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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDY OF SOME NEW BETTI' S PRODUCTS

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ABSTRACT:

Present paper deals with synthesis, characterization and antibacterial study of new coumarin nuclei incorporating derivatives produced by Betti's reaction or protocol. Herein, we report the synthesis, characterization and antibacterial study of 8, 10-bis (4-hydroxyphenyl)-4-methyl-9-phenyl-9, 10-dihydrochromeno [8, 7-e] [1, 3] oxazin-2(8H)-one. All the Betti's products were also synthesized by using green chemistry approach. The proposed structures of the oxazine derivatives were confirmed and characterized by using elemental analysis, H-NMR, C-13 NMR, IR, UV-VIS and mass spectra and tested for antibacterial activity.

Keywords: Betti's reaction, antimicrobial activity, microwave.

INTRODUCTION:

One hundred years ago, Betti reported a straightforward synthesis of 1-(α-aminobenzyl)-2-naphthol (the Betti base, **I**), **[1-5]** starting from 2-naphthol, benzaldehyde and ammonia. The Betti procedure can be interpreted as a specification of the Mannich condensation, in which formaldehyde is replaced by an aromatic aldehyde, secondary amine by ammonia and the C-H acid by an electron-rich aromatic compound such as 2-naphthol. The preparation of substituted Betti base derivatives by the modified Mannich reaction has subsequently become of considerable importance because a C-C bond is formed under mild experimental conditions. In the past decade, interest in the chemistry of the Betti base has intensified. Preparation of the enantiomers of the Betti base and its *N*-substituted derivatives is of significance since they can serve as chiral catalysts. This synthetic strategy originated between the end of the 19th and the beginning of the 20th century when research in several laboratories was performed on reactions between ammonia, or amines,

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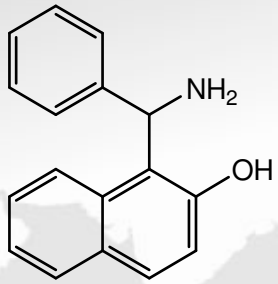
formaldehyde and enolisable carbonyl compounds **[6-8]**. In later years, attention has been paid to the betti's reaction, and a similar reaction can be performed by either using other naphthol **[9]** or quinolinols **[10-11]** or by replacing ammonia with alkyl amine **[12-16]**. In our work, the original synthetic procedure was reconsidered and extended to other reactants. This paper deals with an efficient and expeditious microwave assisted synthesis of novel oxazine derivatives using Betti's protocol in which 7-hydroxy-4-methyl-coumarin as a phenolic moiety reacted with aromatic aldehydes namely 4-hydroxy- benzaldehyde and primary aromatic amines namely aniline, para-chloro aniline, meta nitro aniline, ortho nitro aniline, para nitro aniline.



1-(amino(phenyl)methyl)naphthalen-2-ol

Figure-1

MATERIALS AND METHOD:

1. Generals: All the chemicals and solvents were obtained from EMerck, India (AR grade) and were used without further purification. Melting points were taken in an open capillary tube. IR spectra were recorded on a Shimadzu Dr-8031 instrument. Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer model 2400. 1H NMR spectra of the synthesized compounds were recorded on a Bruker- Avance (300 MHz), Varian-Gemini (200 MHz) spectrophotometer using $CDCl_3$ solvent and TMS as the internal

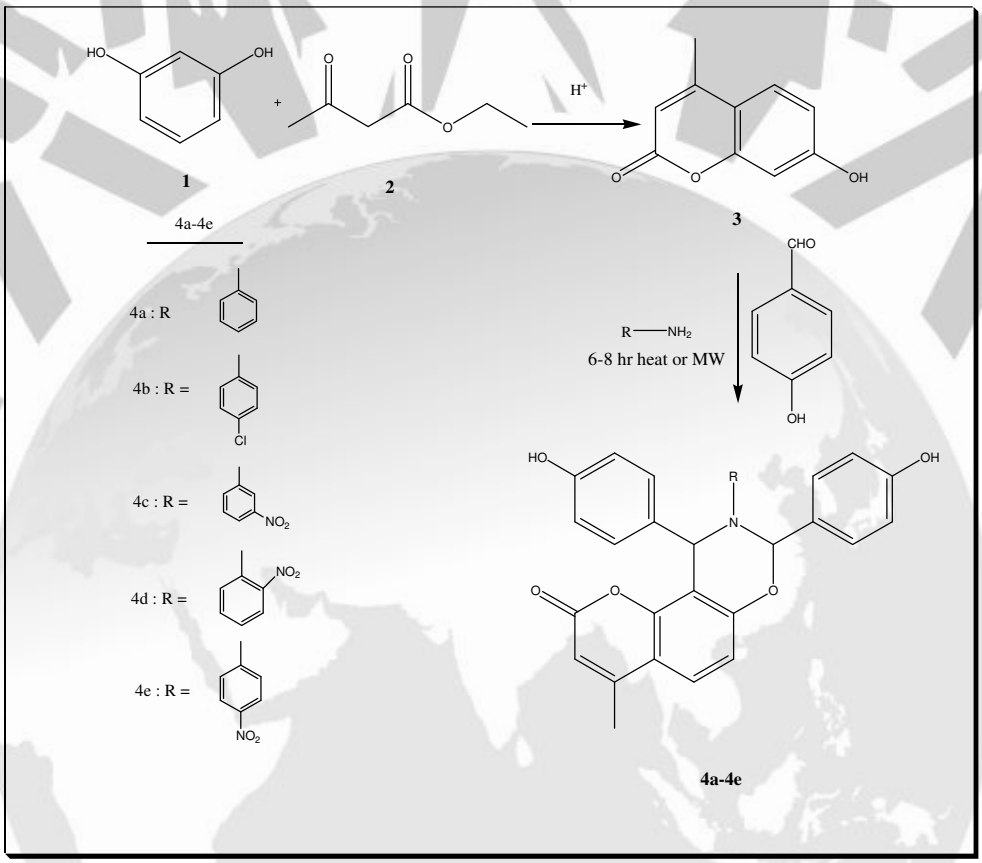
standard. EI-MS spectra were determined on a LCQ ion trap mass spectrometer (Thermo Fisher, San Jose, CA, USA), equipped with an EI source. The microwave assisted reactions were carried out using Kenstar-OM-20 DSP (2450 MHz) with an output energy range of 100 to500 W.

2. General synthesis:

2.1. Synthesis of 7-hydroxy-4-methyl coumarin: 7-Hydroxy-4-methylcoumarin (**3**) was prepared in good yield (Scheme **1**) using a Pechman procedure by condensation of ethylacetoacetate with an equimolar amount of resorcinol in presence of conc. H_2SO_4 [**8**]. The product was isolated with 95% yield. The uncorrected mp was 179- 181 °C and this closely matched the literature value of 180- 182 °C.

2.2. Synthesis of 8, 10-bis (4-hydroxyphenyl)-4-methyl-9-phenyl-9, 10-dihydrochromeno [8, 7-e] [1, 3] oxazin-2(8H)-one (Conventional and Microwave technique): To the mixture of 7-hydroxy-4-methyl-coumarin (10 m mol) in ethanol, aromatic aldehyde i.e. 4-hydroxy benzaldehyde (20 m mol) was added. To this mixture primary aromatic amines (10 m mol) was added and reaction mixture refluxed for 6-8 hours. The completion of the reaction monitored on TLC. However, the same raw materials viz., 7-hydroxy-4- methyl-coumarin (10 m mol), aromatic aldehyde (20 m mol) and primary aromatic amines (10 m mol) were taken in ethanol and irradiated under modified household microwave oven with power from 100-500 W and frequency 2450

MHz. The reaction took few minutes (1-2 min) for the completion as shown in Scheme **1**. All these compounds were recrystallized in absolute alcohol.



Scheme-I

2.3. 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 **4a-4e** 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**.

Table 1 Antibacterial & antifungal activity of 4a-4e.

Compd.	MW g/mol	Gram +		Gram -		Fungal
		B. subtitis	*S. aureus*	*S. typhyl*	*E. coli*	*A. niger*
4a	477	++	+++	+++	++	++
4b	511	+++	+++	++	+++	+++
4c	522	+++	+++	++	++	+
4d	522	++	+++	++	+++	+++
4e	522	++	+++	+++	+	++

STREP: Streptomycin; AMP: Ampicillin; - =. Inactive (inhi+++bition zone <5 mm); + = slightly active (inhibition zone 5-10 mm); ++ = moderately active (inhibition zone 10-15 mm); +++ = highly active (inhibition zone >15 mm

2.4. Spectroscopic characterization of Betti's products:

2.4.1. 8, 10-bis (4-hydroxyphenyl) -4- methyl-9-phenyl-9, 10 dihydrochromeno [8, 7-e] [1, 3] oxazin-2 (8H) - one **(a):**

Yield: 80%, M.P. 141 ^{O} C, FTIR (v cm^{-1}), 1690.25 (C=O, lactone, str.), 3012.00 (-CH3, str), 3221 (Ar-H, str.), 3401 (-OH, str.) 1159.36 (C-N, str.), H-NMR (DMSO-d6, ppm): 2.1524 (s, 3H, CH_3), 6.4840-7.8415 (m, 15H, Ar-H), 9.41 (aromatic C-OH) 5.5429 (s, 1H, Vinylic -CH), 2.5236 (s, 1H, high field hydrogen due to heteroatom O and N), 2.3117 (s, 1H, high field due to heteroatom N only). 13C NMR: 20.25 (CH3), 121.74 (C 1-coumarin), 187.05 (C 2-coumarin), 116.63 (C 3-coumarin), 124.73 (C 4-coumarin), 113.42 (C 5-coumarin), 158.02 (C 6-coumarin), 160.45 (C 7-coumarin), 110.99 (C 8-coumarin), 150.78 (C 9-coumarin), 37.56 (C 10-coumarin), 126.07-160.39 (Phenyl carbon). MS ($C_{30}H_{23}NO_5$): m/z 477 (Mp, 100%). Elemental analysis: calcd. (Found): C, 75.3896 (75.3831), H, 4.8165 (4.8145), N, 2.931 (2.930).

2.4.2. 9-(4-chlorophenyl)-8,10-bis(4-hydroxyphenyl)-4-methyl-9,10 dihydrochromeno [8,7-e][1,3]oxazin-2(8H)-one: **(b)**

Yield: 79%, M.P. 123 ^{O} C, FTIR (v cm^{-1}), 1670.14 (C=O, lactone, str.), 2987.00 (-CH3, str), 3246 (Ar-H, str.), 3435 (-OH, str.) 1152.88 (C-N, str.), H-NMR (DMSO-d6, ppm): 2.1428 (s, 3H, CH_3), 6.4710-7.8015 (m, 14H, Ar-H), 8.89 (aromatic C-OH) 5.214 (s, 1H, Vinylic -CH), 2.56 (s, 1H, high field hydrogen due to heteroatom O and N), 2.350 (s, 1H, high field due to heteroatom N only). 13C NMR: 20.21 (CH3), 121.61 (C 1-coumarin), 187.01 (C 2-coumarin), 117.10 (C

3-coumarin), 124.22 (C 4-coumarin), 113.71 (C 5-coumarin), 158.00 (C 6-coumarin), 160.17 (C 7-coumarin), 110.89 (C 8-coumarin), 150.74 (C 9-coumarin), 37.45 (C 10-coumarin), 126.01-160.38 (Phenyl carbon). MS ($C_{30}H_{22}ClNO_5$): m/z 510 (Mp, 100%). Elemental analysis: calcd. (Found): C, 70.317 (70.314), H, 4.297 (4.294), N, 2.7345 (2.7344).

2.4.3. 8,10-bis(4-hydroxyphenyl)-4-methyl-9-(3-nitrophenyl)-9,10-dihydrochromeno[8,7-e][1,3]oxazin-2(8H)-one: **(c)**

Yield: 63%, M.P. 152 ^{O} C, FTIR (v cm^{-1}), 1690.00 (C=O, lactone, str.), 3011.00 (-CH3, str), 3198 (Ar-H, str.), 3296 (-OH, str.) 1150.36 (C-N, str.), H-NMR (DMSO-d6, ppm): 2.04 (s, 3H, CH_3), 6.3312-7.2105 (m, 14H, Ar-H), 9.53 (aromatic C-OH) 4.9 (s, 1H, Vinylic -CH), 2.56 (s, 1H, high field hydrogen due to heteroatom O and N), 2.3 (s, 1H, high field due to heteroatom N only). 13C NMR: 20.25 (CH3), 121.74 (C 1-coumarin), 187.05 (C 2-coumarin), 116.63 (C 3-coumarin), 124.73 (C 4-coumarin), 113.42 (C 5-coumarin), 158.02 (C 6-coumarin), 160.45 (C 7-coumarin), 110.99 (C 8-coumarin), 150.78 (C 9-coumarin), 37.56 (C 10-coumarin), 126.07-160.39 (Phenyl carbon). MS ($C_{30}H_{22}N_2O_7$): m/z 521 (Mp, 100%). Elemental analysis: calcd. (Found): C, 68.8972 (68.8961), H, 4.2103 (4.2100), N, 5.3586(5.3584).

2.4.4. 8,10-bis (4-hydroxyphenyl)-4-methyl-9-(2-nitrophenyl)-9, 10-dihydrochromeno [8, 7-e] [1, 3] oxazin-2(8H)-one: **(d)**

Yield: 75%, M.P. 129 ^{O} C, FTIR (v cm^{-1}), 1679.02 (C=O, lactone, str.), 2983 (-CH3, str), 3250 (Ar-H, str.), 3369 (-OH, str.) 1151.36 (C-N, str.), H-NMR

(DMSO-d6, ppm): 2.11 (s, 3H, CH_3), 6.0145-7.5291 (m, 14H, Ar-H), 9.09 (aromatic C-OH) 5.70 (s, 1H, Vinylic -CH), 2.55 (s, 1H, high field hydrogen due to heteroatom O and N), 2.307 (s, 1H, high field due to heteroatom N only). 13C NMR: 20.25 (CH3), 121.74 (C 1-coumarin), 187.05 (C 2-coumarin), 116.63 (C 3-coumarin), 124.73 (C 4-coumarin), 113.42 (C 5-coumarin), 158.02 (C 6-coumarin), 160.45 (C 7-coumarin), 110.99 (C 8-coumarin), 150.78 (C 9-coumarin), 37.56 (C 10-coumarin), 126.07-160.39 (Phenyl carbon). MS ($C_{30}H_{22}N_2O_7$): m/z 521 (Mp, 100%). Elemental analysis: calcd. (Found): C, 68.8972 (68.8961), H, 4.2103 (4.2100), N, 5.3586(5.3584).

2.4.5. 8, 10-bis (4-hydroxyphenyl)-4-methyl-9-(4-nitrophenyl)-9, 10-dihydrochromeno [8, 7-e] [1, 3] oxazin-2(8H)-one: **(e)**

Yield: 73%, M.P. 135 ^{O} C, FTIR (v cm^{-1}), 1710.20 (C=O, lactone, str.), 3012.00 (-CH3, str), 3220 (Ar-H, str.), 3406 (-OH, str.) 1149.36 (C-N, str.), H-NMR (DMSO-d6, ppm): 2.1504 (s, 3H, CH_3), 6.1900-7.5734 (m, 14H, Ar-H), 9.41 (aromatic C-OH) 5.53 (s, 1H, Vinylic -CH), 2.523 (s, 1H, high field hydrogen due to heteroatom O and N), 2.317 (s, 1H, high field due to heteroatom N only). 13C NMR: 20.25 (CH3), 121.74 (C 1-coumarin), 187.05 (C 2-coumarin), 116.63 (C 3-coumarin), 124.73 (C 4-coumarin), 113.42 (C 5-coumarin), 158.02 (C 6-coumarin), 160.45 (C 7-coumarin), 110.99 (C 8-coumarin), 150.78 (C 9-coumarin), 37.56 (C 10-coumarin), 126.07-160.39 (Phenyl carbon). MS ($C_{30}H_{22}N_2O_7$): m/z 520 (Mp, 100%). Elemental analysis: calcd. (Found): C, 68.8972 (68.8961), H, 4.2103 (4.2100), N, 5.3586(5.3584).

2.5. Micro-wave assisted synthesis of Oxazine derivatives:

The comparison between conventional and microwave assisted synthesis of oxazine derivative is given in the **table 2**.

Table 2 Synthesis of oxazine derivatives by conventional & using micro-wave technique.

Compounds	Conventional method		Microwave method	
	Time	yield	Time	yield
4a	6-8 hr	80	1-2 minutes	89
4b	6-8 hr.	79	1-2 minutes	92
4c	6-8 hr.	63	1-2 minutes	86
4d	6-8 hr.	75	1-2 minutes	87
4e	6-8 hr.	73	1-2 minutes	91

RESULT AND DISCUSSION:

1. Synthesis of oxazine derivatives.

The novel oxazine derivatives were synthesized by betti's protocol using 7-hydroxy-4-methyl coumarin as phenolic moiety, aromatics aldehydes and aromatics amines following conventional method as well as by microwave technique. In conventional method time required to finish reaction was found to be six to eight hours on the other hand, one to two minutes required in microwave techniques.

2. Spectral study of synthesized oxazine derivatives.

The synthesized oxazine derivatives were characterized by FTIR, 1H-NMR, 13C-NMR and Mass spectroscopic analysis. All the compounds were obtained in excellent yield with crystalline. The presence of lactone carbonyl group is in agreement with their IR value. The IR peak in between 3196-3269 corresponds to aromatics hydrogen stretching. All other peaks in the spectra are in well agreement with the contents of functionalities in the synthesized oxazines derivatives. The 1H NMR spectra were recorded in DMSO-d6 at room temperature using TMS as internal standard. The NMR data of all compounds reveal multiplet peak between 6 and 8 ppm owing to the presence of aromatics proton. 1H NMR spectra also exhibit the peaks at 4.9-5.7 ppm executing the existence of vinylic proton on the coumarin skeleton. Three proton singlets around 2.00-2.2 ppm indicate the presence of -CH3 on the ring. The other signals and peaks of 1H NMR and IR are in complete agreement with the assigned structures. All the compounds have given the satisfactory elemental analysis. The antibacterial and antifungal activities of all synthesized compounds were carried out against some strain of bacteria and fungi as mentioned in the method. The test results presented in **Table 1** suggests that compounds **4a, 4b** and **4c** are highly active against bacterial strain as well as fungi.

CONCLUSION :

Oxazine derivatives synthesized by betti's protocol were founds to be novel. The % yield of prepared products was found to be good. All the synthesized compounds were characterized on the basis of elemental and spectral data. Compounds **4a, 4b** and **4c** were found to be equally potent comparable with Ampicillin and Streptomycin.

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