

Proceeding Paper

# Spectroscopic Investigation, Component Analysis, and DFT Calculations of Tautomeric Forms of Substituted Dihydro-6H-chromeno[4,3-d]pyrazolo[1,5-a]pyrimidin-6-one <sup>†</sup>

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**Abstract:** Chromenopirazolopyrimidinone (CPP) was synthesized using microwave radiation in the absence of solvent by a multicomponent reaction of 4-hydroxy-2H-chromen-2-one with 5-amine-1H-pyrazole and benzaldehyde. CPP exists as a mixture of tautomeric forms, the structure of which was examined by means of spectroscopic methods and quantum chemistry. As was shown by the MILCA algorithm, UV-Vis spectra obtained in different solvents of various polarities contain bands of all tautomeric forms. Decomposition of the absorption spectra of CPP in various solvents on the individual components of the absorption showed the presence of two tautomers. The molar ratios of tautomeric forms depend on the polarity of the solvent used.

**Keywords:** chromenopirazolopyrimidinone; MILCA algorithm; decomposition; tautomers; UV-Vis spectra



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## 1. Introduction

The scientific community faces the challenge of creating complex organic compounds from simple substrates using environmental criteria, including the safety and efficiency of the synthetic procedures [1]. The multicomponent reaction, the Biginelli reaction, is a well-known method of the synthesis of azaheterocycles [2].

One option for the multicomponent synthesis conditions is the microwave exposure in the absence of solvent, with the addition of a Lewis acid catalyst [3]. Currently, microwave activation (Microwave Assisted Organic Synthesis, MAOS) is one of the most rapidly growing trends in organic chemistry. First of all, this is due to the multiple (101–103 times) shortening of the chemical reactions.

The combined experimental and theoretical study of the spectral characteristics of molecular systems is one of the most important modern tools for solving problems of current organic chemistry. Such an approach is relevant for a correct description of the composition and structure of the reaction mixtures of biologically active compounds, especially when it comes to mobile tautomeric equilibrium.

Thus, by combining the techniques of FTIR, NMR, UV-Vis spectroscopy, and by comparison of experimental data with the calculated values, chromenopirazolopyrimidinone (CPP), a product of the multicomponent reaction of 4-hydroxy-2H-chromen-2-one with 5-amine-1H-pyrazole and benzaldehyde, was characterized. CPP exists as a mixture of tautomeric forms, the structure of which was examined using an integrated approach of spectroscopic methods and quantum chemistry.

## 2. Materials and Methods

### 2.1. Synthesis of 7-Phenyl-7, 12-dihydro-6H-chromeno[4,3-d]pyrazolo[1,5-a]pyrimidin-6-one (CPP)

CPP was synthesized using microwave radiation in the absence of solvent by a multicomponent reaction of 4-hydroxy-2H-chromen-2-one with 5-amine-1H-pyrazole and benzaldehyde. Yield 1.5 g (78%), m.p. 125–127 °C; FTIR,  $\text{cm}^{-1}$  3251, 3101, 3099, 3032, 2956, 1660, 1604, 1569, 1518, 1473, 1451, 1409, 1359, 1340, 1230, 1195, 1114, 1076, 1049 1033, 1002, 980, 961, 950, 903, 854, 836, 808, 754, 725, 706, 697, 660, 618, 601, 589, 551, 529, 484, 470, 443, 407. Anal. calc. for  $\text{C}_{19}\text{H}_{13}\text{N}_3\text{O}_2$ , %: C, 72.37; H, 4.16; N, 13.33; found: C, 72.42; H, 4.17; N, 13.26.

### 2.2. NMR Spectra

NMR spectra were recorded on a Varian 400 spectrometer at 20–25 °C. The operating frequency for the  $^1\text{H}$  NMR spectra was 400 MHz, and for the  $^{13}\text{C}$  NMR spectra, it was 100 MHz. The internal standard was TMS, and the solvents were  $\text{CDCl}_3$  and dimethyl sulfoxide- $d_6$ .

### 2.3. LDI-MS

The laser desorption and ionization (LDI) mass spectrum was collected with an AB Sciex 5800 TOF/TOF (Foster City, CA, USA) in reflector mode with positive ion detection using a solid-state Nd: YAG laser (355 nm) and an accelerating voltage of 20 kV.

### 2.4. FTIR Spectra

The FTIR spectrum was recorded using a Nicolet 6700 spectrometer (Thermo Scientific, Waltham, MA, USA) with a KBr pellet (wavenumber range of 4000–400  $\text{cm}^{-1}$ ), with a spectral resolution of 4  $\text{cm}^{-1}$ .

### 2.5. UV-Vis Spectra

The UV-Vis spectra of the CPP solutions were recorded on a Shimadzu-1800 spectrophotometer using 1 cm cuvettes; the scanning step was 1 nm. Solvents and reagents were of special grade. Working solutions were prepared by dissolving exactly weighed samples of the compounds ( $C = 5 \times 10^{-5}$  M) in an appropriate solvent.

### 2.6. Decomposition of UV-Vis Spectra

The computer program of the MILCA algorithm and examples of solving practical tasks are available as independent executables with a MATLAB interface (The MathWorks, Natick, MA, USA).

### 2.7. Quantum Chemical Calculations

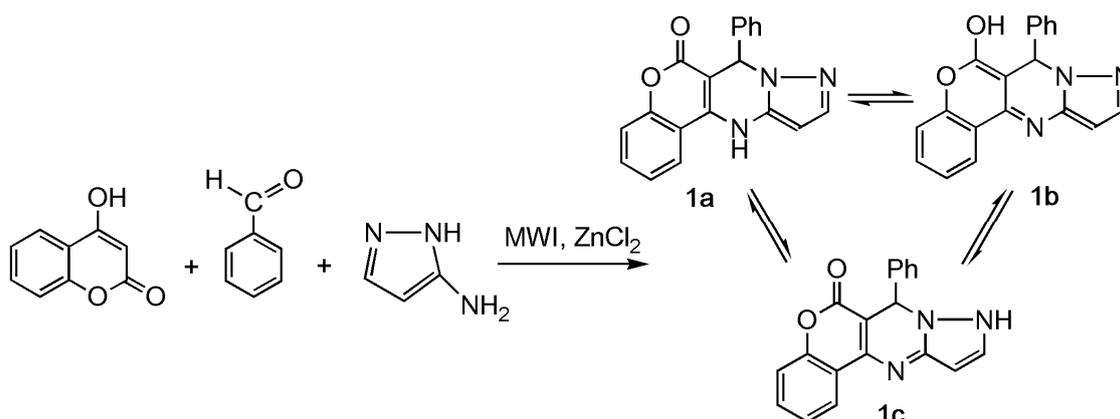
Quantum chemical calculations were performed using density functional theory (DFT) using the hybrid functional Lee–Yang–Parr three-parameter Becke B3LYP, split-valence basis set functions 6-311G, with the inclusion of p-orbitals of the hydrogen atom and d-orbitals for more heavy atoms, as well as the addition of polarization functions (B3LYP/6-311G++ ( $d,p$ )).

## 3. Results and Discussion

### 3.1. Synthesis of CPP

Using microwave radiation and the absence of solvent for a similar reaction results in a product, CPP, that can exist in three forms—as 7-phenyl-7,12-dihydro-6H-chromeno-pyrazolo[1,5-a]pyrimidin-6-one (**1a**), as 7-phenyl-7H-chromeno[4,3-d]pyrazolo[1,5-a]pyrimidin-6-ol (**1b**), and as 7-phenyl-7,9-dihydro-6H-chromeno[4,3-d]pyrazolo[1,5-a]pyrimidin-6-one (**1c**). (Scheme 1).

Theoretically, tautomeric system 1 can be represented by three forms: a, b, c. Forms a and b are realized through keto-enamine-enol-imine tautomerism, and the c form is the result of pyrazole moiety tautomerism.



**Scheme 1.** Synthesis of 7-phenyl-7, 12-dihydro-6H-chromeno[4,3-d]pyrazolo[1,5-a]pyrimidin-6-one (CPP) **1a–c**.

### 3.2. NMR Spectroscopic Data

The  $^1\text{H}$  NMR spectrum indicates the presence of CPP in two tautomeric forms: a and b due to the implementation of keto-enamine-enol-imine tautomerism.

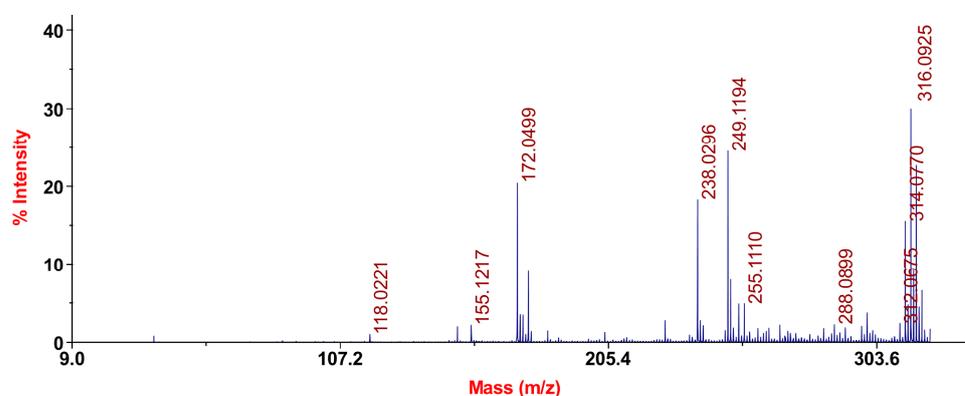
In the  $^1\text{H}$  NMR spectrum of the mixture of tautomers a and b, the singlets NH and OH groups are at 10.58 ppm and 12.31 ppm, respectively. The tertiary proton H7' singlet appears in 4.97 ppm, and the proton H7 appears at 5.23 ppm. The aromatic portion includes 22H and is presented in a doublet 8.31 ppm (H1,1',  $J = 8$  Hz); 7.60 (t., N3,3',  $J = 8$  Hz); 7.42–7.32 (m., N2,2', N4,4'); 7.28–7.25 (m. N10,10' N11,11'); 7.24–7.06 (m., 10 Hz).

To confirm the presence in the  $^1\text{H}$  NMR spectrum signals of tautomeric forms **1a**, we used the nuclear Overhauser effect (NOE 1D), which discovers a spatial interaction ortho-proton H1 chromenone fragment and NH group proton of the pyrimidine moiety.

The HSQC spectrum definitively confirmed the existing of two tautomeric forms **1a** and **b** in the DMSO- $d_6$  solution. It was evidenced by the presence of C7/C7 H7 and ' /H7' cross peaks.

### 3.3. LDI-MS Data

The considered tautomeric mixture of **1a–c** was recorded as a mass spectrum by laser desorption and ionization (LDI). The LDI method is different from the “classical” methods of ionization, such as electron impact or chemical ionization, with a higher probability of the occurrence of photochemical processes, resulting in the ion dimer molecule peregrup-pirovochnye and other more complex cluster ions. In general, LDI mass spectrometry is more complex; however, it can more accurately interpret the structure (Figure 1).



**Figure 1.** Mass spectrum of chromenopirazolopyrimidinone (CPP) **1a–c**.

For the three tautomeric forms **1a–c**, we would expect similar mass-spectrometric patterns as an isomeric structure capable of producing isobaric ions.

The mass spectrum of **1a–c** LDI chromenopirazolopyrimidinone has no molecular ion peak, but there are signals of protonated  $[M + H]^+$  and deprotonated  $[M - H]^+$  forms of molecules with mass-charge numbers  $m/z$  314 and 316, respectively. The instability characteristic of the molecular-ion-fused aromatic systems was often observed in their mass spectra, including rows and heterocyclic compounds.

### 3.4. FTIR Spectra and Vibrational Analysis

The highest frequency vibrations  $3400\text{--}3250\text{ cm}^{-1}$  were assigned to the NH stretching vibrations of forms 1 and 3, and for tautomeric form 2, the highest frequency band was the OH stretching vibration band.

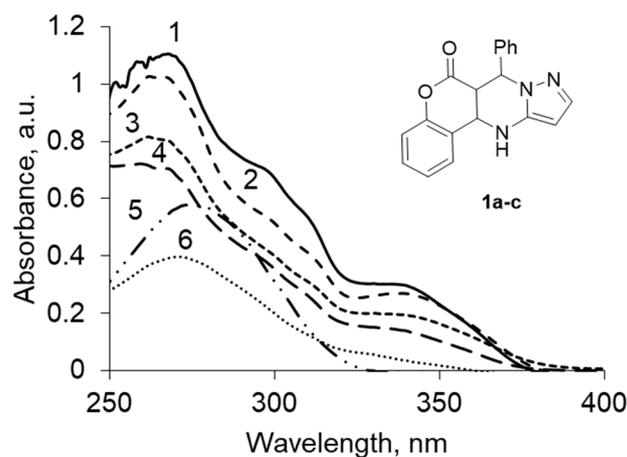
In aromatic heterocycles, C-H ring vibrations are expected to occur in the region of  $3000\text{--}3030\text{ cm}^{-1}$ , and usually the bands are strong. In our case, these bands appear at  $3129\text{ cm}^{-1}$  (HATR) and  $3101\text{ cm}^{-1}$ , while the corresponding scaled calculated frequencies for the third tautomer were at  $3131$  and  $3104\text{ cm}^{-1}$ .

The stretching vibration of the lactone carbonyl group C=O in various cyclic esters differing by the number of atoms in the cycle and the degree of saturation can be observed as a very strong band in the FTIR spectra at  $1770\text{--}1720\text{ cm}^{-1}$ .

### 3.5. UV-Vis Spectra and Their Decomposition

To confirm the assumptions about the form of existence synthesized, we recorded the UV spectra of a mixture of the tautomers **1a–c** in solvents of different polarities.

In the UV spectra of the mixture, chromenopirazolopyrimidinone **1a–c** exhibits two intense bands with  $\lambda_{\text{max}}$  260–273 and 335–345 nm (Figure 2, Table 1).



**Figure 2.** UV spectrum chromenopirazolopyrimidinone (CPP) **1a–c** at (concentration,  $5 \times 10^{-5}$  M) in: (1) dioxane, (2) ethanol, (3) methanol, (4) acetonitrile, (5) hexane, (6) chloroform.

**Table 1.** Values of absorption maxima in UV spectra depending on the solvent of the compound **1a–c**.

Solvent, $\lambda_{\text{max}}$ , nm (lg $\epsilon$ )				
Dioxane	Chloroform	Acetonitrile	Ethanol	Hexane
268 (1.11)	271(0.40)	260 (0.72)	266 (1.02)	273 (0.58)
335 (0.32)	-	335 (0.15)	345 (0.30)	-

Bands with  $\lambda_{\text{max}}$  260–273 nm ( $k = 0.39\text{--}1.15$ ) are forbidden by the symmetry  $n\text{-}\pi^*$  transitions observed in the spectra of compounds in which the supporting structure of the unshared electron pair of the heteroatom connected multiple  $\pi$ -bonds with neighboring atoms. Recognize the strip  $n \rightarrow \pi^*$  transition is possible on hypsochromic shift when changing the nonpolar solvent to polar.

Bands with  $\lambda_{\max}$  335–345 nm ( $k = 0.13$ – $0.36$ ) are characteristic mostly of the enol form. The bathochromic shift of the absorption bands in the transition from nonpolar to polar solvents can be attributed to the  $\pi$ - $\pi^*$  transitions' enamine fragment.

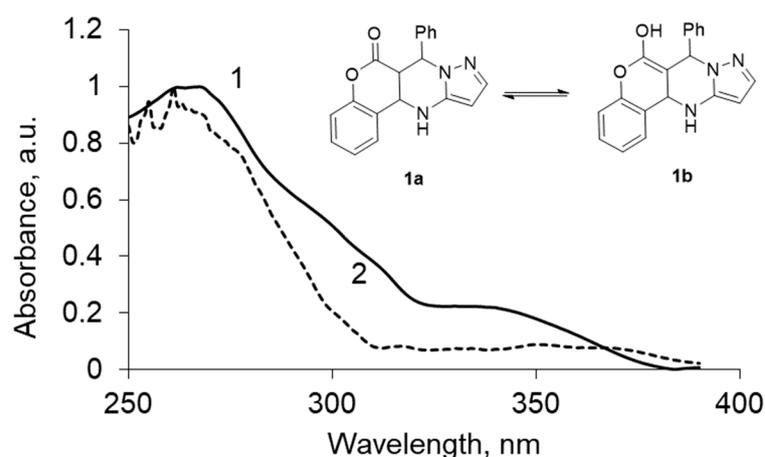
In all the tested solvents, keto-enamine form **1a** was prevalent, but the ratio trend varied in the tautomeric forms depending on the degree of polarity of the solvent.

### 3.6. Decomposition of UV-Vis Spectra

Using chemometrics methods, independent components, implemented in the MILCA algorithm, we have shown the possibility of quantitatively studying tautomeric equilibria with overlapping absorption bands of tautomers in an individual solvent. The essence of the self-similar separation of curves is the decomposition of the matrix of spectra of a multicomponent system  $X$  of size  $M \times N$  ( $N$  is the number of counts by wavelength,  $M$  is the number of spectra of the mixture) into a matrix of spectra of individual components  $S$  of size  $K \times N$  and a matrix of their relative concentrations  $A$  of size  $M \times K$  ( $K$  is the number of components in the system) (Equation (1)):

$$X = A \cdot S. \quad (1)$$

Decomposition of the absorption spectra of compound **1a–c** in various solvents into the individual components of the absorption showed the presence of two tautomeric forms (Figure 3), which allowed calculation of the percentage of each of them in various solvents (Table 2).



**Figure 3.** UV spectrum of the two tautomeric forms obtained by treating the source of the UV spectrum program MILCA, for chromenopirazolopyrimidinone (CPP) **1**: (1) keto-enamine form (**1a**), (2) enol-imine form (**1b**).

**Table 2.** Ratio between keto-enamine and enol-imine forms of **1a–c** depending on the solvent.

Solvent	Keto-Enamine <b>1a</b>	Enol-Imine <b>1b</b>
Dioxane	81	19
Ethanol	98	2
Methanol	86	14
Acetonitrile	99	1
Hexane	100	0
Chloroform	100	0

The analysis is carried out regardless of the number of components, the spectral characteristics of individual compounds, or the quantitative composition of the mixture. Thus, the work of the algorithm consists in isolating the individual spectral contours of the ketone and enol forms and subsequent calculation of the relative content of the enol form in solutions of solvents of different polarities.

### 3.7. Quantum Chemical Calculations

To compare the thermodynamic stability of all three putative tautomeric forms **1a–c** in vacuum and in two solvents, polar methanol and low-polar hexane, we carried out quantum chemical calculations with full geometry optimization. Calculation data indicate that the most thermodynamic stable structure is **1a** regardless of the medium. The **1b** tautomer is 15–19 kcal/mol less stable; so, one can speculate that in solvent, this form will be significantly less represented in tautomeric equilibrium. These data are in harmony with the UV-Vis spectra and their deconvolution by the MILCA algorithm results. Considering solvents of different polarities, we can conclude that all three tautomers prefer the more polar methanol, where their energies tend to a minimum.

## 4. Conclusions

Thus, the existence of all three tautomeric forms is possible: in the solid state (mass and IR), form **1c** predominates; in solution (NMR, UV), form **1a** predominates.

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## References

1. Ruijter, E.; Scheffelaar, R.; Orru, R.V.A. Multicomponent Reaction Design in the Quest for Molecular Complexity and Diversity. *Angew. Chem. Int. Ed.* **2011**, *50*, 6234–6246. [[CrossRef](#)] [[PubMed](#)]
2. Chen, X.-H.; Xu, X.-Y.; Liu, H.; Cun, L.-F.; Gong, L.-Z. Highly Enantioselective Organocatalytic Biginelli Reaction. *J. Am. Chem. Soc.* **2006**, *128*, 14802–14803. [[CrossRef](#)] [[PubMed](#)]
3. Shen, Z.-L.; Xu, X.-P.; Ji, S.-J. Brønsted Base-Catalyzed One-Pot Three-Component Biginelli-Type Reaction: An Efficient Synthesis of 4,5,6-Triaryl-3,4-dihydropyrimidin-2(1H)-one and Mechanistic Study. *J. Org. Chem.* **2010**, *75*, 1162–1167. [[CrossRef](#)] [[PubMed](#)]

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