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DESIGN, SYNTHESIS AND ANTICANCER EVALUATION OF CARBAZOLE COMPRISED WITH 1,3,4-THIADIAZOLE DERIVATIVE

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Keywords:

Carbazole, 1,3,4-thiadiazole, Anticancer activity, MTT assay

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ABSTRACT

The carbazole and 1,3,4-thiadiazole zolo moieties are crucial functionalities because of their wide variety of biological activity and have wide range of therapeutic properties. Keeping in view the importance of these organic moieties, some new compounds were synthesized which contains Carbazole with 1,3,4-thiadiazole. As a part of systematic investigation of synthesis and biological active series of compounds Synthesis of 1-(9H-carbazol-9-yl)-2-(5-aryl-1,3,4-thiadiazol-2-ylamino) ethanone have been synthesized. Structures of all the synthesized compounds were elucidated on the basis of Physical and Spectral characterization. All the synthesized compounds are evaluated for their anticancer activity by MTT assay and compared with standard drugs. The test compounds showed significant anticancer activity.

INTRODUCTION

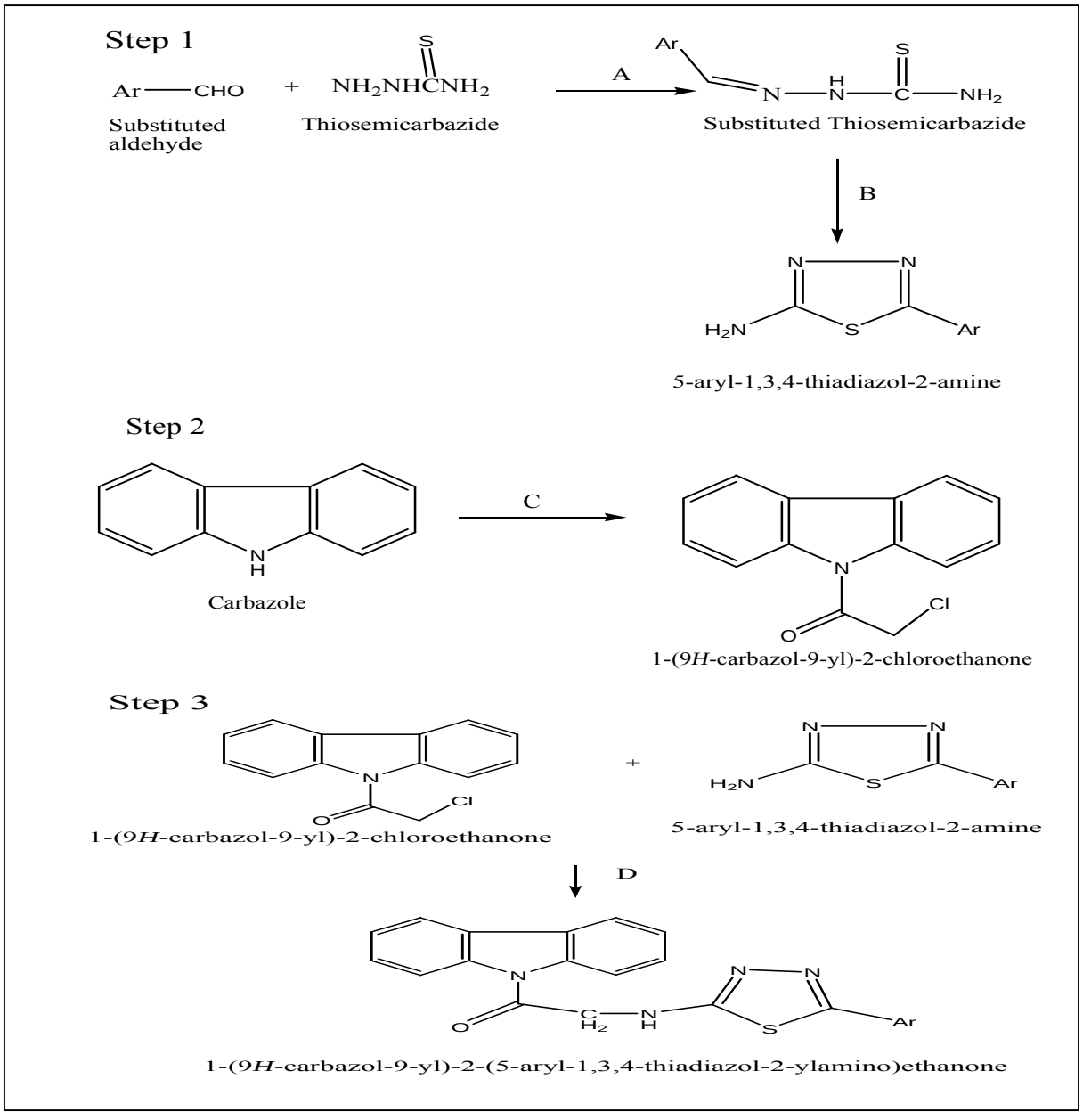
Carbazole is an aromatic heterocyclic organic compound. It has a tricyclic structure, consisting of two six-membered benzene ring fused on either side of a five-membered nitrogen-containing ring. Carbazole alkaloids are known to show a wide range of biological properties such as antitumor, antibiotic, psychotropic, antiinflammatory, and antihistaminic activities.[3] Thiadiazole is a five member heterocyclic ring system having two nitrogen and sulphur atom. Thiadiazole and their derivatives exhibit a wide variety of biological activities like cytotoxic, Antimicrobial, Antiinflammatory,Antitubercular,Analgesic, Antifungal, Antidiabetic, Antiulcer, Antidepressant, and diuretics activity.[19] Carbazole act by preventing cancer growth cells. Carbazole act on the cell cycle transducers, for example cyclins, cyclin-dependent kinases (cdks) or the cdk inhibitors. It also acts like apoptotic machinery that normally disposes of abnormal cells. Thiadiazole is Potent sulfonamide inhibitors of the zinc enzyme carbonic anhydrase and tumour cell growth inhibitors. Carbazole and 1,3,4-thiadiazole fused with acetyl linker. Substitution is done on 1,3,4-thiadiazole electron withdrawing group gives better activity then electron donating group. So, it was thought of interest to synthesize compounds having 1,3,4-Thiadiazole moiety with Carbazole group which might possess potent cytotoxic activity because of synergic activity of tumour cell growth inhibitor. 1,3,4-Thiadiazole a unsaturated form of thiazole, has been considered as a magic moiety which posses almost all types of biological activities. It belongs to an important group of heterocyclic compounds containing sulphur and nitrogen in a five member ring. There are a large number of heterocyclic five member rings are available but 1,3,4-Thiadiazole posses a large number of biological activity. In literature survey it has been found that in combination with many group of heterocyclic ring the spectrum of pharmacological activity associated with 1,3,4-Thiadiazole and its derivatives can be broaden. So on the basis of various literature review conclusion that Carbazole and 1,3,4-Thiadiazole give better Anticancer activity so I would like to choose a my target molecule like 1,3,4-thiadiazole comprised with Carbazole as a Anticancer agent.

MATERIAL AND METHODS

All the Chemicals and Solvents were obtained from E-Merck, India (AR grade) and were used further purification. The melting point of the synthesized compounds were determined in open capillary using VEEGO MELTING POINT APPARATUS model VMP-D and recorded in Celsius without correction. Purity of the compound was verified by precoated TLC plates (E-

Merck Kieselgel 60 F_{254}). The Infrared spectra for the synthesized compounds were recorded using SHIMADZU-FTIR 8400S spectrometer using KBr as a back ground. ^{1}H NMR spectra of the synthesized compounds were taken using BRUKER Advance-II 400 NMR spectrometer using Tetramethyl silane as an internal standard. ^{1}H NMR spectra were recorded with $CDCl_3$ as a solvent & the chemical shift data were expressed as delta values related to TMS. Mass spectra of the synthesized compounds were taken using 2010EV LCMS SHIMADZU instrument at 70 eV.

EXPERIMENTAL PROCEDURE



A= Stirring
B= Fe^{+++}, Sodium citrate, Citric acid, Ammonia, 45 min, 80-90 ^{0}c △
C= $ClCOCH_2Cl$, Acetone, Reflux for 3 hr.
D=Acetone, K_2CO_3, Reflux for 4hr.

Figure 1: Route of synthesis

Step-1: Synthesis of 2-amino 5-aryl 1,3,4-thiadiazole was synthesized following two steps.

Step 1: Synthesis of thiosemicarbazones:

In a dry, clean 500 ml beaker place substituted aldehydes (0.2 mole) in warm alcohol (300 mL) and thiosemicarbazide (0.2 mole) in warm water (300 mL) take in another dry, clean beaker were mixed slowly with continuous stirring for 30 min. Product separated immediately on cooling which was filtered with buckner funnel using suction pump, dried, recrystallize in 75% ethanol.

Step-2: Synthesis of 5-aryl 1,3,4-thiadiazole-2-amine

In a dry, clean 500ml Beaker place Thiosemicarbazones (0.05 mole) was suspended in (300 mL) warm water. In another dry, clean 500ml beaker place $FeCl_3$ (0.15 mole) in (300 mL) water was added quantitatively, Mix these two solution slowly with constant stirring. The reaction mixture was heated at 80-90 ^{0}C for 45 min. Solution was filtered hot. To filtrate add citric acid (0.11 mole) and sodium citrate (0.05 mole). The resulting mixture divided into 4 parts and each part neutralized separately with ammonia (10%). The required amines separated out, filtered with suction, dried and recrystallize with 75% Ethanol.[20]

5-Phenyl-1,3,4-thiadiazole-2-amine (1a)

Yield (%): 64; M.P. (^{O}C):220-222; R_f: 0.76 (Benzene: Ethyl acetate, 9:1); IR (KBr, cm^{-1}): 3350 (NH_2), 1650 (C=N), 700 (C-S).

5-(2-nitrophenyl)-1,3,4-thiadiazol-2-amine (1b)

Yield (%): 66.4; M.P. (^{O}C): 200-202; R_f: 0. 65 (Benzene: Ethyl acetate, 9:1); IR (KBr, cm^{-1}): 3440 (NH_2), 1650 (C=N), 1506 (-NO_2), 650 (C-S).

5-(3-nitrophenyl)-1,3,4-thiadiazol-2-amine (1c)

Yield (%): 62; M.P. (^{O}C): 194-196; R_f: 0.43 (Benzene: Ethyl acetate, 9:1); IR (KBr, cm^{-1}): 3400 (NH_2), 1600 (C=N), 1590 (-NO_2), 650 (C-S).

5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine (1d)

Yield (%): 68.5; M.P. (^{O}C): 196-168; R_f: 0.55 (Benzene: Ethyl acetate, 9:1); IR (KBr, cm^{-1}): 3350 (NH_2), 1650 (C=N), 1550 (-NO_2), 600 (C-S).

5-(2-chlorophenyl)-1,3,4-thiadiazol-2-amine (1e)

Yield (%): 74; M.P. (^{O}C): 230-232; R_f: 0.68 (Benzene: Ethyl acetate, 9:1); IR (KBr, cm^{-1}): 3380 (NH_2), 1600 (C=N), 700 (C-Cl), 630 (C-S).

5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (1f)

Yield (%): 78; M.P. (^{O}C): 234-236; R_f: 0.72 (Benzene: Ethyl acetate, 9:1); IR (KBr, cm^{-1}): 3350 (NH_2), 1650 (C=N), 715.54 (C-Cl), 667.81 (C-S).

2-(5-amino-1,3,4-thiadiazol-2-yl)phenol (1g)

Yield (%): 60; M.P. (^{O}C): 170-172; R_f: 0.72 (Benzene: Ethyl acetate, 9:1); IR (KBr, cm^{-1}): 3400 (NH_2), 1650 (C=N), 1506 (OH), 653.34(C-S)

5-(furan-2-yl)-1,3,4-thiadiazol-2-amine (1h)

Yield (%): 60.6; M.P. (^{O}C): 182-184; R_f: 0.67 (Benzene: Ethyl acetate, 9:1); IR (KBr, cm^{-1}): 3400 (NH_2), 1725(C-O-C), 1610 (-C=N-), 760(C-S)

Step-2: Synthesis of 1-(9*H*-carbazole-9-*yl*)-2-chloroethanone

In 500ml round bottom flask chloroacetyl chloride (10ml, 0.09 mole) was added to a solution of carbazole (5.00 g, 0.02 mole) and acetone (15mL) the reaction mixture refluxed for 3 to 4 hr. The progress of reaction was continuously monitored by TLC. After completion of reaction, the reaction mixture cooled to room temperature.Resultant reaction mixture was concentrated and kept at room temperature for overnight to get crude product. Product was recrystallized from 75% ethanol.[23]

Yield (%): 87.8; M.P. (^{O}C): 220-224; R_f: 0.68 (Benzene: Ethyl acetate, 9:1); IR (KBr, cm^{-1}): 3400, 1570 (Carbazole nucleus), 1600 (C=O), 600 (C-Cl).

Step-3: Synthesis of 1-(9H-carbazol-9-yl)-2-(5-aryl-1,3,4-thiadiazol-2ylamino)ethanone.

In 500ml round bottom flask 5-aryl 2-amino 1,3,4-thiadiazole (0.02 moles) was added to a solution of 1-(9*H*-carbazole-9-*yl*)-2-chloroethanone (5.00 g, 0.02 mole) and acetone (15 mL) the reaction mixture was refluxed for 3 to 4 hr. The progress of reaction was continuously monitored by TLC. After completion of reaction, the resultant mixture was cooled to room temperature. The reaction mixture cool and drain in to cool water the product was precipitated out, filter it. The product was dried and further purified by crystallization from ethyl acetate and water.

1-(9H-carbazol-9-yl)-2-(5-Phenyl-1,3,4-thiadiazol-2-ylamino) ethanone (SK0901)

Yield (%):78; M.P. (^{O}C): 144-146; R_f: 0.70 (Benzene: Ethyl acetate (9:1); IR (KBr, cm^{-1}): 3418, 1559 (Carbazole nucleus), 3350 (-NH_2), 1734 (C=O), 1684(C=N), 742 (C-H); ^{1}H- NMR ($CDCl_3$, ppm): 4.14 δ (s,1H, NH), 4.81δ (s, 2H, CH_2), 7.21-8.17 δ(m, 13H, Ar- H);
Mass spectra (m/z): =385.3 ([M+1]$^{+}$).

1-(9H-carbazol-9-yl)-2-(5-(2-nitrophenyl)-1,3,4-thiadiazol-2-ylamino)ethanone (SK0902)

Yield (%): 72.2; M.P. (^{O}C): 122-124; R_f: 0.76 (Benzene: Ethyl acetate (9:1); IR (KBr, cm^{-1}): 3418, 1559 (Carbazole nucleus), 3250 (NH_2), 1734 (C=O), 1684(C=N), 1490 (NO_2), 667 (C-S), 3250 (NH_2); ^{1}H- NMR ($CDCl_3$, ppm): 4.11 δ (s, 1H, NH), 4.12δ (s, 2H,15-CH_2), 7.21-7.97 δ(m, 12H, Ar-H); Mass spectra (m/z): =430.4 ([M+1]$^{+}$).

1-(9H-carbazol-9-yl)-2-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-ylamino)ethanone (SK0903)

Yield (%): 75; M.P. (^{O}C): 130-132; R_f: 0.67 (Benzene: Ethyl acetate (9:1); IR (KBr, cm^{-1}): 3418, 1559 (Carbazole nucleus), 3350 (-NH_2), 1734 (C=O), 1684(C=N), 742 (C-H); ^{1}H- NMR ($CDCl_3$, ppm): 4.10 δ (s, 1H, NH), 4.23δ (s, 2H,15-CH_2), 7.21-8.27 δ(m, 12H, Ar-H); Mass spectra (m/z): =430.7 ([M+1]$^{+}$).

1-(9H-carbazol-9-yl)-2-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-ylamino)ethanone (SK0904)

Yield (%): 73.6; M.P. (^{O}C): 210-212; R_f: 0.75 (Benzene: Ethyl acetate (9:1); IR (KBr, cm^{-1}): 3400 (Carbazole nucleus), 3250 (NH_2), 1850 (C=O), 1684(C=N), 1490 (NO_2); ^{1}H- NMR ($CDCl_3$, ppm): 4.0 δ (s, 1H, -NH), 4.32- δ (s, 2H, 15-CH2), 7.21-8.16 δ (m, 12H, Ar- H); Mass spectra (m/z): =430.2 ([M+1]$^{+}$).

1-(9H-carbazol-9-yl)-2-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-ylamino)ethanone (SK0905)

Yield (%): 82; M.P. (^{O}C): 154-156; R_f: 0.62 (Benzene: Ethyl acetate (9:1); IR (KBr, cm^{-1}): 3418, 1559 (Carbazole nucleus), 1734 (C=O), 1684(C=N), 742 (C-H), 715(C-Cl), 667 (C-S); ^{1}H- NMR ($CDCl_3$, ppm): 4.45 δ (s, 1H,-NH),4.80 δ (s,2H,15-CH_2),7.22-7.24 δ (m, 12H, Ar- H); Mass spectra (m/z): =419 ([M+1]$^{+}$).

1-(9H-carbazol-9-yl)-2-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-ylamino)ethanone (SK0906)

Yield (%): 87.5; M.P. (^{O}C): 212-214; R_f: 0.60 (Benzene: Ethyl acetate (9:1); IR (KBr, cm^{-1}): 3418, 1559 (Carbazole nucleus), 1734 (C=O), 1684(C=N), 742 (C-H), 715(C-Cl), 667 (C-S); ^{1}H- NMR ($CDCl_3$, ppm): 4.45 δ (s, 1H,-NH), 4.80 δ (s,2H,15-CH_2), 7.22-7.24 δ (m, 12H, Ar-H); Mass spectra (m/z): =419.2 ([M+1]$^{+}$).

1-(9H-carbazol-9-yl)-2-(5-(2-hydroxyphenyl)-1,3,4-thiadiazol-2-yl-amino)ethanone (SK0907)

Yield (%): 54.5; M.P. (^{O}C): 182-184; R_f: 0.55 (Benzene: Ethyl acetate (9:1); IR (KBr, cm^{-1}): 32250, 1506 (Carbazole nucleus), 1734 (C=O), 1652(C=N), 1603 (-OH), 738 (C-H), 667 (C-S); ^{1}H- NMR ($CDCl_3$, ppm): 4.04 δ (s,1H,-NH), 4.20 δ (s,2H,-CH_2), 5.01 δ (s,1H,-OH), 7.22-8.15 δ (m,12H, Ar- H); Mass spectra (m/z): =401.1 ($[M+1]^+$).

1-(9H-carbazol-9-yl)-2-(5-(furan-2-yl)-1,3,4-thiadiazol-2-ylamino) (SK0908)

Yield (%): 56.5; M.P. (^{O}C): 184-186; R_f: 0.71 (Benzene: Ethyl acetate (9:1); IR (KBr, cm^{-1}): 32250, 1506 (Carbazole nucleus), 1734 (C=O) ,1652(C=N), 1603 (-OH), 738 (C-H), 667 (C-S); Mass spectra (m/z): =375.8 ($[M+1]^+$).

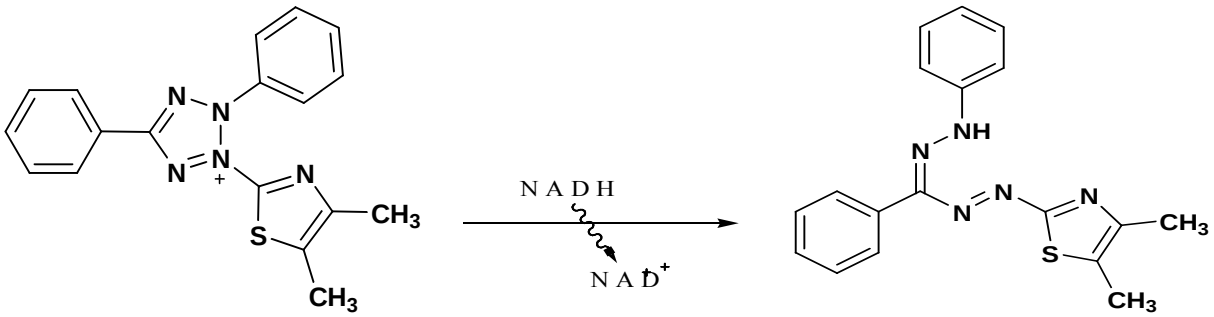
BIOLOGICAL EVALUATION OF ANTIMICROBIAL ACTIVITY

MTT assay[31]

It is a laboratory test and a standard colorimetric assay for measuring cellular growth. It can also be used to determine cytotoxicity of potential medicinal agents and other toxic materials.

This assay is a sensitive, quantitative and reliable colorimetric assay that measures viability, proliferation and activation of cells. The assay is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue formazan product which is insoluble in water. The amount of formazan produced is directly proportional to the cell number in range of cell lines.

MTT Formazan



Yellow color **Blue color**

Principle of MTT assay

Application

MTT used for the non-radioactive, spectrophotometric quantification of cell proliferation and viability in cell populations using the 96-well-plate format. It can be used for:

- Measurement of cell proliferation in response to growth factors, cytokines, mitogens, and nutrients.
- Analysis of cytotoxic and cytostatic compounds, such as anti-cancer drugs and other pharmaceutical compounds.
- Assessment of growth-inhibitory antibodies and physiological mediators.

Advantages of MTT assay

- Rapid, versatile, quantitative and highly reproducible
- Adaptable to large-scale screening; relevant for most cells
- MTT reduction correlates to indices of cellular protein and earlier cell number
- More sensitive and earlier predictor of toxicity than classical LDH or neutral red measurements.

Disadvantage of MTT assay

- Production of the MTT product is dependent on the MTT concentration in the medium. The kinetics and degree of saturation are dependent on cell type.
- Assay is less effective in the absence of cell proliferation.
- MTT cannot distinguish between cytostatic and cytocidal effect.
- Individual cell numbers are not quantitated and results are expressed as a percentage of control absorbance.
- Test is less effective if cells have been cultured in the same media that has supported growth for a few days, which leads to under estimation of control and untreated samples.

To summarize, the design of a screening assay is an array of multiple choices, all of which have significant impacts on the outcome of the overall drug discovery process. Most importantly, the correct selection of the target and assay format, detailed optimization and miniaturization as well as the choice of appropriate detection technology for each individual assay can lead to savings in time, money and labor along with improved data quality in all stages of the drug discovery process.

MTT Material:

Different cell lines:

- HEK293 (Human Epidermal Kidney Cell Line)
- Hep-2 (Laryangeal Cancer Cell Line)
- HELA (Cervical Cancer Cell Line)

- MDA MB 468 (Breast Cancer Cell Line)
- MCF 7 (Breast Cancer Cell Line)
- NCI (Lung Cancer Cell Line)

Reagents

- MTT Dye
- MTT Dye solubilizer
- Acidic Isopropanol – 90 %
- Triton – X
- Fetal Bovine Serum
- Phosphate Buffer Saline
- Antibiotic Antimycotic Solution
- DMEM media

MTT Protocol

Plating Out Cell

- Trypsinize a sub confluent monolayer culture and collect cells in growth medium containing serum
- Centrifuge suspension (5 min at 200 g) to pellet cells. Re-suspend cells in growth medium and count them
- Dilute cells to 2.5 – 50 × 10^3 cells /ml, depending on the growth of cell line and allowing 20ml cell suspension/micro titration plate.
- Transfer cell suspension to a 9 cm Petridis and with a multichannel pipette add 200 µL of suspension into each of central 10 columns of a flat bottomed 96 well plate (80 well per plate) starting with column 2 and ending with column 11, there by placing 0.5 –10 × 10^3 cells into each well)
- Add 200 µL of growth medium to the eight wells in column 1 and 12. Column 1 will be used to blank plate reader, column 12 helps to maintain humidity for column 11 and minimize edge effect. Incubate in the plate in the CO_2 incubator.
- For non-adherent cells, prepare a suspension in fresh growth medium. Dilute cells to 5-100 × 10^3 cells /ml and plate out only 100 µL of suspension into round-bottomed 96-well plates. Add drug immediately to these plates.

Plate Assignment:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.005	0.015	0.045	0.13	0.41	1.23	3.70	11.11	33.33	100	NC	PC
B	0.005	0.015	0.045	0.13	0.41	1.23	3.70	11.11	33.33	100	NC	PC
C	0.005	0.015	0.045	0.13	0.41	1.23	3.70	11.11	33.33	100	NC	PC
D	0.005	0.015	0.045	0.13	0.41	1.23	3.70	11.11	33.33	100	NC	PC
E	0.005	0.015	0.045	0.13	0.41	1.23	3.70	11.11	33.33	100	NC	PC
F	0.005	0.015	0.045	0.13	0.41	1.23	3.70	11.11	33.33	100	NC	PC
G	0.005	0.015	0.045	0.13	0.41	1.23	3.70	11.11	33.33	100	NC	PC
H	0.005	0.015	0.045	0.13	0.41	1.23	3.70	11.11	33.33	100	NC	PC

Where, PC means: positive control; cells + standard drug

NC means: Negative control; only complete media with cells

Figure 2: 96 well plates

Drug addition

- Prepare a serial three fold dilution of cytotoxic drug in growth medium to give eight conc. This set of conc. should be chosen such that highest conc. kills most of cells and lowest kills none of cells. Once toxicity of drug is known smaller range of conc. can be used. Normally three plates are used for each drug to give triplicate determinations within one experiment
- For adherent cells remove medium from wells in columns 2 to 11. Feed cells in eight wells in column 2 and 11 with 200 uL of fresh growth medium, these are the controls
- Add cytotoxic drug to cells in columns 3 to 10. Only four wells are needed for each drug conc. Such that rows A-D can be used for one drug and rows E-H for second drug. Again incubate in CO_2 incubator for 24 hours.

Growth period

- At the end of drug exposure period, remove medium from all of wells containing cells and feed cells with 200 µL of fresh medium. Centrifuge plates containing non-adherent cells (5min at 200g) to pellet cells. Then remove medium, using a fine gauge needle to prevent disturbance of cell pellet. Feed plates daily for 2-3 PDTs

Estimation of surviving cell number

- Feed plate with 200 µL of fresh medium at end of growth period and add 10 µL of MTT to all of wells in columns 1 to 11

- Wrap the plates in aluminium foil, and incubate them for 4 hrs in a CO_2 incubator at 37 °C. note that 4 hrs is a minimum incubation time and plates can be left for up to 8hrs
- Remove the medium and MTT from the well (centrifuge for non-adherent cells) and dissolve the remaining MTT formazon crystals by adding 100 µL of MTT Solubilizer (90% acidified Isopropanol + 10% Triton – X) to all of wells in columns 1 to 11.
- Record absorbance at 540 nM immediately because product is unstable. Use wells in column 1, which contain medium and MTT but no cells, to blank to the plate reader.
- Percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula

% viability = $(A_T-A_B) / (A_C-A_B)$ X 100

A_T=Absorbance of treated cells (drug)

A_B=Absorbance of blank (only media)

A_C=Absorbance of control (untreated)

% cytotoxicity = 100 - % cell survival

RESULTS AND DISCUSSION

Anticancer activity

Table 1: Anticancer activity on HEK 293 (Human Epidermal Kidney Cell Line)

Conc.(µM)	100	33.33	11.11	3.70	1.23	0.41	0.14	0.05	0.02	0.01	IC_{50} (µM)
log Conc.	2	1.52	1.04	0.56	0.09	-0.38	-0.86	-1.33	-1.81	-2.29	-
SK0901	67.99	67.38	60.86	56.34	50.21	46.01	42.01	40	39.21	38.76	**2.309**
SK0902	43.19	41.53	40.88	40.84	40.73	39.82	39.52	39.52	39.16	38.79	10.30
SK0903	39.4	38	37.37	36.8	36.75	34.95	34.89	34.52	33.62	33.48	11.55
SK0904	65.97	41.44	40.65	40.02	38.79	38	37.05	36.83	35.48	35.26	13.700
SK0905	42.63	42.12	39.07	38.31	38.08	37.41	36.03	35.32	35.28	34.91	6.469
SK0906	37.99	37.72	37.02	36.62	36.47	36.11	35.72	35.43	29.46	27.75	**1.478**
SK0907	38.98	38.22	36.05	35.8	35.51	34.78	34.75	33.85	32.57	30.64	12.77
SK0908	41.57	41.53	40.73	39.29	38.41	38.38	37.16	37	36.73	35.9	22.27
MXT	70.66	60.59	59.12	58.08	56.19	54.94	53.34	47.28	43.11	39.34	0.101

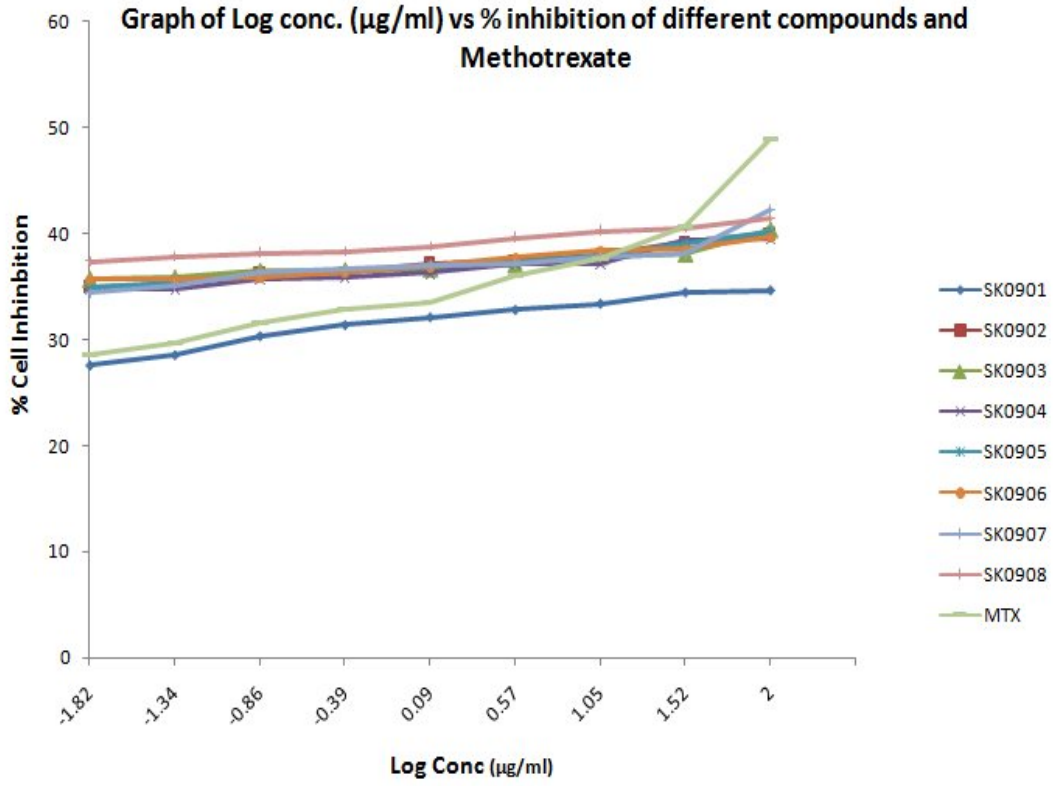


Figure 3: Graph of log Conc. V_s % Cell Inhibition for HEK 293 cell line

Table 2: Anticancer Activity on M468 (Breast Cancer Cell Line)

Conc. (µM)	100	33.33	11.11	3.70	1.23	0.41	0.14	0.05	0.02	0.01	IC_{50} (µM)
log Conc.	2	1.52	1.04	0.56	0.09	-0.38	-0.86	-1.33	-1.81	-2.29	-
SK0901	38.92	38.05	37.19	35.89	35.73	35.66	35.47	34.59	34.31	33.27	6.324
SK0902	39.45	38.12	38.08	37.9	37.77	37.75	36.96	36.76	35.89	35.86	**1.638**
SK0903	35.16	33.55	31.71	30.41	29.88	29.48	28.29	26.87	26.66	26.29	13.904
SK0904	39.18	38.07	37.76	37.58	37.02	36.84	35.85	35.81	35.5	33.54	**2.364**
SK0905	40.27	38.88	38.6	38.21	38.04	37.79	36.59	34.57	34.08	33.23	**1.162**
SK0906	37.59	36.9	36.25	35.73	35.68	35	34.8	34.54	32.93	32.02	**1.829**
SK0907	38.05	37.17	34.38	33.42	32.37	31.11	29.55	28.07	28.07	27.56	16.68
SK0908	38.66	38.57	36.72	35.27	34.95	34.59	34.14	33.97	33.96	33.62	9.215
MXT	70.95	60.98	59.87	59.18	56.43	54.91	54.11	47.28	43.42	39.93	0.3215

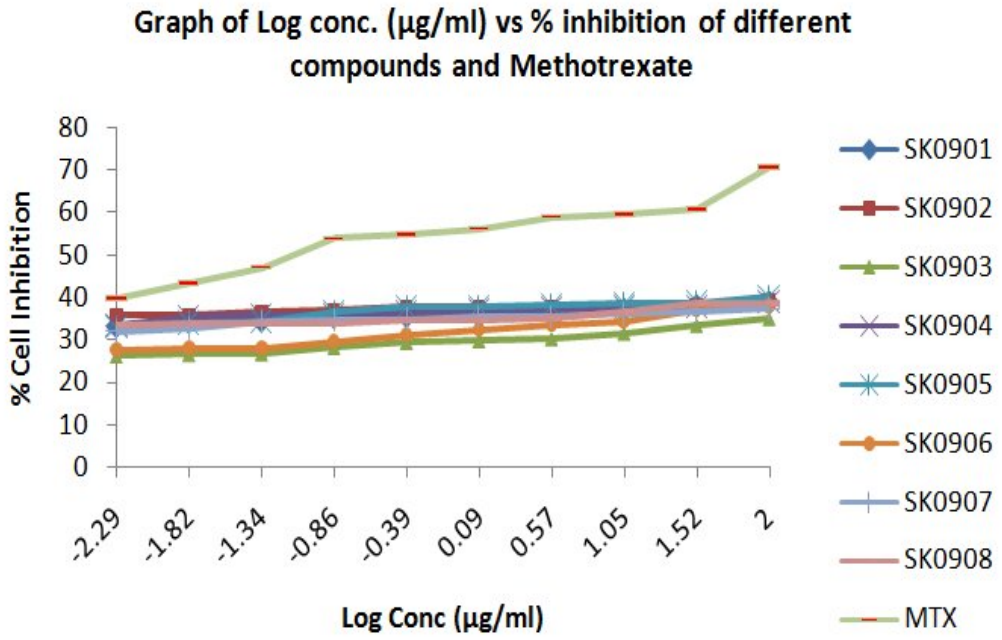


Figure 4: Graph of log Conc. V_s % Cell Inhibition for M468 (Breast Cancer Cell Line)

Table 3: Anticancer activity on NCI (Lung Cancer Cell Line)

Conc. (µM)	**100**	**33.33**	**11.11**	**3.70**	**1.23**	**0.41**	**0.14**	**0.05**	**0.02**	**0.01**	**IC_{50} (µM)**
log Conc.	**2**	**1.52**	**1.04**	**0.56**	**0.09**	**-0.38**	**-0.86**	**-1.33**	**-1.81**	**-2.29**	**-**
SK0901	34.61	34.41	33.35	32.77	32.09	31.35	30.32	28.54	27.57	25.4	22.12
SK0902	39.98	39.25	37.94	37.46	37.24	36.39	36.39	35.35	35.01	32.85	**1.467**
SK0903	40.18	39.05	37.98	37.28	37.07	36.42	36.06	35.5	34.98	33.97	6.871
SK0904	39.52	39.4	37.16	37.07	36.24	35.9	35.72	34.79	34.71	34.18	**2.741**
SK0905	38.09	36.6	36.22	36.16	35.64	35.51	35.35	35.23	32.99	37.28	**2.897**
SK0906	39.66	38.51	38.36	37.78	36.87	36.24	35.85	35.7	35.67	35.21	**2.268**
SK0907	42.3	38.15	37.84	37.19	37.03	36.69	36.32	35.12	34.46	33.04	41.71
SK0908	41.52	40.51	40.31	39.66	38.76	38.33	38.22	37.84	37.35	36.89	6.157
MXT	48.89	40.69	37.58	36.1	33.59	32.89	31.67	29.68	28.6	28.33	0.1615

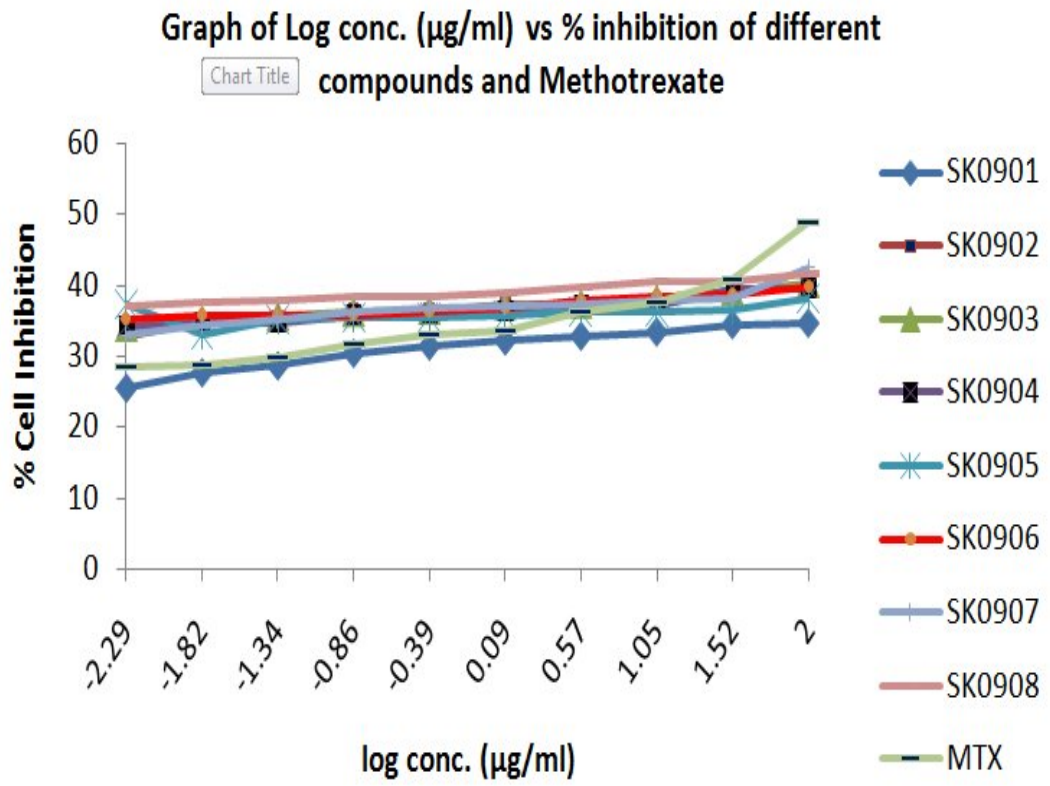


Figure 5: Graph of log Conc. V_s % Cell Inhibition for NCI (Lung Cancer Cell Line) cell line

DISCUSSION

- ✓ A series of carbazole derivatives have been synthesized as anticancer activity. In step-2 carbazole and chloroacetyl chloride which give 1-(9H-carbazole-9-yl)-2-chloroethanone which react with different 2-amino-5-aryl 1,3,4-thiadiazoles and give 1-(9H-carbazol-9-yl)-2-(5-aryl-1,3,4-thiadiazol-2-ylamino)ethanone.
  - ✓ The different derivatives SK0901 – SK0908 were obtained by this reaction.
- ✓ The chemical structure of compound was characterized by IR, MASS, NMR spectral data. The entire synthesized compound was screened for their anticancer activity.

Anticancer activity

All the synthesized compounds were subjected to anticancer activity by MTT assay. IC_{50} was calculated for test and standard compound. HEK 293 (epidermal kidney cell line), M468 (Breast Cancer Cell Line), NCI (lung cancer) cell lines by MTT method. The results were compared with standard drug methotrexate.

HEK 293 (epidermal kidney cell line)

Biological data suggest that compound SK0901 (IC_{50} = 1.11 µM) and SK0906 (IC_{50} = 1.478 µM) were high activity on HEK 293 (epidermal kidney cell line) cell line which have less potent than methotrexate (IC_{50} =0.101 µM). Compound which having NH linking between Carbazole and 1,3,4-thiadiazole and substituted with phenyl ring on 1,3,4-thiadiazole and p-chlorobenzene respectively.

M468 (Breast Cancer Cell Line)

Compound SK0902 (IC_{50} = 1.638 µM), SK0904 (IC_{50} = 2.3640µM), SK0905 (IC_{50} = 1.162µM) and SK0906 (IC_{50} = 1.829 µM) give high activity on M468 (Breast Cancer Cell Line) cell line which produces less activity than methotrexate (IC_{50} = 0.3215 µM). Compound substituted with 2-nitrobenzene, 4-nitrobenzene, o-chlorobenzene, p-chlorobenzene high activity on M468 (Breast Cancer Cell Line) cell line.

NCI (lung cancer)

Compound SK0902 (IC_{50} = 1.467 µM), SK0904 (IC_{50} = 2.741 µM), SK0905 (IC_{50} = 2.897 µM) and SK0906 (IC_{50} = 2.268 µM) give high activity on NCI (lung cancer) cell line which produces less activity than methotrexate (IC_{50} = 0.1615 µM). Compound substituted with 2-nitrobenzene, 4-nitrobenzene, o-chlorobenzene, p-chlorobenzene high activity on NCI (lung cancer) cell line.

CONCLUSION

From above all cell line like HEK 293 (epidermal kidney cell line), M468 (Breast Cancer Cell Line), NCI (lung cancer) cell lines it has been concluded that compound SK0902, SK0904, SK0905, SK0906 are more potent than all synthesized compounds. Compound SK0901 and SK0903 have moderate activity than all synthesized compounds. Compound SK0907 and SK0908 have less activity than all synthesized compound on all cell lines. Structure activity relationship of compounds showed that the presence of NH linker between aryl moiety and 1,3,4-thiadiazole ring has been recognized as potent anticancer agent. Substitution on phenyl ring at 2^{nd} and 4^{th} position with –chloro and nitro group give better anticancer activity. As in the order of electron withdrawing group on phenyl > phenyl > electron donating group on phenyl. Similarly activity on carbazole linked 1,3,4-thiadiazole scaffold give activity better. We can concluded that electron donating group on phenyl ring at 2^{nd} position of 1,3,4-thiadiazole ring is responsible for less activity.

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