Intermolecular Interaction and Complex Formation of Cytoflavin Components: IR Spectroscopy and Molecular Modeling

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Abstract. IR spectra of intermolecular complexes of the main active components of cytoflavin: succinic acid, nicotinamide and inosine are analyzed. The study focuses on interpreting the absorption band maxima associated with hydrogen bonding between the key functional groups of cytoflavin's constituents. The IR spectra were calculated using density functional theory (DFT) with the B3LYP functional, while experimental IR spectra were recorded using the SpectrumTwo FTIR spectrometer. The results showed that succinic acid has the ability to interact effectively with both one and two nicotinamide molecules by forming stable hydrogen bonds, whereas in complexes with inosine, the strength of the hydrogen bonds is weaker. This suggests that inosine acts as a cofactor to increase the overall stability of the complex.

Keywords: succinic acid; nicotinamide; inosine; cytoflavin; density functional theory; IR spectrum.

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1 Introduction

In medical practice, drugs composed of a combination of active substances are widely used. Each substance exerts its own therapeutic effect and can enhance the overall efficacy compared to individual components. Cytoflavin, a metabolic protease known for restoring antioxidant enzyme activity and exhibiting neuroprotective effects, is commonly employed [1]. Cytoflavin contains succinic acid (10%), inosine (2%), nicotinamide (1%), and riboflavin (0.2%) as active components, with succinic acid being the most significant due to its antioxidant properties. Due to the relatively low proportion of the active substance riboflavin, a coenzyme of glutathione reductase, which restores the pool of the most important component of the antioxidant system of the cell [1], the inclusion of this component in the study of intermolecular interaction with the other active substances of cytoflavin was not carried out. Experimental studies have shown [2] that the positive effects of cytoflavin treatment increase from 60% to 80% when combined with standard therapy methods.

Both nicotinamide and isonicotinamide demonstrate a tendency to form cocrystals with carboxylic acids in three ways [3]. Nicotinamide molecules form cocrystals through strong hydrogen bonds (N−H···O=C) (II), while in crystals with succinic acid, strong hydrogen bonds (N−H···O=C and O−H···O=C) (III) are observed. In the case of complex formation between succinic acid and nicotinamide, a soft heteromeric acid-pyridine hydrogen bond (O—H···N) (I) is formed (Fig. 1).

The aim of this research is to investigate the spectral manifestations of intermolecular interactions between the components of the drug cytoflavin, followed by the determination of the parameters of the resulting hydrogen bonds. This will enable an assessment of the stability of the complex through analysis of the calculated structure and comparison of experimentally measured and calculated IR spectra. The key components of cytoflavin, succinic acid, nicotinamide and inosine were selected as the research objects. Understanding these mechanisms will provide deeper insights into the therapeutic effects of cytoflavin.

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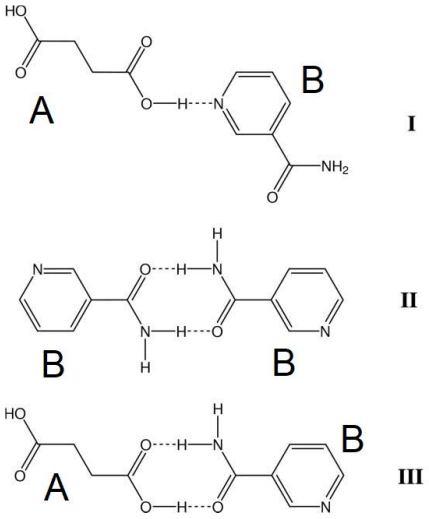


Fig. 1 Potential hydrogen-bond synthons found in acid–amide cocrystals (A − molecule of succinic acid, B − molecule of nicotinamide). (I) Hydrogen bond formed between nicotinamide and succinic acid molecules; (II) Hydrogen bonds formed between two nicotinamide molecules; (III) Hydrogen bonds formed between nicotinamide and succinic acid molecules in the crystals.

2 Methods

The study of intermolecular interactions was conducted by modeling the structures, calculating the normal vibration frequencies, and determining the intensities of IR absorption bands of the molecules and their complexes. These calculations were performed using density functional theory (DFT) [4] with the B3LYP functional [5] and the 6-31G++(d) basis set as this basis set is commonly used in the study of organic molecules [6]. It also includes polarization d-functions for second-row atoms and is supplemented with diffuse functions for heavy atoms and hydrogen [7], which are necessary for the accurate calculation of hydrogen bonding parameters between multiple molecules.

The Chemcraft editor and molecular structure visualizer [8], along with an author-developed program for displaying infrared spectra, were used to recreate the IR spectra based on numerical values obtained from the Gaussian program.

The hydrogen bond energies were calculated using Johansen's empirical formula [9]:

$$-\Delta H = 0.3\sqrt{\Delta v - 40}. \quad (1)$$

Finally, the calculated frequencies were multiplied by an anharmonicity correction factor [10–11]. The following scaling factors were chosen: 0.97 for frequencies between 0 and 2000 cm^{-1} and 0.94 for frequencies between 2000 and 4000 cm^{-1}.

Colleagues from RTA Research Center LLC recorded the IR spectra succinic acid and nicotinamide (Figs. 2−3) using a SpectrumTwo FTIR spectrometer (PerkinElmer, USA) equipped with a diffuse reflectance accessory (without tableting in KBr) in the range of 4000−600 cm^{-1} with a resolution of 2 cm^{-1} at a temperature of 20 °C. In the process of preparing materials for recording IR spectra of succinic acid and nicotinamide, suspensions were used on analytical balances with a sampling rate of 0.01 mg of succinic acid 5.6 mg and nicotinamide 5.76 mg. The suspensions were mixed with 200 µl of water, leading to the formation of a poorly soluble precipitate. The precipitate dissolved completely after the addition of 200 µl EtOH and vigorous stirring of the suspension on a vortex. The final crystalline phase of the substances was obtained after drying on a thermo shaker at 100 °C for 2 h. The experimental IR spectrum of inosine was obtained from the open-access IR spectra database − SpectraBase [12].

3 Results

The optimized structures and calculated IR spectra of succinic acid, nicotinamide, inosine and their complexes are presented in Figs. 2–9.

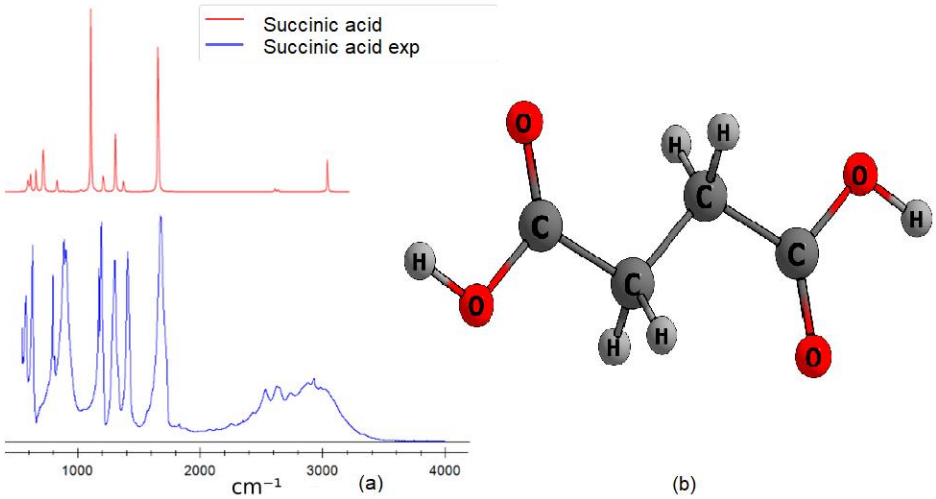


Fig. 2 (a) IR spectrum and (b) calculated structure of succinic acid: $C_4H_6O_4$ (red – calculated, blue – experimental).

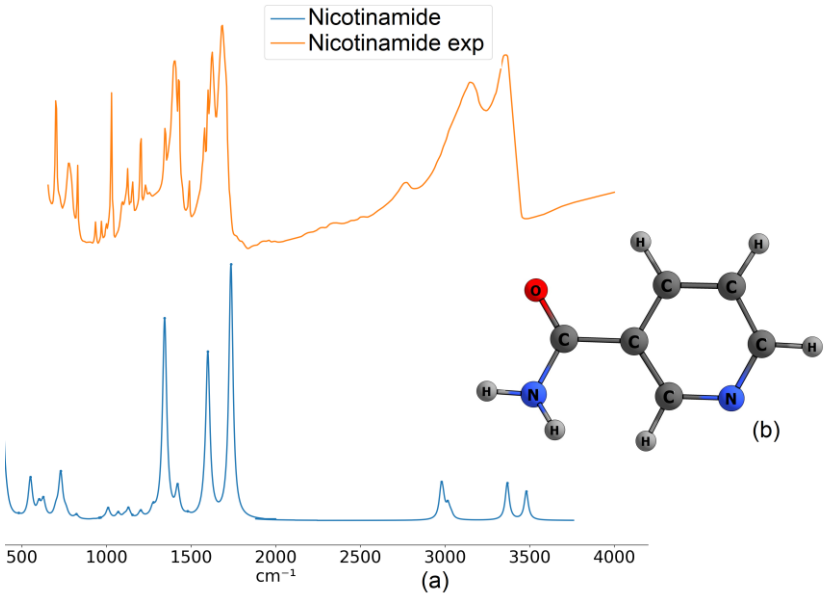


Fig. 3 (a) IR spectrum and (b) calculated structure of nicotinamide: $C_6H_6N_2O$ (orange – experimental, blue – calculated).

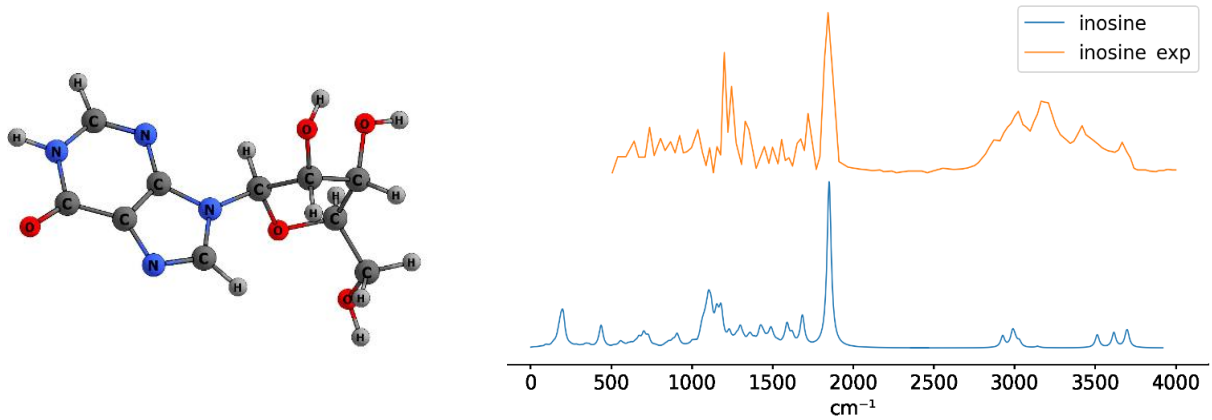


Fig. 4 (a) IR spectrum and (b) calculated structure of inosine: $C_{10}H_{12}N_4O_5$ (orange – experimental, blue – calculated).

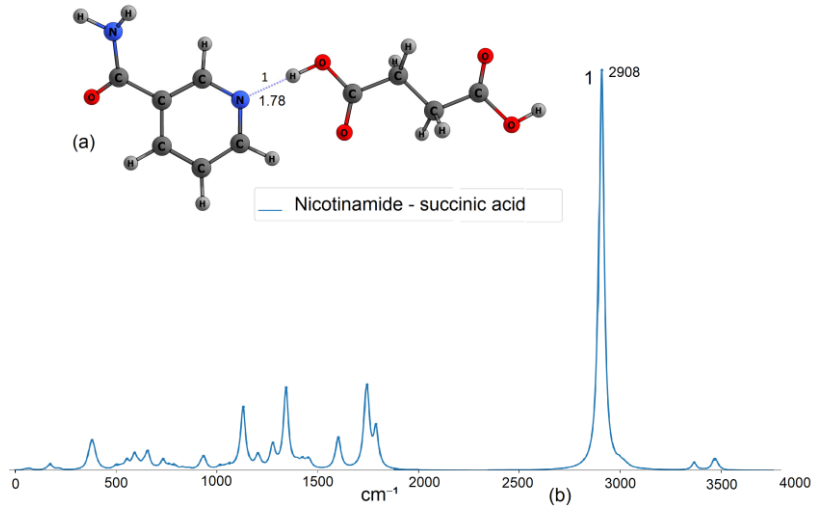
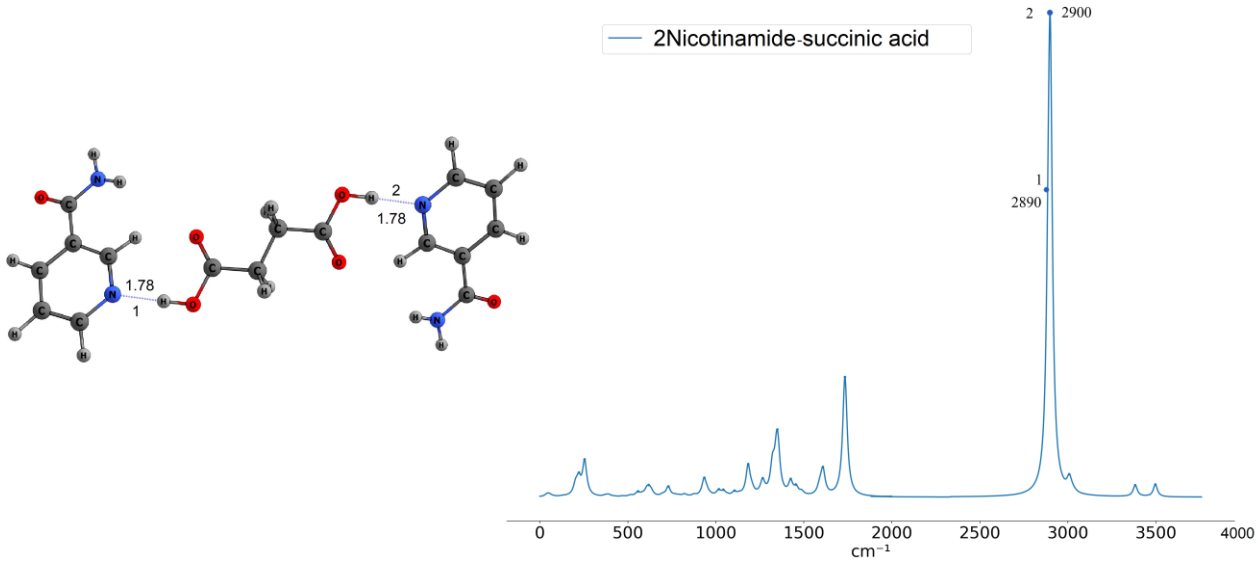
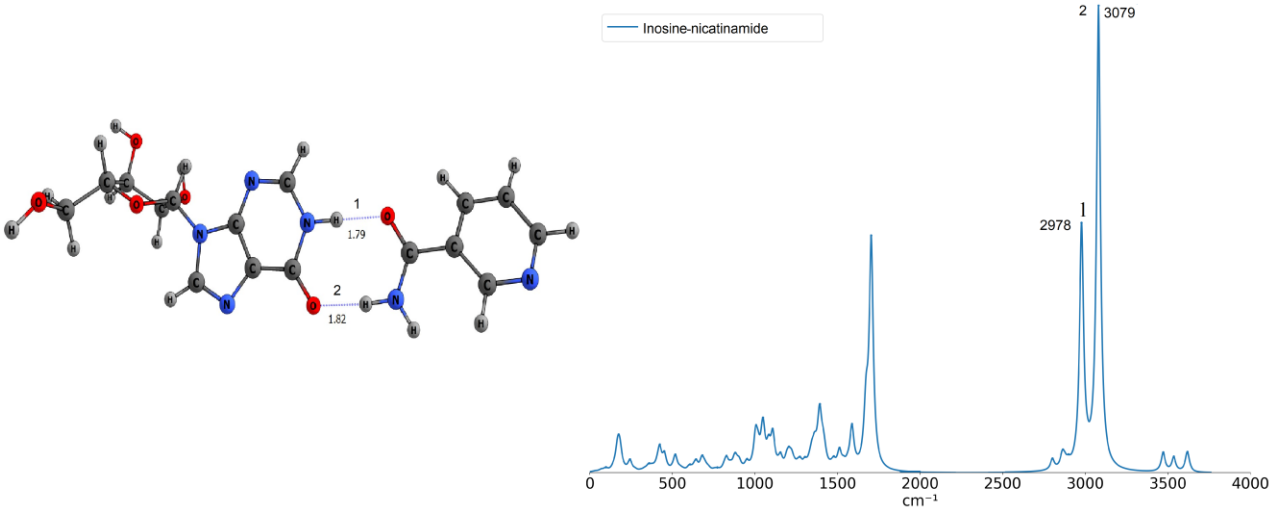


Fig. 5 (a) Calculated structure and (b) IR spectrum of the nicotinamide-succinic acid molecular complex: $C_4H_6O_4$ – $C_6H_6N_2O$. The hydrogen bond lengths are marked 1 in Panel (a). The absorption peaks corresponding to –OH (1) group vibrations are marked with number 1 in Panel (b).



(a) (b)

Fig. 6 (a) Calculated structure and (b) IR spectrum of the 2nicotinamide-succinic acid molecular complex: $C_4H_6O_4 – 2(C_6H_6N_2O)$. The hydrogen bond lengths are marked 1 and 2 in Panel (a). The absorption peaks corresponding to –OH (1, 2) group vibrations are marked with numbers 1−2 in Panel (b).



(a) (b)

Fig. 7 (a) Calculated structure and (b) IR spectrum of the inosine-nicotinamide molecular complex: $C_{10}H_{12}N_4O_5 – C_6H_6N_2O$. The hydrogen bond lengths are marked 1–2 in Panel (a). The absorption peaks corresponding to –NH (1, 2) group vibrations are marked with numbers 1–2 in Panel (b).

Based on research [13], the structures and IR spectra of the succinic acid and nicotinamide complexes were calculated. The characteristic frequencies of the absorption bands in the calculated complexes correspond to those reported in earlier studies [13]: peaks corresponding to C–H bond vibrations are located in the frequency range of 2860–3090 cm^{-1}, peaks in the 2890–2910 cm^{-1} range correspond to the vibrations of hydrogen bonds in succinic acid and nicotinamide complexes, while the stretching vibrations of free N–H bonds are in the 3300–3500 cm^{-1} range, and free O–H bonds are in the 3400–3500 cm^{-1} range. The intensity and frequency of the stretching vibrations of the hydrogen bonds in the succinic acid–nicotinamide-inosine complexes are presented in Table 1.

The strength of the formed hydrogen bonds was assessed according to the classification provided in Ref. [14], where strong hydrogen bonds are considered to have energies of 14.34–28.65 kcal/mol and hydrogen bridge lengths of 2.2–2.5 Å. Medium-strength bonds have energies in the range of 3.82–14.43 kcal/mol and hydrogen bridge lengths of 2.5–3.2 Å, while weak bonds have energies of less than 2.87 kcal/mol and hydrogen bridge lengths of 3.2–4.0 Å.

Table 1 Calculated parameters of hydrogen bonds.

Bond number	Bond type	Length of H– Bond R, Å	Length of hydrogen bridge R_b, Å	Frequency ν, cm^{-1}	Frequency shift $\Delta\nu$, cm^{-1}	Enthalpy, $-\Delta H$, kkal/mol	Intensity, I_{IR}, km/mol
Nicotinamide-succinic acid, E = −873.97 a.u.							
1	O–H···N	1.78	2.79	2908	557	6.82	2232
2Nicotinamide-succinic acid, E = −1290.98 a.u.							
1	O–H···N	1.78	2.79	2890	576	6.95	1152
2	O–H···N	1.78	2.79	2900	566	6.88	3494
Inosine-nicotinamide, E = −1400.5 a.u.							
1	N–H···O	1.79	2.84	2978	389	5.61	1145
2	N–H···O	1.82	2.85	3079	288	4.72	2534
Inosine-succinic acid, E = −1440.44 a.u.							
1	O–H···O	1.76	2.75	3096	369	5.44	852
2	O–H···O	1.84	2.82	3300	165	3.36	773
Inosine-nicotinamide-succinic acid, E = −1857.47 a.u.							
1	N–H···O	1.76	2.83	2997	370	5.45	1453
2	O–H···O	1.79	2.75	3099	367	5.42	783
3	N–H···O	1.85	2.87	3121	246	4.31	2050
4	O–H···O	1.83	2.82	3299	167	3.38	760

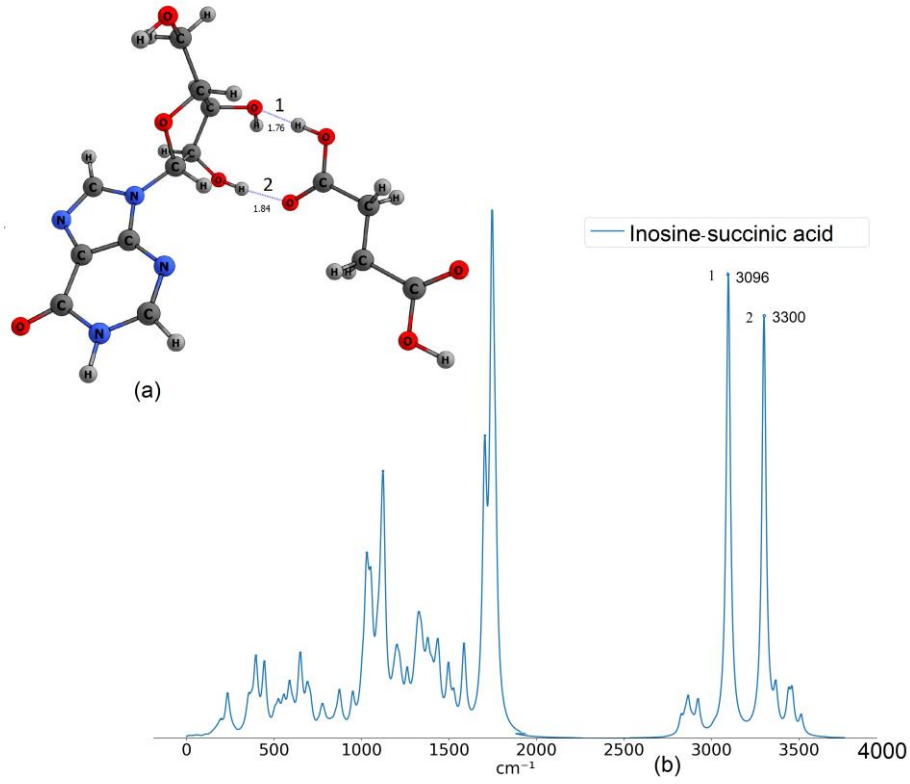


Fig. 8 (a) Calculated structure and (b) IR spectrum of the inosine-succinic acid molecular complex: $C_{10}H_{12}N_4O_5$ – $C_4H_6O_4$. The hydrogen bond lengths are marked 1–2 in Panel (a). The absorption peaks corresponding to –OH (1, 2) group vibrations are marked with numbers 1–2 in Panel (b).

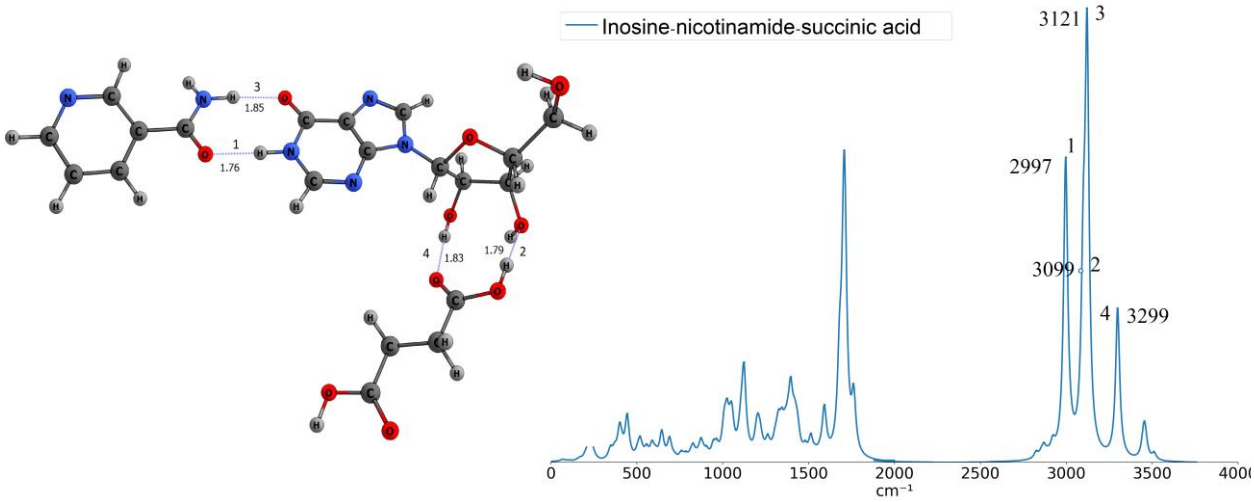


Fig. 9 (a) Calculated structure and (b) IR spectrum of the inosine-nicotinamide-succinic acid molecular complex: $C_{10}H_{12}N_4O_5 – C_6H_6N_2O – C_4H_6O_4$. The hydrogen bond lengths are marked 1–4 in Panel (a). The absorption peaks corresponding to –OH (2, 4) and –NH (1, 3) group vibrations are marked with numbers 1–4 in Panel (b).

The optimization and calculation of the molecular structures of succinic acid and nicotinamide clearly indicate that their interaction ($C_4H_6O_4 – C_6H_6N_2O$) leads to the formation of an O–H···N hydrogen bond. This bond can be characterized as medium-strength, approaching strong, with a frequency shift of 557 cm^{-1} and an intensity of 2232 km/mol.

In the complex formed by two molecules of nicotinamide and one molecule of succinic acid ($C_4H_6O_4 – 2(C_6H_6N_2O)$), two O–H···N bonds are observed. These bonds can be classified as medium-strength, with binding energies of 6.95 and 6.88 kcal/mol, frequency shifts of 576 cm^{-1} and 566 cm^{-1}, and intensities of 1152 and 3494 km/mol, respectively.

When analyzing the experimental and calculated IR spectra of inosine ($C_{10}H_{12}N_4O_5$) (Fig. 4), good agreement was observed in the characteristic absorption band frequencies. Thus, in the frequency range of 2805–3075 cm^{-1}, peaks corresponding to the stretching vibrations of C-H bonds are located. In the interval of 3360–3370 cm^{-1}, stretching vibrations of free N-H bonds are observed, while in the range from 3460 to 3550 cm^{-1}, stretching vibrations of free O–H bonds are present.

In the inosine-nicotinamide complex ($C_{10}H_{12}N_4O_5 – C_6H_6N_2O$), 2 hydrogen bonds of the N–H···O type are formed between the molecules. These bonds can be classified as medium-strength bonds with bond energies of 5.61 and 4.72 kcal/mol, as they exhibit frequency shifts of 389 cm^{-1} and 288 cm^{-1} and intensities of 1145 and 2534 km/mol, respectively.

The calculated structure and IR spectrum of the inosine-succinic acid complex ($C_{10}H_{12}N_4O_5 – C_4H_6O_4$) are shown in Fig. 8. During the complex formation of these molecules, two O–H···O type bonds (1–2) are formed. Bond 1 can be classified as a medium-strength bond, as its bond energy is 5.44 kkal/mol, with a frequency shift of 369 cm^{-1} and an intensity of 852 km/mol. Bond 2 is also classified as a medium-strength bond, with a bond energy of 3.36 kkal/mol, a frequency shift of 165 cm^{-1}, and an intensity of 773 km/mol.

The calculated structure and IR spectrum of the inosine-nicotinamide-succinic acid complex ($C_{10}H_{12}N_4O_5 – C_6H_6N_2O – C_4H_6O_4$) are shown in Fig. 9. During the formation of the molecular ensemble, two hydrogen bonds of the N-H···O type (1, 3) and two hydrogen bonds of the O-H···O type (2, 4) are formed between these molecules. Bond 1 can be classified as a medium-strength bond, as it has a bond energy of 5.45 kcal/mol, a frequency shift of 370 cm^{-1}, and an intensity of 1453 km/mol. Bond 2 is also a medium-strength bond with a bond energy of 5.42 kcal/mol, a frequency shift of 367 cm^{-1}, and an intensity of 783 km/mol. Bonds 3 and 4 are medium-strength bonds with energies of 4.31 and 3.38 kcal/mol, respectively, as their frequency shifts are 246 cm^{-1} and 167 cm^{-1}, and their intensities are 2050 and 760 km/mol, respectively.

The intensity and frequency of the stretching vibrations of hydrogen bonds between nicotinamide, succinic acid and inosine are presented in Table 1.

4 Conclusions

In the course of a comprehensive study of the components of the drug cytoflavin using quantum-chemical methods, it can be concluded that strong hydrogen bonding contributes to a high degree of complex formation between nicotinamide and succinic acid. Inosine, which is part of cytoflavin, exhibits less pronounced hydrogen bonding and is presumably acting as an auxiliary active substance, promoting the optimization of biochemical processes and enhancing the overall effectiveness of the drug.

Furthermore, supramolecular complex formation is of significant importance, as it enhances the interaction between the components of the drug and contributes to

the stabilization of its structure. This suggests that the combination of succinic acid and nicotinamide plays a key role in the manifestation of the therapeutic effect of cytoflavin.

Disclosures

The authors declare no conflict of interest.

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