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PHYSICAL - CHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITY OF NEW THIOUREIDES COMPOUNDS WITH POTENTIALLY ANTIMICROBIAL ACTIVITY

V. Ordeanu, V.A. Voicu, Gh. Ţarălungă, M. Necşulescu, Angela Postoarcă, Diana Popescu, Lucia E. Ionescu, Simona N. Bicheru, Cristina Secară, B. Patranichi

Army Center for Medical Research, Bucharest

Abstract. The study presents a concise selection of applicative research of pharmaceutical microbiology for the purpose of testing the biological activity of certain new thioureides having potentially anti-infectious effects. Objectives: the synthesis, the physical-chemical analysis and biological testing of the thioureides of the 2-(4-ethyl-phenoxymethyl)-benzoic acid (3 series), screening on aerobic bacteria, anaerobic bacteria, viruses, fungi, yeasts, spores and toxins, testing the anti-microbial effect according to the current European norms, acute toxicological testing on laboratory animals, comparing the results and statistical processing for the classification in pharmacological groups. The majority of the tested amides presented anti-bacterial effect; however, this was unequal, varying from very weak to very good; we deemed very weak the average effect occurring by the inhibition area having a diameter smaller than or equal to 6 mm (i.e. the substance application area), weak, good equivalent to the standard sample and very good higher than this value. We registered 6 substances having an effect. The effect is better on the Gram-positive bacteria, rather than on the Gram-negative ones, almost double, which could lead us to conclude that the effect would take place via the alteration of the bacterial wall structure, probably through the inhibition of the peptidoglycan synthesis. On average, the value of the diameter of the inhibition area of thioureides batch 2 as weighed against the standard bacteria tested, as compared with the mean for the phenol sample 5%. The compounds tested do no indicate antifungal, antiviral, sporicidal or anti-toxin effect.
Keywords: antimicrobial, anti-infectious, chemotherapeutic, thioureides

Background

Disinfection is the destruction and elimination of a great part of the microorganisms on surfaces and objects, in the air, water, food etc., with the explicit specification that, following the proper action, certain microorganisms are likely to carry on, particularly spores, and would not put in danger the patients' health. The method tackles with the second link of the epidemiologic practice, means and mechanisms of transmission of the infection. Disinfection uses a set of methods and (mechanical, physical, chemical) means meant to remove or destroy the pathogenic or conditionally pathogenic factors from the environment, which are likely to generate infection. The disinfection is prophylactic when the purpose is to eliminate potential germs (treating water in order to make it drinkable, milk pasteurization, food treatment, disinfection in health units, communities etc.) and "in centre of contagion", when the action concerns known germs eliminated in the environment by the diseased or by germ carriers. The success of disinfection is influenced by many factors: the manner and level of microbial contamination, the extent of exposure to the disinfectant and the concentration

Viorel Ordeanu
37 C.A. Rosetti Str., Bucharest, Romania
email: ordeanu_viorel@yahoo.com

used, the surrounding temperature, the interaction between the disinfectant and the material support on which it is applied etc.

Microbial resistance to antibiotics may be defined as the ability of the pathogenic microorganisms to survive and multiply in the presence of one or more anti-microbial substances. Resistant germs are or become "tolerant" to antibiotics, circumventing by various modalities the expected anti-bacterial outcome, following the administration of therapeutic dosages, non-toxic for the organism. The bacterial sensitivity test to antibiotics, both by the classic, diffusimetric procedure, and by the modern, automated means, by qualitative and quantitative methods, represents the easiest manner of assessing the effect of the antimicrobial substances on the germs. Furthermore, the bacterial sensitivity test to antibiotics, including all of its variations and updates, such as the E-test, provides valuable information with respect to the anti-microbial treatment to be applied in a situation of infection. Moreover, the bacterial sensitivity test to antibiotics is to be read only as "a guide to the selection of the anti-microbial therapy" and not as a "prescribed order", since it is a useful tool as long as "we don't expect from it more than what it can provide".

For such reasons, new anti-microbial chemotherapeutic solutions are always necessary, a genuine competition between drugs and infectious microorganisms. The Department of Pharmaceutical Chemistry synthesized several series of compounds in the group of thioureides carrying potentially anti-microbial action, which we have tested from a microbiological and toxicological perspective.

Material and method

The tests have been carried out according to the indications in "Methods and usual techniques in the clinical laboratory", published by the Ministry of Health and the Academy of Medical Sciences and the Standards "Antiseptiques et desinfectants" AFNOR. We have also tested certain news work techniques for the completion of the standard operation procedures.

The general chemical structure of the tested compounds is represented in Figure 1.

Methyl, chlorine, bromine radicals were added to this structure, and tens of substances were obtained, which were then tested on batches, in order to compare the potential anti-microbial effect, by screening on aerobic bacteria, anaerobic bacteria,

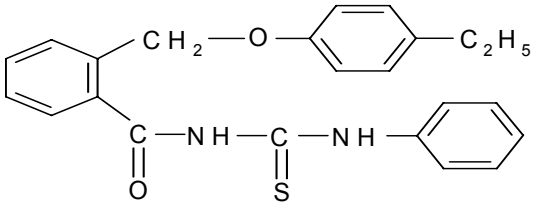


Figure 1. Chemical structure of the tested compounds

viruses, fungi, yeasts, spores and toxins. The testing of the anti-microbial effect was carried out according to the current European norms.

The used method includes an experimental test measuring physical-chemical features, antimicrobial properties and the acute toxicity of the selected compounds.

1. The bactericidal effect. Culture media: Nutrient broth, Nutrient agar, Agar Mueller-Hinton. Bacterial strains used: *Escherichia coli* CIP54 127 (1) (ATCC 10 536), *Pseudomonas aeruginosa* CIP A 22 (1), *Staphylococcus aureus* Oxford CIP 53 154 (1) (ATCC 9 144), *Bacillus anthracis* 34F2 Sterne, *Clostridium botulinum* (strictly anaerobic). The aerobic bacterial strains were cultivated in nutrient broth, static cultures in 16/160 pellets, complying with the following parameters: incubation temperature 37°C, cultivation time 24h; the anaerobic strain was cultivated in VF broth for 72h. Upon the completion of the cultivation, smears were carried out from each bacterial strain (Gram coloration), which were examined microscopically for the purpose of assessing bacterial purity and fungi sterility. The 24h pure cultures were used in testing the bactericidal activity (according to the current norms for the testing of the disinfectants) of the amides researched in dilutions of 1/10; 1/50; 1/100; 1/200; 1/400; and 1/800, after 5 and 10 min of contact (culture + disinfectant). We retain that from the 6 substances, 2 are water-soluble in 1/10, two are sparingly water-soluble in 1/10, and the other two are only partially soluble, resulting in supersaturated solutions with which it was impossible to work for such a concentration. From each dilution, after 5 and 10 min of contact, Petri dish cultures were made with nutrient agar. The dishes were incubated for 48 hours, with examinations at 24 and 48 hours for the purpose of assessing the bacterial growth. The assessment of the bacterial cultures was made according to the following plan: + Growth (culture) on the seeding line = 0 points, ± Growth (culture) in the form of rare colonies = 1 point, L Lack of culture growth = efficient bactericidal activity = 2

points. For the purpose of comparison, we applied a global index, respectively the sum and arithmetic mean of the anti-microbial effect noted.

Following the notice that in other microbiology laboratories the anti-bacterial effect of certain substances from this set had been quantified, we adapted the method of the diffusimetric bacterial sensitivity test to antibiotics. Thus, in 15 cm Petri dishes, with Mueller-Hinton medium for bacterial sensitivity test to antibiotics, cultivated with bacterial suspension of INCDMI Cantacuzino reference strains (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*) and *Bacillus cereus* (vegetative and spore-forming forms), we formed 4 test series. We placed with the sample 19 substances on each dish (3 dishes per series), with a micro-spatula containing approximately 1 mg of pure substance. Following a 37°C aerobic incubation, we read the dishes at 24 and 48 hours, and observed no areas of bacterial growth inhibition. Since it is possible that the effect does not occur because the substances are sparingly soluble, therefore only partially diffusing in the culture medium (which contains 96% water), we made 5% water solutions. With the 20 µl automatic dispensing pipette, we placed the equivalent of 1 mg of active substance, using the same technique (dish, medium, culture, sample, incubation, reading), and no inhibition areas were observed. The tests were made in parallel with the retest in the same conditions of a previously tested series of chemically related substances, anti-microbial C series amides, for which the effect was obvious, particularly on the Gram-positive bacteria, which were used for control. Considering that the substances tested are sparingly soluble, we thought it would be possible that we have real concentrations smaller than those estimated and we made another series of tests, in which the solving agent used was dimethyl sulfoxide (DMSO). In this dissolvent, the solubilization was fast and complete and we repeated the test in the same conditions. With the 50 µl automatic dispensing pipette, we placed 5% solution, equivalent to 2.5 mg of the active substance. The positive control was the standard sample solution of Phenol 5% in distilled water. The other control substances were the distilled water and the DMSO, in order to exclude the potential proper effect of the dissolvent; however, this lacked; using the same technique (dish, medium, culture, sample, incubation, reading) no inhibition areas were noted. The tests were made in parallel. We used blanks in order to check the medium and the bacterial growth. Since we registered no marked anti-bacterial effect, we did not go on with tests for CMI, CMB etc.

2. The antifungal effects. Testing the inhibiting effect of the solutions on the fungi growth in *Aspergillus parasiticus*. The test was made on Petri dishes, ϕ = 10 cm, in solid Czapek-Dox medium. The solutions to be tested, concentration 10%, were added in sterility circumstances, in 0.5 and 1 ml/dish volumes. Following the absorption of the solutions, the fungi suspension of conidia = 1 ml/dish was added, the same volume and spore density and on the control dish we observed the growth of the fungi cultures, following a 26°C incubation during 7 days.

3. The sporicidal effects. *Aspergillus parasiticus* spore-forming cultures were used in order to test the effect of the solutions on spore stability. The 10% concentration solutions tests were applied on an *Aspergillus parasiticus* spore-forming fungi culture, allowing direct contact between the fungus and the solution to be tested for 5, respectively 20 minutes. The fungi spores suspensions was used as inoculation for the cultivation on the solid Czapek Dox medium, monitoring the growth of the strains over a duration of 7 days. The cultivation was made on ϕ = 8 cm Petri dishes; we applied 3 ml of the solution to be tested in each dish. Incubation of the cultures was made at 26°C for 7 days. The following aspects were noted: The fungi cultures from spore suspensions treated for 5, respectively 20 minutes with the 10% solutions, present the same macroscopic morphological features as the Control culture from the untreated spore suspension.

4. The detoxifying effects. Determining the effect of the 10% solutions on the aflatoxins. For this purpose, we used a solution of gross aflatoxin extracted from an afla-toxigenic fungi culture. The 4.1 mg% concentration aflatoxin solution was interacted with the 1 ml aflatoxin solutions + 1 ml solution to be tested. The verification of the detoxifying effect was made after 24 hours, using the method of thin-layer chromatography. The application of the samples on the chromatoplate was made automatically with the Linomat IV applicator in fixed volumes (10 µl/belt). The chromatography conditions were: Chromatographic support – Silicagel 60 F 254 Merck HPTLC, 10/20 cm; Developing - Chloroform: Acetone: Water (140:20:0.3) Migration - unidimensional ascending; Visualisation (qualitative assessment of the fluorescent bands) - UV 254- 366 nm. Tracks 1-6 are the aflatoxin samples treated with the solutions, and track 7 is the untreated aflatoxin sample.

5. The antiviral effects. Infection factors: West Nile virus, tick encephalitis virus and hepatitis B virus. The Flaviviruses are sensitive to high temperatures. In blood or protecting solutions they are deactivated at +56°C in 30 minutes. Temperatures lower than -60°C and freeze-dying merely ensure the conservation of infectivity for very long time spans. Other physical agents - RX, Gamma radiations or ultrasounds – deactivate them. The Flaviviruses, like all enveloped viruses, lose their infectivity after treatment with diluting agents of the lipids: ether, chloroform, Na deoxycholate or with detergents. They are deactivated by 3-8% formaldehyde, 2% glutaraldehyde, 1% iodine and 2% sodium hypochlorite. In general, the tick Flaviviruses are relatively more resistant than mosquito-ones. The Flaviviruses are cultivated in a wide variety of cells, vertebrates, mammals, reptiles, amphibians or arthropods. Currently used: primary cultures of chicken, duck or dog kidney embryo fibroblasts; continuous cell lines: LLC-MK2 (monkey kidneys), BHK-21, FRL-L (rhesus foetal lung), PK (pig kidney), Vero; mosquito cell continuous lines: C6/36 (*A. albopictus*), AP-61 (*A. pseudoscutellaris*), TRA-284 (*Toxorhynchites amboinensis*). The HB virus is one of the most resistant viruses. It preserves its infectivity in ser for 6 months at +30°C and for 10 hours at +60°C. Its destruction is reached through autoclave 30 minutes at 128°C, or through keeping at a pH of 2.4 for 6h. The chemical agents reverse the infectivity by alterations of the viral structures. Thus, deactivation is obtained after the treatment with sodium hypochlorite, for 10 min, 70% isopropylalcohol and 80% ethylalcohol at 11°C, for 2 min. As disinfectants, we can use $1^0/_{00}$ potassium permanganate, 5% formaldehyde, 2% glutaraldehyde, at a pH of 8.4. The HB virus will stay infectious at low temperatures, at -20°C for a period of 15 years, and after freeze-dying at +25°C, for one week. The cytopathic effect is present particularly in cultures from birds and mammals. It will become manifest in 1-7 days, by the rounding of the cells, with the monolayer presenting large areas of cell separations. The cellular lesions are represented by mitochondrial alterations, vacuolation of the cytoplasm and, specific, the hypertrophy of the membranes in the *endoplasmic reticulum*, where viral particles accumulate. In mosquito cells, the cytopathic effect may be minimal or absent (even if the virus is synthesized in high concentrations) or of syncytial type. In the ultra-microscopic exam, we note the hypertrophy of the internal cellular membranes, as well as in the mammal cells. Until now, the cultivation of the HB virus in cellular cultures has not been possible. We used the Vero cell line for the cultivation of the Flaviviruses. From the Vero 186 (1:3) cell line we made subcultures in 25 ml Kolle. For the monolayer separation, we used *Trypsin-Versene*, and for cultivation, the Eagle medium enriched with foetal calf ser (5%). The Kolles were incubated at 37°C for 24 h, and the second day examined with the inverted microscope in order to observe the monolayer formation. The disinfectant substances to be tested were interacted with the WN virus and TBE virus (1 ml virus + 1 ml disinfectant) and maintained at 37°C for 1h. The cultures were checked morphologically at 24, 48 and 72h. The results obtained were conclusive. Both the viruses and the disinfectant substances destroyed the cell monolayer, and by their bringing together we assume their effects cumulated. For this reason, we performed another test, immunoenzymatic, in which we used (WN and TBE) viral suspensions and normal brain suspension. We used the Bioelisa HBsAg kit that outlines the presence of the HBs antigen in various samples. The positive kit control was interacted with the three disinfectant substances. We also used a hepatitis B positive sample (alcohol diluted) that was also mixed with the three disinfectants. All mixes were kept at 37°C for 1h.

6. The acute toxicity screening test is made on white lab mice (mus musculus albicans), young (20 +/- 2g), by single intra-peritoneal injection of 0.5 ml working solution, on batches of 3-5 healthy animals of both sexes. They are clinically monitored for at least 3 days and mortality is registered. If the tested substance is toxic (mortality registered) and presents the expected pharmacodynamic effect (in our situation, anti-microbial effect), it is transmitted to the toxicology Laboratory for pharmaco-toxicological testing. For the purpose of determining the LD_{50} of the selected compound, we used a test pattern of acute toxicity. From the stock solution, working solutions were prepared by dilution.

Biologic material: 100 animals were included in the test, white mice weighing 18-25 g. The animals were distributed in homogenous groups of 10 animals each (equal numbers of groups of male and female mice), kept in appropriate microclimate (vivarium) having free access to water and food, away from insecticides. **Methods of administration of the working solutions:** the tested compound was administered intra-peritoneal, 40 ml/kg, immediately after the weighing of the animals.

Animal monitoring: We monitored the animals' ponderal index and health for 7 days before the launching of the test. One hour before the beginning of the tests, the animals were prevented from having access to food, having free access to water. Animal monitoring during the test meant the monitoring and registration of the moment when the symptoms of intoxication emerged, recording of mortality at 1, 2 and 24 h.

This preliminary study used an experimental pattern of acute toxicity on a rodent species (lab mice) and a way of administration (i.p.) for the evaluation of LD_{50}: 10 study groups of 10 animals each were included in the test (5 females and 5 males):

Study group **1** was represented by 10 animals (5 males and 5 females) in which a 40 ml volume was administered i.p. from the solution S1 of concentration 6.35 mg/ml with a toxic potential of 3.20. The test monitored and recorded the moment when intoxication symptoms and mortality emerged at 60 minutes and 24 hours following the intoxication.

Study group **2** was represented by 10 animals (5 males and 5 females) in which a 40 ml volume was administered i.p. from the solution S2 of concentration 9.56 mg/ml with a toxic potential of 3. The test monitored and recorded the moment when intoxication symptoms and mortality emerged at 60 minutes and 24 hours following the intoxication.

Study group **3** was represented by 10 animals (5 males and 5 females) in which a 40 ml volume was administered i.p. from the solution S3 of concentration 12.75 mg/ml with a toxic potential of 2.90. The test monitored and recorded the moment when intoxication symptoms and mortality emerged at 60 minutes and 24 hours following the intoxication.

Study group **4** was represented by 10 animals (5 males and 5 females) in which a 40 ml volume was administered i.p. from the solution S4 of concentration 25.60 mg/ml with a toxic potential of 2.60. The test monitored and recorded the moment when intoxication symptoms and mortality emerged at 60 minutes and 24 hours following the intoxication.

Study group **5** was represented by 10 animals (5 males and 5 females) in which a 40 ml volume was administered i.p. from the solution S5 of concentration 28.67 mg/ml with a toxic potential of 2.55. The test monitored and recorded the moment when intoxication symptoms and mortality emerged at 60 minutes and 24 hours following the intoxication.

Study group **6** was represented by 10 animals (5 males and 5 females) in which a 40 ml volume was administered i.p. from the solution S6 of concentration 32.11 mg/ml with a toxic potential of 2.5. The test monitored and recorded the moment when intoxication symptoms and mortality emerged at 60 minutes and 24 hours following the intoxication.

Study group **7** was represented by 10 animals (5 males and 5 females) in which a 40 ml volume was administered i.p. from the solution S7 of concentration 51.20 mg/ml with a toxic potential of 2.30. The test monitored and recorded the moment when intoxication symptoms and mortality emerged at 60 minutes and 24 hours following the intoxication.

The batches in which survivors were present were extended to 20 animals as follows:

Study group **8** in which a 40 ml volume was administered i.p. from the solution S4 (administered to batch no. 4) with a toxic potential of 2.60. The test monitored and recorded the moment when intoxication symptoms and mortality emerged at 60 minutes and 24 hours following the intoxication.

Study group **9** in which a 40 ml volume was administered i.p. from the solution S5 (administered to batch no. 5) with a toxic potential of 2.55. The test monitored and recorded the moment when intoxication symptoms and mortality emerged at 60 minutes and 24 hours following the intoxication.

Study group **10** in which a 40 ml volume was administered i.p. from the solution S6 (administered to batch no. 6) with a toxic potential of 2.5. The test monitored and recorded the moment when intoxication symptoms and mortality emerged at 60 minutes and 24 hours following the intoxication.

The statistic analysis of the test results was made by using the Origin 5.0 statistic computation program, leading to a LD_{50} value, an equation of the regression line and the correlation coefficient for $p< 0.05$.

Results and discussions

Physical-chemical features: Sparingly soluble in dilutions of 1/10-1/100 even under heat (at 40-45°C and mixing); soluble in dimethyl sulfoxide.

1. The thioureides tested presented different physical-chemical features, as powders, crystals or flakes of various sizes, white in color;

2. All of them are sparingly soluble in distilled water, but soluble in dimethyl sulfoxide;

3. None of them indicated marked anti-bacterial activity;

4. The animals injected with a dosage of 0.5 ml i.p., in 1/10 dilution, died several minutes after the shot, thus this dose is highly toxic, in fact typical for the majority of the active pharmacological substances.

5. It is necessary to test the anti-bacterial effect under conditions different from those standardized for disinfectants.

Anti-bacterial effect. The standard method was used, with predetermined dilutions and times (table I, II, III).

Crt. no.	Strain	Dilutions				
		1/50	1/100	1/200	1/400	1/800
1	E. coli	+	+	+	+	+
2	P. aeruginosa	+	+	+	+	+
3	S. aureus	+	+	+	+	+
4	B. anthracis	+	+	+	+	+
5	B.ant.(spores)	+	+	+	+	+
6	Cl. bot.	+	+	+	+	+
	SUM	0	0	0	0	0
	MEAN	0	0	0	0	0
	Observations					

Table I. Testing of bactericidal effect – 5 min contact time

Crt. no.	Strain	Dilutions				
		1/50	1/100	1/200	1/400	1/800
1	E. coli	+	+	+	+	+
2	P. aeruginosa	+	+	+	+	+
3	S. aureus	+	+	+	+	+
4	B. anthracis	+	+	+	+	+
5	B.ant.(spores)	+	+	+	+	+
6	Cl. bot.	+	+	+	+	+
	SUM	0	0	0	0	0
	MEAN	0	0	0	0	0
	Observations					

Table II. Testing of bactericidal effect – 10 min contact time

The majority of the thioureides tested through this technique indicated an anti-bacterial effect, however unequal, from very weak to very good; we deemed very weak the average effect occurring via the inhibition areas having a diameter smaller than or equal to 6 mm (i.e. the area of application of the substance), weak between 6 and 10 mm, good between 10 and 17.5 mm (equivalent to the standard sample) and very good when higher than this value. We saw 6 substances having a very weak effect, 3 weak, 5 good and 2 - very good effect. The effect is better on the Gram-positive bacteria, rather than on the Gram-negative ones, almost double, which could lead us to conclude that the effect would take place via the alteration of the bacterial wall structure, probably through the inhibition of the peptidoglycan synthesis.

On average, the value of the diameter of the inhibition area of thioureides batch 2, as weighed against the standard bacteria tested, is 9.1 mm, as compared with the mean for the phenol sample 5%, which is 13.8 mm.

Antifungal effect (table IV)

Sporicidal effect. We may say that the 10% solutions do not have a sporicidal effect. The same results are obtained with the other substances, with no exception.

Detoxifying effect. It can be very clearly outlined that the 2 fluorescent bands represented by AFB1 and AFG1 in the aflatoxin mix on track 7 are unaltered in the case of the samples treated with the solutions to be tested. The same results are obtained with the other substances, without exception.

Antiviral effect. Numerous attempts have been made, using: primary cultures of human adult or foetal hepatocytes and cell hepatoma stabilized lines. The infected cells do not present typical alterations or cytopathic effect, and the virus levels are minimal. Test on arboviruses: the results obtained indicate that the disinfectant substances do not have a definite effect on the WN and TBE viral

Crt. no.	Substance code	Staf.a.	B.cereus	E.coli	Ps.aer.	Total mm	Mean mm	Observations
	C1	8	0	0	0	8	2.0	very weak
	C2	11	6	8	9	34	8.5	weak
	C3	12	9	9	23	53	13.3	good
	C4	6	7	0	6	19	4.8	very weak
	C14	10	6	0	0	16	4.0	very weak
	C15	0	11	0	0	11	2.8	very weak
	C16	6	0	0	0	6	1.5	very weak
	C20	13	11	0	0	24	6.0	very weak
	C21	22	10	12	6	50	12.5	good
	C22	28	15	25	7	75	18.8	very good
	C23	6	20	10	0	36	9.0	weak
	C24	10	12	17	0	39	9.8	weak
	C25	10	11	24	18	63	13.3	good
	C27	14	14	14	7	49	12.3	good
	C28	14	16	17	17	64	16.0	very good
	C29	11	14	11	0	36	9.0	good
	Total mm	181	162	147	93	583	143.6	
	Mean mm	11,31	10,13	9,19	5,81		8.98	
M1	Phenol 5%	11	16	13	15	55	13.8	
M2	AD	0	0	0	0	0	0	
M3	DMSO	0	0	0	0	0	0	

Table III. Results of the tests on standard bacteria (collection I. Cantacuzino)

Solution to be tested (ml/dish)		Macroscopic mycological features after 7 days of incubation
Same results obtained in all substances	0.5	For both volumes, normal mycelium growth is noted, typical to the genus Aspergillus, without modifications or alterations of the spore-forming or pigmentation process for the mycelium. These observations remain valid for all the solutions tested.
	1	We may conclude that the solutions do not have an effect on the growth of the Aspergillus genus fungi.
Aspergillus parasiticus Control Culture		Mycelia web – compact, dense, spore-forming, dark green pigmented.

Table IV. The mycological test

suspensions, the dishes that contained the mixes of suspensions and disinfectants presenting color reaction. For HBV, the samples were made double, and following this test we concluded that disinfectant substances do not have effect on AgHBs.

The **acute toxicity screening test:** for the 1/10 dilution, the mice that were injected intra-peritoneal with 0.5 ml 10% concentrated solution, died in 5-10 min, indicating nervous symptoms. In the necropsy, discrete hemorrhagic lesions were noticed in the peritoneal cavity: congestion and hemorrhages on the bowel area. The same results were obtained with the other substances, without exception. The test pursued the assessment of the acute toxicity of an active pharmacological compound selected from a series of anti-microbial compounds. A chemical compound in the class of thioureides was tested, with a 406 g/mol molecular weight and the chemical structure in figure 2.

Results. The test results representing the mortality of the batches included in the experiment, cor-

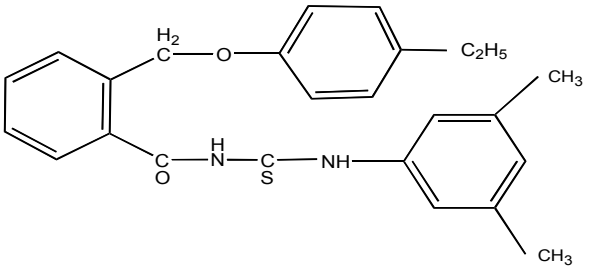


Figure 2. Chemical structure of the tested compound. The value of the intra-peritoneal LD50 in mouse is 1148.15 ± 18.8 mg /kg, corresponding to 2.55 ± 0.05 toxic potential

responding to the dosages administered, registered at 24 h, are given in table V.

Batch no.	Working solution	Concentration of the working solution (mg/ml)	Dosages administered (mg/kg/i.p.)	Volume administered i.p. (ml/kg)	Toxic potential of the solution	Percentage mortality %
1	S1	6.35	256	40	3.20	0
2	S2	9.56	384	40	3	0
3	S3	12.75	512	40	2.90	0
4	S4	25.60	1024	40	2.60	10
5	S5	28.67	1146.88	40	2.55	40
6	S6	32.11	1284.50	40	2.50	80
7	S7	51.20	2048	40	2.30	100

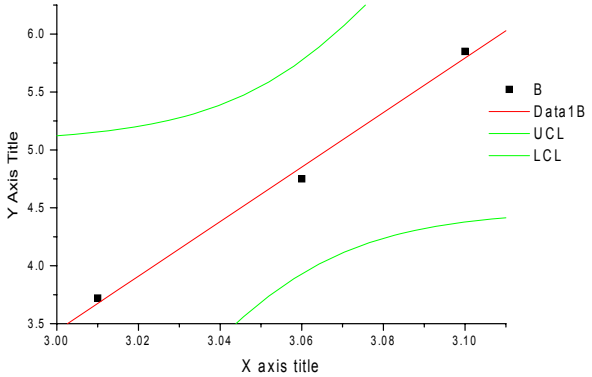
Table V. Test results of batches included in the experiment at 24 h following the intoxication

Logarithm values of the dosages corresponding to the batches in which survivors were present and the probits of the mortalities registered at 24 hours for the same batches were introduced in the computation software (Origin 5.0). Results of the statistical analysis are indicated in table VI.

Animal no./ batch	Dosage administered mg/kg/40ml i.p.	Dosage logarithm	Probit of mortality registered at 24 h
20	1024	3.01	3.72
20	1146.88	3.059	4.75
20	1284.5	3.10	5.85

Table VI. Results of the statistical analysis of the primary data

Graphic of the regression line described by the equation: $Y = A + B * X$



Parameter	Value	Error	
A	-67.18361	5.98907	
B	23.54098	1.95921	
R	SD	N	P
0.99655	0.12494	3	0.0528

The correlation coefficient is 0.9965 for p = 0.05

The equation of the regression line for p ≤ 0.05 is of the form $Y = 23.54X - 67.18$

Y is the probit corresponding to the effect monitored in the experiment, respectively the mortality.

X is the mathematical expression, respectively the logarithm of the dosage of substance that leads to mortality. To different mortality values (Y) resulting from the test, different X values correspond, respectively values of the substance dosages having lethal consequences.

The mathematical correspondence of the biological correlation dosage (logarithm dosage X) - effect (probit Y)s is represented by the equation of the regression line, in the above-given form. The correlation coefficient is a measurement resulting from the computation, representing the correlation between the dosage and the effect. The inferior limit accepted is 0.98.

For Y=5 respectively the probit corresponding to 50% mortality, the value of X respectively value $\log LD_{50}$ is 3.06. By logarithm, we have the value LD_{50} in mouse for the tested substance, of 1148.15 ± 18.8 mg/kg.

The toxic potential corresponding to the LD_{50} computed according to the protocol standardized in the Romanian Pharmacopeia is represented by the following values: 2.55 ± 0.05.

This experimental study has assessed, by determining $LD_{50,}$ the acute toxicity of an active pharmacological compound with an anti-microbial effect. The value of the intra-peritoneal LD_{50} in mouse is 1148.15 ± 18.8 mg/kg, corresponding to 2.55 ± 0.05 toxic potential. Hence, from the point of view of the toxicity, the substance may be included in the category of low toxicity substances.

Conclusions

The present study is a concise selection of the pharmaceutical microbiology issues approached by the research project "Partnership for the synthesis, physical-chemical characterisation and biological

activity testing of new thioureides with potentially anti-infectious action", funded by national competition by the Ministry of Education, Research and Innovation (ANCS/PNCDI/Partnerships, Contract no. P3 41-043 /2007, director Assoc.Prof. Carmen Limban, MD) carried out during 2007-2010 under the management of the University of Medicine and Pharmacy "Carol Davila", Bucharest.

The majority of the tested thioureides ave antibacterial effect; however, this was unequal, varying from very weak to very good; we deemed very weak the average effect occurring by the inhibition area having a diameter smaller than or equal to 6 mm (i.e. the substance application area), weak between 6 and 10 mm, good between 10 and 17.5 mm (equivalent to the standard sample) and very good higher than this value.

We registered 6 substances having a very weak effect, 3 weak, 5 good and 2 with very good effect.

The effect is better on the Gram-positive bacteria, rather than on the Gram-negative ones, almost double, which could lead us to conclude that the effect would take place via the alteration of the bacterial wall structure, probably through the inhibition of the peptidoglycan synthesis.

On average, the value of the diameter of the inhibition area of thioureides batch 2 as weighed against the standard bacteria tested is 9.1 mm, as compared with the mean for the phenol sample 5%, which is 13.8 mm.

The compounds tested do no indicate antifungal, antiviral, sporicidal or anti-toxin effect.

According the LD_{50} value, the tested compound may be considered as a low toxicity substance.

We consider useful the continuation of the series of syntheses and tests for the selection of new anti-microbial substances.

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