



Coupling reactions involving aryldiazonium salt: Part-I. Chemoselective condensation to synthesize 2-(phenylhydrazono)-3-oxo-butyric acid ethylester, its derivatives and their antibacterial activity

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ABSTRACT

The aryldiazonium salt $Ar-N_2^{\oplus}Cl^{\ominus}$ are highly reactive compounds. It is used as an intermediate in the different reactions. These reactions, either, losses nitrogen containing function or without loss of nitrogen function. First category includes replacement by H, -OH, -Br, -F, -I, -CN, -NO₂, Aryl- etc. and the latter involves reduction and diazo coupling type reaction. In the present piece of work we have coupled the aryldiazonium salt with active methylene group(AMG) bearing moiety, β -keto ester viz. Ethyl acetoacetate(EAA). The final product formed has potential to use in some organic reaction to prepare derivatives of benzodiazepines. These compounds were tested for the antibacterial activity against *E. coli* when compared with ampicillin as standard drug.

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

INTRODUCTION

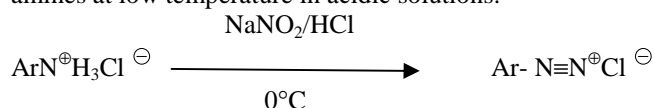
There are many classes of compounds in organic chemistry like carbonyl compounds[1] viz. aldehyde[2] and ketone[3] and imine[4] compounds etc likewise aryldiazonium compounds also plays an important role in synthetic organic chemistry. There are many chemical reactions like reduction[5], oxidation[6], condensation[7], hydrolysis[8], complexation[9] and coupling[10] etc. In the present piece of work we have reacted the aryldiazonium salt, coupled with AMG bearing compound like EAA. These synthesized compounds will be useful for varied applications viz. for synthesis of newer chemicals and pharmaceuticals.

Diazonium Salts

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

Method of Formation:

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



Applications:

The aryldiazonium compound shows a lot of reactions, few to mention here are as discussed below. The reactions, either, losses 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

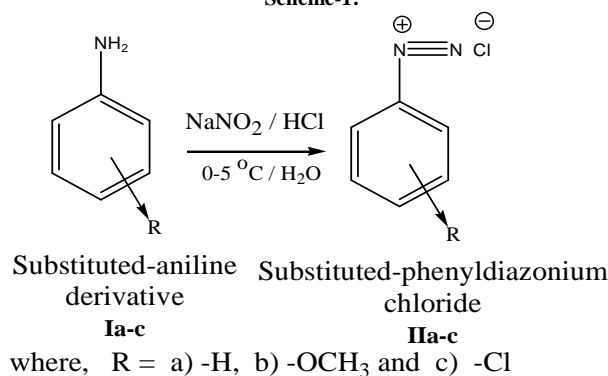
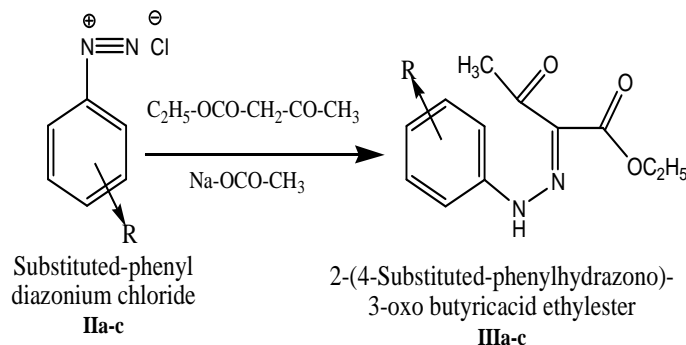
Reaction with ester function

Reduction

First category includes replacement by H, -OH, -Br, -F, -I, -CN, -NO₂, Aryl- etc. and the latter involves reduction and diazo coupling types reaction. In the present piece of work the aryldiazonium salt was reacted with AMG containing compound β -keto ester viz. EAA.

Similar to Sitosterol[11] and Cholesterol etc. Benzodiazepines are widely used class of bioactive compounds due to their remarkable biological and pharmacological property[12-13]. This type of compound requires the intermediates of the type 2-(Phenylhydrazono)-3-oxo-butyricacid ethylester, **IIIa** and its varied derivatives, **IIIb-c**. Such ester derivatives are valuable synthones for the synthesis of oxazino oxadiazolo, triazolo and furano-benzodiazepine[14-16]. In view of synthesis of newer Benzodiazepines and Benzodiazepines of importance, their intermediates are considered worthwhile to study their synthesis. Similar efforts to prepare these type of compounds reported by Mittal and Singhal[17], they have also prepared derivatives using variable in the ester molecule (CH₃-CO-CH₂-CO-OC₂H₅) and (Ph-CO-CH₂-CO-OC₂H₅).

In the present work the type of ester compounds i.e the intermediates of the type 2-(Phenylhydrazono)-3-oxo-butyricacid ethylester and its varied derivatives are prepared(**Scheme-1 and -2**). Review of literature indicated that these ester derivatives are valuable synthones for the synthesis of Benzodiazepines.

Scheme-1:**Scheme-2:**

where, R = a) -H, b) -OCH₃ and c) -Cl

MATERIALS AND METHODS

General: All the chemicals and solvents were obtained from E-Merck, India and are of synthesis and the Spectroscopic grade respectively. They were used without further purification. 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 an 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.

Stage-I. General Procedure for Preparation of Diazonium Salt, **IIa-c:** In a 100 ml capacity beaker add 0.02 M aniline (or its derivative), **IIa-c** and to this add to it mixture of 10 ml con. HCl and 5 ml water and stir with the glass rod to get clear solution, cool the solution to 0°C by keeping in an ice bath. Dissolve (0.025 M) sodium nitrite in 8 ml water. Cool the solution in ice bath to 0°C , after attaining 0°C add sodium nitrite solution into aniline hydrochloride(or derivative's) solution dropwise with constant stirring(Do not allow to rise temperature above 5°C during addition). The reaction mixture shows the positive test of nitrous acid on starch-iodide paper (blue colour is obtained on the potassium-iodide starch paper). Decompose the excess of nitrous acid by adding pinch of urea filter the solution and collect the filtrate which is diazonium salt solution.

Stage-II. General Procedure for Reaction of Diazonium Salt with β -Ketoester, (EAA), **IIIa-c:** Add above prepared diazonium salt solution, **IIa-c** slowly to well cooled mixture of EAA(0.018 M) dissolved in 2.5 ml ethanol and NaOAc, 8-10 gm in 4-5 ml of water(to keep the mixture alkaline to litmus), coloured precipitate is separated then add 20 ml of con. HCl then filter it, check absence of ester by a negative qualitative test and the product, **IIIa-c** obtained is recrystallized by using solvent ethanol. Record the dried weight and the colour with physical constant i.e m.p. range of the compound.

The synthesized compounds were screened for the antibacterial activity against *E. coli* for two different concentration of 50 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$, as per the method described in literature[4] and compared with ampicillin as standard drug.

RESULTS AND DISCUSSION

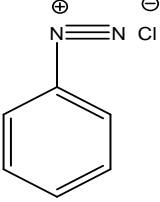
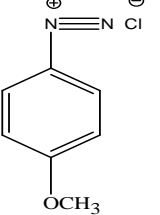
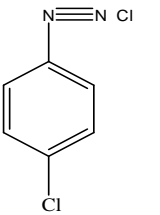
In the present study, diazonium intermediates of aniline and substituted anilines are synthesized, reacted with active methylene compound (β -Ketoester) and screened for antibacterial activity. All the compounds were obtained in high purity. The progress of reactions was monitored by Silica gel-G TLC, 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 are in close agreement with the structures of the synthesized compounds. All compounds gave satisfactory elemental analysis. The elemental analysis values are in the close agreement with the calculated values for expected molecular formulae assigned to these compounds and are in 5 % in statistics.

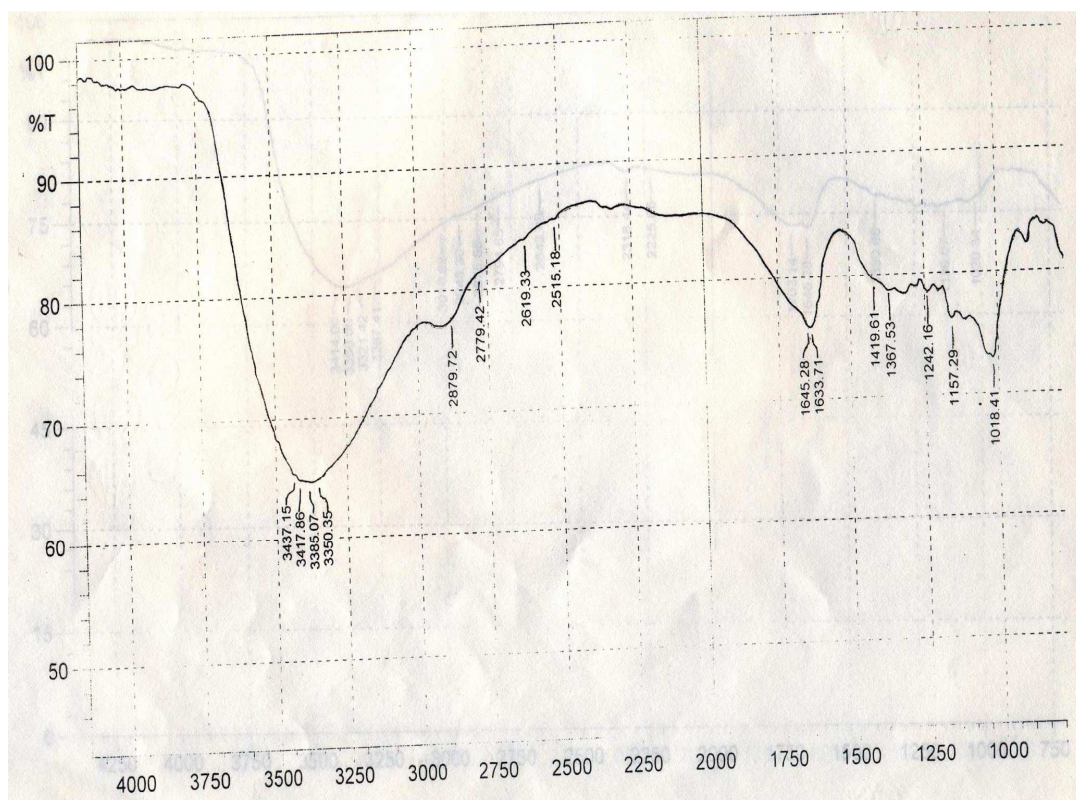
The data showing result of synthesis study and their physical constants, % practical yield and structure of intermediate are depicted in the **Table-1**.

The TLC on completing the synthesis of the final compounds, in the mobile phase of Toluene: Ethyl acetate (9:9:0.1), along with that of the starting raw material, indicated single spot, shows the purity of the compound.

The FTIR spectra of the synthesized final products, **IIIa-c** are depicted below in Fig. **1A** to **1C**. The related data of FTIR characteristic frequency (in cm^{-1}) of the groups indicated in the **Table-2**.

Table-1: The Structures of the Reactant(Ia-c), Intermediate(IIa-c) involved and the Physical Constant(m. p. range °C) and % Practical yield of the products obtained

Sr. No.	Starting Material	Structure of Intermediate and its Name with ID	Physical Constant of Product, m. p. range °C	% Practical Yield of Product
1	Aniline, Ia	 <p>Phenyl diazonium chloride, IIa</p>	114-116	73.64
2	4-Methoxy Aniline, Ib	 <p>4-Methoxyphenyl diazonium chloride, IIb</p>	117-119	60.84
3	4-Chloro Aniline, Ic	 <p>4-Chlorophenyl diazonium chloride, IIc</p>	112-113	60.96

Fig.1A: FTIR spectra for the 2-(Phenylhydrazono)-3-oxo-butyracacid ethylester, **III₁** (P1)

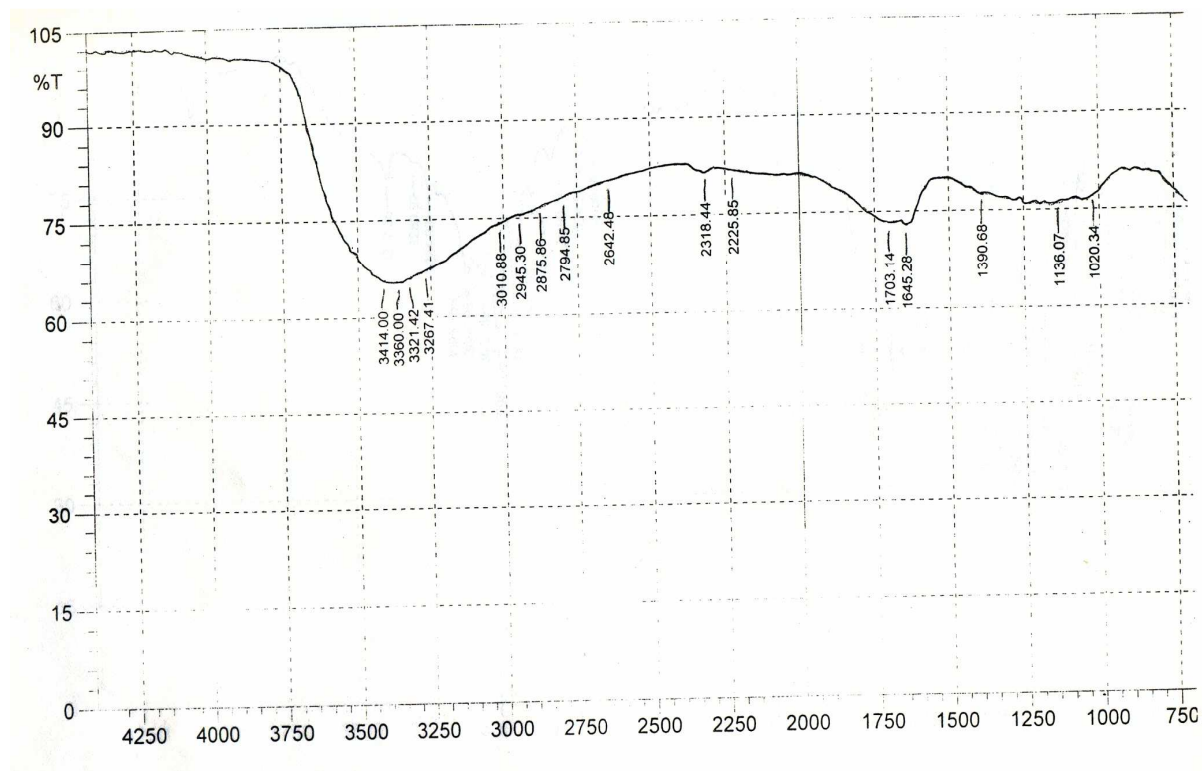


Fig.1B: FTIR spectra for the 2-(4-Methoxyphenylhydrazono)-3-oxo-butiric acid ethylester, III_b (P2)

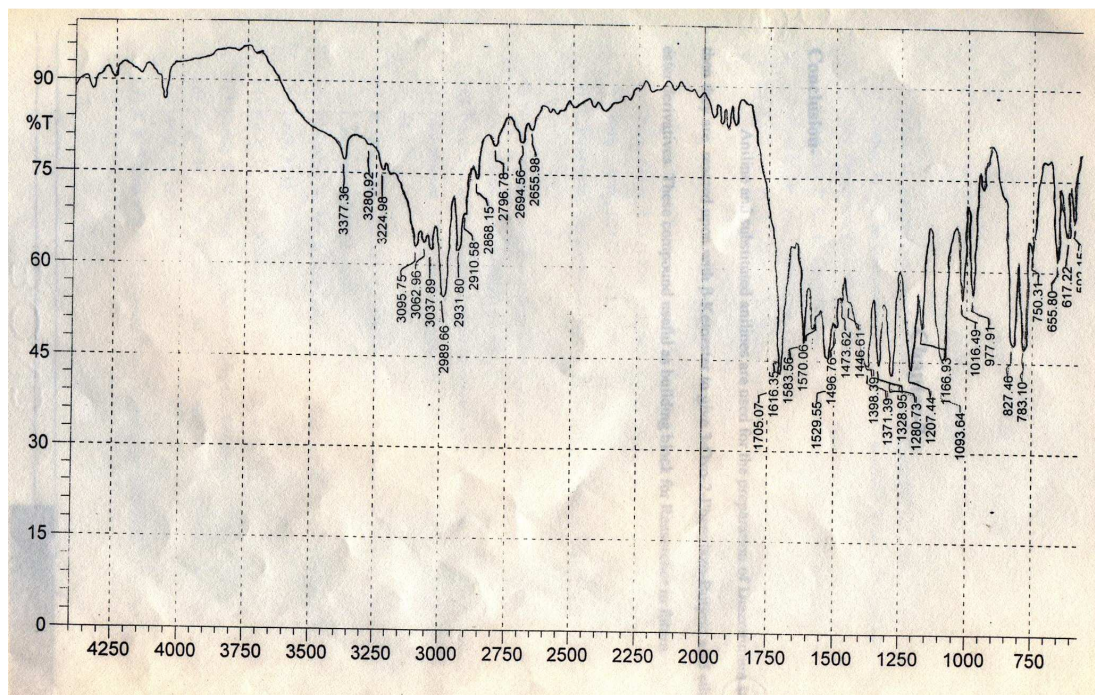
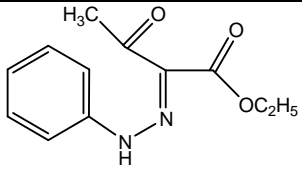
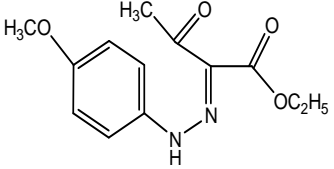
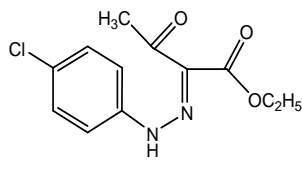


Fig.1C: FTIR spectra for the 2-(4-Chlorophenylhydrazono)-3-oxo-butiric acid ethylester, III_c (P3)

Table-2. The Characteristic FTIR Spectral data of the synthesized compounds, IIIa-c

Sr. No.	Name of the Compound/ MF	Spectral Absorptions (cm ⁻¹)	Structure assigned to Product with Name and ID
IIIa	2-Phenylhydrazono-3-oxo-butyracacid ethylester C ₁₂ H ₁₄ N ₂ O ₃	ν-N-H = 3350-3385 ν-C-H of Ar = 2879 ν-OCH ₂ - = 2779 ν>C=O = 1645 (including ester) ν>C=N- = 1633 ν-CH ₃ , -CH ₂ - = 1367	 <p>2-Phenylhydrazono-3-oxo butyric acid ethylester IIIa</p>
IIIb	2-(4-Methoxy-phenylhydrazono)-3-oxo butyric acid ethylester C ₁₃ H ₁₆ N ₂ O ₄	ν-N-H = 3320-3360 ν-C-H of Ar = 3010 ν-OCH ₂ - = 2794 ν>C=O = 1703 (including ester) ν>C=N- = 1645 ν-CH ₃ , -CH ₂ - = 2945, 1367	 <p>2-(4-Methoxy-phenylhydrazono)-3-oxo butyric acid ethylester IIIb</p>
IIIc	2-(4-Chloro-phenylhydrazono)-3-oxo butyric acid ethylester C ₁₂ H ₁₃ N ₂ ClO ₃	ν-N-H = 3280-3377 ν-C-H of Ar = 3037-3095 ν-OCH ₂ - = 2868 ν>C=O = 1705 (including ester) ν>C=N- = 1616 ν-CH ₃ , -CH ₂ - = 2931, 1371	 <p>2-(4-Chloro-phenylhydrazono)-3-oxo butyric acid ethylester IIIc</p>

Thus the ester compounds viz. 2-(Phenylhydrazono)-3-oxo-butyracacid ethylester and its varied derivatives are prepared and characterized by TLC, physical constant and FTIR spectra and are now available for the further use.

The UV and Visible absorption of the compounds under investigation display mainly three bands observed in ethanol within the range 200-600 nm. The first band in the range 230-255 nm was assigned to the moderate energy $\pi \rightarrow \pi^*$ transition of the aromatic ring, while the second shoulder peak band was observed in the range 310-320 nm(shoulder peak) and the third long wavelength band in the range 450-530 nm was assigned to the $\pi \rightarrow \pi^*$ transition of azo group and the overall effect of methoxy and chloro auxochromes, in analogy to the case of simple azo compound[18].

These structures of were confirmed by elemental analysis and IR spectra showing an absorption band at 1616-1645 cm⁻¹ (C=N), 2879-3095 cm⁻¹ (C-H of Ar.), 2779-2875 cm⁻¹ (-OCH₂), 2931-2945, 1367-1391 cm⁻¹ (-CH₃, -CH₂-), 1645-1703 cm⁻¹ (C=O).

The synthesized compounds were tested for the antimicrobial activity in order to determine their potential application in microbial chemotherapy. The antimicrobial result indicated that all the synthesized compounds found moderately active against *E. coli* on comparison with ampicillin, as standard antibacterial agents.

CONCLUSION

Aniline or substituted aniline, **Ia-c** are used for the preparation of diazonium salt(solution form), **IIa-c** then they are reacted upon with β -Ketoester to give 2-(Substituted-phenylhydrazono)-3-oxo-butyracacid ethyl ester derivatives, **IIIa-c**. These compounds will be useful as synthone or building block by organic researchers in the near future also the compounds shows moderate antibacterial activity when compared to standard drug ampicillin.

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