Research Article

Microwave Assisted and Parallel Synthesis of Novel Substituted Carbazole Derivatives of Biological Interest

Kumar N, Sharma GK and Pathak D*

Department of Pharmaceutical Chemistry, Rajiv Academy for Pharmacy, NH#2, Delhi-Mathura Highway, Chhatikara, Mathura - 281001, Uttar Pradesh, India.

ABSTRACT

Carbazole on reaction with chloroacetyl chloride afforded *N*^{9}-(chloroacetyl)-carbazole **(1)** which on treatment with hydrazine hydrate yielded *N*^{9}-(hydrazinoacetyl)-carbazole **(2)**. Condensation of **(2)** with various substituted aromatic aldehydes afforded *N*^{9}-(arylidine hydrazinoacetyl)-carbazole **(3a-3o)** which on further reaction with benzil/isatin in the presence of ammonium acetate yielded arylated carbazole derivatives containing imidazole moiety **(4a-4o)**. In this paper, a comparative study between the developed microwave method and conventional method is described. The synthesized compounds were analyzed by physical and analytical data. The synthesized compounds were evaluated for their antibacterial and anticancer activity. All the synthesized substituted carbazole derivatives have shown good antibacterial activity against *S. aureus*, *B. subtilis*, *K. pneumoniae* and *E. coli*. Some of the synthesized carbazole derivatives exhibited significant cytotoxic activity against Ehrlich's Ascites Carcinoma (EAC) and HEP_2 cell lines.

Keywords: Carbazole, EAC cell lines, HEP_2 cell lines, SRB assay.

INTRODUCTION

A survey of the pertinent literature reveals that carbazole have been found to possess a wide spectrum of biological activity such as antibacterial[1], antirheumatoid arthritis[2], antitubercular[3], antiviral[4], antiepileptic[5], anti-inflammatory[6] and anticancer[7-9] activities. Since we have made an attempt to synthesize carbazole derivatives incorporated with antibacterial pharmacophore like imidazole[10-15] by using both microwave assisted[16-18] 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 constitute an important class of naturally occurring heterocycles with interesting biological activities including their special affinity toward DNA[19]. Carbazole arrest the tumor cell cycle at the M phase and induce apoptotic cell death by increasing expression of p53 and promoting bcl-2 phosphorylation[20]. Therefore these compounds play a crucial role as potential lead for the discovery of antitumor activity using bioisosteric replacements.

Microwave-assisted organic synthesis (MAOS) is a new and quickly growing technique in 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.

MATERIALS AND METHODS

Experimental section

The laboratory grade chemicals and reagents were used to synthesize all the reported compounds. The melting points were determined in open capillaries and are uncorrected. The temperatures are expressed in ºC and are uncorrected. The IR spectra of newly synthesized compounds were recorded on Perkin-Elmer Infrared–283 FTIR spectrometer by KBr pellet technique and are expressed in

cm^{-1}. ^{1}HNMR spectra were recorded on Bruker DRX-300 (300 MHz, FT-NMR) spectrophotometer using TMS as an internal standard, $CDCl_3$ and DMSO as solvents. Mass spectrum was obtained using LC-MS (Shimadzu-2010AT) under Electrospray Ionization (ESI) technique and elemental analysis was performed using Elemental Vario EL III, Carlo-Erba 1108. TLC was performed to monitor the reactions and to determine the purity of products on a precoated aluminium plates using 10% methanol in chloroform or 20% ethyl acetate in chloroform as a mobile phase.

Synthesis of *N*^{9}-(chloroacetyl)-carbazole (1)
Conventional method
Chloroacetyl chloride (0.04 M) was added to a solution of carbazole (0.04 M) in acetone (60 ml) and the reaction mixture was refluxed on a water bath for 2 hours then the solvent was removed by filtration and the residue was purified over the column of silica gel and eluted with chloroform. The elute was concentrated and the product was recrystallized with ethanol to give compound **1** (yield 75 %, mp.220 °C).

Microwave method
Chloroacetyl chloride (0.04 M) was added to a solution of carbazole (0.04 M) in acetone (60 ml) taken in a round bottom flask and irradiated in a microwave oven for 2 minutes. The completion of the reaction was monitored by TLC. The solvent was removed and the product was recrystallized from ethanol to give compound **(1)** (yield 86 %).

Synthesis of *N*^{9}-(hydrazinoacetyl)-carbazole (2)
Conventional method
A mixture of compound **1** (0.027 M) and hydrazine hydrate (0.027 M) in ethanol : dioxan (9:1 v/v) was refluxed on a water bath for 4 hours then it was cooled, filtered and concentrated to get a solid which was purified through a column of silica gel and eluted with chloroform. The elute was concentrated and the product was recrystallized from chloroform to give compound **2** (yield 61 %, mp. 255 °C).

Microwave method
Hydrazine hydrate (0.027 M) was added to a solution of *N*^{9}-(chloroacetyl)-carbazole compound **1** (0.027 M) in ethanol: dioxane (9:1 v/v) taken in a round bottom flask and irradiated in a microwave oven for 1.30 minutes. The completion of the reaction was monitored by TLC. The solvent was removed and the product was recrystallized from chloroform to give compound (**2**) (yield 73 %).

General procedure for the synthesis of *N*^{9}-(arylidene acetylhydrazino)-carbazole 3(a-o)
Conventional method
A mixture of (**2**) (0.007 M) with various derivatives of benzaldehyde (0.007 M) in ethanol : dioxane (9:1 v/v) was refluxed on a water bath for about 8 hours then it was cooled, filtered and concentrated to get a solid compound which was purified through a column of silica gel and eluted with methanol. The elute was concentrated and the product was recrystallized from chloroform to give compound **3(a-o).**

Microwave method
Various derivatives of benzaldehyde (0.007 M) was added to a solution of compound **2** (0.007 M) in ethanol: dioxane (9:1 v/v) taken in a round bottom flask and irradiated in a microwave oven for about 3 minutes, the completion of the reaction was monitored by TLC. The solvent was removed and the product was recrystallized from chloroform to give compound **3 (a-o).**

General procedure for synthesis of 1-carbazole-9-yl-2-(substituted phenyl-*2H*-imidazole-1-ylamino)-ethanone 4(a-i)
Conventional method
A mixture of compound **3(a-i)** (0.003 M) and benzil (0.003 M) in acetone (50ml) with excess of ammonium acetate was stirred for about 2 hours followed by refluxing on a water bath for about 10 hours then it was cooled, filtered and concentrated to get a solid compound which was purified through a column of silica gel and eluted with ethanol. The elute was concentrated and the product was recrystallized from chloroform to give compound **4(a-i).**

Microwave method
A mixture of compound **3(a-i)** (0.003 M) in acetone (50 ml) was taken in a round bottom flask, benzil (0.003 M) and ammonium acetate (0.003 M) were added followed by stirring for about 2 hours and irradiated in a microwave oven for about 8 minutes. The completion of the reaction was monitored by TLC. The solvent was removed and the product was recrystallized from chloroform to give compound **4(a-i).**

General procedure for synthesis of 1-Carbazole-9-yl-2-(substituted phenyl)-1,4-

dihydroimidazo[4,5-*b*]indole1-ylamino)-ethanone 4(j-o)

Conventional method

A mixture of compound **3(j-o)** (0.003 M) and isatin (0.003 M) in acetone (50ml) with ammonium acetate (0.003 M) was stirred for about 2 hours followed by refluxing on a water bath for about 10 hours then it was cooled, filtered and concentrated to get a solid compound which was purified through a column of silica gel and eluted with ethanol. The elute was concentrated and the product was recrystallized from chloroform to give compound **4(j-o).**

Microwave method

A mixture of compound **3(j-o)** (0.003 M) and isatin (0.003 M) in acetone (45 ml) was taken in a round bottom flask. Then ammonium acetate (0.003 M) was added followed by stirring for about 2 hours and irradiated in a microwave oven for about 8 minutes. The completion of the reaction was monitored by TLC. The solvent was removed and the product was recrystallized from chloroform to give compound **4(j-o).**

Spectral data of the synthesized compounds 4(a-o)

1-Carbazole-9-yl-2-(4-chlorophenyl)-4,5-diphenyl-1*H*-imidazole-1-ylamino)-ethanone (4a)

White amorphous solid; IR (KBr) ν_{max} : 3340 (N-H), 3074 (Ar C-H), 1672 (C=O), 1595 (Ar C=C), 1525 (C=N), 752 (C-Cl); ^{1}HNMR (DMSO-d_6) δ_{ppm} : 2.66 (s, 2H, CH_2), 6.99-7.01 (d, 2H, Ar-H), 7.10-7.26 (d, 2H, Ar-H), 7.28-7.45 (m, 10H, Ar-H), 7.69-7.76 (d, 4H, Ar-H), 7.81-7.89 (dt, 2H, Ar-H), 7.98-8.03 (dt, 2H, Ar-H), Ar-H), 9.99 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 50.7, 111.1 (2), 118.6(2), 119.0 (2), 120.1 (2), 122, 122.2 (2), 127.5 (4), 128.8 (3), 128.9 (2), 129.3 (4), 129.4 (2), 129.5, 130.2 (2), 133.1 (2), 134.3, 136, 200; EIMS (m/z): $[M]^+$ 553.50; Fragments: 329.75, 223.25, 208.20, 194.25, 177.45, 166.25, 68.05; Anal. Calcd for $C_{35}H_{25}ClN_4O$: C, 76.01; H, 4.56; N, 10.13; Found : C, 76.00; H, 4.53; N, 10.11.

1-Carbazole-9-yl-2-(4-nitrophenyl)-4,5-diphenyl-1*H*-imidazole-1-ylamino)-ethanone (4b)

White crystal; IR (KBr) ν_{max}: 3344 (N-H), 3020 (Ar C-H), 1610 (C=O), 1579 (C=N), 1350 (N-O); ^{1}HNMR (DMSO-d_6) δ_{ppm}: 2.74 (s, 2H, CH_2), 6.98-7.00 (d, 2H, Ar-H), 7.11-7.25 (d, 2H, Ar-H), 7.27-7.46 (m, 10H, Ar-H), 7.67-7.75 (d, 4H, Ar-H), 7.78-7.84 (dt, 2H, Ar-H), 7.99-8.02 (dt, 2H, Ar-H), 9.91 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 50.7, 111.1 (2), 118.6 (2), 119.0 (2), 120.1 (2), 121.6 (2), 122, 122.2 (2), 127.5 (4), 128.4 (2), 128.8 (2), 129.3 (4), 129.5, 130.2, 133.1 (2), 136, 136.8, 148.4, 200; EIMS (m/z): $[M]^+$ 563.50; Fragments: 340.75, 223.14, 208.20, 188.00, 166.25, 68.05; Anal. Calcd for $C_{35}H_{25}N_5O_3$: C, 74.59; H, 4.47; N, 12.43; Found : C, 74.57; H, 4.45; N, 12.42.

1-Carbazole-9-yl-2-(4-hydroxyphenyl)-4,5-diphenyl-1*H*-imidazole-1-ylamino)-ethanone (4c)

Yellow crystal; IR (KBr) ν_{max} : 3599 (O-H), 3345 (N-H), 2840 (C-H), 1667 (C=O), 1448 (C=N), 1120 (C-O); ^{1}HNMR (DMSO-d_6) δ_{ppm} : 2.51 (s, 2H, CH_2), 3.55 (s, 1H, OH, D_2O exchangeable), 6.97-7.00 (d, 2H, Ar-H), 7.11-7.27 (d, 2H, Ar-H), 7.30-7.46 (m, 10H, Ar-H), 7.66-7.75 (d, 4H, Ar-H), 7.77-7.89 (dt, 2H, Ar-H), 7.91-7.99 (dt, 2H, Ar-H), 9.59 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 50.7, 111.1 (2), 116.2 (2), 118.6 (2), 119.0 (2), 120.1 (2), 122, 122.2 (2), 123.3, 127.5 (4), 128.8 (2), 128.9 (2), 129.3 (4), 129.5, 130.2 (2), 133.1, 136, 158.5, 200; EIMS (m/z): $[M]^+$ 534.60; Fragments: 311.52, 223.12, 208.95, 194.21, 160.20, 68.02; Anal. Calcd for $C_{35}H_{26}N_4O_2$: C, 78.63; H, 4.90; N, 10.48; Found : C, 78.60; H, 4.88; N, 10.46.

1-Carbazole-9-yl-2-(2-chlorophenyl)-4,5-diphenyl-1*H*-imidazole-1-ylamino)-ethanone (4d)

Pale yelloy crystal; IR (KBr) ν_{max} : 3403 (N-H), 2979 (C-H), 1658 (C=O), 1593 (C=N), 722 (C-Cl); ^{1}HNMR (DMSO-d_6) δ_{ppm}: 2.52 (s, 2H, CH_2), 6.97-7.00 (d, 2H, Ar-H), 7.11-7.27 (d, 2H, Ar-H), 7.30-7.48 (m, 10H, Ar-H), 7.68-7.75 (d, 4H, Ar-H), 7.84-7.86 (d, 1H, Ar-H), 7.86-7.89 (dd, 1H, Ar-H), 7.90-7.93 (d, 1H, Ar-H), 7.94-7.97 (d, 1H, Ar-H), 9.52 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 50.7, 111.1 (2), 118.6 (2), 119.0 (2), 120.1 (2), 122,122.2 (2), 127.4, 127.5 (4), 128.8 (2), 128.9, 129.3 (4), 129.4, 129.5, 130.2 (3), 132.3, 133.1 (2), 136, 138.5, 200; EIMS (m/z): $[M]^+$ 553.55; Fragments: 328.85, 223.45, 208.45, 194.75, 177.20, 160.35, 68.65; Anal. Calcd for $C_{35}H_{25}ClN_4O$: C, 76.01; H, 4.56; N, 10.13; Found : C, 76.00; H, 4.53; N, 10.11.

1-Carbazole-9-yl-2-(4-methoxyphenyl)-4,5-diphenyl-1*H*-imidazole-1-ylamino)-ethanone (4e)

White amorphous solid; IR (KBr) ν_{max} : 3492(N-H), 3061(Ar C-H), 1656 (C=O), 1595 (C=C), 1534 (C=N), 1125 (C-O-C); ^{1}HNMR (DMSO-d_6) δ_{ppm} : 2.16 (s, 2H, CH_2), 3.66 (s, 3H, OCH_3), 6.98-7.01 (d, 2H, Ar-H), 7.11-7.27 (d, 2H, Ar-H), 7.29-7.44 (m, 10H, Ar-H), 7.68-7.75 (d, 4H, Ar-H), 7.78-7.90 (dt, 2H, Ar-H), 7.92-7.99 (dt, 2H, Ar-H), 9.20 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 50.7, 55.9, 111.1 (2), 114.8 (2), 118.6 (2), 119.0 (2), 120.1 (2), 122, 122.2 (2), 123.0, 127.5 (4), 128.5 (2), 128.8 (2), 129.3 (4), 129.5, 130.2 (2), 133.1 (2), 136, 160.7, 200; EIMS (m/z): $[M]^+$ 548.75; Fragments: 325.85, 223.15, 208.24, 194.04, 166.20, 68.07; Anal. Calcd for $C_{36}H_{28}N_4O_2$: C, 78.81; H, 5.14; N, 10.21. Found : C, 78.79; H, 5.13; N, 10.19.

1-Carbazole-9-yl-2-(2-nitrophenyl)-4,5-diphenyl-1*H*-imidazole-1-ylamino)-ethanone (4f)

Pale yellow crystal; IR (KBr) ν_{max}: 3417 (N-H), 2993 (C-H), 1672 (C=O), 1595 (C=N), 1495 (N-O); ^{1}HNMR (DMSO-d_6) δ_{ppm}: 2.53 (s, 2H, CH_2), 6.98-7.01 (d, 2H, Ar-H), 7.10-7.25 (d, 2H, Ar-H), 7.26-7.42 (m, 10H, Ar-H), 7.65-7.74 (d, 4H, Ar-H), 7.83-7.85 (d, 1H, Ar-H), 7.86-7.88 (dd, 1H, Ar-H), 7.91-7.94 (dd, 1H, Ar-H), 7.95-7.97 (d, 1H, Ar-H), 9.91 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 50.7, 111.1 (2), 118.6 (2), 119.0 (2), 120.1 (2), 121.6, 122, 122.2 (2), 127.5 (4), 128.4, 128.8 (2), 129.3 (4), 129.5, 129.7, 130.2 (2), 131.6, 133.1 (2), 135.4, 136, 146.9, 200; EIMS (m/z): $[M]^+$ 563.75; Fragments: 340.20, 223.20, 208.05, 194.04, 188.00, 166.25, 68.05; Anal. Calcd for $C_{35}H_{25}N_5O_3$: C, 74.59; H, 4.47; N, 12.43; Found : C, 74.57; H, 4.45; N, 12.42.

1-Carbazole-9-yl-2-(2-hydroxyphenyl)-4,5-diphenyl-1*H*-imidazole-1-ylamino)-ethanone (4g)

White crystal; IR (KBr) ν_{max}: 3633 (O-H), 3442 (N-H), 2937 (C-H), 1610 (C=O), 1531 (C-C), 1545 (C=N); ^{1}HNMR (DMSO-d_6) δ_{ppm} : 2.16 (s, 2H, CH_2), 4.72 (s, 1H, OH, D_2O exchangeable), 6.96-7.00 (d, 2H, Ar-H), 7.08-7.25 (d, 2H, Ar-H), 7.29-7.44 (m, 10H, Ar-H), 7.68-7.77 (d, 4H, Ar-H), 7.82-7.85 (d, 1H, Ar-H), 7.86-7.90 (dd, 1H, Ar-H), 7.91-7.93 (d, 1H, Ar-H), 7.95-7.99 (dd, 1H, Ar-H), 9.20 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 50.7, 111.1 (2), 116.4, 118.5, 118.6 (2), 119.0 (2), 120.1 (2), 121.9, 122, 122.2 (2), 127.5 (4), 128.8 (2), 128.9, 129.3 (4), 129.5, 130.2 (3), 133.1 (2), 136, 155.3, 200; EIMS (m/z): $[M]^+$ 534.52; Fragments: 311.20, 223.11, 208.11, 194.21, 160.11, 68.08; Anal. Calcd for $C_{35}H_{26}N_4O_2$: C, 78.63; H, 4.90; N, 10.48; Found : C, 78.61; H, 4.87; N, 10.46.

1-Carbazole-9-yl-2-(3-hydroxyphenyl)-4,5-diphenyl-1*H*-imidazole-1-ylamino)-ethanone (4h)

Cream coloured crystal; IR (KBr) ν_{max}: 3614 (O-H), 3346 (N-H), 2920 (Ar C-H), 1610 (C=O), 1579 (C-C, Ar), 1620 (C=N); ^{1}HNMR (DMSO-d_6) δ_{ppm}: 2.55 (s, 2H, CH_2), 4.16 (s, 1H, OH, D_2O exchangeable), 6.98-7.01 (d, 2H, Ar-H), 7.12-7.27 (d, 2H, Ar-H), 7.29-7.46 (m, 10H, Ar-H), 7.70-7.76 (d, 4H, Ar-H), 7.73 (s, 1H, Ar-H), 7.74-7.77 (d, 1H, Ar-H), 7.78-7.83 (d, 1H, Ar-H), 7.84-7.91 (dd, 1H, Ar-H), 9.43 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 50.7, 111.1 (2), 112.9, 115.9, 118.6 (2), 119.0 (2), 120.1 (3), 122, 122.2 (2), 127.5 (4), 128.8 (2), 129.3 (4), 129.5, 130.2 (2), 130.7, 132.1, 133.1 (2), 136, 159.0, 200; Anal. Calcd for $C_{35}H_{26}N_4O_2$: C, 78.63; H, 4.90; N, 10.48; Found : C, 78.62; H, 4.87; N, 10.46.

1-Carbazole-9-yl-2-(3-chlorophenyl)-4,5-diphenyl-1*H*-imidazole-1-ylamino)-ethanone (4i)

Bright yellow crystal; IR (KBr) ν_{max} : 3355 (N-H), 3068 (C-H), 1697 (C=O), 1593 (C=N), 732 (C-Cl); ^{1}HNMR (DMSO-d_6) δ_{ppm}: 1.16 (s, 2H, CH_2), 6.96-7.00 (d, 2H, Ar-H), 7.12-7.28 (d, 2H, Ar-H), 7.28-7.45 (m, 10H, Ar-H), 7.69-7.75 (d, 4H, Ar-H), 7.75 (s, 1H, Ar-H), 7.76-7.82 (d, 1H, Ar-H), 7.84-7.90 (d, 1H, Ar-H), 7.92-7.99 (dd, 1H, Ar-H), 9.56 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 50.7, 111.1 (2), 118.6 (2), 119.0 (2), 120.1 (2), 122, 122.2 (2), 125.6, 127.4, 127.5 (4), 128.8 (2), 128.9, 129.3 (4), 129.5, 130.2 (2), 130.7, 132.1, 133.1 (2), 134.8, 136, 200; Anal. Calcd for $C_{35}H_{25}ClN_4O$: C, 76.01; H, 4.56; N, 10.13; Found: C, 76.00; H, 4.53; N, 10.11.

1-Carbazole-9-yl-2-(4-dimethylaminophenyl)-1,4-dihydroimidazo[4,5-*b*]indole-1-ylamino)-ethanone (4j)

White crystal; IR (KBr) ν_{max}: 3550 (N-H), 2947 (C-H), 1672 (C=O), 1629 (C=N), 1595 (C-N); ^{1}HNMR (DMSO-d_6) δ_{ppm} : 2.37 (s, 2H, CH_2), 2.87 (s, 6H, CH_3), 6.99-7.06 (d, 2H, Ar-H), 7.10-7.26 (d, 2H, Ar-H), 7.28-7.36(t, 4H, Ar-H), 7.40-7.54 (m, 4H, Ar-H), 7.77-7.89 (dt, 2H, Ar-H), 7.91-7.99 (dt, 2H, Ar-H), 9.73 (s, 2H, NH, D_2O exchangeable), 9.86 (s, 2H, NH, D_2O

exchangeable); ^{13}C-NMR δ : 40.3 (2), 49.9, 111.1 (3), 114.8 (2), 118.6 (2), 119.0 (2), 120.1 (4), 120.2, 122.2 (3), 124.1, 128.4 (2), 130.2 (2), 135.5, 136, 136.4, 149.6, 200; EIMS (m/z): $[M]^+$ 498.55; Fragments: 338.75, 223.45, 208.20, 194.75, 186.45, 166.55, 68.55; Anal. Calcd for $C_{31}H_{26}N_6O$: C, 74.68; H, 5.26; N, 16.86; Found: C, 74.66; H, 5.23; N, 16.82.

1-Carbazole-9-yl-2-(3-nitrophenyl)-1,4-dihydroimidazo[4,5-*b*]indole-1-ylamino)-ethanone (4k)

Brown amorphous solid; IR (KBr) ν_{max} : 3344 (N-H), 3051 (Ar C-H), 1610 (C=O), 1579 (C-N), 1498 (N-O); ^{1}HNMR (DMSO-d_6) δ_{ppm} : 2.46 (s, 2H, CH_2), 6.97-7.03 (d, 2H, Ar-H), 7.09-7.25 (d, 2H, Ar-H), 7.26-7.35 (t, 4H, Ar-H), 7.41-7.54 (m, 4H, Ar-H), 7.56-7.60 (dd, 1H, Ar-H), 7.62-7.69 (d, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 7.68-7.71 (d, 1H, Ar-H), 9.43 (s, 2H, NH, D_2O exchangeable), 9.49 (s, 2H, NH, D_2O exchangeable); ^{13}C-NMR δ : 49.9, 111.1 (3), 118.6 (2), 119.0 (3), 120.1 (4), 121.1, 122.1, 122.2 (3), 124.1, 130.2 (3), 131.6, 133.6, 135.5, 136, 136.4, 148.9, 200; Anal. Calcd for $C_{29}H_{20}N_6O_3$: C, 69.59; H, 4.03; N, 16.79; Found : C, 69.57; H, 4.01; N, 16.77.

1-Carbazole-9-yl-2-(3-methoxyphenyl)-1,4-dihydroimidazo[4,5-*b*]indole-1-ylamino)-ethanone (4l)

Yellow amorphous solid; IR (KBr) ν_{max} : 3321 (N-H), 3050 (Ar C-H), 2960(C-H), 1596 (C=O), 1517 (Ar C=C), 1485 (C=N), 1178 (C-O-C); ^{1}HNMR (DMSO-d_6) δ_{ppm} : 2.37 (s, 2H, CH_2), 3.66 (s, 3H, OCH_3), 6.94-7.01 (d, 2H, Ar-H), 7.07-7.24 (d, 2H, Ar-H), 7.24-7.32 (t, 4H, Ar-H), 7.43-7.54 (m, 4H, Ar-H), 7.73 (s, 1H, Ar-H), 7.75-7.79 (d, 1H, Ar-H), 7.81-7.85 (d, 1H, Ar-H), 7.88-7.92 (dd, 1H, Ar-H), 9.20 (s, 1H, NH, D_2O exchangeable), 9.29 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 49.9, 55.9, 111.1 (3), 111.3, 114.3, 118.6 (2), 119.0 (3), 119.8, 120.1 (4), 122.2 (3), 124.1, 130.2 (2), 130.3, 131.7, 135.5, 136, 136.4, 161.2, 200EIMS (m/z): $[M]^+$ 485.27; Fragments: 261.27, 223.15, 208.27, 194.04, 166.20, 68.20; Anal. Calcd for $C_{30}H_{23}N_5O_2$: C, 74.21; H, 4.77; N, 14.42; Found : C, 74.20; H, 4.75; N, 14.40.

1-Carbazole-9-yl-2-(2-methoxyphenyl)-1,4-dihydroimidazo[4,5-*b*]indole-1-ylamino)-ethanone (4m)

White crystal; IR (KBr) ν_{max} : 3328 (N-H), 3026 (Ar C-H), 2960 (C-H), 1650 (C=O), 1573 (C=C), 1521 (C=N), 1085 (C-O-C); ^{1}HNMR (DMSO-d_6) δ_{ppm}: 1.25 (s, 2H, CH_2), 3.66 (s, 3H, OCH_3), 6.95-7.03 (d, 2H, Ar-H), 7.11-7.20 (d, 2H, Ar-H), 7.24-7.31 (t, 4H, Ar-H), 7.41-7.53 (m, 4H, Ar-H), 7.75-7.78 (d, 1H, Ar-H), 7.82-7.86 (dd, 1H, Ar-H), 7.90-7.93 (d, 1H, Ar-H), 7.96-7.99 (dd, 1H, Ar-H), 9.75 (s, 1H, NH, D_2O exchangeable), 9.89 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 49.9, 56.2, 111.1 (3), 114.8, 116.9, 118.6 (2), 119.0 (3), 120.1 (4), 121.6, 122.2 (3), 124.1, 128.5, 129.8, 130.2 (2), 135.5, 136, 136.4, 157.4, 200; Anal. Calcd for $C_{30}H_{23}N_5O_2$: C, 74.21; H, 4.77; N, 14.42; Found : C, 74.19; H, 4.74; N, 14.41.

1-Carbazole-9-yl-2-(4-chlorophenyl)-1,4-dihydroimidazo[4,5-*b*]indole-1-ylamino)-ethanone (4n)

Yellow crystal; IR (KBr) ν_{max} : 3388 (N-H), 3058 (Ar C-H), 1579 (C=O), 1382 (C=N), 779 (C-Cl); ^{1}HNMR (DMSO-d_6) δ_{ppm}: 3.74 (s, 2H, CH_2), 6.97-7.02 (d, 2H, Ar-H), 7.12-7.18 (d, 2H, Ar-H), 7.24-7.30 (t, 4H, Ar-H), 7.42-7.54 (m, 4H, Ar-H), 7.79-7.86 (dt, 2H, Ar-H), 7.98-8.02 (dt, 2H, Ar-H), 9.28 (s, 1H, NH, D_2O exchangeable), 9.76 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR δ : 49.9, 111.1 (3), 118.6 (2), 119.0 (3), 120.1 (4), 122.2 (3), 124.1, 128.8, 128.9 (2), 129.4 (2), 130.2 (2), 134.3, 135.5, 136, 136.4, 200; EIMS (m/z): $[M]^+$ 489.85; Fragments: 265.43, 223.20, 208.20, 194.35, 177.43, 166.20, 68.55; Anal. Calcd for $C_{29}H_{20}ClN_5O$: C, 71.09; H, 4.11; N, 14.29; Found: C, 71.07; H, 4.09; N, 14.27.

1-Carbazole-9-yl-2-(4-nitrophenyl)-1,4-dihydroimidazo[4,5-*b*]indole-1-yl-amino)-ethanone (4o)

Bright yelloy crystal; IR (KBr) ν_{max} : 3382 (N-H), 2904 (Ar C-H), 1632 (C=O), 1579 (C=N), 1473 (N-O); ^{1}HNMR (DMSO-d_6) δ_{ppm}: 2.82 (s, 2H, CH_2), 6.98-7.05 (d, 2H, Ar-H), 7.12-7.19 (d, 2H, Ar-H), 7.22-7.29 (t, 4H, Ar-H), 7.41-7.56 (m, 4H, Ar-H), 7.76-7.82 (dt, 2H, Ar-H), 7.86-7.92 (dt, 2H, Ar-H), 9.56 (s, 1H, NH, D_2O exchangeable), 9.77 (s, 2H, 1H, D_2O exchangeable); ^{13}C-NMR δ : 49.9, 111.1 (3), 118.6 (2), 119.0 (3), 120.1 (4), 121.6 (2), 122.2 (3), 124.1, 128.4 (2), 130.2 (2), 135.5, 136, 136.4, 136.8, 148.4, 200; Anal. Calcd for $C_{29}H_{20}N_6O_3$: C, 69.59; H, 4.03; N, 16.79; Found: C, 69.57; H, 4.01; N, 16.77.

BIOLOGICAL EVALUATION

Antibacterial studies

The synthesized carbazole derivatives were screened for their antibacterial activity against

two gram positive bacterial strains *B. subtilis* (NCIM 2063), *S. aureus* (NCIM 2079) and two gram negative bacterial strains *K. pneumonia* (NCIM 2087), *E. coli* (NCIM 2065) by using modified Kirby-Bauer disc diffusion method. The MIC values of the test compounds were determined by tube dilution technique. All the synthesized compounds were dissolved separately to prepare a stock solution of 1 mg ml^{-1} using DMF. Stock solution was aseptically transferred and suitably diluted with sterile broth medium to have seven different concentrations of each test compound ranging from 200 to 3.1 µg ml^{-1} in different test tubes. All the tubes were inoculated with one loopful of one of the test bacteria. The process was repeated with different test bacteria and different samples. Tubes inoculated with bacterial cultures were incubated at 37 °C for 18 hours and the presence/absence of growth of the bacteria was observed. From these results, MIC of each test compound was determined against each test bacterium. A spore suspension in sterile distilled water was prepared from five-days-old culture of the test bacteria growing on nutrient broth media. About 20 ml of the growth medium was transferred into sterilized petri plates and inoculated with 1.5 ml of the spore suspension (spore concentration 6 X 10^4 spores ml^{-1}). Filter paper disks of 6 mm diameter and 2 mm thickness were sterilized by autoclaving at 121 °C (15 psi) for 15 min. Each petri plate was divided into five equal portions along the diameter to place one disc. Three discs of test sample were placed on three portions together with one disc with reference drug ciprofloxacin and a disk impregnated with the solvent (DMF) as negative control. Test sample and reference drugs were tested at the concentration of 10 µg ml^{-1}. The petri plates inoculated with bacterial cultures were incubated at 37 °C for 18 h. Diameters of the zones of inhibition (mm) were measured and the average diameters for test sample were calculated in triplicate sets. The diameters obtained for the test sample were compared with that produced by the standard drug ciprofloxacin. The results of antibacterial studies are presented in **Table 2.**

Anticancer studies

Anticancer activities of the synthesized compounds were assessed by determining the percentage inhibition of EAC cells by tryphan blue dye exclusion technique. The anticancer activity of all the synthesized compounds were tested at concentration of 500, 250, 125, 62.5, 31.25 µg/ml. The percentage growth inhibition was calculated by using the following formula: % Growth inhibition = [(Total cells – Live cells) × 100]/Total cells.

The study was also done in HEP_2 cell lines by SRB assay method. Sulforhodamine B (SRB) is a bright pink aminoxanthine dye with two sulphonic groups. Under mild acidic conditions, SRB binds to protein basic amino acid residues in trichloroacetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. Colour development in SRB assay is rapid, stable and visible. The developed color can be measured over a broad range of visible wavelength in 96 well plate readers. When TCA-fixed, SRB stained samples are air dried. They can be stored indefinitely without deterioration. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 X 10^5 cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitreplate, 0.1 ml of the diluted suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and µL of different drug concentrations was added to the cells in microtitre plates. The plates were then incubated at 37º for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, 25 µL of 50% trichloroacetic acid was added to the wells gently such that it forms a thin layer over the drug dilutions to form a over all concentration of 10%. The plates were incubated at 4ºC for one h. The plates were flicked and washed five times with tap water to remove traces of medium, drug and serum were then air dried. The air dried plates were stained with Sulforhodamine B, a protein binding dye for 30 minutes. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air dried. 100 µL of 10 Mm tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using microplate recorded at a wavelength at 540 nm. The percentage growth inhibition was calculated. Cyclophosphamide was used as standard drug (CTC_{50}, 12 µg/ml).

The CTC_{50} values were calculated by plotting the graph between concentration versus percentage growth inhibition and by bisecting concentration at the 50% growth inhibition. The CTC_{50} values of the synthesized aryl carbazoles are as shown in **Table 1.**

Scheme: Synthetic pathway for various carbazole derivatives

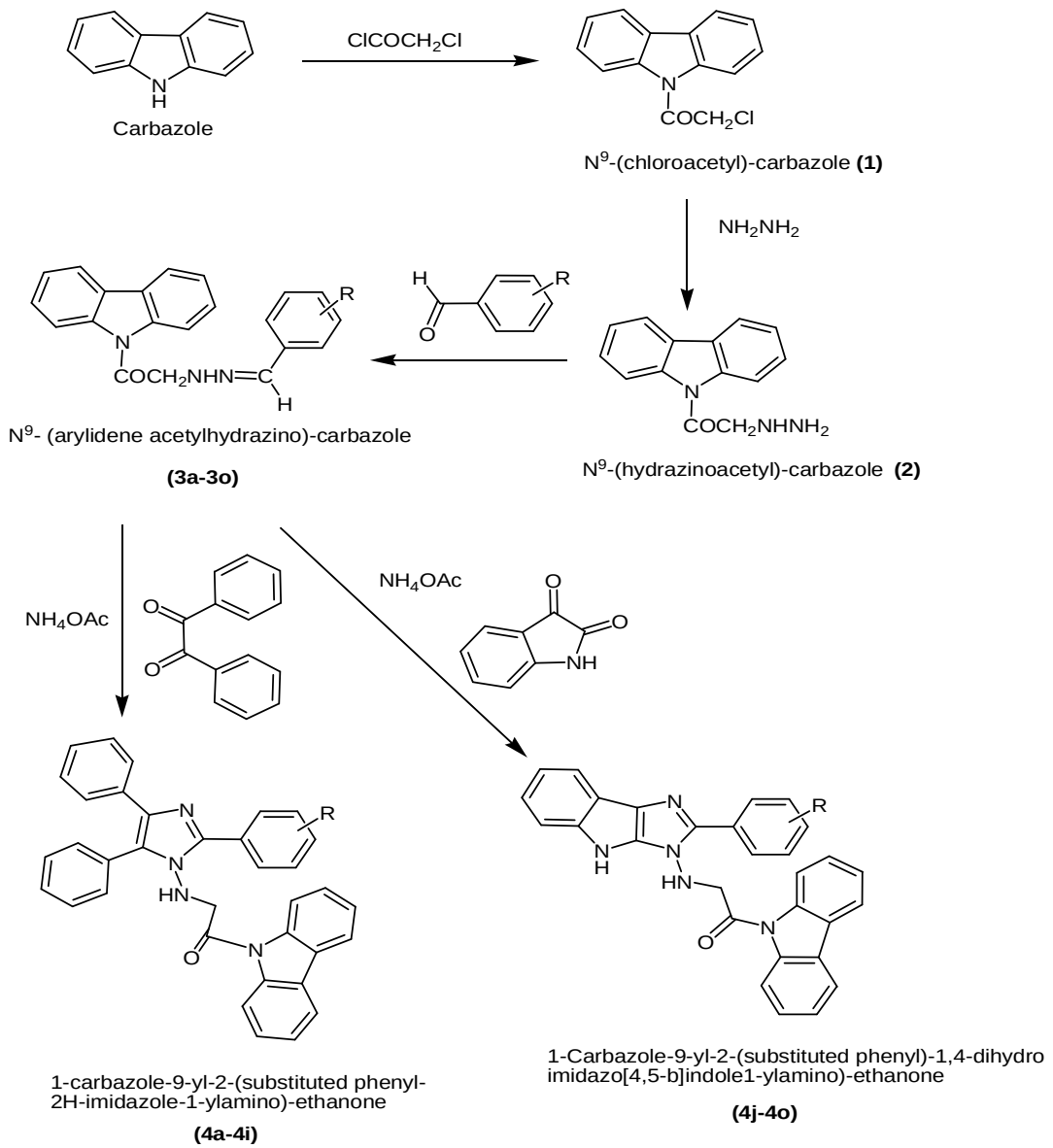
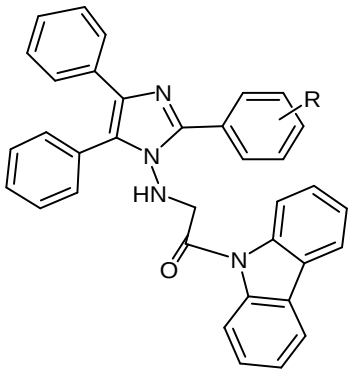
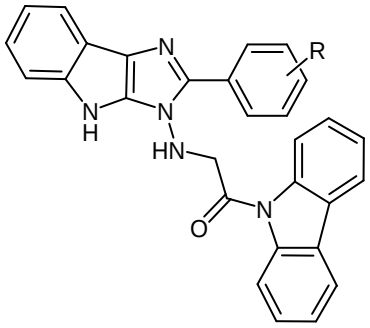


Table 1: Physical, analytical and anticancer data of synthesized compounds



(4a-4i)



(4j-4o)

Comp No.	R	m.p. (^{0}C)	*R_f value	Reaction Time		Yield(%)		EAC cells	HEP_2 cells
				MW (min)	Conven. (h)	MW	Conven.	CTC_{50} µg/ ml	CTC_{50} µg/ ml
4a	*p*-Cl	210-211	0.68	16.3	23.9	83.9	55.8	132.65	195
4b	*p*-NO_2	214-216	0.65	15.8	23.1	85.4	60.0	180.73	168
4c	*p*-OH	180-182	0.70	13.0	22.5	86.5	57.8	>200	165
4d	*o*-Cl	208-209	0.63	14.0	23.0	89.1	58.7	>200	>200
4e	*p*-OCH_3	222-223	0.71	15.0	22.9	82.2	53.7	>200	195
4f	*o*-NO_2	253-254	0.67	14.0	23.6	88.9	59.7	91.61	170
4g	*o*-OH	198-199	0.63	16.0	24.9	89.0	55.0	120.35	165
4h	*m*-OH	196-197	0.61	17.5	25.2	90.0	53.8	156.90	163
4i	*m*-Cl	217-218	0.68	12.5	21.6	83.9	55.0	109.00	197
4j	*p*-$N(CH_3)_2$	233-234	0.78	13.5	22.6	79.7	59.7	167.60	185
4k	*m*-NO_2	198-199	0.65	13.0	21.9	80.8	59.5	109.00	192
4l	*m*-OCH_3	201-202	0.63	13.0	23.0	84.4	58.8	145.68	>200
4m	*o*-OCH_3	234-235	0.71	13.5	23.7	84.7	57.7	159.00	197
4n	*p*-Cl	267-268	0.63	14.5	24.6	87.7	56.5	150.87	31.25
4º	*p*-NO_2	187-188	0.61	17.5	25.6	84.4	55.8	132.90	91.61

CTC_{50} = The cytotoxic concentration (which inhibited 50% of total cells).
*solvent system: (chloroform: methanol::9:1)

Table 2: Antibacterial data of all synthesized aryl carbazole compounds screened against various bacterial strains

Compound	Diameter of zone of inhibition (mm)			
	Gram (+ve)		Gram (-ve)	
	S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*
4a	13.1 (50)	12.9 (50)	8.1 (50)	8.9 (50)
4b	13.6 (50)	12.1 (25)	13.9 (25)	14.1(25)
4c	11.9 (50)	11.3 (25)	9.2 (12.5)	9.5 (12.5)
4d	11.9 (50)	11.3 (25)	11.9 (25)	12.5 (6.2)
4e	9.1 (25)	8.8 (50)	7.6 (100)	7.8 (100)
4f	5.7 (100)	5.9 (100)	6.6 (50)	6.9 (50)
4g	12.5 (50)	12.1 (25)	11.9 (25)	11.6 (25)
4h	12.5 (50)	13.3 (50)	10.9 (100)	10.7 (50)
4i	12.1 (25)	13.8 (50)	14.3 (25)	12.5 (50)
4j	13.1 (25)	12.3 (25)	15.4 (12.5)	11.8 (25)
4k	11.2 (50)	12.4 (25)	13.5 (12.5)	9.1 (50)
4l	6.2 (100)	7.2 (100)	9.2 (50)	7.5 (50)
4m	7.2 (100)	8.7 (50)	10.2 (50)	10.3 (25)
4n	10.3 (25)	12.4 (12.5)	14.5 (6.2)	13.3 (12.5)
4o	12.3 (50)	13.6 (25)	14.6 (25)	14.6 (25)
Control	-	-	-	-
Ciprofloxacin	18 (12.5)	19 (6)	19 (12.5)	17 (6)

Values in bracket are MIC values (µg ml^{-1}).

RESULTS AND DISCUSSION

On the basis of the above facts the novel series of synthesized derivative of aryl carbazoles containing imidazole moiety may yield compounds with high therapeutic potential. All the synthesized compounds were analyzed by TLC, mp, FTIR, ^{1}HNMR, MASS and elemental analysis. In FTIR spectroscopy prominent peaks for N-N and N-C appeared respectively at 1490 and 1170 cm^{-1} which indicates the attachement of imidazole or indole nucleus with carbazole. In ^{1}HNMR spectroscopy a peak appeared between δ 7.00-8.00 ppm which was D_2O exchangeable shows the presence of NH group. Microwave irradiation was done using microwave oven supplied by Catalyst microwave synthesis system, Model: CATA-2R. In conventional method the yield of all the compounds is slightly lower as compared to the synthesis by microwave irradiation technique. Microwave irradiation method facilitates the polarization of the reacting molecule under the irradiation causing fast reaction to occur. The microwave method was found to be better than conventional method in terms of reaction time and yield. The list of synthesized compounds and the comparative yield statement between microwave and conventional method is as shown in **Table 1.**

All the synthesized carbazole derivatives incorporated with chemotherapeutic pharmacophores were evaluated for their *in vitro* antibacterial activity against two-gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative bacteria *Escherichia coli* and *Klebsiella pneumoniae*. Almost all the newly synthesized substituted carbazoles showed good antibacterial activity against gram negative bacterial strains *Escherichia coli* and *Klebsiella pneumoniae*. Results shows that compound **4c**, **4j** and **4l** were most potent against all type of bacterial strains. These compounds contain electron donating group which makes it more basic and made efficient as antibacterial agent.

Anticancer activity of the synthesized compounds was evaluated by determining the percentage growth inhibition of Ehrlich's Ascites Carcinoma (EAC) cells by tryphan blue dye exclusion technique and HEP_2 cell lines by Sulforhodamine B (SRB) assay method. Among all the synthesized compounds containing indole-imidazole nucleus with carbazole were found to be more potent. The CTC_{50} values of the newly synthesized carbazoles are shown in **Table 1.** Compound **4j, 4l and 4m** were found most active against tumor cell line due to the presence of electron donating group which may increase the basicity of the compound.

ACKNOWLEDGEMENTS

The authors are thankful to the Head, Department of Biotechnology, JSS Ooty for performing anticancer activity of synthesized compounds. Authors are also thankful to Panjab University, Chandigarh and IIT, Delhi for spectral studies.

REFERENCES

1. Barbieri V and Ferlin MG. Microwave-assisted one-pot synthesis of substituted tetrahydrocarbazole and 8,9,10,11-tetrahydro-7*H*-pyrido[*α*]carbazoles. Tetrahedron Lett. 2006; 47: 8289-8292.
2. Chakrabarty M, Ghosh N and Harigaya Y. A clay-mediated, regioselective synthesis of 2-(aryl/alkyl) amino-thiozole[4,5-*c*]carbazole. Tetrahedron Lett. 2004; 45: 4955-4957.
3. Henon H, Anizon F, Golsteyn RM et al. Synthesis and biological evaluation of new dipyrrolo[3,4-*α*:3,4-*c*]carbazole-1,3,4,6-tetraones, substituted with various saturated and unsaturated side chains via palladium catalyzed cross-coupling reactions. Bioorg Med Chem. 2006; 14: 3825-3834.
4. Fousteris MA, Papakyriakou A, Koutsourea A et al. Pyrrolo[2,3-*α*]carbazoles as potent cyclin kinase 1 (CDK1) inhibitors, synthesis, biological evaluation and binding mode through docking simulations. J Med Chem. 2008; 51: 1048-1052.
5. Conchon E, Anizon F, Aboab B et al. Synthesis, *in-vitro* antiproliferative activity and Chk1 inhibitory properties of pyrrolo[3,4-α]pyrrolo [3,4-*c*]carbazole-1,3-diones and 2-aminopyridazino[3,4-α]pyrrolo[3,4-*c*]carbazole-1,3,4,7-tetraone. Eur J Med Chem. 2008; 43: 282-292.
6. Guillonneau C, Pierre A, Charton Y et al. Synthesis of 9-*o*-substituted derivatives of 9-hydroxy-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylic acid (2-(dimethylamino)ethyl)amide and their 10- and 11-methyl analogues with

improved antitumor avtivity. J Med Chem. 1999; 42: 2191-2203.
7. Guo R, Hameurlaine A and Dehaen W. Facile one-pot synthesis of 6-monosubstituted and 6,12-disubstituted-5,11-dihydroindolo[3,4-*b*]carbazoles and prepration of various functionalized derivatives. J Org Chem. 2007; 72: 7207-7213.
8. Huang FC, Chang CC, Lou PJ et al. G-Quadruplex Stabilizer 3,6-Bis(1-Methyl-4-Vinylpyridinium)carbazole diiodide induces accelerated senescence and inhibits tumorigenic properties in cancer cells. Mol Cancer Res. 2008; 6: 955-964.
9. Hudkins RL, Diebold JI, Angeles TS and Knight E. Fused enhance human interferon-γ activity. J Med Chem. 1997; 40: 2994-2996.
10. Hudkins RL, Johnson NW, Angeles TS, Gessner GW and Mallamo JP. Synthesis and mixed lineage kinase activity of pyrrolocarbazole and isoindolone analogs of (+) K-252a. J Med Chem. 2007; 50: 433-441.
11. Sako Y, Ichikawa S, Osada A and Matsuda A. Synthesis and evaluation of 5-substituted 9-hydroxypyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-diones as check point 1 kinase inhibitors. Bioorg Med Chem. 2010; 18: 7878-7889.
12. Routier S, Peixoto P, Merour JY et al. Synthesis and biological evaluation of novel naphthocarbazoles as potential anticancer agents. J Med Chem. 2005; 48: 1401-1413.
13. Rajsekaran A and Thampi PP. Synthesis and antinociceptive activity of some substituted-{5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl]tetrazol-1-yl}alkanones. Eur J Med Chem. 2005; 40: 1359-1364.
14. Sangeetha V and Prasad KJR. Synthesis of novel derivatives of 2-acetylfuro[2,3-α]carbazoles,benzo[1,2-*b*]-1,4-thiazepino[2,3-α]carbazoles and 1-acetyloxycarbazole-2-carbaldehydes. Indian J Chem. 2006; 45B: 1951-1954.
15. Das BP, Begum NA, Choudhary DN and Banerji J. Synthesis of some *N*-substituted carbazoles and their larvicidal studies. J Indian Chem Soc. 2005; 82: 158-160.
16. Danish IA and Prasad KJR. A one pot synthesis of 1,2,4,5-tetraazaspiro[5,5]-6,7,8,9-tetrahydrocarbazol-3-thiones and their antibacterial activities. Indian J Heterocycl Chem. 2004; 14: 19-22.
17. Kremser G, Hofmann OT, Sax S et al. Synthesis and photophysical properties of 3,6-Diphenyl-9-hexyl-9*H*-carbazole derivatives bearing electron withdrawing groups. Monatsh Chem. 2008; 139: 223-231.
18. Mulwad VV and Patil CA. Synthesis of some antimicrobial heterocycles from pyranotetrahydrocarbazole. Indian J Chem. 2005; 44B: 2355-2361.
19. Tylinska B, Howorko RJ, Mastalarz H and Wietrzyk J. Synthesis and structure-activity relationship analysis of new olivacine derivatives. Acta Pol Pharm. 2008; 67: 495-502.
20. Mal D, Senapati BK and Pahari P. Anionic [4+2] cycloaddition strategy in the regiospecific synthesis of carbazoles: Formal synthesis of Elipticine and Murrayaquinone A. Tetrahedron. 2007; 63: 3768-3781.