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Synthesis and Characterization of Azo Dyes Incorporating Coumarin Moiety by Betti's Protocol

P. S. Jogi[1], S. S. Shende[2] and J. S. Meshram[3]
[1] Janata Mahavidyalaya, Chandrapur (Ms)-India
[2]Netaji Subhashchandra Science College, Mulchera (Ms)-India
[3]School of Chemical Sciences, NMU, Jalgaon

Abstract:

Coumarins, an old class of compounds, are naturally occurring benzopyrene derivatives. A lot of coumarins have been identified from natural sources, especially green plants. The pharmacological, biochemical and therapeutic applications of simple coumarins depend upon the pattern of substitution. Coumarins have attracted intense interest in recent years because of their diverse pharmacological properties. In this review, a simple and interesting Betti's protocol was used to prepare novel derivatives of coumarin which on diazotization yield new biologically active azo dyes. New azo dyes were obtained by coupling between diazotized aromatic amines and betti's base which were obtained by multi-component reaction of substituted coumarin, benzaldehyde and ammonia. The structure of all azo dyes were confirmed by various physical techniques like 1-H NMR, C-13-NMR, Mass and IR and tested against bacterial strain for biological activity.

Keywords: Betti's reaction, Multi component reaction, Anti-fungal

Introduction:

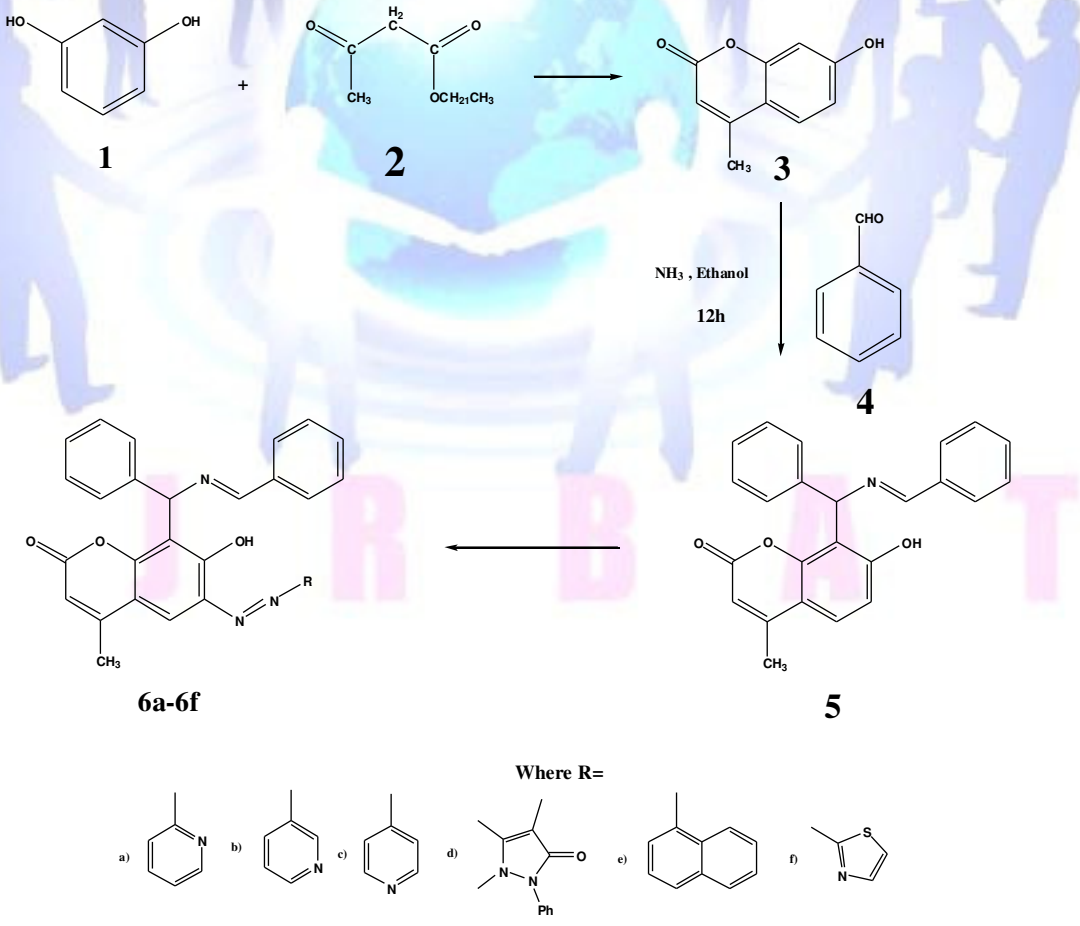
Coumarins are the simplest heterocyclic compounds that naturally occur. A lot of coumarins and their derivatives have been identified from natural sources, especially in the green plants. They are used as additives in foods, perfumes, cigarettes, cosmetics, pharmaceuticals, for optical brighteners, in fluorescent and laser dyes [1, 2]. The synthesis of coumarin dyes has attracted the attention of chemists for many years, as a large number of natural products contain this heterocyclic nucleus. These dyes are also exploited in chemical, biochemical, physical and pharmaceutical applications. The coumarin is not fluorescent, but the introduction of an electron-withdrawing group such as a diazotized aromatic amine or an acetyl group makes it highly fluorescent. The coumarins are generally very convenient compounds for chemical modification due to the ease of synthesis [3]. Literature survey reveals the medicinal importance of coumarins derivatives, which cover the whole gamut of chemotherapeutic agents. Azo compounds play a prominent part in almost every type of application. Azo compounds are the most important class of synthetic colouring materials. The chemistry of azoic dyes has been given much attention by Dorman [4]. There are azo dyes available for the dyeing of cotton, wool, silk, linen, viscose rayon, distemper, printing ink etc. Azo dyes are also available for colouring foodstuffs and a few have valuable medicinal properties. Azo compounds came to prominence in medicinal chemistry after the discovery that sulphanilamide, the active metabolite of the azo dye ProntosilRubrum, had in vivo antibacterial properties. Despite being superseded by the penicillin antibiotics in most antimicrobial applications sulfa drugs such as sulphasalazine still find application in the treatment of Crohn's disease and ulcerative colitis [5].

Materials and Methods

Materials: The solvents and reagents used in the synthetic work were ofanalytical grade obtained from Qualigens India and were purified by distillation or crystallization where necessary and their boiling or melting points were compared with the available literature values. Melting points were determined in open capillaries and are uncorrected.

Apparatus: Compounds were examined by FTIR (KBr pellets) ona Jasco FT-IR 420 spectrometer. ^{1}H and ^{13}C NMR spectra of compound**s** were recorded on a BrukerAvance 400 spectrometeroperating at 400 and 100 MHz, respectively withTMS as internal standard. Massspectra (CI) were performed on a TSQ7000 spectrometerfrom ThermoQuest using DCI/NH3 as the ionization mode(positive mode).

Synthesis: 7-Hydroxy-4-methylcoumarin was prepared in good yield using Pechmann procedure by condensation of ethylacetoacetate with equimolar amount of resorcinol in presence of conc. H_2SO_4 [6]. Novel derivative of 7-Hydroxy-4-methylcoumarin was prepared by multicomponent reaction of 7-Hydroxy-4-methylcoumarin, aromatic aldehydes and ammonia following Betti's protocol. All the six new azo dyes were prepared by following usual processor [7] by coupling of coumarin derivative with diazotized six aromatic amines (Sceme-1)



Scheme-1

In vitro antimicrobial screening

For biological screening, the agar cup plate method using Hi-Media agar medium was employed to study the antibacterial activity of **a-f** against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli*. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Each test compound (50 mg) was dissolved in dimethylformamide (50 mL, 1000 mg/mL), which was used as sample solution. Sample size for all the compounds was fixed at 0.1 mL. Using a sterilized cork borer, cups were scooped out of Agar. Medium contained in a Petri dish which was previously inoculated with the microorganisms. The test compound solution (0.1 mL) was added in the cups and the Petri dishes were subsequently incubated at 37 °C for 48 h. Ampicillin and Streptomycin were used as reference drugs and dimethylformamide as a negative control. Zones of inhibition produced by each compound were measured in millimeters, and the results are listed in Table 1.

Characterisation of synthesized compounds:

Characterisation of Coumarin derivative (4) (E)-8-((benzylideneamino)(phenyl)methyl)-7-hydroxy-4-methyl-2H-chromen-2-one:- Colour: yellow powder. Yield 91%.Mp= 105-107 ◦ C. IR (KBr, cm-1): 1090 (C-O str.), 1715 (lactone, C=O str.), 1750 (C=N str.), 2958 (-CH3 str.), 3015 (Ar-H str.), 3250 (-OH str.). 1H NMR (DMSOd6, ppm): 2.45 (s, 3H, CH3), 6.15-7.25 (m, 13H, Ar-H), 6.45 (s, 1H, vinylic -CH), 8.50 (s, 1H, CH-N), 13.45 (s, 1H, OH). 13C NMR (DMSO-d6, ppm): 125.0 (C1-coumarin),155.4 (C2-coumarin), 115.6 (C3-coumarin), 123.4 (C4-coumarin), 115.7 (C5-coumarin), 155.8(C6- coumarin), 160.0 (C7-coumarin), 112.5 (C8-coumarin), 152.6 (C9-coumarin), 20.4 (C10-coumarin), 160.8 (C=N, Azomethine), 50.8 (C, Methine), 143.6 (C1, Phenyl), 128.2 (C2, Phenyl), 129.2 (C3, Phenyl), 126.2 (C4, Phenyl), 129.2 (C5, Phenyl), 128.2 (C6, Phenyl),136.4 (C1, Phenyl), 129.2 (C2, Phenyl), 128.8 (C3, Phenyl), 131.0 (C4, Phenyl), 128.8 (C5, Phenyl),129.2 (C6, Phenyl). MS (C24H19NO3): m/z 369 (Mþ, 100%). Elemental analysis: calcd. (found): C, 78.03 (78.08), H, 5.18 (5.20), N, 3.79 (3.82).

Characterisation of azo dyes (a-f)

a.8-(((E)-benzylideneamino)(phenyl)methyl)-7-hydroxy-4-methyl-6-(pyridin-4-yldiazenyl)-2H-chromen-2-one :-Colour: Dark Red. Yield 71 %.Mp= 140 ◦ C. *IR (KBr, cm-1):* 3488 (-OH str.), 3216 (Ar-H str.), 2902 (–CH_3 str.), 1671 (lactone, C=O str.), 1641 (C=N str.), 1080 (C-O str.), 1575 (N=N str.). *^{1}H NMR (DMSOd6, ppm):* 2.31(s, 3H, CH_3), 5.87-7.60 (m, 15H, Ar-H), 5.08 (s, 1H, C-H), 8.01 (s, 1H, CH=N), 10.14 (s, 1H, OH). *^{13}C NMR (DMSO-d6, ppm):* 157.8 (C1-coumarin),115.5 (C2-coumarin), 151.9 (C3-coumarin), 122.2 (C4-coumarin), 98.8 (C5-coumarin), 156.8(C6- coumarin), 121 (C7-coumarin), 113.9 (C8-coumarin), 151.4 (C9-coumarin), 20.1(C10-coumarin), 160.9 (C=N, Azomethine), 61.4 (C, Methine), 113.0-159.7 (C-Aromatic)**.** MS (C24H19NO3): m/z 474 (Mþ, 100%). Elemental analysis: calcd. (found): C, 73.33(73.03), H, 4.63 (4.52), N, 11.80 (11.78).

b.8-(((E)-benzylideneamino)(phenyl)methyl)-7-hydroxy-4-methyl-6-(pyridin-3-yldiazenyl)-2H-chromen-2-one :- Colour: Redish brown. Yield 83%. Mp= 153 ◦ C. *IR (KBr, cm-1):* 3398 (-OH str.), 3152 (Ar-H str.), 2930 (–CH_3 str.), 1671 (lactone,

C=O str.), 1648 (C=N str.), 1080 (C-O str.), 1570 (N=N str.). *^{1}H NMR (DMSOd6, ppm):* 2.49(s, 3H, CH_3), 5.17-8.0 (m, 15H, Ar-H), 5.22 (s, 1H, C-H), 8.51 (s, 1H, CH=N), 10.23 (s, 1H, OH). *^{13}C NMR (DMSO-d6, ppm):* 160.8 (C1-coumarin),112.5 (C2-coumarin), 152.7 (C3-coumarin), 124.2 (C4-coumarin), 98.3 (C5-coumarin), 157.4(C6- coumarin), 123 (C7-coumarin), 114.1 (C8-coumarin), 151.0 (C9-coumarin), 19.4(C10-coumarin), 160.8 (C=N, Azomethine), 62.2 (C, Methine), 113.0-159.7 (C-Aromatic).MS (C24H19NO3): m/z 474 (Mþ, 100%). Elemental analysis: calcd. (found): C, 73.33(73.23), H, 4.63 (4.59), N, 11.80 (11.79).

c.8-(((E)-benzylideneamino)(phenyl)methyl)-7-hydroxy-4-methyl-6-(pyridin-2-yldiazenyl)-2H-chromen-2-one:- Colour: Reddish brown. Yield 74%.Mp= 144 ◦ C. *IR (KBr, cm-1):* 3495 (-OH str.), 3161 (Ar-H str.), 2933 (–CH_3 str.), 1674 (lactone, C=O str.), 1643 (C=N str.), 1090 (C-O str.), 1588 (N=N str.). *^{1}H NMR (DMSOd6, ppm):* 2.39(s, 3H, CH_3), 5.97-7.65 (m, 15H, Ar-H), 5.10 (s, 1H, C-H), 8.21 (s, 1H, CH=N), 9.14 (s, 1H, OH). *^{13}C NMR (DMSO-d6, ppm):* 160.8 (C1-coumarin),112.5 (C2-coumarin), 152.7 (C3-coumarin), 124.2 (C4-coumarin), 98.3 (C5-coumarin), 157.4(C6- coumarin), 123 (C7-coumarin), 114.1 (C8-coumarin), 151.0 (C9-coumarin), 19.4(C10-coumarin), 160.8 (C=N, Azomethine), 62.2 (C, Methine), 113.0-164.1 (C-Aromatic) MS (C24H19NO3): m/z 474(Mþ, 100%). Elemental analysis: calcd. (found): C, 73.33(73.25), H, 4.63 (4.60), N, 11.80 (11.74).

d. 1-((E)-(8-(((E)-benzylideneamino)(phenyl)methyl)-7-hydroxy-4-methyl-2-oxo-2H-chromen-6-yl)diazenyl)-5-methyl-2-phenyl-1H-pyrazol-3(2H)-one :- Colour: Brownish. Yield 89 %.Mp= 132 ◦ C. *IR (KBr, cm-1):* 3442 (-OH str.), 3200 (Ar-H str.), 2896 (–CH_3 str.), 1659 (lactone, C=O str.), 1636 (C=N str.), 1069 (C-O str.), 1566 (N=N str.). *^{1}H NMR (DMSOd6, ppm):* 2.30(s, 3H, CH_3), 6.08-7.60 (m, 15H, Ar-H), 5.08 (s, 1H, C-H), 8.11 (s, 1H, CH=N), 09.14 (s, 1H, OH). *^{13}C NMR (DMSO-d6, ppm):* 160.8 (C1-coumarin),112.5 (C2-coumarin), 152.7 (C3-coumarin), 116 (C4-coumarin), 120.5 (C5-coumarin), 149.2(C6- coumarin), 123.1 (C7-coumarin), 113.9 (C8-coumarin), 147 (C9-coumarin), 19.3(C10-coumarin), 160.8 (C=N, Azomethine), 62.8 (C, Methine), 126.2-166.2 (C-Aromatic).MS ($C_{35}H_{29}N_5O_4$): m/z 583 (Mþ, 100%). Elemental analysis: calcd. (found): C, 71.96(71.90), H, 4.96 (4.85), N, 11.99 (11.94).

e. 8-(((E)-benzylideneamino)(phenyl)methyl)-7-hydroxy-4-methyl-6-((E)-naphthalen-1-yldiazenyl)-2H-chromen-2-one:- Colour: Red. Yield 63 %.Mp= 127 ◦ C. *IR (KBr, cm-1):* 3369 (-OH str.), 3225 (Ar-H str.), 2876 (–CH_3 str.), 1650 (lactone, C=O str.), 1625 (C=N str.), 1070 (C-O str.), 1581 (N=N str.). *^{1}H NMR (DMSOd6, ppm):* 2.20(s, 3H, CH_3), 6.28-7.4 (m, 15H, Ar-H), 5.88 (s, 1H, C-H), 8.11 (s, 1H, CH=N), 10.21 (s, 1H, OH). *^{13}C NMR (DMSO-d6, ppm):* 159 (C1-coumarin),116.2 (C2-coumarin), 152.3 (C3-coumarin), 120.8 (C4-coumarin), 102.8 (C5-coumarin), 155.4(C6- coumarin), 120 (C7-coumarin), 113.5 (C8-coumarin), 153.6 (C9-coumarin), 20.2(C10-coumarin), 160.1 (C=N, Azomethine), 61.0 (C, Methine), 126-130 (C-Aromatic).MS ($C_{34}H_{25}N_3O_3$): m/z 523 (Mþ, 100%). Elemental analysis: calcd. (found): C, 77.92(77.84), H, 4.77 (4.73), N, 8.02 (8.00).

f. 8-(((E)-benzylideneamino)(phenyl)methyl)-7-hydroxy-4-methyl-6-((E)-thiazol-2-yldiazenyl)-2H-chromen-2-one: Colour: Brownish. Yield 67 %.Mp= 137 ◦ C. *IR (KBr, cm-1):* 3332 (-OH str.), 3150 (Ar-H str.), 2881 (–CH_3 str.), 1670 (lactone, C=O str.), 1651 (C=N str.), 1070 (C-O str.), 1570 (N=N str.). *^{1}H NMR (DMSOd6,*

ppm): 2.30(s, 3H, CH_3), 6.00-7.70 (m, 15H, Ar-H), 5.98 (s, 1H, C-H), 8.31 (s, 1H, CH=N), 09.84 (s, 1H, OH). *^{13}C NMR (DMSO-d6, ppm):* 159.8 (C1-coumarin),114.5 (C2-coumarin), 150.9 (C3-coumarin), 120.2 (C4-coumarin), 98.8 (C5-coumarin), 156.8(C6- coumarin), 121 (C7-coumarin), 113.9 (C8-coumarin), 151.4 (C9-coumarin), 20.1(C10-coumarin), 160.9 (C=N, Azomethine), 61.4 (C, Methine), 126-153 (C-Aromatic).MS ($C_{27}H_{20}N_4O_3S$): m/z 583 (Mþ, 100%). Elemental analysis: calcd. (found): C, 67.42(67.39), H, 4.16 (4.14), N, 11.65 (11.64), S 6.65 (6.62).

Results and Discussion:

Chemistry

The target compounds were synthesized by three steps protocol. In the first step, starting material 7-hydroxy-4-methlcoumarin 3 was prepared by the condensation reaction between one equivalent of resorcinol 1 and one equivalent of ethylacetoacetate 2 as per reported method (Scheme-1). The product was isolated with 95% yield. The uncorrected mp was 179°C and this closely matched the literature value of 180-182°C. After the generation of coumarin (3) i.e. in the second step of preparation, Betti's methodology was applied for the synthesis of Betti's products 5, which is a condensation reaction between phenol, benzaldehyde and amine in one pot synthesis (Scheme-1). In an approach, one equivalent of coumarin 3, two equivalents substituted aromatic aldehyde 4 and ammonia (up to saturation of system) was kept in 95% of alcohol at room temperature for 12 hours. TLC checked reaction progress duly. The products were isolated by simple and usual work up with 85-90% of yield economy. Thus, this is a reaction between coumarin, substituted aromatic aldehydes and ammonia. It is a special case of Mannich reaction which consisting of an amino alkylation of an acidic proton placed next to a carbonyl functional group with aldehyde and ammonia or any amine. The executed synthetic strategy is highlighted in Scheme1.

At last, the targeted azo dyes 6a-6f was prepared by usual method in third step (Scheme-1). All the azo dyes were obtained by diazotization of aromatic amines followed by coupling with Betti's products 5 incorporating phenolic structure by usual reported methods. The product was isolated with good yield. Here, the coupling of azo group is found to be take place at ortho position of phenolic –OH group of coumarin ring, as shown in structures. All prepared azo compounds have color in between red to brown.

The structures of all the synthesized Betti's base was deduced from their elemental analysis and IR, ^{1}H-NMR and Mass spectral data. All the compounds were obtained in excellent yield as solids melting in the range 105-107°C. The solid state IR spectra of these Betti's bases revealed a characteristic aromatic stretch around 3015 cm^{-1}. Sharp carbonyl (C=O) stretching vibrations for the lactone carbonyl were seen around 1715 cm^{-1}. The stretching vibration for C=N (azomethine) group was present at around 1750 cm^{-1}. Spectra also cleared the information regarding the stretching frequency around 3250 cm^{-1}which corresponds to the presence of phenolic –OH in the skeleton. All other peaks in the spectra are in well agreement with the contents of functionalities in the synthesized molecules. The ^{1}H-NMR spectra of 5 were recorded in DMSO-*d6* at room

temperature using TMS as internal standard. The NMR data reveal multiplets peak between 6.15-7.25 ppm owing to the presence of aromatic protons. The spectra showed characteristic singlet around 6.45 ppm for –CH moiety in the compounds. Presence of characteristic singlet arounds 8.50 ppm assigned to the proton attached to the imine forming carbon confirms the formation of azomethine linkage in the moiety. Presence of singlet around 13.45 ppm reveals the presence of phenol O-H on the ring. Three proton singlet around 2.45 ppm reveals the presence of $–CH_3$ on the ring. The mass spectra of the compounds displayed a molecular ion peak at appropriate m/z value, which was corresponding well with the respected molecular formula.

The structures of synthesized azo dyes were elucidated from their elemental analysis and IR, ^{1}H-NMR and Mass spectral data. The azo linkages (-N=N-) in the synthesized azo dyes were confirmed by presence of IR-peaks in the spectra around 1575 cm^{-1}. In NMR spectra, multiplets peak between 5.76-8.0 ppm owing to the presence of aromatic protons. The presence of sharp peak around 8.21 ppm, conformed –CH=N moiety in azo dye. The structures were further conformed by Mass spectral studies. They gave molecular ion peaks (M^+) corresponding to the correct molecular formulas.

Antimicrobial screening:

The antimicrobial activities of the series (a-f) have been carried out against some strain of bacteria. To determine the antibacterial activity of these agents, Agar cup plate method was used, with ampicillin and streptomycin as the reference antibiotics. The synthesized dyes were examined against two strains of gram positive and gram negative bacteria. The test results, presented in Table 1 suggest that some synthesized compounds are highly active against two strains each of gram positive and gram negative bacteria showing the broadest spectra of antibacterial activity. The rest of the compounds were found to be moderately active, slightly active or inactive against the test microorganism.

Table. 1- Antimicrobial activity of synthesized Azo dyes.

Compd.	MW	Gram +		Gram -	
		B. subtilis	*S. aureus*	*S. typhi*	*E. coli*
a	474	+	+++	++	++
b	474	++	+++	+++	+
c	474	+	++	+	++
d	583	++	++	+	+
e	523	++	+	++	+
f	480	+++	++	+++	++
AMP	-	+	++	+++	+++
STREP	-	+++	++	++	++

Conclusion:

Coumarin based Betti's condensed molecules containing azomenthine linkage was synthesized in good yield utilizing Betti's protocol. All the novel azo dyes were prepared by coupling coumarin derivatives produced by Betti's procedure, in alkaline condition with previously diazotized aromatic amines. All the synthesized Betti's compounds and azo compounds were characterized on the basis of elemental and spectral data. The produced compounds were tested for antimicrobial activity against certain gram positive and gram negative bacteria. Some of the synthesized compounds were found to be equally potent comparable with reference antibiotics.

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References:

[1] **Abramov, R.S., Bankoort, D.L. and Bankoort, (1993):**J.Phys.Chem., 31(1), Pp 23.

[2] **Noroozi-Pesyan, N., Khalafy, J. and Malekpoor, Z.(2009):**Prog.Color Colorants Coat. 2:Pp 61-70.

[3]. **Takadate, A., Masuda, T., Murata, C., Isobe, T.; Shinohara, A., Irikura, M. and Goya, S. (1997)**Analytical Sciences. 13: Pp753.

[4]. **Ito, Kitagawa, Tamaskiand Tsurfugi. (1950)**J. Pharm. Soc. 70.Pp 730.

[5] **Warder, F. W.** Annual Report, Merk'sJahresbericht, 1936, 50, 9.

[6] **Furniss, B.S., Hannaford ,A.J. and Smit P.W.G.(2005)** Vogel's Textbook of practical Organic Chemistry. 5thedition. Pp884-885.

[7] **Ali, P.; Meshrama, J.; Tiwari, V.; Sheik, J.; Dongre, R.; Youssoufi, M. H; Hadda, T. B.**European Journal of Medicinal Chemistry,2010, 45, 4370-4378.