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# Aryl azo 5-arylidene-2,4-thiazolidinone Dyes as Novel Antioxidant and Antibacterial Compounds

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## ABSTRACT

*In the present study, nine bis-azo dyes based on 5-arylidene-2,4-thiazolidinone were obtained in two steps, using Knoevenagel condensation and azo coupling reactions. The structure of the dyes was confirmed on the basis of spectral data. Analysis of spectroscopic data shows that there is equilibrium between the azo and hydrazone tautomers for all dyes in solutions. Investigation of antioxidant activity of compounds was carried out by 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power methods. The antibacterial activity towards three Gram negative and two Gram positive bacteria was also investigated. The activity data show that the synthesized dyes 3a-i have promising antibacterial activity, comparable with their precursor 1. Prog. Color Colorants Coat. 8 (2015), 145-152 © Institute for Color Science and Technology.*

## 1. Introduction

Organic compounds with hydroxyl and sulfur functional groups (-OH and -S), such as phenolic compounds, polyphenols and flavonoids scavenge free radicals, including peroxide and hydroperoxide, thus inhibiting the oxidative mechanisms that lead to degenerative diseases [1, 2]. Antioxidants are of importance due to their ability to retard disease progression by reducing the damage caused by freeradical oxidative stress in a patient. Thesecompounds may act independently or in combination as anti-microbial agents by a variety of mechanisms [3].

Thiazolidine and its derivatives as bioactive heterocycles, play a key role in medicinal chemistry and they have been extensively used as scaffolds for drug development [4-6]. Thiazolidinediones show a

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wide variety of biological activities such as antifungal, antibacterial, antiviral, antitumor, and antidiabetic potentials [7-11].

On the other hand, azo compounds have been extensively used as colorant material and account for over 50 % of all commercial dyes [12, 13]. In addition to their use as colorants, they have also been employed for many applications, such as ink jet printing [14, 15], thermal transfer printing [16], photography [17], as color additives [18], in the biomedical area [19], molecular recognition [20], light controlled polymers [21, 22], and liquid crystal industry [23].

In addition, synthesis and application of such dyes are important in pharmaceutical, food, color and other industries. According to the above potent usefulness of the thiazolidinediones and in continuation of our studies on the synthesis of azo dyes [24], we report here in the synthesis of some novel bis-azo dyes bearing a thiazolidine moiety in order to evaluate their antioxidant and antibacterial activities.

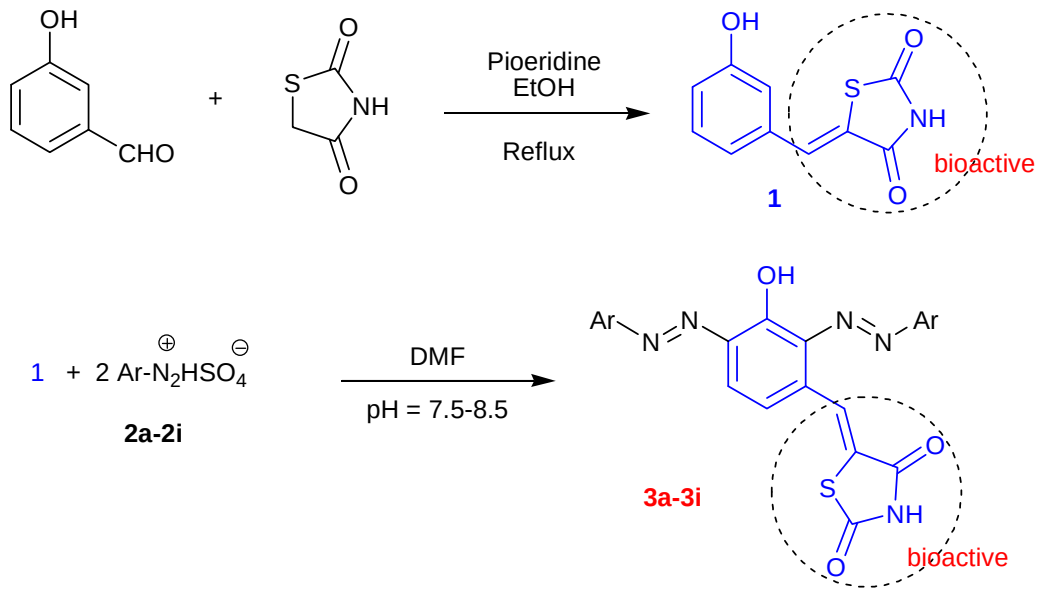
## 2. Experimental

### 2.1. Materials and apparatus

All compounds used in this study were obtained from Merck Chemical Company and were used without further purification. All melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Infrared spectra were recorded on a Shimadzu 8400 FT-IR spectrophotometer (Japan). The Proton nuclear magnetic resonance ($^1$H NMR) spectra were obtained on a FT-NMR (400 MHz) Brucker apparatus spectrometer (Germany), and the chemical shifts are expressed in δ ppm using TMS as an internal standard and *J* values are given in Hz. The visible spectra were measured using a Pharmacia Biotech Spectrophotometer (United States). The purity determination of the substrates and reaction monitoring were accompanied by TLC using silica gel SIL G/UV 254 plates (Merck Chemical Company, Germany).

### 2.2. Synthesis of azo dyes 3a-f

All the investigated dyes in the present work were synthesized by treating the corresponding aryl diazonium salts with 5-(3-hydroxybenzylidene) thiazolidine-2,4-dione (1) in alkaline media using diazotization-coupling reactions, as previously described [25, 26]. Dyes **3a-f** were prepared according to Figure 1.




Ar = (a): 4-$FC_6H_4$, (b): 4-$ClC_6H_4$, (c): 4-$BrC_6H_4$, (d): 4-$IC_6H_4$, (e): 4-$MeOC_6H_4$, (f): 4-$MeC_6H_4$, (g): 4-$CH_3CONHC_6H_4$, (h): 4-$CH_3COC_6H_4$, (i): 4-$O_2NC_6H_4$.

**Figure 1:** General synthetic route for synthesis of dyes **3a-i**.

### 2.3. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The free radical scavenging activity (DPPH assay) was measured according to Brand-Williams et al. (1995) with slight modifications. This method is based on the reduction of stable DPPH$^{\bullet}$, when it accepts a hydrogen from an antioxidant compound. DPPH radicals were prepared in methanol to the final concentration of $1.5 \times 10^{-4}$ mM. Different concentrations of investigated synthetic compounds (3–70 μM) were added and mixed with the DPPH solution. The mixture was incubated at 25 ˚C for 30 min. The absorbance of the samples was measured at 517 nm against a blank, using ascorbic acid as positive control. Radical scavenging activity was calculated by the following equation:

$$\% \text{ Inhibition} = [(A_D - A_S) / A_D] \times 100$$

where $A_D$ is the absorbance of DPPH solution (containing all reagents except the test compound) and $A_S$ the absorbance of the tested sample.

### 2.4. Ferric reducing antioxidant power (FRAP) assay

FRAP was performed according to Benzie and Strain (1996) with minor modifications. The principle of this method is based on the reduction of a ferric 2,4,6-Tris(2-pyridyl)-1,3,5-triazine ($Fe^{3+}$-TPTZ) to ferrous in the presence of synthetic compounds. FRAP reagent was prepared from 0.3 M acetate buffer (pH = 3.6), 10 mmol TPTZ solution in 40 mmol HCl and 20 mmol iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was freshly prepared before analysis and warmed to 37˚C prior to use. 100 μl of diluted compound (20-200 μM) was added to 1.4 mL of the FRAP reagent. The absorbance of the reaction mixture was measured after 5 min against blank at 593 nm. Increased absorbance of the reaction mixture indicated increased reducing power. A standard curve was prepared using different concentrations (0.5-10 mM) of ferrous sulfate. All determinations were performed in triplicate.

### 2.5. Zone inhibition assay

The assay was carried out according to Finegold and Baron (1990), with minor modifications [27]. Five bacterial strains were used for this assay, including: *Micrococcus luteus*, *Bacillus subtilis*, *Salmonella enterica*, *Escherichia coli* and *Pseudomonas aeruginosa.* Tetracycline and chloramphenicol were used as standard antibiotics. The strains grown overnight at 37 ˚C in nutrient broth (Merck) were transferred to nutrient agar (Merck) plates in 40 μl quantities and spread on the surface with a sterile glass spreader. Using sterile Pasteur pipette ends, wells were bored into the agar. A concentration of $2.2 \times 10^{-4}$ M of sample was prepared in DMSO and 30 μl of each sample was added to a well. The plates were incubated at 37 ˚C overnight and zones of inhibition were measured using a ruler.

**Minimum inhibitory concentration (MIC):** Nutrient broth containing the required concentration of compound ($2 \times 10^{-2}$, $9 \times 10^{-2}$ and $16 \times 10^{-2}$ mM) and inoculated with the bacterial strain was incubated at 37 ˚C overnight. Cultures exhibiting no turbidity were taken to indicate MIC.

**Minimum bactericidal concentration (MBC):** 50 μl samples from cultures exhibiting MIC were transferred to nutrient agar plates and incubated overnight as before. MBC was indicated by absence of growth on agar surface.

## 3. Results and discussion

### 3.1. Chemistry

All the investigated compounds were synthesized by treating the corresponding aryl diazonium salts with 5-(3-hydroxybenzylidene) thiazolidine-2,4-dione (1) as the key intermediate in alkaline media using diazotization-coupling reactions. Knoevenagel condensation reaction of commercially available 2,4-thiazolidinedione with 3-hydroxybenzaldehyde in refluxing ethanol in the presence of the piperidine catalyst affords excellent yields of the 5-(3-hydroxybenzylidene)thiazolidine-2,4-dione. In our previous publication [25, 26], the structure of the synthesized dyes was characterized and confirmed by UV-Vis, FT-IR, $^{1}$H NMR and $^{13}$C NMR spectroscopic techniques. Analysis of spectroscopic data showed that the prepared bis-azo compounds exist in three forms; namely, azo-enol-azo ($T_1$), hydrazo-keto-azo ($T_2$) and azo-keto-hydrazo ($T_3$), but other tautomeric forms are possible. The tautomeric forms of the dyes are shown in Figure 2.

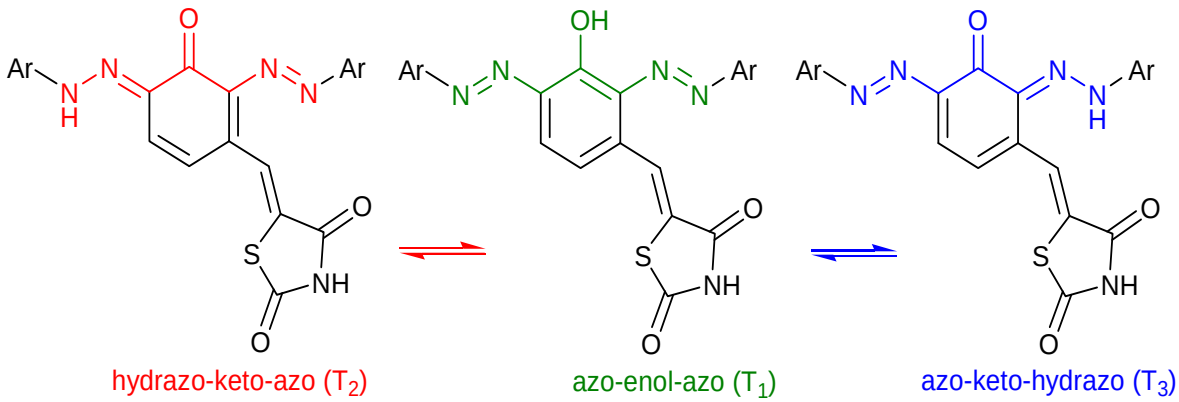



**Figure 2:** Possible tautomeric forms of prepared dyes **3a-i.**

In all the prepared dyes, examination of the $^1$H NMR data shows that the equilibrium between the **T$_1$**, **T$_2$** or **T$_3$** tautomeric forms is predominantly shifted to the **T$_2$** and/or **T$_3$** forms.

### 3.2. Antioxidant activity

The antioxidant activity of prepared compounds **3a-i** and **1** was evaluated using DPPH and FRAP methods. The results obtained from DPPH assay are presented in Table 1. There was significant variation in the percentage inhibition of the DPPH$^{\bullet}$ radical by the compounds **3a-i**. DPPH$^{\bullet}$ scavenging activity was quantified and expressed in terms of inhibitory concentration ($IC_{50}$: the concentration of sample required to scavenge 50 % of the free radicals). There was a clear variation in the $IC_{50}$ values of the prepared compounds. The $IC_{50}$ values followed the order **3f** (1.95 µM) >**3h** (2.1 µM) >**3i=3c** (2.25 µM) >**3g** (3.2 µM) >**3b=3d** (3.5 µM) >**3e** (4.36 µM) >**3a** (6 µM). Compound **3f**, showed the highest activity ($IC_{50}$: 1.95 µM), but lower activity was seen by **3a** ($IC_{50}$: 6 µM).

The antioxidant mechanism of the dyes could be explained on the basis of its chemical structures. The synthesized dyes with hydroxyl group can donate an H atom from its phenol group to DPPH$^{\bullet}$ to form the resonance-stabilized free-radical intermediate. The existence of an aromatic moiety on the oxygen atom would stabilize the phenoxy radical formed, and thus, enhance antioxidant activity of dyes **3a-f**.

The results obtained from the FRAP assay are presented in Table 1. Among the tested dyes, **3f,** with the electron donating group ($CH_3$), displayed the highest FRAP values (159 µM) followed by **3d** (143 µM), **3h** (133 µM), **3g** (107 µM), **3i** (105 µM) and **3a** (82 µM). In general, this notable antioxidant activity may be rationalized on the basis that the prepared azo compounds possess phenolic and thiazolidinone moieties that act as potential antioxidants. The importance of the phenol content of the product lies in the capacity of these compounds to increase the antioxidant capacity.

### 3.3. Antibacterial activity

The antibacterial activity of synthesized compounds **3a–i** and **1** was examined against the selected bacterial strains using the agar well diffusion technique. The results for well diffusion are presented in Table 2. The results revealed that five dyes (**3a**, **3b**, **3c**, **3d**, and **3f**) exhibit strong activities towards the Gram-positive bacteria *M. luteus* and *B. subtilis*.

**Table 1:** Antioxidant Activity of compound **1** and dyes **3a-i** assayed by DPPH and FRAP assays.

| Entry | Compound | DPPH ($IC_{50}$) | FRAP (μM) $Fe^{2+}$ (Con.) |
|---|---|---|---|
| **1** | 1 | 7.81 | 22 |
| **2** | 3a | 5.95 | 82 |
| **3** | 3b | 3.48 | 48 |
| **4** | 3c | 2.22 | 50 |
| **5** | 3d | 3.52 | 143 |
| **6** | 3e | 4.30 | 75 |
| **7** | 3f | 1.91 | 159 |
| **8** | 3g | 3.22 | 107 |
| **9** | 3h | 2.06 | 133 |
| **10** | 3i | 2.19 | 105 |

**Table 2:** Antimicrobial activity of compound **1** and dyes **3a-i**.

| Entry | Compounds ($2.2\times10^{-4}$ M) | Antimicrobial activity (zone of inhibition in mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *M.luteus* | *B.subtilis* | *S. enterica* | *S. enterica* | *E. coli* | *P. aeruginosa* |
| **1** | 1 | 9 | 6 | – | – | – | – |
| **2** | 3a | 27 | 12 | – | – | – | – |
| **3** | 3b | 24 | 19 | 7 | 7 | – | – |
| **4** | 3c | 18 | 11 | – | – | – | – |
| **5** | 3d | 31 | 15 | – | – | – | – |
| **6** | 3e | 16 | 8 | – | – | – | – |
| **7** | 3f | 30 | 14 | – | – | – | – |
| **8** | 3g | 10 | 7 | – | – | – | – |
| **9** | 3h | 9 | 11 | 12 | 12 | – | – |
| **10** | 3i | 17 | 15 | – | – | – | – |
| **11** | Tetracycline (15 μg/well) | 34 | 20 | 26 | 26 | 28 | – |
| **12** | Chloramphenicol (30 μg/well) | 36 | 17 | 28 | 28 | 36 | 20 |

**Table 3**: MIC and MBC of compound 1 and dyes 3a-i against selected Gram positive bacteria.

| Entry | Bacteria | Micrococcus luteus | | | Staphylococcus aureus | | | Bacillus subtilis | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Compound | $2\times10^{-2}$ mM | $9\times10^{-2}$ mM | $16\times10^{2}$ mM | $2\times10^{-2}$ mM | $9\times10^{-2}$ mM | $16\times10^{-2}$ mM | $2\times10^{-2}$ mM | $9\times10^{-2}$ mM | $16\times10^{-2}$ mM |
| **1** | 1 | MIC | ND[a] | MBC | – | – | – | – | MIC | ND[a] |
| **2** | 3a | – | – | MIC | – | – | – | – | – | – |
| **3** | 3b | MIC | MBC | ND[b] | – | – | – | – | – | – |
| **4** | 3c | MBC | ND[b] | ND[b] | – | – | – | – | MIC | ND[a] |
| **5** | 3d | MIC | MBC | ND[b] | – | – | – | – | – | – |
| **6** | 3e | – | – | – | – | – | – | – | – | – |
| **7** | 3f | – | – | – | – | – | – | – | – | – |
| **8** | 3g | – | – | – | – | – | – | – | – | – |
| **9** | 3h | – | – | – | – | MIC | ND[a] | – | – | – |
| **10** | 3i | – | – | – | – | – | – | MIC | MBC | ND[b] |

ND[a]: As the MIC was determined at lower concentrations, it was not determined (ND) at the higher concentration.

ND[b]: As the MBC was determined at lower concentrations, it was not determined (ND) at the higher concentration.

It can also be seen that none of the dyes have antimicrobial activities against the selected Gram-negative bacteria. Although, two dyes, **3b** and **3h**, showed mild activities against *Salmonella enterica* (a Gram negative bacterium). It was decided to determine MIC and MBC against *M. luteus*, *B. subtilis* and a third Gram positive bacterium, *Staphylococcus aureus*. The results obtained are shown in Table 3. As can be seen, compounds **3b**, **3c** and **3d** showed MBC against *M. luteus* at $9\times10^{-2}$ mM. Compound **3h** exhibited MIC at $9\times10^{-2}$ mM against *S. aureus*, whereas compound **3c** showed MIC at $9\times10^{-2}$ mM against *B. subtilis*. In addition, compound **3i** showed MBC at $9\times10^{-2}$ mM against the same bacterium.

It can therefore be concluded that the novel dyes prepared containing the phenolic and thiazolidinone moieties as bioactive components showed promising results and potential for utilization in the pharmaceutical and medicinal industries.

## 4. Conclusions

This paper proposes a convenient and useful method for the synthesis of nine azo dyes with bioactive moieties using Knoevenagel condensation and azo coupling reactions. These dyes were characterized by FT-IR, $^{1}$H NMR and $^{13}$C NMR spectroscopic techniques. Spectroscopic characterization showed that the synthesized tautomeric azo-hydrazone dyes exist mainly in the hydrazone form. The antioxidant and antibacterial properties of the dyes were evaluated. These new classes of colorants exhibit a significant antioxidant and antimicrobial activities as confirmed by DPPH, FRAP and agar well diffusion, respectively. Results obtained from antioxidant tests showed that all the prepared dyes may be considered as new potential antioxidants. In addition, the synthesized dyes provided higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria, which could be attributed to the structural differences between selected bacteria.

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