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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 8-BENZYLIDENEHYDRAZINO-3-METHYL-7-β-METHOXYETHYLXANTHINES

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A simple preparative synthesis of 8-hydrazino-3-methyl-7-β-methoxyethylxanthine was developed. Reaction of the obtained hydrazinoxanthine with aldehydes and ketones produced several previously undescribed 8-ylidenehydrazino derivatives of 3-methyl-7-β-methoxyethylxanthine. PMR spectroscopy confirmed the structures of the synthesized compounds. Biological tests established that several of the synthesized compounds had pronounced antimicrobial, antifungal, and antioxidant activity and could be recommended for further research.

Keywords: xanthine, synthesis, antioxidant activity, antimicrobial activity, antifungal activity.

The present work continues our research on the discovery of biologically active compounds among N-substituted and condensed xanthine derivatives [1 – 4]. The unique biological effects of substituted xanthines consist primarily of their roles as agonists or antagonists of purine receptors [5 – 9]. Therefore, xanthine derivatives can have a regulatory influence on any function of the human body.

Thus, we synthesized previously undescribed 8-benzylidenehydrazino derivatives of 3-methyl-7-β-methoxyethylxanthine (**III-XV**) (Scheme 1) and studied their antioxidant activity (AOA) and their antimicrobial and antifungal effects.

Scheme 1 shows that brief refluxing of 8-bromo-3-methyl-7-β-methoxyethylxanthine (**I**) [4] with an excess of hydrazine hydrate in aqueous dioxane formed 8-hydrazino-3-methyl-7-β-methoxyethylxanthine (**II**). The corresponding 8-benzylidenehydrazinoxanthines **III-XV** were prepared by reacting **II** and aldehydes or *p*-methylacetophenone in aqueous 2-PrOH in the presence of a catalytic amount of HCl for 5 – 10 min.

The structures of the synthesized compounds were confirmed unambiguously using PMR spectroscopy. The presence of the uracil was confirmed by two singlets of the appropriate intensities in the range 10.82 – 10.49 ppm (N_1H) and 3.28 – 3.19 ppm (N_3CH_3) (Table 1). The methylene protons of the 7-methoxyethyl substituent appeared as two triplets in the range 4.74 – 4.46 ppm (N_7CH_2) and 3.68 – 3.65 ppm (OCH_2); the methyl protons, as a strong singlet at 3.34 – 3.32 ppm. It is noteworthy that the methoxyethyl protons of starting **II** were shifted to weak field (Table 1). The presence of the 8-arylmethylidenehydrazine group was confirmed by strong resonances at weak field of 11.84 – 10.22 ppm (C_8NH) and 8.50 – 7.93 ppm (N=CH). Protons of the aromatic rings and substituents were found in the appropriate range with the appropriate intensity and shape, which confirmed their structures.

EXPERIMENTAL CHEMICAL PART

Melting points were determined in open capillaries on a PTP instrument. PMR spectra were recorded from DMSO-d_6 or DMSO-d_6+$CDCl_3$ solutions with TMS internal standard on a Bruker SF-400 spectrometer. Elemental analyses were performed on an Elementar Vario L Cube instrument. Elemental analyses agreed with those calculated.

8-Hydrazinyl-3-methyl-7-(2-methoxyethyl)-3,7-dihydro-1*H*-purine-2,6-dione (II). A mixture of bromoxanthine **I** (3.0 g, 0.01 mol) [4], hydrazine hydrate (5 mL, 0.1 mol), H_2O (30 mL), and dioxane (10 mL) was refluxed for 1 h and

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Scheme

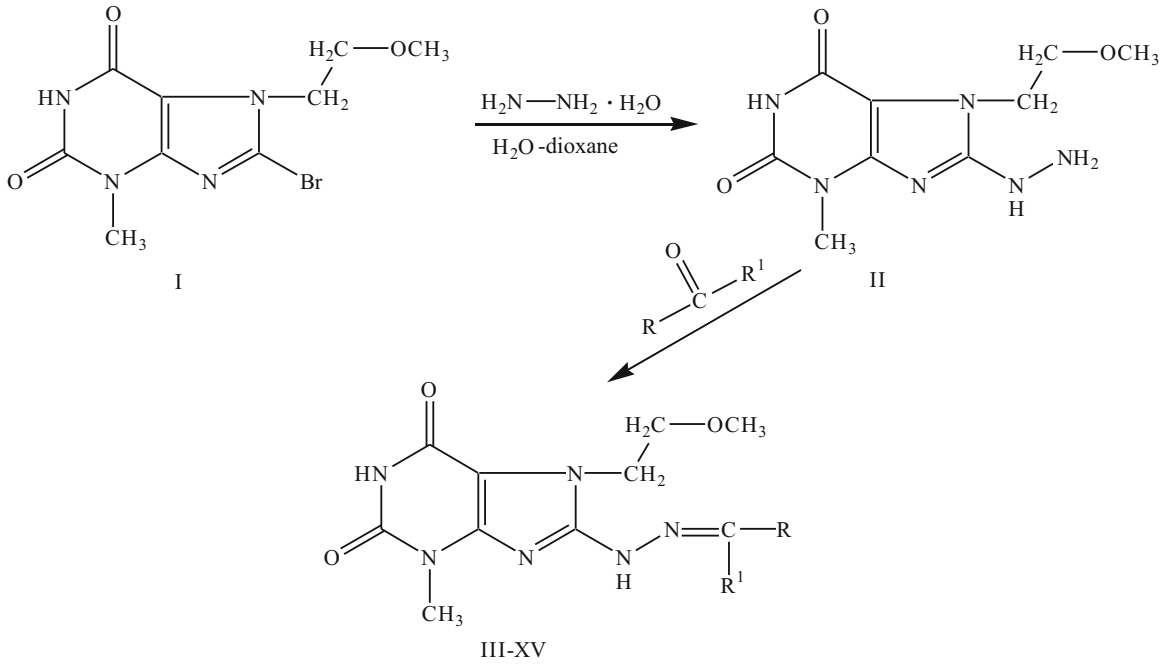


TABLE 1. PMR Spectra of Synthesized Compounds

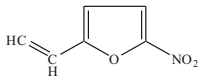
Compound	R	R^1	δ-scale, ppm								
			N_1H (c, 1H)	C_8NH (c, 1H)	N=CH (c, 1H)	CH arom.	N_7CH_2 (t, 2H)	OCH_2 (t, 2H)	OCH_3 (c, 3H)	N_3CH_3 (c, 3H)	Other resonances
II			10.64	7.99	-	-	4.15	3.54	3.30	3.19	4.34 (br. s, 2H)-NH_2
III	H	C_6H_5	10.78	11.47	8.09	7.64 (d, 2H), 7.41 (m, 3H)	4.61	3.67	3.33	3.26	-
IV	H	$C_6H_4CH_3$-*n*	10.76	11.38	8.05	7.52 (d, 2H), 7.23 (d, 2H)	4.60	3.67	3.32	3.26	2.34 (s, 3H)-CH3-*n*
V	H	$C_6H_4OCH_3$-*m*	10.77	11.51	8.04	7.32 (t, 1H), 7.19 (d, 2H), 6.93 (d, 1H)	4.62	3.68	3.80, 3.32	3.26	-
VI	H	$C_6H_4OCH_3$-*n*	10.75	11.31	8.03	7.58 (d, 2H), 6.98 (d, 2H)	4.60	3.67	3.80, 3.32	3.26	-
VII	H	$C_6H_4OC_2H_5$-*n*	10.75	11.30	8.02	7.57 (d, 2H), 6.96 (d, 2H)	4.59	3.67	3.32	3.26	4.06 (q, 2H)-OCH_2, 1.35 (t, 3H)-CCH_3
VIII	H	C_6H_4Cl-*o*	10.81	11.67	8.50	7.93 (m, 1H), 7.49 (m, 1H), 7.39 (m, 2H)	4.56	3.67	3.33	3.25	-
IX	H	C_6H_4OH-*o*	10.78	11.36	8.38	7.53 (d, 1H), 7.22 (t, 1H), 6.88 (m, 2H)	4.46	3.65	3.34	3.25	10.67 (s, 1H)-OH
X	H	$C_6H_4N(CH_3)_2$-*n*	10.69	11.14	7.94	7.45 (d, 2H), 6.72 (d, 2H)	4.61	3.67	3.32	3.26	2.98 (s, 6H)-$N(CH_3)_2$
XI	H	C_6H_3OH(-*n*)OCH_3(-*m*)	10.72	11.29	7.95	7.21 (s, 1H), 7.0 (d, 1H), 6.79 (d, 1H)	4.64	3.68	3.82, 3.32	3.26	9.35 (s, 1H)-OH
XII	CH_3	$C_6H_4CH_3$-*n*	10.80	10.22	-	7.63 (d, 2H), 7.21 (d, 2H)	4.61	3.67	3.34	3.25	2.34 (s, 3H)-CH_3-*n*, 2.29 (s, 3H)-CCH_3
XIII	H	C(Br)=CH-C_6H_5	10.77	11.84	7.93	7.83 (d, 2H), 7.42 (m, 3H)	4.74	3.66	3.32	3.24	7.54 (s, 1H)-ÑHPh
XIV	H	Pyridyl-3	10.82	11.83	8.22	8.90 (s, 1H), 8.64 (d, 1H), 8.27 (d, 1H), 7.65 (t, 1H)	4.59	3.66	3.33	3.25	-
XV	H		10.49	11.38	7,97 (d, 1H)	7.64 (d, 1H), 7,01 (d, 1H)	4.55	3.67	3.34	3.28	7.008 (m, 1H), 6.90 (d, 1H)

TABLE 2. Yields and Melting Points of Synthesized Compounds

Compound	Yield, %	mp, °C	Empirical formula
II	89.4	245 – 247	$C_9H_{14}N_6O_3$
III	93.5	261 – 263	$C_{16}H_{18}N_6O_3$
IV	87.6	266 – 267	$C_{17}H_{20}N_6O_3$
V	88.6	253 – 254	$C_{17}H_{20}N_6O_4$
VI	94.0	266 – 267	$C_{17}H_{20}N_6O_4$
VII	83.3	265 – 266	$C_{18}H_{22}N_6O_4$
VIII	96.6	257 – 258	$C_{16}H_{17}ClN_6O_3$
IX	97.7	257 – 258	$C_{16}H_{18}N_6O_4$
X	67.5	259 – 260	$C_{18}H_{23}N_7O_3$
XI	87.5	246 – 247	$C_{17}H_{20}N_6O_5$
XII	92.9	250 – 252	$C_{18}H_{22}N_6O_3$
XIII	95.2	235 – 237	$C_{18}H_{19}BrN_6O_3$
XIV	41.9	246 – 247	$C_{15}H_{17}N_7O_3$
XV	96.2	248 – 249	$C_{16}H_{17}N_7O_6$

cooled. The precipitate was filtered off, washed with H_2O, and crystallized from aqueous dioxane (Table 2).

Benzaldehyde [7-(2-methoxyethyl)-3-methyl-2,6-dioxo-2,3,4,5,6,7-hexahydro-1*H*-purin-8-yl]hydrazone (III). A solution of **II** (1.27 g, 5 mmol) in H_2O (20 mL) and 2-PrOH (20 mL) with conc. HCl (five drops) was heated to 50 – 60°C, treated with benzaldehyde (0.61 mL, 6 mmol), refluxed for 15 min, and cooled. The precipitate was filtered off, washed with H_2O, 2-PrOH, and Et_2O, and crystallized from aqueous dioxane (Table 2).

Hydrazones **IV**-**XV** were prepared analogously.

EXPERIMENTAL BIOLOGICAL PART

Antimicrobial and antifungal activity was studied using standard test strains of microorganisms that were obtained from the Bacteriological Laboratory of Zaporozhye Regional Laboratory Center of the State Health Service of Ukraine. We used *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 885-653). Antimicrobial and antifungal activities were determined using a double serial dilution method in liquid growth medium. Bacteria were cultivated using Mueller Hinton broth and agar. Fungi were grown on Sabouraud medium. Activities of compounds were estimated from the minimum inhibiting concentration (MIC) and minimum bactericidal concentration (MBC). Observed effects were compared with the literature for reference drugs ampicillin, faringosept (ambazone monohydrate), and nystatin [10].

AOA was studied *in vitro* using non-enzyme-initiated free-radical oxidation. The substrate was a suspension of egg lipoproteins (SEL) [11] that was prepared by homogenization of egg yolk in phosphate buffer (pH 7.4). Alcohol solutions of the test compounds at concentrations 10^{-3}, 10^{-5}, and 10^{-7} M were added to the resulting suspension using a pipette (Biohit Proline). The free-radical oxidation was initiated by adding $FeSO_4 \cdot 7H_2O$ solution (0.025 M) followed by incubation of the mixture for 60 min at 37°C. The reaction was stopped by trichloroacetic acid solution (25%) with Trilon B (EDTA). The supernatant after centrifugation for 30 min was added to thiobarbituric acid (TBA) solution (25%) and heated on a water bath for 60 min. The colored complex of

TABLE 3. Antimicrobial, Antifungal, and Antioxidant Activity of Synthesized Compounds

Compound	*E. coli*		*S. aureus*		*P. aeruginosa*		*C. albicans*		AOA *, %		
	MIC, μg/mL	MBC, μg/mL	MIC, μg/mL	MBC, μg/mL	MIC, μg/mL	MBC, μg/mL	MIC, μg/mL	MBC, μg/mL	$C = 10^{-3}$, M	$C = 10^{-5}$, M	$C = 10^{-7}$, M
III	100	100	50	100	100	200	100	200	57.59	10.34	0.69
IV	200	200	100	200	100	400	100	100	78.46	50.0	48.08
V	100	200	12.5	25	100	200	100	200	67.01	11.68	27.41
VI	100	200	12.5	50	100	200	100	200	52.76	15.86	9.66
VII	100	200	100	200	100	400	100	100	77.3	51.15	53.85
VIII	200	200	100	200	100	100	100	200	92.39	31.47	28.93
IX	100	100	100	100	100	200	100	100	81.38	11.72	4.14
X	200	200	25	50	100	400	100	100	61.15	36.92	35.15
XI	100	200	100	100	100	400	100	100	72.4	34.4	34.4
XII	100	100	200	200	100	400	100	100	78.46	28.25	30.77
XIII	100	200	25	100	50	400	50	100	84.4	50.4	40.4
XIV	200	200	50	50	100	200	100	100	44.16	24.87	25.38
XV	100	200	12.5	25	100	400	50	100	50.8	40.8	33.6
Ascorbic acid	-	-	-	-	-	-	-	-	65.31	39.13	43.59
Dibunol	-	-	-	-	-	-	-	-	25.2	-	-

* AOA results statistically significant for all compounds for $p \le 0.05$ compared with the control.

malondialdehyde with TBA was extracted by adding *n*-BuOH. The concentration of malondialdehyde was determined using spectrophotometry and indicated the extent of the free-radical oxidation. AOA (in percent) was determined using the formula:

$$\text{AOA} = (C_{K1} - C_0/C_{K1} - C_{K2}) \cdot 100\ \%,$$

where C_{K1} and C_{K2} are the contents of TBA in control samples (M); C_0, the contents of TBA in the test sample (M).

The reference standards were drug substances of the known antioxidants [10] ascorbic acid (Asfarma, Russia) and dibunol (Research Institute for Drug Standardization and Control, Russia).

The results were processed by known variational statistics methods using the Student *t* criterion, Windows-2000 software, Excel electronic spreadsheets, and the Mathcad-5.0 program [12].

Table 3 shows that the synthesized compounds in general exhibited moderate antimicrobial and antifungal activity against the studied strains. However, several compounds (**V**, **VI**, **X**, **XIII**-**XV**) were highly selective against *S. aureus*. Their MIC values were 12.5 – 50 μg/mL; MBC, 25 – 100 μg/mL. According to the literature [13], the MBC values of ampicillin and ambazone monohydrate (faringosept) against *S. aureus* were 62.5 μg/mL. Hydrazones **XIII** and **XV**, which contained an α-bromocinnamic aldehyde and 5-nitrofuryl-2-acrolein, were the most active against *C. albicans*. The MIC of the antifungal drug nystatin [13] was 62.5 μg/mL. It is noteworthy that hydrazines **V** and **VI**, which contained a methoxy in the *p*- and *m*-positions of the benzene ring, in addition to hydrazone **XV**, which contained a 5-nitrofuran, had the highest bacteriostatic activity. This was fully reasonable considering the high antimicrobial activity of its derivatives.

An analysis of the AOA results for the synthesized compounds (Table 3) showed that most of them exhibited high AOA. It was found that the AOA decreased or practically disappeared (**III**, **VI**, **IX**) as the concentration decreased. High AOA was retained for most synthesized compounds even at a concentration of 10^{-7} M. The most active compounds were the *p*-methylbenzylidene (**IV**) and *p*-ethoxybenzylidene (**VII**) derivatives. In our opinion, they are extremely promising for further biological testing.

Several of the synthesized compounds could be regarded as potential candidates for designing new chemotherapeutic (**V**, **VI**, **X**, **XIII**, **XV**) and antioxidant (**IV**, **VI**) drugs.

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