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Synthesis and Antimicrobial Activities of Some Acid Dyes

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The present study was taken up to test inherent antimicrobial activity of some newly synthesized acid dyes with a view to develop protective clothing from these. All synthesized dyes were tested against common pathogens *Escherichia coli, Bacillus subtilis* and *Bacillus cerus*. All dyes were effective and showed zone of inhibition thereby indicating sufficient antimicrobial activity against all the microbes tested. Minimum inhibitory concentration was found to be varying from 5 to 40 µg. The textile material impregnated with these acid dyes, however, showed less antimicrobial activity, as uptake of these dyes in textile material is below minimum inhibitory concentration.

Key Words: Imidazolone, Acid dyes, Wool, Antimicrobial activity.

INTRODUCTION

Textile materials and clothing are known to be susceptible to microbial attack, as these provide large surface area and absorb moisture required for microbial growth[1]. Natural fibres have protein (keratin) and cellulose, *etc.*, that provide basic nutrient requirements which along with suitable moisture, oxygen and temperature support bacterial growth and multiplication. This often leads to objectionable odour, dermal infection, product deterioration, allergic responses and other related diseases[2]. This necessitates the development of clothing that could provide a desired antimicrobial effect. Substituted benzylidene imidazolones and its derivatives have been reported for their antibacterial and antifungal properties against gram positive and gram negative bacteria[3,4].

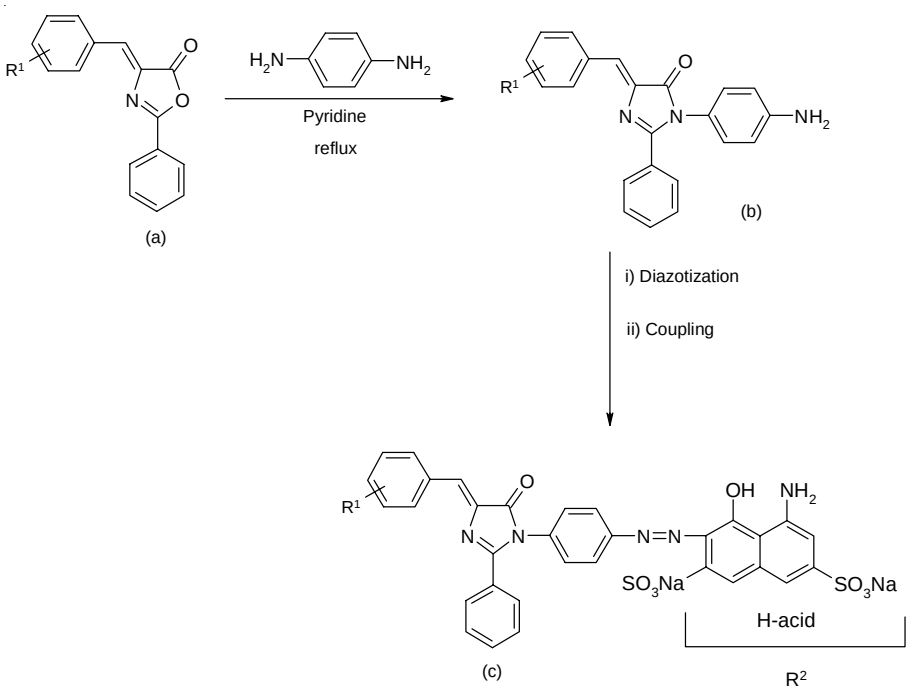
Dyes commonly used in textile are seldom screened for their use as antimicrobial agents for textile finishing. Hence, with a view of this, a series of monoazo acid dyes are synthesized having imidazolones ring system as a diazo component and coupled with various subsititued amino naphthol sulphonic acids. This synthesized dyes were screened for their antimicrobial activities against some common microbes *viz.*, *Escherichia coli*, *Bacillus subtilis* and *Bacillus cerus*.

However, no report including the synthesis and antimicrobial screening of an amino derivative of this heterocyclic compound in the preparation of acid dyes is evident in the literature.

EXPERIMENTAL

All the chemicals and solvents used are of laboratory grade and solvents were purified. Completion of the reaction was monitored by TLC (silica gel GF_{254} (E. Merck), toluene:methanol (8:2). The final dyes were purified by column chromatography using silica gel in increasing percentage of ethyl acetate in carbon tetrachloride. IR (KBr, cm^{-1}) were recorded on a Shimadzu-8400 FT-IR spectrometer, ^1H PMR spectra on a Brucker spectrometer (300 MHz)using TMS as a internal standard (chemical shift in δ, ppm)in $CDCl_3$ and DMSO-d_6 and C, H, N analysis on Perkin-Elmer (USA) 2400 series.

Preparation of 4-arylidene-2-phenyl-5-(4*H*)-oxazolones (a): 4-Arylidene-2-phenyl-5-(4*H*)-oxazolones were prepared according to the reported method[5] (**Scheme-I**).



Dyes	R^1	R^2
D1	4-OCH_3	(γ)-Acid
D2	4-OCH_3	(J)-Acid
D3	4-OCH_3	(H)-Acid
D4	4-OCH_3	(R)-Acid
D5	3,4,5-OCH_3	(γ)-Acid
D6	3,4,5-OCH_3	(J)-Acid
D7	3,4,5-OCH_3	(H)-Acid
D8	3,4,5-OCH_3	(R)-Acid

Scheme-I: Synthesis of imidazolone based acid dyes D1- D8

General procedure for the preparation of 3-(4-amino phenyl)-5-benzylidene-2-substituted phenyl-3,5-dihydro-imidazol-4-one (b): Equimolar amount of 4-arylidene-2-phenyl-5-(4*H*)-oxazolone and *p*-phenylene diamine were taken in a reaction flask attached with a reflux condenser and kept under reflux for 6 h, with dry pyridine as solvent. The content of the reaction mixture was poured in ice-water medium to give coloured precipitates. The completion of reaction was monitored by TLC using toluene:methanol (8:2). Yield 62-70 %. The product was crystallized from ethyl alcohol (**Scheme-I**).

Preparation of 3-(4-amino-phenyl)-5-(4-methoxybenzylidene-2-phenyl-3, 5-dihydro-imidazol-4-one) (b): Equimolar amount of 4-methoxy benzylidene-2-phenyl-5-(4*H*)-oxazolones and *p*-phenylene diamine were taken in a reaction flask attached with a reflux condenser and kept under reflux for 6 h with dry pyridine as solvent. The content of the reaction mixture was poured in ice-water medium to give coloured precipitates. The completion of reaction was monitored by TLC using toluene:methanol (8:2). The product was crystallized from ethyl alcohol[6] (**Scheme-I**).

IR Data: (ν_{max}, cm^{-1}) 3300-3270 (-NH), 3100-3010 (aromatic C-H), 1611 (C=C *str.*), 1690 (C=O, imidazolinone ring), 1274 (C-O-C asymmetric *str.*), 1026 (C-O-C symmetric *str.*).

^{1}H NMR Data: (δ, ppm), 8.04-6.60 (m, 13H, Ar-H), 6.01 (s, 1H, Ph-C=CH), 4.63 (br, 2H, $-NH_2$), 3.78 (s, 3H, $-OCH_3$)[6].

Diazotization of 3-(4-amino-phenyl)-5-(4-methoxybenzylidene-2-phenyl-3,5-dihydro-imidazol-4-one) (b) and coupling with 1-naphthol-8-amino-3,6-disulphonic acid [H-acid] (c): To the 25 mL of water in a beaker 2.96 g (95 % 0.01 mol) of 3-(4-amino-phenyl)-5-(4-methoxybenzylidene-2-phenyl-3,5-dihydro-imidazol-4-one) (b) was added. While stirring the suspension was cooled down to 5 ºC using ice. To this solution 3.67 mL (3 N, 0.011 mol) of $NaNO_2$ and 5 g of ice were added. Then, 2.6 mL of conc. HCl was added to obtain a diazo component of 3-(4-amino-phenyl)-5-(4-methoxybenzylidene-2-phenyl-3, 5-dihydro-imidazol-4-one) (b). Completion of diazotization reaction was checked by starch iodide test. Excess HNO_2 was removed by adding a small amount of sulfamic acid.

To another beaker 3.9 g (82 %, 0.01 mol) of 1-naphthol-8-amino-3,6-disulphonic acid (H-acid) and 40 mL of water was added and the pH was adjusted between 9-10, using 10 % Na_2CO_3. Into this aqueous solution, the above prepared diazo solution was added dropwise using dropping funnel while keeping the temperature 0-5 ºC to complete the coupling reaction. The progress of the reaction was monitored by thin layer chromatography (TLC) using a DMF:water mixture (5:2 by volume) as developing solvent and silica gel TLC plates as the stationary phase. Final dye is purified by column chromatograph[7] (**Scheme-I**).

IR Data: (ν_{max}, cm^{-1}): 1581 (N=N), 1128 (S=O), 3500 (N-H), 3435 (OH), 1700 (C=O) imidazolinone ring, 3000 (aromatic C-H s), 1600-1500s (C=C), below 900 (C-H), 1900-1500s (C=N), 1226 (C-O-C asymmetric *str.*), 1045 (C-O-C symmetric *str.*).

^{1}H NMR Data: (δ, ppm): 7.592-6.608 (m, 16H, Ar-H), 5.850 (s, 1H, -OH), 4.637 (s, 2H, -NH_2), 3.799-3.789 (s, 3H, -OCH_3).

General procedure for dyeing: Knitted wool was initially treated in an aqueous solution with a liquor ratio 50:1 containing 0.5 g/L sodium carbonate and 2 g/L non-ionic detergent at 60 ºC for 0.5 h, after which time it was thoroughly rinsed and dried at room temperature.

Dyeing of the wool fabrics were carried out by know reported methods[8].

The dyeing was carried out at 10 % owf (on weight of fabric), at 1:30 MLR (material to liquor ratio), at 95-100 °C at 4.5-5 pH. Dyed samples were further treated with the non-ionic detergent Lissapol N (0.5 g/L) at 60 ºC for 20 min and rinsed in hot and then cold water.

Test organisms: Cultures of *Escherichia coli*, *Bacillus subtilis* and *Bacillus cerus* were used for antimicrobial activity against these dyes.

Antimicrobial screening test: Nutrient agar medium (g/L: peptone 5.0; beef extract 1.5; yeast extract 1.5; NaCl 5.0; agar 20; pH 7.5) was prepared and autoclaved at 121 °C for 20 min. Sterilized petriplates were prepared with an equal thickness of nutrient agar. Fresh cultures of test organisms were spread on nutrient agar medium with glass spreader and incubated overnight at 37 °C. 10 µg (or 100 µL) of each dye was inoculated into a small 8 mm well on the seeded medium. After overnight incubation at 37 °C, the zones of inhibition were measured.

In the second set of experiments, concentration of dye impregnated (5-40 µg) onto a disc of filter paper was varied to study its effect on the growth of microbes and MIC of dye.

The antimicrobial efficacy of a compound will vary when it is present in solution and when it is held intimately by a textile substrate. In the next set of experiments the antimicrobial activity of dyed wool specimens was tested. The 1 $inch^2$ fabric (dyed and undyed) was introduced in the 100 mL of overnight grown culture (at 37 °C for16 h)[9]. The reduction of bacterial growth by dye was expressed as follows:

$$R = B - A / A \times 100$$

where R = % reduction in bacterial population; B = absorbance (660 nm) of overnight grown culture in presence of undyed fabric; A = absorbance (660 nm) of overnight grown culture in presence of dyed fabric.

RESULTS AND DISCUSSION

Synthesis: Compound (a) 4-arylidene-2-phenyl-5-(4*H*)-oxazolones derivatives were prepared by Erlenmeyer condensation of benzolyglycine with different aldehydes in presence of sodium acetate and acetic anhydride[5,6].

The prepared 5-oxazolone were treated with *p*-phenylene diamine in basic medium to prepared 3-(4-amino phenyl)-5-benzylidene-2-substituted phenyl-3,5-dihydro-imidazol-4-one (b). The IR spectrum of compound (b) showed the NH bands at 3300-3270 cm^{-1} and the C=O band nearly at 1690 cm^{-1}.

The ^{1}H NMR spectrum shows broad peak at 4.63 ppm was due to the protons of the -NH_2 group. The benzylidene proton of (Ph-C=CH) was found in downfield region at δ 6.01 ppm. In the case of 4-OCH_3, singlet is observed at δ 3.73 ppm which corresponds to the three protons of -OCH_3.

3-(4-Amino-phenyl)-5-(4-methoxybenzylidene-2-phenyl-3,5-dihydro-imidazol-4-one) (b) was diazotized and coupled with various substituted napthol mono and di-sulphonic acids with known reported methods to prepare compound (c)[7]. The IR spectrum of compound (c) showed the N=N bands at 1581 cm^{-1}, N-H bands at 3500 cm^{-1}, -OH band at 3435 cm^{-1} and (C=O) imidazolinone ring bands at 1700 cm^{-1}.

The ^{1}H NMR spectrum showed a multiplet at δ 7.592-6.608 ppm due to aromatic protons (16H), the benzylidene proton of (Ph-C=CH) was also found in the same region but was over lapped by multiplet of aromatic protons. The spectrum showed a singlet at δ 4.637 ppm due to the protons of -NH_2 group. The spectrum showed a singlet at δ 5.850 ppm due to the protons of -OH group. The position of protons of -NH_2 and -OH groups depends upon the intramolecular hydrogen bonding into the sample tube. In the case of 4-OCH_3, singlet is observed at δ 3.799-3.789 ppm, which corresponds to the three protons of -OCH_3.

Visible spectral study: The λ_{max} value of acid dyes of H-acid, R-acid are higher than that of J-acid and γ-acid due to the presence of two sulphonic acid group providing the resonance structure in ethanol.

The λ_{max} value for a acid dye based on H-acid, R-acid has shown bathochromic effect as compared to that of acid dye based on J-acid and γ-acid, this is applicable on the basis of presence of a second auxochromic group causing higher electron density in a diazonium component of the acid dye.

The λ_{max} values for acid dyes based on J-acid and γ-acid have shown hypsochromic effect as compared to that of a dye based on H-acid, R-acid This may be due to steric hindrance of the sulphonic acid group, *ortho* to azo group of the dye. The results are shown in Table-1.

Antimicrobial activity of synthesized dyes in solution: Synthesized dyes were screened for their antimicrobial activity against selected microbes (*Escherichia coli, Bacillus subtilis* and *Bacillus cerus*). The preliminary screening showed that all dyes were effective against all the microbes. A clear zone of inhibition can be seen in Fig. 1.

The effect of concentration of dye on antimicrobial activity was studied further and results are summarized in Table-2. The zone of inhibition (diameter) was recorded in each case. It was observed that increase in dye concentration leads to increased inhibition reflected by enhancement in diameter. It may be concluded that the dyes are highly effective antimicrobial agents as the MIC for most of these lies in region of 5-40 μg.

It is evident from Table-2 that with increasing concentrations of dye, the zone of inhibition is increasing almost linearly. From the clear zone of inhibition obtained, it is apparent that the selected dyes are bactericidal in nature and not bacteriostatic.

TABLE-1
CHARACTERIZATION OF DYES D1-D8

Dye	R^1 / R^2	m.f. (m.w.)	Yield ,% (λ_{max}, nm)	E_{max} (dm^3 mol^{-1} cm^{-1})	Elemental analysis %: Found (Calcd.)		
					C	H	N
D1	4-OCH_3 / (γ)-Acid	$C_{35}H_{25}N_5O_6S$ (619.15)	63 (390)	56,485	63.73 (63.96)	4.12 (4.07)	11.13 (11.03)
D2	4-OCH_3 / (J)-Acid	$C_{33}H_{25}N_5O_6S$ (619.15)	65 (405)	59,414	63.71 (63.96)	4.11 (4.07)	11.12 (11.03)
D3	4-OCH_3 / (H)-Acid	$C_{33}H_{25}N_5O_9S_2$ (699.71)	65 (550)	54,550	56.54 (56.65)	3.57 (3.60)	09.89 (10.01)
D4	4-OCH_3 / (R)-Acid	$C_{33}H_{24}N_4O_9S_2$ (684.10)	65 (490)	48,815	57.63 (57.89)	3.44 (3.53)	8.02 (8.18)
D5	3,4,5-OCH_3 / (γ)-Acid	$C_{35}H_{29}N_5O_8S$ (679.17)	62 (445)	48,630	61.69 (61.85)	4.32 (4.30)	10.32 (10.30)
D6	3,4,5-OCH_3 / (J)-Acid	$C_{35}H_{29}N_5O_8S$ (679.17)	55 (452)	55,142	61.72 (61.85)	4.33 (4.30)	10.23 (10.30)
D7	3,4,5-OCH_3 / (H)-Acid	$C_{35}H_{29}N_5O_{11}S_2$ (759.71)	70 (533)	57,982	55.41 (55.33)	3.61 (3.85)	9.19 (9.22)
D8	3,4,5-OCH_3 / (R)-Acid	$C_{35}H_{28}N_4O_{11}S_2$ (744.12)	71 (500)	53,583	56.32 (56.45)	3.90 (3.79)	7.49 (7.52)

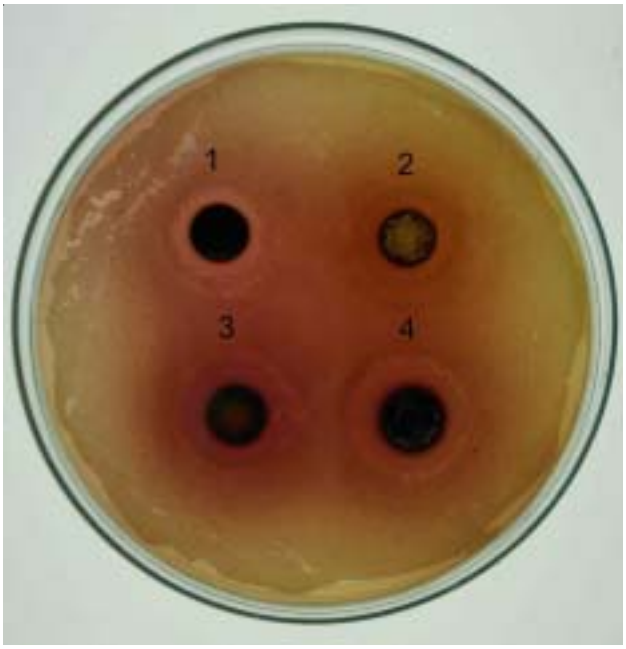


Fig. 1. Antimicrobial activity of some selected dyes D1-D4 (1-4) on *Bacillus subtilis*: Agar diffusion test for the effect of Dyes D1-D4 (1-4) against *Bacillus subtilis* grown on nutrient agar medium

TABLE-2
ZONE OF INHIBITION FOR SYNTHESIZED DYES AGAINST SELECTED MICROBES

Dye	Concentration (µg)	Zone of inhibition (diameter in cm)		
		Escherichia coli	*Bacillus subtilis*	*Bacillus cerus*
D1	5	0.5	0.7	0.5
	10	0.9	1.1	0.8
	20	1.3	1.4	1.1
	40	1.7	1.6	1.3
D2	5	0.6	0.7	0.4
	10	0.8	1.2	0.7
	20	1.3	1.5	1.1
	40	1.5	1.9	1.2
D3	5	0.9	2.0	0.8
	10	1.3	2.2	1.1
	20	1.6	2.4	1.8
	40	1.8	2.6	1.9
D4	5	1.2	2.5	1.3
	10	1.5	2.6	1.5
	20	1.7	2.7	1.6
	40	2.2	2.9	2.0
D5	5	0.6	0.6	0.4
	10	0.8	1.2	0.6
	20	1.4	1.3	1.0
	40	1.5	1.2	1.2
D6	5	0.5	0.6	0.3
	10	0.7	1.4	0.5
	20	1.4	1.5	1.2
	40	1.6	1.8	1.3
D7	5	0.8	1.9	0.7
	10	1.2	2.3	1.2
	20	1.5	2.5	1.6
	40	1.8	2.5	1.7
D8	5	1.1	2.3	1.1
	10	1.5	2.6	1.4
	20	1.6	2.6	1.5
	40	2.1	2.8	1.9

Antimicrobial activity of synthesized dyes on substrate: Since dyes showed good antimicrobial activity against selected microbes in solution, it was thought worthwhile to study their antimicrobial activity on dyed substrate (wool). The wool samples dyed with these dyes were used as model system[9]. A reduction of 10-15 % in bacterial growth was seen on wool (Fig. 2). An interesting observation is that all the dyes, which exhibit high bactericidal activity in solution found to be bacteriostatic when dyed onto wool fibre. This may be because the concentration of dye on fabric samples is not sufficient enough for bactericidal activity. This is an interesting finding and requires more in-depth investigation into the effect of dye structure on antimicrobial property. It is obvious that antimicrobial properties are closely related to the dye structure, especially the presence of functional groups on it.

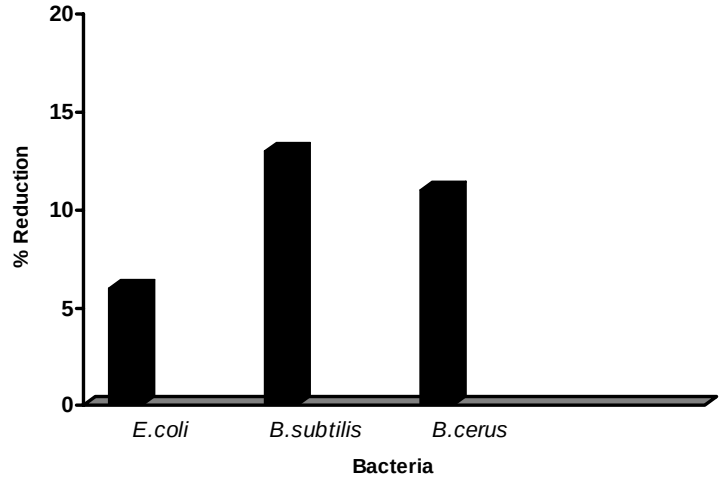


Fig. 2. Antimicrobial activity of textile materials dyed with dye D3: One square inch of sterilized fabric was introduced in 100 mL sterilized nutrient broth. This was aseptically inoculated with the microbe and incubated at 37 ºC for 16 h. A broth without dyed fabric but similarly inoculated was kept as control. The absorbance of test and control was recorded at 660 nm. The antimicrobial activity was calculated as reduction in bacterial growth in sample compared to control using the formula described in experimental section.

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REFERENCES

1. J.M. Cardamone, *AATCC Rev.*, **30**, 5 (2002).
2. M.C. Thiry, *AATCC Rev.*, **11**, 7 (2001).
3. M.M. Hanna, S.E.S. Abbas, A.S.M. Mogahazy, R.M.A. Megid and M.M. Moustafa, *Bull. Fac. Pharm. Cairo Univ.*, **32**, 353 (1994).
4. B. Kadriye, C.K. Ahmet, G.K. Nalan, D. Rayak and G. Klymet, *Arch. Pharm. Res.*, **26**, 773 (2003).
5. A.I. Vogel, A Text Book of Practical Organic Chemistry, Longman, London, p. 909 (1971).
6. P.V. Bhatt, D.N. Wadia, P.M. Patel and R.M. Patel, *Asian J. Chem.*, **18**, 1248 (2006).
7. S.K. Young, K. Yusung, K. Jinsoo and K. Yusung, United States Patent Application Publication, Pub. No. US 2004/0143106 Al (2004).
8. B.G. Skinner and T. Vickerstaff, *J. Soc. Dyer Colour*, **61**, 193 (1945).
9. R. Singh, A. Jain, S. Panwar, D. Gupta and S.K. Khare, *Dyes Pigments*, **66**, 99 (2005).

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