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Qualitative Identification of Synthesized Isoniazid Derivatives by Atemporal Technique: UV-Visible Spectrophotometry

Identificação Qualitativa de Derivados Sintetizados da Isoniazida por uma Técnica Atemporal: Espectrofotometria UV-Visível

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ABSTRACT

Ultramodern analytical techniques must also be hyphenated with cheaper and less complex techniques such as UV-visible spectrophotometry to confirm the formation of organic synthesis products. Here, the synthesis of 4 compounds (HC4ATIOF, HFEC, HCPA4NO2, and HICNM) from a clinically approved antitubercular drug, the Isoniazid, was preliminarily verified by UV-Vis spectrophotometry. Spectra recorded in the UV-Vis region for the four possible isoniazid derivatives allowed us to confirm the synthesis of just one acylhydrazone from isoniazid, the HICNM, since the absorption bands observed in the UV-Vis spectra of the reactants and the product obtained are completely different, indicating the formation of a new substance.

Keywords: Absorption bands; Chalcones; Hydrazide; N-acylhydrazone; Synthesis

RESUMO

Técnicas analíticas ultramodernas também devem ser hifenizadas com técnicas mais baratas e menos complexas como a espectrofotometria UV-visível para confirmar a formação de produtos de síntese orgânica. Aqui, as sínteses de 4 compostos (HC4ATIOF, HFEC, HCPA4NO2 e HICNM) de um medicamento antituberculár clinicamente aprovado, a Isoniazida, foram preliminarmente verificadas por espectrofotometria UV-Vis. Os espectros registados na região UV-Vis para os quatro possíveis derivados da isoniazida permitiram confirmar a síntese de apenas uma acilhidrazona a partir da isoniazida, a HICNM, uma vez que as bandas de absorção observadas nos espectros UV-Vis dos reagentes e do produto obtido são completamente diferentes, indicando a formação de uma nova substância.

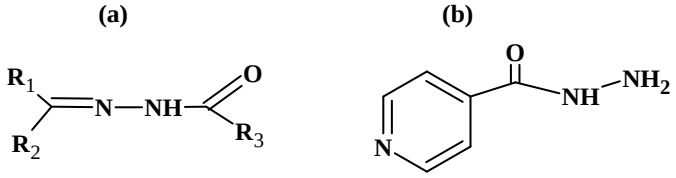
Palavras-chave: Bandas de absorção; Chalconas; Hidrazida; N-acilhidrazona; Síntese

INTRODUCTION

Hydrazones derived from aldehydes form an organic class of compounds whose structures include the functional group $R_1R_2C=N–NH$ (KIVRAK et al., 2018). In the hydrazones, the –C=N azomethine double bond is conjugated to the electron pair of the neighbouring nitrogen, which makes them more resistant to hydrolysis than the common Schiff bases (CUKIERMAN et al., 2021). Organic compounds that have the N-acylhydrazone moiety, figure 1(a), in their chemical structure are of great relevance in the Chemistry.

The drug Isoniazid, figure 1(b), which contains the hydrazide moiety has been widely used in the synthesis of N-acylhydrazones with possible pharmacological actions. Isoniazid is a prodrug used as the first choice for the chemotherapy treatment of tuberculosis, an infection caused by the *Mycobacterium tuberculosis* bacillus (ARRUDA et al., 2020).

Figure 1 – Structural representation of (a) N-acylhydrazone and (b) isoniazid (4-pyridinecarboxylic acid hydrazide)



Source: Julião (2024).

Thus, compounds synthesized from Isoniazid have aroused great interest with wide application in medicinal chemistry, being known in the literature for presenting types of bioactivities such as antibacterial, antifungal (AL-KHATTAF et al., 2021), antitubercular (PATIL et al.., 2020), anti-inflammatory (MOHANRAM; MESHRAM, 2014) and anti-cancer (FIRMINO et al., 2016).

However, it is necessary to urgently develop new Isoniazid derivatives with greater efficacy and fewer side effects that can be made available as a viable strategy in the therapy against infectious diseases, since the resistance of bacterial strains to Isoniazid has been increasingly more frequent (PATIL et al.., 2020). Furthermore, studies with Isoniazid derivatives have shown that they have greater efficacy, less hepatotoxicity, and reduced the resistance of bacteria to the derived drug than the original Isoniazid due to the blockade of the terminal amino group (OLIVEIRA et al., 2017; GUPTA et al., 2023).

Among the optical chemical analysis techniques, ultraviolet-visible (UV-Vis) spectrophotometry is widely used to measure the concentration of various substances on liquid phase (ROCHA et al., 2018), and quantitative concentration can be measured using the optical absorption wavelength of the chemical species in solution. Compared to high-performance liquid chromatography, HPLC (SIDDIQUI; ALOTHMAN; RAHAMAN, 2017; WOLFENDER, 2009) and plasma atomic emission spectrometry, ICP (KUHLIN; STURKENBOOM; GHIMIRE, 2019; KLENCSÁR et al., 2018) techniques, the UV-Vis spectrophotometry makes use of these optical characteristics and does not require pre-processing such as selection column or ionization.

The analytical determination of a chemical species by UV-Vis spectrophotometry is based on Beer's law, which establishes that the absorption rate of a solution is directly proportional to the concentration and optical path length of the absorbing species in the solution (BEER; BEER, 1852). The main advantages of UV-Vis spectrophotometry are: simplicity, versatility, precision, speed and cost-benefit, allowing quantitative and qualitative analyses, observation of reaction rates in the liquid phase and definition of mechanisms at the molecular level (SOYLAK; OZDEMIR; YILMAZ, 2020; ROCHA et al., 2018).

However, the actual operating range of a UV-Vis spectrophotometer is 180 to 700 nm. In the other ranges, the analysis is less effective and must be selected considering the cutoff wavelength depending on the solvent (ROCHA et al., 2018; UPSTONE, 2000). Furthermore, the higher the analyte concentration, the more active interactions occur

between the substances, deviating from Beer's law and causing non-linearity between the analyte concentration and the absorbance value (UPSTONE, 2000).

Although molecular absorption spectra are affected by high concentrations of the analyte and the properties of the solvent used in absorption measurements, there are still technical and financial reasons for using cheaper and more robust methodologies (SYCHEV et al., 2023; CHAPMAN et al., 2021).

In the structural identification of organic compounds, spectroscopic techniques are generally used, such as: infrared with Fourier transform, nuclear magnetic resonance of isotopes of hydrogen (^{1}H) and carbon (^{13}C) and mass spectrometry, which despite their importance and selectivity. In this type of identification, they are very expensive, require specialized personnel to handle them and are often difficult to access for most Brazilian teaching and research institutions.

Nowadays it is a fact that analytical techniques have to be used in a hyphenated form, and therefore it is important to also explore the hyphenation of other cheaper and less complex techniques from an analytical point of view.

Thus, considering the promising results of isoniazid derivatives, a study is presented to verify the feasibility of UV-Vis spectrophotometry in the molecular spectral characterization of hydrazonic compounds obtained from the synthesis reaction between chalcones and the drug isoniazid.

MATERIALS AND METHODS

Reagents

Phosphoric acid (H_3PO_4), sodium bicarbonate ($NaHCO_3$), Isoniazid (4-pyridinecarboxylic acid hydrazide, $C_6H_7N_3O$), and cinnamaldehyde [(2*E*)-3-phenylprop-2-enal, (C_9H_8O)] 95.0% were purchased from Sigma-Aldrich®. Ethanol (C_2H_5OH) 99,5% (v/v) and methanol (CH_3OH) 99.8% (v/v) were purchased from Neon®.

Synthesis of compounds

The synthesis of N-acylhydrazones was carried out in a 25.0 mL reaction flask from a mixture of 0.50 mmol of chalcone and 0. 50 mmol (0.0686 g) of isoniazid, 9.0 mL of distilled water and 1.0 mL of concentrated H_3PO_4. This reaction was subjected to magnetic stirring with heating (100 ^{o}C) for 45 min. Then, 15.0 mL of ethanol (99.8%,

v/v) and 20.0 mL of an ice-cold aqueous solution of $NaHCO_3$ (5.0% m/v) were added to the reaction mixture. The solid formed was filtered through paper under vacuum, washed with ice-cold absolute ethanol (99.8%, v/v) and then dried in an oven for 30 min at 75 ºC. After cooling, the solid was removed from the filter paper, weighed and the gross reaction yield was calculated (ARRUDA et al., 2020).

Instrumental

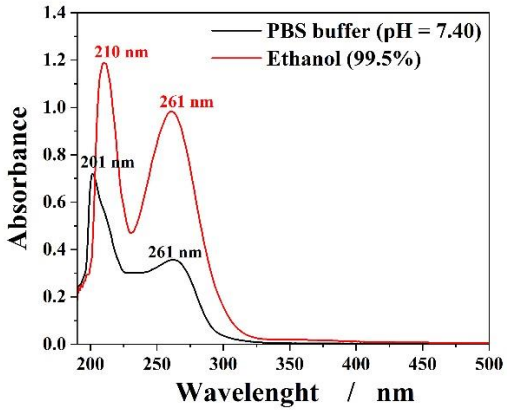
For spectral measurements, all solutions of the reacting substances and probable products were prepared in methanol (99.8% v/v) or ethanol (99.5% v/v) and the spectra were recorded in quartz cuvettes 1.0 cm of optical path in a UV-Vis spectrophotometer (Genesys 10S, Thermo Fisher Scientific) for the spectral range from 190 to 500 nm.

RESULTS AND DISCUSSION

UV-Vis spectra were recorded for Isoniazid 0.10 mmol L^{-1} in two different solvent: ethanol and PBS buffer (pH = 7.40). Isoniazid must present a maximum molecular absorption band on UV-Vis spectrum in the wavelength range $212 \leq \lambda \leq 265$ nm (MHLW, 2021), in this reference the spectra of the Isoniazid solution were recorded in a wavelength range of $200 \leq \lambda \leq 500$ nm, while in this paper the spectra of Isoniazid recorded two bands of maximum absorption in the range of 190 to 500 nm (Figure 2).

In Figure 2 it´s possible to observe the bathochromic effect, as the organic solvent (ethanol) causes the absorption band to shift from 201 nm to 210 nm and in addition there is an increase in the absorption intensity (hyperchromic effect). The bathochromic shift observed upon changing solvent polarity suggests that observed transitions may be assigned to $\pi \rightarrow \pi^*$ transition (GAUTAM et al., 2017). In order to test the nature of transitions, UV-Vis spectra of Isoniazid have been recorded in less polar ethanol than methanol and show the band at 222 nm. This can also be observed in the Isoniazid spectra shown in figures 3(b) – 6(b).

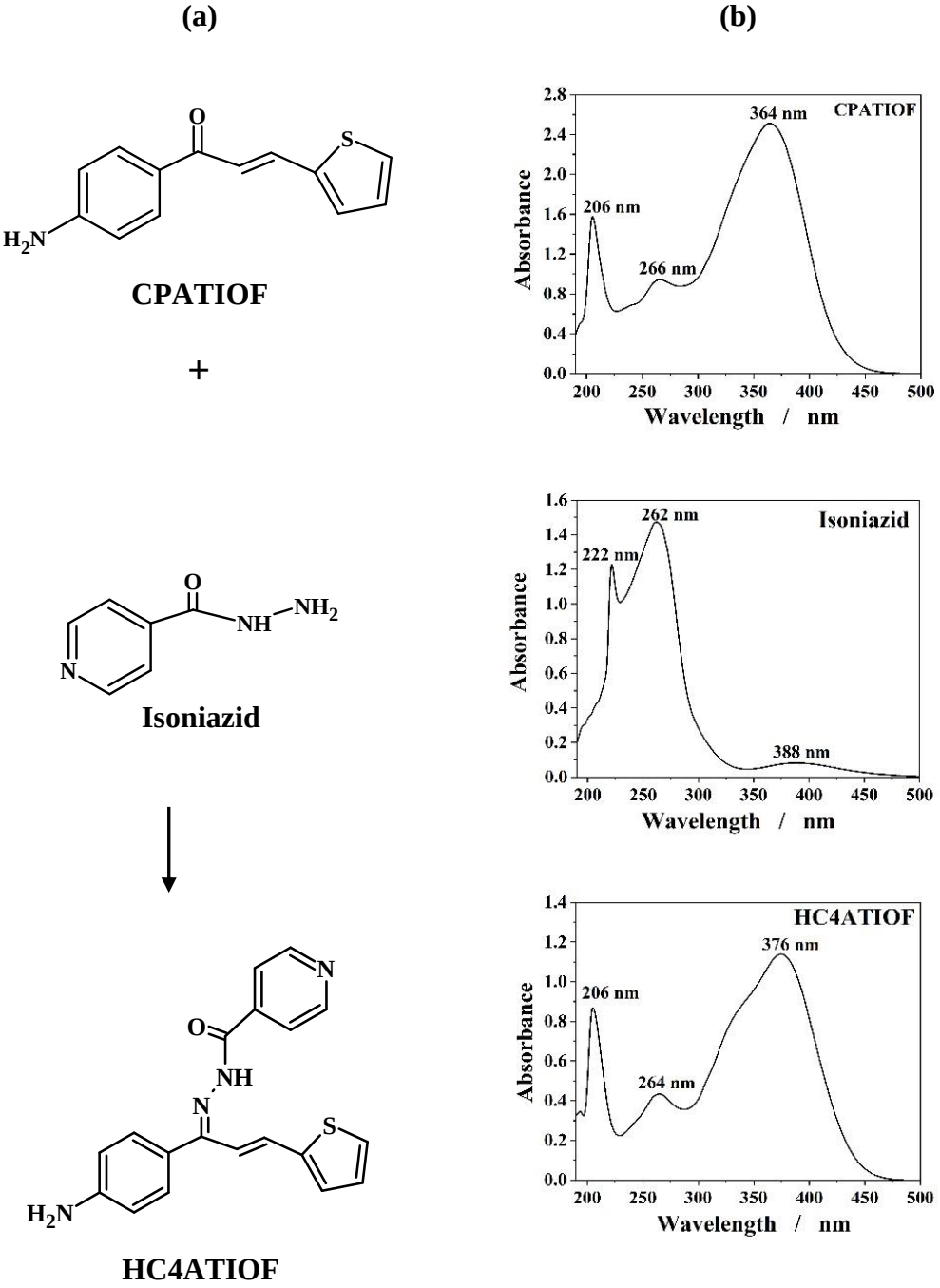
Figure 2 – UV-Vis spectra recorded for Isoniazid 0.10 mmol L^{-1}.



Source: Julião (2024).

Figure 3(a) presents the synthetic route of the compound N'-((1*E*,2*E*)-1-(4-aminophenyl)-3-(thiophen-2-yl)allylidene)isonicotinohydrazide, ($C_{19}H_{16}N_4OS$), here named HC4ATIOF, from the reaction of the chalcone (*E*)-1-(4-aminophenyl)-3-(thiophen-2-yl)prop-2-en-1-one, ($C_{13}H_{11}NO$), named CPATIOF, with the isoniazid ($C_6H_7N_3O$).

Figure 3 – Synthetic route of the compound HC4ATIOF ($C_{19}H_{16}N_4OS$) (a), and UV-Vis spectra recorded for CPATIOF 94.3 μmol L^{-1} (in ethanol); isoniazid 559 μmol L^{-1} (in methanol) and HC4ATIOF 35.0 μmol L^{-1} (in ethanol) solutions (b).

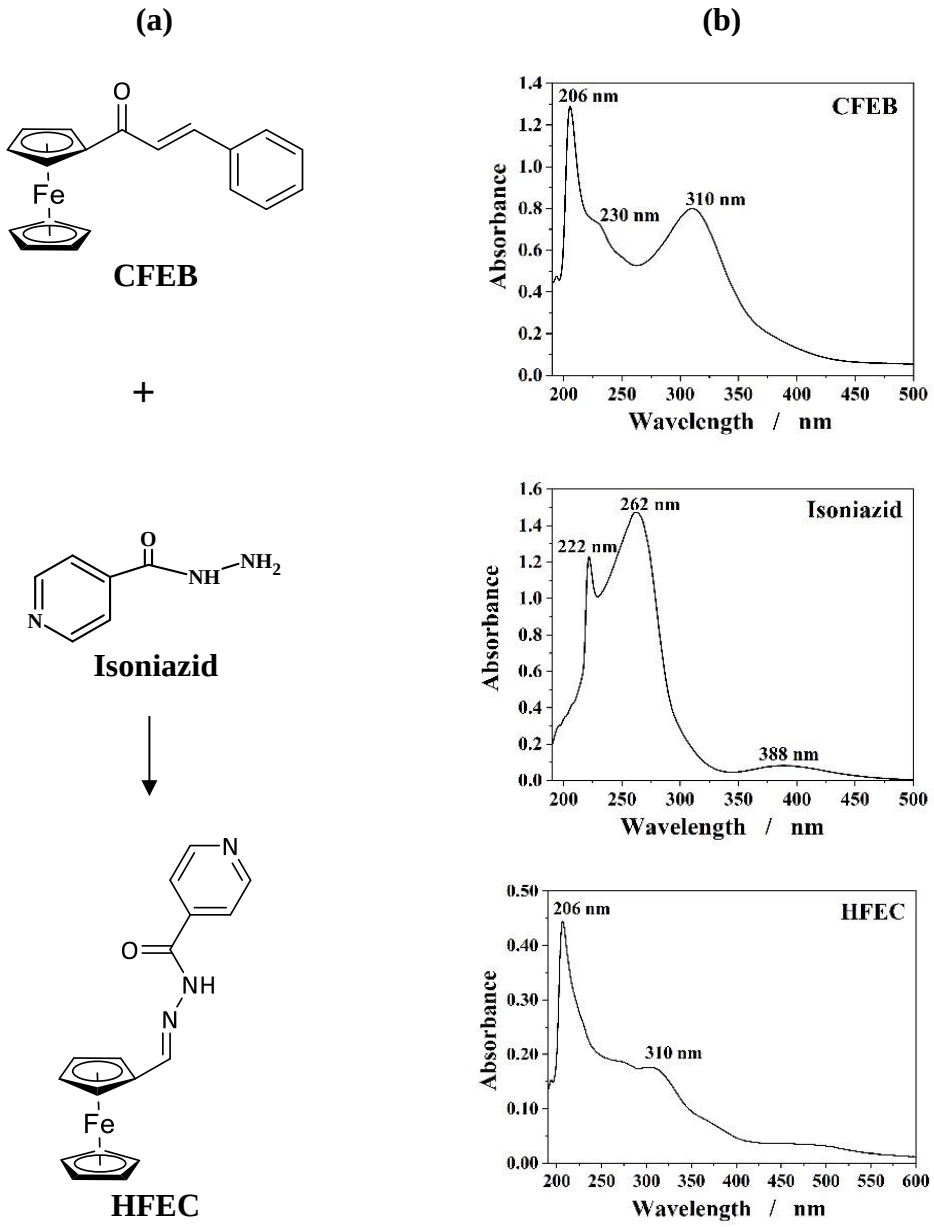


Source: Julião (2024).

However, it is observed through the UV-vis spectra of the reactants and the desired product, figure 3(b), that the absorption bands of the functional groups of the compound HC4ATIOF are actually the same as those of CPATIOF.

Figure 4(a) presents the synthetic route of the compound 1-ferrocenyl-isonicotinehydrazide, ($C_{17}H_{15}FeN_3O$), here called HFEC, from the reaction of the chalcone derived from ferrocene, the (2*E*)-1-ferrocenyl-3-(phenyl)-2-propen-1-one, ($C_{19}H_{16}FeO$), named CFEB, with isoniazid ($C_6H_7N_3O$), observed through the UV-vis spectra of reagents and the desired product. For the CFEB compound, an intense absorption band recorded at 310 nm is observed due to the π-π* transitions.

Figure 4 – Synthetic route of the HFEC compound ($C_{17}H_{15}FeN_3O$) (a), and UV-Vis spectra recorded for CFEB 56.9 μmol L^{-1} (in ethanol); isoniazid 559 μmol L^{-1} (in methanol) and HFEC 50.4 μmol L^{-1} (in ethanol) solutions (b).

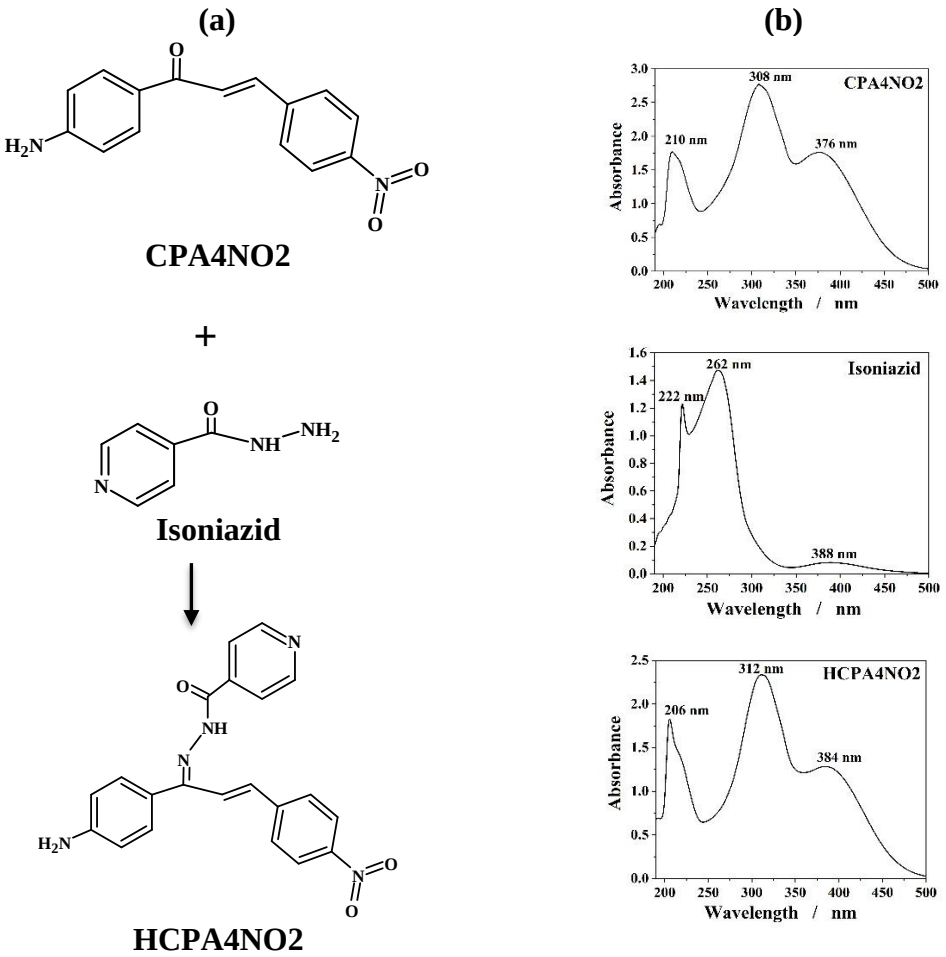


Source: Julião (2024).

Figure 4(b), that the absorption bands of the functional groups of the probable HFEC compound were recorded at practically the same wavelengths as CFEB, again indicating the great difficulty of the azide group (–N–NH_2) in interacting with the carbonyl group of the chalcone derived from ferrocene.

Figure 5(a) shows the synthetic route of the compound N'-((1*E*,2*E*)-1-(4-aminophenyl)-3-(4-nitrophenyl)allylidene)isonicotinehydrazide, ($C_{21}H_{17}N_5O_3$), here called HCPA4NO2, from the reaction of the chalcone [(*E*)-1-(4-aminophenyl)-3-(4-nitrophenyl)prop-2-en-1-one, ($C_{15}H_{12}N_2O_3$), named CPA4NO2, with isoniazid ($C_6H_7N_3O$), observed through of the UV-vis spectra of reactants and the desired product. Figure 5(b), that the three molecular bands of the functional groups of the product formed absorb at practically the same wavelengths as CPA4NO2.

Figure 5 – Synthetic route of the compound HCPA4NO2 ($C_{21}H_{17}N_5O_3$) (a), and UV-Vis spectra recorded for CPA4NO2 53.1 μmol L^{-1} (in ethanol); isoniazid 559 μmol L^{-1} (in methanol) and HCPA4NO2 74.1 μmol L^{-1} (in ethanol) solutions (b).

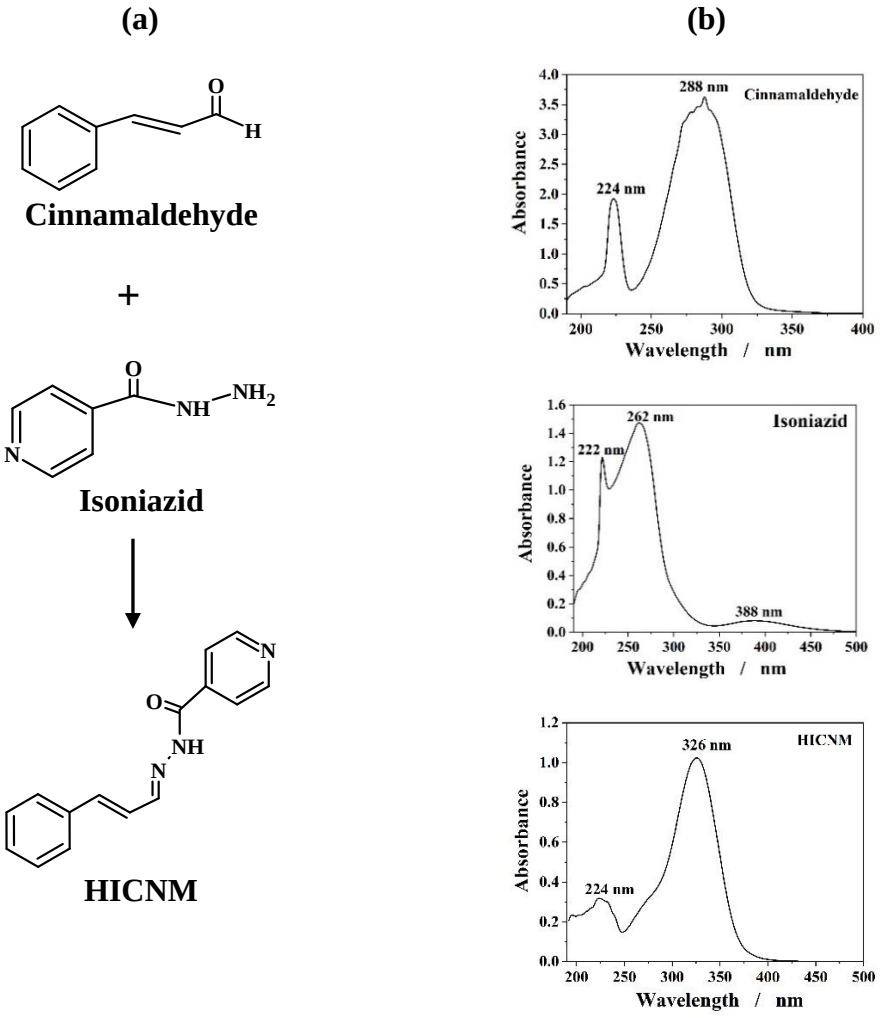


Source: Julião (2024).

From figures 3 to 5, we can observe the great difficulty that the azide group (–N–NH_2) presents in interacting with the carbonyl group of the three aminochalcones, since in none of the spectra of three desired products is it possible observe the bands of absorption recorded on Isoniazid spectrum. Therefore, this difficulty may be related both to the lower reactivity of the conjugated ketone and to the steric hindrance present in the chalcone structure.

Figure 6(a) presents the synthetic route of the compound N'-((1*E*,2*E*)-3-phenylallylidene)isonicotinehydrazide, ($C_{15}H_{13}N_3O$), here called HICNM, from the reaction of cinnamaldehyde (C_9H_8O) with isoniazid ($C_6H_7N_3O$), UV-vis spectra of the desired product.

Figure 6 – Synthetic route of the compound HICNM ($C_{15}H_{13}N_3O$) (a), and UV-Vis spectra recorded for cinnamaldehyde 18.6 μmol L^{-1}; isoniazid 559 μmol L^{-1} and HICNM 29.9 μmol L^{-1} methanolic solutions (b).



Source: Julião (2024).

Figure 6(b) indicate the presence of two absorption bands molecular measurements recorded at 224 and 326 nm, referring to the carbonyl groups and the benzene ring present in cinnamaldehyde, indicating that this synthesis was successful due to the fact that the carbonyl present in the aldehyde is more reactive and is less hindered than the carbonyls of the conjugated ketones of the other three chalcones, for which the reactions were not completed.

For the isoniazid derivative HICNM, the band recorded at higher energy wavelength (224 nm) can be attributed to the π-π∗ transitions of the phthalazine ring, while the lower energy band (326 nm) can be attributed to the n-π∗ electronic transition of the (–CH=N–) azomethine group (SORENSEN; NIELSEN, 2011).

CONCLUSIONS

The spectra recorded in the ultraviolet-visible region for the four possible isoniazid derivatives allowed us to confirm the synthesis of just one acylhydrazone from isoniazid, the product generated from the reaction between cinnamaldehyde and isoniazid, since the absorption bands observed in the UV-Vis spectra of the reactants and the product obtained are completely different, indicating the formation of a new substance, N'-((1*E*,2*E*)-3-phenylallylidene)isonicotinehydrazide, called HICNM.

On the contrary, the syntheses of acylhydrazones from chalcones were not successful, as when comparing the UV-Vis spectra of the reactants with those of the desired products, it is seen that the spectral profiles of the latter are practically identical to those of the chalcones used and this fact may be related both to the lower reactivity of the conjugated ketone and to the steric hindrance present in the chalcone structures.

Based on these premises, it is possible to affirm the feasibility of using a centuries-old analytical technique, such as molecular absorption spectrophotometry in the ultraviolet-visible region for the qualitative identification of organic synthesis products.

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