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## DESIGNED SYNTHESIS, CHARACTERIZATION OF CARBAZOLE-N-PHENYLACETAMIDE ANALGUEOUS EMPLOYED BY Pd (OAc)<sub>2</sub> AS CATALYST

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### ABSTRACT

In this study carried out to a biological potent activity of a series 2-(3-methoxy-9H-carbazol-9-yl)-N-phenylacetamide analogues. These derivatives were synthesized from 2-(3-methoxy-9H-carbazol-9-yl) acetyl chloride treated aromatic primary amines in the presence of strong organic base such as triethylamine and MDC RT condition and the compound (4) also prepared by 3-methoxy-9H-Carbazole (3) with chloroacetylchloride in the presence of potassium carbonate in acetone as solvent at 40°C. The intermediate (3) was obtained by 4-methoxy aniline treated with 1, 2-dichloro benzene in the presence of Pd(OAc)<sub>2</sub> in toluene as a solvent at 100°C. The titled analogues can be evaluated by spectral techniques such as <sup>1</sup>HMR, <sup>13</sup>CNMR and LCMS. The structure of the desired compounds was determined by elemental analysis. In addition to, compounds were examined for *in-vitro* antimicrobial activity against bacterial strain and fungal strains.

### KEYWORDS

N-phenylacetamide analogues, 9H-carbazol-9-yl acetyl chloride, Aromatic primary amines, 3-methoxy-9H-Carbazole, Pd(OAc)<sub>2</sub> and Antimicrobial activity.

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### INTRODUCTION

The investigation of microbial pathogens continues which could not respond to conventional treatments presents a significant threat to global public health. Bacterial and fungal infections are becoming progressively more resistant towards presently available antimicrobial medicines, such as antibiotics. Therefore, there is spontaneous need for new drugs that target these pathogens. Some medical procedures such as diabetes management, organ transplantation and chemotherapy are without effective antimicrobials for treatment and

prevention of infections, routine surgery (e.g. hip replacements or caesarean sections) have a significantly higher risk of morbidity and mortality. The scale of the threat of antimicrobial resistance has been led to the improvement of several strategies to conserve and construct more effective use of existing antibiotics and to promote the development of novel antimicrobials. In various natural medicinal active substances were containing carbazoles ring. Recently, macro cyclic diamides of carbazoles skeleton with thia- and oxy-linkage systems can synthesize.

Microorganism were able to improve the resistance to these chemotherapeutic agents and such strains which are resistance causes major problems in the treatment of microbial infection in the treatment of microbial infections. New antimicrobial agents were searched to continue process for treatment of microbial infections while the great efforts was employed to find new. The derivatives of carbazoles are important type of nitrogen containing condensed hetero cyclic compounds which possesses desirable charge-transport activity and large p-conjugated system. The derivatives of carbazoles have been attracted to considerable attention in medicinal chemistry due to exhibit various type of biological and pharmacological properties and carbazoles analogous having amide linkages are most important significant substances in synthetic organic chemistry and medicinal chemistry.

Carbazoles are the condensed aromatic heterocyclic substances having carbazoles derivatives, are embodied in many naturally occurring compounds which exhibited a wide range of useful biological properties viz; antimitotic and antioxidance<sup>1-5</sup>.

So, there are some of the heterocyclic molecules substituted with a carbazoles unit was reported and the synthesis of such compounds is desirable while the other hand, the oxygen containing fused heterocyclic substances such as benzofurans analogous are the most important class of heterocyclic compounds having to possess important as antimicrobial properties and anticonvulsant, anti-fungal anti-inflammatory and anti-tumor activities<sup>6-8</sup>. Our aim of the present

research work is synthesis of novel titled analogues at position 9.

## MATERIAL AND METHODS

### EXPERIMENTAL

#### Chemistry

All the chemicals, synthetic reagents and solvents were purchased from commercially Sigma Aldrich and they were used without further purification. The reaction mixture were checked by thin-layer chromatography (n-hexane: Ethylacetate) on silica gel plates coated with alumina. The melting points of the titled compounds were determined in open capillary tubes and were uncorrected. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectrum were recorded titled derivatives on a Bruker DRX-400MHz and 100MHz instrument using CDCl<sub>3</sub> as a solvent. The mass spectra were obtained on a Shimadzu 2020A LCMS spectrometer. Elemental analysis of the derivatives was recorded by the instrument.

#### GENERAL PROCEDURE FOR PREPARATION OF 3-METHOXY-9H-CARBAZOLE

The mixture of methoxy aniline (1mmol), 1, 2-dichloro benzene (2mmol) taken in a dry and clean four neck RBF. The catalyst palladium acetate and cesium carbonate added in toluene as solvent RBF. The total set up arranged on the magnetic stirrer and was maintained 6hrs at 100°C. The reaction mixture was monitored by TLC (3:7-Ethyl acetate: n-hexane). After completion of the reaction, catalyst was filtered and the reaction mixture poured into ethyl acetate and washed solution of sodium bicarbonate. The organic layer separated kept side and aqueous layer washed with (2x10mL) after separated. Both of the organic layers combined distilled off u/vacuum. Crude product was separated by columns chromatography and recrystallization from ethanol.

#### 3-METHOXY-9H-CARBAZOLE (3)

Red solid: Yield-87%; Mp-224-216°C; <sup>1</sup>H-NMR (400MHz, CDCl<sub>3</sub>) ppm: 10.558 (s, 1H, N-H, pyrole), 7.884 (s, 1H, indole), 7.537 (d, J=8.9Hz, 1H, Ar-H), 7.491 - 7.126 (m, 5H, Ar-H), 3.728 (s, 3H, -OCH<sub>3</sub>). <sup>13</sup>C-NMR (100MHz, CDCl<sub>3</sub>) ppm: 152.29, 132.08, 131.58, 128.58, 123.34, 122.47,

119.58, 115.56, 112.85, 110.02, 108.91, 99.05, 55.97; LCMS(m/z): 198.54(M+H); Molecular formulae: C<sub>13</sub>H<sub>11</sub>NO; Elemental analysis: Calculated: C-79.15, H-5.62, N-10.07; Obtained: C-79.11, H-5.60, N-10.15.

#### GENERAL PRODUCER OF 2-(3-METHOXY-9H-CARBAZOL-9-YI) ACETYL CHLORIDE

Taken dry and clean 50mL RBF arranged on the Magnetic stirrer. The solvent acetone and base K<sub>2</sub>CO<sub>3</sub> charged in a RBF and 3-methoxy-9H-Carbazole and chloroacetic chloride slowly added into a RBF. The reaction continued and continued 6hrs at 50-55°C. The reaction was identified by TLC ((Ethyl acetate: n-hexane). After completion of the reaction, poured in cold water followed by ethyl acetate and separated organic layer. The separate the organic layer washed with solution of sodium bicarbonate in two times and separated organic layer. The distilled off, separated by columns chromatography.

#### 2-(3-methoxy-9H-carbazol-9-yi) acetyl chloride

Colorless compound Yeild-88%; m.p: 212-214°C: <sup>1</sup>HNMR (400MHz, CDCl<sub>3</sub>) ppm: 8.235 (s,1H, Ar-H), 7.858 (s, 1H, Ar-H),7.723-7.458 (m, 4H, Ar-H),6.940 (d, J=7.2Hz, 1H, Ar-H), 3.648 (s, 3H, -OCH<sub>3</sub>), 2.272 (s, 2H, -CH<sub>2</sub>-). <sup>13</sup>CNMR (100MHz, CDCl<sub>3</sub>) δppm: 169.73, 147.48, 136.81, 133.68, 130.58, 128.75, 128.20, 127.85, 125.12, 123.40, 56.83, 33.52; LCMS (m/z): 275.68 (M+2); Molecular formula: C<sub>15</sub>H<sub>12</sub>ClNO<sub>2</sub>; Elemental analysis: calculated: C-66.70; H-4.32; N-5.20; Obtained: C-65.65; H-4.38; N-5.15.

#### 2-(3-METHOXY-9H-CARBAZOL-9-YL)-N-PHENYLACETAMIDE ANALGUEOUS

Take dry and clean 50mL RBF. The charge methylene dichloride and the strong base such as a triethyl amine into RBF at room temperature which is also arranged on the magnetic stirrer possess hot plate. The introduce a mixture of 2-(3-methoxy-9H-carbazol-9-yi) acetyl chloride (1mmol) and substituted aromatic primary amines (1.1mmol) into a RBF at mixture carried out 35°C. The reaction was continued in 5hrs at same temperature and monitored by TLC (Ethylacetate: n-hexane). After

the completion of the reaction, crude poured into cold water and add 10 mL of 5% HCl into the solution and added with ethyl acetate. The organic layer separated and washed with solution of Brain. Finally separated the organic layer and distilled off. The desired product separated by column chromatography and also recrystallized with ethanol.

#### 2-(3-methoxy-9H-carbazol-9-yl)-N-phenylacetamide (6a)

White solid; Yield-82%; M.p-223-225°C; <sup>1</sup>HNMR (400MHz, CDCl<sub>3</sub>) δppm: 9.854(1H, s, amide), 8.01 (d, J=7.3 Hz); 7.640 (s, 1H, Ar-H), 7.645 (d, J=7.5Hz, 1H, Ar-H), 7.562 (d, J=6.7Hz, 1H, Ar-H), 7.325 (d, J=8.0Hz, 1H, Ar-H),7.423-7.145 (m, 5H, Ar-H), 7.145 (s, 1H, Ar-H), 6.854 (d, J=5.4Hz, 1H, Ar-H), 3.794 (s, 3H,-OCH<sub>3</sub>), 2.212 (s, 2H,-CH<sub>2</sub>-). <sup>13</sup>CNMR (100MHz, CDCl<sub>3</sub>) ppm: 167.54, 151.78, 139.87, 129.14, 128.90, 128.26, 127.45, 125.78, 124.02, 122.06, 120.76, 118.41, 112.55, 110.91, 108.87, 99.45, 52.19, 42.19; LCMS: 331.28(M<sup>+</sup>+1); Molecular formule: C<sub>21</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>; Elemental analysis: Calculated: C-76.34; H-5.49; N-8.48; Obtained: C-76.28; H-5.47; N-8.54.

#### 2-(3-methoxy-9H-carbazol-9-yl)-N-(4-hydroxyphenyl) Acetamide (6b)

White solid; Yield-86%; M.p-235-237°C; <sup>1</sup>HNMR (400MHz, CDCl<sub>3</sub>) δ ppm: 9.915 (1H, s, amide), 9.458 (s,-OH,1H), 8.214 (d, J=7.2 Hz); 7.840 (s,1H,Ar-H), 7.745 (d, J=7.6Hz, 1H, Ar-H), 7.626 (d, J=6.8Hz, 1H, Ar-H), 7.554 (d, J=8.0Hz, 1H,Ar-H), 7.523-7.175 (m, 5H, Ar-H), 7.125 (s, 1H, Ar-H), 6.954 (d, J=5.6Hz, 1H, Ar-H), 3.694 (s, 3H, -OCH<sub>3</sub>), 2.114 (s, 2H, -CH<sub>2</sub>-). <sup>13</sup>CNMR (100MHz, CDCl<sub>3</sub>) ppm: 167.54, 151.78, 139.87, 129.14, 128.90, 128.26, 127.45, 125.78, 124.02, 122.06, 120.76, 118.41, 112.55, 110.91, 108.87, 99.45, 52.19, 42.19; LCMS: 331.28(M<sup>+</sup>+1); Molecular formule: C<sub>21</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>; Elemental analysis: calculated: C-76.34; H-5.49; N-8.48; Obtained: C-76.28; H-5.47; N-8.54.

#### 2-(3-methoxy-9H-carbazol-9-yl)-N-(4-methoxyphenyl) Acetamide (6b)

Colorles ssolid; Yield-90%; M.p-221-223°C. <sup>1</sup>HNMR (400MHz, CDCl<sub>3</sub>) ppm: 9.774 (s, 1H, amide), 8.124 (d, J=7.2Hz, 1H, Ar-H), 7.855 (s, 1H,

Ar-H), 7.675 (d, J=8.8Hz, H, Ar-H), 7.610 (d, J=8.0Hz, 1H, Ar-H), 7.519-7.222 (m, 4H, Ar-H), 6.921-6.714 (m, H, Ar-H), 3.712 (s, 3H, -OCH<sub>3</sub>). 2.356 (s, 2H, -CH<sub>2</sub>-); <sup>13</sup>CNMR (100MHz, CDCl<sub>3</sub>) ppm: 168.64, 156.78, 150.87, 130.49, 128.98, 126.06, 124.88, 123.03, 121.57, 120.60, 118.73, 115.07, 112.67, 110.92, 108.33, 106.78, 100.28, 54.81, 41.96. LCMS: 361.15 (M+H); Molecular formulae: C<sub>22</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>; Elemental Analysis: Calculated: C-73.32, H-5.59, N-7.76: Obtained: C-73.27; H-5.57; N-7.82.

#### **N-(4-Fluorophenyl)-2-(3-methoxy-9H-carbazol-9-yl) Acetamide (6c)**

Colorless solid; Yield-86%; M.p-224-226°C; <sup>1</sup>HNMR (400MHz, CDCl<sub>3</sub>) ppm: 9.984 (s, 1H, amide), 8.176 (d, J=7.4Hz, 1H, Ar-H), 7.846 (s, 1H, Ar-H), 7.677 (d, J=8.0Hz, 2H, Ar-H), 7.684 (d, J=8.8Hz, 1H, Ar-H), 7.567 (d, J=7.6Hz, 2H, Ar-H), 7.489 (t, J=8.4Hz, 1H, Ar-H), 7.276 (t, J=7.2Hz, 1H, Ar-H), 7.210 (d, J=6.4Hz, 1H, Ar-H), 6.841 (d, J=7.5Hz, 1H, Ar-H), 3.625 (s, 3H, -OCH<sub>3</sub>). 2.284 (s, 2H, -CH<sub>2</sub>-); <sup>13</sup>CNMR (100MHz, CDCl<sub>3</sub>) ppm: 169.48, 160.09, 151.34, 1135.88, 129.94, 126.02, 124.77, 122.34, 121.64, 120.22, 118.46, 114.74, 111.88, 109.72, 107.64, 106.34, 100.66, 53.8642.64. LCMS: 348.87(M<sup>+</sup>); Molecular formulae: C<sub>21</sub>H<sub>17</sub>FN<sub>2</sub>O<sub>2</sub>; Elemental analysis: Calculated: C-72.40, H-4.92, N-8.04: Obtained: C-72.34; H-4.91; N-8.12.

#### **N-(4-chlorophenyl)-2-(3-methoxy-9H-carbazol-9-yl) acetoamide (6d)**

Colorless solid; Yield-87%; MP-235-237°C; <sup>1</sup>HNMR (400MHz, CDCl<sub>3</sub>) ppm: 9.746 (s, 1H, amide), 8.124 (d, J=6.8Hz, 1H, Ar-H), 7.834 (s, 1H, Ar-H), 7.785-7.430 (m, 7H, Ar-H), 7.264 (t, J=8.0Hz, 2H, Ar-H), 6.841 (d, J=7.2Hz, 1H, Ar-H), 3.591 (s, 3H, -OCH<sub>3</sub>). 2.301 (s, 2H, -CH<sub>2</sub>-); <sup>13</sup>CNMR (100MHz, CDCl<sub>3</sub>) ppm: 166.04, 150.45, 135.32, 131.39, 129.74, 128.08, 127.19, 126.68, 124.82, 121.91, 120.75, 119.33, 117.45, 112.77, 110.36, 108.44, 103.38, 53.45, 41.09. LCMS: 366.41(M<sup>+</sup>+2); Molecular formulae: C<sub>21</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>2</sub>; Elemental analysis: Calculated: C-69.14, H-4.70, N-7.68: Obtained: C-69.64; H-4.68; N-7.74.

#### **N-(4-bromophenyl)-2-(3-methoxy-9H-carbazol-9-yl) Acetamide (6e)**

Pale yellow solid; Yield-86%, MP:226-228°C; <sup>1</sup>HNMR (400MHz, CDCl<sub>3</sub>) ppm: 9.847 (s, 1H, amide), 8.145 (d, J=7.2Hz, 1H, Ar-H), 7.820 (s, 1H, Ar-H), 7.723 (d, J=7.6Hz, 1H, Ar-H), 7.645 (d, J=8.0Hz, 1H, Ar-H), 7.624 (d, J=8.8Hz, H, Ar-H), 7.540 (d, J=6.0Hz, 1H, Ar-H), 7.472 (t, J=8.4Hz, 2H, Ar-H), 7.227 (t, J=6.8Hz, 2H, Ar-H), 6.952 (d, J=7.6Hz, 1H, Ar-H), 3.714 (s, 3H, -OCH<sub>3</sub>), 2.068 (s, 2H, -CH<sub>2</sub>-); <sup>13</sup>CNMR (100MHz, CDCl<sub>3</sub>) δ ppm: 166.70, 150.84, 135.72, 130.86, 128.92, 127.18, 126.05, 124.55, 121.04, 119.90, 117.38, 113.09, 110.49, 108.68, 103.61, 50.84, 40.22.32; LCMS: 410.78(M+2); Molecular formulae: C<sub>21</sub>H<sub>17</sub>BrN<sub>2</sub>O<sub>2</sub>; Elemental analysis: Calculated: C-61.63, H-4.19, N-6.84: Obtained C-61.56; H-4.18; N-6.91.

#### **N-(4-cyanophenyl)-2-(3-methoxy-9H-carbazol-9-yl) Acetamide (6f)**

Red compound; Yield-87%; MP: 232-234°C; <sup>1</sup>HNMR (400MHz, CDCl<sub>3</sub>) ppm: 9.994 (1H, s, amide), 8.194 (d, J=7.6Hz, 1H, Ar-H), 7.960 (s, 1H, Ar-H), 7.867 (d, J=8.0Hz, 2H, Ar-H), 7.684 (d, J=8.0Hz, 1H, Ar-H), 7.635 (d, J=6.4Hz, 1H, Ar-H), 7.574 (d, J=7.2Hz, 2H, Ar-H), 7.476 (t, J=7.2Hz, 1H, Ar-H), 7.246 (t, J=6.4Hz, 1H, Ar-H), 6.896 (d, J=8.4Hz, 1H, Ar-H), 3.608 (s, 3H, -OCH<sub>3</sub>). 2.250 (s, 2H, -CH<sub>2</sub>-); <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) ppm: 169.45, 152.78, 140.86, 131.49, 128.77, 127.45, 125.62, 123.44, 121.76, 120.55, 118.89, 117.66, 114.78, 111.39, 109.09, 107.54, 99.14, 54.82, 40.74. LCMS: 354.39 (M+H); Molecular formulae: C<sub>22</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>; Elemental analysis: Calculated: C-74.35, H-4.82, N-11.82: Obtained: C-74.29; H-4.81; N-11.90.

#### **2-(3-methoxy-9H-carbazol-9-yl)-N-(4-nitrophenyl) Acetamide (6g)**

Paler solid; Yield-84%; R<sub>f</sub>: .055 (ethylacetate: n-hexane, 45%); mp: 257-259°C <sup>1</sup>HNMR (400MHz, CDCl<sub>3</sub>) ppm: 9.948 (1H, s, amide), 8.219 (d, J=8.0Hz, 2H, Ar-H), 8.015 (d, J=7.0Hz, 1H, Ar-H), 7.815 (s, 1H, Ar-H), 7.764 (d, J=7.2Hz, 2H, Ar-H), 7.614 (d, J=8.4Hz, 2H, Ar-H), 7.605 (d, J=7.0Hz, 1H, Ar-H), 7.584 (d, J=7.2 Hz, 1H, Ar-H), 7.449 (t, J=7.0Hz, 1H, Ar-H), 7.215 (t, J=6.8Hz, 1H, Ar-H), 6.965 (d, J=8.0Hz, 1H, Ar-H), 3.658 (s, 3H, -OCH<sub>3</sub>)

.2.424 (s, 2H, -CH<sub>2</sub>-); <sup>13</sup>CNMR (100MHz, CDCl<sub>3</sub>) ppm: 169.64, 152.76, 145.37, 40.09, 129.44, 128.37, 125.58, 123.12, 122.74, 121.08, 119.94, 117.86, 114.76, 110.39, 108.74, 107.65, 101, 74.54.81, 40.36. LCMS: 374.62 (M-H); Molecular formulae: C<sub>21</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>; Elemental analysis: Calculated: C-67.18, H-4.55, N-11.18: Obtained: C-67.13; H-4.54; N-11.25.

## RESULTS AND DISCUSSION

The reaction sequences scaffold to various titled analgueous is as shown "Scheme-1". The carbazoles analogous were prepared by incorporating new pharmacophore dues amides linkage position at "9" of the carbazoles moiety by using a conventional method in which methoxy aniline and 1, 2-dichloro benzene was taken as a starting material in presence of CuI<sub>2</sub> give the corresponding, 3-methoxy-9H-Carbazole. This compound treated with chloroacetic chloride to leading to 2-(3-methoxy-9H-carbzol-9-yi) acetyl chloride which is reacted with substituted aromatic primary amines to give 2-(3-methoxy-9H-carbazol-9-yl)-N-phenylacetamide analgueous. Titled compounds were identified based on their physical properties i.e., solubility, melting point, thin chromatographic methods (TLC).

The amide analogues can be synthesized from 2-(3-methoxy-9H-carbazole-9-yl) acetyl chloride coupled with substituted aromatic primary amine. Initially, the reaction mixture carried at room temperature the reactant consumes only 50% and obtained the product. The temperature of the reaction mixture raised at reflux, the reaction completed 5 hrs and 90% product obtained.

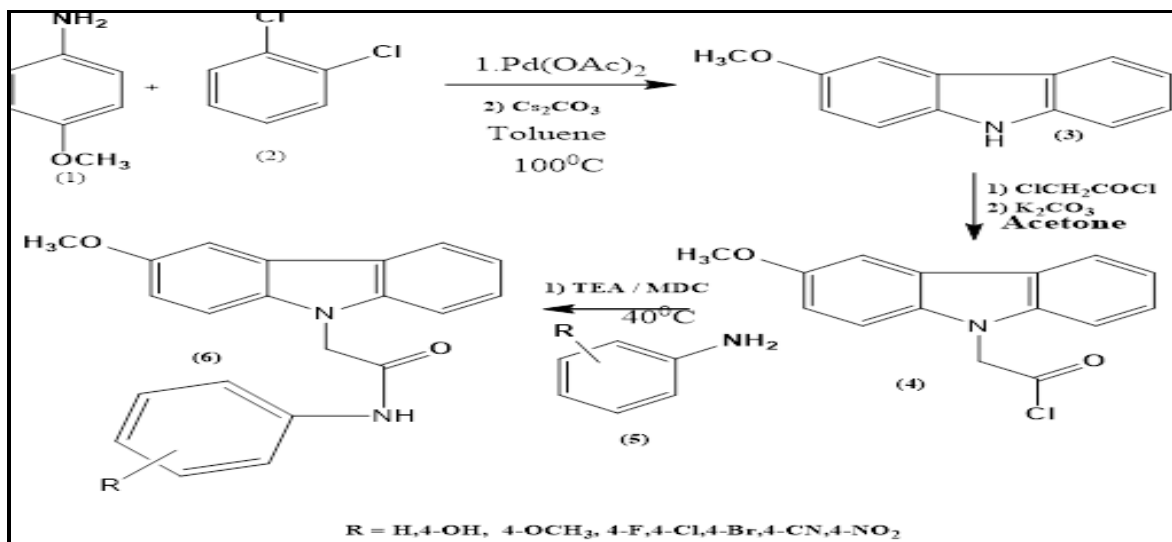
2-(3-methoxy-9H-carbazole-9-yl) acetyl chloride can be obtained from 3-methoxy-9H-carbazole treated with chloroacetic chloride in the presence of potassium carbonate in acetone. The reaction condition started at room temperature and continued 10hrs. The reaction was completed and converts 90% of product was obtained in 5hrs due to slowly heat raise at 50-55°C.

3-methoxy-9H-cabazoles obtained from 4-methoxy aniline with 1, 2-dichlorobenzene in the presence of Copper iodide and alkali base such as Cs<sub>2</sub>CO<sub>3</sub>

carried at elevated temperature (120°C) and reaction completed in 5 hrs. The 79% of product was obtained. In this reaction 1, 2- dichlorobenzene used not only reactants but also solvent.

The spectroscopic methods are submitted in the supplementary material for this article. The <sup>1</sup>HNMR spectra exhibited a peak at 11.174 δppm shown the presence of -COOH proton, a peak between 8.2192-6.667 δppm showed aromatic proton, a peak exhibited between 3.658-3.515 δppm represents methoxy protons, a peak shown between 8.546-8.345 δppm indicates hydroxyl proton(-OH) and a peak exhibited between 2.424-2.054 δppm represents -CH<sub>2</sub>-protons. The <sup>13</sup>CNMR spectra recorded a peak maximum at 169.5 δppm is belongs to the carbonyl group in carboxylic group. The LCMS spectra recorded the molecular weight of halogen compounds represent (M+2) peak. The derivatives "6a, 6c,6g,6i" exhibited (M<sup>+</sup>+H) peacks whereas compounds "6b,6i and 6j" given (M<sup>+</sup>-H) peaks.

The rate of reaction of 2-(3-methoxy-9H-carbazol-9-yl)-N-phenylacetamide analgueous depends on moderate temperature as well as nature of the substitution at phenyl ring. The rate of reaction of the titled derivatives containing Electron withdrawing better than electron releasing group of the derivatives and the yield of these compounds varies with aromatic primary amine having electron releasing group more than that of electron withdrawing group. The result of these analogous indicated that the compounds bearing electron donating groups gives more yield than the electron with drawing groups.



Scheme No.1

## CONCLUSION

To find out this experiment, we prepared the ten 2-(3-methoxy-9H-carbazol-9-yl)-N-phenylacetamide derivatives. The compounds bearing electron releasing groups and electron withdrawing groups including halogen containing derivatives. The percentage of the derivatives acquired electron donating group (92%) compared with electron withdrawing group of the compounds. As shown scheme-1, the compound "3" obtained using CuI<sub>2</sub> is an excellent coupling reagent. The compound "4" prepared using alkali base in neutral polar solvent and derivatives of (6a-6j) synthesized by organic base (TEA) in non-polar solvent (DCM).

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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