

# Coupling Reactions Involving Aryldiazonium Salt: Part-VII. Products of Chemoselective Reaction of Aryldiazonium Chloride with Active Methylene Group containing Moiety

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## Abstract:

**Aim:** Aryldiazonium salts,  $\operatorname{Ar-N_2}^{\oplus} \operatorname{Cl}^{\ominus}$  were used as intermediate in different reactions. To prepare the 3-(Substituted-phenylazo)pentane-2,4-dione from Pentane-2,4-dione or Acetyl acetone (AA) with aryldiazonium salt. and to study their antibacterial activity.

**Method:** These reactions, either, lost nitrogen containing function or without loss of nitrogen function. First category included replacement by -H, -OH, -Br, -F, -I, -CN, -NO<sub>2</sub>, aryl- etc. and the latter involved reduction and diazo coupling type reaction. In the present work we have treated aryldiazonium salt with an Active Methylene Group (**AMG**) containing moiety viz. Pentane-2,4-dione or Acetyl acetone (**AA**) and the antibacterial activity by diffusion cup method.

**Results and Conclusion:** The compounds, 3-(Substituted-phenylazo)pentane-2,4-dione, were tested for the antibacterial activity showed less activity than ciprofloxacin. These compounds will be useful as building block for organic researchers in the near future.

Keywords: Active methylene group, antibacterial activity, aryldiazonium salt, Azo compounds.

# **INTRODUCTION:**

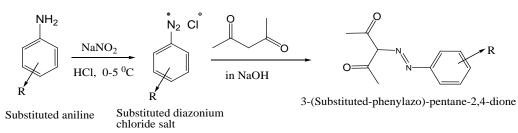
Aryldiazonium salt,  $Ar-N_2^{\oplus}Cl$  are highly reactive class of compounds in synthetic organic chemistry. In chemistry azo dyes of phenolic compounds played a major role in synthesizing many of the commercial dyes and analytical reagent. The dyes were marketed mainly in the form of azo disperse, azo-vat, azo-acid dyes, etc. Due to the simple process of the synthesis, usually an aqueous medium and the almost unlimited choice of starting products, an extremely wide variety of azo dyes skeleton was possible. The number of combination was increased by the fact that a dye molecule can also contain several groups. The practical uses of dyes in various industrial field showed that azo compounds were the largest class of industrial synthesized organic dyes. The azo dyes were a distinct and clearly defined class, characterized by the presence of one or more azo (-N=N-) groups. They were all prepared by a common process involving diazotizing an aromatic primary amine and the formed diazonium salt solution is coupled with a phenol or an aromatic amine.

Diazo coupling reaction products were characterized by chromophoric azo group. Azo dyes were used as corrosion inhibitors for the dissolution of carbon steel in HCl acid solution[1]. Azo compounds have received much attention due to their versatile skeleton, ease of preparation and uses in many practical applications such as colouring fibre, photoelectronic applications, printing systems, optical storage technology and in various analytical techniques[2], as a polymer additive[3] used to colour waxes, oils, petrol, solvents and polishes and successful in textile processing, paper, food, cosmetic medicine, leather, plastics, varnish, automobile[4-6]. The aryldiazonium salt were synthesized and reacted upon with an **AMG** containing organic moiety viz. Pentane-2,4dione or **AA**. Heterocyclic rings[7-8], which were the reason for the activity of Most of the drugs of natural origin lead the discovery of the many synthetic drugs possessing the heterocyclic rings. Heterocyclic nitrogenous[9-10] compounds and their fused analogues represent an important class of heterocyclic compounds exist in numerous natural products displaying a wide range of biological and pharmaceutical activities.

Similar to sitosterol[11] and cholesterol etc. cinnolines were from the class of bioactive compounds due to their remarkable biological and pharmacological property. Cinnoline and its derivatives also showed biological activities such as antihypertensive[12], bacteriocides[13] activity. In view of synthesis of newer 4-Methyl-3acetylcinnoline or derivatives thereof were of importance, their intermediates were considered worthwhile to study their synthesis. Similar types of reactions were also reported by Mittal and Singhal[14] and recently from our laboratory[15-16].

As seen from the above discussion, azo dyes are very important for technical purposes in many types of industries and were easy to prepare. Our previous few reports[15-20] dealt with reactions involving aryldiazonium moiety. Herein we report the part-VII of this series which dealt with the synthesis of 3-(Substituted-phenylazo)-pentane-2,4-diones (**DSP-1** to **DSP-6**), also the antibacterial aspects of these compounds were determined.

Scheme-I:



 $R = -2 - CH_3(DSP-1); -3 - CH_3(DSP-2); -4 - CH_3(DSP-3); -3Br(DSP-4); -4 - Br(DSP-5); -3 - C_2H_5(DSP-6)$ 

In continuation of earlier work[18-21] the present work the prepared aryldiazonium salts were reacted with an **AMG** containing moiety, pentane-2,4-dione viz. **AA** to synthesize 3-(Substituted-phenylazo)pentane-2,4-dione, (**DSP-1** to **DSP-6**) as **Scheme-1**. Review of literature indicated that such ketone derivatives were valuable synthones for the synthesis of 4-Methyl-3-acetylcinnoline or derivatives thereof.

### **EXPERIMENTAL:**

**Materials and Methods:** The materials used and the methods of analysis were employed were as mentioned earlier[18]. The bacterial strains, *E. coli* and *B. subtilis*, were purchased from National Centre for Cell Science(**NCCS**), Pune, India and maintained at Smt. G. G. Khadse College, to determine the antibacterial activity of synthesized compounds.

#### **General Method of Synthesis**

Stage-I. General Procedure for Preparation of Diazonium Salt, (DSP-1 to DSP-6): Charged 0.02 M aniline (or its derivative) in a beaker, the mixture of 10 ml conc. HCl and 5 ml water was added to it and stirred with the glass rod to get clear solution, cooled the solution to 0°C by keeping in an ice bath. Meanwhile (0.025 M) sodium nitrite was dissolved in 8 ml water. Cooled the solution in ice bath to 0°C, after attaining 0°C added NaNO<sub>2</sub> solution in to aniline hydrochloride solution dropwise with constant stirring (The rise in temperature above 5°C during addition was not allowed) test the diazotized solution impart dark blue colour to starch iodide paper(blue colour was obtained on the potassiumiodide starch paper). Decompose the excess of nitrous acid by adding pinch of urea filter the solution and collected the filtrate which was diazonium salt solution.

**Stage-II. General Procedure for Synthesis of** (**Phenylazo)-acetyl acetone**, (**DSP-1** to **DSP-6**): Added aryldiazonium salt solution (from **Stage-I**) slowly, to the well cooled mixture of, Pentane-2,4-dione viz. **AA** (0.018 M) dissolved in 5 ml ethanol and NaOAc, 8-10 gm in 4-5 ml of water(to keep the mixture alkaline to litmus), a coloured precipitate was separated, then added 20 ml of concentrated HCl, filtered and checked the absence of ester and thus the product obtained was recrystallized by using solvent ethanol, dry it. The dried weight (in gms) and the physical constant i.e m. p. range of the compound was recorded.

The synthesized compounds were tested for the antibacterial activity against *E. coli* as per the method

described in literature[19] and compared with ciprofloxacin as standard drug.

The purity of the synthesized compounds, (**DSP-1** to **DSP-6**) was ascertained by TLC method and also characterized by colour, physical constant (melting point range) and by UV-visible and FTIR-spectra.

The solubility of the synthesized compounds was tested using 0.5 gm of the compound and the selected solvent was continuously added, sonicated for 5 min to arrive at the solubility data.

## Method of Antibacterial Activity

Synthesized azo compounds were screened for the antibacterial activity, against E. Coli and B. Subtilis bacterial strain by using the method reported in earlier[18]. To study the antibacterial activity of 3-(Substitutedphenylazo)-pentane-2,4-dione from diazonium chloride salt, following setup was required. The following experiment was adopted. Newly synthesized compounds were screened their antibacterial activities against strain E. coli and B. subtilis using disk diffusion method. Activity of each compound was compared with that of control. The antimicrobial activity of compound (DSP-1 to DSP-6) was studied against two bacterial strains E-coil and B. Subtilis .The bacterial suspension was spread on the surface of sterile nutrient agar medium in the petriplate and then the filter paper disc soaked with compound (DSP-1 to DSP-6) solution was placed in the centre of plate. The plates were incubated at 37 °C for 24 hrs. The

zone of inhibition was measured on the next day in mm.

# **RESULTS AND DISCUSSION:**

In the present study, diazonium intermediates of aniline and substituted anilines were synthesized, reacted with an active methylene compound (acetyl acetone) and screened for antibacterial activity. All the compounds were obtained in high purity. The progress of reactions was monitored by Silica gel-G TLC, visualized by iodine vapour. The purity of the compounds was ascertained by melting point determinations (one end sealed capillary method) and by Silica gel-G TLC. The mobile phases were 10 ml n-Hexane, 1 ml methanol, 2 ml ethylacetate(DSP-1, DSP-3 and DSP-6) and 10 ml n-Hexane, 1 ml Acetic acid (DSP-2, DSP-4 and DSP-5). The structural assignment of the products was based on UV-Vis and FTIR spectral data and elemental (CHN) analyses. The spectral data was in close agreement with the structures of the synthesized compounds. All compounds gave satisfactory elemental analysis. Values were in the close agreement with the values calculated for expected molecular formula assigned to these compounds and were in 5 % in statistics. The results of analytical results and physical constants results were depicted in **Table 1**. The photographic representation

and the characteristic colours of synthesized organic compounds, viz. **DSP-1** to **DSP-6** is shown in **Fig. 1**.

Table 1: The Analytical and Physical data for 3-(Substituted-phenylazo)-pentane-2,4-diones, (DSP-1 to DSP-6).

Code	Aniline derivative	Active methylene Compound used	Colour	Molecular Formula	*m.p. range °C
DSP-1	2-Methyl-aniline	Pentane-2,4-dione	Pale yellow	$C_{12}H_{14}N_2O_2$	70-76
DSP-2	3-Methyl-aniline	Pentane-2,4-dione	Dark brown	$C_{12}H_{14}N_2O_2$	122-128
DSP-3	4-Methyl-aniline	Pentane-2,4-dione	Chocolate Brown	$C_{12}H_{14}N_2O_2$	98-104
DSP-4	3-Bromo-aniline	Pentane-2,4-dione	Shiny orange	$C_{11}H_{11}N_2O_2Br$	84-90
DSP-5	4-Bromo-aniline	Pentane-2,4-dione	Brown	$C_{11}H_{11}N_2O_2Br$	78-84
DSP-6	3-Ethyl-aniline	Pentane-2,4-dione	Orange	$C_{12}H_{14}N_2O_2$	82-86

Physical constants are uncorrected.

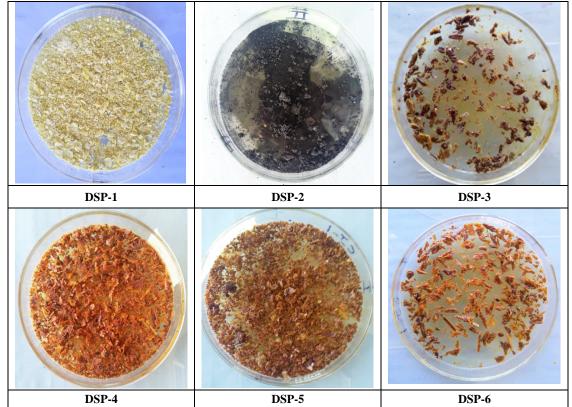


Fig. 1: Colour characteristics for the synthesized 3-(Substituted-phenylazo)-pentane-2,4-diones, (DSP-1 to DSP-6).



Fig. 2A: TLC for 3-(Substituted-phenylazo)pentane-2,4-dione, DSP-1.

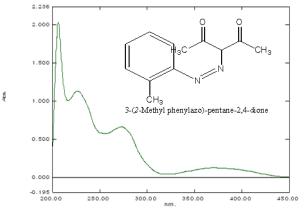


Fig.3: The UV-Vis spectra of DSP-1.

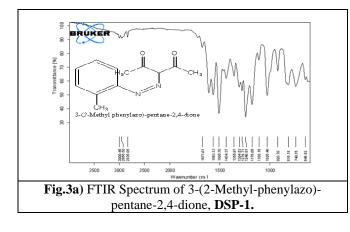
All the studied azo compounds showed four peaks in UV-Vis spectra in ethanol in the studied range 600 nm to 200 nm. In the UV-Vis spectral analysis of azo compound shows the three peaks in the range 397-321 nm, 270-226 nm and 220-203 nm. These were attributed to n \* and \* and \* transitions due to presence of nitro group (auxochrome) and azo group transitions and aromatic phenyl ring transition of moderate energy.

In addition to this the synthesized compounds, **DSP-1** to **DSP-6** were also characterized by spectral analysis viz. UV-Vis, and FTIR and the obtained results were reported in the **Table 2**. The **Table 2** also indicated the assigned structures from the spectral results.

The studied compounds showed absorption frequency in the range 1671-1601cm<sup>-1</sup>, indicated presence of carbonyl group. The studied compounds showed absorption frequency in the range 1680-1613 cm<sup>-1</sup>, indicated presence of Aromatic group >C=C<. The studied compounds showed absorption frequency in the range 3316-3000 cm<sup>-1</sup>, indicated presence of C-H starching. The studied compounds showed absorption frequency in the range 1355-1310 cm<sup>-1</sup>, indicated presence of C-N starching. The studied compounds showed absorption frequency in the range 1563-1513 cm<sup>-1</sup>, indicated presence of -N=Nstarching. These compounds have attracted interest because of their unique structure, in which an electrophilic oxo group is in proximity to two phenylhydrazono groups. Interaction of these groups can give rise to tautomeric phenylhydrazono-phenylazo structures, and the expected -N=N- vibration frequencies in the compound formed are of particular interest. As is known[21, 23-24], the -N=Nvibration frequencies of aromatic and aliphatic azo compounds are difficult to recognize, and therefore they had been the least studied. The presence of the azo (-N=N-) group band in 1563–1513 cm<sup>-1</sup> range confirmed the success of the synthesis[17].

The FITR spectra of the synthesized final products, **DSP-1** to **DSP-6** are depicted below in **Fig. 3a** to **3f**.

The related data of FTIR characteristic frequency (in cm<sup>-1</sup>) of the groups indicated in the **Table 2**. The solubility of the synthesized compounds was decided on the basis of the experiment and the obtained data is depicted in the **Table-3**.



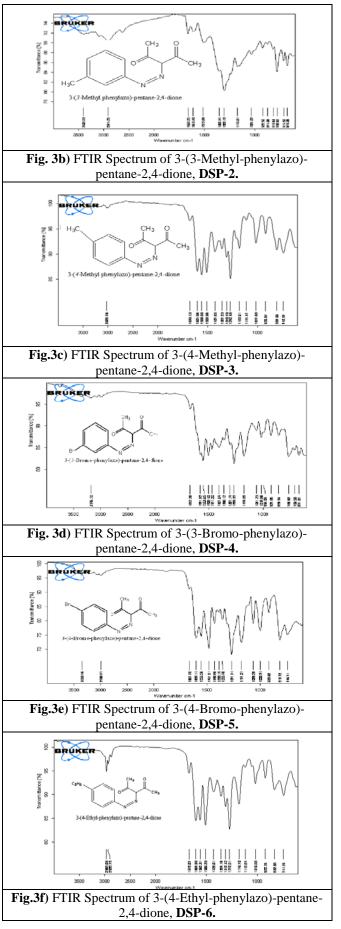


Table 2: Spectral Data for 3-(Substituted-phenylazo)-pentane-2,4-diones, (DSP-1 to DSP-6).

Comp ID	UV (nm)	IR (cm <sup>-1</sup> )	ylazo)-pentane-2,4-diones, ( <b>DSP-1</b> to <b>DSP-6</b> ).
Comp. ID			Assigned Structure
DSP-1	378.20 340.20 273.40 226.00 206.00	$\begin{array}{llllllllllllllllllllllllllllllllllll$	$\nu_{-C-}$ $V_{-C-}$ $H_3C$ $O$ $CH_3$ $GH_3$ $G^2Methyl phenylazo) pentane 2.4 dione$
DSP-2	364.50	$\begin{array}{llllllllllllllllllllllllllllllllllll$	$V_{-C-}$ H <sub>3</sub> C $H_3C$ (3-Methyl phenylazo)-pentane-2,4-dione
DSP-3	556.50 232.00 254.50 225.00 218.00 204.00	$\begin{array}{ll} v_{\text{-C=O}} &= 1601 \\ v_{\text{-C=C-}} &= 1619 \\ v_{\text{-C-H}} &= 3025 \\ v_{\text{-C-N- (Aryl)}} &= 1310 \\ v_{\text{-N=N-}} &= 1560 \\ v_{\text{-C-CH3(p)}} &= 1390 \end{array}$	H <sub>3</sub> C CH <sub>3</sub> O CH <sub>3</sub> O CH <sub>3</sub> CH <sub>3</sub>
DSP-4	331.00 261.00 224.50 205.00	$v_{-C=0}$ =1612 $v_{-C=C}$ =1675 $v_{-C-H}$ =3116 $v_{-C-N- (Aryl)}$ =1311	C-Br(m) C-Br(m
DSP-5	577.50 340.00 226.00 218.00 204.00	$\begin{array}{llllllllllllllllllllllllllllllllllll$	C-Br(p) Br O C-Br(p) 3-(4-Bromo-phenylazo)-pentane-2,4-dione
DSP-6	323.00 269.00 225.00 218.00 204.00	$\begin{array}{llllllllllllllllllllllllllllllllllll$	$C_2H_5$ $C_{H_3}$ $C_{H_$

Table 3: The solubility data for the 3-(Substituted-pl	henylazo)-pentane-2,4-diones, (DSP-1 to DSP-6).
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Compound ID	Solvent used(in ml/0.1 gm of substance)				
Compound ID	Ethyl alcohol Diethyl ether		Acetone		
DSP-1*	24	50**	-		
DSP-2***	6	20**	5		
DSP-3	12	7	2.5		
DSP-4	5	2.5	1.5		
DSP-5	11	1.5	4.0		
DSP-6	14	1.0	6.5		

\* methanol 14 ml required; \*\* Insoluble and coagulated and \*\*\* Benzene 2.5 ml required.

	uioi	ics, (DBI -1 to DBI -0).	~	-	
Nome of the Commound and	ZOI(in mm) of the Compound and Controls				
Name of the Compound and Controls	500 <b>g</b> /ml		1000 <b>g</b> /ml		
Controis	E. coli	B. subtilis	E. coli	B. subtilis	
DSP-1	08	12	10	15	
DSP-2	07	07	08	09	
DSP-3	-	-	-	-	
DSP-4	-	-	-	-	
DSP-5	07	09	06	10	
DSP-6	05	11	07	11	
Std. Drug, (Ciprofloxacin)	21	24	22	25	
Distilled Water (-ve control)			00		

 Table 4: Zone of Inhibition (ZOI, in mm) indicating Antibacterial Activity of 3-(Substituted-phenylazo)-pentane-2,4diones. (DSP-1 to DSP-6).

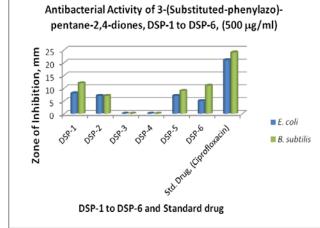


Fig. 4A. The histographic representation of antibacterial activity of Synthesized compound, DSP-1 to DSP-6(500 µg/ml).

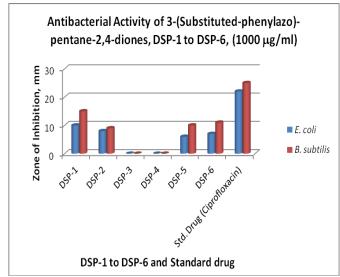


Fig. 4B. The histographic representation of antibacterial activity of Synthesized compound, DSP-1 to DSP-6 (1000 μg/ml).

Antibacterial Activity: Antibacterial activity of all the synthesized compounds were screened against gramnegative bacteria, *E. coli*(NCCS) and *B. subtilis*(NCCS) for three different concentration of 500 and 1000  $\mu$ g/ml, as per the method described in[18]. The results of

antimicrobial testing as zone of inhibition (in mm) are depicted in **Table 4**.

The histographical representation of the antibacterial activity is depicted in Fig. 4A (500  $\mu$ g/ml) and Fig. 4B (1000  $\mu$ g/ml), respectively.

The results showed that the compounds are less active than the standard drug, Ciprofloxacin in case of the studied strains.

Glimpses drawn from the Antibacterial activities of the studied 3-(Substituted-phenylazo)-pentane-2,4-diones were as...

- ✓ All the activity for compound *E. coli* and *B. subtilis* and showed less or no activity as compare to the standard drug, Ciprofloxacin.
- ✓ Compound DSP-1 was active against both *E. coli* and *B. subtilis* (500 and 1000 □g/ml), further it shows little higher activity for *B. subtilis*.
- ✓ Compound DSP-2 was active against both *E. coli* and *B. subtilis* (500 and 1000 □g/ml), further it shows almost same activity for both studied strains.
- ✓ There was no antibacterial activity, shown by compound DSP-3 and DSP-4 for studied concentration and strains.
- ✓ Compound DSP-5 and DSP-6 were active against both *E. coli* and *B. subtilis* (500 and 1000 □g/ml), further it shows little higher activity for *B. subtilis* than for *E. coli*.

# **CONCLUSION:**

Aniline and substituted anilines were used for the preparation of diazoninium salt then they were reacted upon with an active methylene group containing moiety i.e Pentane-2, 4-dione or **AA** to give or derivatives thereof. Their antibacterial activities were also reported. These compounds will be useful as building block for organic researchers in the near future.

**Conflict of Interest Statement:** The authors declares that there is no conflict of interest

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**Dr. Chandrashekhar** has graduated from Pune university, INDIA and post graduated from Pune University of INDIA. He is having 14 years of Industries of different types(perfumery and cosmetics formulation, Agro and Medicine formulation, Halogenation, API in different capacity and 8 yrs of UG and 12 yrs of PG teaching experience at College level. Presently in service at Smt. G. G. Khadse College, Muktainagar. He has hands on experience for different reactions and to handle many of the analytical sophisticated instruments. He has attended more than 54 conferences, seminars and workshops at different level and published about 63 research papers in reputed journals. He has completed two Short Research Projects, guided to 122 students to complete the 54 M.Sc. projects. Under his guidance one research scholar has submitted the thesis and presently 6 students are working for Ph.D. under his guidance. He has completed cGMP certification of a company in Pharma sector.