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SYNTHESIS AND CHARACTERIZATION OF NOVEL N- SUBSTITUTED CONDENSED CARBAZOLE DERIVATIVES FOR ITS ANTITUMOR ACTIVITY

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ABSTRACT

Carbazole is an aromatic heterocyclic organic compound. It has a tricyclic structure, consisting of 2 six membered benzene ring fused on either side with a five membered nitrogen containing ring. Carbazole and its derivatives are an important class of nitrogen containing heterocyclic compounds that are wide spread in nature. The N-substituted derivatives have acquired the attention of researchers due to their therapeutic potential. Hence here an attempt is made to synthesize some novel N- substituted carbazole derivatives for its antitumor activity.

KEYWORDS

Cyclohexanone, N-substituted carbazole derivatives and *In vitro* activity.

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INTRODUCTION

Carbazole are among exclusive types of N containing aromatic heterocycles possessing desirable electronic and charge transport property as well as large pi-conjugated system. The structurally rigid carbazole ring is also found friendly towards the introduction of various functional group. The carbazole nucleus as always stimulated the endeavors to find new pathway for its synthesis and its novel derivatives because of extensive photo physical, photochemical and biological property especially then amazing pharmacological profile and then role as a drug molecule. To date numerous researchers, make effort to develop efficient synthetic avenues to Carbazole and its well modified derivatives. Based on the documented facts which highlighted the incredible potential

application of Carbazole based derivatives in the field of chemistry. According to recent development Carbazole shows very high activity against many organism and bacteria, fungi, parasites. Carbazole derivatives are potential, multifunctional agents for the treatment of neurological disorders and showed promising anti-tumor activity against different cell lines by the variety of mechanism. They work undertaken here relates to the synthesis of some novel N-substituted condensed carbazole derivatives.

MATERIAL AND METHODS

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 described 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 (EMerck).

Procedure

In a 250ml RBF, a mixture of tetrahydro-5H-carbazole (1.0 g, 5.8mmole) and DMF (10ml) was taken. Then added K_2CO_3 with a stirring. After 30 minutes 2-chloroacetyl chloride (1.5ml, 5.8mmloe) was added slowly at room temperature. Then reaction was heated to reflux temperature about 3 hours. After completion of the reaction, (reaction was monitored by TLC/LCMS) reaction, mass was filtered off, filtrate was quenched with water and extracted with toluene. The organic layer was collected and concentrated under vacuum. The desired product was obtained as yellow liquid (1.1 g, 80 % M/z-247.7).

Procedure

A 250ml RBF was charged with solution of stage 01 product (1.1g, 4.4mmloe) and 1, 4- Dioxane. To that added 1.0ml of con. HCl slowly at 18℃ with constant stirring and followed by thiosemicarbazide (0.59g, 4.34mmole) was added. Then reaction was stirred at 100℃ over 6 hours. Reaction was monitored by TLC / LCMS. After completion of the reaction, reaction mass was quenched with bicarbonate solution and extracted with DCM and all organic layer was combined and concentrated under vacuum. Obtained yellow oil (2.0g, 56%, M/z-302.4) was taken for next step.

Stage 03: Reaction Scheme

Procedure

A 100ml RBF was charged with stage 02 product (2.0g, 6.6mmole) and EDC. Then 1-(chloromethyl)-4-methylbenzene was added and reaction was stirred at room temperature over 16 hours. Reaction was monitored by TLC / LCMS. After completion of the reaction, reaction mass was filtered off, filtrate was collected and concentrated under vacuum. The desired product was obtained as a pale brown liquid. (1.5 g, 80%, M/Z- 390).

Procedure

In a 250ml RBF, stage 03 product (1.5 g, 3.88mmloe) was dissolved with Ethanol (20ml). To that added con. H_2SO_4 slowly with a constant stirring at room temperature. Then reaction was kept for reflux over 3 hours. Reaction was monitored by TLC / LCMS. After completion of the reaction, reaction mass was concentrated under vacuum. Obtained product was taken for purification.

General method for the synthesis of N-substituted carbazole derivatives

To the stage o3 product 1-benzylidene -4-(2-(5, 6, 7, 8-tetra hydrocarbazole-9-yl)-2-oxoethyl) thiosemicarbazide (1.5g, 3.88mmole) was dissolved with DCM (50ml). To that add different substitutions slowly with a constant stirring at room temperature. Then reaction was kept for stirring over 1 hour. Reaction was monitered by TLC/ LCMS. After the completion of reaction, reaction mass was quenched with water and extracted with DCM. The organic layer was collected and concentrated under vacuum obtained product was taken for purification.

Principle of assay

This is a colorimetric assay that estimate the reduction of yellow 3-(4, 5-dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and moved into the mitochondria where it is reduced to an insoluble, colored (dark

purple) formazan product. The cells are next solubilized with an organic solvent (eg. DMSO, Isopropanol) and the released, solubilized formazan reagent is measured spectrophotometric ally. Since reduction of MTT can only take place in metabolically active cells the level of activity is a estimate of the viability of the cells

Materials

SL NO CELL LINES MEDIA

A549 (Lung Cancer) DMEM with low glucose (Cat No-22125-0082)

FBS (Gibco, Invitrogen)Cat No -10270106

Antibiotic – Antimycotic 100X solution (Thermofisher Scientific)-Cat No-15240062

Protocol-

Cytotoxicity

The cells were sowed at a density of approximately 5×103cells/well in a 96-well flat-bottom micro plate and maintained at 370C in 95% humidity and 5% CO2 for overnight. Different concentration (500, 250, 125, 62.5, 31.250, 15.125µg/mL) of samples was treated. The cells were incubated for another 48 hours.

The cells in well were washed twofold with phosphate buffer solution, and 20µL of the MTT staining solution (5mg/ml in phosphate buffer solution) was added to each well and plate was incubated at 370C. After 4h, 100 µL of di- methyl sulfoxide (DMSO) was put into each well to dissolve the formazan crystals, and absorbance was recorded with a 570nm using micro plate reader (1, 2). Formula:

Surviving cells (%) = Mean OD of test compound /Mean OD of Negative control ×100

Using graph Pad Prism Version 5.1, we calculate the IC 50 of compounds

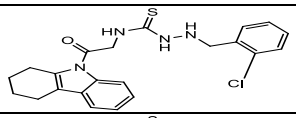
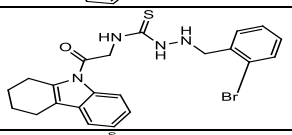
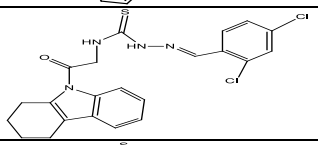
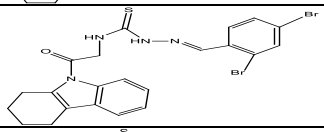
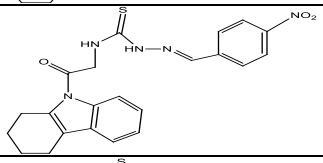
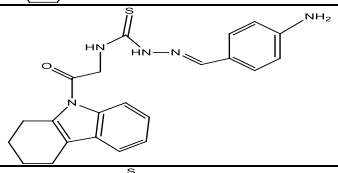
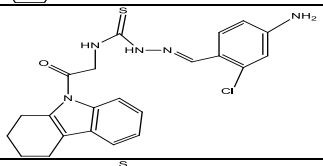
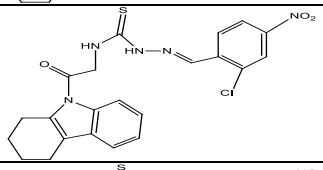
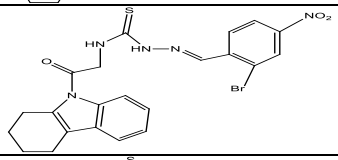
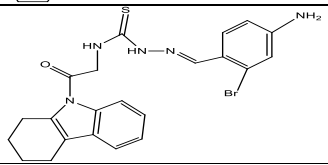
Note – DMSO Concentration is less 1.5% in experiments

Concentrations are in duplicates.

Table No.1: Substitutions of derivatives (A1-A10), the obtained compounds were confirmed by IR, NMR, mass spectroscopy

Compound	R
A_1	n-chloro succinamide
A_2	n-bromo succinamide
A_3	Di chloro succinamide
A_4	Di bromo succinamide
A_5	n- nitro succinamide
A_6	n-amino succinamide
A_7	n-chloro amino succinamide
A_8	n-chloro nitro succinamide
A_9	n-bromo nitro succinamide
A_{10}	n-bromo amino succinamide

Table No.2

Compound	Structure	Chemical Name	Molecular formula	Molecular Weight
A_1		*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] [o-chloro]benzylhydrazinecarbothioamide	$C_{22}H_{22}ClN_4OS$	426.96
A_2		*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] [o-bromo]benzylhydrazinecarbothioamide	$C_{22}H_{22}BrN_4OS$	471.41
A_3		*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] [1,3-dichloro]benzylhydrazinecarbothioamide	$C_{22}H_{22}Cl_2N_4OS$	461.41
A_4		*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] [1,3-dibromo]benzylhydrazinecarbothioamide	$C_{22}H_{22}Br_2N_4OS$	550.31
A_5		*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] [4-nitro]benzylhydrazinecarbothioamide	$C_{22}H_{23}N_5O_3S$	437.51
A_6		*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] [4-amino]benzylhydrazinecarbothioamide	$C_{22}H_{25}N_5OS$	407.53
A_7		*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] [4-amino,2-chloro]benzylhydrazinecarbothioamide	$C_{22}H_{22}ClN_5OS$	441.98
A_8		*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] [2-chloro,4-nitro]benzylhydrazinecarbothioamide	$C_{22}H_{22}ClN_5O_3S$	471.96
A_9		*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] [2-bromo,4-nitro]benzylhydrazinecarbothioamide	$C_{22}H_{22}BrN_5O_3S$	516.41
A_{10}		*N*-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] [4-amino,2-bromo]benzylhydrazinecarbothioamide	$C_{22}H_{24}BrN_5OS$	486.43

Anti-cancer study: **MTT**

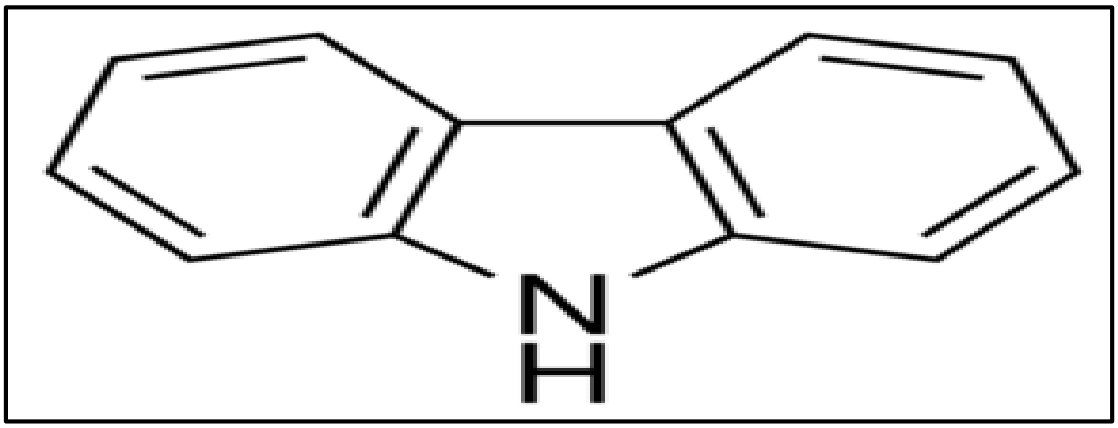
Table No.3: *In-vitro* anti-cancer activity of n-substituted condensed carbazole derivatives (A_1-A_{10}) againstA549 (Lung Cancer)

Compound	A549
A_1	405.90
A_2	304.20
A_3	511.10
A_4	229.30
A_5	659.60
A_6	562.50
A_7	1311.00
A_8	1053.00
A_9	1197.00
A_{10}	262.10
Paclitaxel(µg)	273.25
Paclitaxel(µM)	0.32

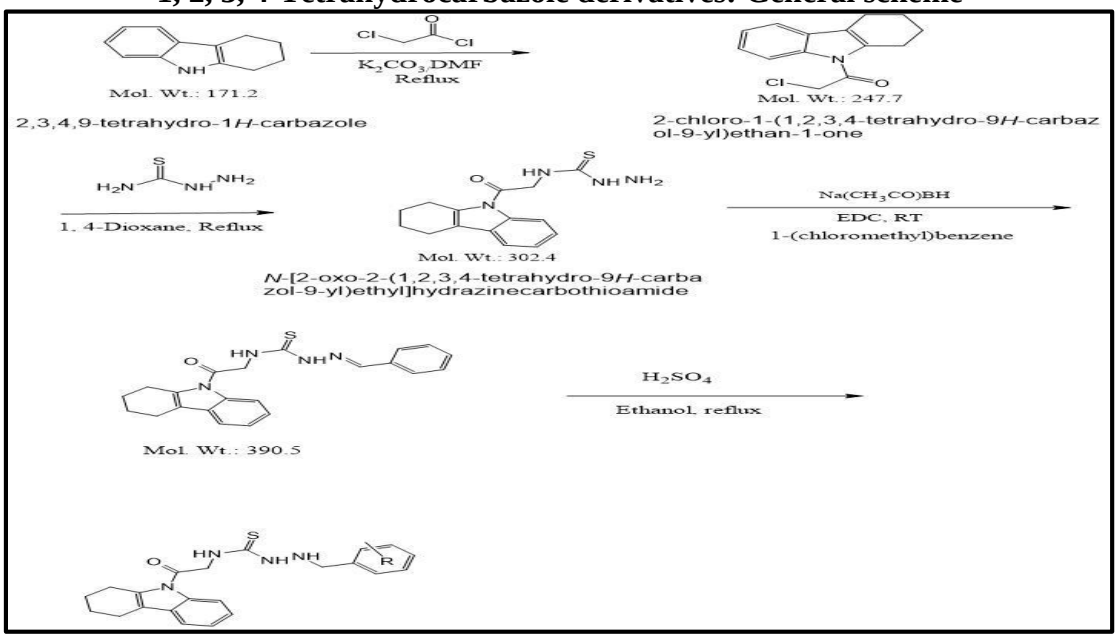
Table No.4

S.No	Conc µl/mL	Mean Cell Viability(A549)									
		A_1	**A_2**	**A_3**	**A_4**	**A_5**	**A_6**	**A_7**	**A_8**	**A_9**	**A_{10}**
1	500.000	32.16	42.16	51.11	29.49	58.07	53.57	68.52	62.71	65.96	37.37
2	250.000	56.66	54.59	62.37	47.58	65.81	65.37	78.89	75.96	77.97	50.72
3	125.000	69.61	64.79	75.48	71.51	76.54	69.82	85.36	83.12	81.45	61.94
4	62.500	81.18	72.19	80.75	74.61	87.57	80.94	90.41	85.15	89.33	82.59
5	31.250	91.48	87.33	88.15	81.09	92.79	91.19	96.50	94.34	94.63	89.50
6	15.625	93.95	96.90	96.95	91.53	98.64	96.90	98.75	97.43	99.46	94.14
7	NC	100									

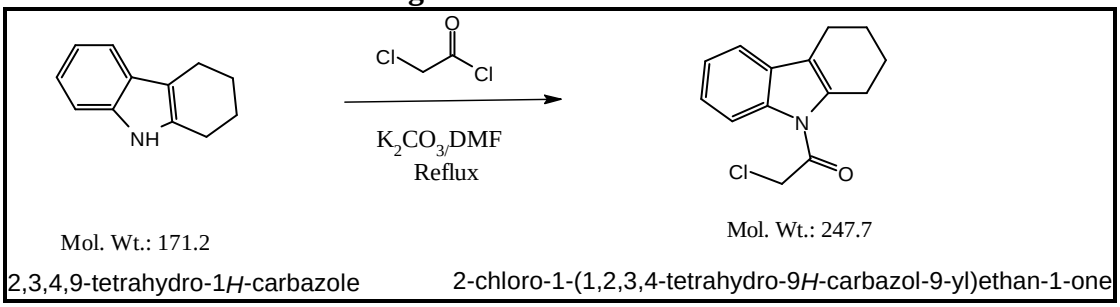
Carbazole structure



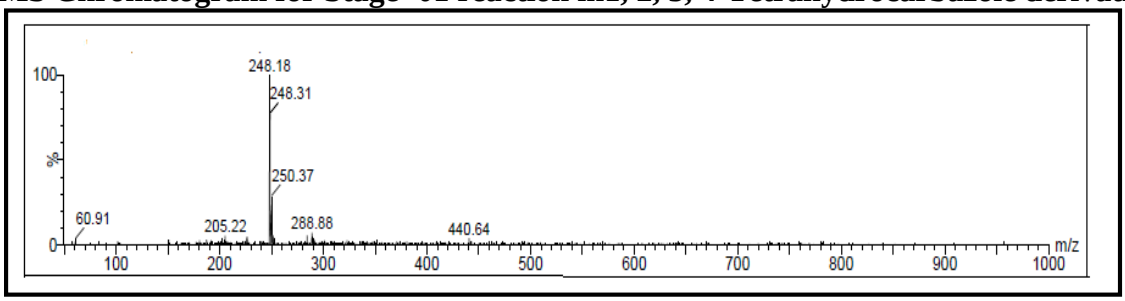
1, 2, 3, 4-Tetrahydrocarbazole derivatives: General scheme



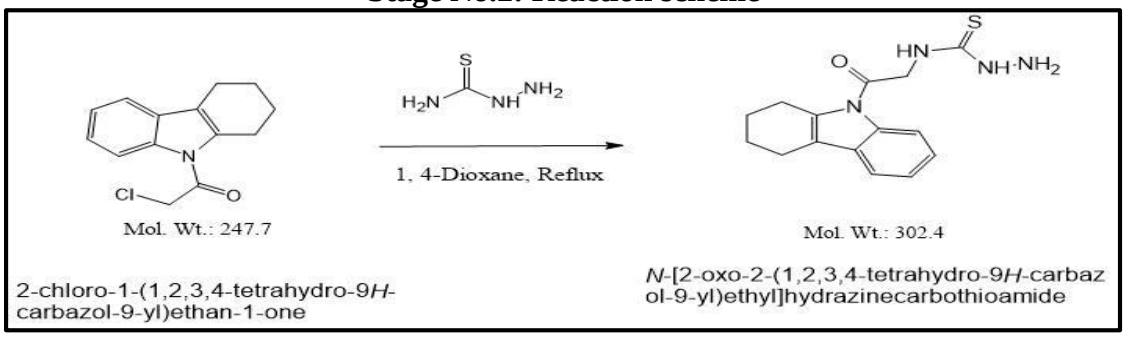
Stage No.1: Reaction scheme



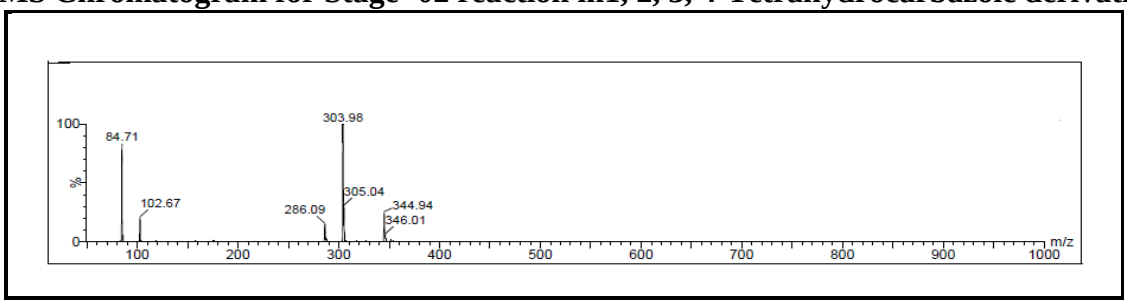
LCMS Chromatogram for Stage -01 reaction in1, 2, 3, 4-Tetrahydrocarbazole derivatives

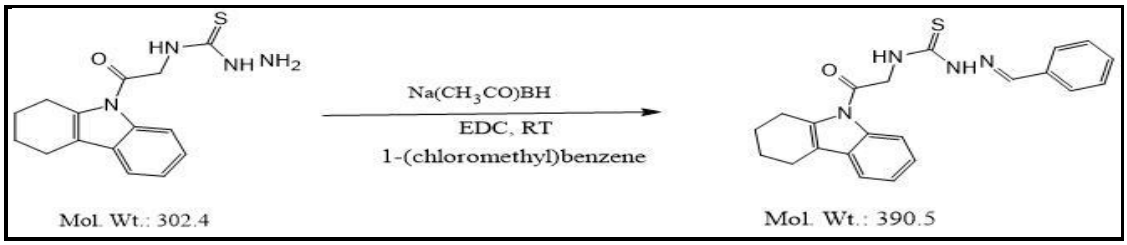


Stage No.2: Reaction scheme



LCMS Chromatogram for Stage -02 reaction in1, 2, 3, 4-Tetrahydrocarbazole derivatives

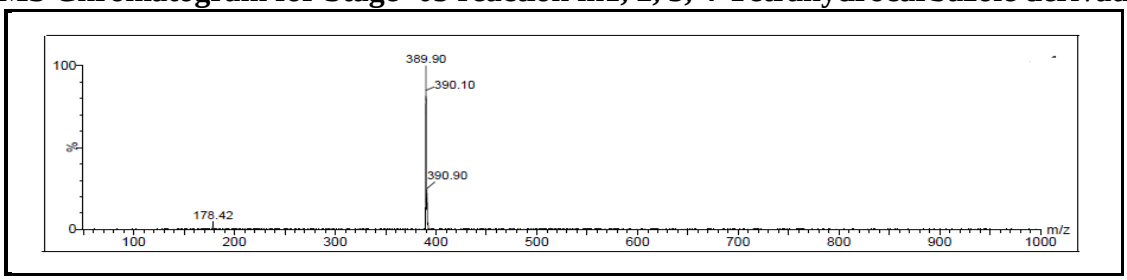




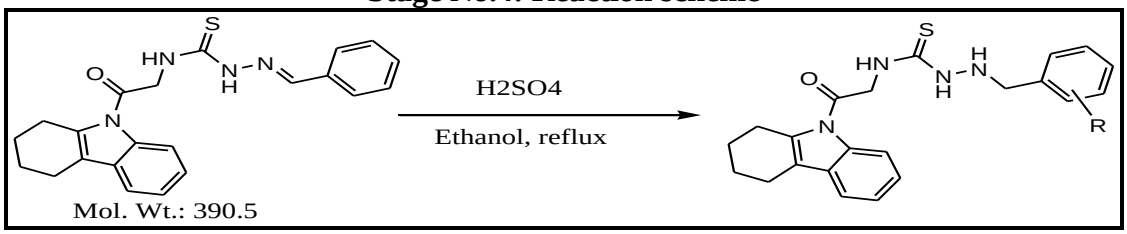
N-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl]hydrazinecarbothioamide

N-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] benzylhydrazinecarbothioamide

LCMS Chromatogram for Stage -03 reaction in1, 2, 3, 4-Tetrahydrocarbazole derivatives



Stage No.4: Reaction scheme



N-[2-oxo-2-(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)ethyl] benzylhydrazinecarbothioamide

CONCLUSION

The synthesized n-substituted carbazole derivatives confirmed their structures by IR, NMR and mass spectroscopic studies. They have shown anticancer potential against A549 (Lung Cancer) cells. It was build out that compounds possess the capability of inducing intrinsic and externsic apoptosis pathway. Our results exhibit that compounds are promising anticancer agents. The substitution of p-chloro, amino and nitro have more cell liability compared to other electro – ve atom in the halogen series. Chloro is moderately active to show cell lysis. But amino and nitro shows good activity. From this we can conclude that N- containing heterocyclic moiety can be used for the treatment of cancer.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

BIBLIOGRAPHY

1. Kanneti Naga Mounika, Angirekula Naga Jyothi. Carbazole derivative a review, *World J Pharm Sci Res,* 4(12), 2015, 420-428.
2. Ghazala Yaqub, Abdul Hannan, Erum Akbar. Synthesis, antibacterial and antifungal activities of novel pyridazino carbazoles Hindawi, *Pub Corp J Chem,* 2(4), 2013, 35-36.
3. Zhang F F. Synthesis, antibacterial and antifungal activities of some carbazole derivatives, *Bio org Med Che Le,* 20(6), 2010, 1881-1420.
4. Chakraborty A, Bhattacharyya P. Carbazole alkaloid with antimicrobial activity from Clausena heptaphylla, *Phy Ch,* 38(3), 1991, 787-789.
5. Haider N. Pyridazine-fused carbazoles: Synthesis, reactivity and antitumor activity, *J Hetero Chem,* 39(3), 2002, 511-521.
6. Knolker H J, Reddy K R. Isolation and synthesis of biologically active carbazole alkaloids, *Chem Rev,* 102(11), 2002, 4303-4328.
7. Yaqub G, Akbar E, Rehman M A, Mateen B. Review on advancements in synthesis of carbazoles, *Asi J Ch,* 21(4), 2009, 2420-2485.
8. Beata Tylinska. Synthesis of new 1-Phenyl-6H-Pyrido [4, 3-b] carbazole derivatives with potential cytostatic activity, *Acta Poloniae Pharm Drug Res,* 68(1), 2011, 31-37.
9. Kumar N, Sharma G K, Pathak D. Microwave assisted and parallel synthesis of novel substituted carbazole derivatives of biological interest, *Inter Jour of Pharma and Chem Sci,* 2(1), 2013, 273-282.
10. Mahadevan. Synthesis and cytotoxic studies of 2, 3-dimethylindoles and tetra hydro carbazoles, *Int J Pha Sci,* 6(2), 2014, 137-140.
11. Parita B. Shah *et al*. Design, synthesis and anticancer evaluation of carbazole comprised with 1, 3, 4-thiadiazole derivative, *Int J Inst Pharm L Sci*, 2(4), 2012, 57-74.
12. Tran Thi Thu Thuy. Synthesis of novel derivatives of murrayafoline A and their inhibitory effect on LPS-stimulated production of pro inflammatory cytokines in bone marrow derived dendritic cells, *Arch Pharm Res,* 36(7), 2013, 832-839.
13. Prudent. Antitumor activity of pyridocarbazole and benzopyridoindole derivatives that inhibit protein kinase CK2, *Cancer Res*, 70(23), 2010, 9865-9875.
14. Devender Pathak. Synthesis of some newer carbazole derivatives and evaluation for their pharmacological activity, *Der Phar Sin,* 3(4), 2012, 470-478.
15. Chen *et al*. BC3EE2, 9B, a synthetic carbazole derivative, up regulates autophagy and synergistically sensitizes human GBM8901 glioblastoma cells to temozolomide ide, *Int J Mol Med,* 36(5), 2015, 1244-1252.

16. Lan-Xiang Liu. Synthesis and antitumor activity of novel N-substituted carbazole imidazolium salt derivatives, *Scientific Reports*, 5, 2015, 13101.
17. Charles S, Vairappan. Biological activity of carbazole alkaloids and essential oil of murraya koenigii against antibiotic resistant microbes and cancer cell lines, *Molecules*, 16(11), 2011, 9651-9664.
18. Bhat S S, Revankar V K, Kumbar V, Bhat K, Kawade V A. Synthesis, crystal structure and biological properties of a cis-dichlorido-bis (diimine) copper(II) complex, *Acta Cryst*, 74(Pt2), 2018, 146-151.
19. Tejas Shah, Kalpana Joshi, Sanjay Mishra, Suhas Otiv, Vijay Kumbar. Molecular and cellular effects of vitamin B12 forms on human trophoblast cells in presence of excessive folate, *Biomedicine and Pharmacotherapy*, 84, 2016, 526-534.

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