ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis And Biological Activity Of 8-Aminosubstituted 7-(2-Hydroxy-3-M-Ethylphenoxypropyl-1)-3-Methylxanthine.

Dmytro Ivanchenko*, Mykola Romanenko, Oleksandr Goloborodko, Oleksandr Kamyshnyi, and Natalia Polishchuk.

Zaporizhzhia state medical university, Maiakovskyi avenue 26, Zaporizhzhia, 69035, Ukraine.

ABSTRACT

The reaction of 8-bromo-3-methylxanthine with m-ethylphenoxymethyloxirane in Propanol-1 medium in the presence of N, N-dimethylbenzylamine leads to formation of the initial 8-bromo-7-(2-hydroxy-3-m-ethylphenoxypropyl-1)-3-methylxanthine. Interactions of the 8-bromo-7-(2-hydroxy-3-m-ethylphenoxypropyl-1)-3-methylxanthine with primary alkyl, oxyalkyl, aminoalkylamines and some dialkyl amines were studied. Structure of synthesized compounds was definitely proved by ^{1}H NMR-spectroscopy. Conducting primary screening research of antimicrobial activity of 8-aminosubstituted 7-(2-hydroxy-3-m-ethylphenoxypropyl-1)-3-methylxanthine, which revealed moderate and weak activity in concentrations 50-200 mcg/ml..

Keywords: xanthine, organic synthesis, ^{1}H NMR-spectroscopy, antibacterial, antifungal agents.

https://doi.org/10.33887/rjpbcs/2019.10.3.50

Corresponding author

ISSN: 0975-8585

INTRODUCTION

It is known that various N- and C_8-substituted xanthine derivatives exhibit antimicrobial and antifungal activity [1-3]. Our earlier researches [4-7] on the search for low-toxic compounds of this action among xanthine derivatives indicates the feasibility and significant prospect of further research.

The aim of this work is development of methods for the synthesis of new 8-alkyl-, aminoalkyl-, oxyalkylaminosubstituted 7-(2-hydroxy-3-m-ethyl-phenoxypropyl-1)-3-methylxanthine and studying of the antimicrobial and antifungal activity of the synthesized compounds.

RESULTS OF THE RESEARCH AND THEIR DISCUSSION

As shown in the scheme 1, the initial 8-bromo-7-(2-hydroxy-3-m-ethylphenoxypropyl-1)-3-methylxanthine (2) was obtained by heating 8-bromo-3-methylxanthine (1) with m-ethylphenoxymethyloxirane in propanol-1 medium in the presence of N, N-dimethylbenzylamine as a catalyst. On the basis of bromoxanthine 2, we have studied its reactions with primary alkyl, oxyalkyl, aminoalkylamines and some dialkyl amines. It was found that optimum conditions of the reaction are short-term heating (1 hour) of synthones in aqueous dioxane medium, which made it possible to obtain 8-aminoxanthine 3-19 with a high yield. The structure of the synthesized compounds is unambiguously proved by the data of the NMR spectroscopy (Table 1). In the spectrum of the output bromoxanthine 2 in a weak field (11.22 ppm) a one-proton singlet of the NH group of the uracil part of the moleecule is recorded. The singlet of the protons of the N^3CH_3 group is recorded at 3.31 ppm (3H). The presence of the substituent in position 7 is confirmed by a triplet and multiplet at 7.16 ppm (1H) and 6.72 ppm (3H), which are caused by the resonance of the protons of the aromatic nucleus. One-proton doublet of OH-groups is observed at 5.43 ppm. Signals of methylene and methine protons of the N^7-propyl residue are recorded in the form of multiplets in the range of 4.55-4.15 ppm (3H) and 3.98 ppm (2H). The multiplicity of signals unambiguously indicates the presence of a chiral Carbon atom in the position 2 of the propyl residue. In the spectrum, classical quartets at 2.55 ppm (2H) and triplet at 1.14 ppm (3H) are also observed due to the presence of an ethyl group in the aromatic nucleus. In the spectra of amino substituted 3-16, resonance absorption of the proton of NH-group in position 8 is fixed in the absorption region of aromatic protons at 6.85-6.63 ppm in addition to compounds 5 and 9, in the spectra of which protons of NH groups are recorded as doublets at 6.50 ppm. (1H) and 6.49 ppm (1H). In the spectra of all received xanthine signals of proton substituents, connected with the Nitrogen atom at the position 8, are registered in the corresponding field, of the corresponding form and intensity. It should be emphasized separately that in the spectrum of 8-N-methyl-N-benzylamine derivative, 19 protons of a methylene group, instead of forming a singlet, form two one-proton doublets at 4.63 ppm and 4.47 ppm, which is connected with the formation of the second chiral center – the Nitrogen at position 8.

According to the results of the study of the antimicrobial activity of the synthesized compounds, 8-aminoxanthine 2-19 does not significantly affect the growth and reproduction of *E. coli*, *S. aureus* and *P. aeruginosa* bacteria (their MIC and MBC is 100-200 μg / ml). The synthesized compounds also showed weak antifungal activity in relation to *C. albicans*. The exception is 8-n-hexylamino(8)- and 8-cyclohexylamino(9)xanthine, with a minimum inhibitory concentration and a minimum fungicidal concentration of 50 μg / ml. That is, these compounds are not inferior to the comparison benchmark – ketoconazole – by the MFC index (MIC – 25 μg / ml, MFC – 50 μg / ml) and require more in-depth study.

Of course, it is too early to make final conclusions, as it is necessary to significantly expand the range of 8-aminocanthians and the library of pathogenic strains of microorganisms, but the prospect of research on the search for new antifungal agents is doubtless.

THE EXPERIMENTAL PART

The melting temperature was determined by the open capillary method with PTP-M device. Elemental analysis was performed on the Elementar Vario L cube device, NMR spectra were taken on a Bruker SF-400 spectrometer (400 MHz operating frequency, DMSO solvent, TMS internal standard). Elemental analysis data is in line with the calculated ones.

Analytical data of synthesized compounds are given in tables 1, 2.

ISSN: 0975-8585

Table 1: The values of the chemical shift in NMR-spectra of synthesized compounds (2-19)

Compound	δ-scale, ppm						
	N^1H (s, 1H)	CH_{arom}	OH (d, 1H)	$N^7CH_2CHCH_2$	N^3CH_3 (s, 3H)	ArC_2H_5	Other signals
2	11.22	7.16 (t, 1H); 6.72 (m, 3H)	5.43	4.55-4.15 (m, 3H); 3.98 (m, 2H)	3.31	2.55 (q, 2H); 1.14 (t, 3H)	–
3	10.57	7.13 (t, 1H); 6.70 (m, 4H)+ C^8NH	5.44	4.23-3.82 (m, 5H)	3.29 (m, 5H)+ NCH_2	2.54 (q, 2H); 1.13 (m, 6H)	–
4	10.56	7.16 (t, 1H); 6.70 (m, 4H)+ C^8NH	5.48	4.21-3.82 (m, 5H)	3.30 (m, 5H)+ NCH_2	2.55 (q, 2H); 1.14 (t, 3H)	1.54 (m, 2H) – CCH_2; 0.88 (t, 3H) – CCH_3
5	10.60	7.17 (t, 1H); 6.77 (d, 1H); 6.68 (m, 2H)	5.55	4.23-3.75 (m, 6H)+ NCH	3.30	2.56 (q, 2H); 1.15 (m, 6H)	6.50 (d, 1H) – C^8NH; 1.52 (m, 2H) – CCH_2; 0.87 (q, 3H) – CCH_3
6	10.54	7.18 (t, 1H); 6.73 (m, 4H)+ C^8NH	5.47	4.25-3.85 (m, 5H)	3.29 (m, 5H)+ NCH_2	2.56 (q, 2H); 1.16 (t, 3H)	1.53 (m, 2H) – CCH_2; 1.30 (m, 4H) – CCH_2; 0.87 (t, 3H) – CCH_3
7	10.59	7.17 (t, 1H); 6.80-6.66 (m, 4H)+ C^8NH	5.49	4.23-3.83 (m, 5H)	3.30 (m, 5H)+ NCH_2	2.56 (q, 2H); 1.17 (t, 3H)	1.62 (m, 1H) – CCH; 1.39 (m, 2H) – CCH_2; 0.87 (d, 6H) – CCH_3
8	10.48	7.16 (t, 1H); 6.78-6.68 (m, 4H)+ C^8NH	5.44	4.22-3.85 (m, 5H)	3.30 (m, 5H)+ NCH_2	2.56 (q, 2H); 1.17 (t, 3H)	1.53 (m, 2H) – CCH_2; 1.28 (m, 6H) – CCH_2; 0.86 (t, 3H) – CCH_3
9	10.58	7.17 (t, 1H); 6.78-6.68 (m, 3H)	5.57	4.23-3.80 (m, 5H)	3.29	2.56 (q, 2H); 1.17 (t, 3H)	6.49 (d, 1H) – C^8NH; 3.64 (m, 1H) – NCH; 1.95-1.52 (m, 5H) – CCH_2; 1.40-1.08 (m, 5H) – CCH_2
10	10.58	7.48 (m, 6H); 6.77 (d, 1H); 6.68 (m, 2H)	5.45	4.23-3.82 (m, 5H)	3.31	2.55 (q, 2H); 1.16 (t, 3H)	3.50 (q, 2H) – NCH_2; 2.84 (t, 2H) – CCH_2
11	10.57	7.17 (t, 1H); 6.80-6.70 (m, 4H)+ C^8NH	5.48; 4.66 (t, 1H)	4.21-3.88 (m, 5H)	3.30	2.57 (q, 2H); 1.17 (t, 3H)	3.55 (q, 2H) – NCH_2; 3.39 (t, 2H) – CCH_2
12	10.65	7.18 (t, 1H); 6.85-6.65 (m, 4H)+ C^8NH	5.51; 4.50 (t, 1H)	4.22-3.85 (m, 5H)	3.29	2.55 (q, 2H); 1.16 (t, 3H)	3.45 (q, 2H) – NCH_2; 3.33 (m, 2H) – OCH_2; 1.69 (m, 2H) – CCH_2
13	10.64	7.18 (t, 1H); 6.85-6.67 (m, 4H)+ C^8NH	5.60 (t, 1H); 4.75 (m, 1H)	4.22-3.77 (m, 6H)+ CHO	3.29	2.55 (q, 2H); 1.16 (t, 3H)	3.35 (m, 2H) – NCH_2; 1.07 (d, 3H) – CCH_3
14	10.62	7.17 (t, 1H); 6.85-6.68 (m, 4H)+ C^8NH	5.48	4.22-3.85 (m, 5H)	3.29	2.54 (q, 2H); 1.16 (t, 3H)	3.35 (m, 4H) – CH_2; 1.77 (m, 2H) – CCH_3
15	10.64	7.15 (t, 1H); 6.85-6.65 (m, 4H)+ C^8NH	5.53	4.22-3.85 (m, 5H)	3.30	2.54 (q, 2H); 1.15 (t, 3H)	3.50 (m, 1H) – CH; 3.35 (m, 4H) – CH_2; 1.07 (d, 6H) – CCH_3
16	10.53	7.16 (t, 1H); 6.80-6.63 (m, 4H)+ C^8NH	5.49	4.22-3.83 (m, 5H)	3.30 (m, 5H)+ NCH_2	2.60-2.40 (m, 8H); 1.17 (t, 3H)	0.97 (t, 6H) – CCH_3
17	10.80	7.17 (t, 1H); 6.83-6.67 (m, 3H)	5.42	4.34-4.17 (m, 3H); 3.87 (m, 2H)	3.31	2.54 (q, 2H); 1.17 (t, 3H)	2.95 (s, 6H) – NCH_3
18	10.84	7.16 (t, 1H); 6.80-6.65 (m, 3H)	5.42	4.34 (m, 1H); 4.17 (m, 2H); 3.87 (m, 2H)	3.30 (m, 7H)+ $N(CH_2)_2$	2.53 (q, 2H); 1.17 (t, 3H)	1.09 (t, 6H) – CCH_3
19	10.84	7.36 (m, 5H); 7.17 (t, 1H); 6.78-6.63 (m, 3H)	5.49	4.35 (m, 1H); 4.25 (m, 2H); 3.85 (m, 2H)	3.31	2.53 (q, 2H); 1.15 (t, 3H)	4.63 (d, 1H) – NCH_2; 4.47 (d, 1H) – NCH_2; 2.88 (s, 3H) – NCH_3

ISSN: 0975-8585

Scheme 1

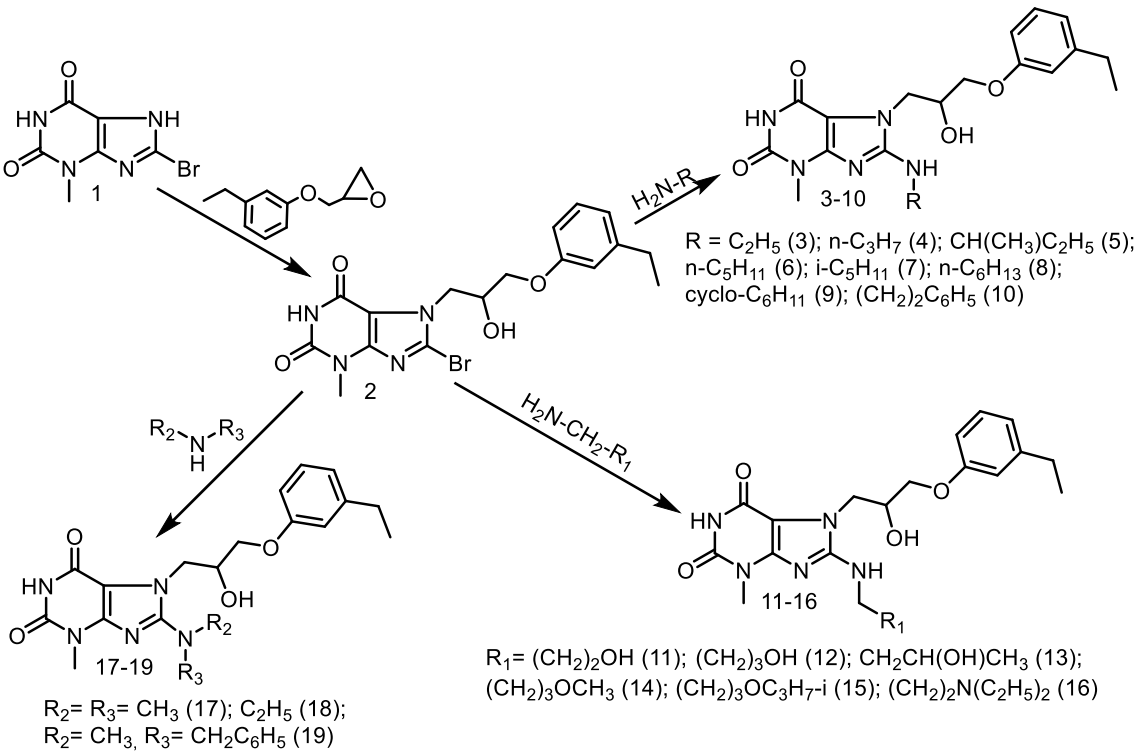

Table 2: The physicochemical characteristics of synthesized compounds (2-19)

Compound	m.p., °C	The empirical formula	Yield, %
2	163-164	$C_{17}H_{19}BrN_4O_4$	59.1
3	216-217	$C_{19}H_{25}N_5O_4$	77.5
4	207-208	$C_{20}H_{27}N_5O_4$	75.0
5	186-187	$C_{21}H_{29}N_5O_4$	82.8
6	196-197	$C_{22}H_{31}N_5O_4$	86.7
7	219-220	$C_{22}H_{31}N_5O_4$	88.3
8	181-182	$C_{23}H_{33}N_5O_4$	90.9
9	215-216	$C_{23}H_{31}N_5O_4$	84.8
10	203-204	$C_{25}H_{29}N_5O_4$	83.6
11	163-164	$C_{17}H_{25}N_5O_5$	85.7
12	218-219	$C_{18}H_{27}N_5O_5$	86.2

ISSN: 0975-8585

13	162-163	$C_{18}H_{27}N_5O_5$	75.9
14	165-166	$C_{21}H_{31}N_5O_5$	70.0
15	173-174	$C_{23}H_{35}N_5O_5$	90.6
16	166-167	$C_{23}H_{34}N_6O_4$	85.7
17	163-164	$C_{19}H_{25}N_5O_4$	53.0
18	120-121	$C_{21}H_{29}N_5O_4$	73.9
19	169-170	$C_{25}H_{29}N_5O_4$	87.5

Table 3: Antibacterial and antifungal activity of synthesized substances

Compound	E. coli		S. aureus		P. aeruginosa		C. albicans	
	MIC, µg / ml	MBC, µg / ml	MIC, µg / ml	MBC, µg / ml	MIC, µg / ml	MBC, µg / ml	MIC, µg / ml	MFC, µg / ml
3	100	200	100	200	100	200	100	200
4	100	200	100	200	100	200	100	200
5	200	200	100	200	100	200	100	200
6	100	200	100	200	100	200	100	100
7	100	200	100	200	100	200	100	100
8	100	200	50	100	100	200	50	50
9	100	200	100	200	100	200	50	50
10	100	100	100	200	100	200	100	200
11	200	200	100	200	100	200	100	100
12	100	200	100	200	100	200	100	100
13	200	200	100	200	100	200	100	200
14	100	200	100	200	100	200	100	200
15	100	200	100	200	100	200	100	100
16	100	200	100	200	100	200	100	200
17	200	200	100	200	100	200	100	200
18	200	200	100	200	100	200	200	200
19	100	200	50	200	100	200	100	100
Furacin	12,5	12,5	6,25	50	50	800	–	–
Ketoconazole	–	–	–	–	–	–	25	50

Synthesis of 8-brom-3-methyl-7-(2-hydroxy-3-m-ethylphenoxypropyl-1-) xanthine (2). A mixture of 49 g (0.2 mole) of 8-bromo-3-methylxanthine (1) [8], 39.2 g (0.22 molees) of m-ethylphenoxymethyloxirane, 300 ml of propanol-1, and 2 ml of N,N-dimethylbenzylamine are boiled for 3 hours and filtered in a hot phase, the filtrate is cooled down and 50 ml of water and 5 ml of NH_4OH are added. A day later, the precipitate is filtered off, washed with water and propanol-2, and dried. Then it is crystallized from aqueous dimethylformamide.

Synthesis of 8-amino substituted 3-methyl-7-(2-hydroxy-3-m-ethylphenoxypropyl-1-xanthine) (3-19). A solution of 4.23 g (0.01 mole) of bromoxanthine 2, 0.03 mole of the corresponding amine (for the synthesis of

compounds 3, 20 and 21, 0.1 mole of amine solution is taken), 20 ml of water and 25 ml of dioxane are boiled for 1 hour, cooled, 30 ml of water are added, the precipitate is filtered off and washed with water and crystallized from aqueous propanol-2.

The antimicrobial and antifungal activity was carried out on standard *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 885-653 strains obtained in bacteriological laboratory of Zaporizhia Regional Laboratory Center of the State Epidemiological Service of Ukraine.

Basic solution was prepared by dissolving 1 mg of investigated substance in 1 mL of DMSO. The 4 mL of Muller-Hinton broth [9] (for 25923 *S. aureus* ATCC, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853) or Sabouraud broth [9] (for *C. albicans* ATCC 885-653) were added to basic solution to obtain the dilution with the concentration of substance 200 μg / mL. After series of two-fold dilutions, three additional solutions were prepared with concentrations 100, 50, 25 μg / mL respectively, each in volume of 1 ml. The suspension of microbial culture on amount of 0.1 mL was added into each tube. The test tubes with crops of *S. aureus*, *E. coli*, *P. aeruginosa* were incubated at 37±1 °C for 16-24 hours, with crops of *C. albicans* – at 28±1 °C for 44-48 hours. MIC was determined on visual absence of bacterial growth in the test tube with minimal concentration of substance investigated [10, 11].

For determination of MBC and MFC, the content of test tube after MIC determination with no bacterial growth was put into 0.1 mL of Muller-Hinton agar [9] (for *S. aureus*, *E. coli*, *P. aeruginosa*) or into 0.1 ml of Sabouraud agar [9] (for *C. albicans*); cups were incubated at 37±1 °C for 16-24 hours and at 28±1 °C for 44-48 hours respectively. Values of MBC and MFC were detected by visual absence of bacterial growth of cultures.

Furacilinum and ketoconazole were used as comparison benchmarks. The results of the study of antimicrobial and antifungal activity are shown in Table 3.

REFERENCES

[1] Hayallah A. M. Design and synthesis of some new theophylline derivatives with bronchodilator and antibacterial activities / A. M. Hayallah, W. A. Elgaher, O. I. Salem, A. A. M. A. Alim // Arch. Pharm. Res. – 2011. – Vol. 34, № 1. – P. 3-21.

[2] Hayallah A. M. Design and synthesis of new 8-anilide theophylline derivatives as bronchodilators and antibacterial agents / A. M. Hayallah, A. A. Talhouni, A. A. M. A. Alim // Arch. Pharm. Res. – 2012. – Vol. 35, № 8. – P. 1355-1368.

[3] Design, synthesis, and antimycobacterial activity of novel theophylline-7-acetic acid derivatives with amino acid moieties / G. Stavrakov, V. Valcheva, Y. Voynikov, I. Philipova, M. Atanasova, S. Konstantinov, P. Peikov, I. Doytchinova // Chem. Biol. Drug. Des. – 2016. – Vol. 87, № 3. – P. 335-341.

[4] The synthesis, physicochemical and biological properties of 8-amino-7-(2-hydroxy-2-phenylethyl)-3-methylxanthines / Romanenko M.I., Ivanchenko D.G., Nazarenko M.V., Diachkov M.V., Kamyshny O.M., Polishchuk N.M. // News of Pharmacy. – 2016. – № 3(87). – C. 17-21.

[5] Synthesis, physical-chemical and biological properties of 7-benzyl-3-methyl-8-thioxanthine derivatives / D. H. Ivanchenko, M. I. Romanenko, O. M. Kamyshnyi, N. M. Polishchuk, K. V. Aleksandrova // Current issues in pharmacy and medicine: science and practice. – 2017. – Vol. 10, №3 (25). – P. 272-277.

[6] Synthesis, physical-chemical and biological properties of derivatives of 3-methyl-7-ethylxanthinyl-8-thioacetic acid hydrazide / M. I. Romanenko, O. P. Dolhikh, D. H. Ivanchenko, K. V. Aleksandrova, N. M. Polishchuk // Current issues in pharmacy and medicine: science and practice. – 2018. – Vol. 11, №3 (28). – P. 264-269.

[7] Synthesis, physical, chemical and biological properties of 7-ethylxanthinyl-8-aminoalkanoic acids' derivatives / O. P. Dolhikh, M. I. Romanenko, D. H. Ivanchenko, K. V. Aleksandrova, N. M. Polishchuk // Current issues in pharmacy and medicine: science and practice. – 2019. – Vol. 12, №1 (29). – P. 10-14.

[8] Preparation of 3-methyl-8-bromoxanthine and its alkylation / B. A. Priimenko, M. I. Romanenko, S. N. Harmash, N. A. Kliuev, I. V. Fedulova, N. I. Hnatov, N. V. Koval // Ukranian chemistry journal. – 1985. – Vol. 51, №6. – P. 660-663.

[9] Atlas R.M. Handbook of Microbiological Media. – 4th Edition. – London: CRC Press, 2010. – 2040 p.

ISSN: 0975-8585

[10] Multicomponent hot-water synthesis of 7-unsubstituted 4,7-dihydro-1,2,4-triazolo[1,5-a]pyrimidines and their antimicrobial and antifungal activity / S. A. Komykhov, I. G. Tkachenko, V. I. Musatov, M. V. Diachkov, V. A. Chebanov, S. M. Desenko // ARKIVOC. – 2016. – Vol. 2016, № 4. – P. 277-287.

[11] (5S,7R)-5-Aryl-7-methyl-4,5,6,7-tetrahydro-[1,2,4]triazolo[1,5-a]pyrimidin-7-ols as products of three-component condensation / S. A. Komykhov, A. A. Bondarenko, V. I. Musatov, M. V. Diachkov, N. Yu. Gorobets, S. M. Desenko // Chem. Heterocycl. Compd. – 2017. – Vol. 53, № 3. – P. 378-380.