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Original article

Novel azo dyes derived from 8-methyl-4-hydroxyl-2-quinolone: Synthesis, UV–vis studies and biological activity

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ABSTRACT

In this study, *N*,*N*′-di-(2-methylphenyl)malonamide was synthesized and reacted with polyphosphoric acid to afford 8-methyl-4-hydroxyl-2-quinolone. Eight novel azo disperse dyes were then synthesized by linking diazotized *p*-substituted aniline derivatives with 8-methyl-4-hydroxyl-2-quinolone. The solvatochromism of these azo dyes in various solvents was evaluated. All the compounds were then evaluated for their antibacterial activity against four bacteria, namely, *Bacillus subtilis*, *Micrococcus luteus*, *Salmonella enterica*, and *Pseudomonas aeruginosa*. The results showed that some of these compounds have high levels of antibacterial activity.

1. Introduction

4-Hydroxy-2-quinolones have attracted great interest in the last years because of their biological and pharmaceutical activities and their use as dye-stuffs [1–6].

Azo dyes are a class of compounds containing a N=N double bond and, due to their ability to absorb visible light and ease of synthesis, have been extensively used in the textile, fiber, leather, paint and printing industries for more than a century [7]. Surprisingly, it has also been found that the hydroxyquinolone azo dye-stuffs have considerable advantages over the known dyestuffs in their fastness properties and solvatochromism behaviors for practical usages and educational aims [8,9]. In particular, the synthesis and tautomerism of aryl and heteroaryl azo dyes derived from 4-hydroxyquinolone and its derivatives have been explored by Sener et al. [10–12].

We therefore became interested in the synthesis of a series of a monoazo dyes derived from 8-methyl-4-hydroxyl-2-quinolone and evaluation of their spectrophotometric and pharmacological (antibacterial) activities. Hence, this paper has focused on the synthesis of novel arylazoquinolone dyes and the examination of their antimicrobial activities in detail. The visible absorption spectroscopic properties in acidic, basic and neutral media are also reported. The structures of coupling component and prepared dyes are shown in Schemes 1 and 2.

2. Experimental

All solvents were dried according to standard procedures. IR spectra were recorded on a Shimadzu 8400 FT-IR spectrophotometer. ^{1}H NMR spectra were obtained by FT-NMR (500 MHz) Brucker apparatus in DMSO-d_6, using TMS as an internal standard. The absorption spectra of the compounds were run on a Cary UV–vis double-beam spectrophotometer (Model 100). The elemental analysis was determined on a Vario EL III elemental analyzer. Melting points were determined with a Barnstead Electrothermal 9100 melting point apparatus in open capillary tubes and uncorrected.

Malonamide (**I**) was obtained using the method described in Ref. [11], by reacting 2-methyl aniline (5.4 mL, 50 mmol) and diethyl malonate (2.85 mL, 25 mmol) in a microwave oven at 320 W for 5 min. After irradiation, the crude product was recrystallized in ethanol to afford the white solid (6.7 g, 95%): Mp: 160–162 °C (reported 158–160 °C [13]); FT-IR (KBr, cm^{-1}): ν 3150 (NH), 1660 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.20 (br, 2H, NH), 7.69 (d, 2H, J = 8.1 Hz), 7.41 (d, 2H, J = 7.7 Hz), 7.12 (dd,

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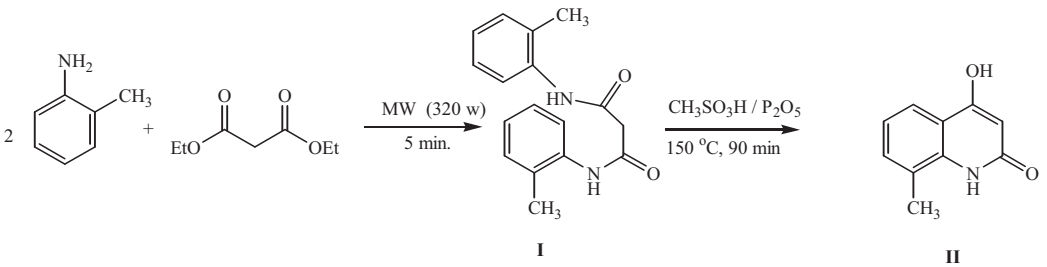
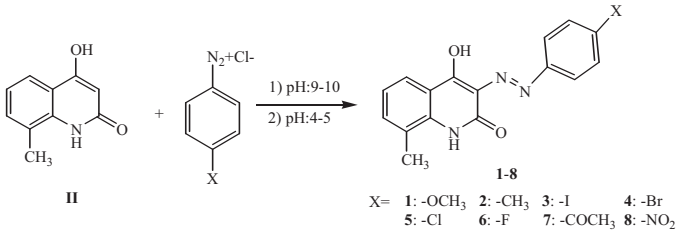


Scheme 1. Preparation of 8-methyl-4-hydroxyl-2-quinolone (**II**).

2H, *J* = 8.1, 7.2 Hz), 7.01 (dd, 2H, *J* = 7.7, 7.2 Hz), 3.58 (s, 2H, $-CH_2-$), 2.34 (s, 6H).

Hydroxyquinolone (**II**) was obtained using the experimental method described in Ref. [14], by heating the *N*,*N*′-di-(2-methylphenyl)malonamide (**I**) (0.56 g, 2.0 mmol) in 3.5 mL methanesulfunic acid, which contains 10% phosphorus pentoxide at 150 °C, for 90 min. The dark viscous solution was allowed to cool. Water was added, and the resultant gum solidified on prolonged standing. The solid was filtered and then dissolved in 100 mL 10% sodium hydroxide. The aqueous solution was filtered to remove insoluble material and slowly acidified to pH < 4 with 10% hydrochloric acid. The resulting crude precipitates were collected, washed with water and dried to afford 8-methyl-4-hydroxyquinoline-2-(1*H*)-one (**II**) as creamy crystals (0.30 g, 87%), mp: 357–358 °C (reported 360 °C [13]); FT-IR (KBr, cm^{-1}): ν 3470 (OH), 3100 (NH), 1660 (C=O); ^1H NMR (500 MHz, DMSO-d_6): δ 11.25 (OH), 10.27 (NH), 7.62 (d, 1H, *J* = 7.8 Hz), 7.30 (d, 1H, *J* = 7.2 Hz), 7.01 (dd, 1H, *J* = 7.8, 7.1 Hz), 5.27 (s, 1H), 2.35 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.75 (C=O), 158.24 (C–OH), 139.14 (C), 134.10 (C–CH_3), 130.78 (CH), 126.20 (C–H), 124.90 (CH), 116.17(C), 99.10 (CH), 17.01 (CH_3). Anal. Calcd. for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00; Found: C, 68.54; H, 5.12; N, 7.78.

A cold solution of aryldiazonium salt (2.0 mmol) was prepared by adding a solution of $NaNO_2$ (2.2 mmol, 0.15 g into 1.0 mL H_2O) to a cold solution of arylamine hydrochloride (2.0 mmol of arylamine in 1.5 mL conc. HCl). The resulting solution of aryldiazonium salt was added drop wise to a mixture of 8-methyl-4-hydroxyquinoline-2-(1*H*)-one (**II**) (0.35 g, 2.0 mmol) in 10 mL aqueous NaOH (20 mmol, 0.8 g) at 0–5 °C. The pH of the reaction mixture was maintained at 9–10 by adding 2.5% sodium hydroxide solution. The resulting mixture was continually stirred at 0–5 °C for 2 h. After completion of the reaction the pH was regulated to 4–5 by simultaneous additions of 10% hydrochloric acid solution. The resulting solid was then filtered off, washed with cold ethanol, dried at 50 °C in an oven and then recrystallized from DMF. The purity of all compounds was evaluated by thin layer chromatography. The physical and spectral data of the purified dyes are available in the supplementary data accompanied with this paper.



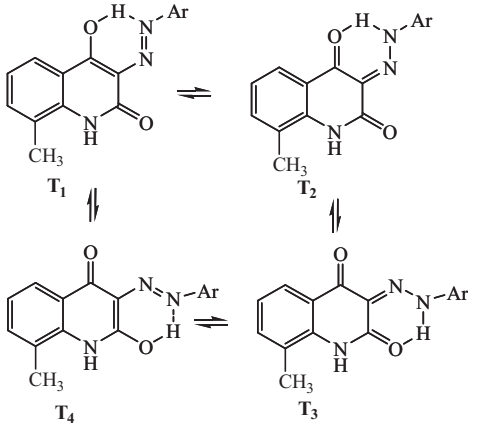
Scheme 2. Synthetic routes for the preparation of azo dyes **1**–**8**.

3. Results and discussion

The arylazoquinololin-2-one dyes **1**–**8** were prepared by coupling reaction of 8-methyl-4-hydroxyquinolin-2-(1*H*)-one with diazotized *p*-substituted aniline derivatives in basic solution (Scheme 2). The chemical structures of these dyes were confirmed by some spectroscopic methods and elemental analysis (see supplementary data). As shown in Scheme 3, azo dyes **1**–**8** can exist as a mixture of four tautomeric forms, namely the azo-enol-keto (**T₁**), hydrazone-keto (**T₂**), hydrazone-keto (**T₃**) and azo-enol-keto (**T₄**).

The infrared spectra of all the compounds (in KBr) showed intense carbonyl bands at 1680–1660 cm^{-1} and showed broad hydroxyl and amide (NH–C=O) bands at 3459–3442 cm^{-1} and 3200–3175 cm^{-1}, respectively. It can be suggested that these compounds do not exist in the hydrazone-keto form in solid state. The FT-IR spectra also show a weak band at 3120–3060 cm^{-1}, which was assigned to aromatic C–H.

^1H NMR spectra measured in DMSO-d_6 at 25 °C are given in the supplementary data. In addition to known aromatic and aliphatic protons, all the prepared azo dyes showed two broad peaks at δ 16.24–15.51 and δ 15.37–14.89 assigned to hydrazone proton signals (=N–NH–). Undoubtedly, these signals correspond to the hydrazone NH proton resonance related to hydrazone-keto forms **T₂** and **T₃** [15,16]. These results are supported by the fact that the hydroxyazo OH proton resonance comes δ 3–5 higher than NH proton resonance; hence, the OH proton resonance signal of enol forms is expected to be in the region δ 9–12 [17,18]. The possibility of tautomers involving ring NH rearrangement can be eliminated by studying the ^1H NMR spectrum of the compounds in DMSO-d_6. The ^1H NMR spectrum of all the dyes showed two singlets at δ 10.45–10.32 and δ 10.30–10.16. The presence of these two broad singlets provides firm evidence for presence of the amide (–NH–C=O) bonds and is related to amide protons of two types of



Scheme 3. Possible tautomeric forms for the synthesized azo dye.

Table 1
Influence of solvents on the λ_{max} of dyes **1**–**8**.

Dye	DMSO	DMF	Acetonitrile	Methanol	Acetic acid	Chloroform
1	437, 408s	445, 412s	443, 410s	447, 416s	450, 415s	441, 411s
2	428, 393s	429, 396s	424, 390s	428, 392s	434, 396s	433, 395s
3	405, 380s	431, 408s	429, 402s	431, 401s	436, 408s	437, 408s
4	402, 378s	424, 400s	423, 398s	422, 398s	431, 406s	434, 402s
5	400, 375s	421, 394s	421, 394s	420, 390s	428, 401s	431, 396s
6	397, 370s	415, 384	418, 388s	419, 385s	424, 394s	427, 392s
7	425, 405s	423, 402s	419, 397s	424, 399s	430, 404s	431, 405s
8	467, 445s	428, 396s	419, 398s	418, 399s	424, 402s	424, 403s

s, shoulder.

tautomeric forms $\mathbf{T_2}$ and $\mathbf{T_3}$. Hence, the spectral data generally led to the conclusion that in DMSO, the tautomeric equilibrium of the arylazoquinolone dyes is a mixture of two hydrazone-keto forms $\mathbf{T_2}$ and $\mathbf{T_3}$.

Further evidence for this assignment was provided by the observation that the quinolone ring NH was extremely affected by solvent species, but the hydrazone NH was not affected so much. For example, in the ^{1}H NMR spectra of dyes **1** and **4**, the quinolone ring NH peak was observed at higher field in chloroform than in DMSO. The quinolone rings NHs of dye **1** were observed at δ 8.11 and δ 8.42 in $CDCl_3$, whereas the corresponding protons were observed at δ 10.32 and δ 10.16 in DMSO-d_6. The downfield chemical shift of the NH proton signal in DMSO-d_6 is larger than in $CDCl_3$, because of the intermolecular hydrogen bonding between the NH and DMSO. Inspection of the hydrazone NHs chemical shifts of those azo dyes which were soluble in both $CDCl_3$ and DMSO-d_6 showed that there was no significant difference in the chemical shifts in the two solvents, provided that the molecules are involved in a strong intramolecular hydrogen bond as shown in Scheme 3 (see supplementary data dyes **1** and **4**).

Absorption spectra of dyes **1**–**8** were recorded in various solvents at a concentration of about 10^{-5} mol/L to 10^{-7} mol/L. The results are given in Table 1. The visible absorption spectra of the dyes did not show regular variation with the polarity of solvents.

All dyes showed two absorption bands in the used solvents (Table 1). It can be suggested that the dyes may exist as a mixture of two tautomeric forms in various solvents. As an example, dye **6** showed two absorbances at 397 nm and 370 nm (in DMSO), 415 and 384 (in DMF), 418 and 388 (in acetonitrile), 419 and 385 (in methanol), 424 and 394s (in acetic acid) and 427, 392 (in chloroform) (Fig. 2). These results were consistent with the findings on ^{1}H NMR spectra and confirmed that all dyes may be a mixture of two tautomeric forms in DMSO. Fig. 1 shows the absorption spectra of the dye **2** in various solvents.

It was also observed that the absorption curves of the dyes were slightly sensitive to acid. The absorption maxima values of all dyes in methanol, on addition of 0.1 mol/L HCl, were nearly the same as those observed in acetic acid; but they are sensitive to base with the exception of dye **8** (see supplementary data Table S1). The λ_{max} of the dyes **1**–**7** showed a hypsochromic shift when a small amount of 0.1 mol/L KOH was added to their methanolic solutions. In the methanolic solution of the dyes containing a trace amount of KOH, both a shoulder and a maximum in the longer wavelength region were disappeared and a new maximum at short wavelength region was observed. These hypsochromic shifts in basic medium are due to deprotonation of dye molecules, which lead to anionic forms of dyes (see supplementary data Scheme S1). A typical example is shown in Fig. 3.

As is apparent in Table 1, the introduction of an electron-donating methoxy group to the benzene ring results in a bathochromic shift in all solvents with respect to electron-accepting fluoro, acetyl, and nitro groups, except for nitro group in DMSO and DMF (for dye **1** $\Delta\lambda = 27$ nm relative to dye **6**, $\Delta\lambda = 25$ nm relative to dye **7**, $\Delta\lambda = 29$ nm relative to dye **8** for spectra in methanol). This behavior can be explained by considering the strong electron-donating groups, which enhance the delocalization of conjugated system of such dyes. In a series of halogen substituents, the absorption maxima of these dyes changed in the following order: I > Br > Cl > F. These variations in absorption maxima (λ_{max}) are in opposition to electronegativity of atoms, which can be attributed to an increase in polarizability of heavy atoms.

To determine the antibacterial activity of the synthesized compounds, the sensitivity of four bacterial strains to the presence of these compounds was measured using the well diffusion method. The four bacteria were: *Salmonella enterica*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Micrococcus luteus*. Briefly, the

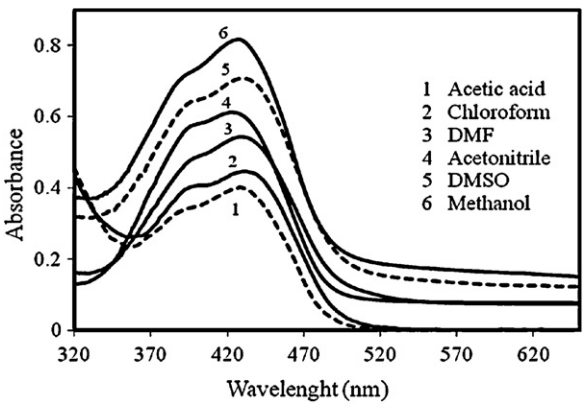


Fig. 1. Absorption spectra of dye **2** various solvents.

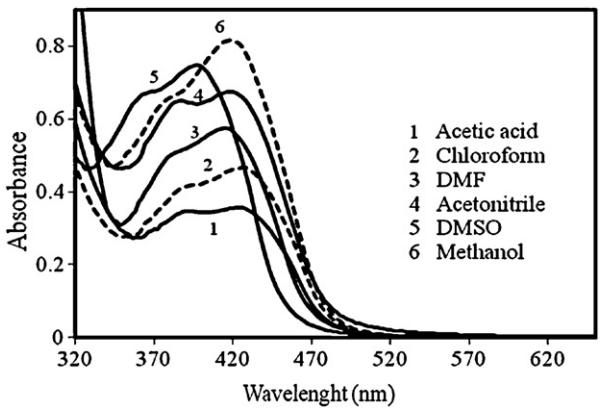


Fig. 2. Absorption spectra of dye **6** in various solvents.

Table 2
Antimicrobial activity of dyes **1**–**8**.

Compound	Conc. of compound (μg/well)	Antimicrobial activity (zone of inhibition in mm)			
		S. enteric	*M. luteus*	*B. subtilis*	*P. aeruginosa*
1	4.38	–	9	11	8
2	4.38	–	9	11	9
3	4.38	–	11	9	10
4	4.38	11	13	14	13
5	4.38	9	12	13	11
6	4.38	–	8	–	–
7	4.38	9	13	9	9
8	4.38	8	9	–	8
Std[a]	15	7	10	12	10
Std[b]	30	8	16	14	18

–, resistant.
[a] Tetracycline is used as standard.
[b] Erythromycin is used as standard.

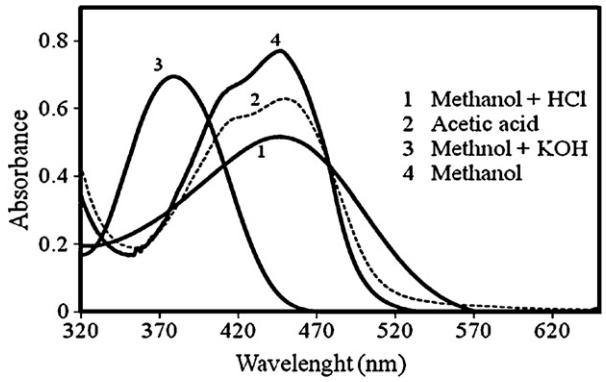


Fig. 3. Absorption spectra of dye **1** in acidic and basic solution.

bacteria were grown on nutrient agar plates (Merck) and aseptically transferred to 3 mL of nutrient broth (Merck). After overnight growth at 37 °C, 50 μL of the suspension was transferred on to nutrient agar plates and spread on the surface using a sterile spreader. Wells were bored in to the agar using sterile Pasteur pipette ends. To each well 40 μL of sample compound was added and after incubation overnight at 37 °C, zones of inhibition were measured. Erythromycin and tetracycline disks were used as positive controls.

The effects of the dyes on growth of bacterial strains are shown in Table 2. Dyes **4**, **5**, **7** and **8** were effective against *S. enterica*, as compared to the antibiotics. The sensitivity of the three other strains to the dyes was similar and considering the lower concentrations of the dyes used, it can be said that the compounds have antibacterial properties. Dyes **4** and **5**, in particular, exhibited higher levels of antibacterial activity against all of the four bacteria.

4. Conclusion

In summary, we have synthesized eight azo dyes **1**–**8** in this paper. It was found that these azo dyes exist in azo-enol-keto forms in solid state, and in hydrazone-keto forms in solvents. It was also observed that the introduction of a methoxy group in the diazo component ring resulted in the bathochromic effect in studied solvents, except for the nitro group in DMSO and DMF. In addition, the antibacterial activities of the dyes prepared were determined against four bacteria species. Some dyes exhibited significant antibacterial activity against the four test strains, which can be further investigated.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2013.03.002.

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