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BIOLOGICALLY ACTIVE COMPOUNDS BASED ON OROTIC ACID

***Ubaydulla Baltabaev, Abdukahhor Djuraev, Abdugafur Khadjimetov, Jahongir abduvakilov, Nusrat Axmadaliev and Feruzbek Ubaydullaev**

Tashkent State Dental Institute, Tashkent, Uzbekistan.

***Corresponding Author: Ubaydulla Baltabaev**

Tashkent State Dental Institute, Tashkent, Uzbekistan.

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ABSTRACT

In this paper, it was of interest to synthesize derivatives of 1,3-bis (aroylthiocarbamoyl) -orotic acid in order to increase the arsenal of biologically active compounds. By the interaction of chloro-, bromo-, iodo- and nitro-substituted benzoyl-isothiocyanates with orotic acid in the environment of pyridine and dimethylformamide, at a temperature of 100-105^{0}C and a reaction time of 5 hours, derivatives of 1,3-bis (aroylthiocarbamoyl) -orotic acid were synthesized. Among the synthesized compounds, effective anti-inflammatory drugs were identified, as well as substances with bactericidal and fungicidal properties, which are of undoubted practical interest.

KEYWORDS: substituted benzoylisothiocyanates, orotic acid, pyridine, dimethylformamide, 1,3-bis (aroylthiocarbamoyl) -orotic acids, anti-inflammatory activity, bactericidal action, fungicidal properties.

INTRODUCTION

The interaction of isothiocyanates with orotic acid has been studied extremely insufficiently, although derivatives of uracil and orotic acid are used as medicines. So, orotic acid and its potassium salt are used for disorders of protein metabolism and as general stimulants of metabolic processes, and methylthiouracil is used in the treatment of diseases associated with impaired thyroid function.[1] Methyluracil as a stimulant of leukopoiesis is prescribed for agranulocytic tonsillitis, nutritional toxic leukemia, chronic benzene poisoning, and leukopenia. Methyluracil is also used for sluggishly healing wounds, burns, and bone fractures.[2]

There is also evidence of the effectiveness of methyluracil and pentoxyl for peptic ulcer of the stomach and duodenum and for chronic gastritis.[3-4]

Antitumor drugs based on uracils,[5] and 5-fluorouracil,[6] are used in oncological practice.

The purpose of the study: based on literature data and continuing research in the field of synthesis of derivatives of aroylisothiocyanates, in this paper it was of interest to synthesize derivatives of 1,3-bis (aroylthiocarbamoyl) -orotic acid in order to increase the arsenal of biologically active compounds.

MATERIAL AND METHODS

Thin-layer chromatography was used to verify the purity and individuality of the resulting substances. As an adsorbent used plates brand Silufol UV-254, the factory AVALIER, as a developer - a pair of iodine.

Various eluent systems were used as solvents: system 1: ether — benzene — hexane (3: 1: 1), system 2: chloroform – hexane (9: 1), and Rf for each compound was determined.

IR spectra were recorded on a UR-20 spectrophotometer in KBr pellets in the region of 3600-500 cm-1, with LiF prisms for the region of 3800-2000 cm-1 and NaCl - 2000-500 cm-1.

Experimental part

1,3-bis (para-chlorobenzoyl-thiocarbamoyl) orotic acid (compound III). In a three-necked flask with a capacity of 250 ml, equipped with a reflux condenser with a calcium chloride tube, a stirrer and a dropping funnel, 3.12 (0.02 mol) of orotic acid in 20 ml of purified dimethylformamide is placed. The contents of the flask are heated in a water bath to 60 ° C, and without stopping stirring, 7.92 g (0.04 mol) of para-chlorobenzoylisothiocyanate in 20 ml of dimethylformamide are added dropwise. The reaction mixture with vigorous stirring is kept for 5 hours at a temperature of 90 ° C.

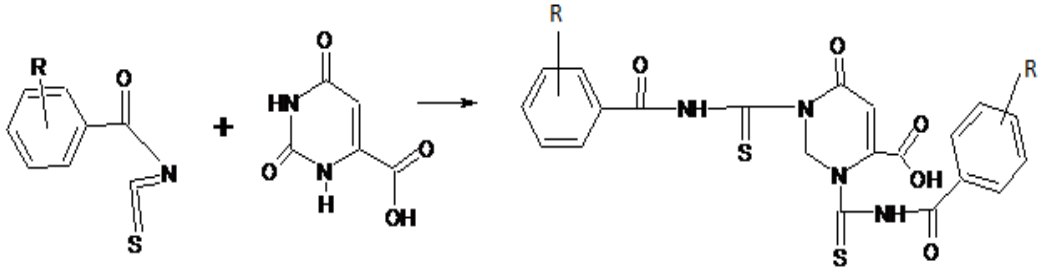
At the end of the reaction, the mixture is cooled and 200-250 ml of water are added. The precipitate formed is filtered off, washed with water and a 10% hydrochloric acid solution. The resulting product is dried in air and purified by adsorption chromatography on a column of

aluminum oxide, eluting in the sulfur ether – benzene – hexane system (3: 1: 1), with a ratio of 1:25 between the product being purified and the adsorbent. The completeness of separation was controlled by thin layer chromatography. The pure product is crystals with melting temperature 230-231°C yield 8.61 g (78% of theory).

In a similar manner, other derivatives of orotic acid were obtained.

RESULTS AND DISCUSSION

Other derivatives of orotic acid, in particular 1,3-bis (halogen or nitro-substituted benzoylthiocarbamoyl) - orotic acid, are of particular interest in this regard. To obtain these compounds, we used the reaction of chloro-, bromo-, iodo- and nitro-substituted benzoylisothiocyanates (BITC) with orotic acid in a medium of dimethylformamide and pyridine, according to the scheme.[7-14]



The value of R and physico-chemical parameters of the obtained compounds are shown in table 1.

Table 1: Physico-chemical characteristics of derivatives of orotic acid.

№	R value	Output, %	Melting temperature, C	R_f	Formula
I	2-Cl	74	215-6	0,60	$C_{21}H_{12}O_6N_4S_2Cl_2$
II	3-Cl	75	251-2	0,65	$C_{21}H_{12}O_6N_4S_2Cl_2$
III	4-Cl	78	230-1	0,63	$C_{21}H_{10}O_6N_4S_2Cl_2$
IV	2,4-Cl	71	198-9	0,67	$C_{21}H_{12}O_6N_4S_2Cl_2$
V	2-J	70	172-3	0,58	$C_{21}H_{12}O_6N_4S_2J_2$
VI	3-J	74	196-7	0,76	$C_{21}H_{12}O_6N_4S_2J_2$
VII	4-J	75	221-2	0,73	$C_{21}H_{12}O_6N_4S_2J_2$
VIII	2-Br	72	207-8	0,78	$C_{21}H_{12}O_6N_4S_2Br_2$
IX	3-Br	73	235-6	0,64	$C_{21}H_{12}O_6N_4S_2Br_2$
X	4-Br	77	218-9	0,6	$C_{21}H_{12}O_6N_4S_2Br_2$
XI	2-NO_2	75	150-1	0,65	$C_{21}H_{12}O_6N_4S_2S_2$
XII	3- NO_2	77	177-8	0,71	$C_{21}H_{12}O_6N_4S_2S_2$
XIII	4- NO_2	81	181-2	0,66	$C_{21}H_{12}O_6N_4S_2$
XIV	2,4-$(NO_2)_2$	72	202-3	0,74	$C_{21}H_{10}O_{14}N_8S_2$
XV	4-CH_3O	68	100-2	0,65	$C_{23}H_{18}O_8N_4S_2$
XVI	4-OH	65	184-5	0,72	$C_{21}H_{14}O_8N_4S_2$

All new derivatives of orotic acid are substances that are readily soluble in many organic solvents: ether, acetone, alcohol, ethyl acetate, dimethyl sulfoxide, and insoluble in water.

For purification and identification of synthesized compounds, in addition to recrystallization from benzene and adsorption chromatography on a column, we used TLC on alumina of the second degree of activity and on a Silufol plate. Various eluent systems were used as solvents: system 1: ether — benzene — hexane (3: 1: 1), system 2: chloroform – hexane (9: 1), and Rf for each compound was determined.

Orotic acid is characterized by lactim-lactam tautomerism. Usually the lactam form prevails in equilibrium. Orotic acid is characterized by acylation of nitrogen atoms in the presence of bases, due to its significant NH-acidity. Given this, we have developed a method in which substituted benzoylisothiocyanates in reactions with orotic acid give good yields of products from 65 to 81%. Thanks to the high reactivity of benzoylisothiocyanates and the creation of favorable reaction conditions: solvent pyridine and dimethylformamide, temperature 100-105 0C, reaction time 5 hours.

The high reactivity of benzoylisothiocyanates can be explained by the fact that the presence of two double bonds at one carbon atom and two heteroatoms N and S with non-generalized electrons makes the electron cloud of the isothiocyanate group very dense and easily mobile, which determines the reactivity of the isothiocyanate

group in benzoylisothiocyanates. Experimental data indicate that the presence of electron-withdrawing substituents in the aromatic ring enhances the reactivity of benzoylisothiocyanates. For example, the yields of 1,3-bis (aroylthiocarbamoyl) orotic acid derivatives in the case of p – NO2 BITC of compound XIII (Table 1) are 81%, which is higher than compounds X (77%) and XI (75%); for p-ClBITC, compound III is 78%, which is higher than compounds II (75%) and I (74%); for p-BrBITZ, compound X - is 77%, which is higher than compounds IX (73%) and VIII (72%).

It should be noted that the yields of compounds I - XVI are observed in the range from 65 to 81%, which can be considered good yields. Higher yields of reaction products (over 90%) could not be obtained, because this, apparently, can be explained by the presence of a carboxyl group in orotic acid, which increases the acidity of the reaction medium, as a result of which the reactivity of the reactants and the rate of the carbamoylation reaction decrease.

The structure of the synthesized compounds is confirmed by analytical data and IR spectroscopy.

The IR spectrum of 1,3-bis (para-chlorobenzoylthiocarbamoyl) orotic acid (III) shows a band at 1017 cm-1, corresponding to fully symmetric vibrations of the pyrimidine ring, and there is an absorption band in the region of 1093 cm-1, characteristic of C stretching vibrations = S connection. The bands in the region of 1283, 1307, 1322 cm – 1 indicate the presence of the N – C (S) –N group; at 1425, 1492 cm – 1 there are absorption bands characteristic of the NH – CS group, in the region of 1575, 1593 cm – 1 - for CH = CH phenyl, at 1689 cm-1 - for C = O bonds, at 2675 cm-1 - for the N – C heterocycle, at 2885 cm-1 - for = CH groups of the pyrimidine ring, at 3052 cm-1 - for NH groups, 3482 cm -1 for OH groups.

The anti-inflammatory activity of compounds I – XVI was studied on white rats of both sexes weighing 140–180 g. Inflammation was caused by a 1% formalin solution, which was administered under the ankle neurosis of the ankle joint in an amount of 0.2 ml. The paw volume of rats was measured oncometrically 3 times before and 3, 6 hours after administration of formalin.

The test compounds were administered orally with a metal probe in the form of a suspension in cottonseed oil at doses of 50, 100 and 200 mg / kg. The drugs were administered 1 to cause inflammation, that is, 2 hours before the introduction of formalin.

For comparison, we took well-known anti-inflammatory drugs - butadion (Butadion), indomethacin (Indometacin) and voltaren (Voltaren). They were used in doses at which they cause the most pronounced anti-inflammatory effect. (tab. 2).

As a result of the studies, it was found that all substances (I – XVI) in certain doses exhibit a distinct anti-inflammatory activity. Among them, compounds (I, II, IV-XII, XIV-XVI) were found to be relatively less active, which within the dose range of 50-100 mg / kg reduce the intensity of the inflammatory process by about 16.8-24%. Compound III and XIII causes a rather strong anti-inflammatory effect, so at doses of 50 and 100 mg / kg after 6 hours it inhibits inflammation by 63.3% and 75.9%, respectively; 63.3% and 70.6%. A further increase in the dose of drugs did not lead to a marked increase in the observed effect. While butadione at a dose of 100 mg / kg inhibits inflammation by 28.1%, indomethacin at a dose of 10 mg / kg - by 36.4%, voltaren at a dose of 25 mg / kg - by 43.2%.

The acute toxicity of the test compounds was determined by calculating the LD50 according to the method of Litchfield and Wilcoxon. White mice weighing 18–25 g were given the studied drug orally. Each dose was checked on 6 animals. Experimental animals were observed for 24 hours.

Tests have shown that the LD50 of compounds is outside the range of 3000 mg / kg, while the LD50 of butadione is 430 mg / kg, indomethacin –47 mg / kg, and voltaren 370 mg / kg.

A comparison of these data shows that test compound VIII was found to be less toxic than butadione by 6.97 times, indomethacin by 63.83 and voltaren by 8.1 times.

Table 2: Anti-inflammatory activity and relative toxicity of 1,3-bis (aroylthiocarbamoyl) -orotic acid derivatives.

№	Chemical compounds	Dose mg/kg	Anti-inflammatory activity,%	Toxicity, mg / kg
1	1,3-Bis (p-chlorobenzoylthiocar-bamoyl) orotic acid (III)	50 100	63.3 75.9	> 3000
2	1,3-Bis (p-nitrobenzoylthio-carbamoyl) orotic acid (XIII)	50 100	59.1 70.6	> 3000
3	Butadion	100	28.1	430
4	Indometacin	10	36,4	47
5	Indometacin	25	43,2	370

Tests of a new drug, 1,3-bis (para-chlorobenzoylthiocarbamoyl) orotic acid (III), were performed to detect bacteriostatic activity.

Bacteriostatic doses of the drug were studied on daily cultures of pyogenic, opportunistic and pathogenic microorganism strains.

Passport data of the used strains of microbes:
1. Staphylococcus aureus - No. 629
2. Proteus vulgaris - No. 260
3. Streprococcus piogenes - Dick I
4. Pseudomonas aerogenosa - CCM - No. 2124
5. Escherichia coli - O111 B4 No. 24
6. Salmonella typhimurium - No. 5215

The bactericidal effect of the drug III was determined by the method of serial dilutions.

As a result of studies, it was found that the minimum bactericidal dose of the drug III was equal to a concentration of 125 γ / ml.

Taken as a control, the well-known drug sulfadimethoxin showed a bacteriostatic effect against pyogenic microbes at a concentration of 2000 γ/ ml, and in relation to the causative agent of intestinal infections at a concentration of 500 γ / ml.

Thus, it was found that the newly synthesized 1,3-bis (para-chlorobenzoylthiocarbamoyl) orotic acid (III) has a pronounced bactericidal activity at a concentration of 125 γ / ml and higher, which is significantly (4-16 times) superior to antimicrobial activity of known sulfadimethoxin.

Tested 1,3-bis (para-chlorobenzoylthiocarbamoyl) orotic acid (III) in order to identify fungicidal action.

Initial tests of a chemical preparation against cotton disease with wilt, hommosis were carried out in laboratory conditions by exposure to the causative agents of the above diseases.

It was revealed that the test drug III has fungicidal activity. This is manifested in a growth retardation of the fungus wilt by 71% and bacteria of hommosis by 43%.

Consequently, 1,3-bis (para-chlorobenzoylthiocarbamoyl) orotic acid (III) has fungicidal properties, destructively acting on the fungus wilt and bacteria of cotton gummosis.

CONCLUSION

Based on the research results, we identified the following conclusions:

- It has been established that the interaction of substituted benzoylisothiocyanates with orotic acid produces derivatives of 1,3-bis (aroylthiocarbamoyl) -orotic acid with high yields.
- It was found that among the studied new derivatives of orotic acid, only compounds 1,3-bis (p-chlorobenzoylthiocarbamoyl) orotic acid (III) and 1,3-bis (p-nitrobenzoylthiocarbamoyl) orotic acid (XIII) have a significantly wider range of anti-inflammatory actions are of undoubted practical interest.
- It was found that the newly synthesized 1,3-bis (para-chlorobenzoylthiocarbamoyl) orotic acid (III) has a pronounced bactericidal activity at a concentration of 125 γ/ml and higher, which is significantly (4-16 times) superior to the antimicrobial activity of sulfadimethoxine.
- It has been established that 1,3-bis (para-chlorobenzoylthiocarbamoyl) orotic acid (III) has fungicidal properties, damaging the wilt fungus and bacteria of cotton gummosis.
- Further in-depth study of the preparations of 1,3-bis (p-chlorobenzoylthio-carbamoyl) -orotic acid (III) and 1,3-bis (p-nitrobenzoylthiocarbamoyl) orotic acid (XIII) is advisable to identify their role as anti-inflammatory, bactericidal and fungicidal agents, for use in medicine and agriculture.

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