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Research Article

SYNTHESIS OF SOME NEW AZO COMPOUNDS AND THEIR ANTIMICROBIAL SCREENING

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Abstract:

In this study, a series of azo compounds were synthesized in excellent yields via the diazotization of different aromatic amines followed by coupling with chloroxylenol (4-chloro-3,5-dimethyl phenol). These compounds were characterized by IR, ^{1}H NMR and Mass spectral techniques. The synthesized compounds have been tested in vitro against of microorganisms *C.albicans, E. coli & S.aureus* in order to assess their antimicrobial properties using cup plate method. Some of the products exhibited comparable activity with known standard drugs at same concentration.

Keywords: Chloroxylenol, Antimicrobial, *C.albicans, E. coli*, *S.aureus* and cup plate method.

Introduction

Nowadays, synthetic azo compounds are widely used in different application fields, such as medicines, cosmetics, food, paints, plastics, shipbuilding, automobile industry, cable manufacture, and in analytical chemistry[1-15]. Biological importance of azo compounds is well known for their use as antineoplastics, antidiabetics, antiseptics, anti-inflammatory, and other useful chemotherapeutic agents [16-19]. Azo compounds are known to be involved in a number of biological reactions such as inhibition of DNA, RNA and protein synthesis, carcinogenesis and nitrogen fixation [20, 21]. Evans blue and Congo Red are being studied as HIV inhibitors of viral replications. This effect is believed to be caused by binding of azo dyes to both protease and reverse transcriptase of this virus [22]. The existence of an azo moiety in different types of compounds has caused them to show antibacterial and pesticidal activity [23, 24]. Since compounds with azo moiety and Chloroxylenol moiety have been extensively used as dyes, but biological activity is less reported.

In the present work, we have synthesized and characterized four azo compounds namely 4-chloro-3, 5-dimethyl-2-(phenyldiazenyl) phenol (A), 4-chloro-3, 5-dimethyl-2-[(4-nitrophenyl)diazenyl]phenol (B), 4-chloro-3, 5-dimethyl-2-[(E)-naphthalen-1-yldiazenyl] phenol (C), 4-chloro-2-[(E)-(2-methoxyphenyl)diazenyl]-3,5-dimethylphenol (D), The antimicrobial activities of the synthesized azo compounds were reported invitro using cup plate method.

Materials and Methods

All chemicals used in the present investigation were of analytical grade. Chloroxylenol and dimethylformamide were purchased from Sigma-Aldrich. The purity of the synthesized compounds was checked by TLC.The melting points were determined in open capillary method and found to be uncorrected. The UV spectra of the azo compounds was reported on Schimadzu® UV visible spectrophotometer (UV-1800). The molecular weight was determined by Mass spectra of the azo compounds were recorded using Micromass Quatto II triple quadrapole Mass Spectrometer SAIF Punjab University, Chandigarh. In the present investigation the IR spectra of azo compounds were recorded on Schimadzu FTIR spectrophotometer model 8400S in KBr pallets at Gurunanak college of pharmacy, Nagpur university, Nagpur, Maharashtra and the ^{1}H-NMR spectra of the azo compounds were recorded using BRUKER AVANCE II 400 Spectrometer SAIF Punjab University, Chandigarh DMSO as solvent and reported relative to TMS as internal standard.

Experimental

Synthesis of azo compounds

Azo compounds were synthesized according to the method reported in literature [25]. There are two steps in the synthesis of azo compounds:

Step I: Diazotization: A mixture of freshly distilled amine (0.016 mol) and concentrated sulphuric acid was stirred until a clear solution was obtained. This solution was cooled to 0–5 ^{O}C, and a solution of sodium nitrite in 10mL water was then added drop wise, maintaining the temperature below 5 ^{O}C. The resulting mixture was stirred for an additional 30 min in an ice bath.

Step II: Coupling : Chloroxylenol (0.016 mol) was dissolved in 8ml 10% potassium hydroxide, and cooled to 0–5 ^{O}C in an ice bath. This solution was then gradually added to the cooled diazonium salt solution, and the resulting mixture

was stirred at 0–5 ^{O}C for 60 min. The resulting crude precipitate was filtered, washed several times with cold water and was recrystallized from hot chloroform to yield azo compound. Azo compounds were synthesized according to following scheme 1.

A) 4-chloro-3, 5-dimethyl-2-(phenyldiazenyl) phenol (A)

Yield 68.54 % ; reddish brown amorphous; mol. wt 261.71 ; m.p.154 °C ; Rf value 0.56 (pet. ether:benzene :: 9:1) ; λmax 401.92; IR (KBr) 3288.63 (Phenolic OH str.), 3030.71(CH-Ar str.), 2920.23 (C-H str. in CH_3), 1585.49(C=C Ar. Str.), 1463.97 (N=N str.), 1024.20 (OH def.), 638.44 (C-Cl str.) ; Mass: molecular ion peak (M+) at m/z = 261.03, and their fragments showed at m/z = 263.03, 243.1, 154.05 102.09; calculated for $C_{14}H_{13}ClN_2O$ was 261.71. ^{1}H-NMR (TMS): 5.3524 (Ar-OH), 2.3441, 2.3442 (C-H), 7.2-7.8 (Ar-H).

B) 4-chloro-3, 5-dimethyl-2-[(4-nitrophenyl)diazenyl]phenol (B)

Yield 65.71 % ; brown amorphous; mol. wt 305.71 ; m.p. 98 °C ; Rf value 0.61 (pet. ether:benzene :: 9:1) ; λmax 399.56; IR (KBr) 3269.34 (Phenolic OH str.), 3000.23 (CH-Ar str.), 2920.23 (C-H str. in CH_3), 1565.49(C=C Ar. Str.), 1465.97 (N=N str.), 1313.52 (N=O, Ar.-NO_2 Str.), 1024.20 (OH def.), 698.24 (C-Cl str.); Mass: molecular ion peak (M+) at m/z = 305.02, and their fragments showed at m/z = 307.02, 288.07, 273.02, 258.25, 138.24; calculated for $C_{14}H_{12}ClN_3O_3$ was 261.71. ^{1}H-NMR (TMS): 5.3502 (Ar-OH), 2.3412, 2.3416 (C-H), 7.8-8.2 (Ar-H).

C) 4-chloro-3, 5-dimethyl-2-[(E)-naphthalen-1-yldiazenyl] phenol (C)

Yield 53.55 % ; red amorphous; mol. wt 310.77 ; m.p. 100 °C ; Rf value 0.66 (pet. ether:benzene :: 9:1) ; λmax 404.22; IR (KBr) 3284.77 (Phenolic OH str.); 2972.31 (CH-Ar str.); 2920.23 (C-H str. in CH_3); 1567.42(C=C Ar. Str.), 1463.97 (N=N str.), 1024.20 (OH def.), 763.8 (C-Cl str.) ; Mass: molecular ion peak (M+) at m/z = 310.02, and their fragments showed at m/z = 312.02, 293.07, 278.02, 263.25, 143.24, 132.09, 106.09; calculated for $C_{15}H_{13}ClN_2O_3$ was 310.77. ^{1}H-NMR (TMS): 5.3524 (Ar-OH), 2.3423, 2.3425 (C-H), 6.92-8.28 (Ar-H).

D) 4-chloro-2-[(E)-(2-methoxyphenyl)diazenyl]-3,5-dimethylphenol (D)

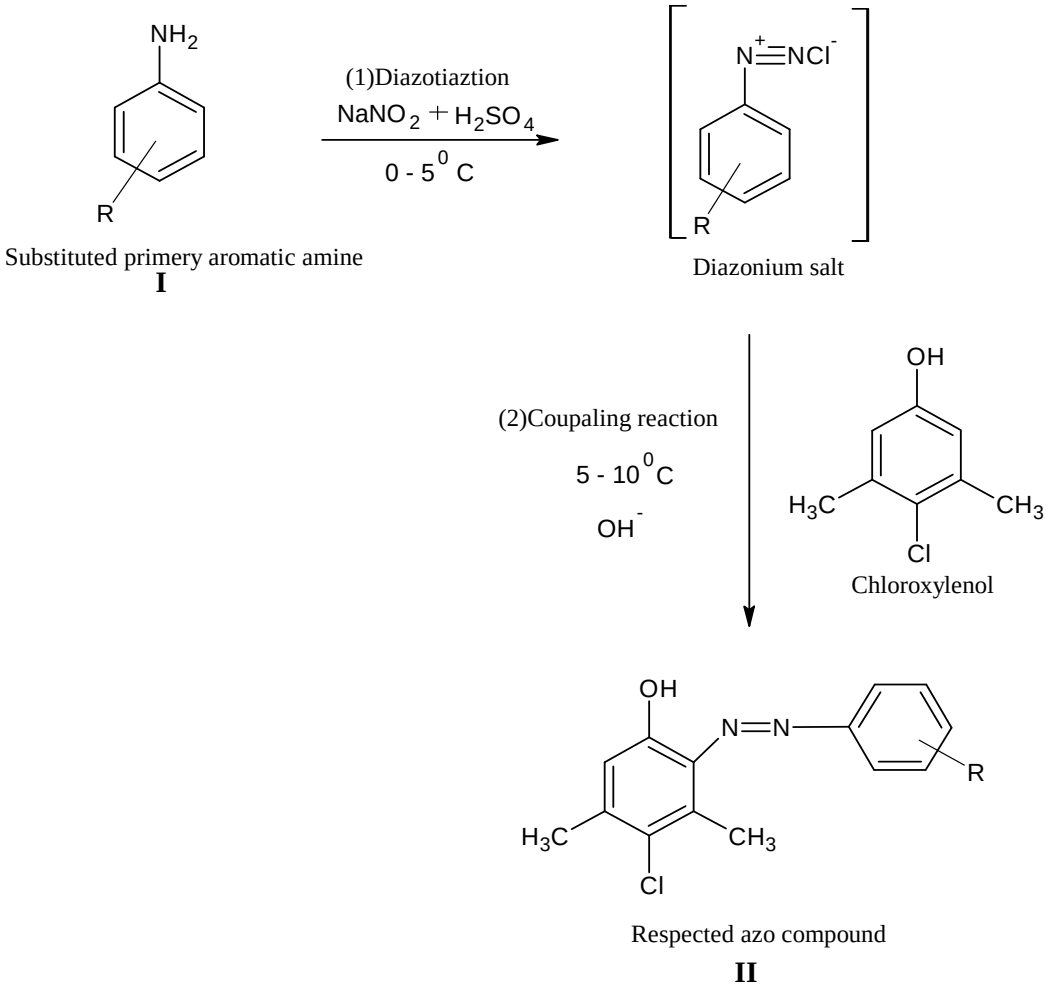
Yield 54.55 % ; yellowish red amorphous; mol. wt 290.77 ; m.p.140 °C ; Rf value 0.59 (pet. ether:benzene :: 9:1) ; λmax 381.65.92; IR (KBr) 3288.63 (Phenolic OH str.), 2922.16 (CH-Ar str.), 2843.07 (C-H str. in CH_3), 1587.42(C=C Ar. Str.), 1463.97 (N=N str.), 1024.20 (OH def.), 753.8 (C-Cl str.); Mass: molecular ion peak (M+) at m/z = 290.07,

and their fragments showed at m/z = 292.05, 273.02, 258.07, 243.02, 143.25, 109.09; calculated for $C_{15}H_{13}ClN_2O_3$ was 290.77. ^{1}H-NMR (TMS): 5.3511 (Ar-OH), 2.3425-8.8322 (C-H), 6.88-7.89 (Ar-H).

Antimicrobial activity[25,26]

All the synthesized compounds were screened for their antibacterial activity against *E Coli, & S. aureus* by using disc diffusion method. Bacteria were cultured in nutrient agar medium and used as inoculum for study. The test compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain a solution of 50,100,300,500µg/ml concentration. The compounds were also screened for their in vitro antifungal activities against *C.albicans.* The zones of inhibition of compounds for bacteria were compared with Ciprofloxacin and for fungi were compared with Miconazole. The zone of inhibition produced by test compounds was measured in mm. The data are given in Table-3.

Scheme



Substituted primery aromatic amine

I

Diazonium salt

Chloroxylenol

Respected azo compound

II

Schem-1: Preparative route of substituted azo compounds.

Table-1: Substituent's Used for Synthesis of (A-D).

Sr. No.	Compounds	R
1	**A**	H
2	**B**	NO_2
3	**C**	OCH_3
4	**D**	C_4H_4

Table-2: Physical Data of Substituted Azo Compounds.

Compound code	Mol. Formula	Mol. Weight	M.P. ^{0}C	% of Yield	Appearance	R_f	λ_{max}
A	$C_{14}H_{13}ClN_2O$	261.71	154	68.54	Reddish brown	0.56	401.92
B	$C_{14}H_{12}ClN_3O_3$	305.71	98	65.71	Brown	0.61	399.56
C	$C_{15}H_{13}ClN_2O_3$	310.77	100	53.55	Reddish	0.66	404.22
D	$C_{15}H_{13}ClN_2O_3$	290.77	140	54.55	Yellowish red	0.59	381.65

Table-2: Evaluation of Antimicrobial Activity of the Compounds.

Compound code	Concentration (µg/ml)	Zone of inhibition (mm)		
		E.coli	*S.aureus*	*C.albicans*
A	50	14	10	-
	100	15	12	-
	300	16	15	12
	500	18	16	14
B	50	-	16	-
	100	7	20	12
	300	12	25	14
	500	33	28	17
C	50	-	-	-
	100	12	6	12
	300	15	12	13
	500	17	20	15
D	50	-	11	-
	100	8	13	10
	300	13	16	11
	500	16	19	12
Std.		Ciprofloxacin		Miconazole
	50	14	6	8
	100	16	8	12
	300	19	12	16
	500	22	22	18

Results and discussion

The purity of the synthesized compounds was checked by performing thin layer chromatography and determining melting points. IR, Mass spectroscopy and 1HNMR spectra were consistent with the assigned structures. Since our titled compounds are known to possess antimicrobial activity, the compounds were screened for their antibacterial and antifungal activity by cup plate method.

Conclusion

Four azo compounds have been prepared and characterized on the basis of analytical and spectral data. Screening of these compounds against pathogenic microorganism reveals that these compounds have the capacity of inhibiting metabolic growth of *some microorganisms* to different extent. The antimicrobial activity of the compounds depends on the nature of substituent present on the aromatic ring.

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