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# Synthesis And Biological Evaluation of Newly Substituted 2-Chloro-9-H-Carbazole-3-Carbaldehyde Derivatives as Potent Anticonvulsants

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**Abstract**

(E)-N-((2-chloro-9H-carbazol-3-yl) methylene) benzylamine derivatives were synthesized by using the vielsmeier-Haack reagent and evaluated for Anticonvulsant activity by using Maximal Electroshock (MES) method. In the first step, 2-chloro-9-H-carbazole-3-carbaldehyde formed via vielsmeier-Hack reaction in the presence of carbazole and vielsmeier reagent (DMF and POCl3). In a second step further reaction of carbaldehyde with different types of substituted aniline(3a-g) for the synthesis of ((E)-N-((2-chloro-9H-carbazol-3-yl) methylene) benzylamine) (4a-g). The substituted derivative was identified by TLC, FT-IR, and 1H NMR. All derivatives were evaluated for anticonvulsant activity in which compound 4e ((E)-N-((2-chloro-9H-carbazol-3-yl) methylene) benzenamine) showed maximum response as compared to standard phenytoin drug. The rest of the compounds (4a-d &f-g) showed mild to moderate response for anticonvulsant activity.

**Keywords**: Carbazole, Schiff base, vielsmeier-hack reaction, anticonvulsant activity.

## Introduction

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The word epilepsy is derived from the Greek word epilambanein (to seize) and was first used by Hippocrates. Epilepsy is the most frequent neurological infection characterized by excessive temporary neuronal discharge. Epilepsy is a disorder of the brain recognized by the event of uncontrolled neuronal firing called seizures. The overall prevalence of the disease is 1.0% of the population and up to 50 million people worldwide [1]. Previous studies showed that a significant percentage of individuals (20%–30%) using anti-epileptic drugs are resistant to the currently used therapeutic agent [2]. Phenytoin, carbamazepine, and Lamotrigine are some antiepileptic drugs that prevent unnecessary neuronal firing by attaching to a site nearest to the inactivation gate, thus delaying the inactivation of Voltage-gated sodium ion channels (VGSCs). Some antiepileptic drugs like Gabapentin and Pregabalin enhance the biosynthesis of GABA by acting on the rate-limiting enzyme L-glutamic acid decarboxylase (GAD) which catalyzes the conversion of L-glutamic acid to GABA.

Thus, there is a continuous need to explore and develop other novel molecules related to the carbazole nucleus for the better treatment of convulsions [3]. It has long been known that carbazole-containing compounds show a broad range of pharmacological activities such as antioxidant [4], anti-inflammatory [1], anti-bacterial [5], antitumor [6], anticonvulsant [7], antitubercular [8], antidiabetic [9], etc. Since the carbazole nucleus exhibits anticonvulsant activity and it has been observed in various kinds of research that the introduction of some other heterocyclic moiety results in enhanced anticonvulsant activity [10].

Taking these observations into account and as a part of an ongoing research program on the development of new anticonvulsant agents, the synthesis and pharmacological activities associated with carbazole moiety are reported here.

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## Experimental

### Material and Methods:

The chemicals and reagents were procured from S.D. Fine and Sigma-Aldrich were used as such. Digital melting point apparatus was used for the determination of melting point that was uncorrected. Progress of the reactions was monitored by thin-layer chromatography on silica gel G plates, using iodine vapors and a UV chamber as visualizing agents. The synthesized compounds were subjected to physical and spectral analysis.

### General procedure:

***General procedure for the synthesis of 2-Chloro-9-H-Carbazole-3-Carbaldehyde (2)***

Dimethyl formamide (DMF)(0.03mole) was placed on an ice bath (0 to -5°C) and then $POCl_3$ (0.09mole) was added dropwise in DMF. After the dropwise addition of $POCl_3$, the vielsmeier reagent was continued stirred for 30 minutes, then 0.01 mole of carbazole and vielsmeier reagent was placed in a round bottom flask. Refluxed the mixture for 8 hours and then the mixture was cooled at room temperature and then pour into the crushed ice with fast stirring, a pale-yellow precipitate was formed immediately. The precipitate was filtered, washed, and recrystallized with ethanol to give Yield 64.5%; eluent n-hexane: diethyl ether (1:1); Rf value 0.69; creamish white amorphous powder; m.p 147-149 ºC; IR spectra (KBr; $cm^{-1}$): 3417(N-H), 3051 (C-H, Ar), 2869 (C-H, Ali),1694 (N=CH, Imine), 1493 (C=C, Ar), 1240 (C-N), 574 (C-Cl).

***General procedure for the synthesis of substituted 2-Chloro-9-H-Carbazole-3-Carbaldehyde derivatives (4a-g)***

2-Chloro-9-H-carbazole-3-carbaldehyde **(2)** (0.01mole) and substituted aromatic amines **(3)** (0.01 mole) were added to 50 ml of ethanol along with the 2ml catalytic amount of hydrochloric acid and reflux the above mixture for 8 hours. The completion of the reaction is checked by using TLC. After the completion refluxing mixture was poured onto crushed ice, formed precipitate was filtered and washed

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with water. Recrystallization of the synthesized compound **(4a-g)** was performed by using ethanol.

**Detection Method:**

All the synthesized compounds were detected at lab scale through thin layer chromatography (TLC) and For the spectral analysis of compounds SHIMADZU IR from GLA University, Mathura, and NMR from CDRI lab Lucknow were used.

**Results and Discussion**

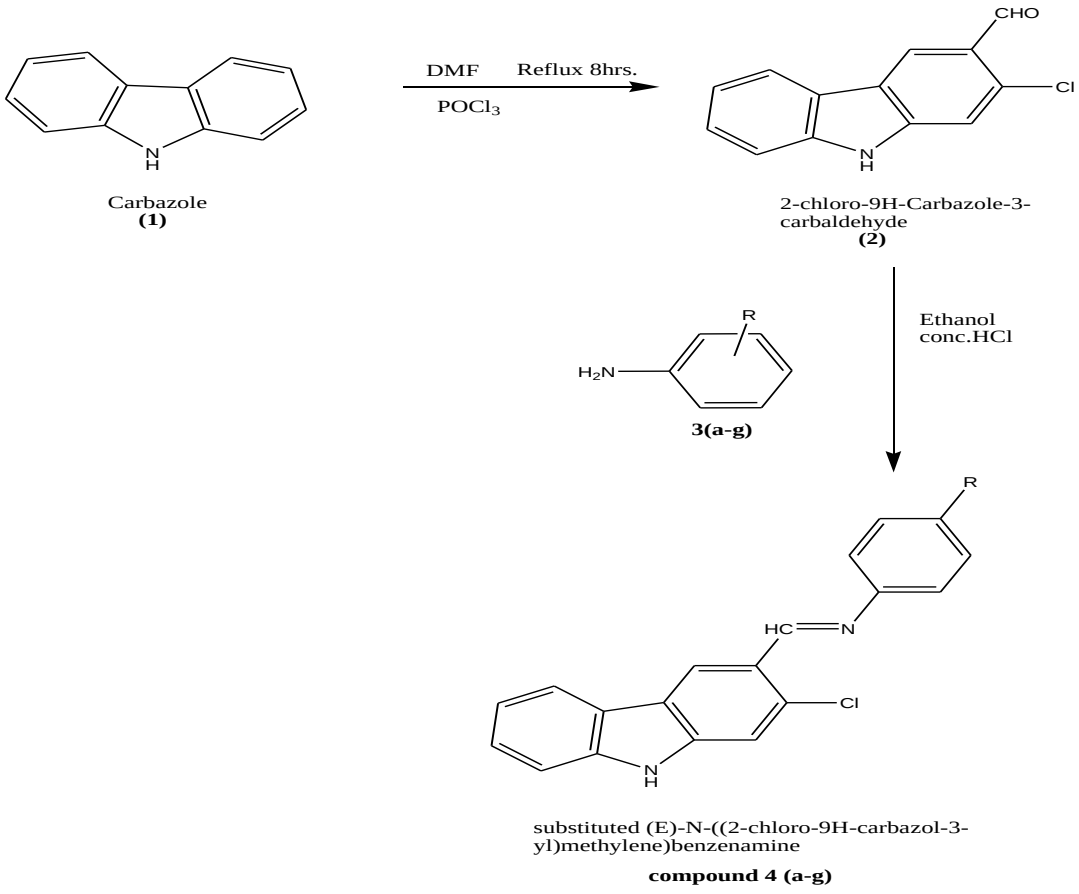


**Fig.1 Scheme for the synthesis of novel carbazole derivatives**

Carbazole is an aromatic heterocyclic organic compound. It has a tricyclic structure, consisting of two six-membered benzene rings fused on either side of a five-membered nitrogen-containing ring. The structure of the compound is based on the indole structure in which a second benzene ring is fused onto the five-membered ring at the 2–3 position of indole. All the desired carbazole derivatives were prepared

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by a two-step reaction, summarized in the scheme. In the first step, carbazole was allowed to react with vielsmeier haack reagent which form 2-chloro-9-H-3-carbaldehyde (2) with 75% yield. The Vilsmeier-Haack reagent was prepared by adding POCl3 dropwise to DMF at 0-5 o C and allowed to stir. Compound 2 was further reacted with different substituted amines to get carbazole derivatives by vielsmeier haack reaction.

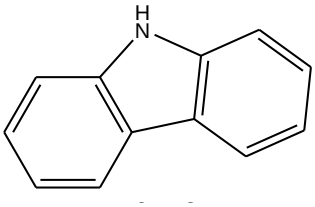


fig 2; Carbazole

All the synthesized compounds were primarily characterized by TLC (Thin Layer Chromatography) for checking the purity of the synthesized compound. In TLC silica gel G (Pore size of 60 Å with gypsum binder) is used for preparing the plate 12. The melting point of the compounds was determined with the help of an open capillary in the digital melting point apparatus 13. NMR characterization was performed using CDCl3 solvent at 400MHz 14.

**Spectral data of 4-chloro-N-((2-chloro-9H-carbazol-3-yl) methylene) benzenamine (4a)** Yield 65.23%; Rf= 0.68; white crystals; m.p 230-232 °C, IR spectra (KBr: cm-1): 3420(N-H), 3051(C-H, Ar), 2839 (C-H, Ali),1646 (N=CH, Imine), 1287 (C-N), 574(C-Cl), 1H NMR (400 MHz, (CDCl3): 3.45-3.46 (m, 3H, indole), 7.24-7.44 (m, 6H, Ar H),8.08(s,1H,C=NH), 8.55(s,1H, NH), Anal. Calcd. for $C_{19}H_{12}Cl_2N_2$ : C, 67.27; H, 3.57; N, 8.26 found- C, 67.57; H, 3.17; N, 8.20.

**Spectral data of N-((2-chloro-9H-carbazol-3-yl) methylene)-2-nitrobenzenamine (4b)**

Yield 69.96%; Rf= 0.71; pale yellow crystals; m.p 233-235 °C, IR spectra (KBr; cm-1): 3420(N-H), 3058(C-H, Ar), 2927 (C-H, Ali), 1642 (N=CH, Imine), 1451(N=O), 1205(C-N), 757(C-Cl), 1H NMR (400 MHz, (CDCl3): 7.21-7.24 (m, 6H, Ar H), 7.42-7.43(m,4H,Ar H), 8.09(s,1H,C=NH), 9.12(s,1H, NH), Anal. Calcd. for $C_{19}H_{12}ClFN_2O_2$ : C, 65.24; H, 3.46; N, 12.01; found- C, 65.14; H, 3.16; N, 12.05.

**Spectral data of 3,4-dichloro-N-((2-chloro-9H-carbazol-3-yl) methylene) benzenamine (4c)** Yield 57.21%; Rf= 0.69; light yellow crystals; m.p 234-236 °C, IR spectra (KBr; cm-1): 3421(N-H),3058(C-H,

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Ar),2927 (C-H, Ali),1642 (N=CH, Imine), 1451(N=O), 1139(C-N), 757(C-Cl), 1H NMR (400 MHz, (CDCl3): 7.18-7.38 (m, 6H, Ar H), 7.42-7.46 (m,3H,Ar H), 8.05(s,1H,C=NH), 9.07(s,1H, NH), Anal. Calcd. for C19H11Cl3N2: C, 61.07; H, 2.97; N, 7.50 found C, 61.02; H, 2.87; N, 7.20.

**Spectral data of N-((2-chloro-9H-carbazol-3-yl) methylene)-2-fluorobenzenamine (4d)** Yield 75.35%; Rf= 0.72; off white crystals; m.p 235-237 °C, IR spectra (KBr; cm-1):3418 (N-H), 3050 (C-H, Ar), 2819 (C-H, Ali),1690 (N=CH, Imine), 1328 (C-N),1240, 573(C-Cl), 1H NMR (400 MHz, (CDCl3): 6.73-6.84 (m, 6H, Ar H), 6.95-7.19 (m,4H, Ar H), 7.20(s,1H, C=NH), 9.08(s,1H, NH), Anal. Calcd. for C19H12ClFN2: C, 70.70; H, 3.75; N, 8.68 found C, 70.20; H, 3.15; N, 8.38.

**Spectral data of N-((2-chloro-9H-carbazol-3-yl) methylene)-2-phenoxybenzenamine (4e)** Yield 55.72%; Rf= 0.69; light brown crystals; m.p 230-232 °C, IR spectra (KBr; cm-1): 3418(N-H),3051(C-H,Ar), 2897(C-H, Ali) 1646 (N=CH, Imine), 1205(C-N), 574(C-Cl), 1H NMR (400 MHz, (CDCl3): 6.94-7.05(m,9H,Phenoxy), 7.32-7.98 (m, 6H, Ar H), 8.00(s,1H,C=NH), 9.12(s,1H, NH), Calcd.for C25H17ClN2O: C, 75.66; H, 4.32; N, 7.06; found C, 75.26; H, 4.12; N, 7.01.

**Spectral data of (Z)-2,3-dichloro-N-((2-chloro-9H-carbazol-3-yl) methylene) benzenamine (4f)** Yield 59.62%; Rf= 0.65; off white crystals; m.p 231-233 °C, IR spectra (KBr; cm-1):3420 (N-H), 3050 (Ar C-H), 2860 (C-H, Ali), 1691 (C=N), 574 (C-Cl), 1353(N=O), 1093 (C-N), 748(C-Cl), 1H NMR (400 MHz, (CDCl3): 6.93-7.69 (m, 9H, Ar H), 7.61(s,1H, C=NH), 10.2(s,1H, NH), Calcd. for C19H11Cl3N2: C, 61.07; H, 2.97; N, 7.50 found C, 61.02; H, 2.25; N, 7.48.

**Spectral data of (Z)-N-((2-chloro-9H-carbazol-3-yl) methylene)-3-nitrobenzenamine (4g)** Yield 61.39%; Rf= 0.68; light brown crystals; m.p 242-244 °C, IR spectra (KBr; cm-1): 3420 (N-H), 3050 (Ar C-H), 2860 (C-H, Ali), 1691 (C=N), 1353(N=O), 1093 (C-N), 748(C-Cl), 574 (C-Cl), 1H NMR (400 MHz, (CDCl3): 7.08-7.30 (m, 6H, Ar H), 7.32-7.97 (m,4H, Ar H), 8.32(s,1H, C=NH), 9.28(s,1H, NH), Anal. Calcd. for C19H12ClN3O2: C, 65.24; H, 3.46; N, 12.01; found C, 65.12; H, 3.22; N, 12.51.

## BIOLOGICAL ACTIVITY

### Anticonvulsant activity

For the anticonvulsant study of synthesized drugs female Swiss albino mice, 25-34g were used. The

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study protocol was approved by the Institutional Animal Ethical Committee, Hygia Institute of Pharmaceutical Education and Research, Lucknow, India with no. HIPERIAEC89022022. The maximal electroshock method in mice is used primarily to determine the potency of drugs for treating grand mal epilepsy. Electric stimulation produces tonic hind limb extension, which is reduced by antiepileptics and some centrally-acting drugs15.

In the experiments, 9 groups were taken, each comprising 2 animals (n=2), and phenytoin, (25mg/kg body weight) was used as the standard drug. Electric current (50mA for 0.2 seconds) was used as the inducing agent for seizures. All of the mice were given doses based on their body weight. The doses include vehicle/test chemicals/and the standard drug.

The potency of the treatment compounds to offer protection to albino mice from convulsions was assessed by their anticonvulsant activity. After 30 minutes of oral administration of vehicle/test compounds/standard drug in 0.5 percent aqueous CMC suspensions, the trial began. The MES apparatus with corneal electrodes was used to produce. Each animal received a 50mA stimulation delivered via corneal electrode for 0.2 seconds. Animals were examined for around 15 minutes, and phases such as extensor, clonus, stupor, flexion, and death were recorded. All the tested compounds were capable to shorten the extensor-tonus phase of convulsion.

**Table 1. Anticonvulsant activity of synthesized compounds in the Maximal Electroshock seizure (MES) model**

| S. No. | Comp. Code | Duration of Various Phases (Time in seconds) | | | |
|---|---|---|---|---|---|
| | | Flexion | Extensor | Clonus | Stupor |
| 1. | Control | 3.18±0.71 | 9.60±0.52 | 18.15±0.94 | 27.39±0.49 |
| 2. | Standard (PHT) | 1.60±0.51 | 2.50±0.50 | 3.58±0.40 | 10.75±0.32 |
| 3. | 4a | 6.5±0.31 | 9.4±0.24 | 13.2±1.01 | 26.2±0.89 |
| 4. | 4b | 7±0.44 | 8.2±0.20 | 11.6±0.78 | 13.6±1.24 |
| 5. | **4c** | **3.90±0.24**** | **6.50±0.20** | **10.65±1.68** | **25.2±0.74** |

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| | | | | | |
|---|---|---|---|---|---|
| **6.** | 4d | 7.8±0.58 | 9±0.36 | 11.2±0.58 | 26.8±0.37 |
| **7.** | **4e** | **3.1±0.24**$^{***}$ | **4.4±0.37** | **6.2±0.60** | **17.4±0.74** |
| **8.** | **4f** | **3.6±0.24**$^{**}$ | **5.40±0.24** | **9.43±0.74** | **21.4±0.74** |
| **9.** | 4g | 11.2±0.24 | 7.4±0.24 | 12.2±0.74 | 27.4±0.74 |

The statistical analysis was carried out using two-way ANOVA followed by Dunette's test where ***p<0.001, **p<0.01,*p<0.05. All the treated groups are compared with standard drugs.

## Conclusions

The present study has identified the anticonvulsant effect of compounds derived from 2-Chloro-9-H-Carbazole-3-Carbaldehyde. The compound 4e and 4f showed potent anticonvulsant activity as compared with standard drug phenytoin. Thus the study revealed that these compounds have potential anticonvulsant activity and can be used for further studies.

## Acknowledgments

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## References

(1) Bashir, M.; Bano, A.; Ijaz, A. S.; Chaudhary, B. A. Recent Developments and Biological Activities of N-Substituted Carbazole Derivatives: A Review. *Molecules (Basel, Switzerland)* **2015**, *20*, 13496-13517.

(2) Neels, H. M.; Sierens, A. C.; Naelaerts, K.; Scharpe, S. L.; Hatfield, G. M.; Lambert, W. E. J. C. C.; Medicine, L. Therapeutic drug monitoring of old and newer anti-epileptic drugs. **2004**, *42*, 1228-1255.

(3) Singh, M.; Kaur, M.; Kukreja, H.; Chugh, R.; Silakari, O.; Singh, D. J. E. j. o. m. c. Acetylcholinesterase inhibitors as Alzheimer therapy: from nerve toxins to neuroprotection. **2013**, *70*, 165-188.

(4) Bhardwaj, N.; Pathania, A.; Kumar, P. J. C. T. M. Naturally available nitrogen-containing fused heterocyclics as prospective lead molecules in medicinal chemistry. **2021**, *7*, 5-27.

ISSN PRINT 2319 1775 Online 2320 7876
  UGC CARE Listed ( Group -I) Journal Volume 11, Iss 4, 2022

(5) Zhou, S.-l.; Tang, H.-l.; Yao, M.; Cao, S.-n.; Zhuang, L.-y.; Cao, C.-s.; Shi, Y.-h. J. C. P. Synthesis and antibacterial activity of fluorinated carbazoles. **2019**, *73*, 2477-2484.

(6) Liu, L.-X.; Wang, X.-Q.; Zhou, B.; Yang, L.-J.; Li, Y.; Zhang, H.-B.; Yang, X.-D. J. S. r. Synthesis and antitumor activity of novel N-substituted carbazole imidazolium salt derivatives. **2015**, *5*, 1-20.

(7) Sharma, D.; Kumar, N.; Pathak, D. J. J. o. t. S. C. s. Synthesis, characterization and biological evaluation of some newer carbazole derivatives. **2014**, *79*, 125-132.

(8) Sellamuthu, S.; Bhat, M. F.; Kumar, A.; Nath, G.; Singh, S. K. J. C. B. C. Design, Synthesis and Biological Evaluation of Carbazole Derivatives as Antitubercular and Antibacterial Agents. **2019**, *15*, 83-97.

(9) Palwankar, S. M.; Kale, P. P.; Kadu, P. K.; Prabhavalkar, K. J. J. o. R. i. P. S. Assessment of antidiabetic activity of combination of Murraya koenigii leaves extract and Vitis vinifera seeds extract in alloxan-induced diabetic rats. **2020**, *9*, 79.

(10) Dalkara, S.; Karakurt, A. J. C. t. i. m. c. Recent progress in anticonvulsant drug research: strategies for anticonvulsant drug development and applications of antiepileptic drugs for non-epileptic central nervous system disorders. **2012**, *12*, 1033-1071.

(11) Arbiser, J. L.; Govindarajan, B.; Battle, T. E.; Lynch, R.; Frank, D. A.; Ushio-Fukai, M.; Perry, B. N.; Stern, D. F.; Bowden, G. T.; Liu, A.; Klein, E.; Kolodziejski, P. J.; Eissa, N. T.; Hossain, C. F.; Nagle, D. G. Carbazole Is a Naturally Occurring Inhibitor of Angiogenesis and Inflammation Isolated from Antipsoriatic Coal Tar. *Journal of Investigative Dermatology* **2006**, *126*, 1396-1402.

(12) Gocan, S. J. J. o. C. S. Stationary phases for thin-layer chromatography. **2002**, *40*, 538-549.

(13) Asma, K. B.; Manju, N.; Chandra, M. M. J. J. M. C. D. D. Synthesis, Antimicrobial, Antioxidant and Molecular Docking Study of Some Novel Bis-1, 2, 4-Triazolo [3, 4-B]-1, 3, 4-Thiadiazoles. **2018**, *1*.

(14) Sankar, R. M.; Roy, T. K.; Jana, T. J. B. o. M. S. Functionalization of terminal carbon atoms of hydroxyl terminated polybutadiene by polyazido nitrogen rich molecules. **2011**, *34*, 745-754.

(15) Castel-Branco, M.; Alves, G.; Figueiredo, I.; Falcão, A.; Caramona, M. The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. **2009**.

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**Table 1. Anticonvulsant activity of synthesized compounds in Maximal Electroshock seizure (MES) model**

| S. No. | Comp. Code | Duration of Various Phases (Time in seconds) | | | |
|---|---|---|---|---|---|
| | | **Flexion** | **Extensor** | **Clonus** | **Stupor** |
| **1.** | Control | 3.18±0.71 | 9.60±0.52 | 18.15±0.94 | 27.39±0.49 |
| **2.** | Standard (PHT) | 1.60±0.51 | 2.50±0.50 | 3.58±0.40 | 10.75±0.32 |
| **3.** | 4a | 6.5±0.31 | 9.4±0.24 | 13.2±1.01 | 26.2±0.89 |
| **4.** | 4b | 7±0.44 | 8.2±0.20 | 11.6±0.78 | 13.6±1.24 |
| **5.** | **4c** | **3.90±0.24**** | **6.50±0.20** | **10.65±1.68** | **25.2±0.74** |
| **6.** | 4d | 7.8±0.58 | 9±0.36 | 11.2±0.58 | 26.8±0.37 |
| **7.** | **4e** | **3.1±0.24***** | **4.4±0.37** | **6.2±0.60** | **17.4±0.74** |
| **8.** | **4f** | **3.6±0.24**** | **5.40±0.24** | **9.43±0.74** | **21.4±0.74** |
| **9.** | 4g | 11.2±0.24 | 7.4±0.24 | 12.2±0.74 | 27.4±0.74 |

## Figure Captions

Fig.1 Scheme for synthesis of novel carbazole derivatives

Fig. 2 Carbazole

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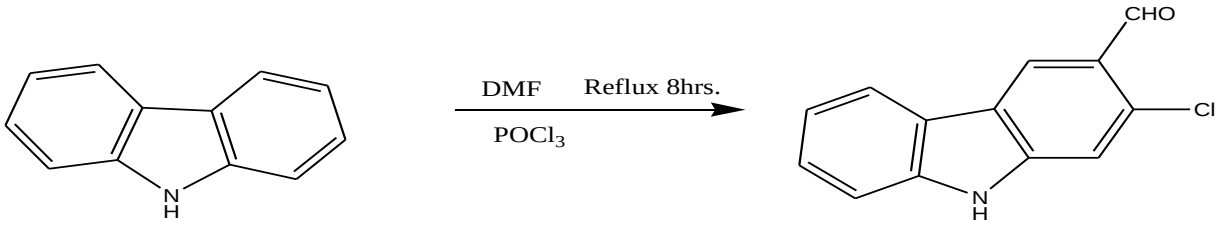
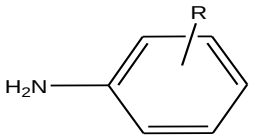


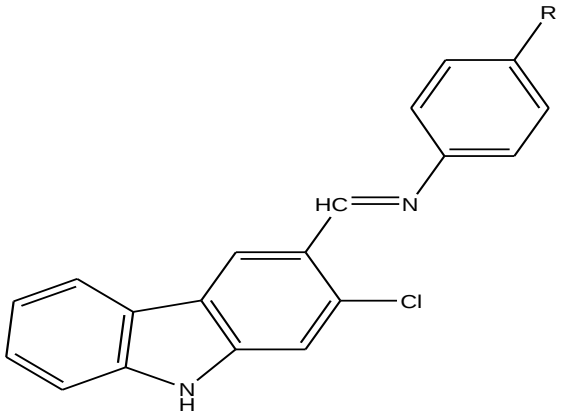

Carbazole
**(1)**

2-chloro-9H-Carbazole-3-carbaldehyde
**(2)**




**3(a-g)**

Ethanol
conc.HCl




substituted (E)-N-((2-chloro-9H-carbazol-3-yl)methylene)benzenamine

**compound 4 (a-g)**