.98 s (1H, NH).

*2-(2-Benzoylhydrazono)-4-methyl-5,5-tetramethylene-2,5-dihydrofuran-3--carboxamide (**3c**)*. Yield 87%; white solid; m.p. 185–186ºC. IR (KBr), *v, cm*$^{-1}$: 3300 (NH_2), 3295 (NH), 1685 (C=O), 1645 (C=N), 1622 (C=C), 1500–1600 (C=$C_{arom.}$). ^{1}H NMR spectrum, *δ, ppm*: 1.42 m (2H) and 1.58–1.85 m (6H, $(CH_2)_4$), 2.33 s (3H, CH_3), 7.44–7.52 m (3H, C_6H_5), 7.80–7.84 m (2H, C_6H_5), 7.40 bs (2H) and 8.34 bs (2H, $N\underline{H}_2$), 9.98 s (1H, NH).

*2-(2-Benzoylhydrazono)-4-methyl-5,5-pentamethylene-2,5-dihydrofuran-3--carboxamide (**3d**)*. Yield 94%; white solid; m.p. 216–217ºC. IR (KBr), *v, cm*$^{-1}$: 3300 (NH_2), 3290 (NH), 1683 (C=O), 1645 (C=N), 1622 (C=C), 1500–1600 (C=$C_{arom.}$). ^{1}H NMR spectrum, *δ, ppm*: 1.27 m (1H), 1.47 m (2H) and 1.58–1.82 m (7H, $(CH_2)_5$), 2.33 s (3H, CH_3), 7.44–7.52 m (3H, C_6H_5), 7.80–7.84 m (2H, C_6H_5), 7.40 br.s (2H), and 8.34 br.s (2H, $N\underline{H}_2$), 9.98 s (1H, NH). ^{13}C NMR spectrum, *δ, ppm*: 12.10 (CH_3), 20.48 (CH_2), 20.91 (CH_2), 24.06 ($2CH_2$), 32.39 ($2CH_2$), 92.81 (C_5), 118.23 (C_3), 127.32 ($2C_{arom.}$), 127.68 ($2C_{arom.}$), 130.52 ($C_{arom.}$), 133.84 ($C_{arom.}$), 161.63 (C_2), 162.17 (C_4), 167.66 (C=O), 171.17 (C=O).

*2-(2-Benzoylhydrazono)-N,4,5,5-tetramethyl-2,5-dihydrofuran-3-carboxamide (**3e**)*. Yield 92%; white solid; m.p. 170–172ºC. IR (KBr), *v, cm*$^{-1}$: 3394 (NH_2), 3294 (NH), 1722 (C=O), 1683 (C=O), 1642 (C=N), 1625 (C=C), 1500–1600 (C=$C_{arom.}$). ^{1}H NMR spectrum, *δ, ppm*: 1.45 s (6H, $2CH_3$), 2.37 s (3H, CH_3), 2.82 d (*J* = 4.9 *Hz*, NCH_3), 7.44–7.52 m (3H) and 7.80–7.84 m (2H, C_6H_5), 9.76 q (*J* =4.8 *Hz*, 1H, NH).

*2-(2-Benzoylhydrazono)-N,4-dimethyl-5,5-pentamethylene-2,5-dihydrofuran--3-carboxamide (**3f**)*. Yield 96%; white solid; m.p. 186–187ºC. IR (KBr), *v, cm*$^{-1}$: 3300 (NH_2), 3290 (NH), 1683 (C=O), 1645 (C=N), 1622 (C=C), 1500–1600 (C=$C_{arom.}$). ^{1}H NMR spectrum, *δ, ppm*: 1.27 m (1H), 1.47 m (2H), 1.58–1.82 m (7H, $(CH_2)_5$), 2.35 s (3H, CH_3), 2.82 d (*J* = 4.9 *Hz*, NCH_3), 7.44–7.52 m (3H), 7.80–7.84 m (2H, C_6H_5), 9.76 q (*J* = 4.8 *Hz*, 1H, NH).

*2-(2-Benzoylhydrazono)-N-benzyl-4,5,5-trimethyl-2,5-dihydrofuran-3-carboxamide (**3g**)*. Yield 84%; white solid; m.p. 134–135ºC. IR (KBr), *v, cm*$^{-1}$: 3394 (NH_2), 3294 (NH), 1722 (C=O), 1683 (C=O), 1642 (C=N), 1625 (C=C), 1500–1600 (C=$C_{arom.}$). ^{1}HNMR spectrum, *δ, ppm*: 1.45 s (6H, $2CH_3$), 2.37 s (3H, CH_3), 4.50 d (2H, *J* = 5.6 *Hz*, $NHC\underline{H}_2$), 7.16–7.42 m (8H), 7.60–7.65 m (2H, $2C_6H_5$), 9.78 br.t (1H, *J* = 5.6 *Hz*, NH), 10.7 s (1H, NH).

Biological Part. Data were analyzed statistically using Student's and Fisher's tests. The antibacterial activities of the starting compounds **1**, **a**–**g** and the obtained compounds **3**, **a**–**g** were evaluated against Gram-positive (*Staphylococcus aureus* – 209p and 1) and Gram-negative (*Shigella Flexneri* 6858, *Esherichia coli* 0–55) bacteria by the agar diffusion technique with microbial loading $20 \cdot 10^6$ microbes per *mL* of medium. Solutions of the tested compounds were prepared in DMSO at a 1 : 20 concentration and poured (0.1 *mL*) into cylinders placed on the surface of an agar medium inoculated with test strain in Petri dishes. The results

were evaluated from the diameter (*d*, *mm*) of the microbe growth inhibition zone at the compound application site after growth for 20 *h* at 37°*C*. The tests were repeated three times. The zones of inhibition were measured in millimetre to estimate the potency of the test compound.

Conclusion. New 2-*N*-substituted 2,5-dihydrofurans were successfully synthesized by the convenient and efficient method, which was based on the reaction of 2-imino-2,5-dihydrofurans with benzohydrazide in glacial acetic acid. Synthesized compounds exhibited moderate to defined antibacterial activities against Gram-positive and Gram-negative bacteria compared to furazolidone.

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