Research Article

Coupling Reactions Involving Reactions of Aryldiazonium Salt: Part-IV. Chemoselective Synthesis and Antibacterial Activity of 3-(Substituted-phenylazo)-pentane-2,4-diones

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Received: 10-04-2017; **Revised:** 23-05-2017; **Accepted:** 12-07-2017.

ABSTRACT

The aryldiazonium salt, Ar-$N_2^{\oplus}Cl^{\ominus}$ are highly reactive compounds. It was used as intermediate in different reactions. 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_2, aryl- etc. and the latter involved reduction and diazo coupling type reaction. In the present piece of work we have reacted the aryldiazonium salt with Active Methylene Group (AMG) containing compound viz. Pentane-2, 4-dione or Acetyl acetone(AA). The final products formed were potentially used as precursor for synthesis of 4-methyl-3-acetylcinnoline or derivatives thereof. These compounds were tested for the antibacterial activity showed less activity than ciprofloxacin.

Keywords: Schiff base, aryldiazonium salt, active methylene group, antibacterial activity.

INTRODUCTION

The aryldiazonium salt, Ar-$N_2^{\oplus}Cl^{\ominus}$ are highly reactive compounds. It also plays an important role 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 and uses in many practical applications such as colouring fibre, photoelectronic applications, printing systems, optical storage technology and in various analytical techniques[2]. The azo compounds also found their wide applications as a polymer additive[3], also the azo dye-additive was mainly 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 AMG containing compound viz. Pentane-2,4-dione or Acetyl acetone(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.

Diazonium Salts

Aromatic diazonium salts, represented by the general formula, Ar-$N_2^{\oplus}X^{\ominus}$, were highly reactive compounds and serves as intermediate in the synthesis of a wide variety of organic aromatic compounds. In fact, they were comparable to Grignard reagents in their versatility i.e ease of processing. They were regarded as salt of the aryldiazonium hydroxide, Ar-N≡$N^{\oplus}OH^{\ominus}$.

Method of Formation:

As described earlier, the aryldiazonium salts were commonly prepared by the diazotization of primary aromatic amines at low temperature in acidic solutions.

$$ArN^{\oplus}H_3Cl^{\ominus} \xrightarrow[0\ ^{\circ}C]{NaNO_2/HCl} Ar\text{-}N{\equiv}N^{\oplus}Cl^{\ominus}$$

Applications

The aryldiazonium compound showed a lot of reactions, few to mention here are as discussed below. The reactions, either, lost nitrogen containing function or without loss of nitrogen function.

a) With loss of N-function:

On Replacement by Bromine group

Replacement by Cyano group

b) Without loss of N-function:

Diazo coupling

Reaction with ester function

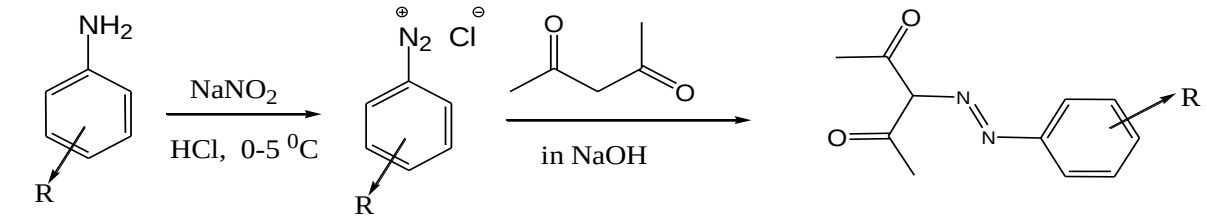
Reduction

First category included replacement by H, -OH, -Br, -F, -I, -CN, $-NO_2$, aryl- etc. and the latter involved reduction and diazo coupling type reaction. In the present piece of work the aryldiazonium salt was reacted with AMG containing compound, Pentane-2,4-dione viz. Acetyl acetone(AA).

Similar to sitosterol[11] and cholesterol etc. cinnolines were from the class of bioactive compounds due to their remarkable biological and pharmacological property[12]. Cinnoline and its derivatives also showed biological activities such as antihypertensive[13], bacteriocides[14] and analgesic[15] activity. This type of compound required the intermediates of the type (Phenylazo)-acetyl acetone or similar compounds. In view of synthesis of newer 4-Methyl-3-acetylcinnoline 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[16] and recently from our laboratory[17].

As seen from the above discussion, azo dyes were easy to prepare and very important for technical purposes in many types of industries. Also, our previous reports[17, 18] dealt with reactions involving aryldiazonium moiety. Herein we report the part-IV of this series which dealt with the synthesis of 3-(Substituted-phenylazo)-pentane-2,4-diones (**DSP-A** to **DSP-G**), also the antibacterial properties of these compounds were determined.

Scheme of Synthesis



Substituted aniline — Substituted diazonium salt — 3-(Substituted-phenylazo)-pentane-2,4-dione

R = -2-OCH_3(DSP-A); -3-OCH_3(DSP-B); -4-OCH_3(DSP-C); -2-F(DSP-D); -4-F(DSP-E); -3-Cl(DSP-F); -3,4-Cl_2(DSP-G)

In the present work the type of ketone compounds i.e the intermediates of the type (Phenylazo)-acetyl acetone, (**DSP-A** to **DSP-G**) and its varied derivatives were synthesized (**Scheme-I**). Review of literature indicated that such ketone derivatives were valuable synthones for the synthesis of 4-Methyl-3-acetylcinnoline or derivatives thereof.

MATERIALS AND METHODS

General

All the chemicals and solvents were obtained from E-Merck, India and were of synthesis and the Spectroscopic grade respectively. They were used without further purification. The reaction involved two steps and the reported procedure[18] was followed here. Silica gel-G was used to monitor the progress of reactions, by TLC and visualized by iodine vapour-chamber. The colour observed was recorded by visual method and melting point range was taken in one end open capillary tube. The purity of the compounds was ascertained by melting point range determination (in one end open capillary method), and by Silica gel-G TLC. The UV-Vis spectra were recorded on a Shimadzu-1800 instrument (wavelength, λ in nm). Quartz cuvette of path length 1 cm was used for measurements in solution. The FTIR spectra were recorded on a Shimadzu FTIR 8400 spectrophotometer (Model-IRAffinity-1) using sample mixed in powder form with KBr powder, the frequency values, '**ν**', are in cm^{-1}. The overall purity and structural assignment of the products was based respectively on the elemental (CHN) analyses, TLC and UV-Vis, FTIR spectral data. 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.

Stage-I. General Procedure for Preparation of Diazonium Salt, (DSP-A to DSP-G)

Charged 0.02 M aniline (or its derivative) in a beaker, the mixture of 10 ml con. 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 $NaNO_2$ 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 potassium-iodide 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-A to DSP-G): Added aryldiazonium salt solution (from **Stage-I**) slowly, to the well cooled mixture of, Pentane-2,4-dione viz. Acetyl acetone(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 con. 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-A** to **DSP-G**) was ascertained by TLC method and were 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[16].

To study the antibacterial activity of 3-(Substituted-phenylazo)-pentane-2,4-dione from Schiff bases, 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-A** to **DSP-G**) 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-A** to **DSP-G**) 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 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 (open capillary method), and by Silica gel-G TLC. 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**.

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

ID. No.	Aniline derivative	Active methylene Compound	Colour	Molecular Formula	*m.p. range °C
1	2-Methoxy-aniline	Pentane-2,4-dione	Wooden brown	$C_{12}H_{14}N_2O_3$	88-94
2	3-Methoxy-aniline	Pentane-2,4-dione	Purplish red	$C_{12}H_{14}N_2O_3$	62-70
3	4-Methoxy-aniline	Pentane-2,4-dione	Coffee brown	$C_{12}H_{14}N_2O_3$	158-164
4	2-Floro-aniline	Pentane-2,4-dione	Coffee brown	$C_{11}H_{11}N_2O_2$	76-80
5	4-Floro-aniline	Pentane-2,4-dione	Golden	$C_{11}H_{13}N_2O_2$	64-78
6	3-Chloro-aniline	Pentane-2,4-dione	Burnt umber	$C_{11}H_{11}N_2O_2$	180-198
7	3,4-Chloroaniline	Pentane-2,4-dione	Cadmium orange	$C_{11}H_{10}N_2O_2$	138-146

* Physical constants are uncorrected.

The photographic representation of synthesized organic compounds, viz. **DSP-A** to **DSP-G** is shown in **Fig. 1**.

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

The homogeneity of the synthesized compounds was carried out by the method of thin-layer chromatography using silica gel G as the stationary phase on glass plates of about 15 cm in height and hexane, methyl acetate as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. 0.1% w/v solution of synthesized compound was applied to **DSP-A** to **DSP-G** separately in hexane, methyl acetate. Develop the plate in hexane, methyl acetate and remove it and mark immediately the solvent front level, allow plate to dry in air and put it in the iodine chamber. The thin layer chromatogram showed only one spot for each compound.

The TLC of the synthesized compounds **DSP-A** to **DSP-G** was as shown (see **Fig. 2A and 2B**) in the following photograph. Thin layer chromatography indicated for the preparation of the final compound, **DSP-A** to **DSP-G**, compared with the starting raw material.

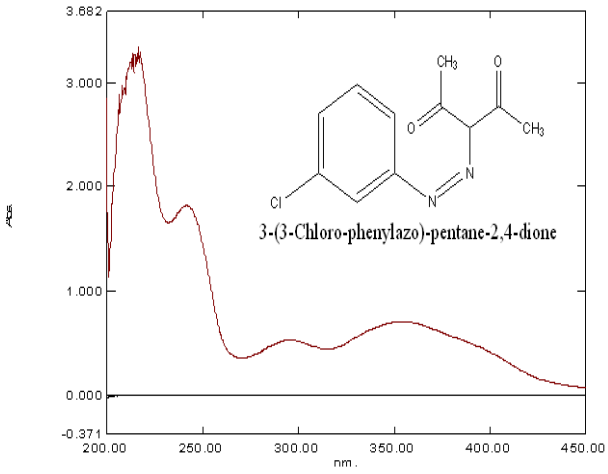


Figure 2: The representative UV-Vis spectra of DSP-A to DSP-G

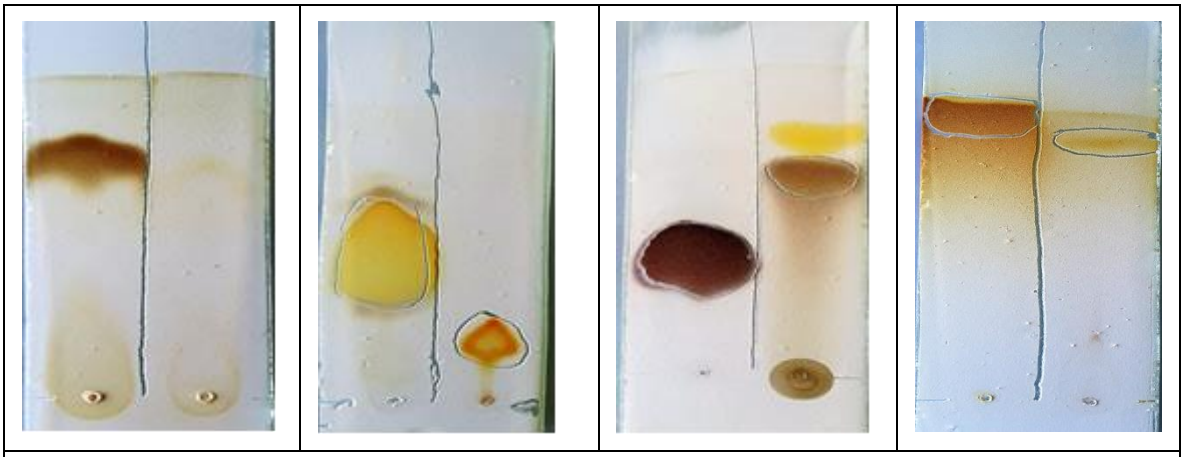


Figure 2A: TLC for 3-(substituted-phenylazo)-pentane-2,4-diones, (**DSP-A** to **DSP-D).**

Figure 2B: TLC for 3-(substituted-phenylazo)-pentane-2,4-diones, (**DSP-E** to **DSP-G).**

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→ π* and π–π* 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-A** to **DSP-G** 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 1665-1610 cm^{-1}, indicated presence of carbonyl group. The studied compounds showed absorption frequency in the range 1630-1563 cm^{-1}, indicated presence of Aromatic group >C=C<. The studied compounds showed absorption frequency in the range 3331-2831 cm^{-1}, indicated presence of C-H starching. The studied compounds showed absorption frequency in the range 1365-1308 cm^{-1}, indicated presence of C-N starching. The studied compounds showed absorption frequency in the range 1567-1497 cm^{-1}, indicated presence of –N=N- stretching. 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[21-22], the -N=N- vibration 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 1,630–1,575 cm^{-1} range confirmed the success of the synthesis[23].

The FITR spectra of the synthesized final products, **DSP-A** to **DSP-G** are depicted below in **Fig. 3a** to **3g**.

Antibacterial Activity: Antibacterial activity of all the synthesized compounds were screened against gram-negative bacteria, *E. coli* and *B. subtilis* for three different concentrations of 100, 500 and 1000 µg/ml, as per the method described in[19]. The results of antimicrobial testing as zone of inhibition (in mm) are depicted in **Table-4**.

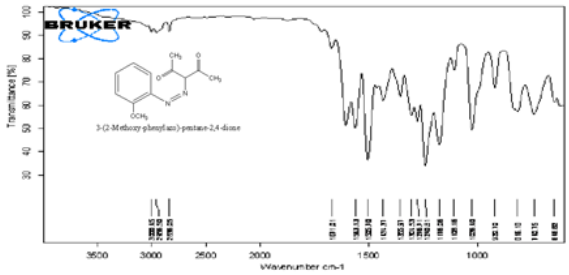


Figure 3a: FTIR Spectrum of 3-(2-Methoxy-phenylazo)-pentane-2,4-dione, **DSP-A.**

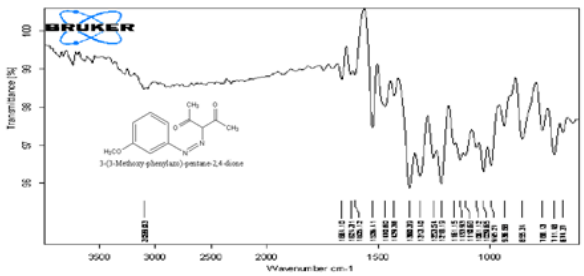


Figure 3b: FTIR Spectrum of 3-(3-Methoxy-phenylazo) pentane-2,4-dione, **DSP-B.**

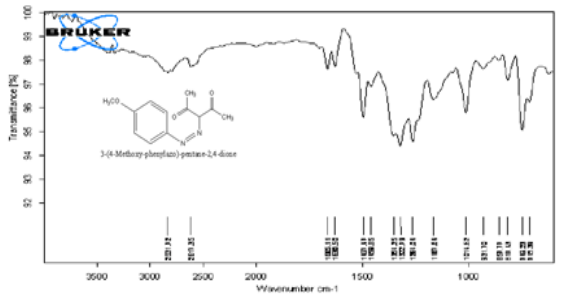


Figure 3c: FTIR Spectrum of 3-(4-Methoxy-phenylazo)-pentane-2,4-dione, **DSP-C.**

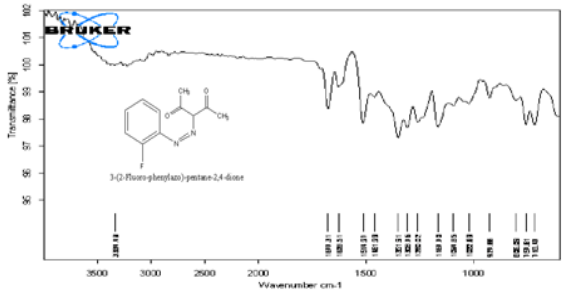


Figure 3d: FTIR Spectrum of 3-(2-Fluro-phenylazo)-pentane-2,4-dione, **DSP-D.**

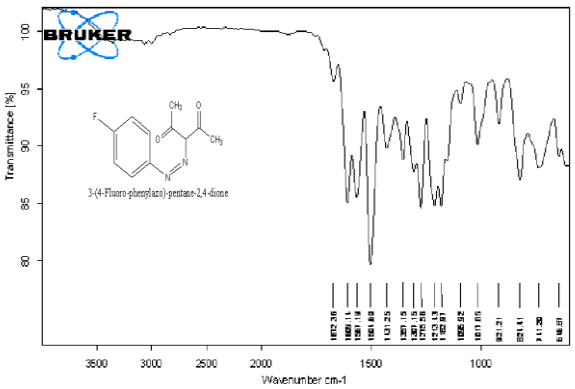


Figure 3e: FTIR Spectrum of 3-(4-Floro-phenylazo)-pentane-2,4-dione, **DSP-E.**

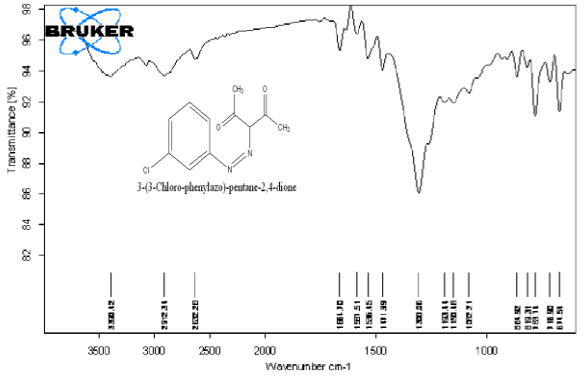


Figure 3f: FTIR Spectrum of 3-(3-Chloro-phenylazo)-pentane-2,4-dione, **DSP-F.**

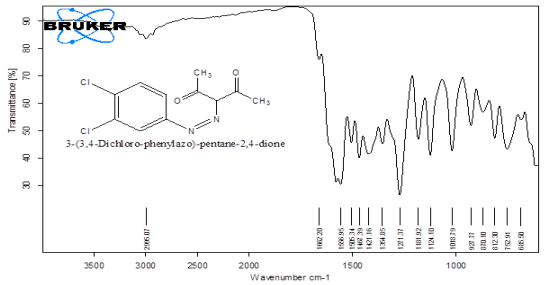
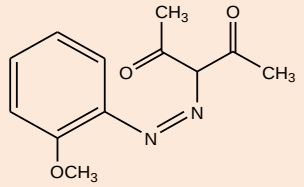
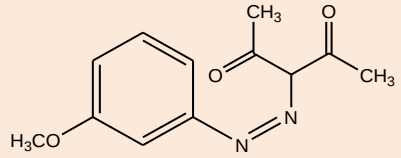
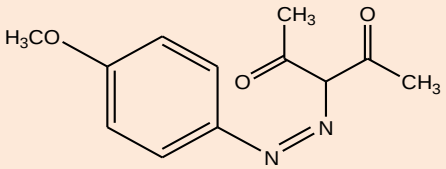
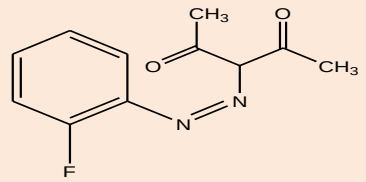
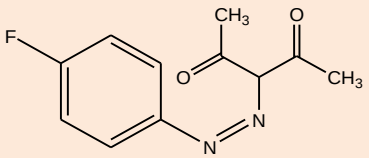
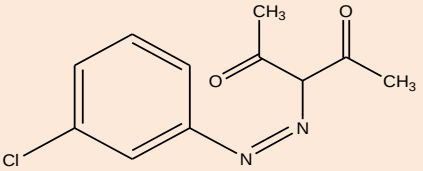
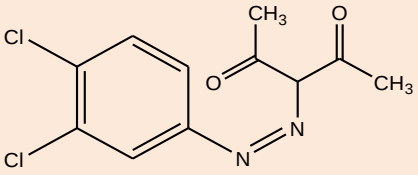


Figure 3g: FTIR Spectrum of 3-(3,4-Dichloro-phenylazo)-pentane-2,4-dione, **DSP-G.**

The related data of FTIR characteristic frequency (in cm^{-1}) of the groups indicated in the **Table-2**.

Table 2: Spectral Data for 3-(Substituted-phenylazo)-pentane-2,4-diones, (**DSP-A** to **DSP-G**).

Comp. ID	UV (nm)	IR (cm^{-1})	Assigned Structure
DSP-A	320.60 203.80	$\nu_{-C=O}$ =1611 $\nu_{-C=C-}$ =1563 ν_{-C-H} =3000 $\nu_{-C-N-(Aryl)}$ =1365 $\nu_{-N=N-}$ =1510 $\nu_{-C-OCH3}$ =740	 3-(2-Methoxy-phenylazo)-pentane-2,4-dione
DSP-B	397.00 244.60 206.60	$\nu_{-C=O}$ =1661 $\nu_{-C=C}$ =1605 ν_{-C-H} =3099 $\nu_{-C-N(Aryl)}$ =1330 $\nu_{-N=N-}$ =1536 $\nu_{-C-OCH3}$ =855	 3-(3-Methoxy-phenylazo)-pentane-2,4-dione
DSP-C	391.20 270.60 217.20	$\nu_{-C=O}$ = 1665 $\nu_{-C=C-}$ = 1630 ν_{-C-H} = 2831 $\nu_{-C-N-(Aryl)}$ = 1351 $\nu_{-N=N-}$ = 1497 $\nu_{-C-OCH3}$ = 851	 3-(4-Methoxy-phenylazo)-pentane-2,4-dione
DSP-D	362.80 209.60	$\nu_{-C=O}$ =1611 $\nu_{-C=C-}$ =1608 ν_{-C-H} =3331 $\nu_{-C-N-(Aryl)}$ =1351 ν-C-$F_{(o-)}$ =1461 $\nu_{-N=N-}$ =1514	 3-(2-Fluoro-phenylazo)-pentane-2,4-dione
DSP-E	320.40 226.80 203.80	$\nu_{-C=O}$ =1612 $\nu_{-C=C-}$ =1609 ν_{-C-H} =3020 $\nu_{-C-N-(Aryl)}$ =1351 $\nu_{-C-F(p-)}$ =1431 $\nu_{-N=N-}$ =1567	 3-(4-Fluoro-phenylazo)-pentane-2,4-dione
DSP-F	355.20 241.20 216.20	$\nu_{-C=O}$ =1661 $\nu_{-C=C-}$ =1581 ν_{-C-H} =3330 $\nu_{-C-N-(Aryl)}$ =1308 $\nu_{-C-Cl(m-)}$ =711 $\nu_{-N=N-}$ =1536	 3-(3-Chloro-phenylazo)-pentane-2,4-dione
DSP-G	350.00 240.80 220.00	$\nu_{-C=O}$ =1662 $\nu_{-C=C-}$ =1566 ν_{-C-H} =2996 $\nu_{-C-N-(Aryl)}$ =1354 ν_{-C-Cl} =752,685 $\nu_{-N=N-}$ =1505	 3-(3,4-Dichloro-phenylazo)-pentane-2,4-dione

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 3: The solubility data for the 3-(Substituted-phenylazo)-pentane-2,4-diones, (DSP-A to DSP-G).

Sr. No.	Compound ID	Solvent used(in ml/0.1 gm of substance)		
		Ethyl alcohol	Diethyl ether	Acetone
1	DSP-A*	25	50**	-
2	DSP-B***	20	40**	30
3	DSP-C	5	16	10
4	DSP-D**	15	25*	15*
5	DSP-E	3	7	15
6	DSP-F	2	15*	30*
7	DSP-G	5	9	15

*** Methanol 14 ml required; ** Insoluble and coagulated and *** Benzene 2.5 ml required.**

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

Strain used ↓	ID of Compound																				
	Ciprofloxacin (Std. Drug, +ve control)	DSP-A			DSP-B			DSP-C			DSP-D			DSP-E			DSP-F			DSP-G		
Concentration	-	100	500	1000	100	500	1000	100	500	1000	100	500	1000	100	500	1000	100	500	1000	100	500	1000
E-Coli	12	0	6	6	0	0	6	0	0	7	0	0	0	7	7	8	0	6	6	6	7	7
B-Subtilis	18	0	0	0	0	6	7	6	7	7	0	0	0	6	6	7	0	7	7	0	0	0
Distilled Water (-ve control)	**0**																					

The graphical representation of the antibacterial activity is depicted in **Fig. 4**.

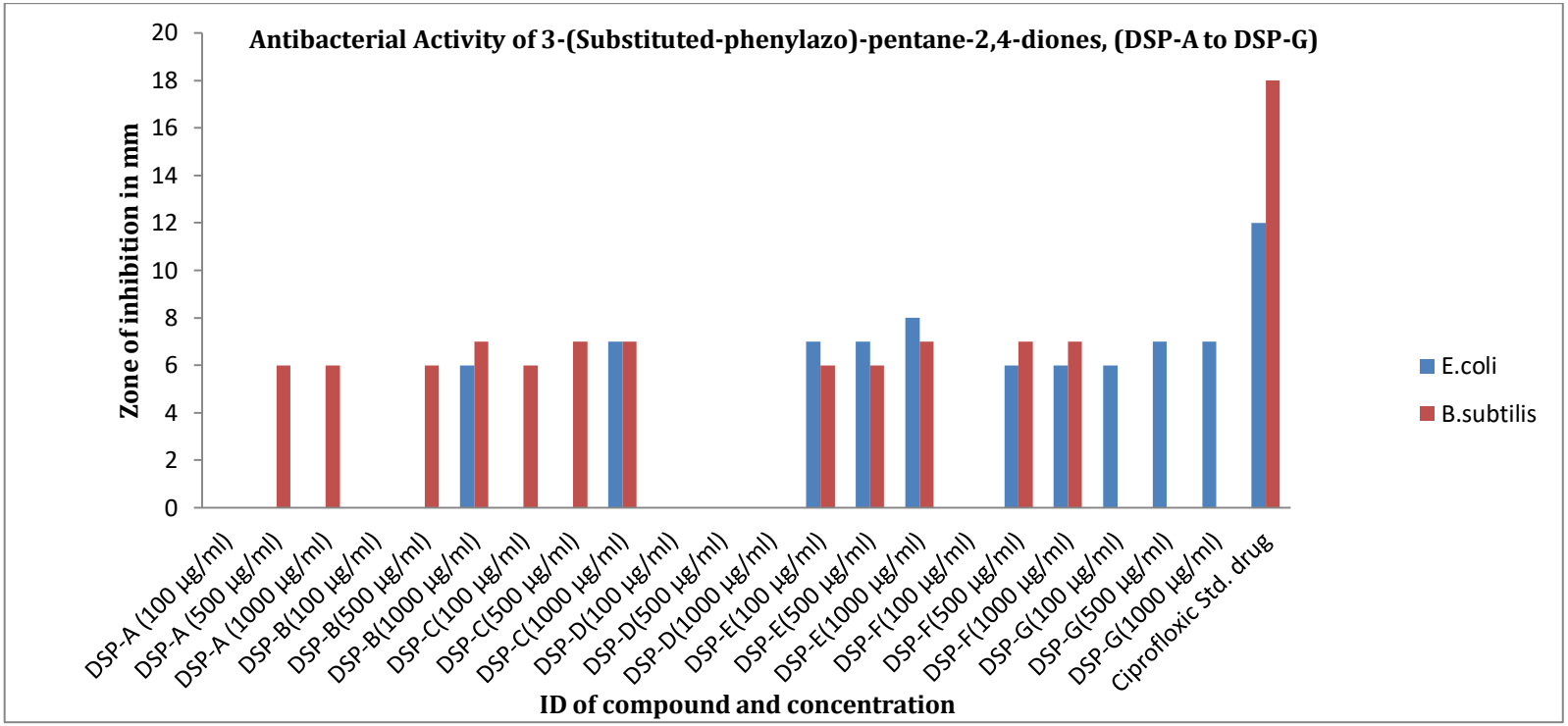


Figure 4: The histographic representation of antibacterial activity of Synthesized compound, DSP-A to DSP-G.

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

Glimpses drawn from the Antifungal activities of the studied Thiazolidin-4-ones were as...

- ✓ All the compounds were active for *E. coli* and *B. subtilis* and showed less activity than the standard drug, Ciprofloxacin.
- ✓ For 100 µg/ml concentration there was no antibacterial activity, except for **DSP-E** and **DSP-G** for *E. coli* and **DSP-C** for *B. subtilis*.
- ✓ Compound **DSP-A** was active against *E. coli* (500 and 1000 µg/ml) and not for *B. subtilis*.
- ✓ Compound **DSP-B** was active against *E. coli* (1000 µg/ml) and also for *B. subtilis* (500 and 1000 µg/ml).
- ✓ Compound **DSP-C** was active against *E. coli* (1000 µg/ml) and also for *B. subtilis* (100, 500 and 1000 µg/ml).
- ✓ Compound **DSP-D** was inactive against *E. coli* as well as *B. subtilis* at all the studied concentration.
- ✓ Compound **DSP-E** was active against *E. oli* as well as *B. subtilis* at all the studied concentration, but the activity is less than the standard drug.
- ✓ Compound **DSP-F** was active against *E. coli* (500 and 1000 µg/ml) and also for *B. subtilis* (500 and 1000 µg/ml).
- ✓ Compound **DSP-G** was active only against *E. coli* and not against *B. subtilis*.

CONCLUSION

Aniline and substituted anilines were used for the preparation of diazoninium salt then they were reacted upon with Pentane-2, 4-dione (Acetyl acetone) to give or derivatives thereof. These compounds will be useful as building block for organic researchers in the near future.

Acknowledgements: The authors are thankful to Chairman, The Muktainagar Taluka Education Society, Muktainagar for continuous encouragement and appreciation of our work and Principal, Smt. G. G. Khadse College, Muktainagar.

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Source of Support: Nil, **Conflict of Interest:** None.

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