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In silico and *in vitro* studies of urea-derived sugars that exhibit potential antitumor properties

Ernazarova Baktygul Kochkorbaevna
Candidate of Chemical Sciences, Associate Professor
Jalal-Abad State University named after B. Osmonov, Republic of Kyrgyzstan
Bakirova Aida Adilbekovna
Candidate of Chemical Sciences, Associate Professor
Jalal-Abad State University named after B. Osmonov, Republic of Kyrgyzstan
Dzhumanazarova Asylkan Zulpukarvna
Doctor of Chemical Sciences, Full Professor
Institute of Chemistry and Phytotechnology, National Academy of Sciences of Kyrgyz Republic, Bishkek, Kyrgyzstan

Abstract: Computer evaluation of the spectrum of biological activity makes it possible to determine the most promising areas for testing the pharmacological action of specific substances. We have studied sugar carbamide derivatives by a computer method (*in silico*) and experimentally (*in vitro*), for which a high probability of antitumor activity is predicted with a low probability of cytotoxic activity. Low cytotoxic activity was confirmed in the MTT test on 5 cell lines. Compounds that are promising for synthesis and experimental study have been identified with a high probability of antitumor activity, presumably not associated with cytotoxic action.

Key words: computer prediction, spectra of biological activity, derivatives of sugar carbamides, synthesis, carbohydrate, heterocyclic compound, antitumor activity, cytotoxic activity.

1. Introduction

The main tasks of modern pharmacology are the search and study of the new drugs mechanisms of action for their subsequent introduction into widespread medical practice. The process of creating medicinal products is quite complex and includes several interrelated stages. At the first stage of drug development, synthetic chemists start to work, synthesizing new chemical compounds with potential biological activity. Usually, synthetic chemists carry out a targeted synthesis of compounds or modify the chemical structure of already known endogenous (produced in the body) biologically active substances.

Purposeful synthesis of medicinal substances implies the creation of biologically active substances with desired pharmacological properties. As a rule, such a synthesis is carried out in a

series of related chemical compounds, in which substances with specific activity were previously identified.

The creation of new anticancer drugs in a series of urea derivatives remains as a promising direction in chemotherapy, including a comparative study of the analog drugs characteristics. These studies provide insight into how a change in chemical structure affects the spectrum of antitumor action.

The *relevance* of the scientific research is, despite the large number of synthesized anticancer drugs recently, the issue of relationship between structure and specific activity remains important, which has not been studied enough yet. Therefore, the search for new compounds with potential antitumor properties and their correlation with the structure is proceeding.

The *aim* of the study is to carry out computer prediction of biologically active compounds from the number of sugar carbamide derivatives and to identify compounds with antitumor activity.

Literature review. This research is of a practical miportance and based on data from scientific articles, the authors of the PASS computer program V.V. Poroikova, D.A. Filimonova, A.A. Lagunina, A.V. Zakharova and D.S. Druzhilovsky. The *methodological basis* of the research is based on *in silico* and *in vitro* methods.

Study results. We hypothesized that inclusion of a glycoside amide bond with an open hydroxyl group as an active metabolite in the molecule of heterocyclic compounds can change the pathway of drug promotion into the tumor and the effect on it. In addition, the presence of an open hydroxyl group in the molecule can lead to a significant improvement in the solubility of the compounds obtained.

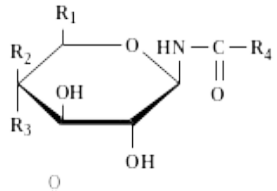
Molecules containing a heterocyclic nucleus of pyrazole and pyridine, along with antibacterial and antiparasitic activities, have other types of biological activity [1-3].

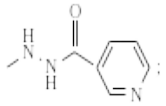
Compounds studied *in silico* were synthesized in the laboratory of organic synthesis at the Institute of Chemistry and Chemical Technology of the National Academy of Sciences of the Kyrgyz Republic and the laboratory of physicochemical analysis of the Jalal-Abad State University [4-6]. *In vitro* experiments of 5 selected out of 23 synthesized compounds were carried out at the Research Institute of EDiTO FSBI “N.I. NN Blokhin "of the Ministry of Health of Russia.

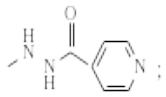
Computer prediction of biological activity was carried out using the PASS system, developed and constantly improved by the employees of the N.N. Gorky Research Institute of Biomedical Chemistry. VN Orekhovich "[7-9]. The used version of PASS 12.06.22 predicts

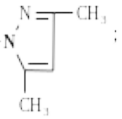
6400 types of biological activity based on the analysis of a training array containing structural formulas and data on the biological activity of 313 345 chemical compounds. The structural formulas of the compounds under study are entered into the system in the form of files in the MOL or SDF format. The average forecast error is 4.9%. Predictions are calculated as the probability of a compound to exhibit a certain biological activity (Pa) and not to exhibit it (Pi). Usually, for new chemical compounds, the threshold for the selection of promising compounds is considered to be Pa> 0.5.

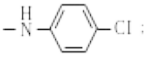
The objects of this study are 23 compounds containing heterocycles with glycosylamide bonds, the formulas of which are shown in Scheme 1:

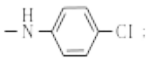


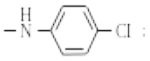
1. R_1=CH_2OH; R_2=OH;R_3=H; R_4=  ;

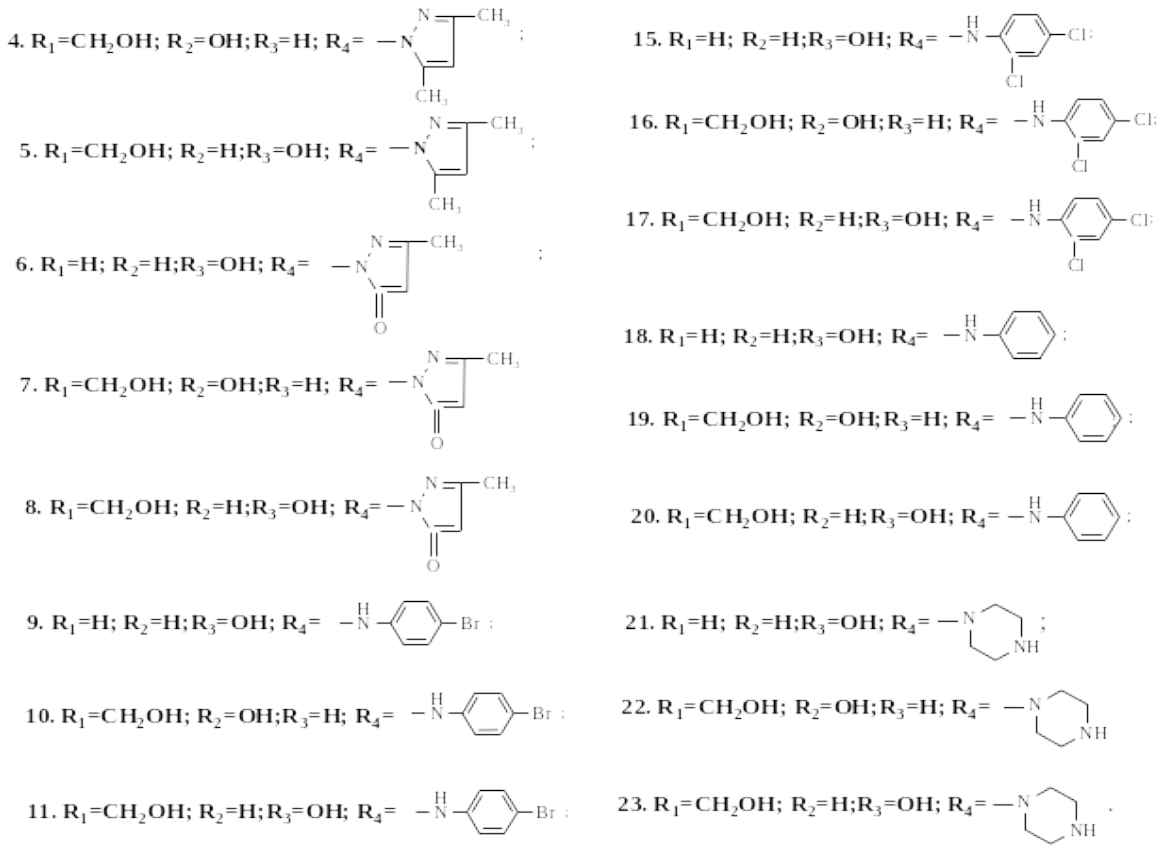
2. R_1=CH_2OH; R_2=OH;R_3=H; R_4=  ;

3. R_1=H; R_2=H;R_3=OH; R_4=  ;

12. R_1=H; R_2=H;R_3=OH; R_4=  ;

13. R_1=CH_2OH; R_2=OH;R_3=H; R_4=  ;

14. R_1=CH_2OH; R_2=H;R_3=OH; R_4=  ;



Scheme 1. General formula of sugar carbamides.

Structural formulas generated in electronic form using the ISIS Draw program from the formulas for the 23 indicated sugar carbamides contained in their paper passports, which were saved in the ISISBASE format database [10]. Molecular weight values retrieved from the ISISBASE database, in which they were automatically calculated when entering the structural formulas of compounds.

Results before experimental (*in silico*) screening

After checking the correctness of the structure of the obtained compounds, the generated structural formulas of the compounds under study were entered into the ISISBASE database, from which they were exported to SDF files for input into the PASS system.

Compounds for which the probability of manifestation of antitumor activity Pa ≥0.5 were obtained during computer analysis qualified as promising for experimental study as potential antitumor substances.

Table 1 shows the results of predicting 9 types of biological activity (antitumor, cytotoxic, general toxicity, antimetastatic activity and a number of mechanisms of action of anticancer drugs) in the form of values of the probability of a compound having antitumor activity (**Pa**) and the probability of the absence of this activity (**Pi**) for 23 studied compounds.

Таблица 1. The results of predicting the biological activity of urea glycoside derivatives.

№	Compound names	Biological activity type								
		Antineo-plastic	**Angioge-nesis inhibitor**	**Antimeta-static**	**Apoptosis agonist**	**Antileu-kemic**	**Cyto-static**	**Cyto-toxic**	**Toxic**	**Anti-mitotic**
		Pa / Pi	Pa / Pi	Pa / Pi	Pa / Pi	Pa / Pi	Pa / Pi	Pa / Pi	Pa / Pi	Pa / Pi
1	N-(-D-galactopyranosyl -1)-2-nicotinoyl-semicarbazide	0,799/ 0,012	0,657/ 0,007	0,407/ 0,044	0,285/ 0,136	0,179/ 0,088	0,216/ 0,117	0,100/ 0,176	0,081/ 0,375	0,019/ 0,621
2	N-(-D-galactopyranosyl-1)-2-isonicotinoyle-semicarbazide	0,779/ 0,014	0,613/ 0,010	0,297/ 0,128	0,384/ 0,051	0,162/ 0,099	0,173/ 0,156	0,084/ 0,199	0,078/ 0,384	0,020/ 0,598
3	1-[(N- –D-xylopyranosyl)-carbomoyl]-3,5-dimethylpyranosyl	0,565/ 0,053	0,457/ 0,026	0,402/ 0,046	0,107/ 0,390	0,010/ 0,743	0,132/ 0,198	0,093/ 0,185	0,190/ 0,213	0,042/ 0,390
4	1-[(N- –D-galactopyranosyl)-carbomoyl]-3,5- dimethylpyranosyl	0,622/ 0,040	0,539/ 0,016	0,502/ 0,019	0,245/ 0,168	0,041/ 0,314	0,182/ 0,148	0,148/ 0,130	0,254/ 0,167	0,034/ 0,463
5	1-[(N- –D-glucopyranosyl)-carbomoyl]-3,5-dimethylpyranosyl	0,599/ 0,045	0,266/ 0,088	0,479/ 0,024	0,122/ 0,351	0,025/ 0,447	0,113/ 0,227	0,172/ 0,112	0,278/ 0,155	0,152/ 0,086
6	1-[(N- –D-xylopyranosyl)-carbomoyl]-]-3-methylpyrazolon-5	0,470/ 0,081	0,329/ 0,058	0,697/ 0,002	0,081/ 0,474	0,008/ 0,826	0,126/ 0,207	0,084/ 0,200	0,143/ 0,263	0,012/ 0,771
7	1-[(N- –D-galactopyranosyl)-carbomoyl]-3- methylpyrazolon -5	0,536/ 0,061	0,417/ 0,034	0,702/ 0,002	0,207/ 0,209	0,030/ 0,405	0,175/ 0,155	0,137/ 0,140	0,196/ 0,207	0,011/ 0,800
8	1-[(N- –D-glucopyranosyl)-carbomoyl]-3- methylpyrazolon -5	0,536/ 0,061	0,417/ 0,034	0,702/ 0,002	0,207/ 0,209	0,030/ 0,405	0,175/ 0,155	0,137/ 0,140	0,196/ 0,207	0,011/ 0,800
9	N-(-D-xylopyranosyl)-p-bromphenylthiourea	0,770/ 0,016	0,699/ 0,005	0,231/ 0,124	0,206/ 0,209	0,040/ 0,324	0,193/ 0,138	0,142/ 0,136	0,152/ 0,251	0,036/ 0,452
10	N-(-D-galactopyranosyl)-p bromphenylthiourea	0,784/ 0,014	0,750/ 0,005	0,422/ 0,040	0,344/ 0,101	0,149/ 0,108	0,241/ 0,100	0,198/ 0,096	0,208/ 0,198	0,027/ 0,524
11	N-(-D-glucopyranosyl)-p-bromphenylthiourea	0,784/ 0,014	0,750/ 0,005	0,422/ 0,040	0,344/ 0,101	0,149/ 0,108	0,241/ 0,100	0,198/ 0,096	0,208/ 0,198	0,027/ 0,524

12	N-(-D-xylopyranosyl)-p-chlorphenyl-thiourea	0,706/ 0,025	0,626/ 0,009	0,304/ 0,080	0,173/ 0,261	0,028/ 0,421	0,186/ 0,144	0,142/ 0,136	0,181/ 0,221	0,020/ 0,600
13	N-(-D-galactopyranosyl)-p-chlorphenyl-thiourea	0,733/ 0,021	0,689/ 0,006	0,459/ 0,030	0,313/ 0,119	0,113/ 0,141	0,233/ 0,105	0,197/ 0,097	0,243/ 0,174	0,017/ 0,657
14	N-(-D-glucopyranosyl)-p-chlorphenyl-thiourea	0,733/ 0,021	0,689/ 0,006	0,459/ 0,030	0,313/ 0,119	0,113/ 0,141	0,233/ 0,105	0,197/ 0,097	0,243/ 0,174	0,017/ 0,657
15	N-(-D-xylopyranosyl)-2,4-dichlorphenyl-thiourea	0,612/ 0,042	0,583/ 0,012	0,267/ 0,098	0,153/ 0,293	0,025/ 0,453	0,169/ 0,161	0,109/ 0,166	0,200/ 0,204	0,014/ 0,712
16	N-(-D-galactopyranosyl)-2,4-dichlorphenyl-thiourea	0,658/ 0,033	0,651/ 0,008	0,436/ 0,036	0,286/ 0,135	0,097/ 0,161	0,215/ 0,118	0,164/ 0,117	0,267/ 0,160	0,013/ 0,750
17	N-(-D-glucopyranosyl)-2,4-dichlorphenyl-thiourea	0,658/ 0,033	0,651/ 0,008	0,436/ 0,036	0,286/ 0,135	0,097/ 0,161	0,215/ 0,118	0,164/ 0,117	0,267/ 0,160	0,013/ 0,750
18	N-(-D-xylopyranosyl)-phenylthiourea	0,782/ 0,014	0,626/ 0,009	0,244/ 0,114	0,148/ 0,300	0,155/ 0,104	0,383/ 0,047	0,084/ 0,199	0,152/ 0,251	0,017/ 0,661
19	N-(-D-galactopyranosyl)-phenylthiourea	0,795/ 0,012	0,689/ 0,006	0,429/ 0,038	0,289/ 0,133	0,312/ 0,041	0,452/ 0,033	0,138/ 0,140	0,208/ 0,198	0,015/ 0,710
20	N-(-D-glucopyranosyl)-phenylthiourea	0,795/ 0,012	0,689/ 0,006	0,429/ 0,038	0,289/ 0,133	0,312/ 0,041	0,452/ 0,033	0,138/ 0,140	0,208/ 0,198	0,015/ 0,710
21	N-(-D-xylopyranosyl-carbomoyl)-diethylenediamine	0,569/ 0,052	0,562/ 0,013	0,347/ 0,063	0,226/ 0,188	0,014/ 0,634	0,206/ 0,126	0,152/ 0,128	0,137/ 0,271	0,024/ 0,556
22	N-(-D-galactopyranosyl-carbomoyl)- diethylenediamine	0,632/ 0,038	0,643/ 0,008	0,488/ 0,022	0,370/ 0,087	0,073/ 0,207	0,257/ 0,090	0,209/ 0,090	0,189/ 0,214	0,019/ 0,624
23	N-(-D-glucopyranosyl -carbomoyl)-diethylenediamine	0,632/ 0,038	0,643/ 0,008	0,488/ 0,022	0,370/ 0,087	0,073/ 0,207	0,257/ 0,090	0,209/ 0,090	0,189/ 0,214	0,019/ 0,624

Table 1 shows that for all studied derivatives of sugar carbamides, low cytostatic activity is predicted. Comparison of the results of predicting cytostatic and antitumor activity suggests that the introduction of glycosylureas into the structure of heterocyclic compounds into the molecule reduces the likelihood of cytostatic activity, does not prevent the high probability of these compounds having antitumor activity.

For most of the compounds from this group, antineoplastic activity prognosed with a high probability and cytotoxic / cytostatic activity with a low probability. For 5 compounds from Table 1 with the highest probability of manifestation of antitumor activity, a prognosis of the cytotoxic effect on tumor cells of 5 lines, used at the Research Institute of EDiTO in in vitro screening, was carried out. The results shown in Table 2.

These compounds recommended for experimental studies as potential anticancer drugs.

Table 2. The results of predicting the cytotoxic effect on tumor cells of 5 lines of 5 urea glycosides (from Table 1., keeping the numbering) with the highest probability of antitumor activity.

№	**Compound name**	**Anti-tumor prognosis activity** ***Pa / Pi***	**Cytostatistical activity prognosis** ***Pa / Pi***
1	N-(β-D-galacto-pyranosylcarbamoyl -1)-2-isonicotinsemicarbazide	0,779 0,014	0,766 0,005 PC-3 cells 0,577 0,029 MCF7 cells 0,502 0,050 A549 cells 0,169 0,190 HCT-116 cells 0,007 0,901 Jurkat cells
4	1-[(N-β-D- galactopyranosyl) carbamoyl -3,5-dimethylpirazol	0,622 0,040	0,585 0,035 A549 cells 0,361 0,087 MCF7 cells 0,213 0,135 PC-3 cells 0,021 0,717 HCT-116 cells 0,006 0,908 Jurkat cells
10	N-(-D-galactopyranosyl)-p-bromphenylthiourea	0,784 0,014	0,561 0,038 A549 cells 0,534 0,018 PC-3 cells 0,249 0,164 MCF7 cells 0,012 0,802 HCT-116 cells 0,015 0,833 Jurkat cells
13	N-(-D-galactopyranosyl)-p-chlorphenylthiourea	0,733 0,021	0,664 0,024 A549 cells 0,501 0,022 PC-3 cells 0,403 0,068 MCF7 cells 0,018 0,799 Jurkat cells 0,008 0,852 HCT-116 cells
14	N-(-D-glucopyranosyl)-p-chlorphenylthiourea	0,733 0,021	0,561 0,038 A549 cells 0,534 0,018 PC-3 cells 0,249 0,164 MCF7 cells 0,012 0,802 HCT-116 cells 0,015 0,833 Jurkat cells

***In vitro* studies.** An experimental study of cytostatic activity of these 5 compounds carried out on the basis of the laboratory of experimental diagnostics and biotherapy of tumor cells in the Research Institute of EDiTO FSBI "N.I. N.N. Blokhin» Ministry of Health in Russia. Cytotoxic activity was investigated on 5 human tumor cell lines: PC-3 prostate adenocarcinoma; colon carcinoma HCT-116; Jurkat T cell lymphoblastic leukemia; breast adenocarcinoma MCF-7; lung carcinoma A549.

Cell lines were cultured in RPMI-1640 medium containing 10% fetal calf serum, 10 mM HEPES (Sigma, USA), 2 mM L-glutamine (Sigma, USA), 40 ng / ml gentamicin (ICN, USA), amino acids and vitamins (PanEko, Russia) at 37˚C in an atmosphere of 5% CO2. The cells were maintained in the logarithmic growth phase by continuous subculture of the culture after 3–4 days. To detach adherent cultures from plastic, Versene solution was used.

For the MTT test, cells were dispensed into 198 μl of complete RPMI-1640 medium into 96-well flat bottom plates (Costar, USA). One day later, the test compounds were added to each well at a concentration of 100 μM, and the cells were incubated for 72 h in 5% CO2 at 37 ° C. Each compound was put in a triplet. Compounds were dissolved in dimethyl sulfoxide so that the concentration of dimethyl sulfoxide in the well did not exceed 1%. Wells with cells with 1% dimethyl sulfoxide in complete growth medium were used as a control [11].

A compound was considered active if, at a concentration of 10 μM, at least on one of the lines, cell survival is ≤50% (IC50 ≤10 μM). The measurement error did not exceed 5%.

The study of cytotoxic activity on all cell lines listed above did not reveal activity in any of the 5 compounds studied (the IC50 value exceeded the specified activity criterion of 10 μM). The results of an experimental study of cytotoxic activity correlate with the results obtained using the PASS system for predicting cytostatic activity.

Conclusion

The investigated derivatives of carbamides of sugars according to the predicted physicochemical properties can be candidates for the development of drugs based on them. Compounds that are promising for synthesis and experimental study have been identified with a high probability of manifestation of antitumor activity, which, presumably, is not associated with cytotoxic action. It is advisable to continue the study of the antitumor activity of sugars containing residues of heterocyclic bases attached to carbohydrate fragments in animals with experimental tumors.

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