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Synthesis and Biological Evaluation of Some New Tetrahydrocarbazole Analogues

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ABSTRACT

Several pharmacological activities like anti-cancer, anti-microbial, antibacterial, antifungal, and anti-viral activity have been attributed to tetrahydrocarbazole. The above observations prompted us to synthesize some novel tetrahydrocarbazole derivatives as possible anticancer agents. A series of novel tetrahydrocarbazole derivatives have been synthesized by the reaction of tetrahydrocarbazole with substituted aromatic aldehydes. The starting material, tetrahydrocarbazole were prepared by Fischer indolisation reaction of cyclohexanone with phenylhydrazine in the presence of acetic acid. The cycloaddition of tetrahydrocarcazole and substituted aromatic aldehydes gives tetrahydrocarbazole derivatives (A_1-A_{10}). The structures of synthesized derivatives were confirmed by IR, ^{1}HNMR and Mass spectrum. The synthesized compounds were screened for their *in-vitro* anticancer activity. The anticancer activity data of the synthesized derivatives were found to be potent activity.

Key words: Phenyl hydrazine, Cyclohexanone, Tetrahydrocarbazole derivatives, *In-vitro* anticancer activity.

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INTRODUCTION

Tetrahydrocarbazole[1] having varied biological features are still of great scientific interest. They are widely found in bio-organic and medicinal chemistry due to its applications in drug discovery and have reported to possess a wide spectrum of various biological properties such as anti-cancer[2-3], anti-fungal[3,4], antimicrobial[4,5], antibacterial[5] and anti-viral[6] activity. Also carbazole nucleus is associated in variety of biological activities. Being a heterocyclic compound, tetrahydrocabazole is found as a starting material for the synthesis of larger number of bioactive pharmacophores. Its aromaticity makes it relatively stable, although as a heterocyclic it has reactive sites which allows for functionalization.

The activity of tetrahydrocarbazole occupies a prominent place in the field of anti cancer agents. With the view of above facts we have planned our work to synthesize and to develop its different tetrahydrocarbazole derivatives.

MATERIAL AND METHOD:

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on SHIMADZU FT-IR 8400 with KBr Pellets. ^{1}H-NMR spectra were recorded on 300 MHz–Bruker DPX 200. The chemical shifts are reported as parts per million down fields from tetramethylsilane. Mass spectra were recorded on LC-MS. The purity of the compounds was checked by TLC on pre-coated SiO2 gel (604 GF 254) aluminium plates (E Merck).

General procedures:

The synthetic strategy leading to the target compounds are illustrated in figure1 scheme and number of substitution is mentioned in Table 1. Tetrahydrocarbazole synthesized by an mixture of 0.1 mol of Cyclohexanone and 0.6 mol of Acetic acid contained in a three necked RBF and through dropping funnel 0.1 mol of Phenyl hydrazine is added during 1 hr. After refluxing and stirring an additional hour, the mixture was cooled to 5 °C and filtered. The crude solid was washed with water, recrystallised from methanol. The percentage yield was found to be 92% and the melting point 114-116 °C.

General method of synthesis of (chloroacetyl)-1,2,3,4-tetrahydro carbazole from 1,2,3,4-Tetrahydrocarbazole:

Equimolar of 1,2,3,4-tetrahydrocarbazole (0.01mol) and chloroacetyl chloride (0.01mol) in dry DMF in presence of potassium carbonate was refluxed for 12 hrs overnight. The contents of the flask poured in cold water and the product is recrystallised by ethanol.

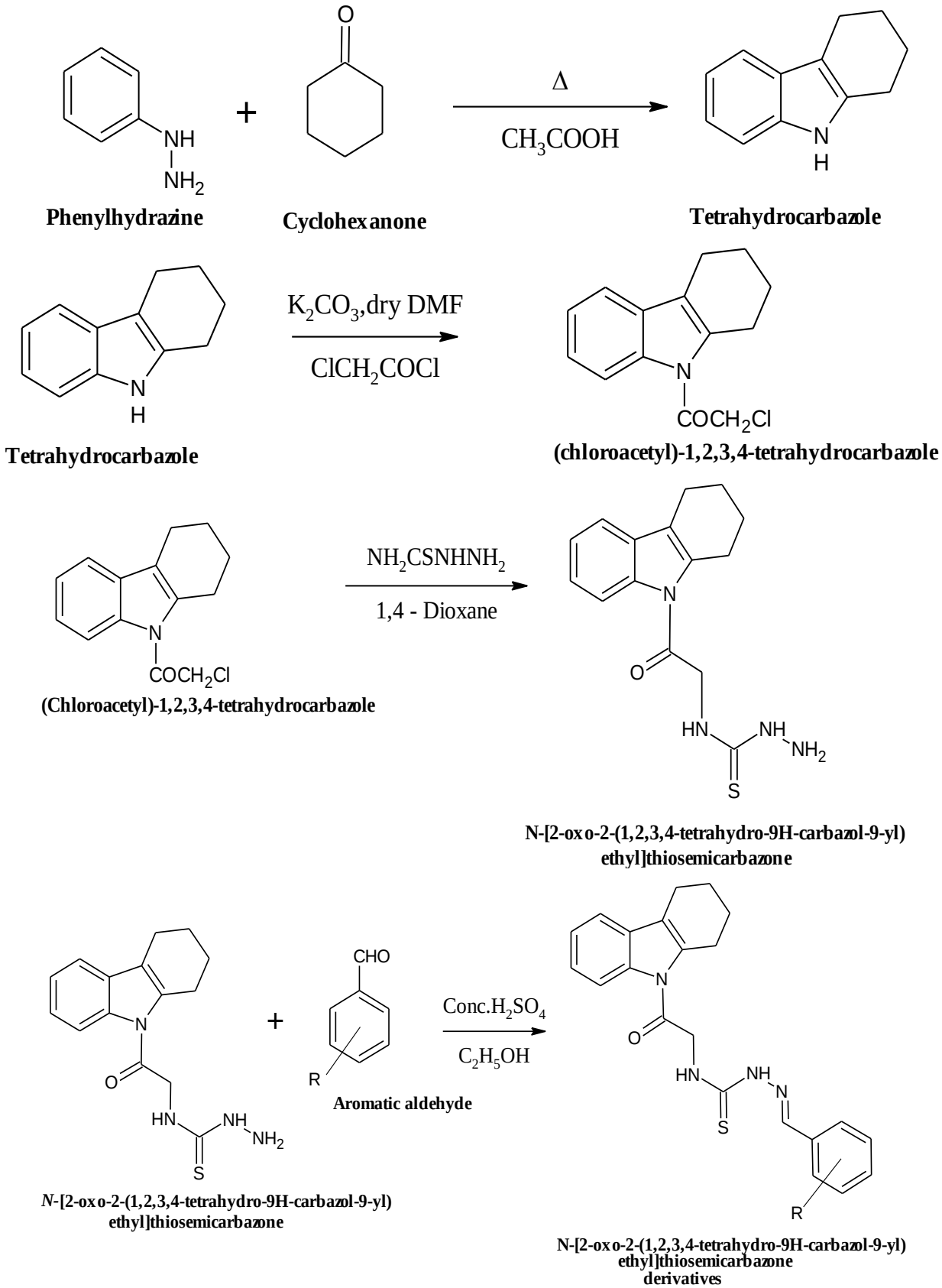
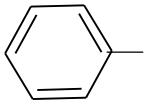
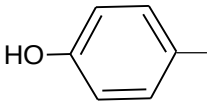
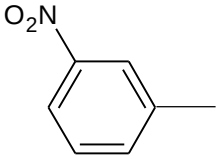
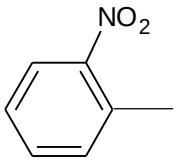
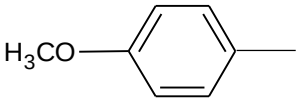
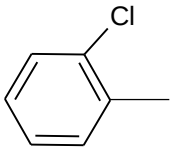
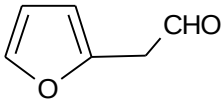
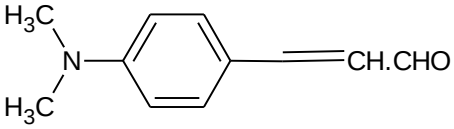
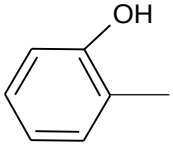


Figure 1: Scheme

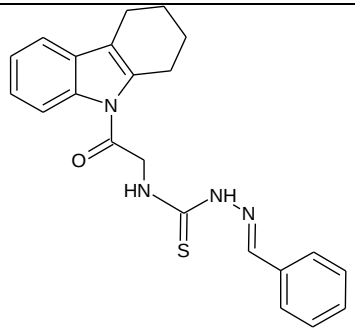
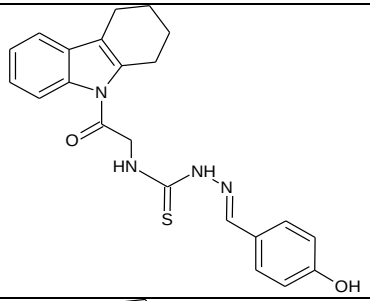
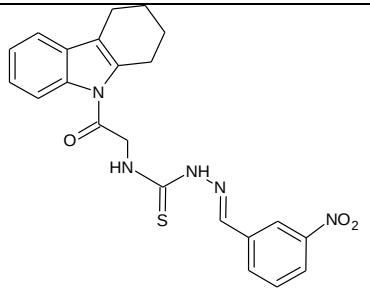
Table 1: Substitutions

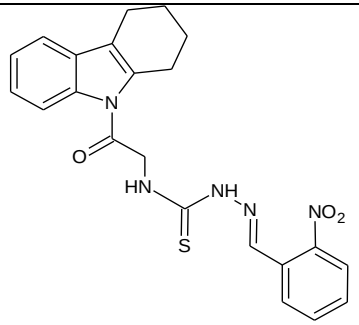
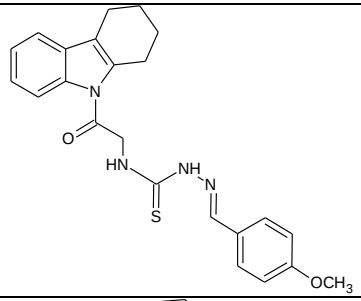
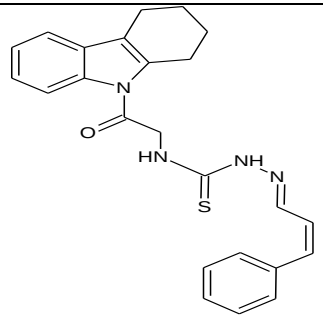
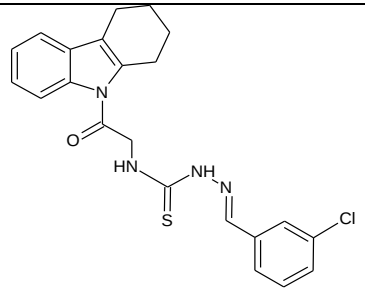
Compound	Structure
A_1	
A_2	
A_3	
A_4	
A_5	
A_6	
A_7	
A_8	
A_9	
A_{10}	

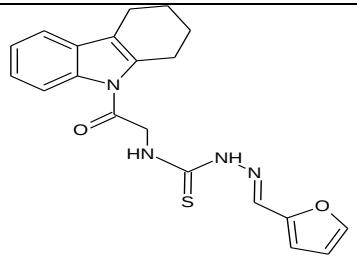
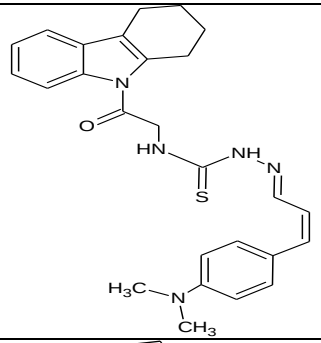
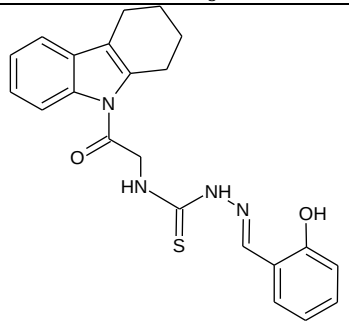
General method of synthesis of *N*-[2-oxo-2-(1,2,3,4)-tetrahydrocarbazole-9-yl) ethyl] thiosemicarbazone from (chloro acetyl) -1,2,3,4 tetrahydro carbazole:

0.03 mol of acyl chloride and 0.01 mol of thiosemicarbazide in 1,4-dioxane (20 ml) was refluxed for 8 hrs on a water bath. The excess solvent was removed under reduced pressure and contents were poured into cold water, recrystallised from ethanol.

Table 2: Physical Characterization Of Derivatives (A_1-A_{10})

SL NO	Compound	Chemical structure	Chemical name	Mol. Formula	Mol. weight	Yield (%)	Colour	MP	Mobile Phase	R_f Value
1.	A_1		phenyl-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone	$C_{22}H_{22}N_4OS$	390.5	75%	Brownish black	182°C	Methanol	0.79
2.	A_2		4-hydroxy phenyl -*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone	$C_{22}H_{22}N_4O_2S$	406.5	68%	Brownish black	185°C	Methanol	0.65
3.	A_3		3-nitro phenyl-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone	$C_{22}H_{21}N_5O_3S$	435.4	70%	Brownish black	192°C	Methanol	0.58

4.	A_4		2-nitro phenyl-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone	$C_{22}H_{21}N_5O_3S$	435.9	78%	Brownish black	198°C	Methanol	0.86
5.	A_5		4-methoxy phenyl -*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone	$C_{23}H_{24}N_4O_2S$	420.5	75%	Brownish black	208°C	Methanol	0.63
6.	A_6		Cinnamaldehyde-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone	$C_{24}H_{24}N_4Os$	416.5	62%	Brown	217°C	Methanol	0.74
7.	A_7		2-chloro phenyl -*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone	$C_{22}H_{21}N_4OSCl$	424.9	55%	Brownish black	210°C	Methanol	0.76

8.	A_8		furfuraldehyde-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone	$C_{20}H_{20}N_4O_2S$	380.4	67%	Yellowish brown	255°C	Methanol	0.69
9.	A_9		4-dimethyl cinnamaldehyde-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone	$C_{26}H_{29}N_5OS$	459.6	72%	Yellowishbrown	229°C	Methanol	0.68
10	A_{10}		2-hydroxy phenyl -*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone	$C_{22}H_{22}N_4O_2S$	406.5	63%	Brownish black	181°C	Methanol	0.84

General method of synthesis of *N*-[2-oxo-2-(1,2,3,4)-tetrahydrocarbazole-9-yl) ethyl] thiosemicarbazone derivatives:

Equimolar quantities of different substituted aromatic aldehyde (0.01mol) and *N*-[2-oxo-2-(1,2,3,4)-tetrahydrocarbazole-9-yl) ethyl] thiosemicarbazone (0.01 mol) were dissolved in 25 ml of alcohol and few drops of conc. sulphuric acid. The reaction mixture was reflux for 5-6 hrs. The contents were poured into cold water. The Schiff's base thus formed was filtered off and recrystalization from ethanol.

Anticancer Study[13,14]:

The anticancer activity of the synthesized compounds was carried out on cancer cell lines namely HT-29 (Colon cancer) and experimental work were done in Nathaji Rao G. Halgekar Institute of Dental Sciences and Research centre, Belgaum, Karnataka, India. The inhibition of the growth of cell lines, *i.e.*, Cytotoxicity was considered as anticancer activity. Toxicity of test compound in cells was determined by MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly coloured blue formazan product which was measured as absorbance at 492 nm on a spectrophotometer (spectra max, Molecular devices) and the IC_{50} values were determined by plotting % inhibition (from control) versus concentration.

RESULTS AND DICUSSION:

Synthesis of *N*-[2-oxo-2-(1,2,3,4)-tetrahydrocarbazole-9-yl) ethyl] thiosemicarbazone derivatives were analyzed physical characterization of derivatives (A_1-A_{10}) shown in Table 2

1. Phenyl-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone.

IR data:3410.5cm^{-1}(-NH str), 2926.01cm^{-1} (Ar-CH), 1448.5cm^{-1}(Ar-C=C str),^{1}H-NMR (DMSO)(d/ppm) 1.044 – 1.242 (m,8H,-CH_2 Cyclo), 2.336 (s,1H,-CH_2), 4.718 (s,1H,-CS-NH), Mass spectroscopy data: m/z 392.3 (M^+).

2. 4-hydroxy phenyl-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone

IR data : 3414.1 cm^{-1} (-NH & Ph-OH Str),2927.9 cm^{-1} (Ar-C-H Str), 1612.4 cm^{-1} (Amide C=O Str), ^{1}H-NMR (DMSO)(d/ppm) δ 1.124 – 1.242 (m,8H,-CH_2 Cyclo), δ 2.506 (s,1H,-CH_2), δ 3.175 (s,1H,Ar-CH=N), δ 4.529(s,1H,-CS-NH),), Mass spectroscopy data: m/z 407.3 (M^+).

3. 3-nitrophenyl-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone

IR data:3404.3 cm^{-1}(-NH Str), 2926.01 cm^{-1}(Ar C-H Str), 1620.2 cm^{-1} (Amide C=O Str),1517.9 cm^{-1}(-NO_2 Str), ^{1}H-NMR (DMSO)(d/ppm) δ 0.079 – 1.4514 (m,8H,-CH_2 Cyclo), δ 2.506 (s,1H,-

CH_2), δ 3.728 (s,1H,Ar-CH=N), δ 4.764 (s,1H,-CS-NH), δ 6.606-8.165 (m,8H,Ar-H), Mass spectroscopy data: m/z 434.3 (M^+).

4. 2-nitrophenyl-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone.

IR data : 3406.2 cm^{-1} (-NH Str), 2922.1 cm^{-1} (Ar –CH Str), 1622.3 cm^{-1} (Amide C=O Str),1519.9 cm^{-1} (-NO_2Str) ^{1}H-NMR (DMSO)(d/ppm) δ 1.114 – 1.602 (m,8H,-CH_2 Cyclo), δ 2.505 (s,1H,-CH_2), δ 3.740 (s,1H,Ar-CH=N), δ 4.601 (s,1H,-CS-NH), δ 7.129-7.926 (m,8H,Ar-H), Mass spectroscopy data: m/z 433.1(M^+).

5. 4-methoxy phenyl-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone.

IR data : 3352.2 cm^{-1} (-NH Str), 2927.9 cm^{-1} (Ar C-H Str), 2032.3 cm^{-1} (-OCH3), ^{1}H-NMR (DMSO)(d/ppm) δ 1.079 – 1.194 (m,8H,-CH_2 Cyclo), δ 2.506 (s,1H,-CH_2), δ 3.402 (s,3H,-OCH_3), δ 4.718 (s,1H,-CS-NH),), Mass spectroscopy data: m/z 421.2 (M^+).

6. Cinnamaldehyde-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone.

IR data :3037.8 cm^{-1} (-NH&AlkenesC-HStr), 2916.3 cm^{-1} (Ar-C-HStr), ^{1}H-NMR (DMSO)(d/ppm) δ 1.066 – 1.431 (m,8H,-CH_2 Cyclo), δ 2.506 (s,1H,-CH_2), δ 3.679 (s,1H,Ar-CH=N), Mass spectroscopy data: m/z 417.2 (M^+).

7. 2-chlorophenyl-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone.

IR data : 3437.1 cm^{-1} (-NH Str), 2924.0 cm^{-1} (Ar C-H Str), 1693.5 cm^{-1} (Amide C=O Str), 744.5 cm^{-1} (C-Cl Str) ^{1}H-NMR (DMSO)(d/ppm) 1.126 – 1.339 (m,8H,-CH_2 Cyclo), δ 2.506 (s,1H,-CH_2), δ 3.524 (s,1H,Ar-CH=N), δ 4.033 (s,1H,-CS-NH), δ 7.333-7.622 (m,8H,Ar-H), Mass spectroscopy data: m/z 381.3(M^+).

8. Furfuraldehyde-*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] thio semicarbazone.

IR data : 3433.2 cm^{-1} (-NH Str), 2856.5 cm^{-1} (Ar-C-H Str), 1675.4 cm^{-1} (Amide C=O str), 1413.8 cm^{-1} (Ar-C=C Str), 1020.3 cm^{-1} (C=S Str) ^{1}H-NMR (DMSO)(d/ppm) δ 1.124 – 1.242 (m,8H,-CH_2 Cyclo), δ 2.506 (s,1H,-CH_2), δ 3.175 (s,1H,Ar-CH=N), δ 4.529 (s,1H,-CS-NH), Mass spectroscopy data: m/z 382.3(M^+).

Anticancer Study[13,14]:

Toxicity of test compound in cells was determined by MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye which was measured as absorbance at 492 nm on a

spectrophotometer and the IC_{50} values were determined by plotting % inhibition (from control) versus concentration. The anticancer (*in-vitro*) studies are summarized Table 3 and 4.

Table-3: *In-vitro* anti-cancer activity of substituted tetrahydrocarbazole derivatives (A_1-A_5) against HT-29 :

Compound	Concenration (µg/ml)	O.D AT 492 nm	(%) OF Cell Lysis	IC_{50}
A_1	10	0.813	25%	20 µg
	20	1.084	50%	
	30	1.109	>75%	
A_2	10	0.532	No lyses	>30 µg
	20	0.566	No lyses	
	30	0.692	No lyses	
A_3	10	0.675	No lyses	30 µg
	20	0.668	No lyses	
	30	0.927	50%	
A_4	10	0.546	No lyses	>30 µg
	20	0.681	No lyses	
	30	0.853	<50%	
A_5	10	0.587	No lyses	>30 µg
	20	0.625	No lyses	
	30	0.821	25%	
	Control	0.586	No lyses	-

Table-4: *In-vitro* anti-cancer activity of substituted tetrahydrocarbazole derivatives (A_6-A_{10}) against HT-29 :

Compound	Concentration (µg/ml)	O.D. AT 492 nm	% of Cell Lysis	IC_{50}
A_6	10	0.810	25%	30 µg
	20	1.085	50%	
	30	1.111	>75%	
A_7	10	0.809	No lyses	>30 µg
	20	1.086	No lyses	
	30	1.111	>75%	
A_8	10	0.540	No lyses	30 µg
	20	0.684	No lyses	
	30	0.856	No lyses	
A_9	10	0.670	No lyses	>30 µg
	20	0.666	No lyses	
	30	0.934	<75%	
A_{10}	10	0.528	No lyses	20 µg
	20	0.564	No lyses	
	30	0.698	25%	
Control	-	0.586	No lyses	-

CONCLUSION :

The cycloaddition reaction of tetrahydrocarbazole with different aromatic aldehydes to obtain tetrahydrocarbazole derivatives (A_1-A_{10}) was attempted by employing various reagents and reaction conditions. However the desired cycloaddition was successful only when the reaction was carried out by using conc.H_2SO_4 as a catalyst and ethanol as a solvent. The desired tetrahydrocarbazole derivatives (A_1-A_{10}) were obtained in a good yield by conventional method. The anticancer activity, of the synthesized tetrahydrocarbazole derivatives revealed that the compounds A_3, A_4, A_5, A_7 and A_9 were effective HT-29 cell lines respectively.

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