NOVEL SYNTHESIS OF ANTI-CANCER ACTIVE SUBSTITUTED CARBAZOLES *BY ONE-POT HANTZSH CONDENSATION*

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Abstract:

Keywords:

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I. INTRODUCTION

During the past 50 years the focus on the synthesis of indole derivatives[1] is due to its undisputable importance in nature, where this particular heterocycle is embedded in countless number of nature products and medicinally relevant compounds[2]. Among the indole alkaloids, the carbazole system is the most explored one. Ever since the first isolation of carbazole alkaloid, murraynine[3], organic chemists have been interested in the synthesis of carbazole and its derivatives[4] due to their promising biological activites. Knolker and Reddy have extensively reviewed[5] the synthesis and biological activities of carbazolealkaloids.

Several annulations strategies based on the Michael addition followed by intramolecular cyclization have been reported[6]. Over the years, the synthesis of large number of carbazole derivatives has been achived through Diel – Alder reaction[7] of 2/3 vinylinodles. Similarly, the synthesis of different types of carbazole derivatives has also been realized through Pd – mediated reactions[8]. Jagtap and Mali have reported[9] an annelation of ethyl – N – methyl – 2 – benzylinodle – 3 – carboxylate.

Microwave assisted organic synthesis (MAOS) is a new and quickly growing technique ion synthetic organic chemistry. This synthetic technique is based on the empirical observation that some organic reactions proceeds much faster and with higher yields under microwave irradiation compared to conventional heating. In many cases, reactions that normally require many hours at reflux temperature under classical conditions can be completed within several minutes or even seconds in a microwave oven, even at comparable reaction temperatures.

Development of new methods for the synthesis of heterocyclo – fused carbazole is currently attracting the organic chemists due to the discovery of many carbazole alkaloids with varied biological activities[10]. Ellipticine and other pyridocarbazoles are usually classified as indole alkaloids[11] and are important owing to their antitumor activity which is in turn due to inhibition of DNA replication and RNA transcription both *invivo* and *invitro*[12.] Many elegant approaches have been developed for the synthesis of benzo and heterocycle – fused carbazoles[13-16] and other related natural products involving annulations of indoles. Ellipticine, in particular has found clinical applications in advanced breast cancer, mieloblastic leukemia and solid tumors[17].

A survey of the pertinent literature reveals that carbazole have been found to possess a wide spectrum of biological activity such as antibactrial[18], antiheumatoid arthritis[19], antibercular[20], antiviral[21], antiepileptic[22], antiinflammatory[23], and anticancer[24-26] activities. Since we have made an attempt to synthesize carbazole derivatives incorporated with antibacterial pharmacophore like imidazole[27-32] by using both microwave assisted[33-35] as well as conventional synthetic method and screened them as potential antibacterial and anticancer agents.

It has been revealed from the literature that carbazole fused with imidazole nucleus possess various biological activities including anticancer activity especially against breast cancer. Carbazole constitutes an important class of naturally occurring heterocycles with interesting biological activites including their special affinity towards DNA[36].

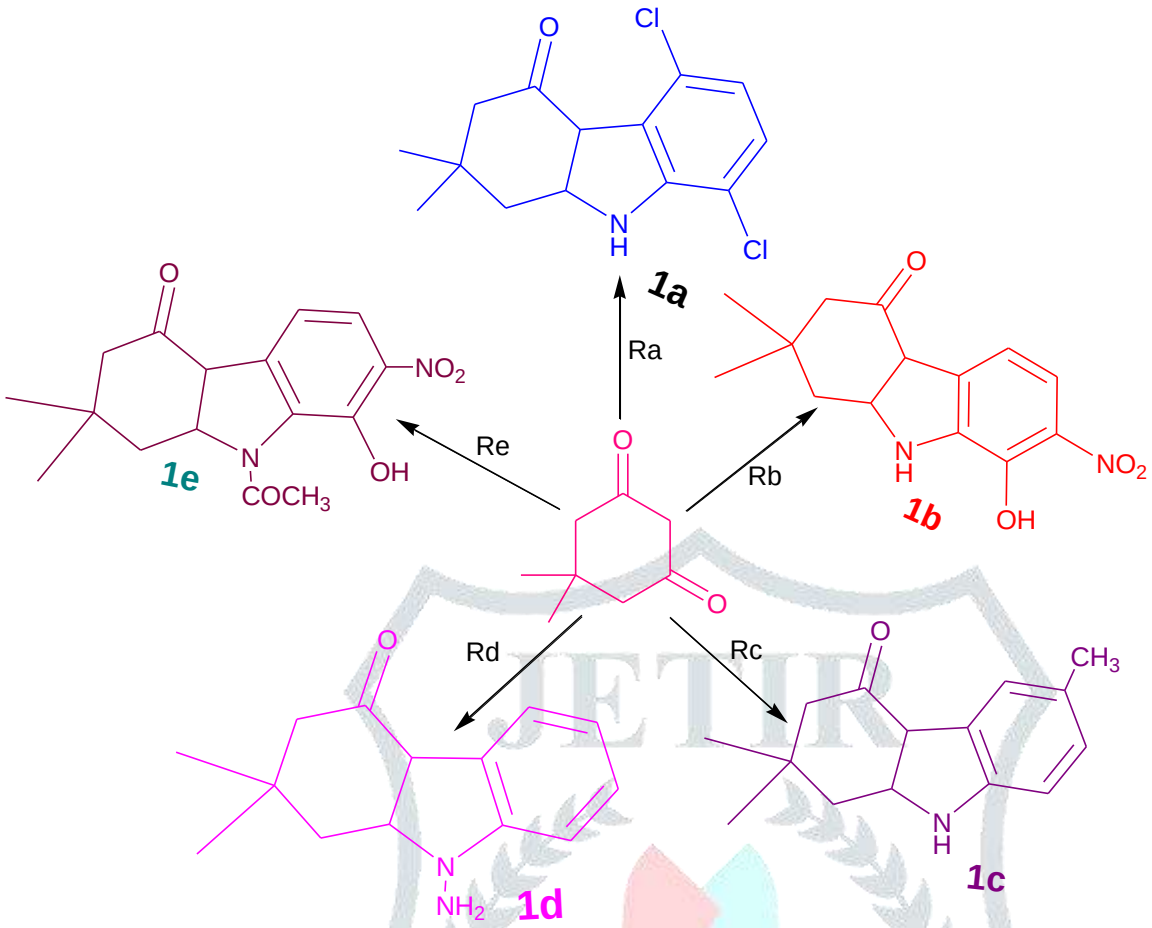
II. MATERIAL AND METHODS

All chemicals and the reagents used in the study were of synthetic grade purity. Aromatic amines and dimedone are purchased from Qualigens Fine Chemicals Company. Solvent used were purified by distillation. All substance prepared for studies were purified by crystallization using appropriate solvents and established procedures. Melting points were measured on a Sigma melting point apparatus using capillary tubes. Analytical TLC was performed on precoated sheets of silica gel to monitor the process of the reaction as well as to check the purity. The spot were visualized by using iodine vapour. NMR spectra were recorded on Jeol – FXQ (90 MHz), Jeol GSX (400 MHz) and DPX 200 (200 MHz).

Microwave irradiation of dimedone and aromatic amines are treated in a solvent free condition for 3 minutes. The formation of orange solid mass, confirm carbazole derivatives in an excellent yield (85 – 90%). Various substituted carbazoles were prepared and reported below.

Synthesis of aromatic amine substituted carbazole derivatives Table I

S. No.	R (Substitution)	
1.	a	$C_6H_3(Cl)_2NH_2$
2.	b	$C_6H_3(OH)(NO_2)_2$
3.	c	$C_6H_4(CH_3)(NO_2)$
4.	d	$C_6H_5NH_2NH_2$
5.	e	$C_6H_5NHCOCH_3$



Comparison of thermal and Microwave method (Yield and reaction time of carbazole derivatives).

Table II

Compound	Thermal method (Hrs.)	Yield %	Microwave method (min)	Yield %	M. P. ^{0}C
1a	6-7	65	3	89	98-100
1b	5-6	60	2	88	108-110
1c	4-5	62	2	90	156-158
1d	3-4	60	1	93	168-170
1e	7-8	55	3	90	141-143

III. RESULT AND DISCUSSION

Chemical and pharmaceutical industries are facing constrains regarding environmental aspects and saving energy. To overcome such problems in organic synthesis, the microwave (MW) irradiation as a source of energy is used. In this study we use as excellent synthetic method for new aromatic amine substituted carbazole derivatives (1a – e) by microwave technique.

Characterization:

(4, 4'- dimethyl) – 6 – keto – (9, 12 – dichloro) - carbazole

^{1}H NMR: δ 1.0 – 1.10 (gem dimethy), 2.25- 2.53 (d, C2 & C4), 3.33- 3.76 (m, 4H), 5.51 (br, 2H), 5.99 (s, 1H) 7.26- 7.33 (m, Ar – H), 8.11- 8.13 (br, 1H, N-H). ^{13}C NMR: δ 28.16, 28.24, 28.35, 30.93, 31.78, 32.72, 34.07, 43.65, 46.21, 46.98, 50.99, 54.12, 57.28 (aliphatic carbons), 76.78, 77.03, 77.29 (aromatic carbons), 103.12, 115.98, 118.12, 119.66, 127.82, 128.53 (aryl carbons), 140.15 (olefinic carbons), 157.77 (amide carbonyl), 201.72, 203.71 (keto carbonyl).

(4, 4' – dimethyl) – 6 – keto – (12- hydroxy) – (11-nitro) - carbazole

^{1}H NMR: δ 1.04-1.16 (gem dimethyl), 2.25-2.53 (d, C2 & C4), 3.33 – 3.69 (m, 4H), 5.48 (br, 2H), 6.01 (s, 1H), 7.26 – 7.30 (m, Ar – H), 8.10 (br, (1H, N-H). ^{13} C NMR: δ 28.15, 28.24, 30.93, 31.76, 32.60, 34.05, 43.63, 44.72, 46.15, 50.92, 51.03, 54.13, 57.27 (aliphatic carbons), 76.77, 77.02, 77.28 (aromatic carbons), 116.01, 128.51 (aryl carbon), 157.37 (amide carbonyl), 201.32, 203.70 (keto carbonyl).

(4, 4' – dimethyl) – 6 – keto – (10 – methyl) - carbazole

^{1}H NMR: δ 1.05 – 1.14 (gem dimethy), 2.18- 2.53 (d, C2 & C4), 3.33- 3.76 (m, 4H), 5.01 (br, 2H), 5.99 (s, 1H) 7.10- 7.26 (m, Ar – H), 8.11- 8.13 (br, 1H, N-H). ^{13}C NMR: δ 28.26, 28.34, 28.35, 30.53, 31.68, 32.52, 34.17, 43.55, 46.31, 46.88, 50.89, 54.22, 57.28 (aliphatic carbons), 76.78, 77.03, 77.29 (aromatic carbons), 103.10, 115.88, 118.22, 119.56, 127.72, 128.43 (aryl carbons), 140.15 (olefinic carbons), 157.77 (amide carbonyl), 201.72, 203.71 (keto carbonyl).

(4, 4' – dimethyl) – 6 – keto - carbazole

^{1}H NMR: δ 1.05-1.12 (gem dimethyl), 2.25-2.53 (d, C2 & C4), 3.33 – 3.69 (m, 4H), 5.56 (br, 2H), 6.78 (s, 1H), 7.13 – 7.30 (m, Ar – H), 8.10 (br, (1H, N-H). ^{13} C NMR: δ 28.15, 28.24, 30.93, 31.76, 32.60, 34.05, 43.63, 44.72, 46.15, 50.92, 51.03, 54.13, 57.27 (aliphatic carbons), 76.77, 77.02, 77.28 (aromatic carbons), 116.01, 128.51 (aryl carbon), 157.37 (amide carbonyl), 201.32, 203.70 (keto carbonyl).

(4, 4' – dimethyl)- 6 – keto – phenyl acetanilide carbazole

^{1}H NMR: δ 1.00 – 1.16 (gem dimethy), 2.25- 2.57 (d, C2 & C4), 3.16- 3.37 (m, 4H), 5.48 (br, 2H), 5.99 (s, 1H) 7.10- 7.26 (m, Ar – H), 8.10- 8.12 (br, 1H, N-H) ^{13}C NMR: δ 28.16, 28.24, 28.35, 30.93, 31.78, 32.72, 34.07, 43.65, 46.21, 46.98, 50.79, 54.32, 57.38 (aliphatic carbons), 76.78, 77.03, 77.29 (aromatic carbons), 103.12 , 115.98, 118.12, 119.66, 127.82, 128.53 (aryl carbons), 140.15 (olefinic carbons), 157.77 (amide carbonyl), 201.72, 203.71 (keto carbonyl).

Biological Studies:

A survey of the pertinent literature reveals that carbazole have been found to possess a wide spectrum of biological activity such as antibactrial[37], antiheumatoid arthritis[38], antibercular[39], antiviral[40], antiepileptic[41], anti-inflammatory[42], and anticancer[43-45]activities. Since we have made an attempt to synthesize carbazole derivatives incorporated with antibacterial pharmacophore like imidazole[46-51] by using both microwave assisted[52-54] as well as conventional synthetic method and screened them as potential antibacterial and anticancer agents.

It has been revealed from the literature that carbazole fused with imidazole nucleus possess various biological activities including anticancer activity especially against breast cancer. Carbazole constitutes an important class of naturally occurring heterocycles with interesting biological activites including their special affinity towards DNA[55]. The antibacterialnactivity of Carbazole derivatives are given in table III .

DNA used: Calf-thymus DNA (50 µg/test)

Agarose gel electrophoresis

Following the treatment of DNA samples, the electrophoresis of the samples weredone according to the following procedure (Sambrook *et al*., 1989)

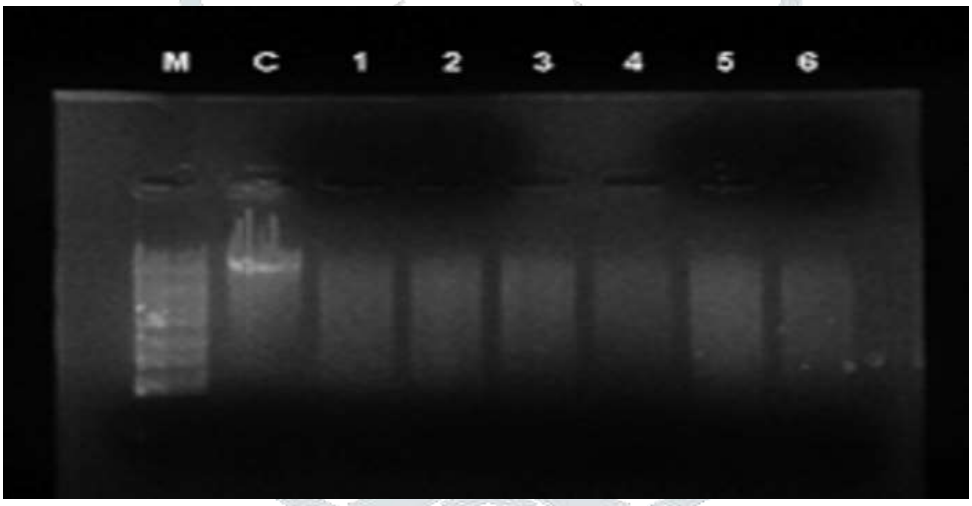
1. Weigh 250mg of agarose and dissolve it in 25 ml of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 ltr) by boiling.

2. When the gel attains ~550C, pour it into the gel cassette fitted with comb. Let the gel to solidify

3. Carefully remove the comb, place the gel in the electrophoresis chamber flooded with TAE buffer.

4. Load 20 µl of DNA sample (mixed with bromophenol blue dye @ 1:1 ratio), care fully into the wells, along with standard DNA marker and pass the constant 50 V of electricity for around 45 min

5. Remove the gel and carefully stain with ETBR solution (10 µg/ml) for 10-15 min and observe the bands under UV transilluminator.



Gel picture-1 showing the cleavage analysis of samples

M- Supermix DNA Ladder (Merck, Cat # MBD21J); C-CT-DNA; 1-6- samples at 50 and 100 µg respectively.

Inference: All samples have shown complete cleavage of DNA at both concentrations screened.

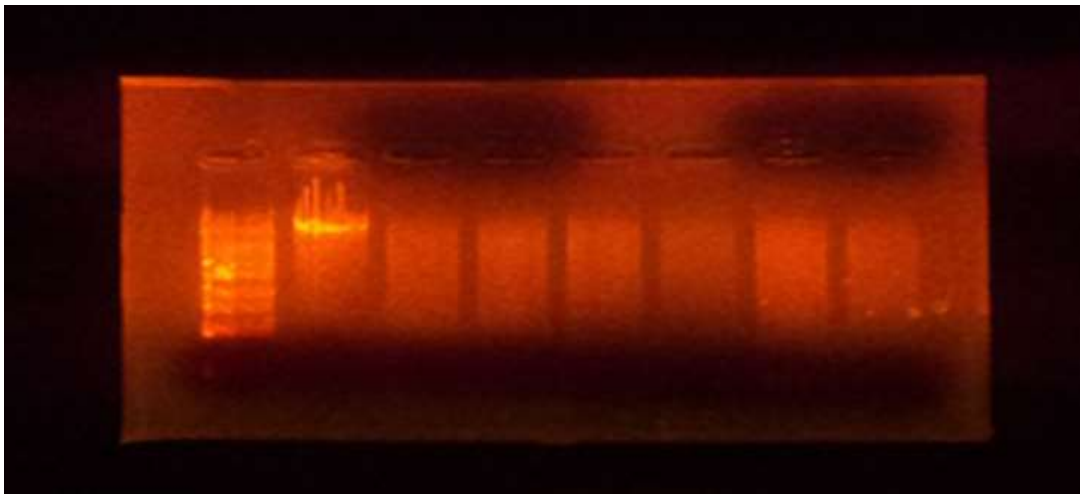


Table III: Antimicrobial activity of the synthesized substituted Carbazole derivatives (**1a-e)**

Compound	Conc. (µ.g/well) In DMF	Zone of inhibition in mm*			
		Antibacterial activity			
		P. aeruginosa	***E. coli***	***B. subtilis***	***S. Aureus***
1a	600	7	6	9	8
1b	600	6	5	10	8
1c	600	7	11	13	9
1d	600	7	7	7	9
1e	600	6	8	8	10
Ciprofloxacin	600	25	26	20	25
	***Diameter of well (bore size) – 6mm**				

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