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ORIGINAL ARTICLE

Study of binary systems of β-cyclodextrin with a highly potential anti-mycobacterial drug

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Abstract The present research paper is dedicated to the obtaining and physicochemical characterization of a highly potential anti-mycobacterial drug candidate with β-cyclodextrin (βCD). The active substance is a 1,3,4-oxadiazole derivative, 2-phenyl-5-{[(2-phenyl-1,3-dioxolan-2-yl) methyl]sulfanyl}-1,3,4-oxadiazole, further named DIOX. DIOX–βCD binary systems were obtained as a physical mixture and a lyophilized product with molar ratio between the main components equal to 1:1 and 1:2. The obtained systems were submitted to physicochemical characterization applying the following instrumental methods: infrared spectrometry, differential scanning calorimetry, and X-ray crystallographic analysis. Besides, a molecular modeling analysis has been performed. The research data suggested certain intermolecular interaction between DIOX and βCD, suggesting formation of a three-molecular inclusion complex DIOX:βCD, including one DIOX molecule and two molecules of βCD. The main parts of the DIOX molecule included in the hydrophobic cavity of the cyclodextrin molecules most probably are dioxolane cycle and two benzene rings.

Keywords β-Cyclodextrin · Anti-mycobacterial drugs · 1,3,4-Oxadiazole derivatives · Drug–cyclodextrin complex · Tuberculosis

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Introduction

According to the WHO surveys and reports, there exists a critical need in efforts to develop new classes of drugs which have the potential of shortening the current standard treatment of tuberculosis and to be effective against multi drug-resistant and extensively drug-resistant forms of the illness [1, 2].

Recently, many groups of researchers have proposed new classes of anti-mycobacterial drugs thiolactomycins [3], benzothiazoles [4], substituted quinolinyl chalcones [5], cinnamic derivatives [6] (Fig. 1a–d).

The studies performed by Macaev et al. [7] have lead to discovery of another group of substances with high anti-mycobacterial activity from the class of 5-aryl-2-thio-1,3,4-oxadiazole derivatives. Among the studied substances, 2-phenyl-5-{[(2-phenyl-1,3-dioxolan-2-yl)methyl] sulfanyl}-1,3,4-oxadiazole (DIOX) (Fig. 1e) has shown the highest level of predicted and in vitro activity. Synthesis of the substance is presented in the paper [7].

The substance's molecular weight is 340.4, it has five freely rotatable bonds, five hydrogen bond acceptors and no hydrogen bond donors that makes it a druggable candidate according to the Lipinski rule [8]. Still, there is one important disadvantage that can influence bioavailability of the drug and reduce its pharmacological activity—a low solubility in water. In order to overcome this shortcoming,

it has been proposed to study the possibility of DIOX inclusion in complexes with β-cyclodextrins (βCD). Besides, in the case of the direct contact of the inclusion complex with the *M. tuberculosis* cell, the presence of cyclodextrins in the formulation may increase permeability of mycobacterial wall for the active substance [9].

The main objective of this study was to determine whether DIOX forms complexes with βCD and what is an approximate ratio between the components in these complexes. For this, two main systems have been prepared, DIOX:βCD = 1:1 and DIOX:βCD = 1:2, as physical mixtures and lyophilized products. The main research methods applied to determine the inclusion complex formation were IR spectrometry, differential scanning calorimetry, and X-ray crystallography.

Experimental

Materials

DIOX 2-phenyl-5-{[(2-phenyl-1,3-DIOXolan-2-yl)methyl] sulfanyl}-1,3,4-oxadiazole, empirical formula $C_{18}H_{16}N_2O_3S$, MW 340) was synthesized by Macaev et al. [7] at the Institute of Chemistry, Academy of Sciences of Moldova [7]. β-Cyclodextrin (empirical formula $(C_6H_{10}O_5)_7$, MW 1,135) was purchased from Sigma-Aldrich.

Preparation of physical mixtures

Equimolar or 1:2 ratio physical mixtures (p.m.) of DIOX and βCD were prepared by homogeneous blending in agate mortar of exactly weighed amounts.

Preparation of lyophilized products

Equimolar or 1:2 ratio amounts of DIOX and βCD were mixed in water and stirred for 2 h at 35 °C. After the equilibration period of 24 h the clear solutions were frozen and subsequently lyophilized in a freeze dryer of Alpha 1-2 LD type.

FT-IR spectrometry

FT-IR spectra of DIOX, βCD, and of their binary systems (physical mixtures and lyophilized products) were obtained using the KBr pellet technique. They were collected with Jasco FTIR 6100 spectrometer in the 4,000–350 cm^{-1} spectral range with the resolution of 2 cm^{-1}.

X-ray diffractometry

Powder XRD patterns were analyzed at room temperature with an automated Bruker D8 Advance powder diffractometer. The patterns were recorded in the 2θ angle range between 3° and 45° and the process parameters were set at scan step size of 0.01 (2θ), scan step time of 1 s. The XRD traces of all raw materials, binary, and ternary systems were compared with regard to peak position, relative intensity, peak shifting, and the presence, and/or absence, of peaks in the certain regions of 2θ values.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis was performed with a Shimadzu DSC-60. Conventional measurements were performed by heating the weighed samples (1.0–1.3 mg), placed in aluminum pans, in a temperature range of 30–300 °C at a heating rate of 10 °C/min under nitrogen flow of 100 cm^3/min.

Molecular modeling

Due to the relatively large number of atoms (more than 330 atoms) which form our supramolecular system, it is difficult to give an adequate description using high-level ab initio methods (including electron correlation effects) and enough large basis set (at least a triple-ζ quality basis set), respectively. Even the widely used DFT method with a well-chosen exchange-correlation functional together with a triple-ζ quality basis set could easily exceeds our computer capacity limits when we apply it for geometry optimization calculation. The density functional-based tight-binding method combined with the self-consistent charge technique (SCC–DFTB) [10] can be considered as an adequate solution for treating large biologically interested or nanoscaled molecular materials with nearly good accuracy as obtained in the case of high-level theoretical methods [11–14]. For the SCC–DFTB method an empirical dispersion correction has also been developed, and it was found to be crucial for predicting reliable nucleic acid base stacking interactions [13]. For the H, C, N, O, S atoms the *mio* [10, 15] atomic parameter suite were used.

Results and discussion

As it can be seen from the DIOX structure (Fig. 1), there are two certain possibilities of its molecule to be accommodated inside the cavity of a CD thanks to presence of two phenyl groups on the opposite sites of the molecule. In order to confirm the formation of DIOX–βCD complex, the binary systems, with molar ratios between the system's components (DIOX:CD) equal to 1:1 and 1:2, respectively, were prepared as physical mixtures and lyophilized products. The FT-IR spectra of the prepared systems have been

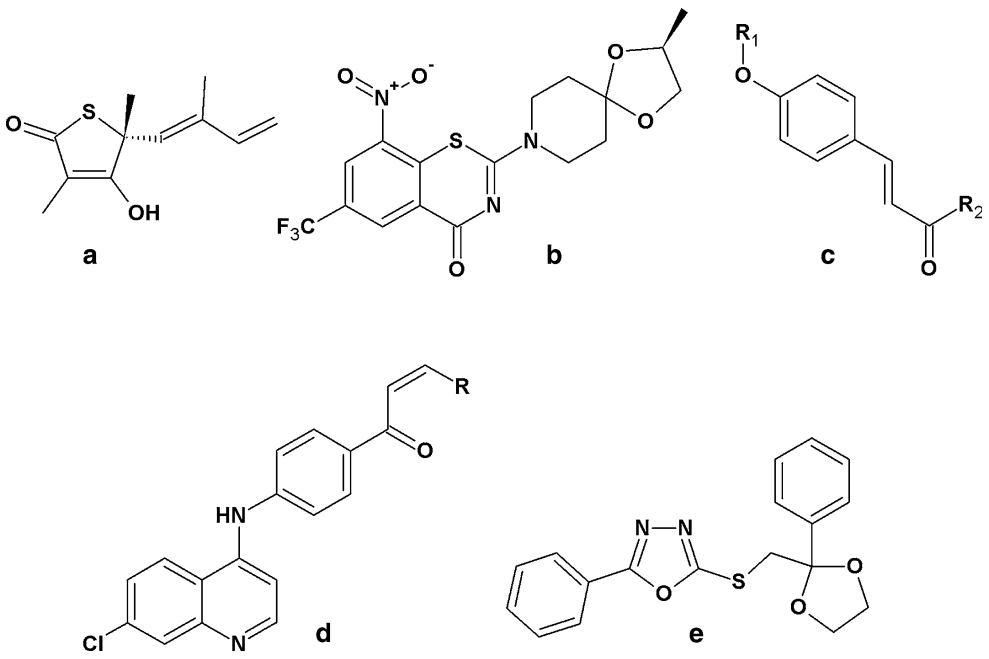

Fig. 1 Novel antituberculosis agent's thiolactomycin (**a**), benzothiazone (**b**), cinnamic derivatives (**c**), substituted quinolinyl chalcones (**d**), DIOX (**e**)

analyzed and compared with those of the individual substances.

The main indicators that confirm the formation of inclusion complexes are changes in peak positions, shapes and intensities observed in the obtained FT-IR spectra of the analyzed systems in comparison with those of the initial substances. These changes can be explained by the implication of different functional groups and parts of the host and guest molecules in the formation of inclusion complex.

Thus, in the 700–710 cm^{-1} region, the absorption peak corresponding to deformational vibrations of benzene rings of DIOX is shifted in the spectrum of the lyophilized product DIOX–βCD = 1:1 (Fig. 2) as compared to that in the spectra the physical mixture DIOX–βCD and pure DIOX. Even larger shifts to higher wave numbers can be observed in the same region of the DIOX–βCD = 1:2 lyophilized product spectrum (Fig. 3).

Benzene ring vibrations, in the region 1,460–1,470 cm^{-1}, are also shifted to higher wave numbers in the spectra of the lyophilized product DIOX–βCD 1:1 (Fig. 4) and the lyophilized product DIOX–βCD 1:2 (Fig. 5) as compared to the same peak in the spectra of the pure DIOX and corresponding physical mixtures. Noteworthy is that the shift in the spectrum of the 1:2 lyophilized product is larger than that in the spectrum of the 1:1 lyophilized product.

Analysis of the obtained IR spectra permits to assume that both benzene rings of the DIOX molecule have changed their vibrations frequency due to interactions with βCD and possibly as a result of inclusion in its

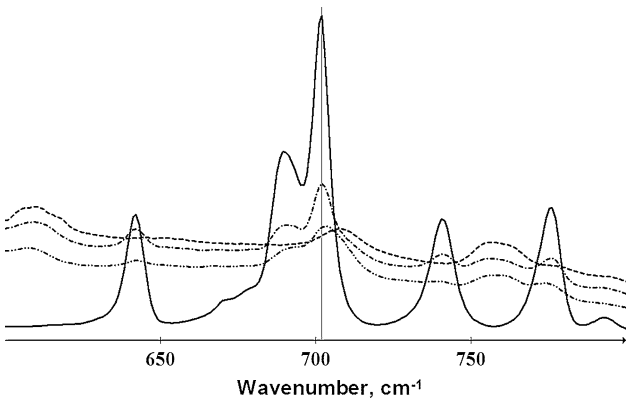

Fig. 2 FT-IR spectra (600–800 cm^{-1}) of DIOX (*solid line*), βCD (*dotted line*), and of their 1:1 binary systems: physical mixture (*solid line with one dotted line*) and lyophilized product (*solid line with two dotted line*)

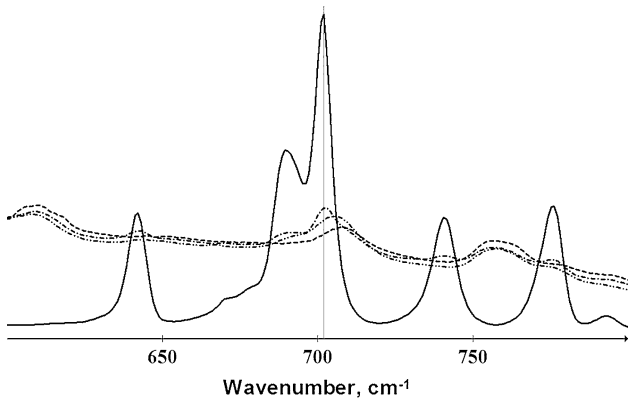

Fig. 3 FT-IR spectra (600–800 cm^{-1}) of DIOX (*solid line*), βCD (*dotted line*), and of their 1:2 binary systems: physical mixture (*solid line with dotted line*) and lyophilized product (*solid line with two dotted line*)

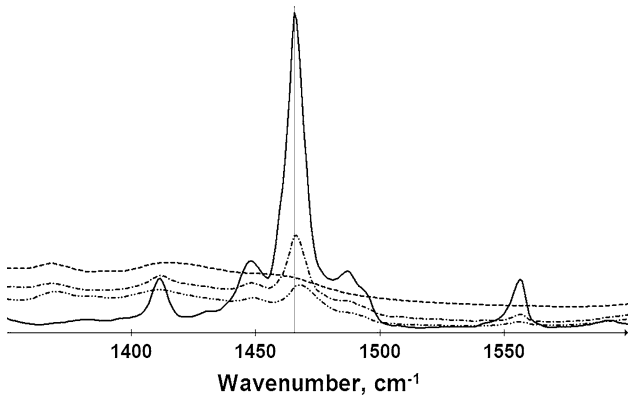

Fig. 4 FT-IR spectra (1,350–1,600 cm^{-1}) of DIOX (*solid line*), βCD (*dotted line*), and of their 1:1 binary systems: physical mixture (*solid line with one dotted line*) and lyophilized product (*solid line with two dotted line*)

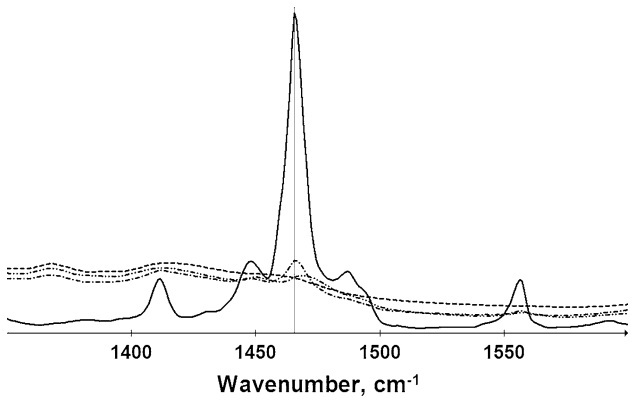

Fig. 5 FT-IR spectra (1,350–1,600 cm^{-1}) of DIOX (*solid line*), βCD (*dotted line*), and of their 1:2 binary systems: physical mixture (*solid line with one dotted line*) and lyophilized product (*solid line with two dotted line*)

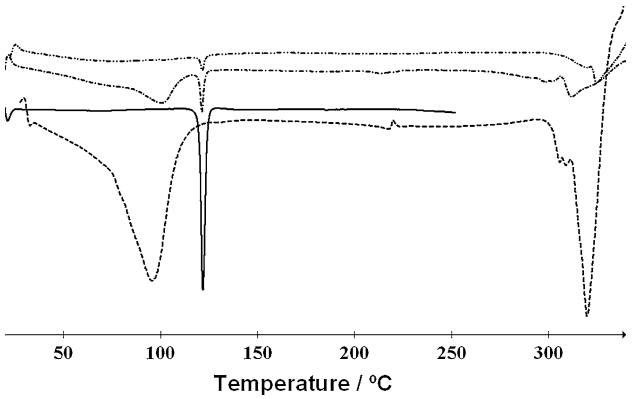

Fig. 6 DSC curves of DIOX (*solid line*), βCD (*dotted line*), and of their 1:2 binary systems: physical mixture (*solid line with one dotted line*) and lyophilized product (*solid line with two dotted line*)

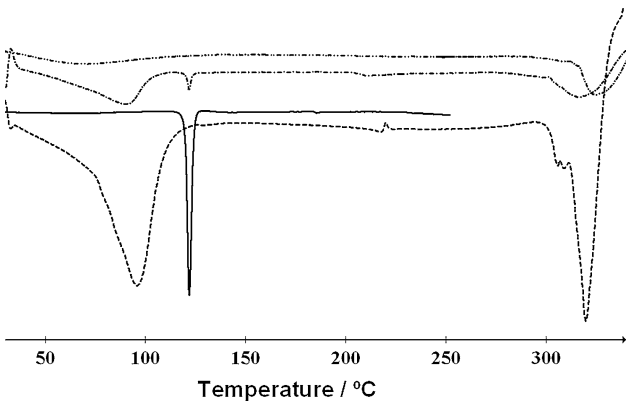

Fig. 7 DSC curves of DIOX (*solid line*), βCD (*dotted line*), and of their 1:2 binary systems: physical mixture (*solid line with one dotted line*) and lyophilized product (*solid line with two dotted line*)

hydrophobic cavity. The fact that there is a significant difference in the shifts between the spectra of the 1:l and 1:2 lyophilized products supports the hypothesis about DIOX:βCD = 1:2 ratio in the complex structure.

In order to verify and confirm the assumptions made above, DSC thermograms of the obtained systems have been analyzed.

The thermal profiles of the pure components and of the respective binary products are presented in Figs. 6 and 7. The DSC curve of DIOX indicates its crystalline state with melting point at 121.83 °C. The curve for βCD reveals a wide band (range approx. 40–110 °C) that corresponds to loss by evaporation of the water molecules existing as residual humidity ($T < 100$ °C) as well as those included in the cavity ($T > 100$ °C). Besides, at 320 °C of the βCD DSC curve one can observe another endotherm corresponding to the melting point of oligosaccharide.

The melting endotherm of DIOX can be observed in the DSC curves of the both physical mixtures DIOX–βCD 1:1 and 1:2. In the thermal profile of the DIOX–βCD 1:1 lyophilized product one can see a reduced endothermal effect characteristic for DIOX as compared to the endothermal effect of the DIOX in the physical mixture 1:1. Comparison was made taking into consideration the quantity of the taken probes.

At the same time, the thermal profile of the DIOX–βCD 1:2 lyophilized product does not present any endothermal effect in the zone characteristic for the DIOX melting process. The last fact indicates the absence of crystalline substance and its amorphization as a result of DIOX intermolecular interaction with βCD including possible formation of an inclusion complex.

The XRD patterns of DIOX, βCD, and their physical mixtures and lyophilized products, in the range of 3°–45° 2θ, are shown in Fig. 8 for the systems with the molar ratio

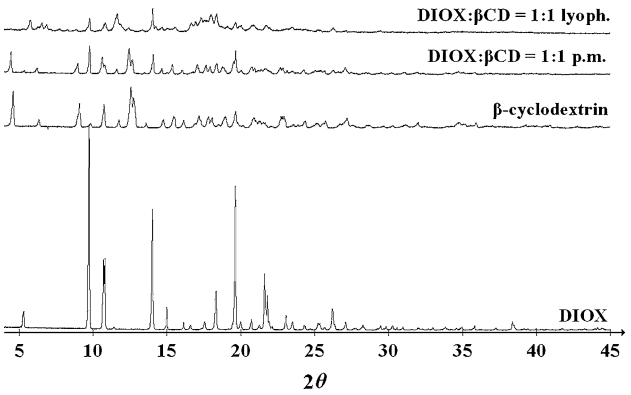

Fig. 8 Powder XRD patterns of DIOX, βCD, physical mixture (p.m.) DIOX–βCD (1:1), and lyophilized (lyoph.) product DIOX–βCD (1:1)

1:1, and in the Fig. 9 for the systems with the molar ratio 1:2. The XRD pattern of DIOX revealed several high-intensity reflections corresponding to the diffraction peaks 9.7°, 10.7°, 10.8°, 14.0°, 19.6°, 21.5°, and 21.8° (2θ), which were indicative of its crystalline character. The XRD pattern of βCD reveals its crystalline nature, as well as patterns for DIOX–βCD physical mixtures with molar ratio 1:1 and 1:2 (Figs. 8, 9).

A close analysis of the XRD pattern for the 1:1 lyophilized product showed the presence of peaks characteristic for the DIOX crystalline structure, while there were no peaks characteristic for the βCD crystals. Also, the ratio between the intensity of DIOX peaks at 9.7°, 14.0°, and 19.6°, has changed in the pattern of the lyophilized product as compared to the physical mixture, and pure DIOX. Respective intensity ratios are 1:0.59:0.70 (DIOX), 1:0.71:0.85 (DIOX–βCD 1:1 p.m.), and 1:1.59:0.78 (DIOX–βCD 1:1 lyoph.). All these indicated that while there was no crystalline βCD left in the lyophilized product, there was still present crystalline form of DIOX, though, some part of it had been transformed in other form, possibly due formation of the inclusion form.

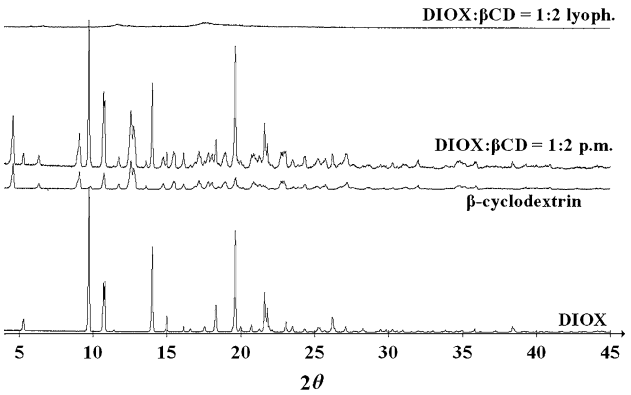

Fig. 9 Powder XRD patterns of DIOX, βCD, physical mixture (p.m.) DIOX–βCD (1:2), and lyophilized (lyoph.) product DIOX–βCD (1:2)

The analysis of the XRD pattern for the 1:2 lyophilized product (Fig. 9) showed that it did not include any peaks characteristic for the crystalline structure of DIOX or βCD. This was another observation that suggested a stronger intermolecular interaction in DIOX–βCD 1:2 system than in DIOX–βCD 1:1 system.

Infrared spectrometry, DSC, and XRD measurements have demonstrated a strong intermolecular interaction in DIOX–βCD system with possible formation of inclusion complex with 1:2 stoichiometry. According to this finding, we have performed geometry optimization for the DIOX–βCD dimer system using the SCC-DFTB theory. The optimized geometry structure of the βCD dimer and the DIOX molecule located inside the dimer is presented in Fig. 10. Analyzing the position of the DIOX molecule inside the βCD dimer one can see that the sulfur bridge, the dioxolane fragment and one of the benzene fragments are situated in the cavity of the first βCD monomer, while the other benzene ring belongs to the cavity of the second βCD monomer. The total intermolecular interaction energy between the DIOX molecule and the βCD dimer ($\Delta E^{(\mathrm{DIOX}-2\beta\mathrm{CD})}$) is −58.00 kcal/mol. If one removes one by one the βCD monomers and then calculates intermolecular interaction energy between the DIOX molecule and the remaining βCD monomer, the following energy values: $\Delta E_{\mathrm{A}}^{(\mathrm{DIOX}-\beta\mathrm{CD})} = -32.07$ kcal/mol, and $\Delta E_{\mathrm{B}}^{(\mathrm{DIOX}-\beta\mathrm{CD})} = -26.02$ kcal/mol are obtained. At the same time, we have also estimated the interaction energy between the two βCD monomers, which is −59.78 kcal/mol. The deformation energies, which are defined as the differences between the energies of the optimized molecular constituents and the energy of the same molecular constituents only inside the supramolecular complex are follows: −2.00 kcal/mol for the DIOX molecule and −2.58 and −5.42 kcal/mol for the two βCD monomers, respectively. All these energy values show us, that the DIOX–βCD dimer system could forms a quite stable supramolecular complex.

On the other hand, not every DIOX molecule can form a closed supramolecular system with two βCD monomers. Therefore, we have performed further geometry optimizations where instead of the βCD dimer we have considered only one βCD molecule. The initial geometries were prepared considering two different orientation of the DIOX molecule compared with the βCD cavity. The geometry structures of the DIOX–βCD binary systems are presented in Fig. 11. The intermolecular energy for case (a) is −41.22 kcal/mol, while for configuration (b) is −44.92 kcal/mol.

Conclusions

The analyzes performed have shown that 2-phenyl-5-{[(2-phenyl-1,3-dioxolan-2-yl)methyl]sulfanyl}-1,3,4-oxadiazole

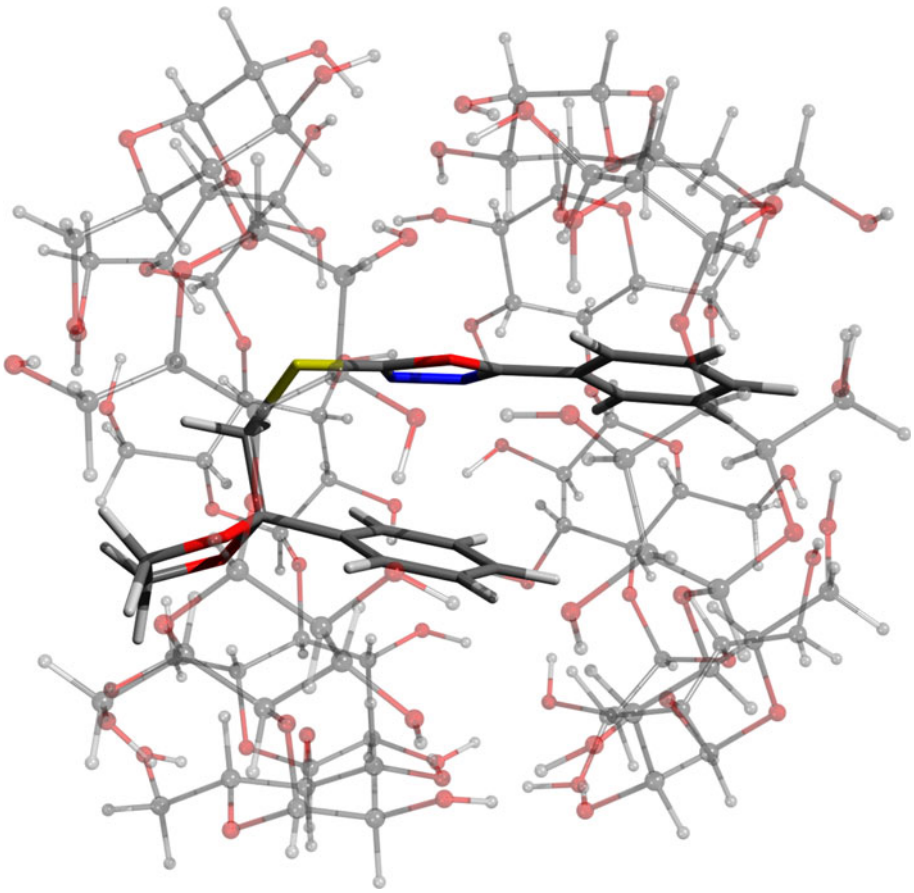

Fig. 10 The optimized geometry structure of DIOX–βCD dimer system

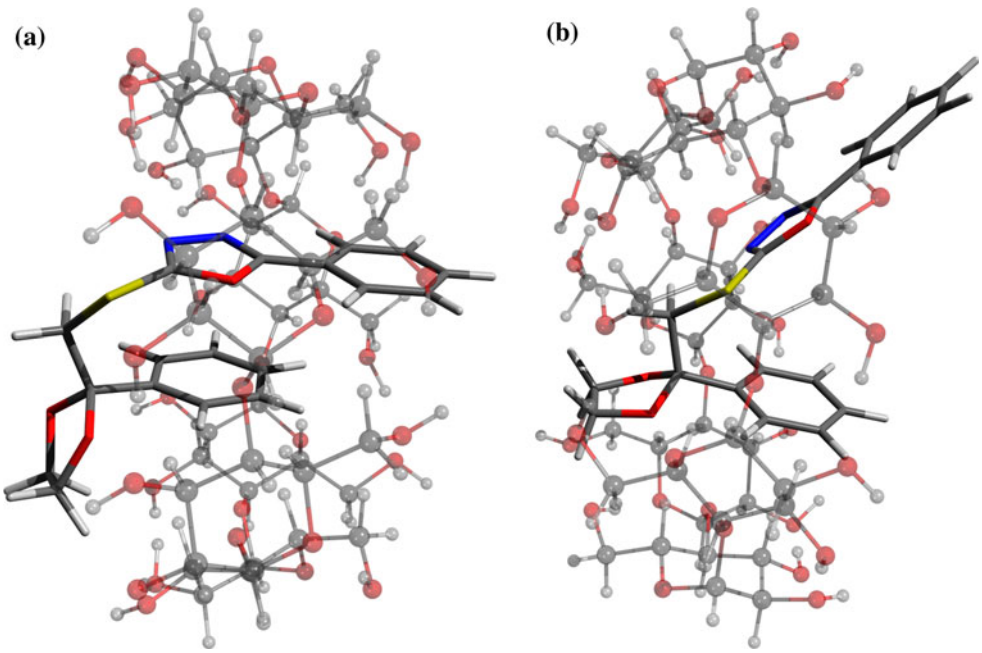

Fig. 11 The optimized geometry structures of DIOX–βCD system

(DIOX) interacts with βCD and the main parts of the DIOX molecule involved in the interaction process are dioxolane and benzene rings. Data obtained through molecular modeling suggest that the interaction might be of the inclusion complex type.

The molecular interaction between DIOX and βCD makes possible its future study in new types of antituberculosis treatment where the presence of βCD does not only provide better bioavailability and stability characteristics to the active ingredient, but also plays the role of promoter of

the active substance transportation through the bacterial cell wall, increasing its permeability for the substance. The latest is possible in case of direct interaction of the complex with the bacterial cell.

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