THE SYNTHESIS, PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF 8-AMINO-7-(2-HYDROXY-2-PHENYLETHYL)-3-METHYLXANTHINES

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The work is devoted to development of the method for the synthesis of 8-amino-7-(2-hydroxy-2-phenylethyl)-3-methylxanthines and the study of their physicoc hemical and biological properties. Purine derivatives have long been used as antiviral agents (acyclovir, ganciclovir, etc.) in medical practice, and further research to find new antimicrobial and antiviral agents among purine derivatives are justified and promising. Reactions of 8-bromo-7-(2-hydroxy-2-phenylethyl)-3-methylxanthine with the primary and secondary amines proceed when boiling in the aqueous dioxane medium with formation of the corresponding 8-amino derivatives. The structure of the compounds synthesized has been confirmed by the results of elemental analysis and the data of $^1$H NMR-spectrometry. Proton signals of the uracil moiety of the molecule in the spectra of all compounds are recorded as two singlets of the corresponding intensity in the range of 10.98-10.54 ppm (NH) and 3.38-3.26 ppm (NCH$_3$). Protons of the substituent in position 7 form several groups of signals with the corresponding intensity. Aromatic proton signals are recorded as multiplets in the range of 7.46-7.18 ppm. The proton signal of the secondary alcohol group is fixed in the form of a doublet at 5.78-5.45 ppm. Intensity, shape and location of other proton signals fully correspond to the structure of the compounds synthesized. For the initial screening study of the new substances synthesized the reference testing cultures of both gram-positive and gram-negative bacteria and fungi, which belong to clinically significant groups of causative agents of infectious diseases that are different by their morphophysiological properties, have been used. 7-(2-Hydroxy-2-phenylethyl)-8-(4-methylpiperidine-1-yl)-3-methylxanthine has exhibited the antimicrobial and antifungal activity against Staphylococcus aureus and Candida albicans, and it is higher than that of the reference drugs – ampicillin and nistatine. The regularities in the «chemical structure – biological activity» relationship have been determined.

It is well known that the most promising direction for creation of new synthetic drugs is the structural modification of the known drugs of the natural origin. The xanthine derivatives play a special part in this regard since a great number of effective drugs (diprophyllinum, etofylline, theofibrate, trental, etc.) has been created on the basis of 1,3-dimethylxanthine (theophylline), and 3,7-dimethylxanthine (theobromine) [3]. Recent studies indicate that 7,8-disubstituted xanthines reveal a wide range of the pharmacological activity [2, 5-10], therefore, further synthetic and biological studies in this series of heterocyclic compounds are well justified.

The aim of our work was the synthesis of xanthine derivatives previously undescribed and the study of their biological action.

Materials and Methods

The melting point was determined by the open capillary method on a PTP-M device (a device for determining the melting temperature of solid substances, Russia). Elemental analysis was performed on an Elementar Vario L cube device (Germany). The PMR-spectra were taken on a Bruker SF-400 spectrometer (Germany) (the working frequency – 400 MHz, the solvent – dimethylsulfoxide (DMSO), the internal standard – tetramethylsilane). The data of elemental analysis corresponded to the calculated ones.

The analytical data of the compounds synthesized are given in Tab. 1, 2.

The assessment of the antimicrobial and antifungal activity was carried out using the standard test strains of microorganisms obtained in the bacteriological laboratory of the SI “Zaporizhzhya Regional Laboratory Centre of the State Sanitary and Epidemiological Service of Ukraine”. Such strains as Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), Candida albicans (ATCC 885-653) were used in our studies. The sensitivity of microorganisms to the new promising antimicrobial compounds synthesized was determined according to the methodical recommendations [1]. To incubate bacteria the Mueller-Hinton broth and agar (pH 7.2-7.4) were used, and for fungi the Sabouraud’s medium (pH 6.0-6.8) was applied.

The minimal inhibitory concentration (MIC) was determined. Dimethylsulfoxide (DMSO) was used as a solvent for the compounds in our studies, the initial solutions were adjusted to the concentration of 1 mg/ml. Additionally, monitoring of nutrient media and the solvent was performed using conventional methods. As the reference drugs ampicillin (PJS “Kyivmedpreparat”, Ukraine) and nistatine (PJSIC “Borschchahivskiy CPP”, Ukraine) were used.
Results and Discussion

8-Bromo-7-(2-hydroxy-2-phenylethyl)-3-methylxanthine (1) previously synthesized [4] by the interaction of 8-bromo-3-methylxanthine with phenyloxirane was selected as a starting compound. The reactions of bromoxanthine 1 with the primary and secondary amines were studied. It has been found that regardless of the amine structure boiling of the synthons specified in the aqueous dioxane medium leads to formation of the corresponding 8-amino derivatives 2-16 (Scheme). The compounds obtained are white crystalline substances, insoluble in water and soluble in hot ethanol, propanol-2, dioxane-1,4, dimethylformamide (DMF), DMSO. Compounds 5, 6, 15, 16 containing the basic nitrogen atoms are readily soluble in dilute acids, and this fact can be used in further studies.

The structure of the compounds synthesized has been proven on the basis of the data of PMR-spectroscopy (Tab. 1). Proton signals of the uracil moiety of the molecule in the spectra of all compounds are recorded as two singlets of the corresponding intensity in the range of 10.98-10.54 ppm (NH) and 3.38-3.26 ppm (N\textsubscript{3}CH\textsubscript{3}). Protons of the substituent in position 7 form several groups of signals with the corresponding intensity. Aromatic proton signals are recorded as multiplets in the range of 7.46-7.18 ppm. The proton signal of the secondary alcohol group is fixed in the form of a doublet at 5.78-5.45 ppm. The presence of the chiral centre conditions multiplicity of signals of the methylene and methine protons of the substituent, they are fixed at 5.22-4.87 ppm (СНО) and 4.15-4.0 ppm (N\textsubscript{7}CH\textsubscript{2}). The exception is 8-dimethylaminoxanthine 7, in its spectrum the methylene and methine protons are registered as a classical doublet and triplet, respectively. In the spectrum of 8-N-methylbenzylamino derivative 9 the methylene protons of the substituent in position 7 form two doublets at 4.65 ppm (1H) and 4.46 ppm (1H). The methylene protons of 8-N-pyrolidiniumxanthine 10 are registered as a doublet at

| Compound | N\textsubscript{1}H (c, 1H) | CH\textsubscript{aom} | C\textsubscript{NH} | OH (d, 1H) | OCH (m, 1H) | N\textsubscript{7}CH\textsubscript{2} (m, 2H) | N\textsubscript{3}CH\textsubscript{3} (s, 3H) | Other signals |
|----------|------------------|-----------------|-----------------|-----------|-------------|------------------|-----------------|----------------|
| 1        | 11.40            | 7.38 (m, 5Н)    | –               | 5.92      | 5.11        | 4.40-4.15        | 3.23            | –              |
| 2        | 10.66            | 7.42 (d, 2Н); 7.36 (t, 2Н); 7.29 (t, 1Н) | 6.52 (d, 1Н) | 5.51      | 4.94        | 4.17-4.12        | 3.30            | 1.94-1.59 (m, 5Н); 1.33-1.29 (m, 5Н); 3.61 (m, 1Н) |
| 3        | 10.66            | 7.44-7.22 (m, 10 Н) | 6.98 (t, 1Н) | 5.72      | 4.94        | 4.17-4.05        | 3.26            | 4.53 (t, 2Н)   |
| 4        | 10.73            | 7.46 (d, 2Н); 7.38 (m, 3Н) | 7.29 (t, 1Н) | 5.72      | 4.96        | 4.14-4.10        | 3.32            | 7.63 (s, 1Н); 6.43 (t, 1Н); 6.36 (d, 1Н); 4.54 (t, 2Н) |
| 5        | 10.60            | 7.40-7.21 (m, 5Н) | 6.73 (t, 1Н) | 5.78      | 4.92        | 4.20-3.93        | 3.30            | 3.60 (m, 4Н); 3.38 (m, 2Н); 2.41 (m, 6Н) |
| 6        | 10.61            | 7.44-7.27 (m, 5Н) | 6.74 (t, 1Н) | 5.69      | 4.92        | 4.12-4.03        | 3.30            | 3.60 (m, 4Н); 3.37 (m, 2Н); 2.34 (m, 6Н); 1.69 (m, 2Н) |
| 7        | 10.87            | 7.38-7.35 (m, 5Н) | –              | 5.67      | 5.06 (t, 1Н) | 4.19 (d, 2Н) | 3.32            | 2.96 (s, 6Н)   |
| 8        | 10.90            | 7.36-7.26 (m, 5Н) | –              | 5.67      | 5.12        | 4.12-4.09        | 3.32            | 3.24 (qv, 4Н); 1.08 (t, 6Н) |
| 9        | 10.88            | 7.36-7.23 (m, 10Н) | –              | 5.75      | 5.09        | 4.65 (d, 1Н); 4.46 (d, 1Н) | 3.32            | 4.14 (s, 2Н); 2.87 (s, 3Н) |
| 10       | 10.65            | 7.36-7.23 (m, 5Н) | –              | 5.65      | 4.87        | 4.26 (d, 2Н) | 3.28            | 3.62 (t, 2Н); 3.47 (t, 2Н); 1.85 (m, 4Н) |
| 11       | 10.95            | 7.33-7.22 (m, 5Н) | –              | 5.68      | 5.08        | 4.15-4.01        | 3.32            | 3.19 (m, 2Н); 2.94 (m, 2Н); 1.53 (m, 6Н) |
| 12       | 10.97            | 7.37-7.18 (m, 5Н) | –              | 5.71      | 5.11        | 4.12-4.03        | 3.30            | 3.65 (d, 1Н); 3.18 (d, 1Н); 2.80 (m, 2Н); 1.70-1.09 (m, 5Н); 0.93 (d, 3Н) |
| 13       | 10.57            | 7.32-7.22 (m, 5Н) | –              | 5.59      | 4.98        | 4.20-4.13        | 3.30            | 3.49 (m, 4Н); 1.75 (m, 4Н); 1.59 (m, 4Н) |
| 14       | 10.98            | 7.39-7.20 (m, 5Н) | –              | 5.72      | 5.13        | 4.17-4.10        | 3.30            | 3.65 (m, 4Н); 3.29 (m, 2Н); 2.95 (m, 2Н) |
| 15       | 10.92            | 7.30 (m, 5Н)    | –              | 5.69      | 5.12        | 4.18-4.05        | 3.30            | 3.28 (m, 2Н); 2.98 (m, 2Н); 2.39 (m, 4Н); 2.20 (s, 3Н) |
| 16       | 10.54            | 7.42-7.18 (m, 5Н) | –              | 5.45      | 5.22        | 4.15-4.0        | 3.38 (m, 5Н)+ NCH\textsubscript{2} | 3.07 (m, 2Н); 2.45 (m, 4Н); 2.40 (qv, 2Н); 1.08 (t, 3Н) |

Table 1

The values of the chemical shift in PMR-spectra of 8-amino-7-(2-hydroxy-2-phenylethyl)-3-methylxanthines
4.26 ppm (2H). In the spectra of aminoxanthine 2-6 the protons of the NH-group in position 8 are clearly recorded in a weak field at 6.74-6.52 ppm (1H) as triplets or a doublet in the case of the cyclohexylamino substituent. Intensity, shape and location of other proton signals of the amine residue in position 8 fully correspond to their structure.

The analysis of the data concerning the study of the antimicrobial and antifungal action of the compounds under research (Tab. 3) shows that the amino alcohols synthesized exhibit a moderate inhibitory action against the strains studied. It should be noted that 8-(4-methyl-piperidine-1-yl)xanthine 12 shows the high activity against *S. aureus* and *C. Albicans*, whereas unsubstituted piperidinoxanthine 11 does not almost show any activity. Extension of the heterocyclic ring (13), introduction of atoms of oxygen (14) or nitrogen (15, 16) does not lead to increase in the antimicrobial or antifungal activity. Only N-methylpiperazine xanthine 15 reveals a marked inhibitory effect on the growth of *Ps. aeruginosa* and

**Table 2**

The physicochemical characteristics of the compounds synthesized (2-8)

| Compound | M. p., °C | The empirical formula | Yield, % |
|----------|-----------|-----------------------|---------|
| 2        | 259       | C_{20}H_{25}N_{5}O_{3} | 51.8    |
| 3        | 220-221   | C_{21}H_{21}N_{5}O_{3} | 64.1    |
| 4        | 196-197   | C_{19}H_{19}N_{5}O_{4} | 34.6    |
| 5        | 205-206   | C_{20}H_{26}N_{6}O_{4} | 43.9    |
| 6        | 200-201   | C_{21}H_{28}N_{6}O_{4} | 48.0    |
| 7        | 221-222   | C_{21}H_{24}N_{6}O_{3} | 32.5    |
| 8        | 203-204   | C_{20}H_{25}N_{5}O_{3} | 69.6    |
| 9        | 206-207   | C_{21}H_{25}N_{5}O_{3} | 50.0    |
| 10       | 239-240   | C_{19}H_{19}N_{5}O_{3} | 45.0    |
| 11       | 215       | C_{21}H_{25}N_{5}O_{3} | 51.1    |
| 12       | 217-218   | C_{21}H_{23}N_{5}O_{3} | 65.0    |
| 13       | 209-210   | C_{20}H_{22}N_{5}O_{3} | 50.0    |
| 14       | 207-208   | C_{20}H_{25}N_{5}O_{3} | 40.5    |
| 15       | 229-230   | C_{20}H_{26}N_{5}O_{4} | 47.3    |
| 16       | 212-213   | C_{20}H_{25}N_{5}O_{3} | 45.0    |

**Table 3**

The biological activity of the compounds synthesized

| Compound | MIC, µg/ml |
|----------|------------|
|          | *E. coli* | *S. aureus* | *P. aeruginosa* | *C. albicans* |
| 3        | 100       | 50         | 100             | 100          |
| 5        | 100       | 200        | 100             | 100          |
| 6        | 100       | 100        | 100             | 25           |
| 9        | 200       | 200        | 100             | 100          |
| 10       | 200       | 25         | 100             | 50           |
| 11       | 100       | 400        | 100             | 100          |
| 12       | 100       | 6.25       | 50              | 25           |
| 13       | 100       | 200        | 100             | 100          |
| 14       | 100       | 200        | 100             | 100          |
| 15       | 100       | 100        | 50              | 50           |
| 16       | 100       | 100        | 100             | 100          |

Ampicillin 12.5 50 25 –

Nistatine 200 100 100 50

**Scheme**
C. albicans in the concentration of 50 µg/ml. 8-Pyrolidinium xanthine 10 (25 µg/ml) also has the high inhibitory activity against S. aureus. In addition to the most active compound 12, morpholinopropylaminoxanthine 6 (25 µg/ml) exhibits the high antifungal activity. Reduction of the carbon chain by one methylene group (5), or a direct bond of the morpholine core with a carbon atom in position 8 (14) lead to a sharp decrease in the antifungal activity.

The above facts clearly demonstrate reasonability and prospects for further search of antimicrobial and antifungal agents in the series of xanthines, especially among their 8-heteryl substituents. For final conclusions it is necessary to significantly expand both the spectrum of pathogenic microorganisms, and the number of the compounds synthesized.

CONCLUSIONS

1. The simple laboratory method for the synthesis of 8-amino-7-(2-hydroxy-2-phenylethyl)-3-methylxanthines – potential biologically active compounds and the initial synths has been developed for further structural modification of the xanthine molecule.

2. The PMR-spectroscopic studies of the compounds synthesized have been conducted, and their structure has been confirmed.

3. The activity of the substances obtained against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans has been studied.

REFERENCES

1. Волянський Ю.Л., Гриценко І.С., Широбоков В.П. та ін. Вивчення специфічної активності антимікробних лікарських засобів: Метод. рекоменд. – К.: ДФЦ МОЗ України, 2004. – 38 с.

2. Киреев И.В. // Вісник Сумського державного університету. Серія Медицина. – 2009. – №1. – С. 22-29.

3. Машковский М.Д. Лекарственные средства. – М.: ООО «Издательство Новая волна», 2012. – 1216 с.

4. Прийменко Б.А., Романенко Н.И., Гармаш С.Н. и др. // Укра. хим. журн. – 1985. – Т. 51, №6. – С. 660-663.

5. A. Alafeefy A.M., Alqasoumi S.I., Abdel Hamid S.G. et al. // J. Enzyme Inhib. Med. Chem. – 2014. – Vol. 29, №3. – P. 443-448.

6. Aleksandrova K.V., Levich S.V., Belenichev I.F., Shkoda A.S. // Intern. J. of Pharmacy. – 2015. – №5 (17). – Р. 1-4.

7. Belenichev I.F., Aleksandrova K.V., Nosach S.G. et al. // Elixir Pharmacy. – 2014. – Vol. 76. – P. 28286-28292.

8. Hayallah A.M., Talhouni A.A., Alim A.A.M.A. // Arch. Pharm. Res. – 2012. – Vol. 35, №8. – Р. 1355-1368.

9. Ran Y., Pei H., Shao M., Chen L. // Chem. Biol. Drug Des. – 2016. – Vol. 87, №2. – Р. 290-295.

10. Vojnikova Yu., Valchevab V., Momekova G. et al. // Bioorg. Med. Chem. Lett. – 2014. – Vol. 24, №14. – P. 3043-3045.
СИНТЕЗ, ФИЗИКО-ХИМИЧЕСКИЕ И БИОЛОГИЧЕСКИЕ СВОЙСТВА 8-АМИНО-7-{2-
ГИДРОКСИ-2-ФЕНИЛЭТИЛ}-3-МЕТИЛКСАНТИНОВ

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Ключевые слова: ксантин; органический синтез; ПМР-спектроскопия;
антабиотическая, противовирусная средства

Работа посвящена разработке методики синтеза неописанных в литературе 8-амино-7-(2-
гидроксии-2-фенилэтил)-3-метилксантинов и изучению их физико-химических и биологиче-
ских свойств. Производные пурина давно используются в медицинской практике в качестве
противовирусных средств (ацикловир, ганцикловир и др.), а, следовательно, дальнейшие ис-
следования по поиску новых противомикробных и противовирусных средств в ряду пуриновых
производных оправданы и перспективны. Реакции 8-бром-7-{2-гидроксии-2-фенилэтил}-
3-метилксантин с первичными и вторичными аминами протекают при кипячении в сре-
де водного диоксана с образованием соответствующих 8-аминопроизводных. Структура
синтезированных веществ подтверждена результатами элементного анализа и данными
ЯМР 'H-спектрометрии. В спектрах всех соединений сигналы протонов урациловой части
молекулы регистрируются в виде двух синглетов соответствующей интенсивности в ин-
тервалах 10,98-10,54 м.д. (NH) и 3,38-3,26 м.д. (N3CH3). Протоны заместителя в положении 7
образуют несколько групп сигналов соответствующей интенсивности. Сигналы аромати-
ческих протонов регистрируются в виде мультиплетов в диапазоне 7,46-7,18 м.д. Сигнал
протона вторичной спиртовой группы фиксируется в виде дубleta в области 5,78-5,45 м.д.
Интенсивность, форма и местоположение других сигналов протонов полностью отвеча-
ют структуре синтезированных соединений. Для первичного скринингового исследования
новосинтезированных веществ использовался этапный тест-культуры как грамположи-
тельных, так и грамотрицательных бактерий и грибов, принадлежащих к разным по мор-
фофункциональным свойствам клинически значимых групп возбудителей инфекционных
заболеваний. 7-(2-Гидроксии-2-фенилэтил)-8-(4-метилпиперидин-1-ил)-3-метилксантин показал
значительную антимикробную и противогрибковую активность, которая превысила препа-
раты сравнения ампициллин и нистатин по отношению к штаммам Staphylococcus aureus и
Candida albicans. Установлены определенные закономерности в ряду “химическая структу-
ра – биологическая активность”. 
