

Theoretical and Experimental Study of Inclusion Complex Formation of β -Cyclodextrin with Some 1,4-Diazepine Derivatives

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Benzodiazepines belong to a group of heterocyclic compounds that are widely used as pharmaceuticals. They have a wide spectrum of pharmacological effects including anxiolytic, sedative, hypnotic, anticonvulsant, muscle relaxant and amnesic activity. Ten novel fused 1,4-diazepines were examined using the PASS Online service in order to evaluate their anticipated biological activity. Virtual screening check list was composed of 6 essential psychotropic activities such as antipsychotic (neuroleptic), antidepressant, tranquilizing (anxiolytic), muscle relaxant, nootropic and anticonvulsant. Screening results showed that 1-methyl-4,5-dihydro-6H-pyrrolo[1,2-a][1,4]benzodiazepin-6-one the most likely would have nootropic effect while the N-(tert-butyl)-2-(1-methyl-6-oxo-4H-pyrrolo[1,2-a][1,4]benzodiazepin-5(6H)-yl)acetamide has been recognized as neuroleptic and 8,9-dimethoxy-1-methyl-4,5-dihydro-6H-pyrrolo[1,2-a][1,4]benzodiazepine-6-tione was expected to have an anticonvulsant effect. Drug delivery systems, especially those based on cyclodextrin inclusion complexes are the most promising ways towards enhanced pharmaceutical forms. Cyclodextrins are cyclic oligosaccharides composed of six to eight dextrose units joined through one to four α -D bonds. They have hydrophilic outer surface and a hydrophobic axial open cavity, which is capable to encapsulate a great variety of organic and inorganic compounds through the formation of inclusion complexes. This property of cyclodextrins has been used in the pharmaceutical industry to improve the physical and chemical properties (water solubility, stability, dissolution rate) of various drug molecules. The complexes of β -cyclodextrin with non-steroidal anti-inflammatory drugs (e.g., paracetamol, ibuprofen, ketoprofen, flufenamic and mefenamic acids, etc.), steroids, prostaglandins and prostacyclins, barbiturates, sulfonamides, cardiac glycosides and many other drugs are known. Moreover, cyclodextrins are nontoxic and inexpensive substances. The inclusion complex of 1-methyl-4,5-dihydro-6H-pyrrolo[1,2-a][1,4]benzodiazepin-6-one with β -cyclodextrin was synthesized by kneading method. The obtained complex compound was characterized by solid state NMR, IR spectroscopy and thermal analysis. The formation of inclusion complex leads to changes in the IR spectrum. The $\nu(\text{OH})$ band shifts from 3257 cm^{-1} for β -cyclodextrin to 3278 cm^{-1} for complex compound which indicates that hydroxyl groups of β -cyclodextrin participate in hydrogen bonding. Similar changes exhibit stretching and bending vibration bands of the amino group but the effect is less than for OH. Thus, $\delta(\text{N-H})$ frequency changed from 1624 cm^{-1} in the ligand up to 1629 cm^{-1} in the complex compound. The frequency of characteristic stretching vibration of carboxyl group $\nu(\text{C=O})$ is decreased from 1657 cm^{-1} to 1648 cm^{-1} which could also be considered as a proof that the formation of inclusion complex takes place. Such a minor changes are common for the non-covalent nature of the interaction in the inclusion complexes. The results of thermal analysis showed that the formation of inclusion complex leads to decreasing of thermal stability both the β -cyclodextrin and the ligand. The obtained inclusion complex was studied by solid state NMR. The changes of chemical shifts of the β -cyclodextrin signals caused by the complexation are -0.9 ppm for the C2, C3 and C5 atoms and 0.512 ppm for C4. The main change in the spectrum of complex is that the signals of the individual conformations visible in pure β -CD as multiplets are converged to broad single peaks in complex. In the "guest" molecule the atoms closest to the β -cyclodextrin ring exhibit the largest shifts. This is consistent with the results of quantum chemical calculations. To find the most probable geometry of inclusion complex, molecular docking study was carried out using the AutoDock program. As a result, a number of the most preferred conformations were obtained. The geometry of the obtained conformations was optimized using the semi-empirical method AM1. Based on the docking data, the geometry of the most probable structure of the inclusion complex compound was suggested.

Keywords: Inclusion complexes, biological activity, 1,4-diazepines, β -cyclodextrin, IR spectroscopy, NMR spectroscopy, molecular docking.

Теоретическое и экспериментальное исследование комплексов β -циклодекстрина с некоторыми производными 1,4-дiazепина

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Проведен скрининг потенциальной биологической активности для ряда соединений бензодиазепиновой группы. На основании полученных данных синтезирован комплекс включения наиболее перспективного соединения из исследованного ряда с β -циклодекстрином. Состав и структура полученного комплекса включения подтверждены методами ИК и ЯМР спектроскопии, а также квантово-химическими расчетами.

Ключевые слова: Комплексы включения, биологическая активность, 1,4-дiazепины, β -циклодекстрин, ИК спектроскопия, ЯМР спектроскопия, молекулярный докинг.

Introduction

Benzodiazepines are a group of heterocyclic compounds that have been known and widely using as pharmaceuticals for a long time. They reveal a wide spectrum of pharmacological effects including anxiolytic, sedative, hypnotic, anticonvulsant, muscle relaxant and amnesic activity.^[1] The duration of action of the drug usually include the length of time while that active substance is effective as well as the length of time while its metabolites are active. In addition, many benzodiazepines were reported to have so called aftereffect.

An important factor affecting the duration of action and selectivity of the drug is its dosage form. Drug delivery systems, especially those based on cyclodextrin inclusion complexes are the most promising ways towards enhanced pharmaceutical forms. Drug delivery system represents a purposely developed mechanism for the release of a drug in a specific organ of a living organism in order to increase its biological effects.^[2]

The use of cyclodextrins as a kind of transport for this purpose is a very attractive approach for a number of reasons. Cyclodextrins are cyclic oligosaccharides composed of six to eight dextrose units joined through one to four α -D bonds. They have hydrophilic outer surface and a hydrophobic axial open cavity, which is capable to encapsulate a great variety of organic and inorganic compounds through the formation of inclusion complexes. This property of cyclodextrins has been used in the pharmaceutical industry to improve the physical and chemical characteristics (water solubility, stability, dissolution rate) of various drug molecules. The complexes of β -cyclodextrin with non-steroidal anti-inflammatory drugs (e.g., paracetamol, ibuprofen, ketoprofen, flufenamic and mefenamic acids, etc.), steroids, prostaglandins and prostacyclins, barbiturates,

sulfonamides, cardiac glycosides and many other drugs were reported.^[3] Moreover, cyclodextrins are nontoxic and inexpensive compounds.^[4-12] We examined *in silico* ten fused 1,4-diazepines synthesized by us earlier,^[4-12] using the prediction software to evaluate their possible biological activity. For the most promising compound in the series, the inclusion complex with β -cyclodextrin was synthesized, the composition and structure were determined.

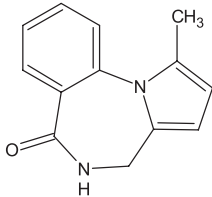
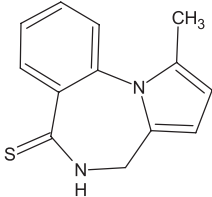
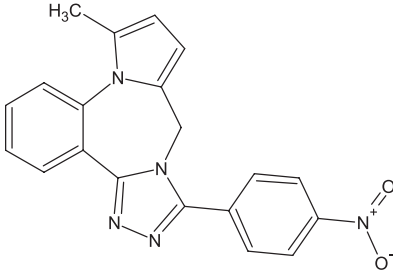
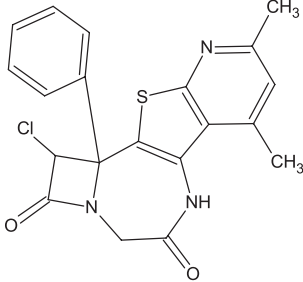
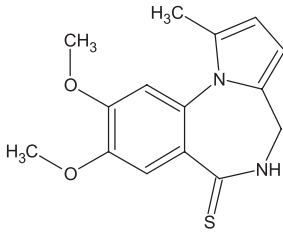
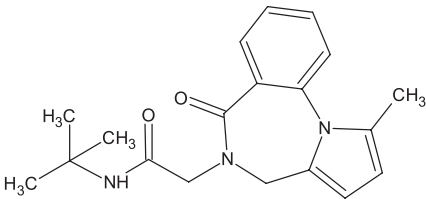
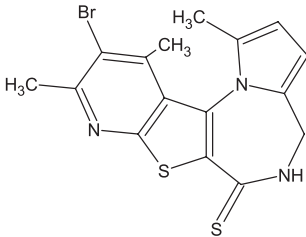
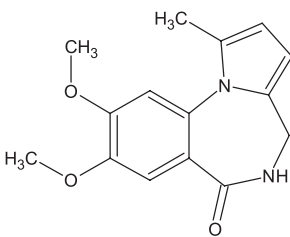
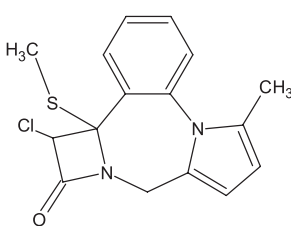
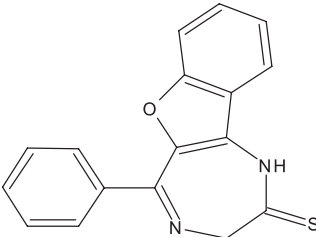
Experimental

Solid state ¹³C NMR spectra (CP-MAS) were recorded on a JEOL JNM-ECA-400 NMR spectrometer at 100 MHz in 4 mm rotor at 10 kHz spinning rate. Contact time was 2 ms, relaxation delay 5 seconds, the number of scans was 1300 for β -cyclodextrin and 3000 for the ligand and complex. Adamantane (38.48 ppm) was used as the external standard. IR spectra were recorded on a Bruker VERTEX 70 Fourier transform IR spectrometer using the ATR method.

Virtual screening was run using the PASS Online service.^[13] Molecular docking of the 1-methyl-4,5-dihydro-6H-pyrrolo[1,2-a][1,4]benzodiazepin-6-one inclusion complex with β -cyclodextrin was carried out using the AutoDock Version 4.2 program.^[14] Quantum chemical calculations were carried out using the HyperChem 8.0 program.^[15]

β -Cyclodextrin was purified by recrystallization from water. The synthesis and spectral data of 1-methyl-4,5-dihydro-6H-pyrrolo[1,2-a][1,4]benzodiazepin-6-one are given in the paper.^[12] The complex compound was prepared by the solid phase method (kneading). Thus, β -cyclodextrin and 1-methyl-4,5-dihydro-6H-pyrrolo[1,2-a][1,4]benzodiazepin-6-one was grinded in agate mortar for 10 min and then the substances were mixed in a molar ratio of 1:1. The compounds were kneaded in a mortar with a little ethanol for 60 min at the room temperature (24 °C). Ethanol was added by 0.2 ml portions during the first 30 minutes.^[16] The resulting complex was dried for 24 hours in desiccator at the room temperature.

Table 1. The compounds selected for *in silico* prediction of biological effects.

<p style="text-align: center;">1</p>  <p style="text-align: center;">1-Methyl-4,5-dihydro-6H-pyrrolo[1,2-a] [1,4]benzodiazepin-6-one</p>	<p style="text-align: center;">2</p>  <p style="text-align: center;">1-Methyl-4,5-dihydro-6H-pyrrolo[1,2-a] [1,4]benzodiazepin-6-thione</p>
<p style="text-align: center;">3</p>  <p style="text-align: center;">12-Methyl-7-(4-nitrophenyl)-9H-pyrrolo[1,2-a][1,2,4] triazolo[4,3-d][1,4]benzodiazepine</p>	<p style="text-align: center;">4</p>  <p style="text-align: center;">1-Chloro-7,9-dimethyl-11b-phenyl-6,11b-dihydroazeto[1,2-d] pyrido[3',2':4,5]thieno[2,3-f][1,4]diazepine-2,5(1H,4H)-dione</p>
<p style="text-align: center;">5</p>  <p style="text-align: center;">8,9-Dimethoxy-1-methyl-4,5-dihydro-6H-pyrrolo[1,2-a][1,2-a] [1,4]benzodiazepine-6-thione</p>	<p style="text-align: center;">6</p>  <p style="text-align: center;"><i>N</i>-(<i>tert</i>-Butyl)-2-(1-methyl-6-oxo-4H-pyrrolo[1,2-a] [1,4]benzodiazepine-5(6H)-yl)acetamide</p>
<p style="text-align: center;">7</p>  <p style="text-align: center;">10-Bromo-1,9,11-trimethyl-4,5-dihydro-6H-pyrrolo[1,2-a][1,4] diazepine-6-thione</p>	<p style="text-align: center;">8</p>  <p style="text-align: center;">8,9-Dimethoxy-1-methyl-4,5-dihydro-6H-pyrrolo[1,2-a] [1,4]benzodiazepin-6-one</p>
<p style="text-align: center;">9</p>  <p style="text-align: center;">12-Chloro-6-methyl-12a-(methylthio)-12,12a-dihydro-9H,11H- azeto[1,2-d]pyrrolo[1,2-a][1,4]benzodiazepin-11-one</p>	<p style="text-align: center;">10</p>  <p style="text-align: center;">5-Phenyl-1,3-dihydro-2H-[1]benzofuro[3,2-e] [1,4]diazepine-2-thione</p>

Thermal analysis of the samples was carried out on a Netzsch STA 409 PC/PG instrument in open platinum-rhodium crucible in air at 25–1000 °C using Al₂O₃ as an inert standard. Heating rate was 10 °C/min, analyte sample weights: complex – 24.310 mg, physical mixture – 23.730 mg, β -cyclodextrin – 23.462 mg, 1-methyl-4,5-dihydro-6*H*-pyrrolo[1,2-*a*][1,4]benzodiazepin-6-one – 26.888 mg.

Results and Discussion

Virtual Screening

The structures of fused 1,4-diazepines – benzodiazepines **1–3**, **5**, **6**, **8**, **10** and pyridothienodiazepines **4**, **7** selected for *in silico* screening are given in Table 1.

The selection of the most promising candidates with desired pharmacological properties is a common practice in the development of pharmaceuticals.^[1] Nowadays, a preliminary search is carried out using virtual screening tools *in silico*.

Virtual screening was performed using the Way2Drug online service.^[13] Each compound in the study was evaluated for 6 essential psychotropic effects, namely, antipsychotic (neuroleptic), antidepressant, tranquilizing (anxiolytic), muscle relaxant, nootropic and anticonvulsant. Using the program database (about 250000 reference compounds), the expected activity f was calculated as a difference of the P_a value (probability that compound will exhibit the certain type of activity) and P_i value (probability that compound will be inactive), $f = P_a - P_i$. Calculated values are given in Table 2.

The estimation results in the Table 2 show that the most promising in this series are compounds **1**, **3**, **6** and **8**. Compounds **6** and **8** may exhibit antipsychotic properties (by blocking dopamine D2-receptors, $f = 0.362$) as well as tranquilizing properties (by increasing the GABA-like inhibition in the central nervous system, $f = 0.256$). Antidepressant properties (by reuptake of monoamines, $f = 0.164$) are expected for compounds **1**, **2**, **6–9**; compounds **6** and **9** may be muscle relaxants (due to the blockade of acetylcholine, $f = 0.305$); nootropic properties are highly likely for

compounds **1**, **2**, and **6** (GABA-receptor antagonist that enhances brain cognitive abilities, $f = 0.274$). Compound **10** seems do not exhibit psychotropic properties, probably due to the influence of the furan ring.

Based on the screening results, 1-methyl-4,5-dihydro-6*H*-pyrrolo[1,2-*a*][1,4]benzodiazepin-6-one (compound **1**) (hereinafter MDPB) was selected as a “guest” to prepare an inclusion complex. The inclusion complex of MDPB with β -cyclodextrin (hereinafter β -CyD) was synthesized by the kneading method.^[17,18]

IR Spectroscopy

The spectra of β -CyD, MDPB, its physical mixture and the inclusion complex were recorded.^[19,20] In the case of a physical mixture of the “guest” and β -CyD, the spectrum is the superposition of the spectra of individual compounds without any changes in absorption bands frequencies. The formation of the inclusion complex leads to the changes in the IR spectrum compared to the spectra of individual substances. The characteristic absorption bands are shown in the Table 3.

The frequency and the shape of $\nu(\text{OH})$ stretching vibration band depend on the degree of hydroxyl group involvement in hydrogen bonding. The formation of hydrogen bond decreases the O–H force constant and, consequently, leads to a change in the vibration frequency.^[21,22] The $\nu(\text{OH})$ β -CyD frequency in the complex shifts from 3257 cm^{−1} to 3278 cm^{−1}, thus confirming the participation of the hydroxyl group in hydrogen bond. Stretching and deformation frequencies of the amino group also are influenced by hydrogen bond, but to a lesser extent than for OH. As a result, the frequency $\delta(\text{N-H})$ shifts from 1624 cm^{−1} in MDPB to 1629 cm^{−1} in the complex.

A shift in the characteristic band of the carbonyl group $\nu(\text{C=O})$ also might point to the complex formation.^[22] Thus, the strong band corresponding to the C=O stretching vibrations shifts from 1657 cm^{−1} in the ligand to 1648 cm^{−1} in the inclusion complex. Small changes are characteristic^[19] for non-covalent interactions in the inclusion complex of β -CyD with MDPB.

Table 2. Estimated psychotropic activity of 1,4-diazepines **1–10**.

Compound	Psychotropic Activity					
	antipsychotic	antidepressant	anxiolytic	muscle relaxant	nootropic	anticonvulsant
1	–	0.205	–	–	0.470	0.276
2	–	0.040	–	–	0.029	0.003
3	–	–	–	–	–	0.506
4	–	–	–	–	–	0.098
5	–	–	–	–	–	–
6	0.362	0.164	0.256	0.305	0.274	–
7	–	0.137	–	–	–	–
8	0.319	0.033	0.065	–	–	–
9	–	0.064	–	0.129	–	0.201
10	–	–	–	–	–	–

Table 3. The characteristic absorption bands in the IR spectra of MDPB, β -CyD and their inclusion complex.

Vibration	The characteristic absorption band, cm^{-1}		
	MDPB	β -CyD	Inclusion complex
$\nu(\text{N-H})$ of amino group	3259	—	—
$\nu(\text{O-H})$ in β -CyD	—	3257	3278
$\nu(\text{C-H})$ of CH_2 group	—	2926	2929
$\nu(\text{C=O})$ of carbonyl group	1657	—	1648
$\nu(\text{C=C})$ in benzene ring	1520	—	1516
$\nu(\text{C-N})$ of amino group connected with benzene ring	1211	—	1211
$\delta(\text{N-H})$ of amino group	1624	—	1629
$\delta(\text{C-H})$ of benzodiazepine ring	1406	—	1406

NMR Spectroscopy

The prepared inclusion compound was studied by solid state NMR. In the Figure 1, a comparison is given for ^{13}C solid state NMR spectra (CP-MAS) of MDPB, β -CyD and the resulted inclusion complex.

In general, chemical shifts in ^{13}C NMR spectrum of β -CyD in the solid state correspond to the ones in a liquid state (C1: 100–105 ppm, C4: 80–85 ppm, C6: 60 ppm, C2, C3, C5: 70–80 ppm). The main difference is that every carbon has multiple signals instead of averaged signals for the liquid state. The existence of several resonances for each carbon of β -CyD is mainly correlated with different torsion angles about the 1–4 linkages for C1 and C4, and with torsion angles describing the orientation of the hydroxyl groups.^[23] Most clearly this effect exhibit C1, which is usually appears as quartet.

The chemical shifts caused by the complex formation in β -CyD are small (-0.904 ppm for the C2, C3, C5 group and 0.512 ppm for C4). The main difference in the spectrum of the inclusion complex is that the multiple signals of the individual conformations are merged together. This is a common fact usually explained by the ongoing structural rearrangement which accompanies the inclusion of the “guest” into the β -CyD cavity. During the inclusion a part of water molecules inside the cavity are replaced by “guest”, destroying the system of existing hydrogen bonds in β -CyD ring. As a result, glucopyranose links in the complex turn to a more symmetrical conformation.^[23,24] For the “guest”, atoms that are closest to the β -CyD atoms revealed the most prominent shifts (C5: 0.162 ppm, C7: -0.161 ppm, C8: -0.04 ppm, C3: -0.08 ppm, C5: 0.162 ppm). Apparently, so small chemical shifts can be explained by the fact that the geometry of “guest” in complex remains almost unchanged. This is in a good agreement with the results of quantum chemical calculations given below.

Thermal Analysis

The thermal destruction of β -CyD is characterized by several effects on the DTA curve accompanying by mass loss on the TG and DTG curves (Figure 2). Due to the fact that β -CyD is a nonstoichiometric hydrate, the inner H_2O content may vary over a rather wide range (from 12.3 to 9.4

H_2O molecules at 100 % and 15 % humidity, respectively) with no changes in crystal structure. There are two endo effects and one exo effect on the DSC curve of β -CyD, accompanied by mass loss on the TG and DTG curves.^[25,26] The endo effect at 134.6 °C corresponds to the dehydration of β -CyD and is accompanied by the weight loss (13.30 %) in the range of 20–150 °C. The calculation shows that this β -CyD sample contained 9.7 water molecules per β -CyD cavity in average, both solvated and adsorbed.

According to the literature data, β -CyD destruction starts at 230–280 °C. Such a broad range is explained by a different preparation, purification and storage. The thermogram shows one more endo effect at 291.1 °C, which, obviously, corresponds to the β -CyD melting. The melting of β -CyD is accompanied by oxidative degradation, which leads to the elimination of hydroxyl groups and the destruction of glucopyranose units of a cyclic oligosaccharide. Two intense exothermic peaks at 376 and 502.7 °C (with approximately equal energy/enthalpy) with a mass loss of 59.34 and 26.37 %, respectively, as well as a narrow peak of the exo effect at 324 °C, are corresponded to these two stages.

The MDPB thermogram (Figure 3) shows a clearly visible endo effect at a temperature of 238.9 °C, which can be attributed to the melting point of the substance. Another effect with 66.49 % weight loss is present at a temperature of 596.6 °C.

The thermogram of the β -CyD complex with MDPB (Figure 4) has an endo effect caused by water desorption (9.15 %) at 108.9 °C, which is 24.7 °C lower than for the β -CyD itself. The total loss of mass corresponds to 7.5 water molecules for the 1:1 complex (and 13.9 molecules of water, if we assume the formation of a complex with β -CyD to MDPB ratio of 2:1), which is explained by the displacement of water from the internal cavity of β -CyD in the formation of inclusion complexes.

The effects associated with the thermal decomposition of the complex appear at a lower temperature and are of a substantially different nature than for the initial β -CyD. The initial exoeffect at 358 °C has lower intensity; the mass loss at this stage is 62.05 %. Next, an intense peak of the exo effect with a maximum at 492.2 °C was observed on the DSC curve with a mass loss of 25.21 %. A weak exo effect at 556 °C which belongs to the “guest” was also observed. As

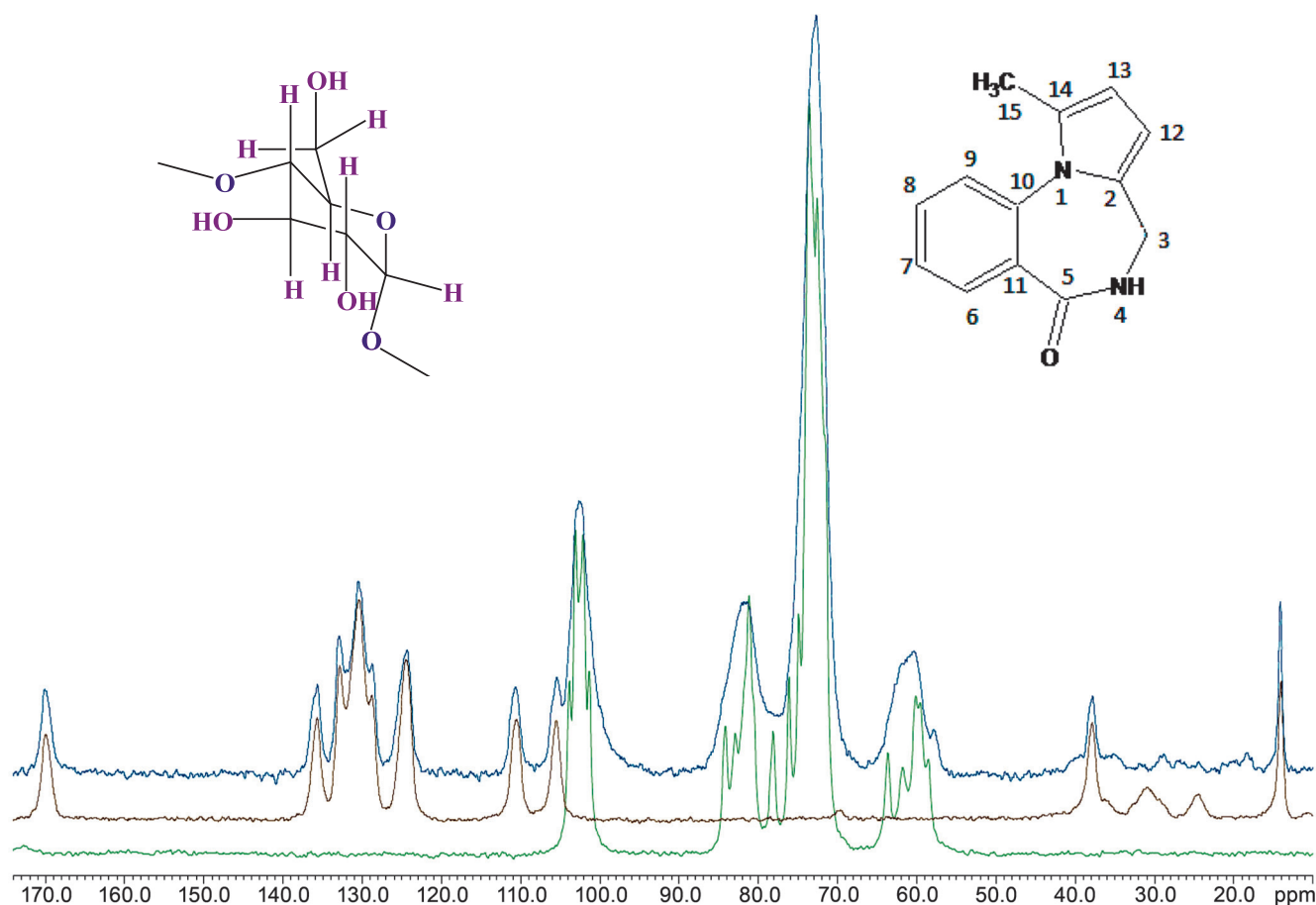


Figure 1. The fragments of ^{13}C CP-MAS NMR spectra of β -CyD, MDPB and the inclusion complex (from bottom to top) overlapped.

we can see from the thermogram, the stability of MDPB is decreased by 40.6 °C. The formation of an inclusion complex decreases the thermal stability of both β -CyD and MDPB. The Figures 4 and 5 show a clear difference between the thermogram of β -CyD–MDPB physical mixture and the thermogram of the complex. The physical mixture thermogram (Figure 5) shows an endothermic melting peak of MDPB at 232.6 °C. It has another character of the water desorption process, but the temperatures of exothermic peaks of the oxidative degradation process are differ from those observed both for the inclusion complex and individual substances. This is probably due to the formation of an inclusion complex during analysis when heating the physical mixture over the melting point.^[27]

Quantum Chemical Calculations

When modeling inclusion complexes, an important point is the search for optimal guest-host binding geometry. The search can be implemented by various methods, including direct generation of conformations using the molecular dynamics method, or by molecular docking. Molecular docking is a molecular modeling method, the purpose of which is to find the most reliable orientation and conformation of the ligand in the binding site of the target protein.^[29] The docking predicts the structure of an intermolecular complex formed between two or more molecules^[30,31] and is often used

for virtual high-throughput screening (vHTS) of biologically active compounds. The search for the most probable conformations of the intermolecular complex is carried out using the so-called scoring functions (SF).^[32] The SF is used in the docking process to calculate the approximate energy of the complexes and to rank the various estimated ligand conformations at each step of the conformational search. As a result, we obtain a set of ligand (ligands) conformations that are optimally located at the receptor binding site.

Molecular docking of the prepared inclusion complex of MDPB with β -CyD was performed using the AutoDock program.^[31] For the each possible conformation the program calculates the energy as the sum of the scoring function terms, final interaction energy ΔG (kcal/mol) and the inhibition constant K_i . As a result, a number of the most preferred conformations were selected (numbered 47, 49, 22, 11, 23, 10, 5, 1).

Obviously, the real structure of the resulting complex depends on many factors. For example, it can be affected by the use of various buffer solutions and ligands, as well as solvent molecules. In the standard docking methods, their presence is neglected. Therefore, to refine the structure, the geometry of the obtained conformations was optimized using the semi-empirical method AM1^[33] both *in vacuo* and in aqueous media.

The calculation results showed that conformations 22, 11, 23, 10, 5, 1 are not realized in aqueous solution. As

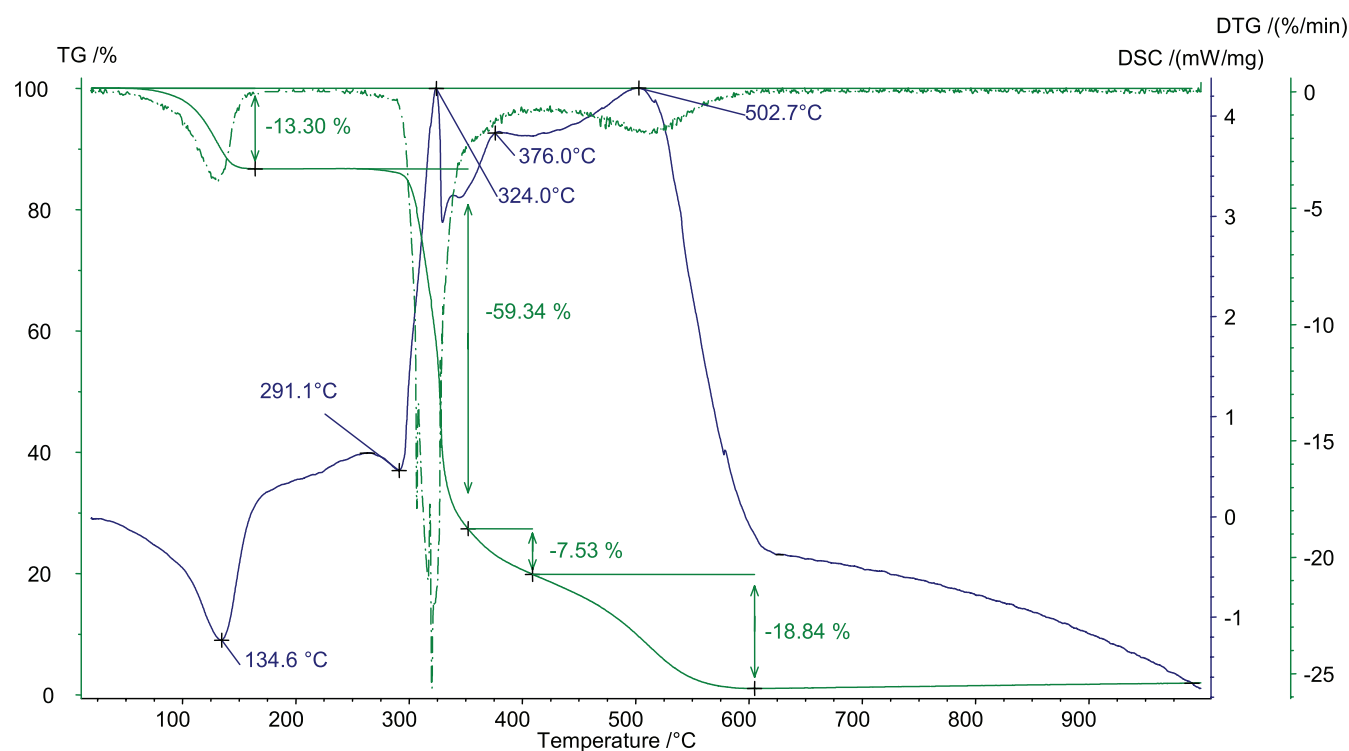


Figure 2. Thermogram of β -cyclodextrin.

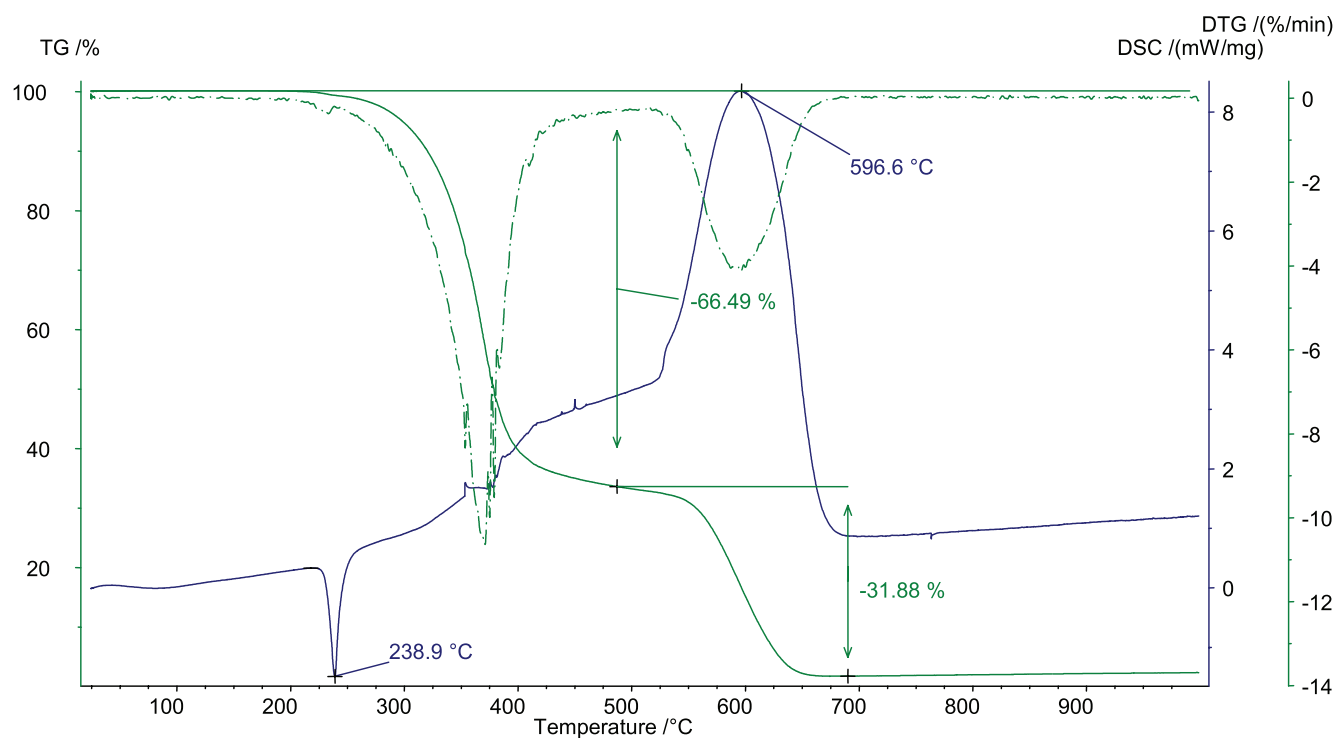


Figure 3. Thermogram of MDPB.

a result of the geometry optimization, the structures were greatly distorted; in some cases, the “guest” was “pushed out” of the CyD cavity. The lowest energy of the system was obtained for the conformation **49** (Table 4).

Although being calculated to exist, conformations **47** and **49** have low stability in aqueous solution. This

is in agreement with the fact that the ordinal method of coprecipitation^[17] from water–ethanol mixture gave a negative result and the kneading method was used instead to prepare the inclusion complex. During the kneading, hydrophobic molecules of the “guest” tend to occupy the cavity of β -CyD molecules and avoid contacting with a solvent as

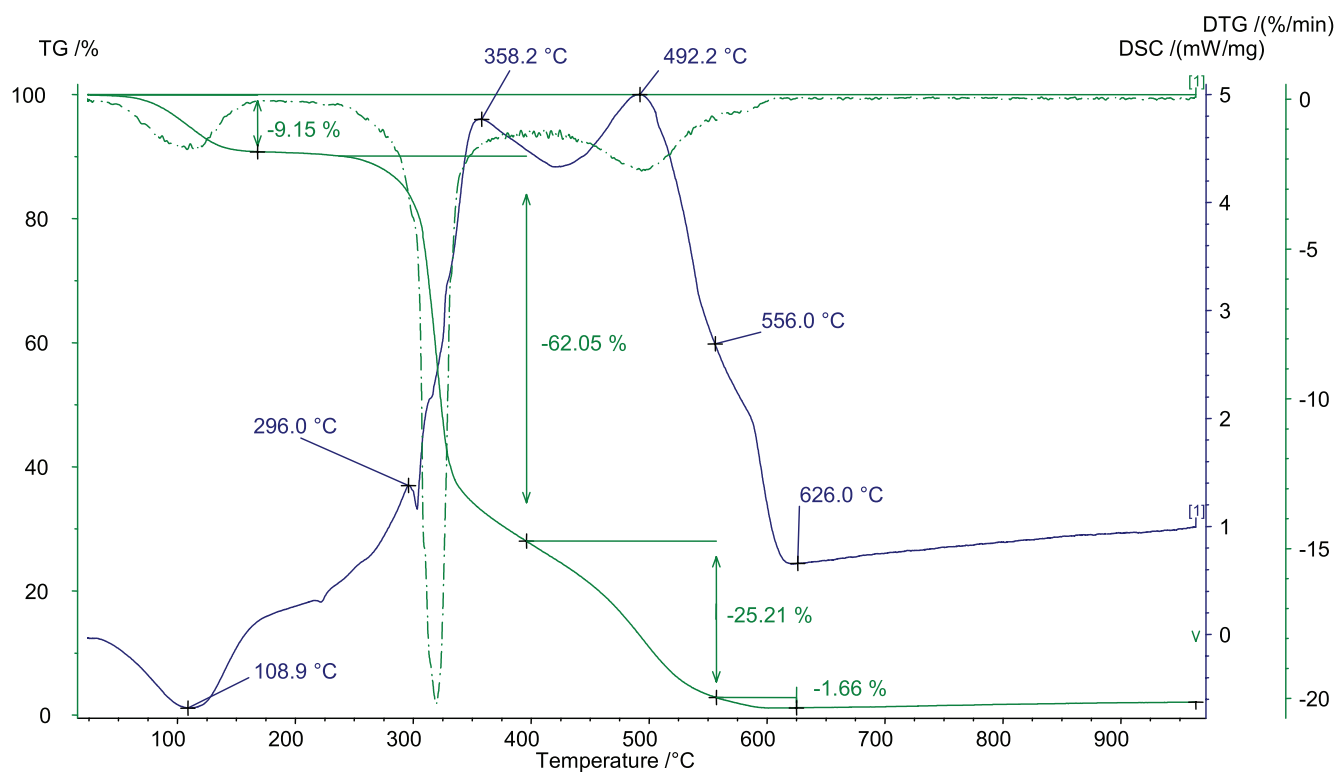


Figure 4. Thermogram of inclusion complex of β -CyD with MDPB.

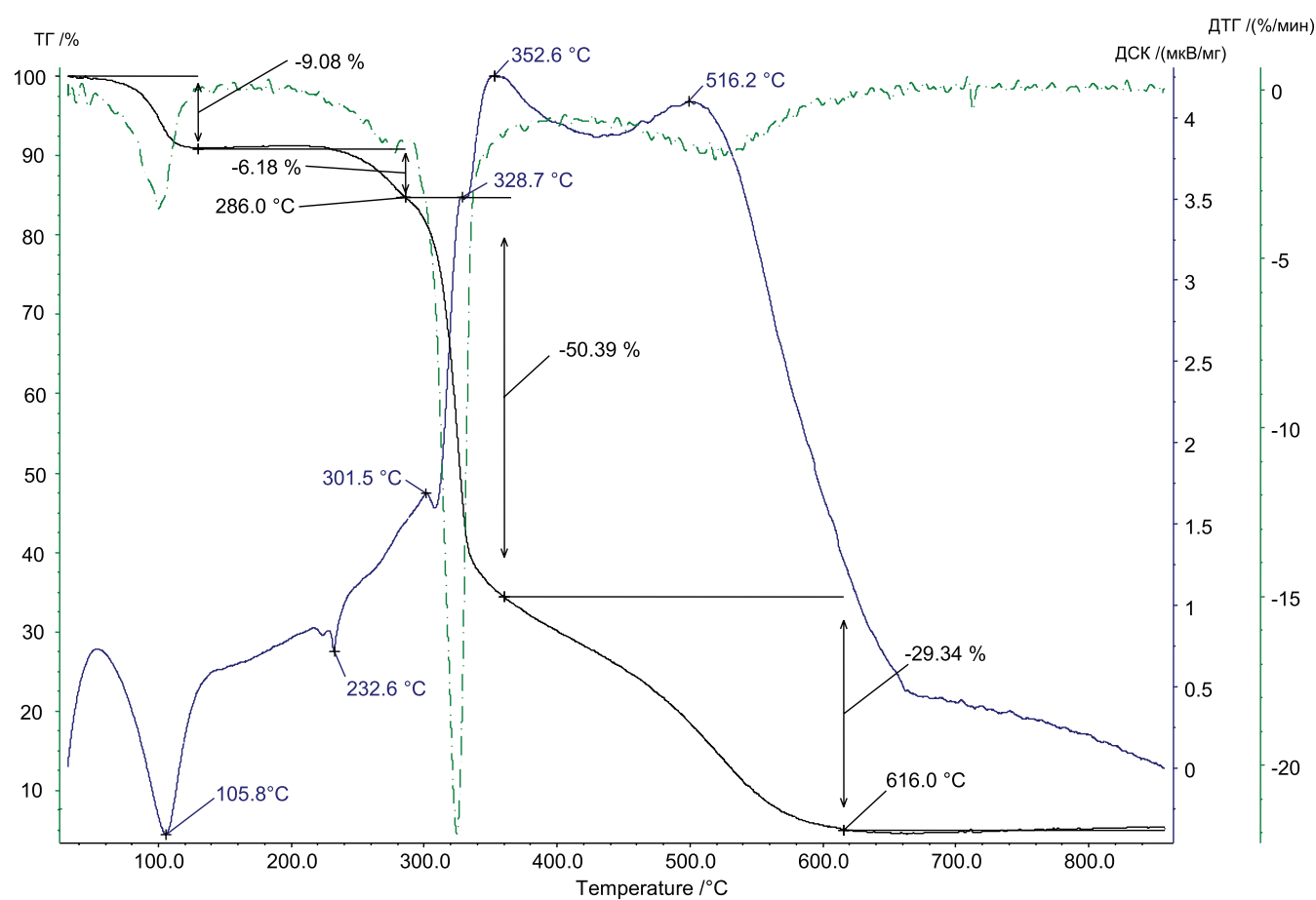
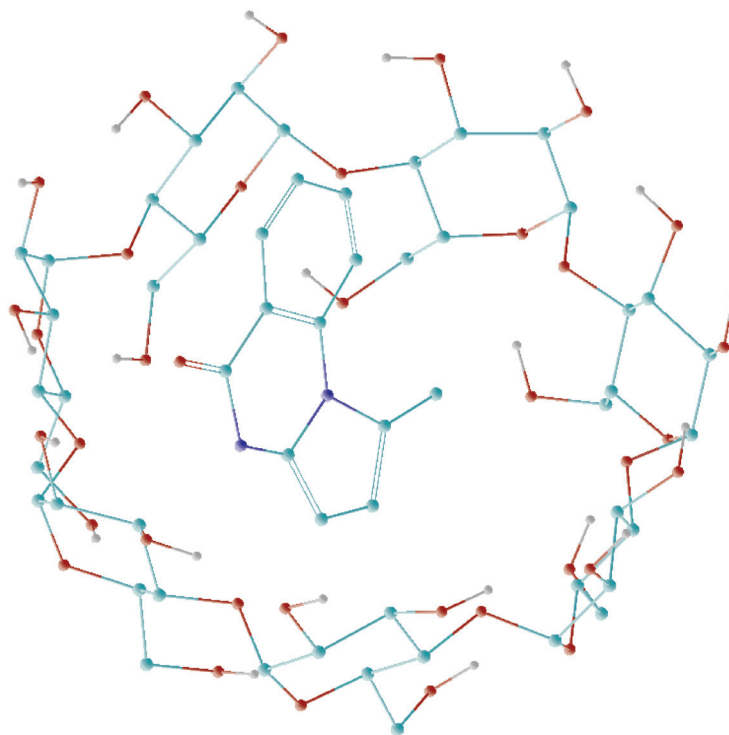


Figure 5. Thermogram of physical mixture of β -CyD with MDPB.

Table 4. AutoDock report for the conformations **47** and **49**.

Parameter	Conformation	
	47	49
Estimated Free energy of binding	−6.10 kcal/mol	−6.97 kcal/mol
Estimated Inhibition constant, K_i (298.15 K)	33.63 μ M	7.74 μ M
Final Intermolecular energy (vdW + Hbond + desolv energy)	−6.10 kcal/mol	−6.97 kcal/mol
Final Total internal energy	−6.10 kcal/mol	−6.97 kcal/mol
Torsional free energy	0.0 kcal/mol	0.0 kcal/mol
Unbound system's energy	0.0 kcal/mol	0.0 kcal/mol

**Figure 6.** The most favorable calculated structure for the inclusion complex of β -CyD with MDPB (1:1).

much as possible, that provides the formation and stability of the complex.^[26]

The geometry optimization for conformation **49** gives the following results: the binding energy is −27149.9 kcal/mol, the heat of formation is −4572.5 kcal/mol. The energy of complex formation, calculated as the difference of the heat of formation of the complex compound and initial compounds,^[25,28] is −26.5 kcal/mol. The optimized geometry of the complex is shown in Figure 6.

Conclusion

Ten novel fused diazepines were examined *in silico* using the PASS Online service to evaluate their biological activity. Virtual screening check list consists of 6 essential psychotropic effects such as antipsychotic (neuroleptic), antidepressant, tranquilizing (anxiolytic), muscle relaxant, nootropic and anticonvulsant activity. Screening results

showed that 1-methyl-4,5-dihydro-6*H*-pyrrolo[1,2-*a*][1,4]benzodiazepin-6-one most likely would have nootropic effect while the *N*-(*tert*-butyl)-2-(1-methyl-6-oxo-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepin-5(6*H*)-yl)acetamide has been recognized as possible neuroleptic and 8,9-dimethoxy-1-methyl-4,5-dihydro-6*H*-pyrrolo[1,2-*a*][1,4]benzodiazepine-6-thione was expected to have an anticonvulsant effect.

The inclusion complex of 1-methyl-4,5-dihydro-6*H*-pyrrolo[1,2-*a*][1,4]benzodiazepin-6-one with β -cyclodextrin was synthesized by kneading method. The obtained complex compound was characterized by solid state ^{13}C NMR, IR spectroscopy and thermal analysis.

A complex compound of β -CyD inclusion with MDPB was obtained by the solid-phase synthesis method. The structure of the inclusion complex was confirmed by means of NMR, IR spectroscopy and thermal analysis.

To find the most possible geometry of inclusion complex, the molecular docking studies were carried out using the AutoDock program. As a result, a number

of the most preferred conformations was obtained. The geometry of the obtained conformations was optimized using the semi-empirical method AM1. Based on the docking data, the geometry of the most probable structure of the inclusion complex compound was suggested.

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