Химия растительного сырья. 2024. №1. С. 162–169.
Khimiya rastitel'nogo syr'ya, 2024, no. 1, pp. 162–169.
DOI: 10.14258/jcprm.20240112023

UDC 547.562.1.+615:

COMPLEXES OF WATER-SOLUBLE LOCAL PLANT POLYPHENOL DERIVATIVES AND THEIR BIOLOGICAL ACTIVITY

© ***K.Zh. Rezhepov*, Sh.B. Alimbayeva***

A.S. Sadykov Institute of Bioorganic Chemistry, Uzbekistan Academy of Sciences, Mirzo Ulugbeka str. 83, Tashkent, 100125, Republic of Uzbekistan, r_k_zh@bk.ru

The article is devoted to obtaining water-soluble complexes of iminoazo derivatives of gossypol, studying their physicochemical properties and biological activity. Aromatic, heterocyclic amines and sulfanilamide preparations were used as amine components in obtaining iminoazo derivatives of gossypol and their water-soluble complexes. The maximum values of wavelengths and the corresponding optical densities were determined in the UV spectra of compounds in acetone solvent. In order to determine the structure of the compounds, the infrared spectrum was taken and analyzed: the shift of the fundamental vibrational frequencies up to 31 cm^{-1} showed that the water-poly-N-vinylpyrrolidone in the compound is connected to a lesser extent as a result of hydrogen bonding. Obtaining complexes of azoderivatives of gossypol imines with poly-N-vinylpyrrolidone is related to the multi-functionality of the reactive groups of the ligand compound; formed hydrogen bonds due to the oxygen of the cycloamide group. For the first time, six new water-soluble complexes iminoazo derivatives of gossypol with poly-N-vinylpyrrolidone were obtained. The results of determination of interferon-inducing activity of compounds were analyzed. The activity was compared with the effectiveness of azo-, iminoazo derivatives of gossypol. According to the obtained results, it was shown that the effectiveness of water-soluble complexes iminoazo derivatives of gossypol.

Keywords: gossypol, Schiff bases, azo derivatives of gossypol, iminoazo derivatives of gossypol, poly-N-vinylpyrrolidone, UV and IR spectroscopy methods, interferon-inducing activity.

For citing: Rezhepov K.Zh., Alimbayeva Sh.B. *Khimiya Rastitel'nogo Syr'ya*, 2024, no. 1, pp. 162–169. (in Russ.). DOI: 10.14258/jcprm.20240112023.

Introduction

As shown in Figure 1, gossypol is a polyphenolic substance containing two aldehyde and six hydroxyl groups. The active groups of gossypol also contribute to its reactivity and biological activity. Gossypol – for the treatment of leukemia [1], lymphoma [2], colon carcinoma [3, 4], breast cancer [5, 6], myoma, prostate cancer [7] and other malignant tumors is a promising agent [8–12] and has broad-spectrum antiviral, antitumor, and antifungal activity.

The biological activity of many plant polyphenols is mainly determined by their ability to inhibit free radical processes [13]. Polyphenols are secondary metabolites of plants, usually involved in protection against UV radiation or pathogen aggression.

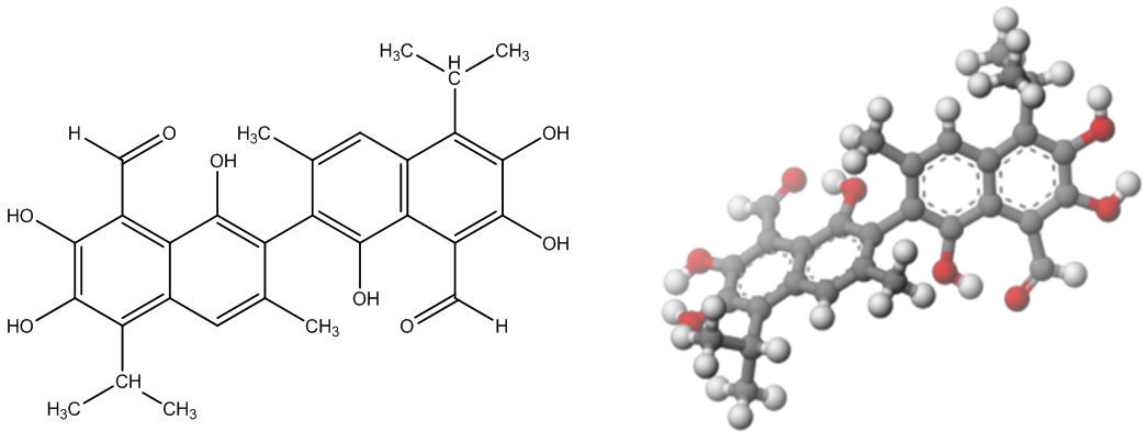
The purpose of our research is to obtain water-soluble complexes of iminoazo derivatives of gossypol, to study their physicochemical properties and biological activity. Some aromatic, heterocyclic amines and sulfonamide preparations were selected as attached amine components to react with the C-4 atom and aldehyde group of gossypol.

Experimental part

Azo-, iminoazo-derivatives of gossypol and their water-soluble complexes with poly-N-vinylpyrrolidone were selected as the *object of research*.

Research methods. The following equipment was used to study the spectral properties of substances: Shimadzu UV-1280 UV spectrophotometer (Japan) (1x1 cuvette), IR spectrometer.

* Corresponding author.



A *B*

Fig. 1. The chemical structure (*A*) and the structural formula of the gossypol molecule built using the package (program) HyperChem (*B*)

The interferon-inducing activity of the compounds was carried out in white mice weighing 10–12 *g*. The drugs were injected intraperitoneally at a dose of 100 *mg/kg* and 200 *mg/kg*. The content of interferon was determined after 24 and 48 hours by titration in homologous cells according to the level of protection against the cytopathic effect of mouse encephalomyocarditis virus.

Preparation of complexes of azo derivatives of gossypol imines with poly-N-vinylpyrrolidone. 0.005 mol of poly-N-vinylpyrrolidone was added to a solution of 0.01 mol of the azo derivative of gossypol imines in 100 ml of chloroform and stirred on a magnetic stirrer at a temperature of 25–30 °C for 24 hours. The reaction mixture was then filtered, and the filtrate was evaporated in a rotary evaporator until it became dry powder. The obtained powder residue was dried.

Discussion of results

Continuing the study of nitrogenous derivatives of gossypol, six azo derivatives of gossypol, whose physicochemical properties, structures, and biological activities were studied [14-19] were resynthesized in the presence of primary amines. Their general structural formula is presented in Figure 2.

In order to carry out our main research work, six iminoazo derivatives of gossypol were synthesized with the presence of the same aryl-, heterylamines and sulfanilamide prepates to the C-4 atom of gossypol and the aldehyde group (Fig. 3).

The development of methods for creating water-soluble polymer carriers of biologically active substances is important at the intersection of scientific fields that study polymer chemistry, biology and medicine. In most cases, biologically active substances are substances that are poorly soluble in water and contain hydrophobic fragments, so the possibility of using them, for example, as therapeutic drugs, is limited. The use of polymer derivatives of biologically active substances allows to give them completely new properties, to minimize side effects, and in some cases to increase efficiency [20–28].

Reference information on the use of polymers as carriers of biologically active substances appeared half a century ago. The first studies were carried out by Yatskevich in 1955, who added mescaline to poly-N-vinylpyrrolidone via a dipeptide spacer to increase its effectiveness [29]. In the 1960s and 1970s, the followers of Ushakov and Panarin synthesized a number of water-soluble polymer derivatives of antibiotics with various types of polymer-biologically active compounds [30–32]. Carrier – the necessary requirements for the polymer are its solubility in water, biocompatibility (lack of toxic effects, antigenicity, carcinogenicity, etc.), long enough circulation of the system in the blood, but at the same time, the polymer is liquid in the kidney organs (when administered intravenously). is the possibility of expulsion through In addition, the carrier polymer should contain functional groups and ligands with which biologically active substances can be covalently or non-covalently bound.

Poly-N-vinylpyrrolidone was chosen to obtain complexes iminoazo derivatives of gossypol. The substance is mixed with the polymer at room temperature on a magnetic stirrer at in non-aqueous solvent systems, and then the solvent is dried by rotary evaporation. The UV spectroscopy method was used to characterize the obtained complex compounds.

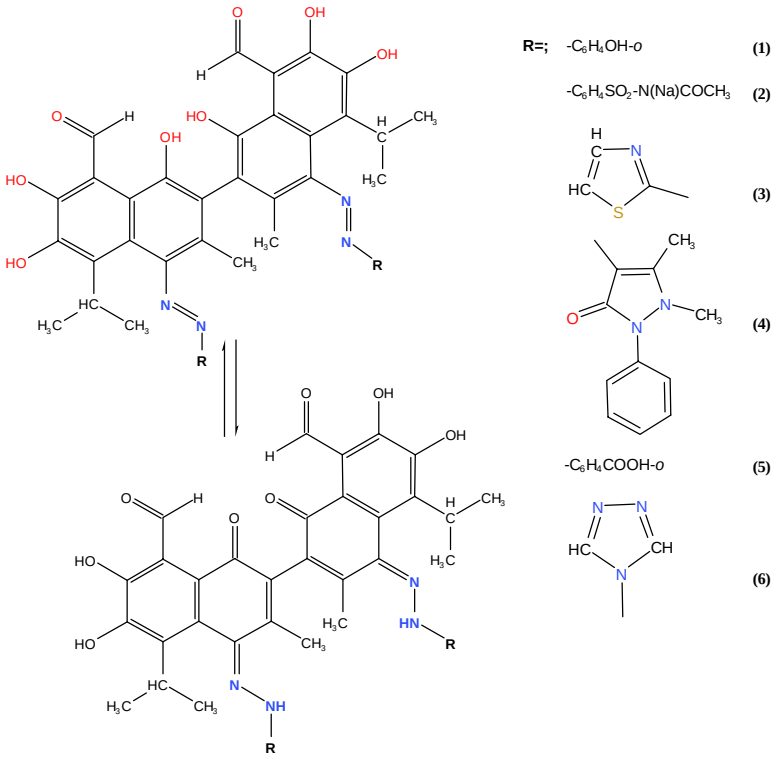


Fig. 2. General structural formula azo derivatives of gossypol

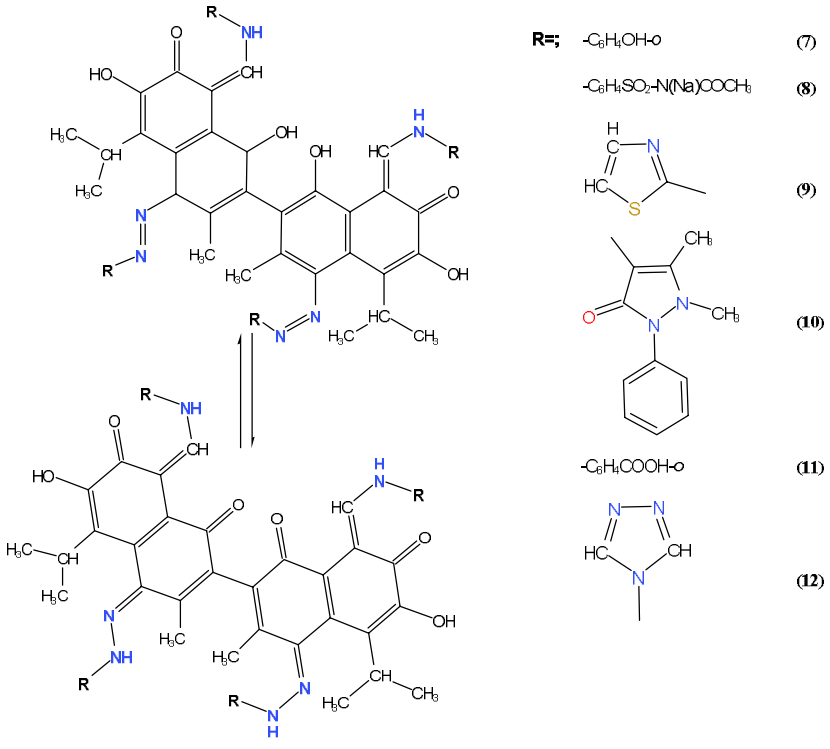


Fig. 3. General structural formula iminoazo derivatives of gossypol

Table, which presents the parameters of the electronic spectra of compounds 13–18, showed that the most characteristic of them is the absorption maximum in the region of 440–475 nm.

Physico-chemical properties of water-soluble complexes iminoazo derivatives of gossypol formed as a result of the reaction are presented in Table.

The resulting complexes are bright colored powdery substances that are completely soluble in water.

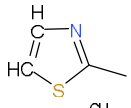
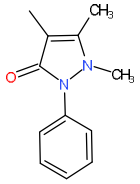
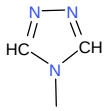
To confirm the structure of the complexes of azo derivatives of gossypol imines with poly-N-vinylpyrrolidone, a comparative IR spectroscopic study of compound **15** with compound **9** was carried out.

In the IR spectrum of compound **9** (Fig. 4), the absorption band at a frequency of 3429 cm^{-1} originates from stretching vibrations of the hydroxyl groups of the gossypole part of the molecule. The absorption band at a frequency of 1727 cm^{-1} is absent both in the IR spectrum of gossypol and in the IR spectrum of 2-amino-1,3-thiazole. This absorption band may be due to vibrations in the C=N bond, which probably occurs when gossypol is combined with 2-amino-1,3-thiazole. The absorption band at a frequency of 1635 cm^{-1} comes from vibrations of the chain of conjugated bonds in the gossypole part of the compound **9** molecule. The indicated absorption band is shifted towards high frequencies compared to a similar absorption band in the IR spectrum of gossypol, which indicates a decrease in the degree of conjugation. Therefore, the gossypole part of compound **9** is in the quinoid state. This is also confirmed by the fact that the region of the spectrum with frequencies below 1495 cm^{-1} is similar to the same region in the IR spectrum of gossypolone.

In the IR spectrum of compound **15** (Fig. 5), the absorption band at a frequency of 3443 cm^{-1} comes from vibrations of water molecules, which is contained in Poly-N-vinylpyrrolidone. The specified absorption band in the IR spectrum of Poly-N-vinylpyrrolidone is at a frequency of 3412 cm^{-1}. The frequency shift from 3412 cm^{-1} to 3443 cm^{-1} indicates that water in compound **15** is hydrogen bonded to the polymer to a lesser extent than in Poly-N-vinylpyrrolidone itself. Therefore, in compound **15**, compound **9** forms a hydrogen bond with the polymer instead of water. Moreover, hydrogen bonds are formed with carbonyl groups of Poly-N-vinylpyrrolidone, since in the IR spectrum of compound **15** the corresponding absorption band is at a frequency of 1653 cm^{-1}, and in the IR spectrum of Poly-N-vinylpyrrolidone at a frequency of 1637 cm^{-1}.

Thus, obtaining complexes of azo derivatives of gossypol imines with Poly-N-vinylpyrrolidone is due to the multi-functionality of the reactive groups of the ligand compound and hydrogen bonds formed at the expense of the oxygen of the cycloamide group. The mesomeric structure of Poly-N-vinylpyrrolidone was formed by electrostatic donor-acceptor interaction. The methylene and methine groups of Poly-N-vinylpyrrolidone chains and rings also formed hydrophobic interactions. Based on the above information, the water-soluble complexes of gossypol iminoazo derivatives with Poly-N-vinylpyrrolidone were expressed according to the structural formula (Fig. 6).

Physicochemical properties of water-soluble complexes of iminoazo derivatives of gossypol

Comp. No	R	Solubility, g/ml (at 30 °C)			Melting temperature, °C	UV spectrum, nm, λ_{max} (*log* ε), acetone	Rf*	Yield, %
		H_2O	CH_3Cl	CH_3-C(O)-CH_3				
13	-C_6H_4OH-*o*	1 : 25	1 : 210	>1 : 10000	175–179	450 (4.21)	0.71	80.25
14	-$C_6H_4SO_2$-N(Na)$COCH_3$	1 : 23	1 : 205	>1 : 10000	187–190	465 (4.29)	0.76	86.78
15		1 : 19	1 : 220	>1 : 10000	170–172	450 (4.01)	0.83	76.89
16		1 : 18	1 : 230	>1 : 10000	190–193	455 (3.97)	0.79	83.47
17	-C_6H_4COOH-*o*	1 : 24	1 : 240	>1 : 10000	171–174	475 (3.85)	0.85	79.63
18		1 : 20	1 : 215	>1 : 10000	189–191	440 (4.12)	0.80	88.56

*System: acetone-toluene (6.0 : 4.0).

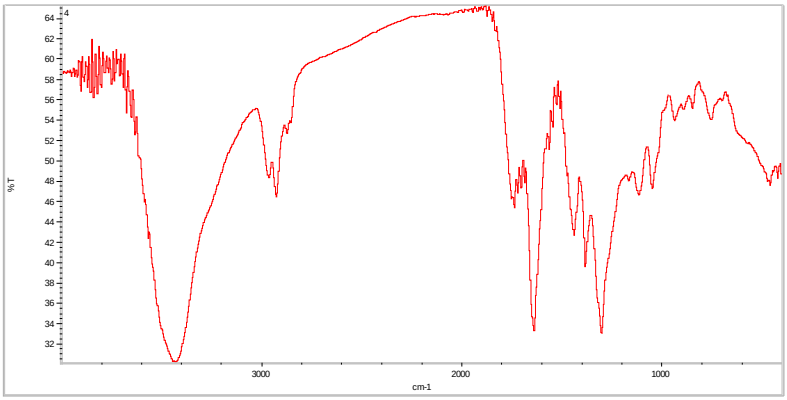


Fig. 4. IR spectrum of compound **9**

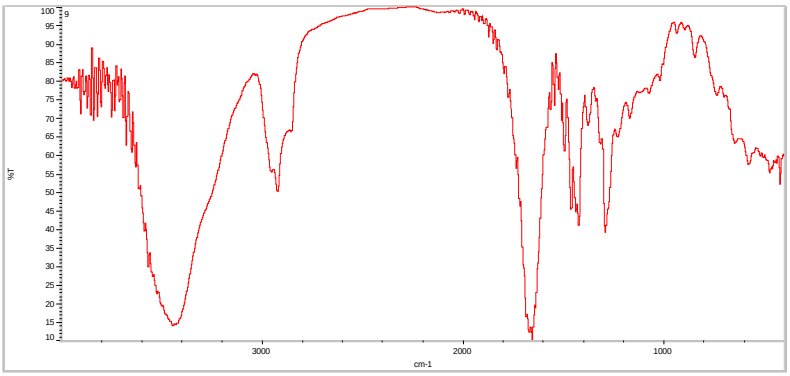


Fig. 5. IR spectrum of compound **15**

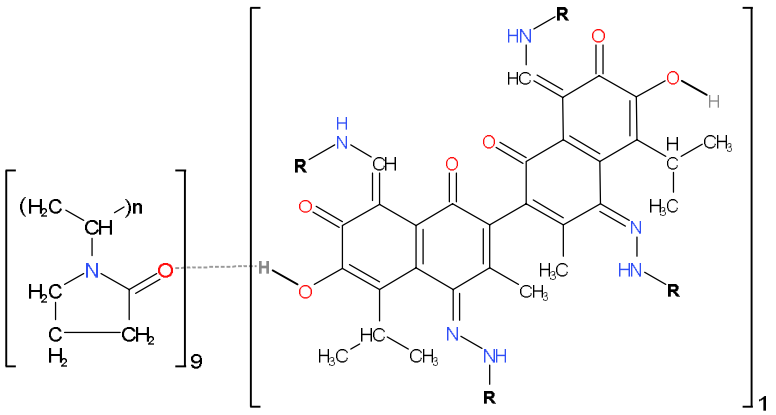


Fig. 6. Structural formula of water-soluble complexes of iminoazo derivatives of gossypol

Biological activity of synthesized gossypol imino derivatives and their water-soluble complexes with poly-N-vinylpyrrolidone. The biological activity of a chemical substance can be related to a certain set of chemical reactions that occur with substances in a number of organisms. The level of biological activity is determined by the ability of organs to undergo certain types of chemical reactions. In general, molecules of a biologically active substance undergo a number of physico-chemical and chemical changes in the body. These include dissolution, sorption, distribution, binding, chemical reaction, modification, etc. In this regard, it is known from the literature that long-term research was carried out in cooperation with the polyphenols laboratory of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan and scientists of the Research Institute of Epidemiology and Microbiology of the Russian Federation named after N.F. Gamaleya. For example, the Institute of Virology of the Russian Federation named after D.I. Ivanovsky showed that not only gossypol itself, but also its

derivatives have high interferon-inducing activity [30–33]. According to the analysis of this literature, the interferon-inducing activity of most of the imine derivatives of gossypol synthesized on the basis of Schiff and azo-derivatives attached to the C-4 atom of gossypol has not been fully studied, as it was done in the determination of immunomodulatory activity.

In our research, it was of great interest to check whether the interferon-inducing activity is characteristic of azo derivatives of gossypol or whether the activity depends on other factors. Considering the above, the interferon-inducing activity of newly synthesized iminoazo derivatives of gossypol and their complexes with poly-N-vinylpyrrolidone was studied. The study of the interferon-inducing activity of substances was carried out by Professor A.M. Sayitkulov, doctor of biological sciences, at the Scientific Research Institute of Virology of the Ministry of Health of the Republic of Uzbekistan.

A comparative structural-functional analysis of the results obtained in the study of interferon-inducing activity azo derivatives of gossypol and iminoazo derivatives of gossypol was performed and presented in figures 7, 8.

Comparing the data presented in Figure 7, it was observed that the titer of interferon induced in the body also depends on the time of contact with the substance. For example: in compounds **8** and **10**, we can see a significant increase after 200 *mg/kg* dose compared to other synthesized compounds after 48 hours.

Note: when water-soluble complexes of iminoazo derivatives of gossypol are used in a dose of 10–20 *mg/kg*, the titers of interferon induced in experimental animals correspond to (or exceed) a dose of iminoazo derivatives of gossypol 100–200 *mg/kg* (Fig. 8).

In the analysis of the results obtained in determining the interferon-inducing activity of water-soluble complexes of iminoazo derivatives of gossypol with poly-N-vinylpyrrolidone, it was shown that the effectiveness of iminoazo derivatives of water-insoluble gossypol doubled.

In addition, we can assume that the interferon-inducing activity of iminoazo derivatives of gossypol synthesized with primary amines of different structures depends on the nature of the substituents introduced into the structure of gossypol and the type of interaction reaction.

In conclusion, for our further scientific research, the fact that the iminoazo derivatives of gossypol (compounds **8** and **10**) synthesized on the basis of some heterylamines and sulfonamide drugs and their water-soluble complexes with poly-N-vinylpyrrolidone (compound **15**) showed high interferon-inducing activity aroused great interest in us, and a deep pharmacotoxicological study was recommended in order to conduct studies.

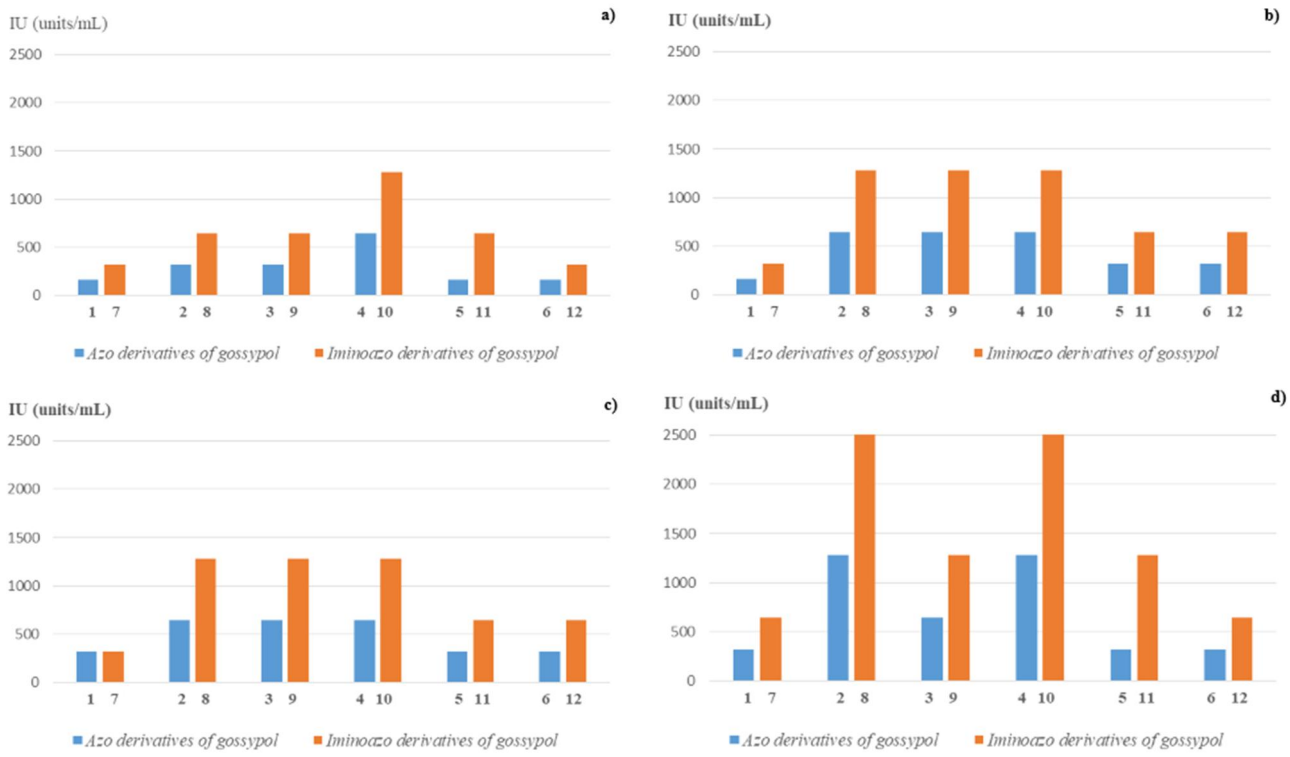


Fig. 7. Interferon-inducing activity of gossypol derivatives; **a)** dose 100 *mg/kg*, after 24 h, **b)** dose 100 *mg/kg*, after 48 h, **c)** dose 200 *mg/kg*, after 24 h, **d)** 200 *mg/kg*, after 48 h

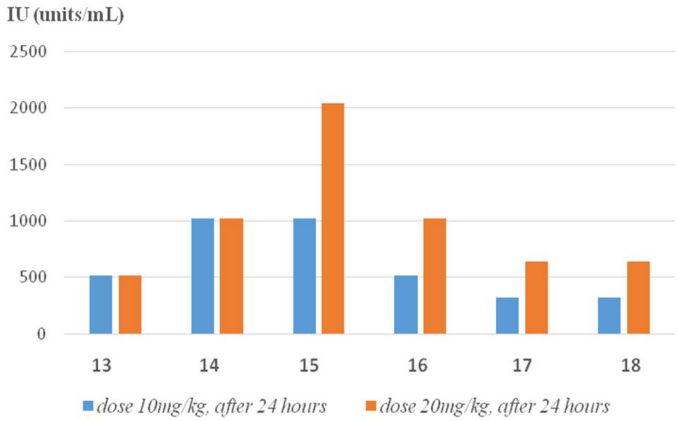


Fig. 8. Interferon-inducing activity of water-soluble iminoazo derivatives of gossypol

Thus, such multifaceted scientific approaches to the study of structural and functional analyzes of gossypol derivatives help to target the most promising compounds from the point of view of creating interferon inducers with a wide range of antiviral activity.

Conclusions

1. For the first time, six new water-soluble complexes iminoazo derivatives of gossypol with poly-N-vinylpyrrolidone were obtained, their physicochemical properties, structures and biological activities were studied.

2. Modern UV- and IR-spectroscopy methods were used to describe the structure of the obtained complex compounds. Absorption maxima corresponding to intensive and characteristic electronic transitions were determined in the UV-spectrum of the complexes.

3. As a result of the comparative IR spectroscopic analysis of the complex formed from the specified values of the initial substances, the structural formula of the water-soluble complexes of gossypol iminoazo derivatives was expressed.

4. The analysis of the results obtained in the study of interferon-inducing activity of water-soluble complexes with poly-N-vinylpyrrolidone showed that the effectivenes s was doubled compared to water-insoluble iminoazo derivatives of gossypol.

5. Compounds **8** and **10** iminoazo derivatives of gossypol, which showed high interferon-inducing activity, and compound **15** of their new water-soluble complexes with poly-N-vinylpyrrolidone were recommended for further pharmacotoxicological research in our future research.

Funding
This work was supported funding Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan named after academician A.S. Sadikov and the Ministry of Innovative Development of the Republic of Uzbekistan. No additional grants to carry out or direct this particular research were obtained.

Conflict of Interest
The authors of this work declare that they have no conflicts of interest.

References

1. Balakrishnan K., Wierda W.G., Keating M.J., Gandhi V. *Blood*, 2008, vol. 112, no. 5, pp. 1971–1980.
2. Johnson P.W.M. *Annals of Oncology*, 2008, vol. 19, no. 4, pp. 56–59.
3. Wang X., Wang J., Wong S.C.H. et al. *Life Sciences*, 2000, vol. 67, no. 22, pp. 2663–2671.
4. Ping C., Li Z., Jing-Wen L. et al. *Biomedical Research*, 2016, vol. 27, no. 2, pp. 419–423.
5. Van Poznak C., Seidman A.D., Reidenberg M.M. et al. *Breast Cancer Research and Treatment*, 2001, vol. 66, no. 2, pp. 239–248.
6. Ye W., Chang H.L., Wang L.S. et al. *Anticancer Research*, 2007, vol. 27, no. 1, pp. 107–116.
7. Jiang J., Slivova V., Jedinak A., Sliva D. *Clinical and Experimental Metastasis*, 2012, vol. 29, no. 2, pp. 165–178.

8. Badawy S.Z.A., Souid A.K., Cuenca V. et al. *Asian Journal of Andrology*, 2007, vol. 9, no. 3, pp. 388–393.
9. Ko C.H., Shen S.C., Yang L.Y. et al. *International Journal of Cancer*, 2007, vol. 121, no. 8, pp. 1670–1679.
10. Chien C.C., Ko C.H., Shen S.C. et al. *Journal of Cellular Physiology*, 2012, vol. 227, no. 8, pp. 3128–3137.
11. Hsiao W.T., Tsai M.D., Jow G.M. et al. *Molecular Vision*, 2012, vol. 18, pp. 2033–2042.
12. Wong F.Y., Liem N., Xie C. et al. *PLoS ONE*, 2012, vol. 7, no. 12, pp. 1–10.
13. Il'kevich N.S., Rybachenko V.I., Shreder G. i dr. *Naukovi pratsi donets'koho natsional'noho tekhnichnoho universytetu. Seriya: "Khimiya I khimichna tekhnolohiya"*, 2009, no. 13, pp. 110–117. (in Russ.).
14. Baram N.I., Ismailov A.I. *Chemistry of Natural Compounds*, 1993, vol. 29, p. 275. DOI: 10.1007/BF00630521.
15. Baram N.I., Ismailov A.I., Ziyaev Kh.L., Rezhepov K.Zh. *Chemistry of Natural Compounds*, 2004, vol. 40, p. 3. DOI: 10.1023/B:CONC.0000039123.09208.79.
16. Rezhepov K.Zh., Ziyaev Kh.L., Baram N.I., Ismailov A.I., Kamaev F.G., Saiitkulov A.M. *Chemistry of Natural Compounds*, 2002, vol. 38, p. 4. DOI: 10.1023/A:1021613705698
17. Rezhepov K.Zh., Ziyaev Kh.L., Baram N.I., Kamaev F.G., Levkovich M.G., Saiitkulov A.M., Ismailov A.I. *Chemistry of Natural Compounds*, 2003, vol. 39, p. 4. DOI: 10.1023/B:CONC.0000003416.49975.25
18. Rezhepov K.Zh., Ziyaev Kh.L., Alimbayeva Sh.B. *Khimiya rastitel'nogo syr'ya*, 2022, pp. 277–282. DOI: 10.14258/jcprm.20220410648
19. Rezhepov K.Zh., Alimbayeva Sh.B. *Khimiya rastitel'nogo syr'ya*, 2023, pp. 135–143. DOI: 10.14258/jcprm.20230110881
20. Plate N.A. *Fiziologicheski aktivnyye polimery*. [Physiologically active polymers]. Moscow, 1986, 296 p. (in Russ.).
21. Pan H. *Multifunctional Pharmaceutical Nanocarriers*. Springer: NY, 2008, vol. 4, pp. 81–142.
22. Liu S. *Macromolecules*, 2009, vol. 42, no. 1, pp. 3–13.
23. Duncan R. *Nat. Rev. Cancer*., 2006, vol. 6, no. 9, pp. 688–701.
24. Dhal P.K. *Adv. Drug Deliver Rev*., 2009, vol. 61, no. 13, pp. 1121–1130.
25. Chadha R. *J. Sci. Ind. Res*., 2008, vol. 67, no. 3, pp. 185–197.
26. Vilar G. *Curr. Drug Deliver*., 2012, vol. 9, no. 4, pp. 367–394.
27. Markovsky E. *J. Controlled Release*, 2012, vol. 161, no. 2, pp. 446–460.
28. Kopeček J. *J. Controlled Release*, 2001, vol. 74, no. 1-3, pp. 147–158.
29. Jatzkewits H. *Z. Naturforsch*., 1955, vol. 10b, pp. 27–31.
30. Ushakov S.N. *Dokl. AN SSSR*, 1962, vol. 147, no. 5, pp. 1102–1107. (in Russ.).
31. Givental' N.I., Ushakov S.N., Panarin Ye.F., Popova G.O. *Antibiotiki*, 1965, vol. 10, no. 8, pp. 701–706. (in Russ.).
32. Panarin Ye.F. Shumikhina K.I. *Khimiko-farmatsevticheskiy zhurnal*, 1974, no. 9, pp. 16–18. (in Russ.).

Received October 22, 2022

Revised January 11, 2023

Accepted September 6, 2023

Information about authors

Rezhepov Kuralbay Zhadigerovich – Candidate of Chemical Sciences, Senior Researcher, Laboratories of low molecular weight biologically active compounds, r_k_zh@bk.ru

Alimbayeva Shiyrin Bekniyaz kizi – research intern, r_k_zh@bk.ru