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Short Communication

ANTICANCER ACTIVITY OF MICROWAVE ASSISTED NEWLY SYNTHESIZED 2,3,4,9-TETRAHYDRO-1H-CARBAZOLE DERIVATIVES

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ABSTRACT

Objective: The objective of this study was to synthesize 2,3,4,9-tetrahydro-1H-carbazole derivatives and evaluation of their anticancer activity by MTT assay.

Methods: We have synthesized a series of novel 2,3,4,9-tetrahydro-1H-carbazole derivatives of 1-4(substituted phenyl)-3-1-9 (2,3,4,9-tetrahydro-1H-carbazole-9yl)-propane-1-one, by Indolization of 2 ml (0.1 mol) cyclohexanone and 2 ml (0.1 mol) phenyl hydrazine in the presence of a few drops of glacial acetic acid. The mixture was placed in a microwave for 5 min. to give 2, 3, 4, 9-tetrahydro-1H-carbazole (J). Then titled compounds were prepared by Mannich reaction in which compound (J) condensed with formaldehyde and various aromatic acetophenone (J-1 to J-5). Finally, all synthesized products were characterized on the basis of melting point, R_f value, NMR, IR and mass spectral analysis. All newly synthesized compounds were evaluated for their *in vitro* anticancer activity against A-549 cell line by MTT assay.

Results: Among the newly synthesized compounds, only J-3 (p-Br acetophenone) and J-4 (p-Nitro acetophenone) exhibited significant activity against A-549 cell line at concentration 1000µg/ml, 500 µg/ml, and 250µg/ml. Other compounds J-1, J-2 and J-5 have not shown the activity.

Conclusion: Hence we conclude that newly synthesized derivatives (1-(4-bromophenyl)-3-1(1,2,3,4-tetrahydro-9H-carbazole-9yl)propan-1-one) J-3 and (1-(4-nitrophenyl)-3-1(1,2,3,4-tetrahydro-9H-carbazole-9yl)propan-1-one) J-4 possess considerable anticancer activity.

Keywords: Microwave-assisted synthesis, 2,3,4,9-tetrahydro-1H-carbazole, Anticancer activity, MTT assay.

Carbazole are probably the most widely spread nitrogen heterocycles in nature [1-2]. It is commercially prepared from coal tar. Carbazoles represent one of the most valuable ring systems in the modern synthetic chemistry, because of their widely recognizes versatility with biological and pharmacological activities such as antibacterial [3] antimicrobial activity [4-5] antiviral [6] antiepileptic [7] anti-inflammatory [8] antioxidant and anticancer [9-10] activities. Carbazole arrest the tumor cell cycle at the M phase and induce apoptotic cell death by increasing expression of p-53 and promoting bcl-2 phosphorylation [11-12]. From the SAR studies of the carbazole ring system in the various literature suggest that substitution at position3, 6 and 9 are very much important for better pharmacological activities. In view of the above considerations, we planned to present work to design novel microwave-assisted synthesis and evaluation of novel 2,3,4,9-tetrahydro-1H-carbazole derivatives as anticancer agents.

Synthesis and characterization of 2,3,4,9-tetrahydro-1H-carbazole derivatives: J 2 ml of Cyclohexanone (0.1 mol) and 2 ml of Phenylhydrazine (0.1 mol) was taken into a conical flask. To this, few drops of glacial acetic acid were added, and the mixture was placed in a microwave in 5 min and mid temperature. The product was filtered and recrystallized from methanol (J).

Preparation of titled compounds (J-1 to J-5): Compound (J) (0.01 mol) was allowed to the Mannich reaction with various aromatic acetophenone (0.01 mol) in the presence of paraformaldehyde (0.01 mol). The reaction mixture was kept into a microwave in 10 min and mid temperature. The product was filtered and recrystallized from methanol (J-1 to J-5).

J: IR (KBr) cm-^{1}: N-H str-2921.53, C-H str-2853.49, C=C—1460,1376,1154,N-H-bending 1600,^{1}H NMR($CDCl_3$) (δppm): 2.5-3.35 (s, Ar-C-NH), 6.6-6.7 (4H, Ar-C-C), 7.0-7.1(4H, Ar-C-H), 8.1-9.5 (s, Ar-N-C), Mass: m/e 171, base peak 115.

J-1: IR (KBr)cm-^{1}:N-H str-2920.82, C-H str 2731.6, C=O str-1747.2, N=Ostr-1460.18, CH_2bending-1376.9, NMR ($CDCl_3$) (δppm): 1.9-2.16(m, C=O-CH_2), 4.06-4.08 (4H,Ar-N-CH), 6.6-6.9 (4H, cyclohexadiene), 7.0-7.9 (4H, Ar-N-C-C), 8.0-8.2(4H, Ar-N-C=O), Mass: m/e 336, base peak 335 other dominant peak 231,313

J-2: IR (KBr) cm-^{1}: N-H str-2921.06, C-Hstr-2853.25, CH_2 bending-1461,1376,721.91,NMR($CDCl_3$) (δppm): 1.3-1.98(m, C=O-CH_2), 2.0-2.6 (m, C=O-CH2), 6.67-6.9 (4H, cyclohexadiene), 7.0-7.89 (4H, Ar-N-C-C), 8.0-8.2(4H, Ar-N-C=O), MASS: m/e 290, base peak 231.5.

J-3: IR (KBr)cm-^{1}:N-H str—2921.11, C-H str-2853.2, C=Ostr-1588.92,1695.13, CH_2-1376.87, C-Ostr-1011.05, Br-747.87, ^{1}HNMR($CDCl_3$) (δppm): 1.7-1.97 (4H, Ar-CH), 2.02-2.6 (4H, cyclohexadiene), 4.10 (4H, N-CH_2), 6.02-6.9 (4H, cyclohexadiene), 7.1-7.94 (4H, Ar-N-C-C), 8.2(4H, Ar-N-C=O), Mass: m/e 463, base peak 335 other dominant peak 353,313.

J-4: IR(KBr)cm-^{1}: N-Hstr-2921.93, C-Hstr-1603, C=O-1462, CH_2-1035, C-Ostr-1304.9,1HNMR($CDCl_3$) (δppm): 1.6-1.97 (4H,CH_3), 2.02-2.6 (4H, cyclohexadiene), 3.7 (2H, N-CH_2), 6.5-6.82 (4H, cyclohexadiene), 7.1-7.94 (4H, Ar-N-C-C), 8.4(4H, Ar-N-C=O), Mass: m/e 334, base peak 333.

J-5: IR(KBr) cm-^{1}: N-H str. 2924.16, C-H str. 2843.5, C=O str—1601,O-H—1459, C-O str. 1045.2. Mass**:** m/e 317, base peak 313.

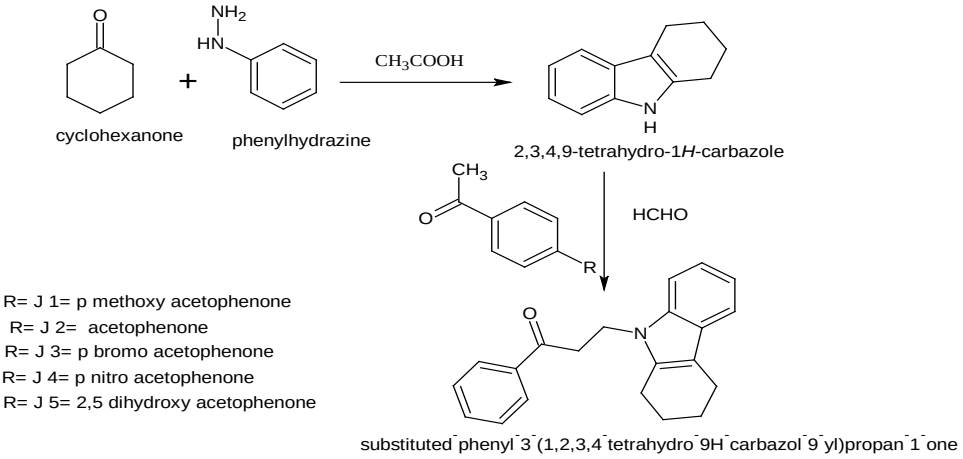
All synthesized derivatives were J-1-J-5 evaluated for anticancer activity against A-549 cell line. Stock cells of A-549 were cultured in DMEM supplement with 10 % inactivated Fetal Bovine Serum (FBS), Penicillin(100 IU/ml), Streptomycin (100µg/ml) and amphotericin B(5µg/ml) in a humidified atmosphere of 5 % CO_2 at 37°C until confluent. The cells were dissociated with a TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The stock cultures were flasks, and all experiments were carried out in 96 microtiter plates (Tarsons India PVT. Ltd., Kolkata, India).

For cytotoxicity studies, each weighs test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2 % inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two-fold dilutions were prepared for this for carrying out cytotoxic studies.

The monolayer cell culture was adjusted to 1.0×10^5cells/ml using DMEM containing 10 % FBS. To each well of the 96 well microtiter plates, 0.1 ml of the diluted cell suspension (approximately10, 000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37 °c for 3 d in 5 % CO_2 atmosphere, and microscopic examination was carried out, and observations were noted every 24-hour interval. After 72 h, the drugs solutions in the wells were discarded, and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3h at 37 °c in 50 % CO_2 atmosphere the supernatant was removed, and 100 µl of propanol was added, and the plates were gently shaken to solubilize the formed Formazan. The absorbance was measured using a micro plate's reader at a wavelength of 540 nm. The % growth inhibition was calculated using the following formula concentration of test drug needed to inhibition cell growth by 50 % (CTC_{50}) values are generated from the dose response curves for each cell line [13].

% Growth inhibition= 100-[(test absorbance/control absorbance) x 100

The procedure outlined in Scheme 1 illustrates the microwave assisted synthesis of a series of novel 2,3,4,9-tetrahydro-1H-carbazole derivatives of 1-4(substituted phenyl)-3-1-9 (2,3,4,9-tetrahydro-1H-carbazole-9yl)-propane-1-one, by Indolization of 2 ml (0.1 mol) cyclohexanone and 2 ml (0.1 mol) phenyl hydrazine in the presence of a few drops of glacial acetic acid. The mixture was placed in a microwave for 5 min to give 2,3,4,9-tetrahydro-1H-carbazole (J) [14]. Then titled compounds were prepared by Mannich reaction in which compound (J) condensed with formaldehyde and various aromatic acetophenone (J-1 to J-5). All the newly synthesized compounds were characterized on the basis of melting point, R_f value, NMR, IR and mass spectral analysis. All newly synthesized compounds were evaluated for their *in vitro* anticancer activity against A-549 cell line by MTT assay. Only p-Br acetophenone substituted tetrahydro carbazole (J-3) and p-Nitro acetophenone (J-4) exhibit significant activity against A-549 cell line at concentration 1000µg/ml, 500µg/ml, and 250µg/ml. The anticancer activity may be attributed due to the presence of electron donating group which may increase the basicity of the compound. Other three compounds didn't show anticancer activity at the tested dose. Hence, we can conclude that among of five synthesized compounds only J-3 having bromophenyl & J-4 having nitrophenyl as side chain possess anticancer activity against A-549 cell line with CTC_{50} value ranging between 190.00±to 514.00±22.22 µg/ml. So, further research is required to determine the specific mode of their anticancer activity. The test result is expressed as the concentration of test compound shows in table 1.



Scheme 1: Synthesis of Novel 2,3,4,9-Tetrahydro-1H-Carbazole Derivatives as Anticancer agent

Table 1: Pharmacological evaluation of novel 2,3,4,9-Tetrahydro-1H-carbazole derivatives as anticancer agent

Drug name	Test conc.(µg/ml)	%cytotoxicity MTT	CTC_{50} (µg/ml)	Average CTC_{50}
J-1	1000, 500, 250	60.19, 59.13, 53.28	240	265.00±35
J-2	1000,500,250	50.19,50.9, 53.19	240	4915.50±2
J-3	1000,500,250	76.71,76.51, 55.34	190	190±0.00
J-4	1000,500,250	76.71,76.51, 55.50	190	190±0.00
J-5	1000,500,250	-	510	>1000

n= 5, *The values obtained in at least three separate assays done in triplicate±SD–Standard deviation, The CTC_{50} value defined as the concentration at which 50 % survival of cells was observed

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CONFLICT OF INTERESTS

Declare none

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