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ODREĐIVANJE FARMAKOLOŠKE AKTIVNOSTI SINTETSKIH BOJA NA BAZI PIRIDONA

ASSESSING THE PHARMACOLOGICAL POTENTIAL OF SYNTHETIC COLORANTS WITH PYRIDONE CORE

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Azo boje predstavljaju strukturno najraznovrsniju klasu organskih jedinjenja koja sadrže jednu ili više azo grupa (–N=N–) koje premošćavaju dva organska motiva od kojih je bar jedan aromatičan. Ova grupa sintetskih boja dobija se jednostavnom sintezom koja uključuje reakciju diazo-kuplovanja u visokom prinosu. Da su azo boje od neprocenjivog značaja u različitim granama industrije gde se primenjuju za nijansiranje tekstila, kože, plastike i kozmetičkih proizvoda, govori i činjenica da su zastupljene sa preko 60%. Pored tradicionalne primene, azo boje su poznate i po sve većoj upotrebu u biomedicini i farmaceutskoj industriji. Na osnovu navedeneg, cilj ovog rada je da se detaljnije ispita veza između hemijske strukture četiri azo piridonske boje i njihove farmakološke aktivnosti uzimajući u obzir tautomerni oblik boje. Ova veza određena je empirijski korišćenjem odgovarajućih softverskih paketa kao i in vitro određivanjem njihove antioksidativne aktivnosti primenom ABTS metode. Rezultati in silico predikcije pokazuju da sva ispitivana jedinjenja poseduju dobru oralnu bioraspoloživost i nisku do odličnu antioksidativnu aktivnost u zavisnosti od supstituenta u fenilnom jezgru. Uzimajući u obzir širok spektar primene arilazo piridonskih boja kao i činjenicu da će njihov značaj konstantno rasti, rezultati ostvareni u ovom radu predstavljaju interesantnu osnovu za buduća istraživanja.

Ključne reči: *azo boje; piridon; hemoinformatički modeli; antioksidativna aktivnost*

Azo dyes are known as structurally diverse class of organic compounds bearing one or more azo groups (–N=N–) as a bridge between organic residues of which at least one is an aromatic moiety. This group of synthetic dyes is obtained easily by the reaction of diazo coupling with high yield. The importance of azo dyes is reflected in the fact that they account for 60 % of the total number of the dye structures known to be manufactured and used in the coloration of textiles, leather, plastics and cosmetics. Aside from their traditional usage, azo dyes are known for their therapeutic properties and wide range of applications in biomedicine and pharmaceutical industry. Hereby, the present study aims to investigate the relationship between the chemical structure and pharmacological activity of four azo pyridone dyes taking into account the tautomeric form of dyes. The relationship was

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determined empirically using appropriate software packages as well as in vitro using the 2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method. The results of in silico prediction suggested that all investigated compounds possess good oral bioavailability, while the results of the ABTS assay indicate poor to excellent antioxidant activity depending on the substituent in the phenyl ring. Considering the broad applications of arylazo pyridone dyes, as well as the fact that their relative importance may increase in the future, results obtained in this study serve as a basis for further investigations.

Key words: *azo dyes; pyridone; chemoinformatic prediction models; antioxidant activity*

1. Introduction

The ever-growing drug resistance is becoming a significant challenge in contemporary times due to the widespread availability and misuse of medications. Consequently, continuous efforts have been made in developing novel bioactive compounds and modifying existing ones with proven efficacy. A significant class of compounds integral to numerous drugs and bioactive substances is represented by azo dyes. The main structural motif incorporated into azo dyes is conjugated azo linkage (–N=N–) connecting two aryl/heteroaryl rings. Numerous azo compounds bearing heterocyclic moiety have been recognized for their therapeutic potential and admitted antimicrobial, antitumor, anti-inflammatory, anticonvulsant and antidiabetic activities [1]. Apart from being utilized as colorants in over 60% of all commercial dyes, they find applications in various fields such as printing systems, non-linear optics, color additives, light-controlled polymers, and the liquid crystal industry [2].

Among the heterocycle-containing azo derivatives, dyes with 2-pyridone scaffold have gained significant attention in recent years. In addition to their notable coloration properties, azo pyridone dyes exhibit significant antibacterial and antioxidant activity and represent promising anticancer agents [3]. In this paper, four azo pyridone dyes (Figure 1) are assessed for their pharmacological potential using cheminformatic prediction models and *in vitro* evaluation of antioxidant activity. One of the phenomena inherent to this class of pyridone dyes is azo-hydrazone tautomerism (Figure 1). The structural characterization of the dyes is pivotal, as the tautomeric forms vary in physical properties, consequently influencing their biological activity. In light of that, *in silico* study is performed for both tautomeric forms in order to establish the relationship between the structure of the particular tautomer and pharmacological properties. The tautomeric forms are profiled for physicochemical parameters, druglikeness and ADME (absorption, distribution, metabolism, and excretion) properties. Moreover, the antioxidant properties of the compounds are evaluated *in vitro* by ABTS assay.

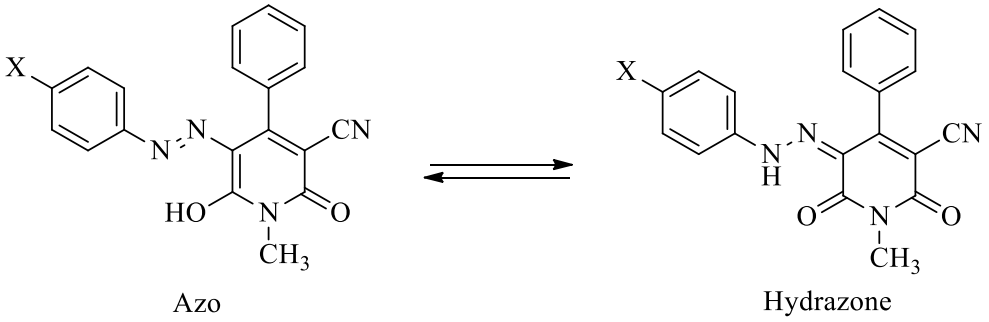

*Figure 1. Azo-hydrazone tautomerism of the investigated dyes (X = –H (**1**), –OMe (**2**), –NO_2 (**3**) and –Cl (**4**))*

2. Experimental part

2.1. In-silico assessment of physicochemical and ADMET properties

Determination of the relevant parameters for both azo and hydrazone tautomers of the investigated compounds was assessed employing the following software packages: SwissADME (Swiss Institute of Bioinformatics, Switzerland [4] and PreADMET [5]. The physicochemical properties determined are molecular weight (MW), number of hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), rotatable bonds (nrb), topological polar surface area (TPSA), octanol-water partition coefficient (logP) and molar refractivity (MR), while pharmacokinetic parameters include human intestinal absorption (HIA) and plasma protein binding (PPB).

2.2. Determination of antioxidant activity

The antioxidant potential of investigated compounds **1**–**4** was determined using ABTS radical-scavenging assay according to the procedure described in the literature [3].

3. Results and discussion

The synthesis and the structural characterization of compounds **1**–**4** are presented in our previous report [6]. The study showed that dyes exist in hydrazone form in the solid state, and predominantly in this form in most of the investigated solvents. The dyes are synthesized in a simplistic synthesis approach that can afford a wide range of derivatives which is beneficial from pharmaceutical and drug industry standpoints. The initial step in the selection or identification of the potential drugs is the assessment of the bioavailability and ADME properties.

3.1. Multiparametric optimization of molecular descriptors

Druglikeness represents a key consideration when selecting compounds during the early stages of drug discovery [7]. This biochemical property, which is often used as a proxy for oral bioavailability, is commonly defined by its physicochemical and structural similarity to a set of known commercially available drugs. It could be rationalized by consideration of how simple physicochemical properties, such as MW, polarity and lipophilicity impact molecular behavior *in vivo* with special respect to solubility, permeability, metabolic stability and transport effects. A frequent category of druglikeness evaluation methods is property-based rules defined as the acceptable thresholds on the physicochemical properties of investigated compounds. Therefore, those methods could be defined by comparing the structural features of an investigated compound to those of a set of known drugs from different perspectives [8]. The original and most well-known of this empirical rule is Lipinski's rule of five (RO5), which describes that an investigated compound is more likely to possess good absorption or permeation when following physicochemical criteria are fulfilled: MW < 500 g/mol, log P < 5, HBD < 5 and HBA < 10 [7]. Thereafter, additional empirical druglikeness rules have been developed based on the analysis of physicochemical properties of drugs such as Veber's (nrb ≤ 10 and TPSA ≤ 140 Å^2), Egan's (WlogP ≤ 5.88 and TPSA ≤ 131.6 Å^2) and Ghose's criteria (160 ≤ MW ≤ 500; –0.4 ≤ WlogP < 5.6; 40 ≤ MR ≤ 130; 20 ≤ number of atoms ≤ 70). Compounds that fulfill these empirical criteria are described as "druglike". The design and development of these compounds is approached on their ability to form strong intermolecular interactions with crucial biomolecules, such as proteins and enzymes, involved in disease progression [9]. Compliance of all investigated dyes in both azo and hydrazone forms with RO5 increases oral availability of the drug molecule (Tables 1 and 2). Molecular weights of dyes range from 330.34–375.34 g/mol, the number of HBD in all cases is 1, while the number of HBA varies from 4–7 rendering these molecules for oral drug

development. The value of logP is the log of the octanol/water partition coefficient with optimal values in the range of 1–3 [10], and for the investigated dyes these values are presented in Table 2. Values of WLOGP for the azo tautomer of all compounds are above the optimal value of 3, while for the corresponding hydrazone form these values are significantly lower and in the optimal range. On the other hand, MLOGP values fall in the optimal range for both tautomeric forms while exhibiting lower lipophilicity of hydrazone form.

The TPSA value which in the case of all four compounds doesn't exceed 140 $Å^2$ and the number of rotatable bonds in all cases being less than 10 meet the criteria of Veber's rule indicating efficient permeability. The values of TPSA are lower for hydrazone form than for the azo form. On the other hand, among the investigated forms only the azo form of compound **3** violates Egan's rule for TPSA, while parameters of other forms are within the limits of this rule.

According to Table 1, it could be observed that all compounds in both tautomeric forms meet the criteria of Ghose's rule. Furthermore, hydrazone form for all compounds exhibits a slightly higher value for MR.

Compared with unsubstituted derivative **1**, it could be concluded that the introduction of strong electron-donor in **2** and electron-acceptor groups in **3** and **4**, increased the molar refractivity and TPSA (except in the case of **4**) of investigated compounds.

Table 1. Physico-chemical properties of the investigated compounds [4,5].

			Azo tautomer				*Hydrazone tautomer*			
No.	*MW [g/mol]*	*nrb*	*HBD*	*HBA*	*MR*	*TPSA [Å²]*	*HBD*	*HBA*	*MR*	*TPSA[Å²]*
1	330.34	3	1	5	94.76	90.74	1	4	97.93	85.56
2	360.37	4	1	6	101.25	99.97	1	5	104.42	94.79
3	375.34	4	1	7	103.58	136.56	1	6	106.75	131.38
4	364.79	3	1	5	99.77	90.74	1	4	102.94	85.56

Table 2. Values of the partition coefficient of the investigated compounds [4,5].

	Azo tautomer		*Hydrazone tautomer*	
No.	*$logP_{o/w}$ (WLOGP)*	*$logP_{o/w}$ (MLOGP)*	*$logP_{o/w}$ (WLOGP)*	*$logP_{o/w}$ (MLOGP)*
1	4.04	2.25	1.86	1.78
2	4.05	1.95	1.87	1.48
3	3.95	1.37	1.77	0.90
4	4.70	2.75	2.51	2.28

According to PreADMET (Table 3), high HIA (84.02–97.34%) and high PPB (89.71–96.36 %) qualify those pharmacologically active compounds for the further stages of drug design and development. The values for different azo and hydrazone tautomers are similar indicating that tautomeric form does not have a significant impact on the pharmacokinetic profile of the dyes.

Table 3. Pharmacokinetic profiles of the investigated compounds related to absorption properties

	Azo tautomer		*Hydrazone tautomer*	
No.	*HIA, %*	*PPB, %*	*HIA, %*	*PPB,%*
1	97.19	96.36	96.85	96.86
2	97.34	92.99	97.20	93.42
3	84.02	92.83	89.91	92.94
4	96.98	89.71	96.57	90.64

3.2. Antioxidant activity

Given that antioxidants provide hydrogen atoms or electrons to counteract the lone electron generated by free radicals, the quest for potent antioxidants in the field of medicinal chemistry is of paramount importance. Evaluation of the antioxidant properties of the synthesized compounds was assessed using the ABTS assay at the concentration of 5 mM and the scavenging activity was further compared to the activity of ascorbic acid (Figure 2). Among the investigated dyes, only dye **2** exhibits good antioxidant activity comparable to the activity of the ascorbic acid. On the other hand, the results imply that the introduction of the substituents into the *para*-position of the phenyl ring, both electron-donating and electron-accepting, induces enhancement of the antioxidant activity compared to un-substituted dye **1**. It should be emphasized that higher scavenging ability is more pronounced in the presence of electron-donating substituents such as methoxy group. Furthermore, IC_{50} value of dye **2** is evaluated (2.25 mM) and compared to the value of the ascorbic acid (1.55 mM). These results suggest that dye **2** could be qualified as a potential antioxidant molecule and could be used in the development of new drugs for the treatment of pathological disorders caused by oxidative stress.

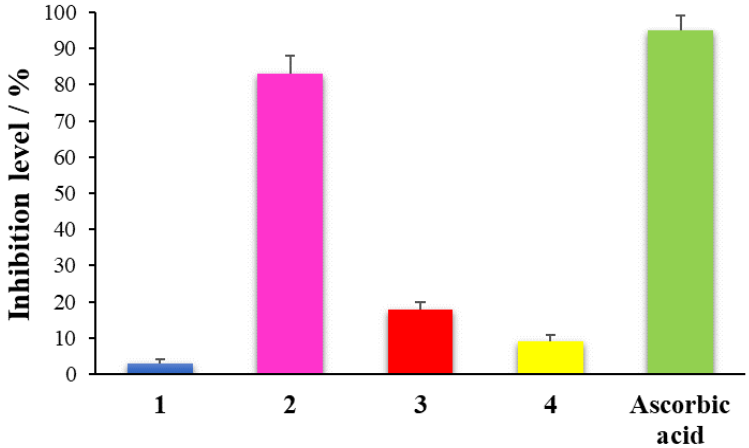

Figure 2. The antioxidant properties of investigated compounds compared to the ascorbic acid

4. Conclusion

Nowadays, there is considerable focus on the synthesis of heterocycle-incorporated azo dye derivatives as foundational scaffolds in pharmaceutical development. In this context, four azo pyridone dyes are evaluated for their pharmacological potential with respect to the tautomeric form of the

dyes. *In silico* study revealed that all dyes comply with empirical rules indicating their good bioavailability. According to these criteria, no significant impact of the tautomeric structure on the pharmacological impact is observed for the investigated dyes. The most notable difference between tautomers is observed for the partition coefficients where lower values are obtained for the hydrazone form. Evaluation of antioxidant activity has revealed that methoxy substituent in the para-position of the phenyl ring significantly affects the activity of the dyes with IC_{50} value comparable with standard ascorbic acid. The excellent antioxidant ability associated with good oral bioavailability of dye **2** qualifies this compound into the further stages of drug design and development.

5. Acknowledgement

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