Journal of Agricultural Chemistry and Environment, 2022, 11, 15-23
https://www.scirp.org/journal/jacen
ISSN Online: 2325-744X
ISSN Print: 2325-7458

Study of Biological Activity and Toxicity of Thiosemicarbazides Carbohydrate Derivatives by *in Silico*, *in Vitro* and *in Vivo* Methods

Baktygul Ernazarova[1], Taitokur Zhusubaliev[1], Zarylkan Asilbek kyzy[1], Aida Bakirova[1], Gulsara Zhusupbaeva[2], Orozby Akparalieva[1], Zhypargul Abdullaeva[3*], Nasibakhon Razykova[1], Asilkan Dzhumanazarova[4], Galina Apryshko[5], Alina Orozmatova[1]

[1]Department of Pharmacy and Medical-Biological Disciplines, Zhalal-Abad State University, Zhalal-Abad, Kyrgyzstan
[2]Zhalal-Abad Scientific Center, South Department Academy of Sciences, Zhalal-Abad, Kyrgyzstan
[3]Science and Research Department, Osh State University, Osh, Kyrgyzstan
[4]Institute of Chemistry and Phytotechnology, National Academy of Sciences, Bishkek, Kyrgyzstan
[5]National Medical Research Center of Oncology Named after N.N. Blokhin, Moscow, Russia
Email: *jypar.science@oshsu.kg

How to cite this paper: Ernazarova, B., Zhusubaliev, T., Asilbek kyzy, Z., Bakirova, A., Zhusupbaeva, G., Akparalieva, O., Abdullaeva, Z., Razykova, N., Dzhumanazarova, A., Apryshko, G. and Orozmatova, A. (2022) Study of Biological Activity and Toxicity of Thiosemicarbazides Carbohydrate Derivatives by *in Silico*, *in Vitro* and *in Vivo* Methods. *Journal of Agricultural Chemistry and Environment*, **11**, 15-23.
https://doi.org/10.4236/jacen.2022.111002

Received: December 13, 2021
Accepted: January 17, 2022
Published: January 20, 2022

Abstract

Computer analysis of N-(β-D-galactopyranosyl)-thiosemicarbazide compounds by *in silico* method revealed high probability of antibacterial (antimycobacterial), anti-tuberculosis (antituberculosic), antiviral (Influenza), antitumor (antineoplastic) 9 > Pa > 0.5 and with a low probability of cytotoxic/cytostatic (cytostatic/cytotoxic) activities. An experimental study by *in vitro* and *in vivo* methods allowed us to conclude that studied new synthetic compound N-(β-D-galactopyranosyl)-thiosemicarbazide in the studied concentrations has a pronounced bactericidal and bacteriostatic effects.

Keywords

Biological Activity, Synthesis, Urea, Carbohydrate, Compounds, Hyperurcemia, Ototoxicity, Testing, Toxicity, Anti Tuberculosis, Antitumor

1. Introduction

In recent years, computer programs have been widely used to predict biological activity and toxic effects of organic compounds. Among the programs that can be used to predict various types of biological activity is the PASS program developed by the V.N. Orekhovich RAMS [1].

The use of the PASS program makes it possible, among a wide group of analyzed compounds, to select those that with a high degree of probability have the

required types of biological activity and, at the same time, with a low degree of probability, give undesirable toxic effects. When choosing promising compounds, not only main, but also side pharmacological effects were considered.

The fundamental problem of the relationship between the biological activity and the structure of chemical compounds and the search on this basis for new highly active medicinal substances is of fundamental importance for modern pharmacology. For this purpose, we have developed methods for the preparation of carbohydrate derivatives of thiosemicarbazides using the Lawesson reagent [2].

Molecule structure have an important role in the pharmacological activity of thiosemicarbazide derivatives [3], compounds having a pyridine ring and a thiosemicarbazide system as a well-known carrier antituberculosis agent with biological action were synthesized [4].

2. Research Methods and Materials

In this article, study of biological activity and toxicity of thiosemicarbazides carbohydrate derivatives was conducetd by *in silico*, *in vitro* and *in vivo* methods based on our previous works [5] [6]. Acute toxicity of N-(*β*-D-galactopyranosyl)-thiosemicarbazide was tested in the Biotechnology and Chemistry, Bacteriology Departments of Kyrgyz Republican Center for Diagnostics and Expertise.

3. Results and Discussions

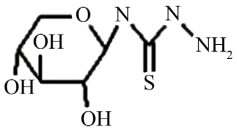
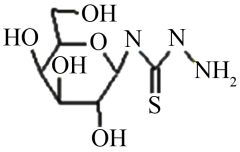
3.1. Possibility Assessment in Using the PASS Computer System for Biological Activity

The results of assessing the possibility of using the PASS computer system to predict biological activity by structural formula for chemical compounds as a stage in pre-experimental screening of new substances are presented below. **Table 1** shows the results of predicting 14 types of biological activity in the form of values of the probability of presence (Pa) and the probability of absence of this activity (Pi).

With the studied virtual compounds in N-(*β*-D-galactopyranosyl)-thiosemicarbazide, antibacterial (antimycobacterial), anti-tuberculosic (antituberculosic), antiviral (antiviral (Influenza)), antitumor (antineoplastic >) activity of the compounds are predicted with a high probability (0.9 > 0.5) and with a low probability of cytotoxic/cytostatic (Cytostatic/Cytotoxic) activity. Next, we analyzed possible toxic effects with a probability Pa > 0.5 for compounds 1, 2 based on their structural formulas (**Table 2**).

The results of computer predictions, we found that compounds 1.2 are predicted to have increased side effects, the ability to cause hyperuricemia an increased level of uric acid in the blood. Among the studied compounds, promising substances for experimental research identified with a high probability of antibacterial activity in N-(*β*-D-galactopyranosyl)-thiosemicarbazide compounds (Pa/Pi 0.799/0.004 antimycobacterial).

Table 1. Results of glycosylthiosemicarbazides biological activity.

No.	Compound name	Structure	Pharmacological effect results Pa Pi activity
1	N-(*β*-D-xylopyranosil)-thiosemicarbazide		6 of 464 Possible Pharmacological Effects at Pa > 0.500 0.654 0.007 Antimycobacterial 0.637 0.037 Antineoplastic 0.593 0.005 Antidiabetic symptomatic 0.576 0.004 Antineoplastic (small cell lung cancer) 0.548 0.005 Restenosis treatment 0.523 0.010 Antituberculosic
2	N-(*β*-D-galactopyranosil)-thiosemicarbazide		14 of 464 Possible Pharmacological Effects at Pa > 0.500 0.799 0.004 Antimycobacterial 0.718 0.004 Antituberculosic 0.700 0.004 Restenosis treatment 0.679 0.007 Antiviral (Influenza) 0.685 0.029 Antineoplastic 0.628 0.013 Antiviral (Poxvirus) 0.586 0.005 Antidiabetic symptomatic 0.543 0.005 Antiviral 0.564 0.028 Immunostimulant 0.549 0.014 DNA synthesis inhibitor 0.539 0.005 Antioxidant 0.544 0.013 Antibacterial 0.511 0.024 Cytostatic 0.508 0.022 Antidiabetic

3.2. Studies on Acute Toxicity of N-(*β*-D-Galactopyranosyl)-Thiosemicarbazide

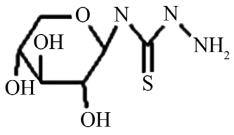
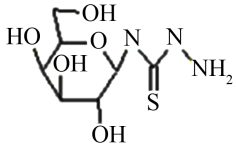
The acute toxicity of N-(*β*-D-galactopyranosyl)-thiosemicarbazide was tested in the Biotechnology and Chemistry, Bacteriology Departments of Kyrgyz Republican Center for Diagnostics and Expertise. Study aim of acute toxicity was to determine tolerable, toxic and lethal doses of a pharmacological substance and cause of animals' death [6].

When studying the toxicology of newly synthesized compounds, the first prerequisite is to determine the parameters of acute toxicity. These indicators are necessary to establish the degree of hazard of a chemical, as well as, for further research, where knowledge of the degree of acute toxicity is required.

Acute toxicity of pharmacological substances [7] [8] is determined by the following parameters: LD_0 is the maximum tolerated dose, LD_{50} is an average lethal dose, LD_{100} is minimum lethal dose [9]. LD_{16} and LD_{84} are also determined to establish the confidence limits of LD_{50} the average lethal dose.

There are a number of classifications of chemical substances for the assessment of acute toxicity [10] [11] [12]. To assess the toxicity of antiparasitic drugs, the classification according to [12] [13] is more suitable. Based on the foregoing,

Table 2. Results of glycosylthiosemicarbazides toxic effects.

No.	Compound name	Structure	Pharmacological effect results Pa Pi activity
1	N-(*β*-D-xylopyranosil)-thiosemicarbazide		9 of 321 Possible Toxic and Adverse Effects at Pa > 0.500 0.747 0.010 Hyperuricemia 0.680 0.046 Neuritis 0.664 0.052 Renal insufficiency 0.643 0.031 Acidosis, metabolic 0.628 0.042 Acidosis 0.640 0.067 Anemia, hemolytic 0.640 0.067 Hyperactivity 0.574 0.111 Dysesthesia 0,.512 0.089 Optic neuritis
2	N-(*β*-D-galactopyranosil)-thiosemicarbazide		23 of 321 Possible Toxic and Adverse Effects at Pa > 0.500 0.818 0.005 Hyperuricemia 0.689 0.039 Diarrhea 0.682 0.047 Renal insufficiency 0.682 0.049 Myocardial ischemia 0.652 0.048 Hypoxia 0.628 0.035 Optic neuritis 0.620 0.042 Psychoses 0.605 0.027 Peripheral neuropathy 0.599 0.046 Optic neuropathy 0,596 0.080 Hyperactivity 0.549 0.051 Ototoxicity 0.679 0.007 Antiviral (Influenza) 0.685 0.029 Antineoplastic 0.628 0.013 Antiviral (Poxvirus) 0.586 0.005 Antidiabetic symptomatic 0.543 0.005 Antiviral 0.564 0.028 Immunostimulant 0,549 0.014 DNA synthesis inhibitor 0.539 0.005 Antioxidant 0.544 0.013 Antibacterial 0.511 0.024 Cytostatic 0.508 0.022 Antidiabetic

we studied the acute toxicity parameters of a new carbohydrate derivative of thiosemicarbazides N-(*β*-D-galactopyranosyl)-thiosemicarbazide.

The experiments were carried out on 36 clinically healthy white mice of both sexes with a live weight 18 to 22 grams. The substance was administered to animals orally in the form of a 10% solution using a syringe equipped with a special metal probe, in various doses. Control animals received an appropriate volume of sodium chloride saline solution.

During the experiment, the animals were not limited to feeding and watering. The experiments lasted 12 days, during which the general condition, the nature and degree of chemical toxicity, the time of death of the experimental and con-

trol mice were observed. The corpses of the dead experimental animals were subjected to a visual pathoanatomical autopsy to establish the degree and nature of organ damage and the causes of death.

Statistical processing of digital materials was carried out by the method [14], modified [15], using ordinary graph paper [16].

The results of the experiments showed that the nature and degree of chemical toxicity in white mice were in direct proportion to the doses of the studied compound. Signs of poisoning when giving large doses appeared for the first time within minutes and were often fatal within 1 to 2 hours after giving. They were mainly expressed in the manifestation of an excited state (anxiety, increased reaction to external stimuli, tachycardia, rapid breathing), from feed tons of water.

Then this condition passed on to progressive oppression, leading to complete prostration. Breathing is shallow, frequent, and intermittent. Rapid palpitations, sometimes turning into arrhythmias. The death of animals that received large toxic doses of the compound occurred mainly on the first day of administration of the substance. The surviving animals showed mild diarrhea and poor appetite, which soon subsided.

The postmortem autopsy of dead white mice revealed: the back of corpses is soiled with liquid feces; the brain is swollen, hyperemic; the mucous membrane of the stomach and intestines is strongly hyperemic, in some places there are areas of extensive hemorrhage and necrosis; the liver is enlarged, dark red in color, its parenchyma is softened; kidneys of normal size, multiple punctate hemorrhages under the membrane; the heart is flabby, the myocardium is soft, the ventricles contain dark red blood clots, there are punctate hemorrhages on the epicardium and endocardium; the lungs are swollen of a dark red color, the blood vessels are dilated.

The results of statistical processing of digital experimental data showed that (**Table 3**) the maximum tolerated dose (LD_{50}) of N-(β-D-galactopyranosil)-thiosemicarbazide for white mice was 400 mg/kg, LD_{16} was 754 mg/kg, the average lethal dose LD_{50} was 1134 (957 ± 1311) mg/kg, LD_{84} was 1534 mg/kg and absolutely lethal dose (LD_{100}) was 2000 mg/kg.

Table 3. Acute toxicity parameters of N-(β-D-galactopyranosyl)–thiosemicarbazide tested on mice.

Dose, ml/mice quantity	Mice quanlity	Results		Acute toxicity parameters, mg/kg
		Extinct	Survived	
400	6	0	6	
800	6	2	5	$LD_0 = 400$
1200	6	3	4	$LD_{16} = 754$
1600	6	4	2	$LD_{50} = 1134$ (957 ± 1311)
2000	6	6	0	$LD_{84} = 1534$
Control	6	0	6	$LD_{100} = 2000$

The results obtained indicate that, according to the current classification of the hazard of chemotherapeutic drugs according to the degree of impact on the body (GOST 12.1.007-76), N-(*β*-D-galactopyranosyl)-thiosemicarbazide belongs to substances of III class of moderate hazard [17] confirms the prospects for further study of this compound in this direction.

3.3. Study of Antibacterial Activity of N-(*β*-D-Galactopyranosyl)-Thiosemicarbazide

The experiments were carried out at the Department of Biotechnology and Chemistry and at the Department of Bacteriology of the Kyrgyz Republican Center for Diagnostics and Expertise by the *in vitro* method using generally accepted bacteriology techniques [18] [19] [20] [21] [22].

For this, by means of serial dilutions with distilled water (1:10; 1:20; 1:40 … 1:2650), various concentrations of the substance were prepared, the bactericidal effect of which was studied by plating Salmonella infection of lambs on agar-agar in Petri dishes (Salmonellatyphimurium, 04), salmonellosis of calves (Salmonella Dublin, 09) and colipathogenic serotypes of Escherichia coli (Escherichiacoli 055, 026) and the addition of 0.1 ml of each dilution of the substance.

The results were taken into account after daily cultivation in a thermostat at t = 37˚C, by measuring the diameters of the zones (in mm), and by the absence of microorganism growth at the place where the compound was applied. Distilled water (*i.e.*, solvent) served as a control.

The results of experiments carried out on a solid nutrient medium show (**Table 4**) that the test compound showed bactericidal activity against the selected microbial cultures, although no pronounced species specificity was observed in its action.

Table 4. Bactericidal activity of N-(*β*-D-galactopyranosyl)-thiosemicarbazide in a dense nutrient medium (agar-agar).

Microorganisms	Studied substance dilutions								
	1:10	1:20	1:40	1:80	1:160	1:380	1:640	1:1280	1:2560
N-(*β*-D-galactopyranosil)-thiosemicarbazide									
Sal. typhimurium 04	22*	20	18	16	14	7	3	1	0
Sal. dublin 09	20	19	18	17	13	6	2	1	0
Esch. coli 055	21	20	19	16	13	7	4	2	0
Esch. coli 026	20	19	17	14	12	8	4	2	1
Distilled water									
Sal. typhimurium 04	-	-	-	-	-	-	-	-	-
Sal. dublin 09	-	-	-	-	-	-	-	-	-
Esch. coli 055	-	-	-	-	-	-	-	-	-
Esch. coli 026	-	-	-	-	-	-	-	-	-

*numbers show diameter of microorganisms' growth zones (in mm).

Table 5. Bacteriostatic activity of N-(*β*-D-galactopyranosyl)-thiosemicarbazide in meat peptone broth.

Microorganisms	Studied substance dilutions								
	1:10	1:20	1:40	1:80	1:160	1:380	1:640	1:1280	1:2560
N-(*β*-D-galactopyranosil)-thiosemicarbazide									
Sal. typhimurium 04	–	–	–	–	–	–	+	+	+
Sal. dublin 09	–	–	–	–	–	–	+	+	+
Esch. coli 055	–	–	–	–	–	+	+	+	+
Esch. coli 026	–	–	–	–	–	–	+	+	+
Distilled water									
Sal. typhimurium 04	+	+	+	+	+	+	+	+	+
Sal. dublin 09	+	+	+	+	+	+	+	+	+
Esch. coli 055	+	+	+	+	+	+	+	+	+
Esch. coli 026	+	+	+	+	+	+	+	+	+

– lack of growth, + presence of growth.

The bacteriostatic activity of the substance was studied by diluting it in mesopatamia broth at the same concentrations as in the previous experiment, followed by sowing pure cultures in it. The results of this series of experiments showed (Table 5) that the bacteriostatic substance acts on *Esch. coli* 055 from a dilution of 1:380, for other cultures from a dilution of 1:640.

4. Conclusion

Experiment results after analyses allow concluding, that studied new synthetic compound N-(*β*-D-galactopyranosyl)-thiosemicarbazide in studied concentrations has a pronounced bactericidal and bacteriostatic effects. Thus, it was proved that experimental study of biological activity coincided with the prediction data, which is an average accuracy of computer prediction with sliding control about 90%.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Understanding Chemical-Biological Interactions. PASS Online. http://way2drug.com/PassOnline/index.php

[2] Ernazarova, B.K. (2013) Thionation of Carbohydrate Derivatives of Semicarbazides. *Collection of Materials of the XXI International Scientific and Practical Conference* "*Science and Modernity*-2013", Novosibirsk, 182-186.

[3] Popovici, C., Pavel, C.M., Sunel, V., Cheptea, C., Dimitriu, D.G., Dorohoi, D.O.,

David, D., *et al.* (2021) Optimized Synthesis of New Thiosemicarbazide Derivatives with Tuberculostatic Activity. *International Journal of Molecular Sciences*, **22**, Article ID: 12139. https://doi.org/10.3390/ijms222212139

[4] Pitucha, M., Karczmarzyk, Z., Swatko-Ossor, M., Wysocki, W., Wos, M., Chudzik, K., Ginalska, G. and Fruzinski, A. (2019) Synthesis, *in Vitro* Screening and Docking Studies of New Thiosemicarbazide Derivatives as Antitubercular Agents. *Molecules*, **24**, Article No. 251. https://doi.org/10.3390/molecules24020251

[5] Ernazarova, B., Bakirova, A., Dzhumanazarova, A., Abdullaeva, Z., Berkmamatov, S. and Zhusupbaeva, G. (2020) Thionization Method of Glycosyl Urea and Carbamide Sugars. *International Journal of Organic Chemistry*, **10**, 111-122. https://doi.org/10.4236/ijoc.2020.103008

[6] Ernazarova, B., Dzhumanazarova, A., Bakirova, A., Abdullaeva, Z., Zhusupbaeva, G., Asylbek Kyzy, Z. and Arzybaev, M. (2020) Synthesis, Assessment of Biological Activity and Toxicity for N-(*β*-D-Glycopyranosyl)-Thiosemicarbazides. *International Journal of Organic Chemistry*, **10**, 159-169. https://doi.org/10.4236/ijoc.2020.104012

[7] Turner, R.A. (1967) Screening Methods of Pharmacology. Academic Press, New York, 111-112.

[8] Guengerich, F.P. (2011) Mechanisms of Drug Toxicity and Relevance to Pharmaceutical Development. *Drug Metabolism and Pharmacokinetics*, **26**, 3-14. https://doi.org/10.2133/dmpk.dmpk-10-rv-062

[9] Liakoni, E., Dolder, P.C., Rentsch, K.M. and Liechti, M.E. (2016) Presentations Due to Acute Toxicity of Psychoactive Substances in an Urban Emergency Department in Switzerland: A Case Series. *BMC Pharmacology & Toxicology*, **17**, Article No. 25. https://doi.org/10.1186/s40360-016-0068-7

[10] Walum, E. (1998) Acute Oral Toxicity. *Environmental Health Perspectives*, **106**, 497-503. https://doi.org/10.1289/ehp.98106497

[11] Barenboim, G.M. and Malenkov, A.G. (1986) Biological Active Substances. Science, Moscow, 284 p.

[12] Gimenez-Bastida, J.A., Martinez Carreras, L., Moya-Pérez, A. and Laparra Llopis, J.M. (2018) Pharmacological Efficacy/Toxicity of Drugs: A Comprehensive Update about the Dynamic Interplay of Microbes. *Journal of Pharmaceutical Sciences*, **107**, 778-784. https://doi.org/10.1016/j.xphs.2017.10.031

[13] Belenky, M.L. (1963) Elements of a Quantitative Assessment of the Pharmacological Effect. Science, Leningrad, 146 p.

[14] Albert, A. (1987) Selective Toxicity, Part II. Medicine, Moscow, 49-56.

[15] Sanotsky, I.V. (1970) Methods for Determining the Toxicity and Hazard of Chemicals (Toxicometry). Medicine, Moscow, 219-225.

[16] Sanotsky, I.V. and Ulanova, I.P. (1976) The Criterion of Harm in Hygiene and Toxicology in Assessing the Hazard of Chemical Compounds. Medicine, Moscow, 76-81.

[17] Bear, L.I. (1977) Pesticide Handbook. Harvest, Kiev, 448-450.

[18] Litchfield, J.T. and Wilcoxon, F.J. (1949) Asimplified Method of Evaluating Dose-Effect Experiments. *Journal of Pharmacology and Experimental Therapeutics*, **96**, 99-113.

[19] Roth, Z. (1960) Physiologia Bogtmoslovenica. Praga, 125-128.

[20] Kudrin, A.N. and Ponamareva, G.T. (1967) Application of Mathematics in Experimental and Clinical Medicine. Meditsina Publishers, Moscow, 26-142.

[21] Izmerov, N.F., Sonotsky, N.V. and Sidorov, K.K. (1977) Parameters of Toxometry

of Industrial Poisons with a Single Exposure (Reference Book). Medicine, Moscow, 34-36.

[22] Vasiliev, D.A., Shcherbakov, A.A., Karpunina, L.V., Zolotukhin, S.N. and Shvidenko, I.G. (2003) Methods of General Bacteriology. Ulyanovsk, 17-23.