ISPITIVANJE ANTIMIKROBNIH SVOJSTAVA NEKIH BIĐINELI-AZO PIRIDONSKIH BOJA

THE ANTIMICROBIAL EVALUATION OF SOME BIGINELLI-BASED AZO PYRIDONE DYES

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Heterociklična azo jedinjenja predstavljaju značajnu klasnu organskih sintetskih boja. Pored njihovih izuzetnih svojstava za bojenje, heterociklična azo jedinjenja imaju i biološku aktivnost. Mnoge studije pokazuju da azo boje sa heterocikličnim fragmentima u strukturi mogu ispoljavati antibakterijska, antifungalna, antivirusna, antiinflamatorna i antikancerogena svojstva. Rezistencija mikroorganizama na postojeće antimikrobne agense predstavlja jedan od najvećih problema u svetskom zdravstvu. Zbog toga je razvijanje novih molekula sa antimikrobnim svojstvima kako značajno, tako i neophodno za globalno očuvanje zdravlja. U ovom radu, ispitivanje antimikrobne aktivnosti dve Biđineli-azo piridonske boje je urađeno pomoću metode difuzije na agarnoj podlozi. Antimikrobna svojstva su ispitana prema patogenim sojevima Staphylococcus aureus (Gram-pozitivna bakterija), Escherichia coli (Gram-negativna bakterija) i Candida albicans (oportunistički kvasac). Preliminarni rezultati istraživanja ukazali su na antimikrobni potencijal ispitivanih azo jedinjenja.

Ključne reči: *Candida albicans; dihidropirimidinon; hidrazon*

The heterocyclic azo dyes represent significant synthetic organic colorants. In addition to their exceptional coloration properties, heterocyclic azo compounds exhibit antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory and anticancer properties. The antimicrobial resistance is one of the major problems for global public health. Therefore, finding new organic structures with antimicrobial properties represents a challenging research field. In this study, antimicrobial screening of two Biginelli-based azo pyridone dyes has been carried out. The antimicrobial activity of synthetized dyes was studied against bacteria (Staphylococcus aureus, Escherichia coli) and yeast (Candida albicans), using agar diffusion method. The preliminary research results indicate the antimicrobial potential of the investigated compounds.

Key words: *Candida Albicans; dihydropyrimidinone; hydrazone*

1 Introduction

Nowadays, heterocyclic azo dyes have gained growing attention, considering their broad spectrum of applications in both traditional and high-tech industry [1]. Besides their excellent coloration ability, it is no longer surprising that many heterocyclic azo compounds are ecologically acceptable, and moreover, promising candidates for new antibacterial, antifungal, antiviral, anti-inflammatory and anticancer agents [2]. On the other side, especial interest can be noted in synthesis of 3,4-dihydropyrimidin-2-(1*H*)-ones (DHPM), *i.e.* products of Biginelli reaction, related to their pharmacological and therapeutic properties [3]. Having in mind that microbial resistance to the medicaments, in standard use, is becoming more prevalent, development of new antimicrobial agents represents challenging and significant research field [4].

In our previously published study, we have investigated anticancer and antioxidant properties of some Biginelli-based azo pyridone compounds [5], and in this work we present the evaluation of

their antimicrobial properties. Two azo compounds consisted of 2-pyridone and 3,4-dihydropyrimidin-2-(1*H*)-one pharmacophores have been synthesized starting from DHPM derivative, as diazo-component, and two different 2-pyridones as coupling-components. The antimicrobial activity of synthetized compounds was studied against Gram positive bacteria *Staphylococcus aureus*, Gram negative bacteria *Escherichia coli*, and yeast *Candida albicans*, by the agar diffusion method.

2 Experimental

2.1 Synthesis

All chemicals were obtained from Merck, Fluka and Acros and were used without further purification. Overall synthesis of dyes **1** and **2** (Fig. 1) was presented in our published study [5]. In brief, **a** (1 mmol) was dissolved in the diluted hydrochloric acid, then sodium nitrite (1.1 mmol) was dissolved in cold water and added dropwise to the DHPM-acid solution. The mixture was stirred in an ice bath for 1 hour to obtain diazonium chloride (**b**). The corresponding 2-pyridone (**c**) (1 mmol) was dissolved in an aqueous solution of potassium hydroxide (1 mmol) and then cooled to 0–5 °C. The obtained diazonium chloride (**b**) was added dropwise to the corresponding pyridone solution. The resulting reaction mixture was stirred for 3 hours and maintained at 0–5 °C. When the reaction was completed the obtained azo dyes (**1** and **2**) were filtered, washed with water, air dried and recrystallized from ethanol.

The spectroscopic data (FT-IR, NMR, ESI-MS, UV-Vis) for synthetized compounds are given within our published work [5].

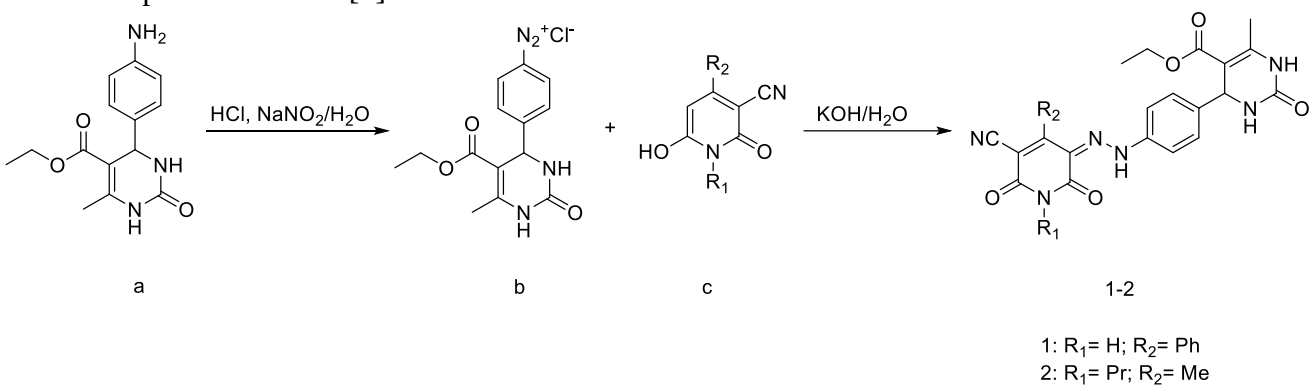

*Figure 1. The synthesis of Biginelli-based azo pyridone dyes **1** and **2***

2.2 The antimicrobial assay

The antimicrobial activity of dyes **1** and **2** was tested by the agar diffusion method [6]. The assay was performed using *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 24433. In brief, TSB (Tryptic soy broth) and TSA (Tryptic soy agar) were used to prepare the agar nutrient medium. Nutrient media were poured onto Petri dishes and incubated for 24 hours at 30 °C. After incubation, 8 mm tubes were placed in Petri dishes, and a soft TOP agar medium, inoculated with corresponding pathogen (2×105 CFU/ml, 200 µl of pathogen in 6 ml of TOP agar), was added. Subsequently, tubes were removed to form 8 mm wells. 100 µl of each sample with a concentration of 125 µg/ml of compounds **1** and **2**, dissolved in DMSO, was introduced into the wells. After incubation for 24 h at 37 °C, the size of inhibition zones was measured (in mm), regarding the control well (neat DMSO).

3 Results and discussion

3.1 Structure of the investigated dyes

The synthesized pyridone dyes **1** and **2**, contain hydroxy group in the pyridone moiety, in the *ortho*- position to the azo bond, which enables intramolecular proton transfer, and thus the existence of azo and hydrazone tautomeric forms (Fig. 2) [5].

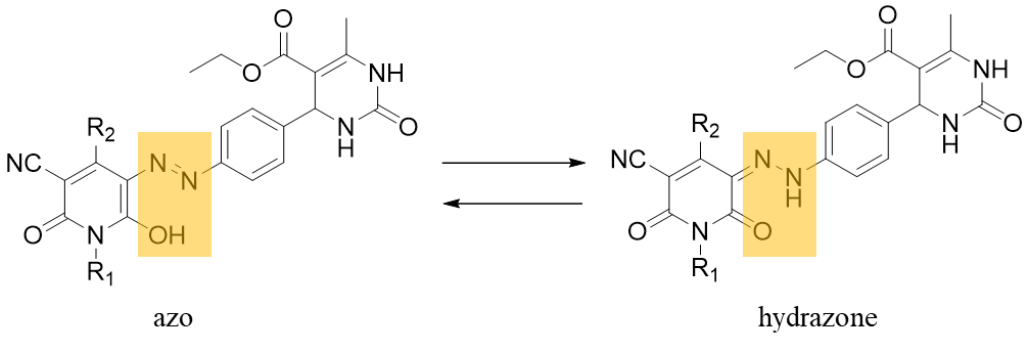

Figure 2. Azo and hydrazone tautomeric forms

The ATR-FTIR and NMR spectra of investigated dyes suggest the existence of the hydrazone tautomeric form in the solid state, as well as, in the DMSO-d_6 solution [5]. The stretching vibrations of the carbonyl groups appear in the ATR-FTIR spectra in the region of 1709–1651 cm^{-1}. The N-H stretching vibrations of the hydrazone group appear in the region of 3221–3210 cm^{-1}. Additional confirmation of the presence of the hydrazone form is intensive band appearing in the region of 1514–1505 cm^{-1} which is ascribed to mutual stretching of C=N and bending of N–H vibrations. The ^1H NMR spectra of dyes **1** and **2**, obtained in DMSO-d_6 solution, contain the signal of the hydrazone N–H group in the range of 14.62–14.60 ppm [5].

Since that hydrazone derivatives display a wide variety of biological activities, i.e., antibacterial, antifungal, anticancer and anti-inflammatory actions [7], the antimicrobial properties of the synthesized hydrazones have been studied.

3.2 The evaluation of antimicrobial activity

In order to evaluate the antimicrobial activity of compounds **1** and **2**, an antimicrobial assay was performed by the agar diffusion method, as previously described. The antimicrobial properties were tested against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Table 1 shows the obtained results, i.e., the diameter of the inhibition zones, regarding the control sample (neat DMSO).

*Table 1. Antimicrobial activity of dyes **1** and **2***

Sample	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
Inhibition zone [mm]			
1	11	-	-
2	-	-	10
DMSO	-	-	-

The presented results indicate that compound **1** exhibits antibacterial properties against *Staphylococcus aureus*, while compound **2** causes the growth inhibition of yeast *Candida albicans*. However, *Escherichia coli* was resistant to the action of the tested compounds.

4 Conclusion

In this work, the antimicrobial properties of two Biginelli-based azo pyridone dyes were investigated to discover new antimicrobial agents. The spectroscopic data, published in our previous study, confirmed that synthetized dyes **1** and **2** exist in hydrazone tautomeric form, in solid state, as well as in DMSO-d_6 solution. The conducted antimicrobial assay evinced that studied Biginelli-based dyes have promising antimicrobial properties against *Staphylococcus aureus* and *Candida albicans*. These results represent a good starting point for further development of new antimicrobial Biginelli-based azo pyridone compounds.

5 Acknowledgment

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