

AZO PIRIDONSKE BOJE ZA VIŠENAMENSKU UPOTREBU: SINTEZA, KAKRAKTERIZACIJA, ANTIOKSIDATIVNA I ANTIMIKROBNA AKTIVNOST

MULTIPURPOSE AZO PYRIDONE DYES: SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY

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*Široka komercijalna upotreba piridonskih azo boja zasniva se na njihovoj relativno jeftinoj i jednostavnoj pripremi, kao i činjenici da se veliki broj kuplujućih i diazo komponenti mogu međusobno kombinovati kako bi se dobile boje odgovarajućih svojstava. Veliki značaj ovih boja poslednjih nekoliko decenija, ogleda se u njihovim istaknutim svojstvima kao što su živopisna obojenja i dobra postojanost. Ove boje, takođe, nalaze primenu u ink-džet štampi i LCD ekranima. Sa druge strane, ove boje predstavljaju značajna jedinjenja u biološkim studijama, jer poseduju antioksidativnu, antimikrobnu i antiproliferativnu aktivnost. U ovom radu, sintetisane su tri boje koje u svojoj strukturi obuhvataju tri bioaktivna heterociklična jezgra, pri čemu je kao kuplujuća komponenta korišćen 6-hidroksi-4-metil-1-piridinijum-2-piridon, a kao diazo komponente anilini na bazi tiazola (2-aminotiazol, 2-aminobenzotiazol i 6-metoksi-2-aminobenzotiazol). Jedinjenja su okarakterisana temperaturom topljenja, NMR i FTIR spektroskopijama. Solvatohromna svojstva su ispitana u rastvaračima različitih svojstava. Antioksidativna aktivnost jedinjenja ispitana je ABTS (2,2'-azino-bis-3-etilbenzotiazolin-6-sulfonat) testom, dok je minimalna inhibitorna koncentracija ispitana primenom mikrodilucione metode u bujonu prema 5 bakterijskih sojeva (*S. aureus*, *E. faecalis*, *B. subtilis*, *E. coli* i *K. pneumoniae*), kao i prema oportunističkoj gljivici *C. albicans*.*

Ključne reči: Azo boje; 2-piridon; tiazol; antioksidativna aktivnost; antimikrobna aktivost

*Wide commercial application of azo pyridone dyes rests on the cost-effectiveness of their preparation and almost innumerable possibilities for the synthesis by combining various diazo and coupling pyridone components that enable tuning of targeted properties. The great significance of these dyes in the last few decades is due to their outstanding properties, such as vivid colors and good color fastness properties. Azo pyridone dyes are also applicable in different coloration related processes such as inkjet printing and liquid crystal displays. On the other hand, they are recognized as valuable compounds in biological studies, as they possess antioxidant, antimicrobial and anti-proliferative properties. In this work, three novel 6-hydroxy-4-methyl-1-pyridinium-2-pyridone based dyes bearing three bioactive heterocyclic rings in their structures are synthesized, wherein thiazole based anilines (2-aminothiazole, 2-aminobenzothiazole, 6-methoxy-2-aminobenzothiazole) are used as diazo components. The compounds are characterized by melting points, NMR and FTIR spectroscopy. The solvatochromic properties are investigated in solvents of different properties. Furthermore, the antioxidant capacities of the compounds are evaluated by ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay, while antimicrobial activity is determined by broth microdilution method against five bacterial strains (*S. aureus*, *E. faecalis*, *B. subtilis*, *E. coli* and *K. pneumoniae*) and one fungal strain (*C. albicans*).*

Key words: Azo dye; 2-pyridone; thiazole; antioxidant activity; antimicrobial activity

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1 Introduction

Is our colorful closet one of the main reasons for water pollution? According to Ellen MacArthur Foundation, the fashion industry uses around 93 billion cubic meters of water annually which makes dyeing of clothes one of the most polluting and energy-intensive processes [1]. Dye chemistry nowadays is in a constant search of finding solutions to overcome fast fashion disadvantages without leaving our closets colorless. As one of the most promising coloring organic compounds, azo dyes arise, with ease in preparation, intense color and diverse innovative application [2]. The application of azo dyes is not limited only on textile dyeing as it expands on usage in advanced technology products such as LCD screens [3], different storage devices [2], dye sensitized solar cells [4], sensors and optical recording systems [2]. In the last decade the attention has shifted towards heterocyclic azo dyes, which besides versatile application, possess an advantage of an easy design for donor- π -acceptor (D- π -A) molecular structure. Due to the presence of azo π -conjugated system there is an innumerable number of colors that can be synthesized by replacing one of the substituents of the azo linkage, either donating (D) or accepting (A) one [2,5]. On the other side, azo bond in the architecture of heterocyclic systems is shown to be responsible for remarkable biological application. The literature reports heterocyclic azo dyes with antimicrobial, antioxidant, antituberculosis, anti-inflammatory and anticancer activities [6,7]. As economic and ecological aspects play an important part in the development of azo dyes, the need of developing multifunctional colorants becomes a great challenge. In this manner heterocyclic azo dyes possessing pyridone frameworks become of a great interest from a synthetic aspect due to their color deepening effect, simplicity of preparation and high molar extinction coefficients [8]. Pyridone containing azo dyes are considered a class of heterocyclic compounds that are rapidly replacing industrial dyes like C.I. Disperse Yellows 114, 119, 211 with distinctive properties as well as the presence of several biological activities [9].

Inspired to an above stated literature review, in this work three novel dyes incorporating three bioactive heterocyclic moieties have been synthesized. The coupling component was 6-hydroxy-4-methyl-1-pyridinium-2-pyridone wherein thiazole based anilines (2-aminothiazole, 2-aminobenzothiazole and 6-methoxy-2-aminobenzothiazole) are used as diazo components. Synthesized compounds were characterized by melting points, NMR and FTIR spectroscopy wherein the solvatochromic properties were investigated in solvents of different properties. Furthermore, the antioxidant capacities of the compounds are evaluated by ABTS assay, while antimicrobial activity is determined by broth microdilution method against five bacterial strains (*S. aureus*, *E. faecalis*, *B. subtilis*, *E. coli* and *K. pneumoniae*) and one fungal strain (*C. albicans*).

2 Experimental section

2.1 Synthesis

The synthesis of the novel pyridone azo dyes **1**, **2** and **3** (Figure 1) was performed following classical diazo-coupling reaction [8]. Corresponding heterocyclic amines 2-aminothiazole (**A**), 2-aminobenzothiazole (**B**) and 6-methoxy-2-aminobenzothiazole (**C**) were used for the diazotisation reaction, while 6-hydroxy-4-methyl-3-pyridinium-2-pyridone (**D**) [9] was employed as coupling component. The spectral analyses confirmed the structure of the dyes and revealed that dyes acquire hydrazone form, rather than azo tautomeric form.

Dye **1** (5-((thiazol-2-yl)diazenyl)-6-hydroxy-4-methyl-3-pyridinium-2-pyridone). Dark purple solid. Yield: 48%; mp. 305.4–306.7 °C; ATR-FTIR (ν/cm^{-1}): 3357 (N–H hydrazone), 3190 (N–H pyridone), 1677, 1628 (C=O); ^1H NMR (400 MHz, DMSO- d_6 , δ/ppm): 14.32 (1H, s, NH hydrazone), 12.51 (1H, s, NH pyridone), 9.16 (2H, d, $J = 5.6$ Hz, pyridinium), 8.92 (1H, t, $J = 9$ Hz, pyridinium), 8.48 (2H, t, $J = 7.2$ Hz, pyridinium), 7.62 (1H, d, $J = 3.4$ Hz, thiazole), 7.48 (1H, d, $J = 3.4$ Hz, thiazole) 2.41 (3H, s, $-\text{CH}_3$).

Dye **2** (5-((benzothiazol-2-yl)diazenyl)-6-hydroxy-4-methyl-3-pyridinium-2-pyridone). Red solid. Yield: 55%; mp. 329.5–330.5 °C; ATR-FTIR (ν/cm^{-1}): 3420 (N–H hydrazone), 3097 (N–H pyridone), 1693, 1645 (C=O); ^1H NMR (400 MHz, DMSO- d_6 , δ/ppm): 14.51 (1H, s, NH hydra-

zone), 12.31 (1H, s, NH pyridone), 9.15 (2H, d, $J = 5.6$ Hz, pyridinium), 8.95 (1H, t, $J = 7.8$ Hz, pyridinium), 8.44 (2H, t, $J = 7$ Hz, pyridinium), 8.08 (1H, d, $J = 7.8$ Hz, benzothiazole), 7.95 (1H, d, $J = 7.8$ Hz, benzothiazole), 7.32 (1H, t, $J = 7.8$ Hz, benzothiazole), 7.15 (1H, t, $J = 7.8$ Hz, benzothiazole), 2.49 (3H, s, CH₃).

Dye **3** ((5-((6-methoxy-benzothiazol-2-yl)diazenyl)-6-hydroxy-4-methyl-3-pyridinium-2-pyridone). Dark red solid. Yield: 44%; mp. 327.3–328.5°C; ATR-FTIR (ν/cm^{-1}): 3380 (N–H hydrazone), 3105 (N–H pyridone), 1678, 1634 (C=O); ¹H NMR (400 MHz, DMSO-*d*₆, δ/ppm): 14.33 (1H, s, NH hydrazone), 12.23 (1H, s, NH pyridone), 9.11 (2H, d, $J = 5.6$ Hz, pyridinium), 8.88 (1H, t, $J = 7.8$ Hz, pyridinium), 8.44 (2H, t, $J = 7$ Hz, pyridinium), 7.58–7.33 (3H, m, benzothiazole), 3.84 (3H, s, OCH₃), 2.50 (3H, s, CH₃).

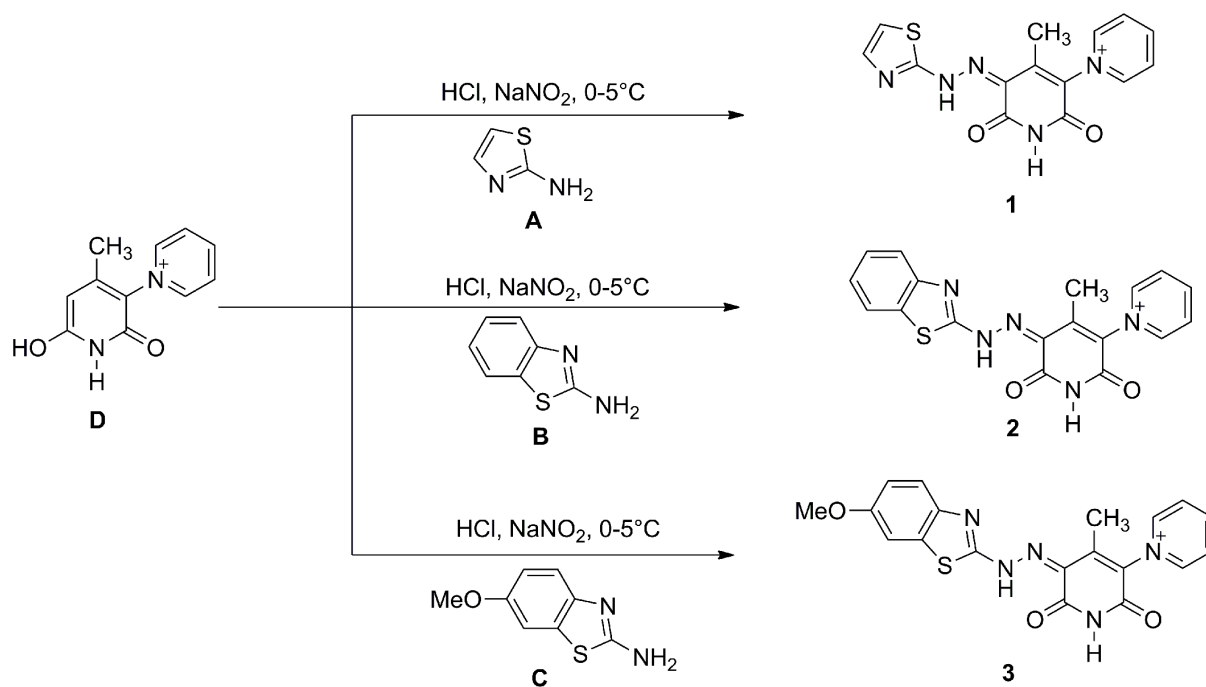


Figure 1. Synthesis of the novel dyes

2.2 Determination of the antioxidant activity

Antioxidant activity of the synthesized compounds was assayed according to the previously described procedure [8]. Ascorbic acid was used as a reference antioxidant molecule.

2.3 Determination of the antimicrobial activity

Antimicrobial activity of was determined by the broth microdilution method [10] against the Gram-positive *S. aureus* (ATCC 6538), *E. faecalis* (ATCC 29212), *B. subtilis* (ATCC6633); the Gram-negative *E. coli* (ATCC 8739) and *K. pneumoniae* (NCIMB 9111) and one yeast, *C. albicans* (ATCC 10231). The results are expressed as minimum inhibitory concentration (MIC).

3 Results and discussion

Specific nature of azo pyridone dyes is manifested through the tautomerization phenomenon. It is worth mentioning that azo dyes are also referred to as hydrazone dyes since they can be either in the azo form or in the tautomeric hydrazone one. Tautomerism is important not only to chemists but also to dye manufactures, as tautomers in distinctive isomeric forms not only differ in color, but also have different tinctorial strengths and properties such as color fastness to washing and light fastness, sublimation, perspiration, and so on. Azo dyes and corresponding hydrazone ones in the tautomeric system appear to be quite interesting from structures to properties [11]. The literature findings and the experience of the authors has shown that the more stable and dominant form is hydrazone one. On the other hand, azo form of pyridone dyes is rare and deprotonation of the hydroxyl group of the pyridone scaffold occurs in most cases, wherein there is an acid/base equilibri-

um with azo anion and hydrazone forms [8]. The acid/base equilibrium is present in the case of synthesized compounds **1–3**, wherein azo anion (**Ai**)–hydrazone (**H**) tautomeric forms are presented in Figure 2.

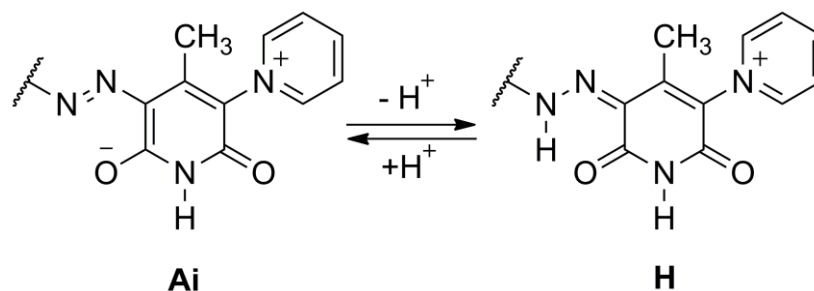


Figure 2. Azo anion-hydrazone tautomeric equilibrium

The pioneer method, mostly used for determination of the dominant tautomeric form in solution, is UV-Vis spectroscopy. In this manner, and in aspiration to determine the dominant tautomeric form, UV-Vis spectra of compounds **1–3** have been recorded in ethanol (Figure 3a). As seen from Figure 3a compound **1** exhibit a peak centered at 480.5 nm as well as another one at 445.5 nm. Furthermore, compound **2** has a distinctive peak at 474.5 nm and a broad shoulder around 444 nm, wherein compound **3** has only one distinctive peak at 457 nm. Dominant peaks can be ascribed to a π - π^* transition of the π conjugated system of the hydrazone form [8].

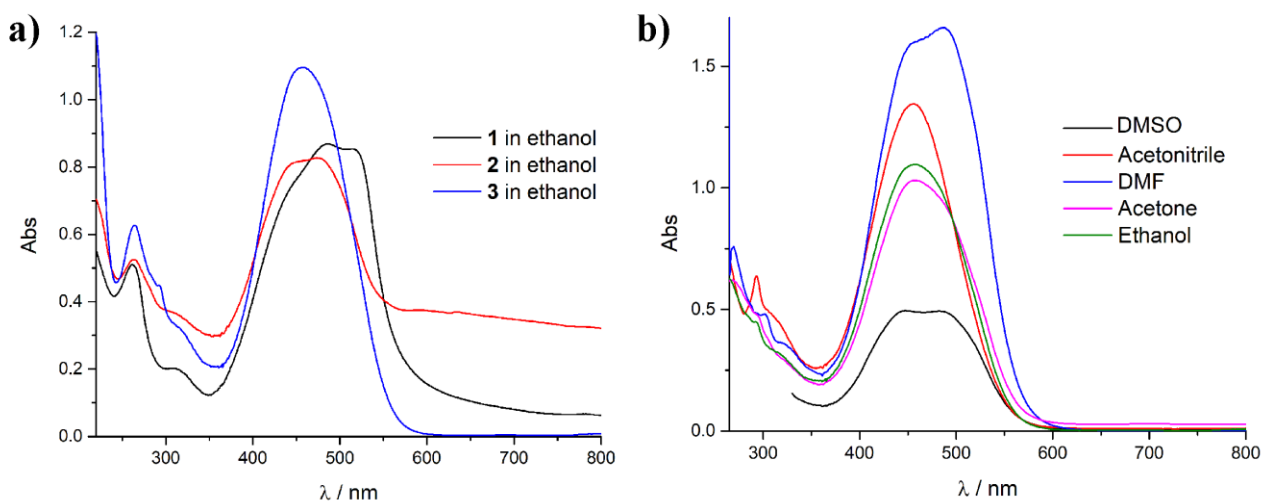


Figure 3. UV-Vis spectra of compounds **1–3** in ethanol (a) and compound **3** in different solvents (b)

The appearance of a shoulder or another peak in the recorded spectra of the compounds **1** and **2** suggest the existence of azo-anion hydrazone equilibrium in ethanol. To examine the solvent effect on azo anion-hydrazone equilibrium of compound **3**, which, according to one dominant band, appeared to have only one form in ethanol. As seen from Figure 3b, UV-Vis spectra of compound **3** in more polar and solvents of high basicity and permittivity (DMF (*N,N*-dimethylformamide) and DMSO (dimethylsulfoxide)), there is a slight bathochromic shift of a dominant peak and another peak at higher wavelengths arises. A new peak can be ascribed to the azo anionic structure, formed by deprotonation of the hydrazone form in the alkaline environment. Therefore, it can be concluded that the equilibrium of the azo anion-hydrazone form is present, wherein anionic form is positioned at higher wavelengths with respect to hydrazone form [12].

Antioxidant activity of the dyes was estimated by ABTS assay at the concentration of 3 mM and the results are presented in Figure 4. The results show that only dye **1** showed significant antioxidant activity comparable to the activity of the reference ascorbic acid. IC_{50} values of the dye **1** and ascorbic values are determined and are 1.95 and 1.48 mM, respectively, indicating that although

dye has lower antioxidant capacity than ascorbic acid, it is still a candidate for good antioxidant molecule.

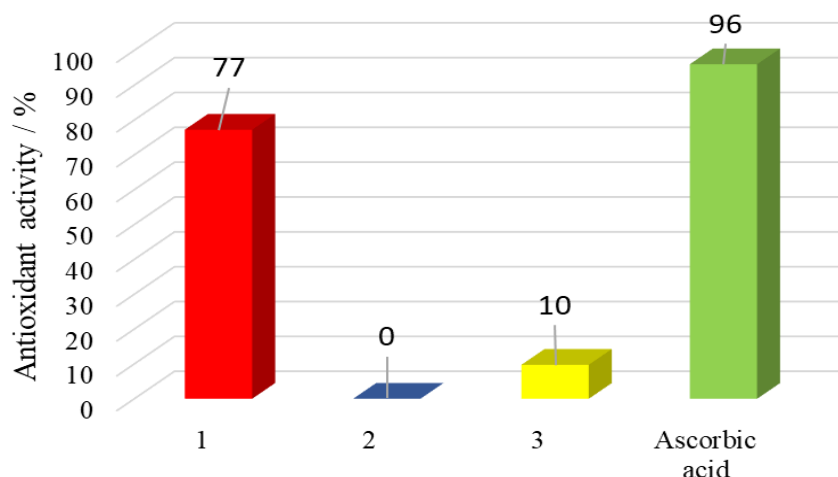


Figure 4. Antioxidant activity of the synthesized dyes

Antimicrobial activity of the synthesized compounds was studied based on a determination MIC value and are presented in Table 1. The results show that dyes **1** and **3** do not exhibit antimicrobial activity against all strains, while dye **2** showed moderate activity against fungal *C. albicans* and causes significant inhibition of the bacterial strain *K. pneumoniae*. However, *S. aureus*, *E. faecalis*, *B. subtilis* *E. coli*, were resistant to the action of the dye **2**.

Table 1. MIC values [$\mu\text{g mL}^{-1}$] for antimicrobial activity for the synthesized dyes

Microorganisms	1	2	3
<i>S. aureus</i> (ATCC 6538)	>1000	>1000	>1000
<i>E. faecalis</i> (ATCC 29212)	>1000	>1000	>1000
<i>B. subtilis</i> (ATCC 6633)	>1000	>1000	>1000
<i>E. coli</i> (ATCC 8739)	>1000	>1000	>1000
<i>K. pneumoniae</i> (NCIMB 9111)	>1000	250	>1000
<i>C. albicans</i> (ATCC 10231)	>1000	500	>1000

4 Conclusion

Inspired by the lack of multifunctional colorants, in this work three novel dyes **1**, **2** and **3**, incorporating three bioactive heterocyclic moieties have been synthesized. NMR and ATR-FTIR characterization of the synthesized compounds lead to a conclusion that dyes are found in hydrazone, rather than azo anionic form. The other conclusion derived from UV-Vis spectra of these compounds is that the azo anion-hydrazone equilibrium of compounds is found when in solution. Basic solvents deprotonate hydroxyl group of the pyridone scaffold and therefore increase the share of azo anionic form in the mixture, which is evidence by the bathochromic shift in the spectra. Moreover, the results obtain by ABTS screening assay revealed that only dye **1** showed significant antioxidant activity comparable to the activity of the reference ascorbic acid. IC_{50} values of the dye **1** and ascorbic values are determined and are 1.95 and 1.48 mM, respectively, which makes this dye a prominent antioxidant agent. Antimicrobial activity of the dyes showed that dyes **1** and **3** do not exhibit antimicrobial activity against tested strains. On the other hand, dye **2** showed moderate activity against fungal *C. albicans* and causes significant inhibition of the bacterial strain *K. pneumoniae*, but *S. aureus*, *E. faecalis*, *B. subtilis* *E. coli*. To conclude, it can be stated that from three synthesized dyes dye **1** has potential to be used as antioxidant and dye **2** has potential to be used as

antifungal and antibacterial agent. These properties can, hopefully be transferred on the textile material when dyeing is employed and therefore lead to some novel, protective textile materials.

4.1 Acknowledgement

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-47/2023-01/200135 and 451-03-47/2023-01/200287)

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