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Research Article

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Synthesis, characterization and biological studies of some azo compounds

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ABSTRACT

*In continuation of our earlier work [1], we report a series of 2-(substituted phenyl)azo-4,6-diacetylresorcinol derivatives (**I-VI**) synthesized by diazotisation of substituted aniline followed by coupling with 4,6-diacetylresorcinol. All the synthesized compounds have been characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. In general azo resorcinol compounds can exist in azo hydrazone tautomeric forms. But in our study, all the spectral data show that 2-(substituted phenyl)azo-4,6-diacetylresorcinol derivatives (**I-VI**) exist in the azo form. Besides, In vitro antimicrobial activity of all the synthesized compounds has been evaluated against four strains of bacteria viz., Escherichia coli, Proteus vulgaris , Vibrio cholera and Salmonella typhi and two fungi viz., Aspergillus niger and Candida albicans. The study showed that all the synthesised compounds have potent activity.*

Key words: azo, diacetylresorcinol, azo - hydrazone, *in vitro*, antibacterial, antifungal.

INTRODUCTION

Azo compounds are considered as class of organic colorants which consist of at least a conjugated chromophore azo (-N=N-) group in association with one or more aromatic or heterocyclic system [2]. They are capable of providing high intensity color and have reasonably good technical properties, including light and weather fastness and resistance to solvents and water. The biological importance of azo compounds is well known due to their use as inflammatory [3,4] anticancer [5,6], antibacterial [7-9] and antifungal [10-15]. Azo compounds have received much attention due to their versatile use in many practical applications such as coloring fiber [16]. Azo dyes show better stability than a natural dyes in the whole pH range of foods, are heat stable and do not fade when exposed to light or oxygen. Because of low toxicity, less allergic reactions and no hyperactivity effect, azo dyes are used in food stuffs. In the present work, the investigator has made an attempt to synthesise, characterize and to study the *in vitro* antimicrobial activity of azo compounds of 4,6-diacetylresorcinol (**I-VI**).

EXPERIMENTAL SECTION

The purity of the compounds was checked by TLC using silica gel-G plates and visualized in iodine vapours. Melting points were recorded in open capillary tubes in sulfuric acid bath and were uncorrected. FT-IR spectra were obtained on SHIMADZU FT-IR Affinity-I instrument using KBr pellets. ^{1}H and ^{13}C NMR spectra were taken in BRUKER 400MHz instrument in DMSO-d_6 / $CDCl_3$ using TMS as internal standard. SHIMADZU mass spectrometer operating at an ionization potential of 70 eV was used to record Mass spectra.

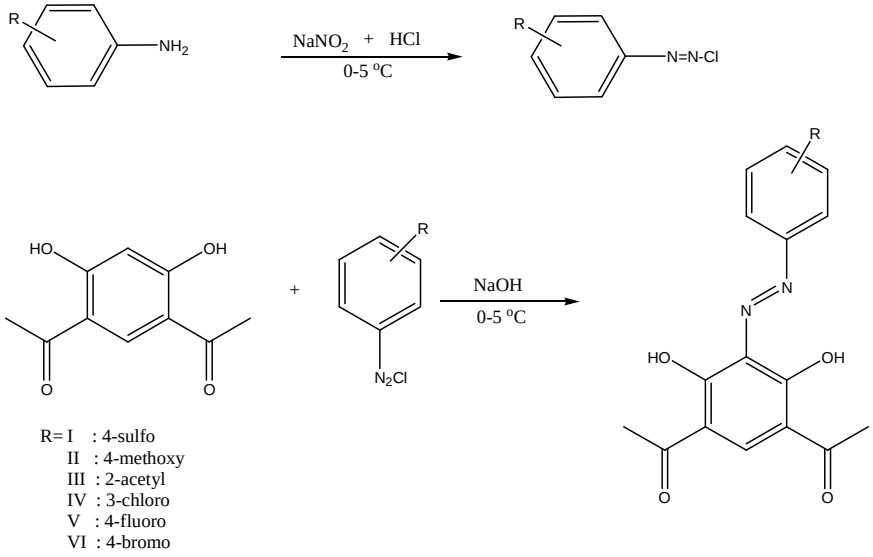
4,6-diacetylresorcinol

The required starting material 4,6-diacetylresorcinol was prepared by the procedure reported in literature [17-19].

2-(substituted phenyl)azo-4,6-diacetylresorcinol

To a stirred ice cold solution of substituted aniline (0.001mol) in 2 ml Conc. HCl, a solution of sodium nitrite (0.002 mol) in water (2 ml) was added in drops at a temperature of 0-5 ˚C in an ice bath. To this diazotized mixture,

a solution of 4,6-diacetylresorcinol (0.001 mol, 0.194 g) in 10% NaOH was added dropwise with constant stirring. (Scheme **1**). The precipitated product, thus formed was filtered, washed with water and then dried. It was then recrystallised from appropriate solvent.



Scheme 1

RESULTS AND DISCUSSION

All the six compounds gave satisfactory elemental analysis and the spectral data are in agreement with literature values. The presence of various functional groups in the molecules, i.e., -OH, C=O, N=N and C⎓C are evidenced by the IR spectra. Due to chelating effect [20-22] or intra molecular hydrogen bonding between C=O and –OH group, low absorption frequency is observed for C=O and are shown in Table-**1**.

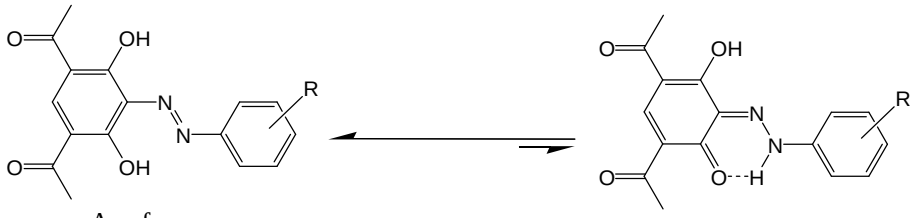
Other spectral data also support the expected structure of the compound, for instance, in ^{1}H NMR spectrum, 2-(2-acetylphenyl)azo-4,6-diacetylresorcinol (III) gives eight signals. In the up field alkyl region, three protons of acetyl group of phenyl moiety and six protons of two acetyl groups of resorcinol moiety give the signals at δ 2.6 and δ 2.7 as singlets respectively. $C_{5'}$-H, $C_{4'}$-H, $C_{6'}$-H and $C_{3'}$-H of phenyl moiety resonate at δ 7.5, δ 7.7, δ 7.9 and δ 8.2 respectively. C_5 hydrogen of resorcinol moiety is clearly evidenced by the appearance of a singlet peak integrating for one hydrogen atom at δ 8.5.

Due to the possibility of azo - hydrazone tautomerism, the hydroxy proton which involves in such tautomerism shows a hump at δ 15.4 [23-28]. The peak of another -OH proton would be extremely broad at room temperature. Hence it cannot be distinguished from the base line. It is because the -OH proton may involve in intermolecular hydrogen bond with -OH of other molecules or the acidic impurities present in $CDCl_3$ solvent used in NMR study.

In ^{13}C NMR spectrum, methyl carbon of three acetyl groups appears at δ 28.8. In the down field carbonyl carbon region, three acetyl carbonyl carbons resonate and give a NMR peak at δ 200.4. In ESI negative mode mass spectrum, the compound shows a high intense peak at m/z = 339 (100%) accounting for both the molecular ion peak ($M^{+\bullet}$- H) and base peak.

Table 1: Characterization data of compounds I-VI

Compound	Molecular Formula	Molecular Weight	Melting Point (˚C)	IR (KBr) (cm^{-1})				Purification Solvent
				-OH	C=O	N=N	C⎓C	
I	$C_{16}H_{14}N_2O_7S$	378	>300	3439	1656	1587	1520	Dimethyl sulfoxide
II	$C_{17}H_{16}N_2O_5$	328	200	3454	1674	1579	1502	Methanol
III	$C_{18}H_{16}N_2O_5$	340	210	3419	1666	1595	1516	Acetonitrile
IV	$C_{16}H_{13}ClN_2O_4$	332	195	3423	1668	1585	1527	Ethanol
V	$C_{16}H_{13}FN_2O_4$	316	220	3446	1656	1587	1492	Ethanol
VI	$C_{16}H_{13}BrN_2O_4$	376	170	3448	1672	1591	1529	Methanol



Scheme II

In general azo resorcinol compounds exist in azo-hydrazone tautomeric forms [29-31]. In compounds **I-VI**, absence of a band at N-H region of the IR spectrum shows the absence of N-H hydrazone formation and absence of N-H hydrogen peak in the ^{1}H NMR spectrum also confirms the absence of N-H hydrazone formation. This shows that compounds **I-VI** exist in the azo form (Scheme **II)**.

2-(4-sulfophenyl)azo-4,6-diacetylresorcinol (I)
IR (cm^{-1}) : 3439(O-H), 3039 (Ar-H), 2929 (CH_3), 1656 (C=O), 1587 (N=N), 1520 (C=C), 1382 (C–N), 1238 (C–O) **^{1}H NMR (δ):** 2.6 (s, 6H, 2-$COCH_3$), 7.8 (t $C_{3',5'}$-H, 2H), 7.9 (t, $C_{2',6'}$-H, 2H), 8.4 (s, 1H, C_5-H), 15.4 (hump, -OH), **Mass:** m/e 378, $C_{16}H_{14}N_2O_7S$.

2-(4-methoxyphenyl)azo-4,6-diacetylresorcinol (II)
IR (cm^{-1}) : 3454 (O-H), 3076 (Ar-H), 2995 (CH_3), 1674 (C=O), 1579 (N=N), 1502 (C=C), 1365 (C–N), 1232 (C–O) **^{1}H NMR (δ) :** 2.6 (s, 6H, 2-$COCH_3$), 3.9 (s, 3H, -OCH_3), 7.0 (dd, $C_{3',5'}$-H, 2H), 7.9 (dd, $C_{2',6'}$-H, 2H), 8.4 (s,1H,C_5-H), 14.1 (hump, -OH), **Mass:** m/e 328**,** $C_{17}H_{16}N_2O_5$.

2-(2-acetylphenyl)azo-4,6-diacetylresorcinol (III)
IR (cm^{-1}) : 3419 (O-H), 3066 (Ar-H), 2924 (CH_3), 1666 (C=O), 1595 (N=N), 1516 (C=C), 1379 (C–N), 1249 (C–O) **^{1}H NMR (δ) :** 2.6 (s, 3H,$C_{2'}$-$COCH_3$), 2.7 (s, 6H, C_4, C_6 -$COCH_3$) 7.5 (m, $C_{5'}$-H, 1H), 7.7 (m, $C_{4'}$-H, 1H), 7.9 (m, $C_{6'}$-H, 1H), 8.2 (m, $C_{3'}$-H, 1H), 8.5 (s,1H, C_5-H), 15.4 (hump, -OH), **Mass:** m/e 340, $C_{18}H_{16}N_2O_5$.

2-(3-chlorophenyl)azo-4,6-diacetylresorcinol (IV)
IR (cm^{-1}) : 3423 (O-H), 3055 (Ar-H), 2922 (CH_3), 1668 (C=O), 1585 (N=N), 1527 (C=C), 1365 (C–N), 1230(C–O) 678 (C-Cl), **^{1}H NMR (δ) :** 2.6(s, 6H, 2-$COCH_3$), 7.4 (m, 2H, $C_{4',5'}$-H), 7.7 (m, 1H, $C_{6'}$-H), 7.9 (s, 1H, $C_{2'}$-H), 8.5 (s, 1H, C_5-H), 14.3 (hump, -OH), **Mass:** m/e 332, $C_{16}H_{13}ClN_2O_4$.

2-(4-fluorophenyl)azo-4,6-diacetylresorcinol (V)
IR (cm^{-1}) : 3446 (O-H), 3064 (Ar-H), 2985 (CH_3), 1656 (C=O), 1587 (N=N), 1371 (C–N), 1269 (C-Cl), 1203(C–O), **^{1}H NMR (δ) :** 2.6 (s, 6H, 2-$COCH_3$), 7.2 (t, $C_{3',4'}$-H, 2H), 7.9 (t, $C_{2',5'}$-H, 2H), 8.5 (s, 1H, C_5-H), 14.2 (hump, -OH), **Mass:** m/e 316, $C_{16}H_{13}FN_2O_4$.

2-(4-bromophenyl)azo-4,6-diacetylresorcinol (VI)
IR (cm^{-1}) : 3448 (O-H), 3051(Ar-H), 2924 (CH_3), 1672 (C=O), 1591(N=N), 1529 (C=C), 1369 (C–N), 1238 (C–O), 580 (C-Br), **^{1}H NMR (δ) :** 2.6 (s, 6H, 2-$COCH_3$), 7.8(d, 2H, $C_{3',5'}$-H), 7.9 (d, 2H, $C_{2',6'}$-H), 8.4 (s, 1H, C_5-H), 14.6(hump, –OH), **Mass:** m/e 376, $C_{16}H_{13}BrN_2O_4$.

Antibacterial activity:
The purified products were screened for their antibacterial activity by well diffusion method using Gentamycin as a standard antibiotic drug. The nutrient agar prepared by the usual method, was inoculated aseptically with 0.5 ml of overnight subculture of *Escherichia coli*, *Proteus vulgaris* , *Vibrio cholera* and *Salmonella typhi* in separate conical flasks at 30 ºC and mixed well by gentle shaking. About 25 ml of the contents of the flasks were poured and evenly spread in petridish (90 mm in diameter) and allowed to set for 2 h. Mueller Hinton agar (beef infusion solids 4.0 g, starch 1.5 g, casein hydrolysate 17.5 g, agar 15.0 g, final pH 7.4 ± 0.2 at 37 °C) was used for antimicrobial assay. The assay plates were prepared by spread plate technique with appropriate pathogen inoculums (~104 CFU). Using a sterile cork borer, a 7 mm well was made and filled with 0.05 ml (50 µg/ml of solution of sample in DMSO). The plates were incubated at 37 ºC for 24 h and the control was also maintained with 0.05 ml of DMSO in similar manner and the zones of inhibition of the bacterial growth were measured in millimeter and recorded in Table **2**. Compounds **I** and **III** have found to possess appreciable activity against all the chosen organisms. Compound **IV** has shown no remarkable effect against the chosen organisms except *Escherichia coli* and compound V has shown no remarkable effect against the chosen organisms except *Proteus vulgaris.*

Antifungal activity: *Aspergillus niger* and *Candida albicans* were used for testing antifungal activity by well diffusion method. The culture was maintained on Sabouraud dextrose agar (SDA) slants. SDA medium was inoculated with 72 h old 0.5 ml suspension of fungal spores in a separate flask. About 25 ml of the inoculated medium was evenly spread in a sterilized petridish and allowed to set for 2 h. The well (7 mm in diameter) were punched in petridish and loaded with 0.05 ml (50µg/ml) of solution of sample in DMSO. The plates were incubated at 30^{o}C for 4-8 hours after the completion of incubation period, the zones of inhibition of growth in the form of diameter in mm was measured. Along the test solution in each petridish on well was filled up with solvent which acts as control. The zones of inhibition are recorded in Table **2**. Compounds **I** and **III** have found to posses excellent activity against *A. niger and C. albicans*. Compounds IV and **V** have shown no remarkable effect against the chosen organisms.

Table 2: Antimicrobial activity of compounds (I-VI)

Compounds	Antibacterial activity zones of inhibition (mm)*				Antifungal activity zones of inhibition (mm)*	
	Escherichia coli	*Proteus vulgaris*	*Vibrio cholera*	*Salmonella typhi*	*Aspergillus niger*	*Candida albicans*
I	30	35	32	31	35	34
II	-	17	10	10	-	30
III	20	20	22	23	24	24
IV	10	-	-	-	-	-
V	-	23	-	-	-	-
VI	10	15	18	10	10	22
+ve Control	25	28	25	23	18	28
-ve Control	-	-	-	-	-	-

**Zone of inhibition is obtained as a mean value of triplicates*
+ve Control : Standard Gentamycin (50 µg/ml) was used as positive control dissolved in DMSO.
-ve Control : Solvent DMSO alone was used to study the physical inhibition

CONCLUSION

2-(substituted phenyl)azo-4,6-diacetylresorcinol derivatives (**I-VI**) have been synthesized and characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. The spectral data reveal that 2-(substituted phenyl)azo-4,6-diacetylresorcinol derivatives (**I-VI**) exist in the azo form. All the compounds except IV and V were found to possess appreciable activity against the chosen bacteria and fungi. The compound II has no remarkable effect against *E. coli* and *A. niger*.

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