Introduction. For the joint use of L-tryptophan with thiotriazoline, it is necessary to solve the problem of the possibility of formation of sufficiently stable intermolecular complexes of these compounds. Therefore, it seemed interesting to consider the possible structure and energetic characteristics of complexes formed by L-tryptophan, 3-methyl-1,2,4-triazolyl-5-thioacetate (MTTA) and morpholine to create a dosage form.

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Study results. Quantum-chemical calculations show that L-tryptophan and thiotriazoline are capable to form three-component complexes, the molecules in which are connected by a lot of intermolecular hydrogen bonds. Each of the molecules is linked by intermolecular hydrogen bonds to the other two in one complex only. All other complexes contain the components which are linked sequentially: morpholine – MTTA – tryptophan. The results of quantum-chemical calculations make it possible to assume that the studied complexes are thermodynamically unstable in an infinitely dilute solution. The formation energies of the complexes are positive, despite the presence of a lot of charge assisted intermolecular hydrogen bonds. This can be caused by the high conformational flexibility of the molecules in which the groups participating in the formation of hydrogen bonds are separated by bridge containing several methylene groups and a fairly easy transfer of protons participating in the formation of hydrogen bonds.

Conclusions. The results of quantum-chemical study of a system consisting of three components (L-tryptophan, MTTA and morpholine) showed that the most thermodynamically stable three-component complexes have a positive energy of formation in infinitely dilute solutions. Despite the possibility of the formation of intermolecular hydrogen bonds between the components, some of which are charge-assisted, the L-tryptophan, MTTA and morpholine system is a mixture of substances, which makes it possible to use it in a single dosage form.

Key words:
L-tryptophan, thiotriazoline, quantum chemical calculations, energy of complex formation, combination drugs.

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Вопросы фармации

Theoretical study of the possibility of L-tryptophan with thiotriazoline complex formation

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Теоретичне існуювання можливості обрання комплексів L-триптофана с тиотриазолином

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Для совміщеного призначення L-триптофана з тиотриазолином необхідно розглядати питання стабільності міжмолекулярних комплексів L-триптофана з тиотриазолином і морфоліном – сполуки, що мають складні структурні зв'язки. Такі комплекси можуть мати високу конформаційну гнучкість і стабільність структур.

Цель работы — методами квантовой химии исследовать строение и оценить энергию образования трехкомпонентных комплексов L-триптофана с тиотриазолином и морфолином для создания лекарственной формы.

Материалы и методы. Объектом исследования являлись L-триптофан и тиотриазолин. Исходное приближение к геометрии комплексов получено с помощью программы AutoDock Vina. Для построения трехкомпонентных комплексов на первом этапе проводился докинг МТТА и морфолина, по результатам которого отобрано 50 наиболее стабильных комплексов. Далее проведён докинг полученных комплексов CSTA и морфолина с молекулой L-триптофана, по результатам которого также отобраны 50 наиболее стабильных комплексов. Таким образом, для каждого из трехкомпонентных комплексов получено 2500 возможных вариантов геометрии.

Результаты. Квантово-химические расчеты показывают, что L-триптофан и тиотриазолин способны образовывать трехкомпонентные комплексы, молекулы в которых связаны за счет множественных водородных связей. Только в одном комплексе каждая из молекул связана межмолекулярными водородными связями с двумя другими. Все остальные комплексы содержат компоненты, связанные последовательно: морфолин–МТТА–триптофан. Проведенные квантово-химические расчеты позволяют предполагать, что изученные комплексы термодинамически нестабильны в бесконечно разбавленном растворе. Энергии образования комплексов положительные, несмотря на зарядовое усиление ряда межмолекулярных водородных связей. Это обусловлено большей конформационной гибкостью молекул.

Выводы. Проведенное квантово-химическое исследование системы, состоящей из трех компонентов (L-триптофан, МТТА и морфолин), показало, что все комплексы в равной степени могут создать комплексную систему, в которой будет обеспечена стабильность комплексов и сохранение всех составляющих. Комплекс образования комплексов, состоящий из трех компонентов, может находиться в одной лекарственной форме.

Introduction

In connection with the high prevalence of mental pathologies, the development of inadequate adaptive reactions in a large part of the population, the pathological response of the human body to stressful effects, and the further development of severe disadaptation of various mental and somatic pathologies in a large part of the population are one of the typical problems of modern clinical medicine. The reason for this is the development of not only mental disorders, such as depression, emotional stress, amnesic disorders, but also other pathologies that arise precisely as a result of stress. Examples include hypertension, coronary heart disease, bronchial asthma, hyperthermial, dermatosis, peptic ulcer and duodenal ulcer, functional irritable bowel syndrome, etc. [1]. Despite constant efforts to optimize the treatment of diseases of the central nervous system, the problem remains not fully resolved. An important element of solving this complex problem is the creation of new highly effective and safe drugs, the use of which would lead to a decrease in the prevalence of mental disorders and diseases, improvement of quality and longevity.

Consequently, the development of neuropsychotropic substances with anxiolytic, stress-protective, nootropic activity is an actual problem of pharmacy and medicine. In modern pharmacy and medicine, substances of the class of neurotransmitter amino acids are increasingly used. They regulate and correct the course of mental disorders in patients who have undergone stress, psychological stress by controlling the main functions of the body, including movement, emotional reactions and physical ability to feel pleasure and pain [2]. Arginines, glycine, GABA, taurine, tryptophan, lysine, glutamic acid, etc. are the most known neurotransmitter amino acids that affect mood regulation.

Currently, the creation of drug based on fixes combination of L-tryptophan with antioxidants is one of the promising approaches in the development of new drugs. Thiotriazine is one of the striking examples of the domestic preparations of the group of antioxidants, which was developed by the employees of SPA «Farma-tron», as well as by the specialists of the Department of Pharmaceutical Chemistry of the Zaporozhye State Medical University under the guidance of prof. Mazur I. A. This drug has antioxidant, membrane stabilizing, anti-ischemic, anti-arrhythmic, immunomodulating, anti-inflammatory, hepatoprotective, cardioprotective effect. Therefore, it is of interest to create a new combined drug in the form of tablets containing L-tryptophan (Fig. 1) and thiotriazine (Fig. 2) [3]. For the joint use of L-tryptophan with thiotriazo-line, it is necessary to solve the problem of the possibility of formation of sufficiently stable intermolecular complexes of these compounds. Therefore, it seemed interesting to
consider the possible structure and energetic characteristics of complexes formed by L-tryptophan, 3-methyl-1,2,4-triazolyl-5-thioacetate (MTTA) and morpholine.

**Objective:** to investigate the structure and evaluate the formation energy of three-component complexes of L-tryptophan, 3-methyl-1,2,4-triazolyl-5-thioacetate and morpholine compounds using quantum-chemical methods and to study the possibility of combining of these substances into a single dosage form.

**Materials and methods**

Tiotriazoline (Fig. 1) and L-tryptophan (Fig. 2) were used in the studies.

![Fig. 1. Structural formula of L-tryptophan.](image)

![Fig. 2. Structural formula of thiotriazoline.](image)

The procedure which is similar to that used earlier in the study of thiotriazoline and isoniazide complexes was used to simulate the structure and stability of L-tryptophan and thiotriazoline complexes [4].

The initial approximation to the geometry of the complexes was obtained using the molecular docking technique within the AutoDock Vina program. At the first stage docking of MTTA and morpholine was carried out, according to the results of which 50 most stable three-component complexes were separated out. Further, docking of obtained complexes of MTTA and morpholine with the L-tryptophan molecule was carried out, and also the 50 most stable complexes were selected. Thus, 2500 initial geometries were obtained for the three-component complexes. This procedure was carried out for each of the possible tautomeric forms of the three-component complexes [5].

At the second stage, the obtained three-component complexes were preliminarily optimized by the semi-empirical PM7 method with modeling of environment influence by COSMO method. The calculations were carried out using MOPAC2012 program [6]. Based on the results of calculations, 100 most stable structures were selected for each of the complexes, which were then optimized by the density functional method with B97-D3/SVP+COSMO (Water) empirical dispersion correction [7] using geometric correction for incompleteness of the gCP basic set [8]. 10 complexes with the lowest energy were selected from the obtained structures of the three-component complexes for which the final calculation of the geometry and thermodynamic parameters in the hard rotor approximation by the B97-D3/TZVP+COSMO method was carried out. A more accurate calculation of the energy of solvation was carried out by SMD method. Calculations by the density functional method were carried out using ORCA 3.0.3 program [9]. The formation energy of the complexes in solution was calculated as the difference between the free Gibbs energies of the solvated complex and its solvated individual components.

The chosen technique, which relies on the study of the complete conformational space of complexes by molecular docking methods and a series of sequential quantum chemical calculations using increasingly higher methods, guarantees with a high degree of probability the most stable three-component complexes.

**Results and discussion**

Previous studies of thiotriazoline [10] have shown that MTTA is easily deprotonated by converting to an organic anion, and morpholine, respectively, becomes a cation by attaching a hydrogen atom. Thus, the MTTA and morpholine compound is an organic salt. On the other hand, L-tryptophan, being an amino acid, must exist as a zwitterion (Fig. 1). In this case, the zwitterionic form of L-tryptophan acts simultaneously as both a proton donor and an proton acceptor in the formation of charge-assisted intermolecular hydrogen bonds with the morpholinium cation and the MTTA anion. This suggests the possibility of forming of both two-component complexes involving L-tryptophan and one of the components of thiotriazoline, and three-component complexes involving all three molecules. The multiplicity of donor and acceptor properties in all three molecules under study makes it possible to form a sufficiently large number of possible complexes.

The structure of isolated molecules and the atoms numbering are shown in Fig. 3.

![Fig. 3. The structure of isolated molecules of L-tryptophan (1), MTTA (2), morpholine (3) and their atomic numbering used in quantum chemical calculations.](image)
multiple hydrogen bonds. From 4 to 5 intermolecular hydrogen bonds are formed in the most stable complexes. The structure of the most stable complexes with L-tryptophan with thiotriazole and morpholine, their formation energies (kcal/mol) and hydrogen bond characteristics (H...A, Å and D-H...A, deg.) obtained by the method of B97-D3/TZVP+SMD (Water) are given in Table 1.

The analysis of the binding order of components in the 10 most stable three-component complexes (Fig. 4) showed some regularities. Only in complex 1 each of the molecules is linked by intermolecular hydrogen bonds with other two. All other complexes contain the components which are linked sequentially: morpholine-MTTA-tryptophan.

It should be noted that the protonated amino group and the deprotonated carboxyl group of tryptophan located at the same carbon atom form an intramolecular hydrogen bond in almost all cases. In the formation of intermolecular hydrogen bonds, the protonated amino group of amino acid is most active proton donor, providing from two (complexes 2, 6–10) to three (complexes 3 and 5) of hydrogen bonds with MTTA in 8 of 10 complexes. The morpholine molecule proved to be a much less active donor of the proton, in spite of the protonation of the nitrogen atom and the positive charge on it. Moreover, in complex 7, the morpholine molecule is not only a proton donor, but also a proton acceptor in hydrogen bonding. NH group of triazole ring shows proton donor properties only in complexes 3 and 7. NH group of bicyclic amino acid fragment (complexes 3, 5, 6 and 8) also showed the properties of the proton donor in the intermolecular hydrogen-bonding. In turn, deprotonated carboxyl group MTTA is the most active proton acceptor which is also involved in the formation of two simultaneous intermolecular hydrogen bonds in complexes 2, 3, 5, 6, 8–10. Deprotonated carboxyl group of amino acid forms an intermolecular hydrogen bond as a proton acceptor in only three out of ten complexes (complexes 1, 3, 7). It can be assumed that this is caused by its involvement in the formation of an intramolecular hydrogen bond. The triazole MCTA cycle, despite the absence of intermolecular hydrogen bonds.

The results of quantum-chemical calculations make it possible to assume that studied complexes are thermodynamically unstable in an infinitely dilute solution. The formation energies of the complexes are positive, despite the charge-assisted character of a number of intermolecular hydrogen bonds.

Table 1. The structure of the most stable complexes with L-tryptophan with thiotriazole and morpholine, their formation energies (kcal/mol) and hydrogen bond characteristics (H...A, Å and D-H...A, deg.) obtained by the method of B97-D3/TZVP+SMD (Water)

| Complex | Complex 1 | Complex 2 |
|---------|-----------|-----------|
| ΔG_{298} = 6.5 kcal/mol | N4-H...O1 | N4-H...O1 |
| N4-H...O2 | 1.61 | 1.61 |
| N4-H...O5 | 1.75 | 1.78 |
| N5-H...O2 | 1.70 | 1.99 |
| N5-H...N1 | 1.70 | 1.66 |
| ΔG_{298} = 6.9 kcal/mol | N4-H...O1 | N4-H...O1 |
| N2-H...O2 | 1.68 | 1.59 |
| N1-H...N1 | 1.90 | 1.88 |
| N4-H...O2 | 1.58 | 1.77 |
| N5-H...O1 | 1.76 | 1.97 |
| Complex 3 | Complex 4 |
| ΔG_{298} = 8.0 kcal/mol | N2-H...O2 | N4-H...O1 |
| N1-H...N1 | 1.90 | 1.88 |
| N4-H...O2 | 1.58 | 1.77 |
| N5-H...O1 | 1.76 | 1.97 |
| ΔG_{298} = 8.1 kcal/mol | N4-H...O1 | N4-H...O1 |
| N4-H...O1 | 1.55 | 1.69 |
| N4-H...N1 | 1.92 | 1.86 |
| N5-H...O1 | 1.92 | 1.80 |
| N5-H...O1 | 2.39 | 1.82 |
| N5-H...O2 | 1.72 | 1.92 |
| Complex 5 | Complex 6 |
| ΔG_{298} = 8.6 kcal/mol | N4-H...O1 | N4-H...O2 |
| N4-H...N1 | 1.92 | 1.86 |
| N5-H...O1 | 1.92 | 1.80 |
| N5-H...O1 | 2.39 | 1.82 |
| N5-H...O2 | 1.72 | 1.92 |
| ΔG_{298} = 9.3 kcal/mol | N4-H...O1 | N4-H...O1 |
| N2-H...O3 | 1.86 | 1.59 |
| N4-H...O5 | 1.64 | 1.86 |
| N6-H...O2 | 1.60 | 1.76 |
| N6-H...N3 | 1.99 | 2.02 |
| Complex 7 | Complex 8 |
| ΔG_{298} = 9.4 kcal/mol | N2-H...O3 | N4-H...O1 |
| N4-H...O5 | 1.64 | 1.86 |
| N6-H...O2 | 1.60 | 1.76 |
| N6-H...N3 | 1.99 | 2.02 |

It should be noted that the solvation energies were calculated in the approximation of infinite dilution. In solutions of a finite concentration, greater stability of molecular complexes should be expected due to the enhancement of intermolecular interactions.
Conclusions

The results of the quantum-chemical study of the system consisting of three components (L-tryptophan, MTTA and morpholine) showed that the most thermodynamically stable three-component complexes have a positive energy of formation in infinitely dilute solutions.

Despite the possibility of the formation of intermolecular hydrogen bonds between the components, some of which are charge-assisted, the L-tryptophan, MTTA and morpholine system is a mixture of substances, which makes it possible to use them in a single dosage form.

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