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# THERMAL/MICROWAVE/DESMEDIATED SYNTHESIS OF IMINOTETRAHYDROCARBAZOLES AND THEIR BIOLOGICAL EVALUATION

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**ABSTRACT**

A new series of iminotetrahydrocarbazoles **(3a-g** and**3′a-g)** were synthesized via conventional, microwave and DES approach. All compounds were characterized through FTIR, $^{1}$H-NMR and MS. The synthesized iminotetrahydrocarbazoles were tested for various biological activities like anti-tumor activity using potato disc assay as well as toxicity assays. It was found that among all the screened derivatives. **3′b**, **3′e**,**3′f** and **3′g**exhibited 90, 96, 92 and 93% anti-tumor activity as compared to the parent compound at 1000ppm. Most of the compounds were cyto-friendly at 10ppm while at 1000ppm only **3′b**, **3′e**, **3′f** and **3′g**showed biologically friendly behavior (60, 80, 70 and 50% survival). Phytotoxicity showed that none of the compounds were totally suppressing the root length and there was no toxic effect on the cell division and germination of seeds.

**Keywords: Iminotetrahydrocarbazole, microwave, DES, anti-tumor activity, toxicity**

## 1. INTRODUCTION

Among the malignant tumors, cancer is most life threatening worldwide. Recent developments in cancer research have been focused on designing potent heterocyclic motifs because phytochemicals have limited the prehistoric remedial use of natural products. Today's pressing need is the modification of known potential compounds into novel and pharmacologically active units. Carbazole being naturally occurring condensed heterocyclic system is used for the treatment of many routine and complex diseases [1]. They have demonstrated excellent anticancer, anti-HIV, antihypertensive, anti-inflammatory, antimalarial and anti-microbial activities [2]. Azomethine moiety when associated with carbazole nucleus further broadens the bio-spectrum being potent motif [3]. A

series of Schiff bases possess potent anti-tumor activity[4].

Relative ease of preparation, synthetic flexibility with bioactive features made C=N group perceptible and thus turned our interest to produce much better carbazole based Schiff derivatives. Deep eutectic solvents (DES) are now attention grabbing set of eutectics which have been recently explored in many organic reactions[5-6].These eutectics are non-toxic, easy to store and cost effective than most ionic liquids. In addition to being eco-friendly, another advantage of using choline chloride and urea based eutectics is the ability of urea to catalyze reactions via hydrogen bond catalysis [7], therefore we explored the catalytic activity of deep eutectic solvent (choline chloride-urea) in methanol for rapid and facile synthesis of compounds[8].We have already reported many carbazoles with unique functionalities[9] and this time, we are presenting novel analogues **(3a-g, 3′a-g)** furnished by electrophilic substitution of acetyl derivatives with various amino compounds and their anti-tumor activity.

## 2. MATERIALS AND METHODS

Analytical grade solvents and reagents were used throughout experimental. Silica coated Al supported TLC sheets (Merck 60 $F_{254}$, 0.2 mm thick) were run in various eluting systems and visualized under UV light of 254/365 nm (CAMAG scientific Inc). Melting points were uncorrected and determined by open capillary method. FTIR spectra were recorded on M 2000 spectrophotometer using KBr disks.$^1$H-NMR spectra was measured on 300 MHz Bruker AXS spectrometer in Acetone-$d_6$(δ, ppm).

### 2.1.General procedure for the synthesis of imine derivatives (3a-g, 3′a-g)

**2.1.1. Conventional approach:** Compound**1**or **1′** (1 mmol), amine analogue **2a-g** (1 mmol) catalyzed by NaOH (10-15%) were refluxed for suitable time as mention in table 1 and reaction progress was monitored by TLC (10% *n*-hexane: ethyl acetate and 12% dichloromethane:methanol). The flask contents were allowed to cool in ice bathand filtered. Crude product was washed and recrystallized from methanol to afford pure product **3a-g** and**3′a-g**.

**2.1.2. Microwave approach:** Compound**1** or **1′**(1 mmol) was dissolved in 1mL ethanol having 1 drop of glacial acetic acid to get clear solution followed by addition of amine analogue **2a-g**(1 mmol) and irradiated for appropriate time (Table 1). After completion of reaction, the flask contents were allowed to cool in ice bath and filtered to get crude solid.

**2.1.3. DES approach:** Compound **1** or **1′**(1 mmol) and amine analogues **2a-g** (1 mmol,

Table 1) were stirred at 60-75°C in the presence of % DES in methanol. After completion of reaction, chilled water was added to mixture and filtered to get crude solid. DES was recovered from filtrate under vacuum and reused for next run.

**2.2.Spectral data**

**1-(2,3,4,9-Tetrahydro-6-nitro-9*H*-carbazol-1-yl)ethanone(1′)**

FTIR(KBr, $\upsilon_{max}$. $cm^{-1}$):3452 (aromatic N-H), 3145 (aromatic C-H), 2990 (aliphatic C-H), 1691 (C=O), 1590 (aromatic C=C), 846 (aromatic C-CH); UV/Vis (MeOH, $\lambda_{max}$, nm): 295, 280,229;$^1$H NMR($d_6$-Acetone): δ, ppm:10.52 (s, 1H, H-9), 8.09 (dd,J = 8.8, 2.4Hz 1H, H-7), 7.51 (d, J =8.8Hz, 1H, H-8), 7.32 (d, J = 2.4Hz, 1H, H-5),2.85 (s, 3H, $COCH_3$), 2.36 (t, J = 6.4Hz, 1H, H-1), 1.98-1.84 (m, 2H, Cyclo);MS (EI): *m/z*258.1206 [$M^+$].

**(*E*)-1-(1-(2,3,4,9-Tetrahydro-9*H*-carbazol-1-yl)ethylidene)urea(3a)**

FTIR(KBr, $\upsilon_{max}$. $cm^{-1}$):3452 (aromatic N-H), 3370 ($NH_2$), 3145 (aromatic C-H), 2990 (aliphatic C-H), 1670 (C=N); UV/Vis (MeOH, $\lambda_{max}$, nm): 291, 283,227;$^1$H NMR ($d_6$-Acetone): δ, ppm:10.30 (s, 1H, H-9), 7.46-7.28 (m, Ar-H),2.74 (s, 3H, $CH_3$), 2.28 (t, J = 6.4Hz, 1H, H-1), 1.85-1.79 (m, 2H, Cyclo);MS (EI): *m/z*255.1518 [$M^+$].

**(*E*)-1-(1-(2,3,4,9-Tetrahydro-6-nitro-9*H*-carbazol-1-yl)ethylidene)urea(3′a)**

FTIR (KBr, $\upsilon_{max}$. $cm^{-1}$):3440 (aromatic N-H), 3360 ($NH_2$), 3150 (aromatic C-H), 2980 (aliphatic C-H), 1665 (C=N); UV/Vis (MeOH, $\lambda_{max}$, nm): 381, 350,341; $^1$H NMR ($d_6$-Acetone): δ, ppm:10.42 (s, 1H, H-9), 8.10 (dd,J = 8.8, 2.4Hz 1H, H-7), 7.63 (d, J =8.8Hz, 1H, H-8), 7.46 (d, J = 2.4Hz, 1H, H-5),2.70 (s, 3H, $CH_3$), 2.24 (t, J = 6.4Hz,1H, H-1), 1.82-1.73 (m, 2H, Cyclo);MS (EI): *m/z*300.1502[$M^+$].

**(*E*)-1-(1-(2,3,4,9-Tetrahydro-6-nitro-9*H*-carbazol-1-yl)thiourea(3′b)**

FTIR(KBr, $\upsilon_{max}$. $cm^{-1}$): 3415 (aromatic N-H), 3370 ($NH_2$), 3145 (aromatic C-H), 2990 (aliphatic C-H),1660 (C=N), 1340 (C=S); UV/Vis (MeOH, $\lambda_{max}$, nm): 381, 350,341; $^1$H NMR ($d_6$-Acetone): δ, ppm:10.32 (s, 1H, H-9),8.08 (dd,J = 8.7, 2.3Hz, 1H, H-7), 7.48 (d, J =8.7Hz, 1H, H-8), 7.22 (d, J = 2.3Hz, 1H, H-5),2.60 (s, 3H,$CH_3$,), 1.80-1.69 (m, 2H, Cyclo); MS (EI):*m/z*316.2150 [$M^+$].

**(*E*)-1-(1-(6,7,8,9-Tetrahydro-5*H*-carbazol-8-yl)ethylidene)-2-phenylhydrazine(3c)**

FTIR(KBr, $\upsilon_{max}$. $cm^{-1}$):3450 (Aromatic N-H), 3344 (N-H), 3145 (aromatic C-H), 2940 (aliphatic C-H),1668 (C=N);UV/Vis (MeOH, $\lambda_{max}$, nm): 376, 291, 283, 227;$^1$H NMR ($d_6$-Acetone): δ, ppm:11.15 (s, 1H, Hydrazine N-H), 10.25 (s, 1H, H-9), 8.24-7.33(m, 5H, Ar-H), 6.84-7.47(m, 4H, Ar-H), 2.74 (s, 3H, $CH_3$), 2.21 (t,J = 6.2Hz,

1H, H-1), 1.72-1.84 (m, 2H, Cyclo);MS (EI): *m/z*303.1862 [$M^+$].

**(*E*)-1-(1--(2,3,4,9-Tetrahydro-6-nitro-9*H*-carbazol-1-yl)-2-phenylhydrazine (3′c)**

FTIR(KBr, $\upsilon_{max}$. $cm^{-1}$):3445 (Aromatic N-H), 3340 (N-H), 3140 (aromatic C-H), 2970 (aliphatic C-H), 1664 (C=N);UV/Vis (MeOH, $\lambda_{max}$, nm): 341, 224; $^1$H NMR ($d_6$-Acetone): δ, ppm: 11.10 (s, 1H, Hydrazine N-H),10.34 (s, 1H, H-9), 8.45-7.92 (m, 5H, Ar-H), 8.13 (dd,J = 8.7, 2.3Hz, 1H, H-7), 7.75(d, J = 2.3Hz, 1H, H-5), 7.46 (d, J =8.7 Hz, 1H, H-8), 2.68 (s, 3H,$CH_3$),2.25 (t, J = 6.2Hz, 1H, H-1),1.78-1.64 (m, 2H, Cyclo);MS (EI):*m/z*348.1816 [$M^+$].

**(*E*)-1-(1--(2,3,4,9-Tetrahydro-6-nitro-9*H*-carbazol-1-yl)-2-(2,4-dinitrophenyl)hydrazine(3′d)**

FTIR(KBr, $\upsilon_{max}$. $cm^{-1}$): 3438 (Aromatic N-H), 3340 (N-H), 3142 (aromatic C-H), 2976 (aliphatic C-H), 1664 (C=N), 1585 (aromatic C=C); UV/Vis (MeOH, $\lambda_{max}$, nm): 334, 225; $^1$H NMR ($d_6$-Acetone): δ, ppm:11.18 (s, 1H, Hydrazine N-H), 10.48(s, 1H, H-9), 8.62(dd, J = 8.6, 2.8Hz, 1H, H-5′), 8.43 (d, J = 2.8 Hz, CH, H-3′), 8.15(dd,J = 8.8, 2.4Hz, 1H, H-7), 7.98(d, J = 8.6Hz, 1H, H-6′), 7.67(d, J =8.8Hz, 1H, H-8),7.58(d, J = 2.4Hz, 1H, H-5), 2.31(t, J = 6.2Hz, 1H, H-1), 2.09 (s, 3H, $CH_3$);1.88-1.67(m, 2H, Cyclo), MS (EI):*m/z*438.1756[$M^+$].

**(*E*)-2-(1-(2,3,4,9-Tetrahydro-6-nitro-9*H*-carbazol-1-yl)ethylideneamino)acetic acid(3′e)**

FTIR(KBr, $\upsilon_{max}$. $cm^{-1}$):3455 (Aromatic N-H), 3140 (aromatic C-H), 2990 (aliphatic C-H), 2923 (OH), 1745 (acid C=O), 1658 (C=N);UV/Vis (MeOH, $\lambda_{max}$, nm): 381, 341; $^1$H NMR ($d_6$-Acetone): δ, ppm:11.12 (s, 1H, Hydrazine N-H), 10.30(s, 1H, H-9), 8.21(dd,J = 8.8, 2.3Hz, 1H, H-7), 7.58(d, J =8.8Hz, 1H, H-8), 7.14(d, J = 2.3Hz, CH, H-5), 3.48 (s, 2H), 2.79 (s, 3H, $CH_3$), 2.18 (t, J = 6.4Hz, 1H, H-1), 1.76-1.64 (m, 2H, Cyclo);MS (EI):*m/z*315.1508[$M^+$].

**(*E*)-2-(1-(2,3,4,9-Tetrahydro-9*H*-carbazol-1-yl)ethylideneamino)propanoic acid(3f)**

FTIR(KBr, $\upsilon_{max}$. $cm^{-1}$):3438 (Aromatic N-H), 3145 (aromatic C-H), 2950 (aliphatic C-H), 2923 (OH), 1745 (acid C=O), 1660 (C=N); UV/Vis (MeOH, $\lambda_{max}$, nm): 348, 226; $^1$H NMR ($d_6$-Acetone): δ, ppm:10.43(s, 1H, H-9), 7.32-8.14(m, 4H),7.26-6.61 (m, Ar-ring, 4H), 2.57 (s, 3H, $CH_3$),2.42 (s, 1H), 1.83-1.75 (m, 2H, Cyclo),1.12 (s, 3H, $CH_3$);MS (EI):*m/z*284.1710[$M^+$].

**(*E*)-2-(1-(2,3,4,9-Tetrahydro-6-nitro-9*H*-carbazol-1-yl)ethylideneamino)propanoic acid(3′f)**

FTIR(KBr, $\upsilon_{max}$. $cm^{-1}$):3442 (Aromatic N-H), 3145 (aromatic C-H), 2960 (aliphatic C-H), 2930 (OH), 1745 (acid C=O), 1660

(C=N); UV/Vis (MeOH, $\lambda_{max}$, nm): 224; $^1$H NMR ($d_6$-Acetone): δ, ppm:10.36 (s, 1H, H-9),8.33(dd,J = 8.8, 2.3Hz, 1H, H-7), 7.49 (d, J =8.8 Hz, 1H, H-8),7.18(d, J = 2.3Hz, CH, H-5),2.46 (s, 1H,), 2.24(t, J = 6.4Hz, 1H, H-1), 2.21 (s, 3H, $CH_3$), 1.09 (s, 3H, $CH_3$);MS (EI): *m/z*329.1658[$M^+$].

**(2*E*)-2-(1-(-(2,3,4,9-Tetrahydro-9*H*-carbazol-1-yl)ethylideneamino)-2-(4*H*-imidazol-4-yl)acetic acid(3g)**

FTIR(KBr, $\upsilon_{max}$. $cm^{-1}$):3400 (aromatic N-H), 3036 (aromatic C-H),2986 (aliphatic C-H),2938 (OH),1740 (C=O), 1645 (C=N);UV/Vis (MeOH, $\lambda_{max}$, nm): 291,283, 353, 227; $^1$H NMR ($d_6$-Acetone): δ, ppm:10.38(s, 1H, H-9),10.20 (s, 1H, H-9), 8.79 (s,1H, Imidazole-CH), 7.59(s, 1H, Imidazole-CH), 7.38-8.37(m, 4H, Ar-H), 2.76 (s, 3H, $CH_3$), 2.29(s, 1H), 2.27(t, J = 6,4Hz, 1H, H-1), 1.88-1.67 (m, 2H, Cyclo), 2.14(d, J = 2.8Hz, 1H, H-1′);MS (EI):*m/z*336.1820[$M^+$].

**(2*E*)-2-(1-(2,3,4,9-tetrahydro-6-nitro-9*H*-carbazol-1-yl)ethylideneamino)-2-(4*H*-imidazol-4-yl)acetic acid(3′g)**

FTIR(KBr, $\upsilon_{max}$. $cm^{-1}$):3448 (aromatic N-H), 3030 (aromatic C-H),2950 (aliphatic C-H),2940 (OH),1735 (C=O), 1638 (C=N);UV/Vis (MeOH, $\lambda_{max}$, nm): 381, 350,342;$^1$HNMR ($d_6$-Acetone): δ, ppm: δ 10.39 (s, 1H, H-9),8.74 (s,1H, Imidazole-CH), 7.62 (s, 1H, Imidazole-CH), 8.45(dd,J = 8.8, 2.4Hz, 1H, H-7);7.79 (d, J =8.8 Hz, 1H, H-8), 7.24(d, J = 2.4Hz, 1H, H-5); 2.91 (s, $CH_3$, 3H), 1.85-1.64 (m, 2H, Cyclo);MS (EI): *m/z*381.1802 [$M^+$].

**2.3.Biological evaluation of the iminocarbazole derivatives**

Shaking incubator, eppendorf tubes, cork borer, autoclaved distilled water, agar plates, Lugol's solution (10% KI, 5% $I_2$). Brine shrimp, artificial seawater, 3X magnifying glass, petriplatesand sterilized filter paper.

The iminocarbazole derivatives **(3a-g,3′a-g)** were screened forbiological activities including, anti-tumorand toxicity assays (cytotoxicity and phytotoxicity). All theexperiments were carried out in triplicate and the data was statistically analyzed by applying ANOVA (Analysis of Variance) on SPSS 20.0.

**2.3.1.Anti-tumor assay**

Potato disc assay was carried out in order to screen the synthesized iminocarbazoles**(3a-g, 3′a-g)** for their anti-tumor activity[11,12],*Agrobacterium tumefaciens*(AT10) was inoculated to nutrient broth medium at 28°C in shaking incubator at 100 rpm. The inoculums were prepared by centrifuged to harvest the cells. The cells were diluted in 1mL of each three dilutions (1000, 100 and 10ppm) of all compounds in eppendorf tubes. The sterilized fresh potatoes were used to make 8mm/5mm cylinders. These potato discs

were placed on solidified nutrient agar plates (five discs per plate) and inoculums (40µL) were poured over each disc of the respective concentration using different controls. Anti-cancerous drug vincristine (10µL, 100ppm) was used as a positive control, while negative control was pure *A. tumefaciens*culture. The plates were incubated at 28°C for three weeks. The discs were stained withLugol's solution and the number of tumors was counted under dissecting microscope. The percent tumor inhibition was calculated by:

%tumor formation = No. of tumors formed by the sample/ No. of tumors formed by control x 100

**2.3.2.Cytotoxicity study**

Cytotoxicity was carried out using brine shrimp assay [13-14].Brine shrimp (*Artemiasalina*) eggs (Sera, Heidelberg, Germany) were sprinkled in 100mL of artificial seawater, (Harvest Co. H.K.). The flask was left for four days under illumination. The eggs hatched and shrimps nauplii were observed swimming near the light source. Samples (1000, 100 and 10 ppm),were poured (2 mL each) in individual vials. Ten shrimps with 4mL seawaterwere transferred to each vial without solvent. Theseawater was used as negative control while MS-222 (Tricainemethanesulfonate, Aquatic Eco-systems, Inc.), a common fish anesthetizer, as positive control. The vials were left for 24h at room temperature under illumination. Survivors were counted with the aid of 3X magnifying glass. The $LC_{50}$ was calculated by using probitanalysis[15].

**2.3.3.Phytotoxicity assay**

The phytotoxic assay using radish (*Raphanussativus* L) seeds was carried out[16-17].Two different parameters were determined independently, the effect of compounds on the seed germinationand root length inhibition. Different dilutions (100, 10 and 1ppm) of all compounds were tested and each dilution (2 mL) waspoured on the autoclaved petriplates having double layerfilter paper followed by autoclaved distilled water 2mL.About 50 sterilized radish seeds were placed on each plate. The petriplates were kept in dark at room temperature (25$\pm$ 2°C) and the germinated seeds were counted every day from 1$^{st}$to 5$^{th}$ day.

For root length determination, ten sterilized seeds were placed on the filter paper prepared as in previous experiment. The petri plates were kept in dark at room temperature and the root length of individual seed was measured on the day five with the help of scale.

In conclusion, we have reported the synthesis of several acetyltetrahydrocarbazole and nitroacetyltetrahydrocarbazole imines

through simple condensation reaction using conventional, microwave and DES method. The DES approach has been proposed which avoids VOSs. In addition, operational simplicity, mild conditions, high yields, short reaction time, reusability of catalyst with cost effectiveness make this protocol an important addition to existing methods of iminotetrahydrocarbazole derivatives. The compounds were having anti-mutagenic activity while they were nontoxic for cell life and the processes of cell division, differentiation and elongation occurring during germination and root length elongation.

## 3.RESULTS AND DISCUSSION

The interesting chemical properties of acetyltetrahydrocarbazole have already inspired researchers to design and synthesize its variety of biologically compelling derivatives[3,10].Versatility of Schiff base ligands pertaining biological, analytical and industrial applicationsto make further investigation in this area is highly desirable. Encouraged by its characteristics, new molecules bearing tetrahydrocarbazole nucleus with imine moiety have been devised**(3a-g)** by three different approaches; conventional, microwave and DES. Most exciting results were observed in microwave method with respect to time. However, DES seems to be more advantageous due to its reusability although it showed comparatively low yield and took more time than microwave mediated synthesis.Furthermore, initial biological screening derived us to prepare mononitrotetrahydrocarbazolesbased imines **(3′a-g)**to strengthen carbazole profile.

According to literature methods, we prepared starting acetyltetrahydrocarbazole**1**which was in complete spectral agreement as reported[3].To the best of our knowledge, nitro group was introduced at C-6 to afford**1′** in glacial acetic acidfor first time. In $^{1}$H-NMR, a single was appeared for methyl protons of acetyl group at δ2.8andalso supported –C=O group by sharp absorption band at 1691 $cm^{-1}$. Methylene protons were observed at δ 1.8-1.9 attributed to H-2 to H-4. Aromatic protons were confirmed by two doublets at δ7.3 and 8.09 ($J$ = 2.8 Hz) for H-5 and H-8 indicating the *meta* substitution at position 6.A singlet at δ 10.20 represent amine proton which was further confirmed by –NH stretching at 3450 $cm^{-1}$ in FTIR spectrum.

In next stage, imineswere synthesized by reacting compound**1** or **1′** with different amines. Reaction conditions were optimized.In thermal method, ethanol was best in terms of reaction time and yield as compared to acetic acid probably due to

hydrogen bonding in ethanol that catches moisture from the air providing less chance of collision among the molecules which results in longer reaction time.This formation is slow at high and low pH but attain maximum rate at pH 4-5. This pH dependence can be justified as catalyst is required to protonate carbinolamineintermediate, thereby causing dehydration. This is why, reaction will be slow if not enough acid is present (high pH). Moreover, it facilitateswater removaland hence resulting in faster reaction rate.At low acidic pH, amine nucleophile is completely protonated making initial nucleophilic addition impossible. Nitro containingImines(**3′a-g**) have high yields than (**3a-g**) in conventional approach.

In $^{1}$H-NMR, compound **3a**amultiplet at δ 7.46 to 7.28were attributed to phenyl ring which was in support of absorption bands at 1585 $cm^{-1}$ for aromatic C-H stretch with an additional band at 1670 $cm^{-1}$for -C-N functionality. Compound **3c**, multiplet at δ8.24 to 7.33resonated for additional aromatic protons showing the presence of aryl group of hydrazine along with a singlet at δ11.15 whichwas attributed to new secondary amine that is further corroborated by absorption band at 3344 $cm^{-1}$.Compound **3f**, twosinglets at δ2.57 and 1.12 were attributed to aliphatic methyl and methane attached to amino acid group.The carbonyl stretch of acid was observed at 1745 $cm^{-1}$whileabsorption band at 1660$cm^{-1}$corresponds to iminemoietiesin FTIR thereby ensuring the successful Schiff base formation.Compound **3g**, threesingletsresonated at δ8.79, 7.59 and 2.29 were assigned to imidazolemoiety while characteristic absorption bands at 1660, 1740, 2923, 2990 $cm^{-1}$ were found for -C=N, -C=O, -OH, aliphatic -$CH_2$ groups in FTIR. All the molecular masses were determined by EIMS.

Another approach for this one pot condensation reaction was achieved by using 40, 60and 80% DES in methanol. Initially, reaction was explored in pure DES (choline chloride–urea) and products were afforded in quiet low yield. Therefore, catalytic amount of methanol was added in reaction mixture to initiate chemical process. This provided strong *in situ* methoxide ions and thus proof that imine formation must require acidic or basic catalyst (use of acetic acid in conventional approach). DES in acidic medium also furnished imines but it did not support the reaction in terms of recyclability of DES for next run due to its interaction with acetate ion.The reaction conditions were optimized using various concentrations of DES in methanol (20, 40, 60, 80%) at 25-75$^{o}$C.In 40 and 80% DES, high yield was

observed for compounds **3a-g**and **3′a-g,** respectivelywhileother trials gave inappreciable results. This catalytic system is reused in three consecutive runs, after activation at 80$^{o}$C under vacuum in each cycle.

**3.2. Biological evaluation**

The antitumor assay of different dilutions of each compound was carried out after full confirmation that the compounds were nontoxic, non-lethal for *Agrobacteriumtumefaciens* itself. So before antitumor assay, the antibacterial test was performed and none of the compounds gave any zone of inhibition except antibiotic (Cefexime 100ppm, 10 µL, 18mm zone of inhibition).

Different dilutions of compounds were applied on potato disc along with *A. tumefaciens*. The experiment was kept at 28°C and the number of tumors formed was counted after staining with Lugol's solution after 3 weeks.

It was observed that the tumor inhibition effect was concentration dependent in almost all compounds (Figure 1). Most of the compounds havemaximum anti-tumor efficiency at 1000ppm. The highest activity observed was 96% by**3′e**followed by **3′g**(93%) while the minimum inhibition is 48 and 60% by **3′a** and **3b**,respectively.At 100ppm again **3′e**and **3′g**gave maximum results (96 and 88%) and even at minimum concentration reasonable inhibition (91 to 31%) was observed. The results were statistically analyzed by ANOVA and demonstrated a significant difference in inhibition among all the compounds tested. When different dilutions of the same compound were analyzed significant results were obtained ($p<0.05$ at $\alpha$ 5%).

In order to recommend any synthetic or natural compound as a pharmaceutical agent, its nontoxic behavior (cyto-friendly nature) towards the eukaryotic cells is required to test. The cytotoxic effect of different dilutions of the compounds was studied by using the model organism brine shrimps. However, **3b, 3c, 3f** and **3g** were found to be toxic for the organism even at 10ppm (Table 2). The results varied from 0-80% at 1000 and 100ppm. The maximum survival (80, 70, 60 and 50%) was observed by the **3′e, 3′f, 3′b** and **3′g**at 1000ppm. At 10ppm, maximum survival was observed, here 90% survival by **3′e**, 70% by both **3′b** and **3′g**, 50% by **3′f**, 30% by **3e** and 20% by **1,1′, 3′a** and **3′c**. As the probit analysis was applied on the results, for most of the compounds $LC_{50}$was more than 200ppm.

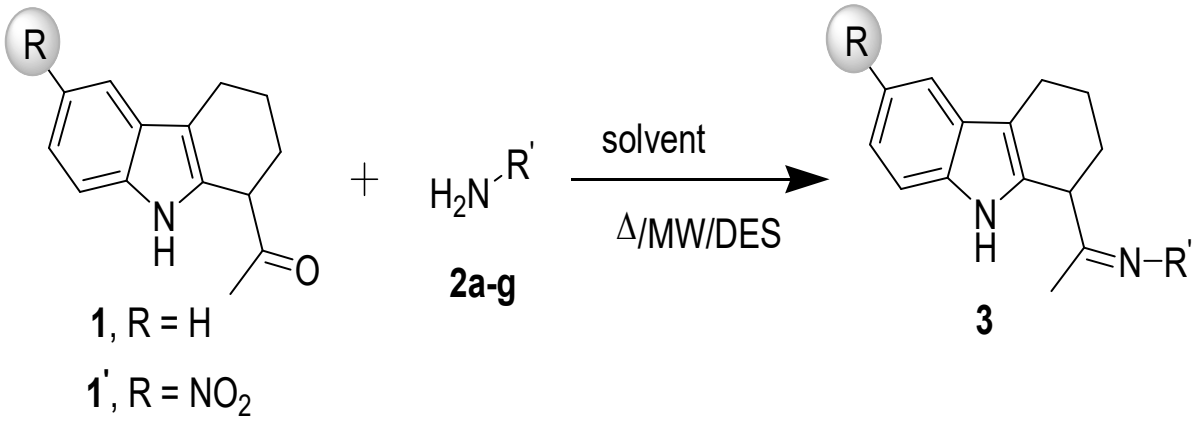
**3.2.1. Seed germination**

Two dilutions of**3a-g** and **3′a-g** (100 and 10ppm) were tested for their effect on seed germination. No significant effect of concentration on germination and dormancy of the seedswere observed

(Figure2 and 3). At 100ppm **3′b**, **3′e**, **3′f** and **3′g**, showedmaximum germination 90, 95, 100 and 100%, respectivelywhile at 10ppm,they furnished95, 100, 95 and 100%, respectively on 5th day. The minimum value was 62% observed by compound **1** and **3′c** concluding nontoxic behavior towards germination (Figure 2). The results when statistically analyzed gave a significant effect ($p<0.05$) of compounds whereas non-significant ($p>0.05$) effect of their concentrations.

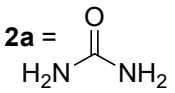
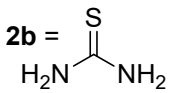
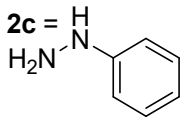
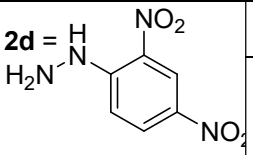
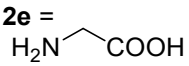
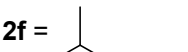
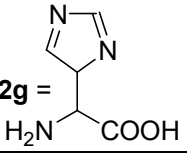
**3.2.2.Root length**

In order to observe the effect of different dilutions of the compounds**3a-g** and **3′a-g** on the rapid cell division and cell elongation occurring at root tips, their effect on root length was studied. Three dilutions (100, 10 and 1ppm) of each compound were used. The root length of ten seeds was measured after five days and mean was recorded (Figure 4). Most of the compoundsshowedaconcentration dependent effect on root length and none of them were toxic enough to totally suppress the cellular processes occurring at the root tips. At 100ppm, the root lengths obtained were33-70mm while at 10ppm a length between 53-100mm, was obtained showing less suppression as compared to higher concentrations. The control (distilled water) gave 65mm length on the day five.




**Scheme. Synthesis of iminocarbazole derivative**

**Table 1: Comprehensive representation of iminocarbazolederivatives(3a-g,3′a-g)**

| Entry | $NH_2$-substitution | Product | Mol. Formula | Solvent | Time/Yield | | | | | | m.p |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Conv. hr | % | MW min | % | DES hr | %** | |
| 1 |  **2a** = | 3a | $C_{15}H_{17}N_3O$ | EtOH | 4.5 | 76 | 4 | 78 | 4:00/4:30/6:25 | 56/49/42 | sticky |
| 2 | | 3′a | $C_{15}H_{16}N_4O_3$ | EtOH | 5 | 54 | 4 | 65 | 3:25/3:50/4:45 | 47/44/42 | sticky |
| 3 |  **2b** = | 3b* | $C_{15}H_{17}N_3S$ | EtOH | - | - | 3 | 83 | 1:10/ 1:30/3:45 | 59/44/29 | 190 |
| 4 | | 3′b | $C_{15}H_{16}N_4O_2S$ | EtOH | 4 | 67 | 1.30 | 85 | 3:30/ 4:50/5:25 | 56/50/43 | 89 |
| 5 |  **2c** = | 3c | $C_{20}H_{21}N_3$ | GAA | 3 | 51 | 4.33 | 60 | 2:45/ 3:30/4:20 | 60/56/53 | 105 |
| 6 | | 3′c | $C_{20}H_{20}N_4O_2$ | GAA | 3 | 29 | 3.40 | 67 | 2:45/3:15/4:00 | 65/61/58 | 96 |
| 7 |  **2d** = | 3d | $C_{20}H_{19}N_5O_4$ | GAA | 3.5 | 73 | 5 | 80 | 2:45/3:15/4:00 | 73/64/55 | 155 |
| 8 | | 3′d | $C_{20}H_{18}N_6O_6$ | GAA | 3.5 | 53 | 1 | 69 | 2:30/2:55/3:30 | 60/56/52 | 125 |
| 9 |  **2e** = | 3e* | $C_{16}H_{18}N_2O_2$ | EtOH | 4 | 45 | 1.40 | 48 | 3:00/4:20/5:00 | 74/70/66 | 170 |
| 10 | | 3′e | $C_{16}H_{17}N_3O_4$ | EtOH | 4 | 70 | 1.20 | 73 | 2:30/ 3:45/5:40 | 49/44/38 | 78 |
| 11 |  **2f** = | 3f | $C_{17}H_{20}N_2O_2$ | EtOH | 2.45 | 66 | 3 | 79 | 2:30/ 3:45/4:50 | 74/69/64 | 102 |
| 12 | | 3′f | $C_{17}H_{19}N_3O_4$ | EtOH | 4 | 79 | 1.20 | 84 | 3:00/ 3:45/4:15 | 62/57/52 | 58 |
| 13 |  **2g** = | 3g | $C_{19}H_{20}N_4O_2$ | GAA | 3 | 77 | 3 | 80 | 4:50/ 5:00/5:30 | 53/50/47 | 115 |
| 14 | | 3′g | $C_{19}H_{19}N_5O_4$ | GAA | 3 | 55 | 5 | 79 | 3:30/ 4:30/6:00 | 10/8/5 | 86 |

** = reported3b, 3e[3], ** = three consecutive runs*

**Table 2: Percent survival of Brine shrimps**

| | 1000 ppm | 100 ppm | 10 ppm | $LC_{50}$ (ppm) |
|---|---|---|---|---|
| 1 | 0 | 10 | 20 | <200 |
| 1′ | 0 | 0 | 20 | <200 |
| 3′a | 0 | 20 | 20 | <200 |
| 3b | 0 | 0 | 0 | >200 |
| 3′b | 60 | 60 | 70 | <200 |
| 3c | 0 | 0 | 0 | >200 |
| 3′c | 0 | 10 | 20 | <200 |
| 3e | 0 | 20 | 30 | <200 |
| 3′e | 80 | 80 | 90 | <200 |
| 3f | 0 | 0 | 0 | >200 |
| 3′f | 70 | 50 | 50 | <200 |
| 3g | 0 | 0 | 0 | >200 |
| 3′g | 50 | 70 | 70 | <200 |

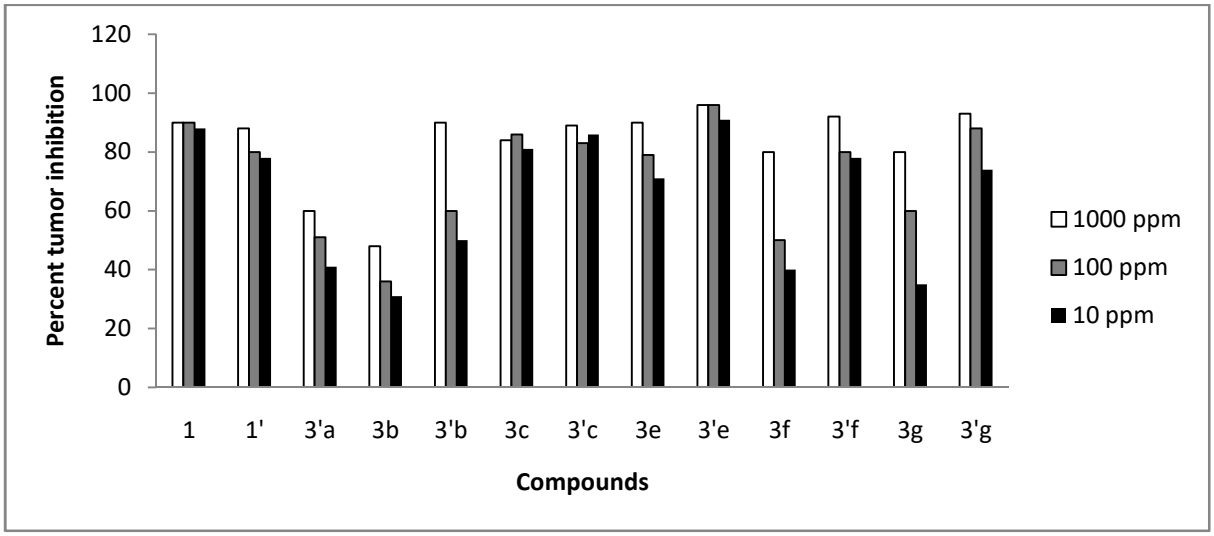



**Figure 1: Percent tumor inhibition by different dilutions of the compounds**

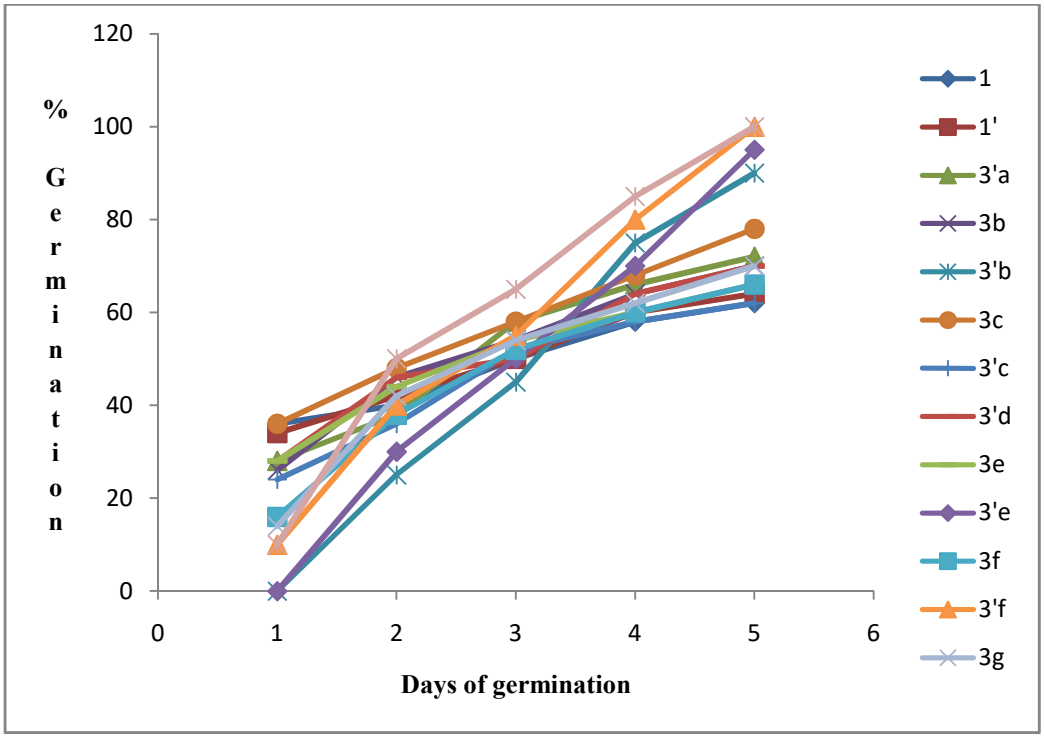



**Figure 2: Effect of 100 ppm of 3a-g, 3′a-g on seed germination**

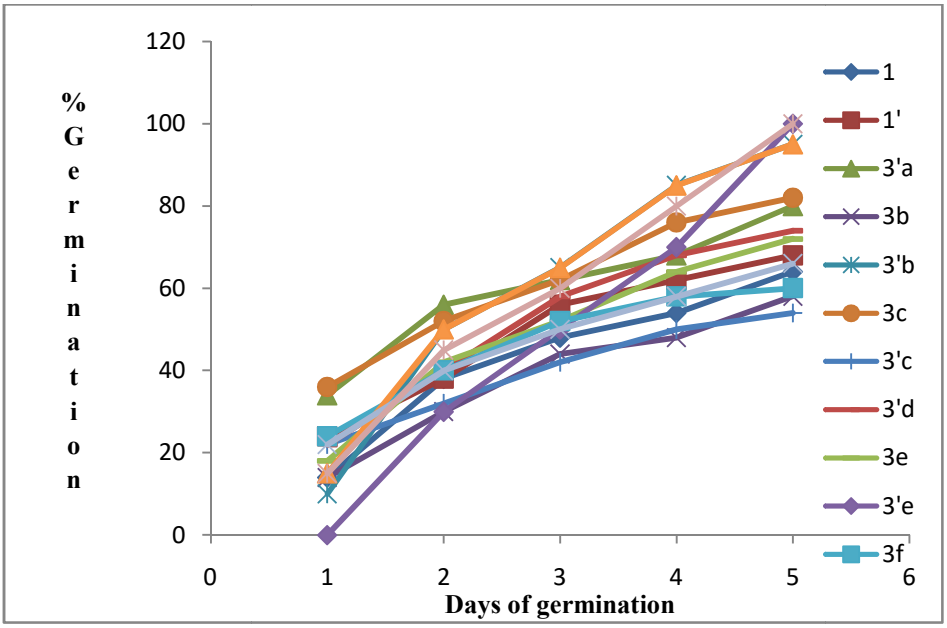



**Figure 3: Effect of 10 ppm of 3a-g, 3′a-g on seed germination**

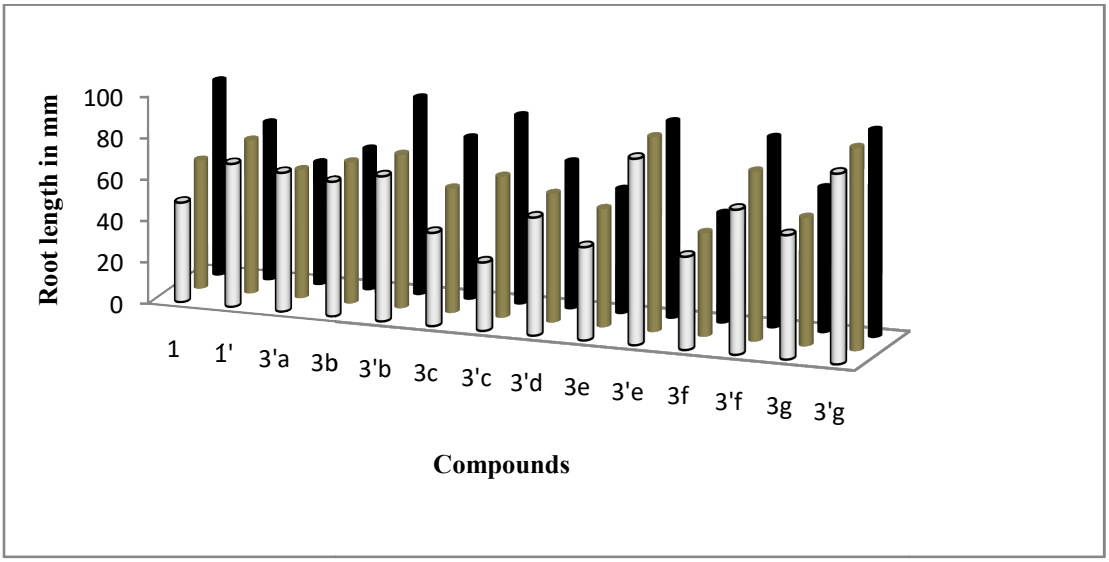
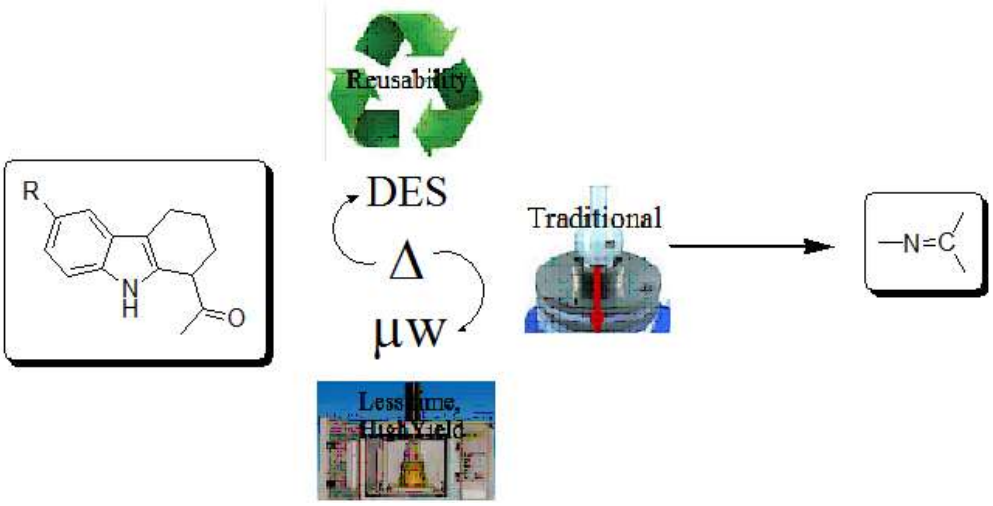



**Figure 4: The average root length obtained at different dilutions**




**Graphical Abstract**

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