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Solvent-free synthesis and characterization of antibacterial azo dyes in the presence of Bronsted-acid ionic liquid as a green catalyst

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ABSTRACT

A convenient and efficient direct protocol for the preparation of antibacterial azo dyes by the reaction of 2-naphthol with aromatic amines in the presence of catalytic amount of *N*-methyl-2-pyrrolidonium hydrogen sulfate ([H-NMP]HSO_4) was carried out under solvent free conditions. This method has some advantages such as: easy work-up and easy separation of catalyst from the reaction mixture. The *in vitro* antibacterial activities of some compounds were studied using gram positive and negative microorganisms.

Keywords*: Ionic liquid, Azo dyes, Antibacterial, Bronsted acid, 2-Naphthol*.

1. Introduction

Organic color chemistry is undergoing very exciting development as a result of the opportunities presented by dye applications in high technology fields such as, pharmaceutical, cosmetic, textile and leather industries, electronic devices, linear and non linear optics [1-4]. Azo dyes are formed via condensation of diazonium salts with a strong nucleophile such as naphthoxide. Diazonium salts are prepared by the reaction of nitrosonium ion (NO^+) and aniline derivatives in low temperature (0-5 °C). NO^+ is achieved via reaction of sodium nitrite and strong liquid acids [5]. Furthermore, azo dyes can be obtained with silica sulfuric acid [6], sulfanilic acid [7], *p*-toluene sulfonic acid [8], potassium hydrogen sulfate under solvent free condition [9], nano sized iron-promoted [10], zinc and ammonium salts [11]. It is worth noting that most of these techniques have problems such as long reaction time, toxic materials and tedious work-up. Using ionic liquids have many advantages such as recyclable/reclaimable solvents, easy work-up and are less toxic than typical organic solvents. These advantages led to the use of ionic liquids in the synthesis of azo dyes [12-14]. In pharmaceutical, azo dyes are well known for antiseptic activity [15,16] and some are useful as chemotherapeutic agents [17]. A number of them have been investigated at various times for possible the rapeutic activities. For example, direct red 75 (chlorazol fast pink) has been used for its anticoagulant activity as it was shown to inhibit the thrombin-fibrinogen reaction [18]. Congo red can also be used as nonpeptidic inhibitors of HIV [19,20]. The azo dyes which inform sulfonamides anti-bacterial drugs are totally inactive *in vitro* but possess excellent activity *in vivo*. This effect is believed to be caused by broken azo links [21]. Azo compounds with naphthalene core have been extensively used as dyes and their biological and antibacterial activities are less reported [22,23]. As part of our ongoing research program for exploring the bi-functional catalytic properties [24-27], in this article we wish to report the reaction conditions for preparation of some antibacterial phenylazonaphthols in acid ionic liquid as reusable catalyst using a couple reactions of diazonium salts with 2-naphthols under solvent-free condition.

2. Experimental

IR spectra were recorded as KBr pellets on a Perkin-Elmer 781 spectrophotometer and an Impact 400 Nicolet FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-400 spectrometer with tetramethylsilane as internal reference. Melting points were obtained by a Yanagimoto micro melting point apparatus. The purity determination of the substrates and reaction monitoring were accomplished by TLC on silica-gel polygram SILG/UV 254 plates (from Merck Company). The catalyst Bronsted-acid ionic liquid was prepared by the reported method [28].

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2.1. Typical procedure for azo dye synthesis

A mixture of sodium nitrite (2 mmol, 0.138 g) and *N*-methyl-2-pyrrolidonium hydrogen sulfate ([H-NMP] HSO_4) (0.3 g) was stirred at 0-5 °C for 2-3 minutes. 4-Methyl aniline (1 mmol, 0.107 g) was added to the mixture and mixed for 5 minutes in at same temperature. 2-Naphthole (1 mmol, 0.144 g) was added to mixture and stirred for 10 minutes. The reaction progress was monitored by thin layer chromatography (TLC) using a mixture of ethyl acetate and n-hexane (3:7; v/v) as a solvent. After completion of the reaction, the mixture was washed by water (20 mL). The aqueous layer was further extracted with ethyl acetate (20 mL). The combined organic layer was dried with $CaCl_2$. The organic solvent was evaporated under reduced pressure and the crude product was purified by flash column chromatography. Through this procedure, azo dye was obtained with 94% isolated yield; m.p. 141 °C (lit: 142 °C [29]). The pure product was characterized by physical and spectroscopic data.

2.2. Biological assays of azo dyes

Preliminary antimicrobial activities of some compounds (Table 2, entries 1,4,7 and 10) were tested against various gram-negative (Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 27853) and gram-positive bacteria (Staphylococcus aureus ATCC 9144, Bacillus cereus ATCC 709 ROMA) by agar disk diffusion method. The 6 mm diameter disks were made in the agar and moistened with the test compound 500 ppm solution in DMF. These agar disks were carefully placed on the agar culture medium which had been previously inoculated separately by microorganisms. The disks were inoculated at 37 °C and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria. Ciprofloxacin (5 mcg/disc for bacteria) was used as reference antibiotic and DMF as control.

2.3. Minimum Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) was determined by broth microdilution method [30]. For MIC determination, the inoculums were prepared using 4.6 h broth culture of each bacterial strains adjusted to a turbidity equivalent to a 0.5 McFarland standard, diluted in nutrient broth media to give concentration of ≈106 cfu/mL for bacteria. Two fold serial dilutions of compounds were prepared in nutrient broth in 96-well plates starting from a stock solution of compounds (2.00 mg/mL DMF). DMF had no effect on the microorganism in the studied concentrations. An equal volume of bacterial inoculums were added to each well on the microtitre plate. In this manner final concentration of compounds range 0.49-500 μg/mL and 5 ×105 cfu/mL for bacteria in each well (last wells are broth only control well). The inoculated microtiter plates were then inoculated at 37 °C for 24 h. The MIC value was defined as the lowest concentration of compounds whose absorbance was comparable with the negative control wells (broth only, without inoculum).

Table 1. Synthesis of 1-((4-methylphenyl)azo)-2-naphthol in the presence of various amount of [H-NMP] HSO_4 as catalyst at 0-5 °C.

Entry	Time (min)	IL (g)	Yield (%)[a]
1	23	0.05	45
2	18	0.1	60
3	15	0.2	75
4	10	0.3	94
5	10	0.4	94

[a]Isolated yields.

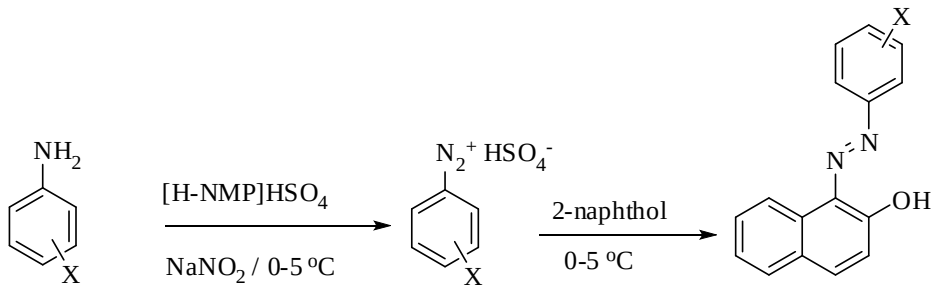
2.4. The Selected spectral data

Entry 1, Table 2: UV-Vis: λ_{max}(nm), $CHCl_3$= 490, 318. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 16.05 (s, 1H), 8.37 (d, *J* = 8 Hz, 1 H), 7.58 (d, *J* = 8.4 Hz, 2 H), 7.56 (d, *J* = 9.2 Hz, 1 H), 7.42 (d, *J* = 8 Hz, 1 H), 7.36 (t, *J* = 8Hz, 1 H), 7.30 (t, *J* = 7.2 Hz, 2 H), 7.21(t, *J* = 8 Hz, 1 H), 7.11(t, *J* = 7.2 Hz, 1 H), 6.68 (d, *J* = 9.2 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 177.88, 144.80, 140.06, 133.61, 130.08, 129.59, 128.86, 128.62, 128.07, 127.42, 125.72, 124.82, 121.73, 118.60. IR (KBr, cm^{-1}): 3434 (w), 3031 (w), 1617 (s), 1447 (s), 1207, 1261 (s), 839 (s), 751 (s).

Entry 7, Table 2: UV-Vis: λ_{max}(nm), $CHCl_3$= 488, 320 nm. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 15.73 (s, 1H), 8.73 (d, *J* = 8.4 Hz, 1H), 7.85 (d, *J* = 9.2 Hz, 2H), 7.78 (d, *J* = 9.2 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.6 (t, *J* = 8 Hz, 1H), 7.41 (t, *J* = 8 Hz, 1 H), 7.06 (d, *J* = 9.2 Hz, 2H), 7.04 (d, *J* = 9.2 Hz, 1 H), 3.91(s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 172.65, 141.84, 136.71, 133.31, 129.53, 128.33, 128.15, 127.11 124.80, 122.19, 122.06, 121.61, 114.78, 55.64. IR (KBr, cm^{-1}): 3433 (w), 3025, 2938, 1601 (s), 1521 (s), 1441 (s), 1159, 1246 (s), 828 (s), 753 (s).

Entry 8, Table 2: UV-Vis: λ_{max}(nm), $CHCl_3$= 490, 324 nm. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 15.60 (s,1H), 8.65 (d, *J*= 8 Hz 1H), 7.95 (d, *J* = 9.6 Hz, 1H), 7.88 (d, *J* = 8.6 Hz, 2H), 7.78 (d, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 8.6 Hz, 2H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 9.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 170.59, 144.33, 140.10, 133.39, 133.01, 129.76, 128.94, 128.69, 128.19, 126.42, 125.89, 124.33, 121.75, 119.93. IR (KBr, cm^{-1}): 3432 (w), 2931 (m), 1621 (s), 1492 (s), 1451 (s), 1210, 1255 (s), 821 (s), 749 (s).

Entry 10, Table 2: UV-Vis: λmax(nm) $CHCl_3$= 496, 322. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 16.2 (s,1H), 8.62 (d, *J*= 8 Hz, 1H), 7.74 (d, *J* = 9.6 Hz, 1H), 7.69 (d, *J* = 8 Hz, 2H), 7.64 (d, *J* = 8 Hz 1H), 7.57 (t, *J* = 7.6 Hz, 1H), 7.40 (t, *J* =



Scheme 1.

Table 2. Synthesis of azo dyes based on 2-naphthol[a].

Entry (Product No.)	X	Yield (%)[b]	m.p. (°C)	
			Found	Reported [29]
1	H	90	135	134
2	4-Cl	83	161	161
3	4-Br	85	156	154
4	2-NO_2	92	246	245
5	3-NO_2	91	195	194
6	4-NO_2	88	254	256
7	2-Cl	85	163	166
8	2-CH_3	91	132	131
9	4-CH_3	94	142	142
10	4-OCH_3	85	179	180

[a]Aniline derivatives (1mmol), $NaNO_2$ (2 mmol), 2-naphthol (1 mmol) and [H-NMP] HSO_4 (0.3 g) were used.
[b]Isolated yields

7.6 Hz, 1H), 7.30 (d, *J* = 8 Hz, 2H), 6.94 (d, *J* = 9.6 Hz, 1H), 2.43 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 168.56, 143.51, 138.92, 138.35, 133.54, 130.20, 129.77, 128.61, 128.52, 128.02, 125.34, 124.04, 121.64, 119.15, 21.32. IR (KBr, cm^{-1}): 3437 (w), 3030 (w), 2935 (m), 1616 (s), 1501 (s), 1447 (s), 1207, 1266 (s), 814 (s), 748 (s).

3. Results and Discussion

An attempted was made to prepare *N*-methyl-2-pyrrolidonium hydrogen sulfate ([H-NMP] HSO_4) quite analogous to the procedure described by Xinli Tong and Yongdan Li *via* easy combination of two reaction components: *N*-methyl-2-pyrrolidone with concentrated sulfuric acid [22]. Initially, to optimize the reaction conditions, the reaction of 2-naphthol (1 mmol) and *p*-methyl aniline (1 mmol) were studied as a simple model using different quantities of catalyst (Table 1). It was found that the best result was obtained when the reaction was carried out in the presence of 0.3 g of catalyst (Table 1, Entry 4).

After optimization of the reaction conditions, the generality of this method was investigated. Sodium nitrite was added to the catalyst and stirred for a few minute. After this procedure, aniline was selected as a model compound and was added to the mixture under stirring conditions at 0-5 °C(ice bath). Diazonium salt was formed and 2-naphthol was added to it and stirring was continued for 10 minutes. The whole process of diazotization and azo coupling was performed in 15 minutes. The crude product was purified by

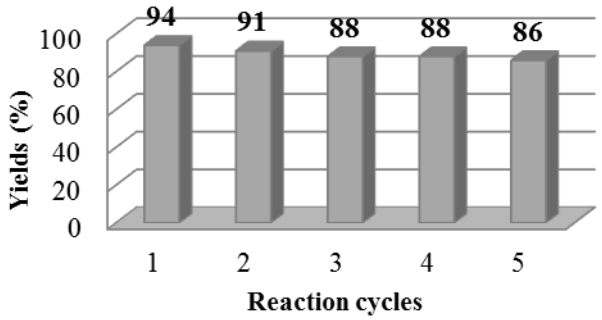


Fig. 1. Reusability of catalyst.

Table 3. Antibacterial screening data (zone of inhibition in mm) of some azo dyes.

Product No.	*E. coli* (Gram negative)	*P. aeruginosa* (Gram negative)	*S. aureus* (Gram positive)	*B. cereus* (Gram positive)
1	11	10	17	9
4	20	18	20	20
7	9	11	16	10
10	20	19	22	19
Standard	22	19	25	21

Table 4. Minimum Inhibitory Concentration (MIC) in μg/mL of some azo dyes

Compound	*E. coli* (Gram negative)	*P. aeruginosa* (Gram negative)	*S. aureus* (Gram positive)	*B. cereus* (Gram positive)
1	65	70	75	80
4	55	55	67	65
7	65	75	75	80
10	63	72	73	82

short column chromatography. By using this method, 1-((4-methylphenyl)azo)-2-naphthol was obtained with 94% isolated yield. The same conditions were used as the conversion of other aromatic amines to the corresponding azo dyes (Scheme 1) in good to excellent yields (Table 2).

For reusability of the catalyst, after the complete separation of solid products by water, the water containing Brønsted acid ionic liquids was evaporated under vacuum condition and the catalyst was recycled for several times with a few decrease in catalytic activity (Fig. 1).

According to FT-IR spectra, azo group (N=N) was appeared in about 1500 cm^{-1} and phenolic (O-H) group at about 3437 cm^{-1}. In UV-Vis. spectra of products, two signals around 496 nm ($\pi \rightarrow \pi^*$) and 320 ($n \rightarrow \pi^*$) nm were recorded. In ^1H NMR spectra, the signal of OH/NH proton was appeared about δ = 15-16 ppm due to tautomeric forms in azo dyes derivatives.

Bactericidal activities of some synthesized azo compounds against pathogenic bacteria were recorded by disc diffusion method and results were given in Table 3. Azo compounds which were shown remarkable activity against used microbes and results were compared with standard drugs. From the results (Table 3), it is concluded that insertion of substituent at the *ortho* position of compound 4 was increased its antimicrobial activity but *o*-chloride substituent involved in the compound 7 do not modify the antibacterial effect. MIC is the lowest amount of drug at which it is able to inhibit the growth of specified microorganism. MIC value of the synthesized azo compounds were calculated against two strains of gram positive (S. aureus and B. cereus) and two strain of gram negative bacteria (E. coli and P. aeruginosa) using broath microdilution method. The comparison of MIC values (Table 4) of azo compounds indicates that the presence of a chloro substituent group on the phenyl rings contributes almost nothing to the antimicrobial activity.

4. Conclusion

In conclusion, various azo dyes based on 2-naphthol were synthesized using *N*-methyl-2-pyrrolidonium hydrogen sulfate ([H-NMP] HSO_4) as acid catalyst and solvent in good to excellent yields. Novelty, high efficiency and eco-friendly are some advantages of this protocol. Meanwhile, the *in vitro* antibacterial activity study of some azo dyes have shown good positive results.

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