CHIMIE FARMACEUTICĂ ŞI CONTROLUL MEDICAMENTULUI

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EVALUATION OF SOME PHYSICO-CHEMICAL PROPERTIES OF PROPYLTIODIAZOLOCHINAZOLIN-ONE DERIVATIVES

EVALUAREA UNOR PROPRIETĂŢI FIZICO-CHIMICE ALE DERIVAŢILOR DE PROPILTIODIAZOLOCHINAZOLIN-ONĂ

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Rezumat. Apariţia unor noi forme de tuberculoză, inclusiv rezistente la tratament, precum şi complexitatea tratamentului antituberculos rămân factori de provocare în obţinerea şi cercetarea de agenţi antimicobacterieni noi din diferite clase, cu efecte maxime chiar şi împotriva bacilului drog-rezistent. În cadrul echipei de sinteză a Laboratorului de Sinteză Organică şi Biofarmaceutică de la Institutul de Chimie a fost realizată sinteza unui şir de oxadiazoli, pentru care a fost demonstrată acţiunea antimicobacteriană pronunţată *in vitro* şi toxicitatea mică a acestora. Au fost obţinuţi compuşi noi - derivaţi de propiltiodiazolochinazolin-onă sub formă de sare anorganică (sulfat) şi complex cu ciclodextrina, pentru care s-a efectuat un screening toxicologic, antimicrobian şi antituberculos. Totodată, s-au evaluat proprietăţile fizico-chimice ale compuşilor: punctul de topire, solubilitatea în diverşi solvenţi la trei nivele de pH, pierderile la uscare (umiditate) şi higroscopicitatea, analiza spectrală în IR. Studiile, redate în acest articol vor contribui la selectarea compusului optimal obţinut din acest grup, cu perspectiva elaborării unui nou remediu antituberculos.

Cuvinte cheie: tuberculoză, propiltiodiazolochinazolin-onă, proprietăţi fizico-chimice.

Abstract. The emergence of new forms of tuberculosis, including resistant to treatment, as well as the complexity of antituberculous treatment remain challenging factors in obtaining and researching new antimycobacterial agents of different classes, with maximum effects even against the drug-resistant bacillus. Within the synthesis team of the Organic and Biopharmaceutical Synthesis Laboratory of the Institute of Chemistry, a series of oxadiazoles was synthesized for which the pronounced antimycobacterial action in vitro was proved. The low toxicity was demonstrated as well. New compounds of propylthiodiazolokinazolin-one derivatives in the form of inorganic salt (sulfate) and cyclodextrin complex were obtained. The toxicological, antimicrobial and antituberculous screening on these substances was performed. At the same time, the physico-chemical properties of the compounds were evaluated: melting point, solubility in various solvents at three pH levels, drying losses (humidity) and hygroscopicity, spectral analysis in IR. The studies presented in this article will contribute to the selection of the optimal compound obtained from this group, with the perspective to develop a new antituberculosis product.

Keywords: tuberculosis, propylthiodiazolokinazolin-one, physicochemical properties.

Introduction

Nowadays, one third of the people in the all world is infected with Mycobacterium tuberculosis (MTB) and, therefore, they are at risk of developing active tuberculosis (TB). More than 99% of deaths and 95% of new TB cases happen in low- and middle-developed countries. The Republic of Moldova is placed by the World Health Organization (WHO) in the top of 18 priority countries fighting against tuberculosis in the European region. At the same time, the Republic of Moldova is one of the 27-th countries with a high incidence of multidrug-resistant tuberculosis [1]. Thus, one of the main aims of the National Tuberculosis Control Program for 2016-2020 is to elaborate the antitubercular treatment regimens by using the new antituberculosis medications, applying in children [2].

The bacterial resistance to antibiotics is constantly growing both in Europe and in the world. It represents an extremely complex and important problem in the whole world. According to the recent data, there are more than 240000 people annually exposed to the bacterial infection resistant to standard antibiotic treatment lines in Europe and the USA. Unfortunately, about 50000 of them culminated with death. Due to COVID-19 this situation is getting worse [3, 4]. Despite the urgent need for new antibacterial treatment options, there are a small number of new antimycobacterial medications used in clinical trials. Unfortunately, infections caused by surgical interventions or hospitalizations still represent the essential problem that can lead to reducing the effectiveness of the most advanced therapies for treating initial medical problems. Moreover, the cost of drug-resistant infections treatment is increasing by 1.5 billion euros annually. As a result, the design and synthesis of new compounds with effective antibacterial activity on multi-drug-resistant bacteria (MDR) is not only a desideratum but an obligation [3, 4].

According to the recent reports, it was found that in the Republic of Moldova 10 people were diagnosed with tuberculosis daily, every tenth was suffering from multi-drug-resistant tuberculosis, (MDR TB) and one person died daily caused by tuberculosis progresses, unless health services maintained and strengthened [5]. Tuberculosis is among the top causes of death as a result of infectious diseases in our country. Nowadays, there are no original autochthonous antimycobacterial drugs. On the other hand, the remedies used have a number of disadvantages: they are expensive, the spectrum of activity is limited, drug-resistance is developed by bacilli. The advanced bibliography analysis of the recent years leads to the conclusion that the development and appearance of new antimycobacterial classes of medications were behind the development of mycobacterial resistance. As a result, the therapeutic arsenal for treating MDR bacterial infections requires new molecules with superior efficacy to conventional ones. This is also highlighted in the World Health Organization's 2020 report recommending "stimulating innovation, research and development of new tools" to control bacterial resistance [6].

The COVID-19 is a pandemic that seriously threatens those small recent advances in reducing the incidence of TB disease. Due to disrupted health services, it has been estimated that the overall number of deaths from TB could increase by about 0.2-0.4 million just in 2020, caused by decreasing the number of diagnosed and treated people by about 25-50% in only 3 months [4].

The research of new compounds with anti-tuberculosis activity is a priority direction in the international and national scientific world. Thus, due to synthesis team of the Laboratory of Organic Synthesis and Biopharmaceuticals within the Institute of Chemistry, a series of aryl-oxadiazoles were obtained, including propylthiohinothiadiazolol, that represents one of the most active substances in this group [7, 8, 9, 10, 11, 12]. Obtaining inorganic salts, complexing with cyclodextrins are attempts to optimize bioavailability and to use these new derivatives as medicinal substances for the antituberculosis treatment, including multi-drug resistant forms.

The purpose of this study was to evaluate some physico-chemical parameters of three compounds from the propylthiodiazoloquinazolinone group.

Materials and Methods

Test substances. Three synthetic compounds from the propylthiodiazolokinazolinone group, obtained in the Laboratory of Organic Synthesis and Biopharmaceuticals within the Institute of Chemistry from the Republic of Moldova, were used as a test material: Propylthiohinothiadiazole (MF-001); Propylthiohinothiadiazole sulfate (MF-001-H2SO4); Propylthiohinothiadiazole complex with cyclodextrins (β-CD-MF-001); as internal reference standard was used 2- (propylthio) 5H- [1, 3, 4] thiadiasolo- [2,3-b] quinasolin-5-one substance, purified by recrystallization in the Laboratory of Analysis, Standardization and Control of Medications (LASCM) within the Scientific Center of Medicines of the Nicolae Testemitanu State University of Medicine and Pharmacy from the Republic of Moldova (concentration 99.98%) [13].

Melting behavior/Melting point. The melting point was determined in the LASCM using A. KRÜSS KPS Melting-Point Meter KPS II device. Each substance was measured three times, according to the requirements of the European Pharmacopoeia (Ph. Eur.) 10.0 [14]. The propriety of MF-001 and β-CD-MF-001 in melting was also studied by the thermal analysis method – differential scanning calorimetry (DSC), using the Mettler Toledo STARe Thermal Analysis system, version 6.0 (Schwerzenbach, Switzerland). About 2-5 mg of substances were examined in the temperature range from 25 to 300°C in argon flux (10 L/h). The heating rate was 5°C / min.

IR spectral analysis. The βCD + MF-001 binary complex was analyzed by the FT-IR (Fourier-transform infrared) spectrophotometer: SPECTRUM 100 (PERKIN ELMER, USA) at the Institute of Chemistry. Due to this method, which makes use of totally attenuated reflection, the substance was described and characterized. The binary mixtures were prepared by weighing the appropriate amounts of analyzed substances and homogenizing in agate mortar for 20 minutes. The FT-IR spectrum of MF-001 was compared to the spectrum of the binary mixture βCD + MF-001 (1: 1) and βCD + 1MF-001 + H2O (1: 1: 18). All FTIR spectra were recorded in the range from 4000 cm^{-1} to 400 cm^{-1}.

Solubility. Solubility determination was measured by the department of pharmaceutical technology of the educational institution "Belarusian State Medical University" (BSMU). The tests were carried out at temperatures of 20^{0C} and $37^{0}C$; solvents used: purified water, ethyl alcohol, dimethylsulfoxide (DMSO), hexane, chloroform, 0,1 M sodium hydroxide solution, 0,1 M hydrochloric acid solution. Dissolving media used: 0,1 M hydrochloric acid buffer M, with pH = 1.2, phosphate buffer with pH = 4.5, phosphate

buffer with pH = 6.8. The measurements were performed on the ERWEKA DT 800 Dissolution tester; the volume of the dissolution medium 250, 500 and 900 ml; temperature of the dissolution medium 37 ± 0.5 ° C; rotational speed 50 rotations per minute; sampling interval: 15, 30, 45, 60, 90, 120 minutes; number of samples tested in each dissolution medium: 6. Quantitative evaluation of the substances was performed spectrophotometrically on the Sagu 50 spectrophotometer, using buffer solutions with different pH values as comparison solutions.

The ability of PEG to solubilize the substances in the suspensions was determined. The suspensions were prepared by stirring 0.01 g of substances in 4 ml of water in a graduated tube. PEG was added dropwise until the volume doubled (8 ml).

Loss on drying. The determination of humidity was performed by the drying method, according to the requirements of Ph. Eur. 10 [14]. The exact amounts of substances were dried to a constant mass in the drying cabinet model 2B-151. Drying losses were calculated in %.

Hygroscopicity. The exact masses of the substances were placed in plastic ampoules and placed for 6 hours in a desiccator at the bottom where water was poured. The desiccator was hermetically covered with a lid to prevent the entering water from the outside. The ampoules were weighed every 2 hours. The amount of water absorbed was expressed in %.

Results and discussions

Melting behavior / Melting point.

The simplest parameter to estimate the purity of a solid organic substance is the melting point. The melting point is defined as the temperature at which the solid is in equilibrium with its melt. The pure substances have a fixed melting temperature, its exact determination (approx. 0.01°C) being possible only by plotting the melting curves. Impurities, even in very small quantities, lower the melting temperature and widen the melting range (1°C). This phenomenon is used to determine the identity of two substances with the same melting temperature.

The melting point was determined for all three test substances, according to the requirements of Ph. Eur. 10 [14]. As a result of the carried out investigations the close values of the melting range for MF-001 and β-CD-MF-001 were found. The β-CD-MF-001 melted with carbonization and the MF-001-H2SO4 melted under structural changes (the value of the melting point is not stable). The results of the determinations are shown in table 1.

To determine the degree of MF-001 capture by cyclodextrins and the formation of the β-CD-Mf-001 complex, the DSC curves of the pure substance and the 1: 1 binary mixture were recorded. It is known that when the „guest" molecule is captured in the cyclodextrin cavity, its melting, boiling or sublimation point is usually shifted to another temperature, during which the cyclodextrin decomposes. Thus, for a 1: 1 molar ratio, certain endothermic or exothermic peaks can be observed in the DSC curves, which

Table 1. The results of determining the values of melting points and range

Test substance	Melting point ^{0}C		
	Determination 1	Determination 2	Average
MF-001	116.2	116.4	116.3
MF-001-H_2SO_4	146.2	144.1	145.1
β-CD-MF-001	114.4 with carbonization	113.9 with carbonization	114.15 with carbonization

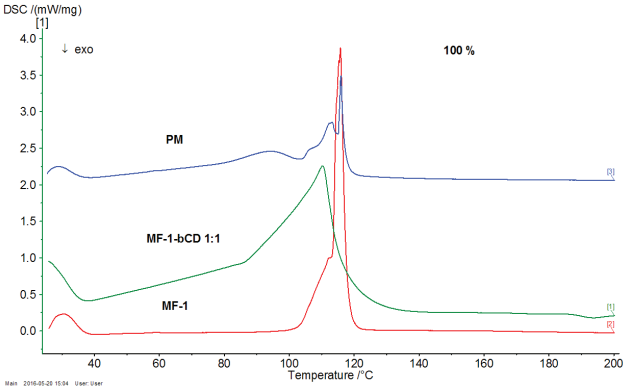

Fig. 1. DSC thermograms of substance MF-001 and β-CD-MF-001 complex

means that the physical mixing method does not provide a complete encapsulation of the binary mixture in the cyclodextrin cavity (fig. 1).

The thermograms showed very fine changes in the melting temperature of the binary system at lower values, and the reduction in the melting enthalpies at decreasing amounts of the substance. This phenomenon may be an indication of the dispersion of molecule crystals in the β-CD matrix, and the formation of very energetic crystals of MF-001. At the same time, the substance maintains a certain level of crystallinity in the crushed product.

FT-IR spectral analysis.

FT-IR spectroscopy is a modern spectral optical method, which is used to identify the molecular structures of medicinal substances based on the interactions of infrared light with some functional groups, bonds and structural units that are reflected in spectra. The interpretation of the FT-IR spectra of the test substances is a very important part. The identification of the chemical structure is based on the values of vibration frequencies characteristic of functional groups, shown in the table [16]. In particular, there is an increased interest for the molecules of MF-001 and the β-CD-MF-001 complex, because valence displacements that characterize the formation bonds of the complex are illustrated in the spectra.

According to the fig. 2 and fig.3, it was found that the

system spectrum presents mainly the spectra sum of its components. However, some of the absorption bands of the individual compounds may disappear from the system spectrum, for example, the band at 756 cm^{-1} in the β-CD spectra, which corresponds to the vibrations δCCO + δCCH. The FT-IR spectra recorded for the MF-001 + βCD binary system shows displacements of the absorption bands characteristic of the constituents. Thus, the absorption band characteristic of the –OH functional group, observed in the complex spectrum, has been shifted to a lower frequency. On the other hand, for the deformation vibrations of the C-H bonds in the βCD structure (1407, 1335, 1296, 1251 cm^{-1}) a displacement can be observed at higher wave numbers (table 2).

Table 2. Characteristic infrared absorption bands of functional groups

Group	Assignment	Range of absorption	Intensity of bands
-C-S-	ν C-S	683,9	medium narrow
$-CH_2-$	Scissoring βs	1463,4	medium narrow
-C=O	ν –C=O	1704,9	strong
-C=N-	ν -C=N	1608,3	strong
C_6H_5	ν -CH	1484,7 &3070,6	different
CH_3	ν -CH	1332,6	medium

Solubility.

The studies were made of the complete characterization of the degree of solubilization of the test substances by using polar, organic, polymeric, greasy solvents. These studies are particularly important for predicting bioavailability, but also for the preformulation stage of a possible pharmaceutical form. The results of the determinations are shown in tables 3 and 4. According to tables 3 and 4, it was found that the dissolution behavior of substances in different solvents has varied as a result of the different chemical structure. Thus, MF-001 is freely soluble in DMSO, PEG 400 and Polysorbate 20, is sparingly soluble in chloroform, while in the rest of the solvents it is very slightly soluble or practically insoluble.

Due to the form of salt of an inorganic acid, a good dissolution of MF-001-H2SO4 in water, alcohol and dissolution media with predetermined pH was expected, but it did not happen. The substance is freely soluble only in Polysorbate 20 and moderately soluble in DMSO, in the rest of the solvents it does not dissolve.

The β-CD-MF-001 complex is close in solubility to MF-001, being more advantageous in solubility in DMSO, Sunflower Oil and Polypropylene.

It is well-known that water-cosolvent mixtures are widely used in pharmaceutical technology to increase the solubility of poorly water-soluble medications during the pharmaceutical forms development. PEG 400 is most com-

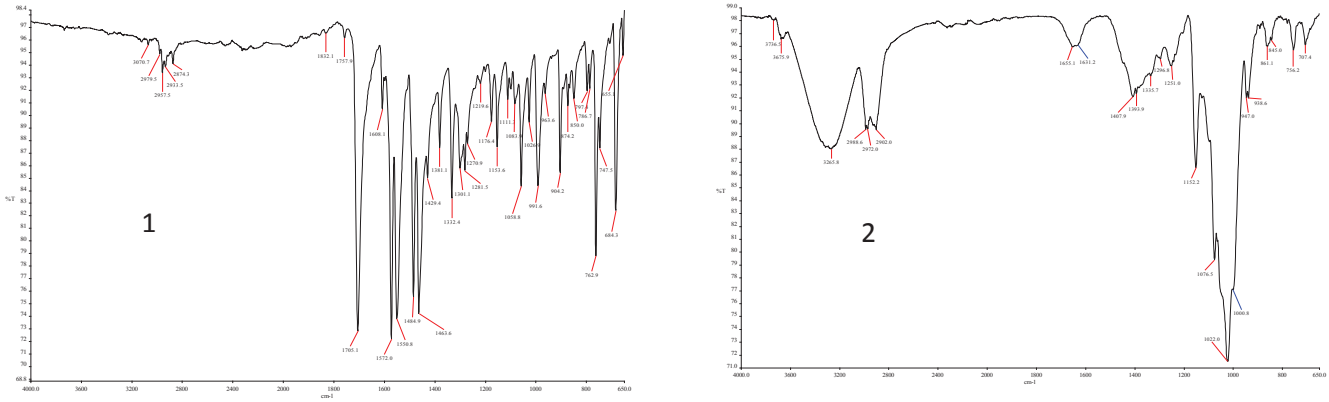

Fig.2. FT-IR spectra of MF-001 (1) and β-CD (2)

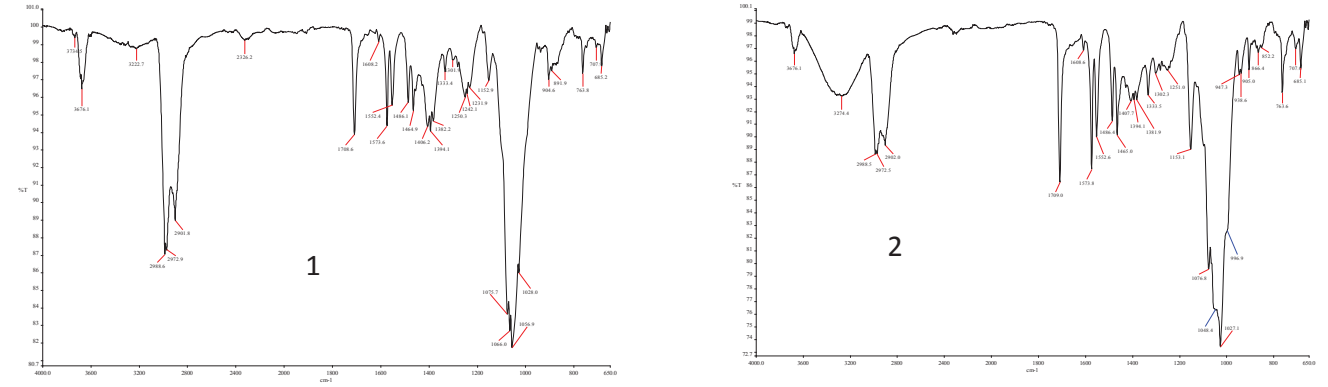

Fig. 3. FT-IR spectra of βCD + MF-001 (1: 1) (1) and βCD + 1MF-001 + H2O (1: 1: 18) (2)

Table 3. The results of the determination of solubility in BSMU

Solvent	MF-001	MF-001-H_2SO_4	β-CD-MF-001
[1]Purified water	Practically insoluble	Practically insoluble	Practically insoluble
[1]Ethyl alcohol	Practically insoluble	Slightly soluble	Practically insoluble
[1]DMSO	Freely soluble	Moderate soluble	Very soluble
[1]Hexane	Practically insoluble	Slightly soluble	Practically insoluble
[1]Chloroform	Sparingly soluble	Slightly soluble	Practically insoluble
[1]0.1 M sodium hydroxide solution	Practically insoluble	Practically insoluble	Practically insoluble
[1]0.1 M hydrochloric acid solution	Practically insoluble	Practically insoluble	Practically insoluble
[2]0.1 M hydrochloric acid buffer with pH = 1.2	0,08 mg/ml	0,08 mg/ml	0,075 mg/ml
[2]Phosphate buffer solution with pH = 4.5	0,075 mg/ml	0,075 mg/ml	0,07 mg/ml
[2]Phosphate buffer solution with pH = 6.8	0,07 mg/ml	0,07 mg/ml	0,065 mg/ml
[1]determinations performed at 20^{0}C; [2]determinations performed at 37^{0}C;			

Table 4. The results of the determination of solubility in BSMU

Solvent	MF-001	MF-001-H_2SO_4	β-CD-MF-001
Sunflower oil	Sparingly soluble	Practically insoluble	Freely soluble
Polyethylene glycol (PEG) 400	Freely soluble	Practically insoluble	Freely soluble
Polypropylene	Sparingly soluble	Practically insoluble	Freely soluble
Polysorbate 20	Freely soluble	Freely soluble	Freely soluble

Table 5. The results of the determination of PEG's ability to influence the dissolution degree of test substances in water

The added amount of PEG	MF-001	MF-001-H_2SO_4	β-CD-MF-001
1 ml	Sparingly soluble	Practically insoluble	Freely soluble, complete solubilization
2 ml	Moderate soluble	Practically insoluble	-
3 ml	Soluble	Practically insoluble	-
4 ml	Freely soluble	Sparingly soluble	-

monly used as a co-solvent in the development of oral and parenteral solutions. The ability of PEG to influence the dissolution degree in water of the test substances was studied (table 5). Thus, the compound β-CD-MF-001 is solubilized immediately after the addition of the first portions of PEG 400, MF-001 becomes soluble after the addition of 3 ml of PEG 400, while MF-001-H2SO4 remains insoluble.

The results obtained from the solubility study of the studied compounds represent as a basis for selecting the optimal solvents to develop the methods of analysis and for the technological stages of research of the test compounds.

Loss on drying.

The ability of substances to absorb water molecules from the air is an extremely important and decisive physical parameter in the selection and formulation of storage conditions.

The moisture content of the substances was determined by the drying method. MF-001 was found to be lost on drying 0,70 %, MF-001-H_2SO_4 – 5,77 % and β-CD-MF-001 – 11,52 %.

Hygroscopicity.

The kinetics of water absorption capacity by the test substances were studied (table 6).

According to fig. 4 it was found that the phenomenon of moisture absorption by MF-001-H2SO4 was described by a linear function while in the case of MF-001 and β-CD-MF-001 the absorption was described by a logarithmic function, that provided evidence for stronger hygroscopic properties of MF-001-H2SO4 than others. The studies of the influence of water on the integrity of substances have made it possible to establish preconditions for their store.

Table 6. Determination of moisture absorption degree for the test substances

t, hours		0	2	4	6	% water
MF-001	mass, g	0,10006	0,12993	0,12997	0,12992	29,84
MF-001-H_2SO_4	mass, g	0,10016	0,12153	0,13618	0,14956	49,32
β-CD-MF-001	mass, g	0,10015	0,11135	0,11250	0,11258	12,41

During 6 hours the substances were weighed (every 2 hours of storage).

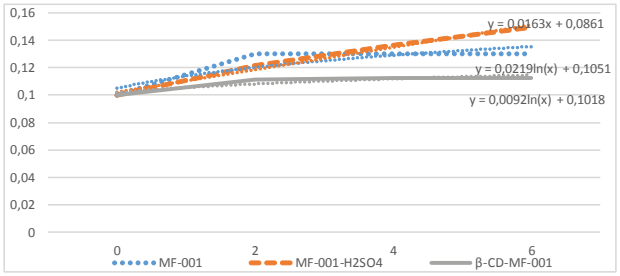

Fig. 4. The curves describing the kinetics of water absorption by the test substances

Conclusions

Evaluation of physicochemical parameters of the compounds, such as, MF-001, MF-001-H2SO4 and β-CD-MF-001 from the propylthiodiazolokinazolinone group - by determining the melting point and thermal behavior, analyzing the spectral behavior in the IR, determining the solubility in various solvents and dissolution media and the possibility of using PEG 400 as a co-solvent, evaluating the moisture and hygroscopicity of substances - highlighted the distinct properties for each substance.

These studies allow the continuation of research in order to determine the bioavailability of these compounds, the selection of the substance with optimal properties and the development of a pharmaceutical form with antituberculous effect.

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