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# Synthesis and anticancer activity evaluation of substituted carbazole bearing thiosemicarbazide derivatives against human glioma U87 MG cell line

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## Abstract

Both carbazole and thiosemicarbazide scaffold showed various potential biological activities in medicinal chemistry. In this research work, we synthesized a series of carbazole bearing thiosemicarbazide derivatives (17-28) and evaluated thier *in vitro* cytotoxicity (IC50) profile on the U87 MG cell line using MTT assay. All target compounds were well characterized by NMR and HRMS mass spectroscopy. In series, six compounds (17, 20, 21, 22, 23, 28) found better *in vitro* cytotoxicity (IC50) values were 26.50 µM, 34.0 µM, 39 µM, 80 µM, 62 µM, 50 µM respectively compare to standard drug Temozolomide (IC50 = 100 µM). The SAR study of all final compounds (17-28) were also analyzed on the basis of different substituents on 6th position of carbazole scaffold bearing thiosemicarbazide derivatives against U87 MG cell line. All of the above studies showed that carbazole bearing thiosemicarbazide derivatives (17-28) showed anticipated anticancer activity against U87 MG glioma cell line.

**Keywords:** GBM (Glioblastoma); Carbazole; Thiosemicarbazide; TMZ (Temozolomide); Compound 17((E)-2-((1,4-dimethyl-9-H-carbazol-3-yl)methylene)hydrazinecarbothioamide); SAR (Structure activity relationship); MTT (tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

## 1 Introduction

Glioblastoma multiforme (GBM) is a type IV grade tumor according to WHO grading and classification [1,2]. GBM is a most deadly, aggressive and undifferentiated type of tumor originated from glial cell in the brain. The current treatment standard is a multimodal approach combining neurosurgery, fractionated radiation therapy, and chemotherapy [3, 4]. However, GBM patients usually have a median survival of approximately 14 to 15 months from the diagnosis [3]. Temozolomide (TMZ), is the only anticancer drug has been reported to improve the survival in a phase III study of GBM when administered concomitant with the radiotherapy [5].

Carbazole derivatives showed potential biological and photophysical properties [6-11]. Carbazole derivatives can target DNA structures and has prospective for development into anticancer drugs [6, 9]. Carbazole, a tricyclic planar structure, makes it prone to interact with DNA either via intercalation or minor groove binding well established in literature[9-11]. 1,4-Dimethyl-9-H-carbazole structure part is present in Ellipticine (5,11-dimethyl-6-H-pyrido[4,3-b]carbazole), acts as an intercalative agent and blocks topoisomerase II activity only [6, 9, 12] Anna Caruso et al. designed and synthesized 3,6 substituted carbazole linked to guanidine derivatives (**1**) showed potential *in vitro* cytotoxicity ($IC_{50}$) value 3.1 µM against HL-60 cell line [12]. Earlier our group reported substituted bis carbazole derivatives (2) showed potential *in vitro* cytotoxicity ($IC_{50}$) against human glioma U87 MG cell line[9]. G.A. Ciftci et al. reported apoptotic effect of compound 2-[(9-ethyl-9*H*-carbazol-3-yl)amino]-2-oxoethyl *N*,*N*-disubstituted dithiocarbamates (3) showed good *in*

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*vitro* cytotoxicity value ($IC_{50}$) 3.9 µg/mL on C6 (glioma) cell line [13]. Bis(carbazole-2,9N-benzyl)-3-ethyl ethanoate (BC3EE2, 9B) **4** showed potential significant *in vitro* antitumor activities on the two glioma GBM8401 ($IC_{50}$ = 5 µM) and GBM8901 ($IC_{50}$ = 7 µM) cell lines[14]. Philippe Diaz and co-workers reported modified carbazoles derivatives (**5**) showed potential *in vitro* anticancer activity ($IC_{50}$ = 80 nM) against glioma cell line [15] (Figure.1)

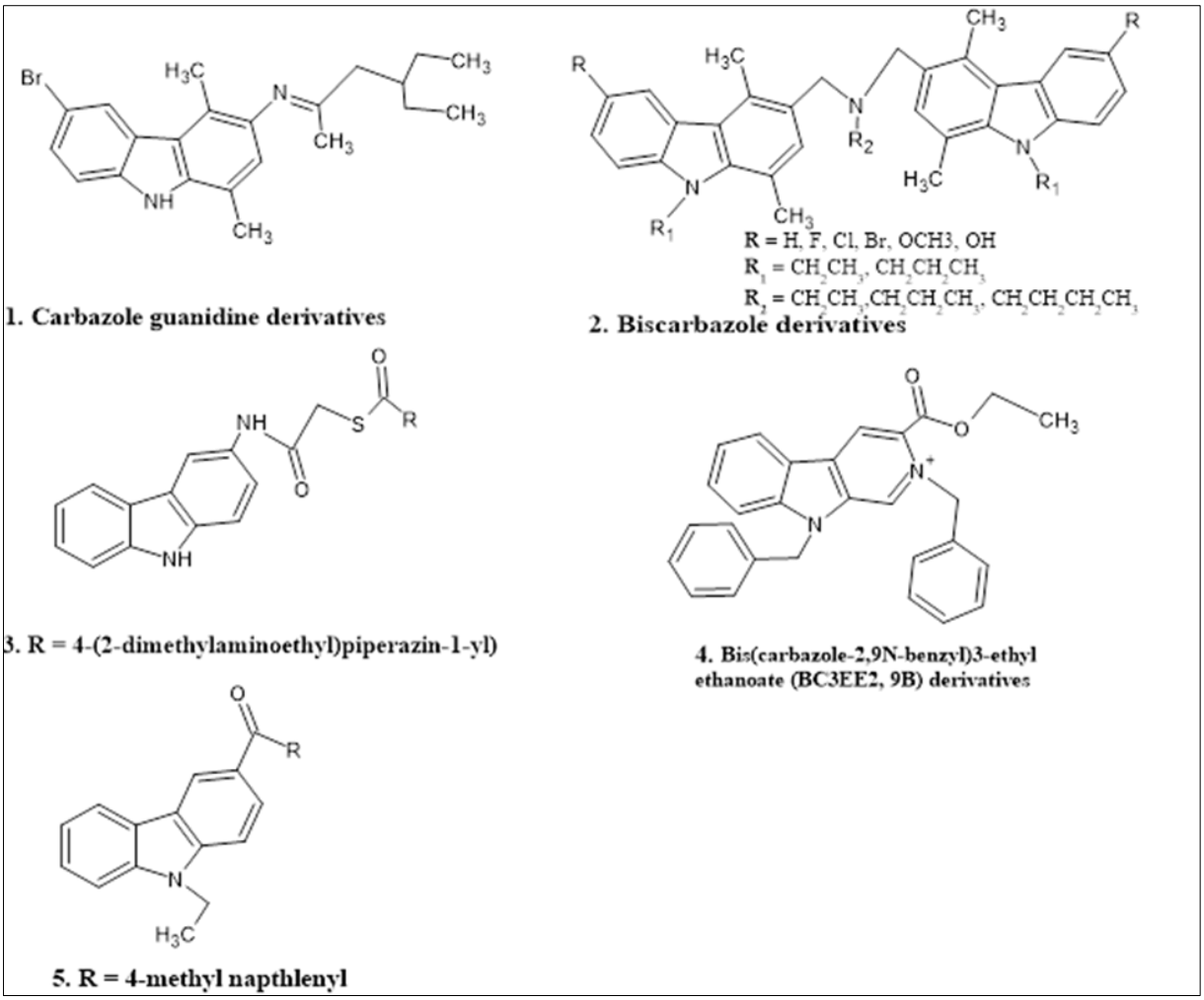



**Figure 1** Structure of some carbazole scaffold based anticancer potential molecules against glioma cell line (1-5)

During the last decades, thiosemicarbazones structure based compounds have been clinically developed for a variety of diseases including tuberculosis, viral infections, malaria and cancer [16, 17]. Thiosemicarbazones have a thiourea moiety contain conjugated N–N–S tridentate ligand system ($NH_2$–CS–NH–$NH_2$), which is crucial role for anticancer activity [18]. In 1956 Brockman et al. reported anti-leukemic effect of 2-formylpyridine thiosemicarbazone (PT) **6** but this compound was found to be cumulatively toxic[19]. In 1963 French and col. formulated hypotheses about the mode of action of the α(N)-heterocyclic thiosemicarbazones (**7**). The first was that they were acting as tridentate ligands and the second was that modifying the ring system while retaining the ligand pattern could lead to improved activity and decreased toxicity. The electron densities, substituent's and geometry could have critical effects on activity [20] Triapine (**8**) has potential anticancer activity and is undergoing phase-2 clinical trial for the treatment of solid tumors such as cervical and vaginal cancer [21, 22] (Figure 2). COTI-2 (9) entered a clinical phase I trial for treatment of advanced or recurrent gynecologic malignancies. DpC(di-2-pyridylketone4-cyclohexyl-4-methyl-3-thiosemicarbazone 10 also entered in clinical phase I evaluation since January 2016 [21, 22] (Figure. 2).

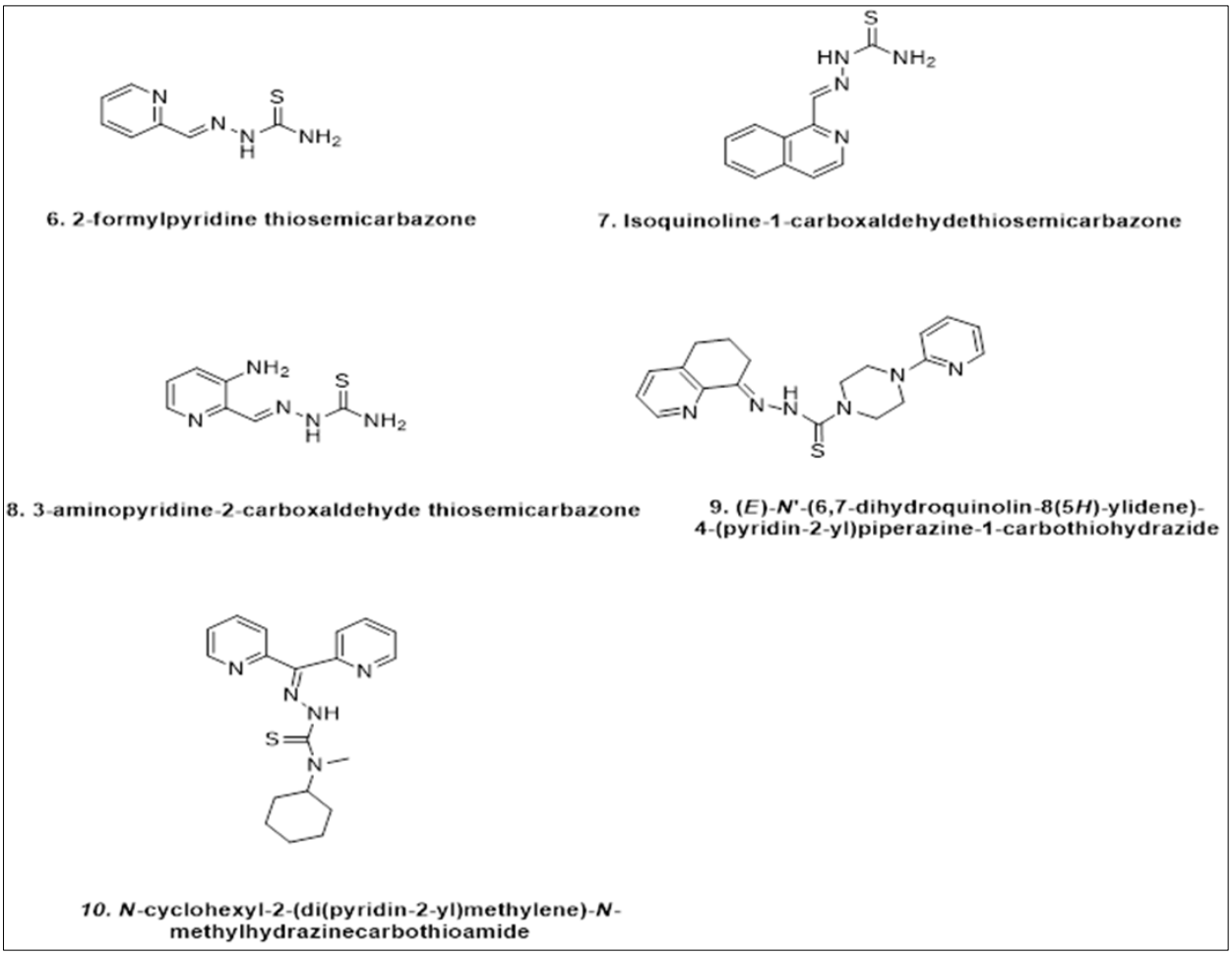



**Figure 2** Structure of potent anticancer thiosemicarbazides based derivatives (6-10)

Schiff bases containing azomethine (–C=N-) or imine functional group and are establish to be a versatile pharmacophore for design and development of various bioactive lead compounds [23-24].

Considering the important biological activities of carbazole and thiosemicarbazide derivatives, herein we report design, synthesis and *in vitro* anticancer activity of substituted carbazole bearing thiosemicarbazides derivatives. All final compounds (**17-28**) were evaluated for *in vitro* anticancer activity ($IC_{50}$) against the U87 MG cell line using the MTT assay for 24 time period.

## 2 Material and methods

All organic and biological chemicals were purchased from Spectrochem Private ltd. India, Sigma–Aldrich Co. USA, Hi-Media, Alpha Aesar and used without further purification.

### 2.1 MTT Assay

Treatment of cells ($5\times10^3$ U87MG) with various concentrations (1μM to 250 μM) of compound (17-28) for 24 h time period. The $IC_{50}$ value was calculated using the MTT assay reported formula in previous papers [9, 25]. All experiments were performed in triplicate, and the relative cell viability (%) was expressed as a percentage relative to the untreated control cells.

### 2.2 Synthesis of compound (17-28)

Starting material 6-substituted 1,4-dimethyl-9-*H*-carbazol-3-carbaldehyde (R=H,F,Cl, Br, $OCH_3$,OH) (11-16) intermidate reported in our and other reseach groups previous method [9, 26, 27]. Equimolar amount of 6-substituted 1,4-dimethyl-9-H-carbazol-3-carbaldehyde (11-16) intermediate and substituted thiosemicarbazides (R=H, $CH_3$) were dissolved in ethanol reflux for 4-6 h (SCHEME 1) (Figure 3) [28]. Progress of the reaction was monitored by TLC using, petroleum

ether: ethyl acetate (7:3) or chloroform: methanol (9:1) system. After completion of the reaction, the solid product that appeared out on cooling overnight was filtered, washed twice with ethanol: water (2:8), dried it and used without any further purification.

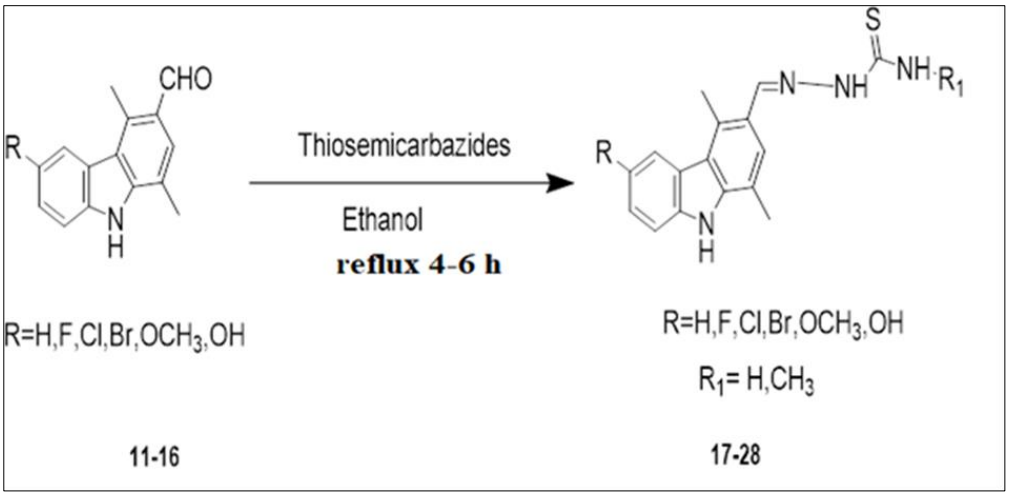



**Figure 3** Reagent & condition (C) Substituted thiosemicarbazides, Ethanol, reflux, 4-6 h (**Scheme 1)**

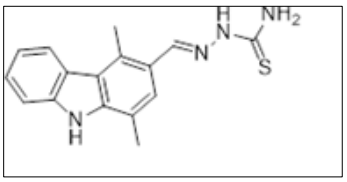
- (E)-2-((1,4-dimethyl-9-*H*-carbazol-3yl)methylene)hydrazinecarbothioamide **(17).** Yield: 80 %; off white solid; mp:224ºC;$^{1}$HNMR(400MHz-dmso-d6):δ(ppm)11.35(1-NH-N,br-s),11.25(1-NH-indole,br-s),8.69(CH=N,s), 8.01(H,s, aromatic), 7.90 & 8.123 (2H,$NH_2$, br-s), 7.45 (d,1H, aromatic), 7.522(m,2-H,aroamtic) ,2.77(3H,s,$CH_3$), 2.56(3H,s,$CH_3$), HRMS(ESI-Q-TOF):$C_{16}H_{16}N_4S$ [M + H]$^+$ calcd *m/z*, 296.1095, found m/z(M+1) 297.1165
- (E)-2-((6-fluro-1,4-dimethyl-9-*H*-carbazol-3-yl)methylene)hydrazinecarbo thioamide **(18).** Yield: 70%. Off white solid; mp: 285ºC. $^{1}$H NMR(400 MHz-dmso-d6): δ(ppm);11.35(1-NH,br-s),11.00(1-NH-N,br-s), 8.5(CH=N,s), 8.10and 7.90 (2H,$NH_2$br, s),8.18(1-H,d,aromatic),7.89(1H,s, aromatic),7.51(d,1-H,aromatic),7.40(d,1H, aromatic),7.15(1-H,aromatic),2.77(3H,s,$CH_3$),2.56(3-H,s,$CH_3$),HRMS(ESI-Q-TOF):.$C_{16}H_{15}FN_4S$ [M+H]$^+$ calcd *m/z*,314.1001, foundm/z(M+1) 315.1256.
- (E)-2-((6-cholro-1,4-dimethyl-9-*H*-carbazol-3-yl)methylene)hydrazinecarbothioamide **(19).** Yield :70%. Off white solid; mp: 235ºC. $^{1}$H NMR(400 MHz-dmso-d6):δ(ppm)11.70(1-NH,br-s),11.35(1-NH-N,br-s), 8.5(CH=N,s), 8.18(1-H,d,aromatic), 8.23 & 7.90 (2H, -$NH_2$, brs), 7.89(1-H,s, aromatic), 7.51(d,1-H, aromatic),7.40( d,1-H), 7.15(1-H,Aromatic), 2.77(3-H,s), 2.56(3-H,s). HRMS (ESI-Q-TOF): $C_{16}H_{15}ClN_4S$ [M + H]$^+$ calcd *m/z* 330.0705 found m/z(M+1) 331.0806
- (E)-2-((6-bromo-1,4-dimethyl-9-*H*-carbazol-3-yl)methylene)hydrazinecarbothioamide**(20**). Yield: 80%. Pale yellow solid. mp: 254 ºC. $^{-1}$HNMR;11.645(1-NH, br-s),11.257(1-NH-N,br-s),8.68 (CH=N,s) 8.274(1-H,s,aromatic) ,8.144 and 7.92 (2H,-$NH_2$-brs),8.102(1-H, s, aromatic) ,7522(m,2-H, aromatic), 2.77(3-H,s),2.56(3-H,s).$^{13}$CNMR: 177.65,142.37, 140.92, 139.13, 131.37, 127.84, 125.63, 125.20, 124.95, 123.48, 120.27,119.01,113.60, 111.67, 17.06, 15.46. HRMS (ESI-Q-TOF):$C_{16}H_{15}BrN_4S$ [M+H]$^+$ calcd *m/z*,374.0200, found m/z(M+1) 375.0252.
- (E)-2-((6-methoxy1,4-dimethyl-9-*H*-carbazol-3-yl)methylene)hydrazine carbothioamide **(21)** Yield: 70%. Pale yellow solid; mp: 244º C. IR (KBr): 3422.63, 3348, 3287.32, 2825, 1588.13,1537.96,1495,1316,1208,1040 cm$^{-1}$. $^{1}$HNMR: 11.35(1- Indole N-H br-s), 11.25(1-NH-N, br s) ,8.68(1H,CH=N,s), 8.12 and 7.85 (2H-$NH_2$-C=S, 2 br-s), 8.00(1-H,s aromatic), 7.7(1-H,s, aromatic),7.45(d,1H,aromatic),7.05(d,1-H,V),3.85(3H,$OCH_3$),2.82(3-H,s),2.49(3-H,s).HRMS (ESI-Q-TOF):$C_{17}H_{18}N_4OS$ [M +H]$^+$ calcd *m/z* 326.1201, found m/z (M+1) 327.1294
- (E)-2-((6-hydroxy-1,4-dimethyl-9-*H*-carbazol-3-yl)methylene)hydrazine carbothioamide **(22).** Yield: 80%. Off White solid. $^{1}$H NMR;11.659(1indole-NH,brs),11.286(1-NH-N,brs),8.48(CH=N,s), 8.406(1-H,s,aromatic), 8.144 and 7.92 (2H,-$NH_2$-brs),8.28(d,1-H),8.070(1-H,d),7.531(2-H,d),3.10(3-H,N-CH3,s),2.77(3-H,s),2.56(3-H,s). $^{13}$CNMR: 177.65,141.95, 140.92, 139.15,131.31,127.88,125.62,.62,124.98,123.32, 120.32,118.96, 113.61, 111.67, 31.0,17.14,15.46. HRMS(ESI-Q-TOF): $C_{16}H_{16}N_4OS$ [M+H]$^+$ calcd *m/z*, 312.1044 , found m/z(M+1) 313.1129
- (E)-2-((1,4-dimethyl-9-*H*-carbazol-3-yl)methylene)N-methylhydrazinecarbothio amide **(23).**Yield: 70%. Light blue solid. HNMR: 11.35(1- Indole N-H br s), 11.25(1-NH-N, br s), 8.68(CH=N,s) , 8.12 and 7.89 (-$NH_2$, 2 br-s) 8.00(1-H,s aromatic),7.7(1-H,saromatic),7.45(d,1H),7.05(d,1-H),3.85(3-H,$OCH_3$),2.82(3-H,s),2.49(3-H,s), $^{13}$CNMR: 177.30, 177.1221, 141.8346, 139.9849, 124.95, 123.80, 123.39,122.64, 122.44, 120.71,119.41, 118.16, 111.25,30.85, 16.75, 15.70 HRMS(ESI-Q-TOF) $C_{17}H_{18}N_4S$ calcd *m/z* 310.1252 found m/z(M+1) 311.1324
- (E)-2-((6-fluro-1,4-dimethyl-9-*H*-carbazol-3-yl)Nmethylene)N-methyl hydrazine carbothioamide **(24)**. Yield: 80%, off white solid. $^{1}$HNMR: 11.49(1-Indole N-H br s), 11.24(1-NH-N, br s), 8.66 (CH=N,s), 8.36 (1H,aromatic),

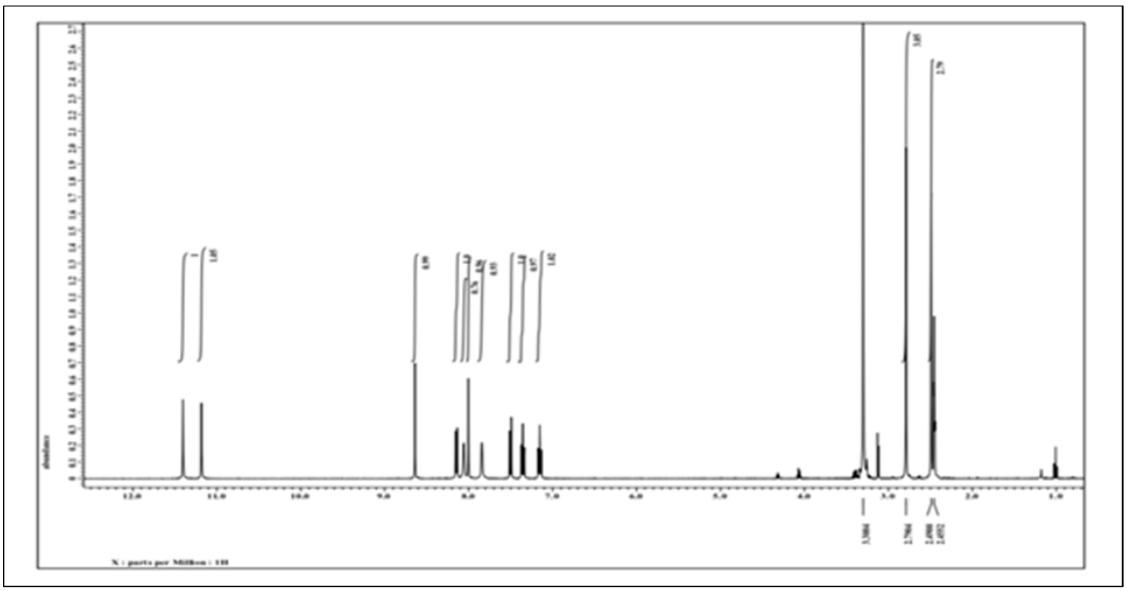
8.01(NH=CS, s, aromatic), 7.91(1-H,d aromatic),7.51(dd,2H,aromatic),7.25(1-H,t,aromatic),3.33(3H,N-$CH_3$), 2.78(3-H,s,$CH_3$),2.49(3-H,s,$CH_3$).HRMS (ESI-Q-TOF):.$C_{17}H_{17}FN4S$ $[M+H]^+$ calcd $m/z$ ,328.1158, found m/z 329.1034

- (E)-2-((6-bromo-1,4-dimethyl-9-*H*-carbazol-3-yl)Nmethylene)N-methyl hydrazine carbothioamide **(26)**. Yield: 80%, off white solid. $^1$HNMR: 11.65(1-Indole N-H br s), 11.28(1-NH-N, br s), 8.67 (CH=N,s), 8.40 (1H,aromatic), 8.03(NH=CS,s, aromatic), 8.25(1-H,d aromatic),7.51(dd,2H,aromatic), 3.33(3H,N-$CH_3$),2.78(3-H,s,$CH_3$),2.49(3-H,s,$CH_3$).HRMS (ESI-Q-TOF):.$C_{17}H_{17}FN4S$ $[M+H]^+$ calcd $m/z$ ,388.0357, found m/z 389.0410
- (E)-2-((6-methoxy-1,4-dimethyl-9-*H*-carbazol-3-yl)methylene)N-methyl-hydrazine carbothioamide **(27).** Yield: 80%, light yellow solid.$^1$HNMR(dmso-d6): 11.30(1-Indole N-H br-s), 11.22(1-NH-N, br s), 8.63(1H,CH=N,s), 8.05(NH-C=S, br-s) 8.00(1-H,s aromatic),7.6(1-H,s, aromatic), 7.45(d,1H, aromatic), 7.05(d,1-H,aromatic),3.86(3H,$OCH_3$), 3.05(3-H,N-$CH_3$,s), 2.80(3-H,s), 2.49(3-H,s). HRMS (ESI-Q-TOF): $C_{16}H_{16}N_4S$ $[M + H]^+$ calcd $m/z$ , 296.11found m/z 297.1165
- (E)-2-((6-hydroxy-1,4-dimethyl-9-*H*-carbazol-3-yl)methylene)N-methylhydrazine carbothioamide **(28).** Yield: 75%. Off white solid.$^1$HNMR(dmso-d6):11.24(1-Indole-N-H, br, s), 11.13(1-NH-N,brs), 8.97(1-OH,s),7.96(NH-C=S,),8.63(CH=N,s),8.35(1H,d,aromtic),7.96(1-H,s,NH-$CH_3$),7.55(1-H,aromatic,d),7.34(1-H,aromatic,d),6.91(1-H,aromatic,dd),3.05(3-H,N-$CH_3$,s),2.78(3-H,$CH_3$),2.51(3-H,$CH_3$).HRMS(ESI-Q-TOF):.$C_{17}H_{18}N_4OS$ $[M + H]^+$ calcd $m/z$ 326.1201 found m/z(M+1) 327.1240

### 2.3 Analysis of synthesized compounds

All the compounds were well confirmed by NMR, and HRMS. $^1$H and $^{13}$CNMR spectra were recorded on a Bruker Avance II and JEOL 400 MHz NMR instrument. Molecular mass of the compounds was determined by HRMS MASS spectroscopy using a Micromass, Q-Tof micro (Water make) spectrophotometer.



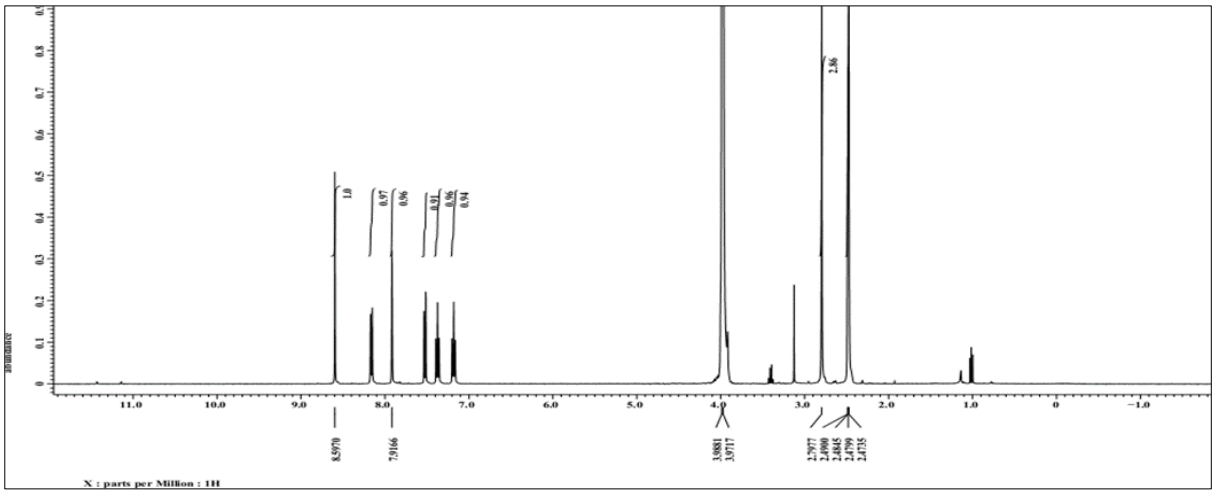



**Figure 4** Proton NMR spectra of compound (E)-2-((1,4-dimethyl-9-*H*-carbazol- yl)methylene)hydrazinecarbothioamide (17) in Deuterated DMSO solvent (above) and DMSO-D6 + D20 solvent (below)

## 3 Results and discussion

### 3.1 Chemistry

Synthesis of 6th position substituted carbazole (R=H,F,Cl,Br, $OCH_3$,OH) bearing with or without substituted thiosemicarbazide ($R_1$=H,$CH_3$) 17-28 done from starting material 6-substituted 1,4-dimethyl-9-*H*-carbazol-3-carbaldehyde (R=H,F,Cl, Br, $OCH_3$,OH) (11-16) intermidate reported in our previous reserach paper method [9]. All final compounds (17-28) were well analyzed by proton NMR experiment (Figure 4) where appearance of six aromatic proton of 1,4-dimethyl-9-H-carbazole structure around 7-8.5 ppm. Three separate broad singlet peaks around δ = 11.5, 11.25 and 8.20-7.90 ppm respectively were assigned to indole 9-NH of carbazole scaffold, N-NH groups, $NH_2$-C=S, proton respectively. The two characteristic methyl peaks in 1,4-dimethyl-9-H-carbazole found around δ value 2.50 and 2.71 ppm respectively in proton NMR spectra. In the $^1$H NMR spectra of compounds 17-28, the signal due to the CH=N proton was observed in the NMR spectra at 8.5– 8.6 ppm gives Schiff base formation i.e., linking of carbazole scaffold to thiosemicarbazide part formation. All exchangeable proton likes indole 9-NH, N-NH and $NH_2$-C=S groups were also disappeared on addition of deuterated solvent $D_2O$ in DMSO-d6 proton NMR spectra further proven existence of these final compounds (Figure 4). In the HRMS spectroscopy of some few compounds, the M+1 peak was observed for the compounds (17= 297.1165, 18 = 315.1256., 19 = 331.0806, 20 = 375.0252, 21 = 327.1294, 22 = 313.1129, 23 = 311.1324, 24 = 329.1034, 26 = 389.0410, 27 = 297.1165, 28 = 327.1240 further confirmed the formation of 17-28.

### 3.2 *In vitro* anticancer study

MTT assay was carried to determine their *in vitro* anticancer activity ($IC_{50}$) of all synthesized compounds (17-28) on the U87 human glioma cell line for 24 h time period. Results of *in vitro* anticancer profile were analysed on the basis of $IC_{50}$ values were compared to standard drug TMZ shown in **Table -1**[9, 29]. The $IC_{50}$ values of Mahanine, a pyranocarbazole alkaloid showed potential *in vitro* anticancer activity ($IC_{50}$) in a range of 12- 15 μM against glioma cell lines viz. LN229 and U87MG cells respectively after 48 h using MTT assay by Bhattacharya K et al.[ 30, 31]. All final compounds (17-28) divided in two series (A, B) showed $IC_{50}$ values in the range of 21 μM-215 μM (Table 2). In series A, $IC_{50}$ values of 6-substituted-1,4-dimethyl-9-*H*-thiosemicarbazide derivative (R= F, Br, Cl, $OCH_3$, OH and $R_1$ = H) 17-22 were found to be 26.50 μM (17), 160 μM (18), 140 μM (19), 34.00 μM (20), 39.00 μM (21), 80.00 μM (22) respectively. *In vitro* anticancer activity profile was found in the order of 17 (H) >20 ($OCH_3$) >21 (Br) > 22 (OH) >19 (Cl) > 18 (F). In series A, four compounds 17, 20, 21, 22 showed better anticancer activites on the human glioma U87 MG cell line than temozolomide (100 μM) [9, 29, 32]. Electronegative elements like fluoro, chloro substitution imparts inductive and mesomeric effect, whereas bromo substitution exhibits mostly mesomeric effect found play an important role for potent *in vitro* anticancer activity in series A[9, 33]. In series B, $IC_{50}$ values of 6-substituted-1,4-dimethyl-N-methyl-thiosemicarbazide derivative (R= F, Br, Cl, $OCH_3$, OH and $R_1$ = $CH_3$) 23-28 were found to be 62 μM (23), 215 μM (24), 198 μM (25), 161 μM (26), 183.32 μM (27), 50.00 μM (28) respectively, and their activity profile was in the order of 23 (H) >28(OH)>26 ($OCH_3$) >27 (Br) > 25 (Cl) > 24 (F). Methyl substitution on thiosemicarbazide part or varying substitution on 6th position on carbazole core based thiosemicarbazide derivatives (23-28) showed loss of anticancer activities on the U87MG cell line were

observed (**Figure 5**). Based on the *in vitro* anticancer data of all compounds (**17-28**) showed that series A, were found better than series B on the U87MG cell line

**Table 1** *In vitro* cytotoxicity (IC50) profile for the compounds (17-28)

| **S.NO.** | **R** | **$R_1$** | **$IC_{50}$ (µM)** |
|---|---|---|---|
| 17 | H | H | 26.50 ±6.24 |
| 18 | F | H | 160 ±12.24 |
| 19 | Cl | H | 140 ±12.24 |
| 20 | Br | H | 34.00 ±12.24 |
| 21 | $OCH_3$ | H | 39.00 ±12.24 |
| 22 | OH | H | 80.00 ±12.24 |
| 23 | H | $CH_3$ | 62.00 ±12.24 |
| 24 | F | $CH_3$ | 215 ±12.24 |
| 25 | Cl | $CH_3$ | 198 ±12.24 |
| 26 | Br | $CH_3$ | 161.00 ±12.24 |
| 27 | $OCH_3$ | $CH_3$ | 183.32 ±12.24 |
| 28 | OH | $CH_3$ | 50.00 |
| Temozolomide | ---------- | ---------- | 100 |
| Carmustine | ---------- | ---------- | 18.24 |

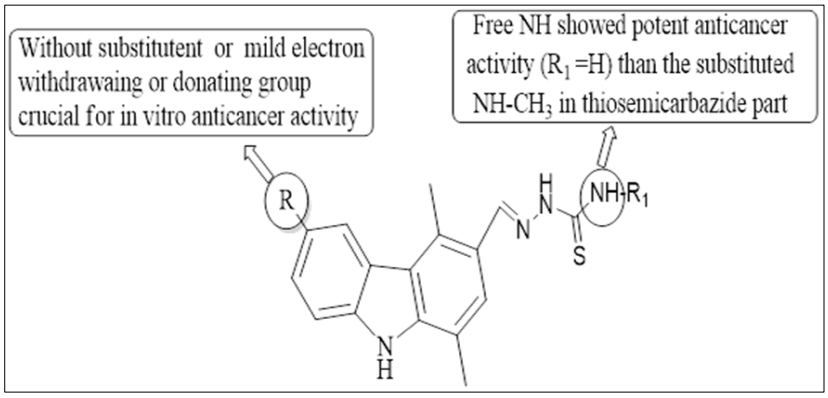



**Figure 5** SAR analysis based on *in vitro* anticancer activity of final compounds against U87 MG human glioma cell line

## 4 Conclusion

In this research work, we synthesized substituted carbazole bearing thiosemicarbazides Schiff based derivatives (17-28) incorporating different electron-donating and withdrawing groups was prepared and their structure were well characterized by different analytical spectroscopy techniques. SAR study was also well established based on *in vitro* cytotoxicity ($IC_{50}$) data of all final compounds (17-28) evaluated against human glioma U87 MG cell line using MTT assay. SAR studies of all carbazoles bearing thiosemicarbazide derivatives showed that no substitutent on $6^{th}$ position of carbazole scffaold (R =H) or bromo or methoxy group and also no substitutent on thiosemicarbazide moiety ($R_1$ =H) necessary for potent anticancer activity on the U87MG cell line. Mesomeric or inductive effect due to electron donating or electron withdrawing group on 6th position of carbazole group bearing with thiosemicarbazide moiety also found crucial role for anticancer activities. *In vitro* anticancer studies of carbazole bearing thiosemicarbazide derivatives (17-28) can be helpful to further in vivo of these potential novel anticancer agents against brain tumors.

## Compliance with ethical standards

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*Disclosure of conflict of interest*

The authors declared that article content has no conflicts of interest.

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