International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 2(4): July-August 2012

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 08-07-2012; Revised; Accepted: 10-07-2012

DESIGN, SYNTHESIS AND ANTICANCER ACTIVITY OF 2-PHENYL-1*H* INDOLE DERIVATIVES

Hardik M. Patel*, Nilesh Darji, Jagath Pillai, Bhagirath Patel

Sat Kaival College of Pharmacy, Sarsa, Anand, Gujarat, India

Keywords:

Anticancer, Indole, Pyridine

For Correspondence:

Hardik M. Patel

Sat Kaival College of Pharmacy, Sarsa, Anand, Gujarat, India

E-mail:

hardik_pharmacist@yahoo.com

ABSTRACT

In recent years, a wide range of research has been done in the field of anticancer drug development. Since indole nucleus has shown quite good response as an anticancer agent, hence this nucleus has become an interest in the field of research. Indole is present in Vinca alkaloid which can be used as an anticancer agent. Indole has been used for better anticancer activity of target compound. The pyridine nucleus is an essential part of many anticancer derivatives. So, it was thought of interest to synthesize compounds having Indole moiety with pyridine which might possess potent anticancer activity. Novel series of Indole derivatives with Pyridine moiety have been synthesized by the formylation of indole and formation of chalcone by Claisen-Schmidt reaction, and then it is react with Malononitirile to yield indole derivatives with pyridine moiety. The purity of all compounds have been checked by the TLC monitoring and the conformation of structure will be checked by different spectra like IR, Mass and NMR and evaluated for anticancer activity by MTT assay using Methotraxate as a standard. 4-(2-(4-bromophenyl)-1H-indol-3-yl)-2-methoxy-6-(4-bromophenyl) nicotinonitrile was found to be the most potent derivative compared to other compound but less potent as compared to standard drug.

INTRODUCTION

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Cancer cells can spread to other parts of the body through the blood and lymph systems. Cancer (medical term: malignant neoplasm) is a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood). Most cancers form a tumor but some, like leukemia, do not. The branch of medicine concerned with the study, diagnosis, treatment and prevention of cancer is oncology.Cancer may affect people at all ages, even fetuses, but the risk for most varieties increases with age.[1]

Rationale behind this work is to develop a molecule having good anticancer activity. Indole show a wide range of biological activities like anticancer, antibacterial, antifungal, antimalarial, anticonvulsant, antiinflammatory. Many anticancer drug show side effect and toxicity.Indole compounds can potentially have major clinical implications because these non-toxic compounds can reduce the toxicity associated with available chemotherapies.Indole reduces problem i.e.toxicity. So, indole might be give better activity.

1.2) Indole

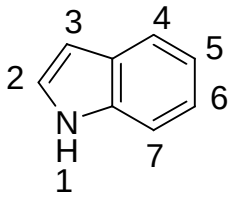
- The name indole is deriving from the words ‘indigo’ and ‘oleum’, since indole was first isolated by treatment of the indigo dye with oleum. Indole chemistry began to develop with the study of the dye indigo.
- Indole is a benzopyrrole in which the benzene and pyrrole ring are fused through the 2nd and 3rd positions of the pyrrole nucleus. The indole ring is also found in many natural products such as the indole alkaloids, fungal metabolites and marine natural products.
- Indole derivatives are found to contain several biological activities those antibiotic, anti-inflammatory, analgesic, anticonvulsant, antimalarial, anticancer, antiulcer.[11]

1.2.1) Reactivity of indole

Indoles are aromatic heterocycle, but exhibit very distinctive reactivity. Here are some general rules:

- The nitrogen is not basic. (pKa -3.6)
- Indole can readily undergo aromatic electrophillic substitution. The C3 position is the most nucleophillic, followed by the N and C2 positions.

- The C2 – C3 bond can often react like alkenes.
- Indole can be deprotonated at nitrogen. The resulting salts can be good nucleophiles.
  1) Highly ionic salts (e.g. Li^+, K^+) favours N substitution.
  2) Softer counter ions favours C3 substitution.
- When N is substituted, C2 can be deprotonated.[12]

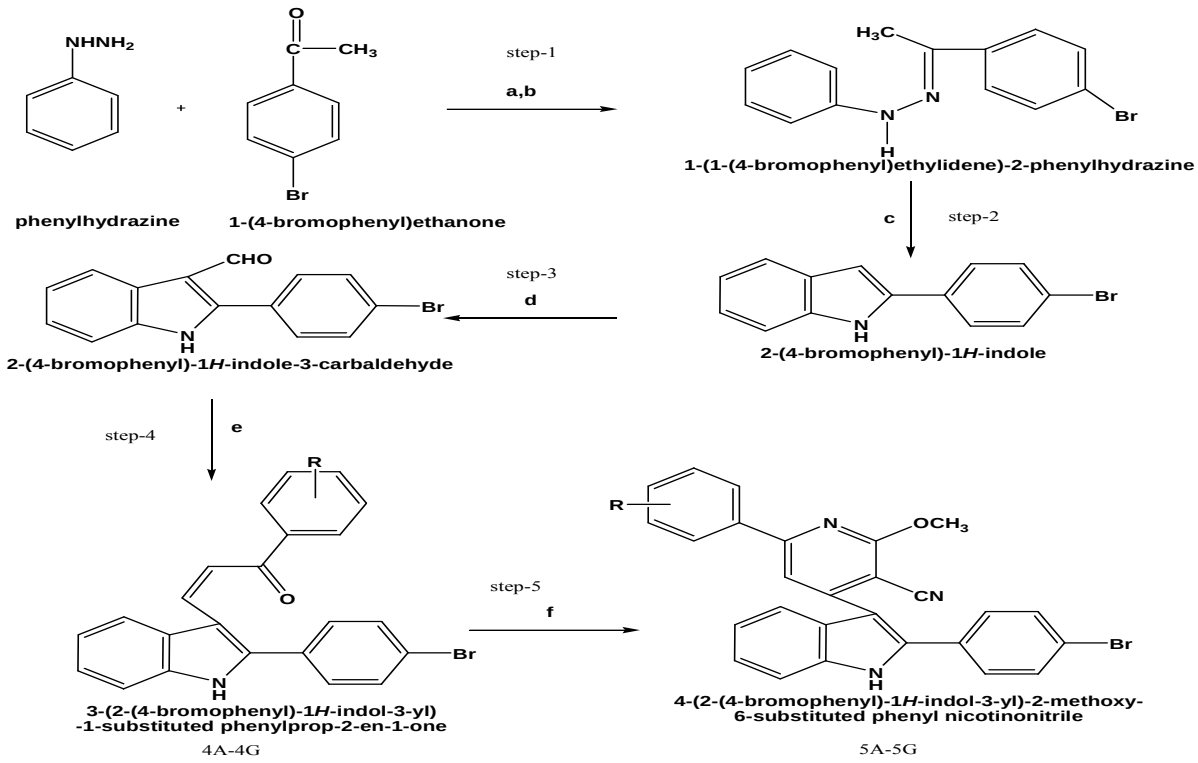


Indole

MATERIAL AND METHODS

Chemical:- Phenyl hydrazine, 4-bromo acetophenone, poly phosphoric acid, DMF, $POCl_3$, Glacial acetic acid, methanol, ethanol, malononitrile, NaOH, Substituted acetophenone.

Scheme:-



a = Ethanol,
b = Glacial acetic acid,
c = Poly phosphoric acid,
d = Phosphorus oxychloride($POCl_3$), Dimethyl formamide (DMF), NaOH ,
e = Substituted acetophenone, NaOH, HCl ,
f = Malononitirile,NaOH,Methanole

R= -H,-NH_2, 2-OH,4-OH, -Br,3-OH, -CH_3

Methods:-

Step-1 Synthesis of 4-bromoacetophenone phenylhydrazone

- Place 5.6 gm. (0.028 mol) of 4- bromoacetophenone and 5.0 gm. (0.046 mol) of phenyl-hydrazine in 250 ml beaker. Add 2 drop of glacial acetic acid. Mix well and heat this solution on a boiling water bath for one hour.
- Pour this mixture into a 25-mL ethanol which has stirr with a stirring rod for an additional 10 min.
- Cool the mixture in an ice bath, allow the product to crystallize and collect the phenylhydrazone by filtration on a Buchner funnel. Wash the crystals with a few mL of 1 M aqueous hydrochloric acid, followed by a few ml of ice cold 95% ethanol, and allow it to air dry on the filter.
- Yield (%): 86.2; M.P. (^{O}C): 104-106; R_f: 0.75 (Hexane : Ethyl acetate, 9:1)

Step-2 Synthesis of 2-(4-bromophenyl)-1H-indole.

- Weigh 20 gm. of the syrupy polyphosphoric acid into a *dry* 50 ml beaker. Place the beaker on a steam bath, and clamp a thermometer so that it measures the temperature of the liquid near the wall of the beaker.
- Warm the acid to 50 ^{0}C, and slowly stirr in the 4-bromoacetophenone phenylhydrazone 7.0 gm. , taking care not to hit the thermometer bulb with the stirring rod. After the addition is complete, continue stirring, and heat the mixture strongly on the steam bath for 15 min with stirring.
- Cautiously pour the hot mixture into 30 ml of ice and water, washing the beaker with a few ml of water. Stirr until all the acid has dissolved in the water. Collect the precipitated crude product on a Buchner funnel, washing it with cold methanol, and allow it to air dry on the filter.
- Recrystallize the crude product from ethanol-water, using about 0.1 gm. of decolorizing carbon. (Dissolve it in hot ethanol, add the activated carbon, and filter hot).

- Reheat the filtrate to boiling, and add water to the cloudy point. Add a drop or two of ethanol to redissolve the fine cloudy precipitate, and allow the solution to cool undisturbed. Collect the crystals on a Buchner funnel, and allow them to air dry at room temperature.
- Yield (%): 82.86; M.P. (^{O}C): 212-214; R_f: 0.46 (Hexane : Ethyl acetate, 9:1)

Step-3 Synthesis of 2-(4-bromophenyl)-1H-indole-3-carbaldehyde

- Phosphorus oxychloride (3 gm) was added in portions to N,N-dimethyl-formamide (6 mL) with stirring at 0 ºC.
- After addition of phosphorus oxychloride, the mixture was stirred for 60 minutes at the same temperature. And then, a solution of 2-(4-bromophenyl)-1H-indole (3 gm) in minimum quantity of N,N-dimethylformamide was added and the resulting mixture was stirred at 0-5 ºC for 1 h.
- The reaction mixture was allowed to stir at 35 ºC for 60 minutes and then poured into ice-cold water (90 mL) while a clear red coloured solution was obtained. A 10% sodium hydroxide solution was added till precipitate obtain and , boiled it till all precipitate dissolved and filtered. Upon cooling the filtrate, crystals were formed, which were collected by filtration and subsequently recrystallized from aqueous DMF.
- Yield (%): 62.5; M.P. (^{O}C): 266-268; R_f: 0.64 (Hexane : Ethyl acetate, 9:1)

Step-4 Synthesis of 3-(2-(4-bromophenyl)-1H-indol-3-yl) substituted phenyl prop-2-en- 1-one

- 2-(4-bromophenyl)-1H-indole-3-carbaldehyde (3 gm) and substituted acetophenones (-H, 2-OH, 3-OH, 4-OH, 4-Br, 4-CH_3, 4-NH_2) (3 gm) were taken in conical flask and dissolved in minimum quntity of DMF .
- To this suspension of NaOH (2.80 gm) in minimum quantity of water was added and the resulting mixture was refluxed for 8-10 hrs. After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature and the crude product was collected by filtration and washed with cold ethanol. The final compound was recrystallized from ethanol.

  3-(2-(4-bromophenyl)-1H-indol-3-yl) phenyl prop-2-en- 1-one(4A)

  Yield (%): 82; M.P. (^{O}C): 156-158; R_f: 0.72 (Hexane : Ethyl acetate, 9:1)

  3-(2-(4-bromophenyl)-1H-indol-3-yl) 4-aminophenyl prop-2-en- 1-one(4B)

  Yield (%): 84.48; M.P. (^{O}C): 196-198; R_f: 0.58 (Hexane : Ethyl acetate, 9:1)

  3-(2-(4-bromophenyl)-1H-indol-3-yl) 2-hydroxyphenyl prop-2-en- 1-one(4C)

Yield (%): 74.20; M.P. (^{O}C): 140-142; R_f: 0.38 (Hexane : Ethyl acetate, 9:1)

3-(2-(4-bromophenyl)-1H-indol-3-yl) 4-hydroxyphenyl prop-2-en- 1-one(4D)

Yield (%): 67.55; M.P. (^{O}C): 144-146; R_f: 0.54 (Hexane : Ethyl acetate, 9:1)

3-(2-(4-bromophenyl)-1H-indol-3-yl) 4-bromophenyl prop-2-en- 1-one(4E)

Yield (%): 55.35; M.P. (^{O}C): 152-154; R_f: 0.78 (Hexane : Ethyl acetate, 9:1)

3-(2-(4-bromophenyl)-1H-indol-3-yl) 3-hydroxyphenyl prop-2-en- 1-one(4F)

Yield (%): 70.20; M.P. (^{O}C): 148-150; R_f: 0.56 (Hexane : Ethyl acetate, 9:1)

3-(2-(4-bromophenyl)-1H-indol-3-yl) 4-methylphenyl prop-2-en- 1-one(4G)

Yield (%): 65.20; M.P. (^{O}C): 132-134; R_f: 0.60 (Hexane : Ethyl acetate, 9:1)

Step-5 Synthesis of 4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy Substituted phenyl nicotinonitrile

- A mixture of 3-(2-(4-bromophenyl)-1H-indole-3-yl)1- substituted phenyl(-H, 2-OH, 3-OH, 4-OH, 4-Br, 4-CH_3, 4-NH_2) prop-2-en-1-one (2 gm) and malononitrile (2gm) in 5% methanolic sodium hydroxide (30ml) was refluxed for 8 hr. The reaction mixture was cooled, poured onto ice/cold water the formed precipitate was filtered, dried and recrystallized to give target molecule.

4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-phenyl nicotinonitrile (5A)

Yield (%): 72; M.P. (^{O}C): 232-234; R_f: 0.80 (Hexane : Ethyl acetate, 9:1)

4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(4-aminophenyl) nicotinonitrile(5B)

Yield (%): 60.25; M.P. (^{O}C): 250-252; R_f: 0.67 (Hexane : Ethyl acetate, 9:1)

4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(2-hydroxyphenyl) nicotinonitrile(5C)

Yield (%): 50; M.P. (^{O}C): 276-278; R_f: 0.72 (Hexane : Ethyl acetate, 9:1)

4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(4-hydroxyphenyl) nicotinonitrile(5D)

Yield (%): 39.40; M.P. (^{O}C): 278-280; R_f: 0.75 (Hexane : Ethyl acetate, 9:1)

4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(4-bromophenyl) nicotinonitrile(5E)

Yield (%): 65.64; M.P. (^{O}C): 228-230; R_f: 0.64 (Hexane : Ethyl acetate, 9:1)

4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(3-hydroxyphenyl) nicotinonitrile(5F)

Yield (%): 52.5; M.P. (^{O}C): 274-276; R_f: 0.78 (Hexane : Ethyl acetate, 9:1)

4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(4-methylphenyl) nicotinonitrile(5G)

Yield (%): 70.5; M.P. (^{O}C): 180-182; R_f: 0.55 (Hexane : Ethyl acetate, 9:1)

BIOLOGICAL ACTIVITY

Anticancer Activity (*In vitro*)

Material and method[31]

Different cell lines:

- HEK293 (Human Epidermal Kidney Cell Line)
- HELA (Cervical Cancer Cell Line)
- MDA MB 468 (Breast Cancer Cell Line)

Reagents:

- MTT Dye
- MTT Dye solubilizer
  - Acidic Isopropanol – 90 %
  - Triton – X
- Fetal Bovine Serum
- Phosphate Buffer Saline
- Antibiotic Antimycotic Solution
- DMEM and FBS media
- Anticancer drug (methotrexate)

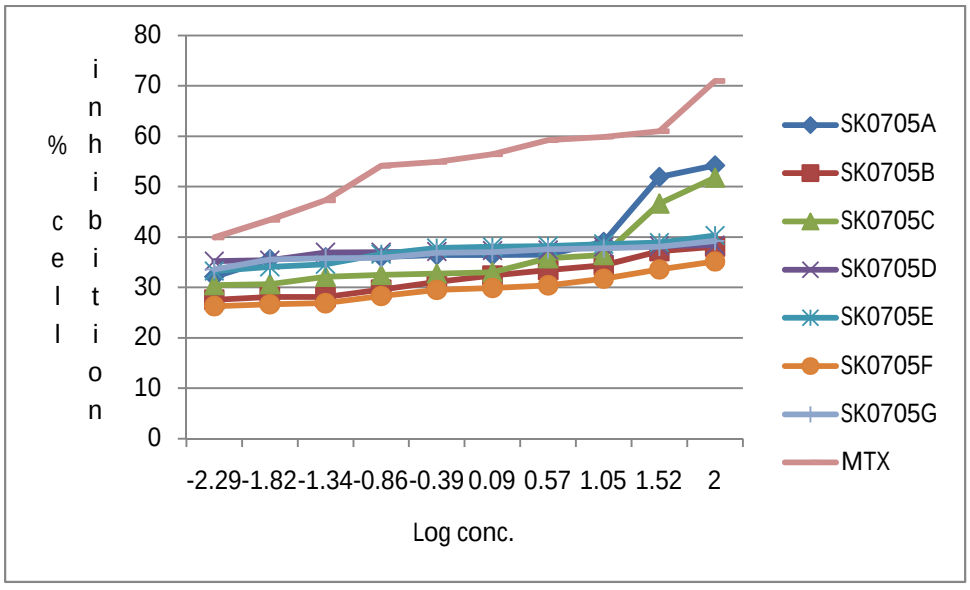
MTT Protocol:

- Cells were preincubated at a concentration of 1×10^6 cells / ml in culture medium for 3 hrs at 37 °C and 6.5 % CO_2, 75 % Relative Humidity.
- Cells were seeded at a concentration of 5×10^4 cells / well in 100 µl culture medium and various amounts of compound (final concentration *e.g.* 100 µM – 0.005 µM) were added into microplates (tissue culture grade, 96 wells, flat bottom).
- Cell cultures were incubated for 24 hrs at 37 °C and 6.5% CO_2.
- 10µl MTT labeling mixture was added,incubated for 4 hrs at 37 °C,6.5%CO_2,75% Relative Humidity
- 100 µl of solubilization solution was added to each well and incubate for overnight
- Absorbance of the samples was measured using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product is between 550 and 600 nm according to the filters available for the ELISA reader, used. (The reference wavelength should be more than 650 nm)

RESULTS AND DISCUSSION

Table 1: Anticancer activity on MDA MB468 cell line (Breast cancer)

Conc. (in µM)	Log Conc.	% Cell Inhibition							
		SK0705A	**SK0705B**	**SK0705C**	**SK0705D**	**SK0705E**	**SK0705F**	**SK0705G**	**MTX**
0.01	-2.29	32.10	27.56	30.45	35.22	33.23	26.29	33.54	39.93
0.02	-1.82	35.58	28.07	30.66	35.42	34.08	26.66	35.50	43.42
0.05	-1.34	35.93	28.07	32.11	36.95	34.57	26.87	35.81	47.28
0.14	-0.86	36.02	29.55	32.50	36.99	36.59	28.29	35.85	54.11
0.41	-0.39	36.40	31.11	32.74	37.13	37.79	29.48	36.84	54.91
1.23	0.09	36.44	32.37	33.02	37.32	38.04	29.88	37.02	56.43
3.70	0.57	36.45	33.42	35.77	37.45	38.21	30.41	37.58	59.18
11.11	1.05	39.00	34.38	36.41	38.00	38.60	31.71	37.76	59.87
33.33	1.52	51.93	37.17	46.61	38.22	38.88	33.55	38.07	60.98
100.00	2.00	54.20	38.05	51.75	38.39	40.27	35.16	39.18	70.95
IC_{50}		32.00	**1.829**	33.15	14.012	**1.162**	13.904	**2.364**	0.3215

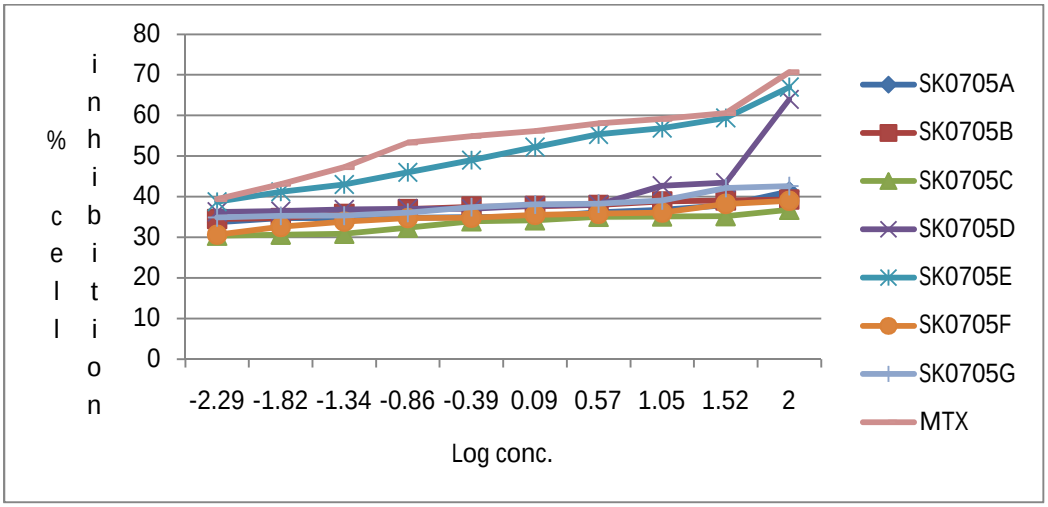


MTX= Methotrexate

Figure 1: Graph of log conc. VS % cell inhibition for MDA MB468 cell line

Table 2: Anticancer activity on HEK 293 cell line (Human Epidermal Kidney Cell Line)

Conc. (in µM)	Log Conc.	% Cell Inhibition							
		SK0705A	**SK0705B**	**SK0705C**	**SK0705D**	**SK0705E**	**SK0705F**	**SK0705G**	**MTX**
0.01	-2.29	33.70	34.48	30.39	36.26	38.76	30.64	34.91	39.34
0.02	-1.82	34.76	34.72	30.65	36.48	41.21	32.57	35.28	43.11
0.05	-1.34	34.76	35.74	30.90	36.83	43.00	33.85	35.32	47.28
0.14	-0.86	34.83	36.90	32.37	37.05	46.01	34.75	36.03	53.34
0.41	-0.39	34.94	37.51	33.94	37.00	49.01	34.78	37.41	54.94
1.23	0.09	35.06	37.67	34.15	37.79	52.21	35.51	38.08	56.19
3.70	0.57	36.12	37.94	35.02	38.02	55.34	35.80	38.31	58.08
11.11	1.05	36.74	38.84	35.15	42.65	56.86	36.05	39.07	59.12
33.33	1.52	37.91	39.09	35.22	43.44	59.38	38.22	42.12	60.59
100.00	2.00	41.25	39.28	36.75	63.97	66.99	38.98	42.63	70.66
IC_{50}		53.39	13.194	11.51	13.700	**2.309**	12.77	**6.469**	0.101

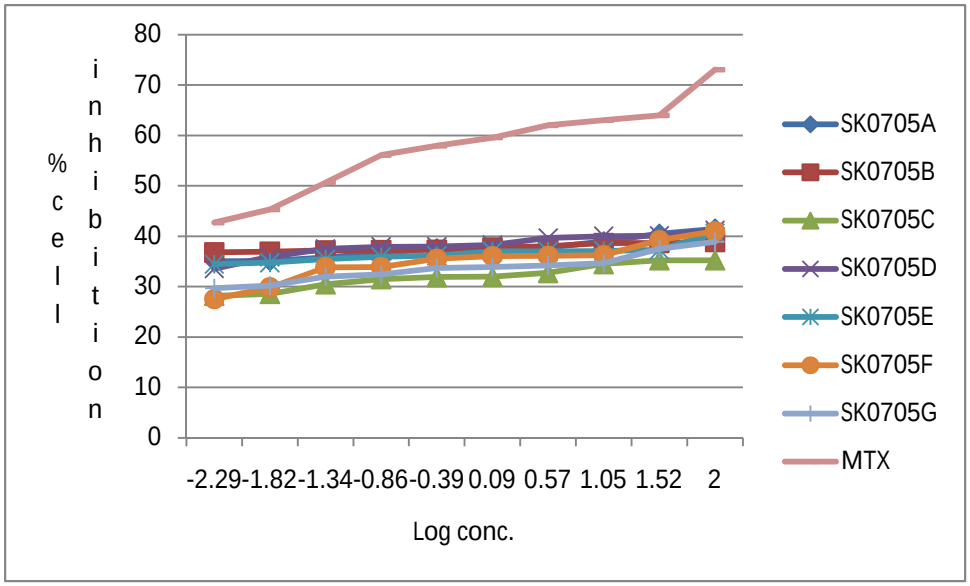


MTX= Methotrexate

Figure 2: Graph of log conc. VS % cell inhibition for HEK 293 cell line

Table 3: Anticancer activity on HELA cell line (Cervical Cancer Cell Line)

Conc. (in µM)	Log Conc.	% Cell Inhibition							
		SK0705A	**SK0705B**	**SK0705C**	**SK0705D**	**SK0705E**	**SK0705F**	**SK0705G**	**MTX**
0.01	-2.29	35.01	36.77	28.19	33.58	34.44	27.51	29.67	42.72
0.02	-1.82	35.08	36.94	28.54	35.89	34.74	29.84	30.19	45.28
0.05	-1.34	35.79	37.18	30.48	37.50	35.47	33.84	31.96	50.66
0.14	-0.86	36.66	37.22	31.48	37.90	35.91	33.86	32.42	56.11
0.41	-0.39	37.53	37.31	31.90	37.91	36.14	35.50	33.67	57.93
1.23	0.09	37.68	37.73	31.97	38.27	36.99	36.04	33.89	59.55
3.70	0.57	37.72	38.03	32.71	39.64	36.99	36.11	34.18	62.02
11.11	1.05	38.94	38.61	34.49	40.00	37.08	36.20	34.61	62.99
33.33	1.52	40.46	38.63	35.23	40.11	37.33	39.23	37.51	63.96
100.00	2.00	41.46	38.85	35.23	41.16	40.59	41.00	38.90	73.02
IC_{50}		5.961	**2.028**	4.604	**2.915**	**1.314**	14.405	7.885	0.204



MTX= Methotrexate

Figure 3: Graph of log conc. VS % cell inhibition for HELA cell line

1. **4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-phenyl nicotinonitrile (5A)**

   Yield: 72 %. **m.p.** 232-234˚C.

   MS m/z: 482.3$(M+2)^+$

   IR (KBr) cm^{-1}:3350, 1542 (NH); 2200 (—C≡N); 1242 (C-N); 501 (C-Br)

   H^1 NMR (400 MHz, DMSO): 11.76 (s,1H,NH- of indole), 6.7-7.9 (m,14H,Ar-H), 3.9 (s,3H,OCH_3)

2. **4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(4-aminophenyl) nicotinonitrile (5B)**

   Yield: 60.25%. **m.p**. 250-252˚C.

   MS m/z: 495$(M)^+$

   IR (KBr) cm^{-1}:3390, 1535 (NH); 2200(—C≡N); 1635 (C=N); 555 (C-Br)

   H^1 NMR (400 MHz, DMSO): 11.69 (s,1H,NH- of indole), 6.5-7.8 (m,13H,Ar-H), 3.9 (s,3H,OCH_3),4.1(s,2H,NH_2)

3. **4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(2-hydroxyphenyl)nicotinonitrile (5C) Yield:** 50%. **m.p**. 276-278˚C.

   MS m/z: 496$(M)^+$

   IR (KBr) cm^{-1}:3571(NH); 2214 (—C≡N); 1288 (C-N); 1203(-OH); 493 (C-Br)

   H^1 NMR (400 MHz, DMSO): 11.76 (s,1H, NH- of indole), 6.9-7.8 (m,13H,Ar-H), 5.2(S,1H,OH);3.98 (s,3H,OCH_3)

4. **4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(4-hydroxyphenyl)nicotinonitrile (5D) Yield:** 39.40%. **m.p**. 278-280˚C.

   IR (KBr) cm^{-1}:3409, 1550 (NH); 2206(—C≡N); 1288 (C-N);1203 (-OH); 408 (C-Br)

   H^1 NMR (400 MHz, DMSO): 11.87 (s,1H,NH- of indole), 6.9-7.8 (m,13H,Ar-H),5.12 (s,1H,OH), 3.9 (s,3H,OCH_3)

5. **4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(4-bromophenyl)nicotinonitrile(5E)**

   Yield: 65.64%. **m.p.** 228-230˚C.

   MS m/z: 561.2$(M+2)^+$

   IR (KBr) cm^{-1}:3340, 1542 (NH); 2210(—C≡N); 1620 (C=N); 416 (C-Br)

   H^1 NMR (400 MHz, DMSO): 11.74 (s,1H,NH- of indole), 6.7-7.9 (m,13H,Ar-H), 3.98 (s,3H,OCH_3)

6. **4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(3-hydroxyphenyl)nicotinonitrile(5F)**

   Yield: 52.5%. **m.p.** 274-276˚C.

IR (KBr) cm^{-1}:3386 (NH); 2215(—C≡N); 1288 (C-N); 555 (C-Br)

H^1 NMR (400 MHz, DMSO): 10.5 (s,1H,NH- of indole), 6.8-7.7 (m,13H,Ar-H),5.3 (s,1H,OH), 3.9 (s,3H,OCH_3)

7. **4-(2-(4-bromophenyl)-1H-indole-3-yl)-2-methoxy-6-(4-methylphenyl) nicotinonitrile (5G)**

Yield: 70.5%. **m.p.** 180-182˚C.

MS m/z: 495.3$(M+1)^+$

IR (KBr) cm^{-1}:3390, 1550 (NH); 2220(—C≡N); 501 (C-Br)

H^1 NMR (400 MHz, DMSO): 10.45 (s,1H,NH- of indole), 6.8-7.7 (m,13H,Ar-H), 3.9 (s,3H,OCH_3), 2.5 (s,3H,CH_3)

CONCLUSION

- Compound 5E is more potent than all synthesized compound.
- Compound 5A, 5C, 5D, 5F have less activity than 5E, 5B compound and standard drug on all cell lines.
- So, I concluded that halogenated phenyl ring on 6^{th} position of pyridine ring is responsible for more activity.
- Substitution at phenyl ring on 6^{th} position of pyridine ring gives good anticancer activity as in the order of Br>NH_2>CH_3>OH>H.

ACKNOWLEDGEMENT

The authors wish to thank Lord Swaminarayan and my sweet parents with whose blessings, I have been able to complete my research work in time.

REFERENCES

1. Kinzler, Kenneth, W.Vogelstein (2002), reported "Introduction", the genetic basis of human cancer, *Medical Pub. Division,* 5, 234-247.
2. Tannock IF, Hill RP, The Basic Science of Oncology (2005), 4^{th} edition, published by McGraw-Hill, 940-965.
3. http://www.cancer.gov/cancrtopics/understndingcancer/cancer
4. Rang H.P., Dale M.M. (2003), Ritt “Pharmacology”, Fifth edition, Churchill Livings -tones pub., New York, 217-243.
5. Kleinsmith, LJ (2006), Principles of Cancer Biology, 225-230.
6. Donald J., Burger's Medicinal Chemistry and Drug Discovery (2003), Sixth edition, Published by John Wiley and Sons Publication, 6, 52-67.

7. Report sees 7.6 million global (2007) cancer deaths, American Cancer Society, Retrieved on 2008-08-07.
8. Mondragón L. *J. Med. Chem. (*2008), 51, 521–529.
9. Skeel, Roland T. (2007), Handbook of cancer Chemotherapy, Seventh edition, Lippincott Williams and Wilkins, 1, 15-16.
10. Foye William O., Lemake Thomas L. William David A., Principles of Medicinal Chemistry, published by B.I. Waverly Pvt. Ltd., New Delhi, 4th edition,882-907.
11. Srivastava and Pandeya S.N. (2010), "Indole a versatile nucleus in pharmaceutical field", *International Journal of current pharmaceutical review and research*, 1(3),1-2.
12. http://www.ch.ic.ac.uk/mimi/group%20meeting/Indole020307.pdf
13. Doris Kaufmann et al. (2007), "Antimitotic activities of 2-phenyl indole-3-carbaldehyde in human breast cancer cell", *Bioorganic and Medicinal chemistry,* 15, 5122-5136.
14. Ulrich Jacquemard et al. (2008), Synthesized 3,5-bis(2-indolyl)pyridine and 3-[(2-indolyl)-5-phenyl]-pyridine derivatives as CDK inhibitors and cytotoxic agents", *Bioorganic and Medicinal chemistry*,16, 4932-4953.
15. Ekhlass Nassar et al. (2010), "Synthesis (in vitro) Antitumor and Antimicrobial Activity of some Pyrazoline, Pyridine, and Pyrimidine Derivatives Linked to Indole Moiety", *Journal Of American Science*, 6(8), 463-471.
16. Huasheng Ding et al. (2005), "Novel indole α-methylene-γ-lactones as potent inhibitors for AKT –mTOR signaling pathway kinase," *Bioorganic and Medicinal chemistry Letters*, 15 , 4799-4802.
17. Magdy A H Zahran et al. (2010), synthesized antitumor activity of indolylchalcones and their pyrazoline analogs.", *Journal Of Chemistry science*; 122, 587-595.
18. Abdel-Rahman Farghaly et al. (2010), synthesized indole derivatives containing pyrazoles with potential antitumor activity", *ARKIVOC,* 11, 177-187.
19. Ahmed Kamal et al. (2010), "Synthesis of 3,3-diindolyl oxyindoles efficiently catalysed by $FeCl_3$ and their in vitro evaluation for anticancer activity", *Bioorganic and Medicinal chemistry Letters*, 20,5229-5231.
20. Yu-Shan Wu et al. (2009), "Synthesis and Evaluation of 3-Aroylindoles as Anticancer Agents" *Journal of Medicinal Chemistry*, 52, 4941-4945.

21. Palwinder Singh et al. (2009), "Design, synthesis and anticancer activities of hybrids of indole and barbituric acids", *Bioorganic and Medicinal chemistry Letters*, 19, 3054-3058.
22. Dharmendra Kumar et al. (2010),"Synthesis of pharmacologically active 2-phenyl sulpha/substituted Indoles", *Int. J.Eng. Sci. and Tech*., 2((7), 2553-2557.
23. Mohammad Amir et al. (2008), "Synthesis and biological evaluation of some 4-(1*H*-indol-3-yl)-6-phenyl-1,2,3,4-tetrahydro-pyrimidin-2ones/thiones as potent anti-inflammatory agents", *Acta pharm.*, 58, 467–477.
24. Radwan, M.A. et al. (1997), "synthesis and evaluated the analgesic activity of 3-substituted indole derivatives", *Bioorganic and Medicinal chemistry,*15, 3832.
25. Nadeem Siddiqui et al.(2008),"Synthesis, anticonvulsant and toxicity evaluation of 2-(1*H*-indol-3-yl)acetyl-*N*-(substituted phenyl)hydrazine carbothioamides and their related heterocyclic derivatives" *Acta Pharm.,*58,445–454.
26. Dun Wang et al. (2004), " Synthesis and *In Vitro* Antiviral Activities of Some New 2-Arylthiomethyl-4- tertiaryaminomethyl substituted Derivatives of 6-Bromo-3-ethoxy carbonyl-5- hydroxyindoles", *Chinese Chemical Letters*,15(1), 19 – 22.
27. Li,Y.Y. et al. (2007), "synthesis the indole derivatives which were evaluated for their insulin sensitizing and glucose lowering effects",*J. Pharmacol Res,* 56,335.
28. Enien et al. (2010), "synthesis of indole-2 and 3-carboxamides and evaluated their biological activities as antioxidant by chemoluminesence and electron spin response spin trapping", *International Journal of Current Pharmaceutical Review and Research*, 1(3),10.
29. Talaz et al. (2009) "synthesis of 5,10- dihydroindolo[1,2-b]indoles containing sub-stituents such as methoxy, hydroxyl, and halogens on indeno part and their antioxidant activities were assayed by various in vitro assays", *Bioorganic and Medicinal chemistry,*17,6583.
30. S. H. Kaufmann; W. C. Earnshaw (2000), MTT assay *Cell Research*, 256, 42-49.
31. Prakash sukhramani, Maulik suthar et al. (2011), In-vitro cytotoxicity evaluation of novel N- substituted bis-benzimidazole derivatives for anti-lung and anti-breast cancer activity, *Annals of biological research*, 2, 1, 51-59
32. Furniss B.S., Vogel's text book of practical organic chemistry 5th edition, Experiment no.8.26, Britesh library cataloguing in publication data, London, 1989 , pp-1161.

33. Jaime N. D.,William A.R., Wilson and Gisvold's Textbook of organic Medicinal and Pharmaceutical Chemistry ,Antineoplastic agents Ch. 12,11th edition, Lippincott Williams and Wilkins, 2004 , pp-390-448.
34. Http://en.Wikipedia.org/Wiki/cancer