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Synthetic Novel 1, 4-Dihydropyridine Derivatives Act as Potential Anticancer Agent Against Both Human Small Cell Lung DMS 114 Cancer Cell Line and Human Colon Cancer Cell Line HCC 2998

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ABSTRACT

The objective of the present work was the synthesis of 1-[5-acetyl-4 (4-substituted phenyl)-2,6-dimethyl-1,4-dihydroxypyridine-3-yl]-ethan-1-one and evaluation of *in vitro* anticancer activity. Based on this a new series of compound had been planned to synthesize by reacting acetyl acetone with various aromatic aldehydes in the presence of ammonium acetate. The *in vitro* anticancer activities were carried out against Human small cell lung DMS 114 cell line and Human colon cancer cell line HCC 2998 and MTT assay was used to analyze the cell growth inhibition of the both. The results had been displayed that compound B1-B4 were possessed an excellent anticancer activity (at 25 µg/ml) against both Human small cell lung DMS 114 cancer cell line and Human colon cancer cell line HCC 2998 and doxorubicin (at 10µg/ml) was used as a standard drug for Human small cell lung DMS 114 cancer cell line and 5-Fluro uracil (5-FU) for Human colon cancer cell line HCC 2998. In the present study IC_{50} values below 4 µg/ml were displayed by the compound B1 (IC_{50} of 4.0 µg/ml), B2 (IC_{50} of 3.4 µg/ml), B3 (IC_{50} of 3.2 µg/ml) and B4 (IC_{50} of 3.1 µg/ml) against Human small cell lung DMS 114 cancer cell line and B1(IC_{50} of 3.5 µg/ml), B2 (IC_{50} of 3.2 µg/ml), B3 (IC_{50} of 2.9 µg/ml) and B4 (IC_{50} of 2.9 µg/ml) against Human colon cancer cell line HCC 2998. The IC_{50} values of standard drugs doxorubicin and 5-FU were found to be 1. 2 µg/ml and 1.1 µg/ml.

Keywords: IR, NMR, Anticancer activity, DMS 114, HCC 2998, MTT assay, IC_{50}

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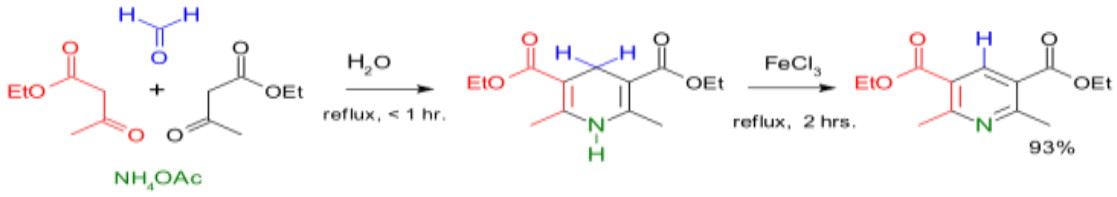
INTRODUCTION

Dihydropyridine is a molecule based upon pyridine, and the parent of a class of molecules that have been semi-saturated with two substituents replacing one double bond. They are particularly well known in pharmacology as L-type calcium channel blockers, used in the treatment of hypertension. Compared with certain other L-type calcium channel blockers (for example those of the phenylalkylamine class such as Verapamil) which have significant action at the heart, they are relatively vascular selective in their mechanism of action in lowering blood pressure[1]. The Hantzsch pyridine synthesis or Hantzsch dihydropyridine synthesis is a multi-component organic reaction between an aldehyde such as formaldehyde, 2 equivalents of a β-keto ester such as ethyl acetoacetate and a nitrogen donor such as ammonium acetate or ammonia[2]. The initial reaction product is a dihydropyridine which can be oxidized in a subsequent step to a pyridine. The driving force for this second reaction step is aromatization. This reaction was reported in 1881 by Arthur Rudolf Hantzsch. A 1, 4-dihydropyridine dicarboxylate is also called a 1, 4-DHP compound or a Hantzsch compound. These compounds are an important class of calcium channel blockers and as such commercialized in for instance nifedipine, amlodipine or nimodipine. The reaction has been demonstrated to proceed in water as reaction solvent and with direct aromatization by ferric chloride, Manganese Dioxide or potassium permanganate in a one-pot synthesis[3].



The Hantzsch dihydropyridine synthesis is found to benefit from microwave chemistry[4]. Calcium channel blockers (CCB), calcium channel antagonists or calcium antagonists[5] are several medications that disrupt the movement of calcium (Ca2+) through calcium channels[6] Calcium channel blockers are used as antihypertensive drugs, i.e., as medications to decrease blood pressure in patients with hypertension. CCBs are particularly effective against large vessel stiffness, one of the common causes of elevated systolic blood pressure in elderly patients[7] Calcium channel blockers are also frequently used to alter heart rate, to prevent cerebral vasospasm, and to reduce chest pain caused by angina pectoris. N-type, L-type, and T-type voltage-dependent calcium channels are present in the zona glomerulosa of the human adrenal, and CCBs can directly influence the biosynthesis of aldosterone in adrenocortical cells, with

consequent impact on the clinical treatment of hypertension with these agents[8]. Dihydropyridine (DHP) calcium channel blockers are derived from the molecule dihydropyridine and often used to reduce systemic vascular resistance and arterial pressure. Sometimes when they are used to treat angina, the vasodilation and hypotension can lead to reflex tachycardia, which can be detrimental for patients with ischemic symptoms because of the resulting increase in myocardial oxygen demand. Dihydropyridine calcium channel blockers can worsen proteinuria in patients with nephropathy[9]. Moreover 4-phenyl substituted 3,5–diacetyl–1,4-dihydro- pyridines Showed cytotoxic activity against human oral suamous carcinoma (HSC-2) cells [10, 11]. The objective of the present work is the synthesis of 1-[5-acetyl-4 (4-substituted phenyl)-2,6-dimethyl-1,4-dihydroxypyridine-3-yl]-ethan-1-one and evaluation of *in vitro* anticancer activity. Based on this a new series of compound have been planned to synthesize by reacting acetyl acetone with various aromatic aldehydes in the presence of ammonium acetate which involved Knoevenagel Condensation and Michael Addition reaction.

MATERIALS AND METHODS

The all chemicals used for the synthesis were of laboratory grade and analytical grade. The melting points of newly synthesized 1,4-dihydrpyridine compounds were determined by open capillary method. The IR spectra of synthesized compounds were recorded by ABB Bomen FT-IR spectrometer MB 104 IR spectra recorder with KBr pellets**.** The H^1-NMR spectra of synthesized compounds were recorded by BRUKER NMR spectrometer in $CDCl_3$. The Mass spectra of synthesized compounds were recorded by JEOL GC mate. The purification of newly synthesiszed compounds were done by TLC method.TLC plates are pre-coated silica gel(HF254-200 mesh) aluminium plate using ethyl acetate and n-hexane as an solvent system and spots were visualized under U.V chamber. The IR, H^1-NMR and Mass spectra were assigned to elucidate the structure of synthesized compounds (B1-B4).

General procedure for the synthesis of target compounds [12, 13]

To a stirred mixture of aromatic aldehydes (0.318 gm) and acetyl acetone (0.696 gm), ammonium acetate (0.231 gm) was added; the reaction mixture was homogenized by stirring to a viscous liquid. The progress of the reaction was monitored by TLC. The mobile phase for the synthesized compounds B1, B2, B3, and B4 was ethyl acetate and n-hexane in the ratio of 6:4. After completion of reaction a small amount of ethanol was added to the viscous liquid and stirred for 5 min. Ice cold water was added to the mixture, the solid thus obtained was filtered. The crude product was purified by crystallization from ethanol: water (95:5) mixture.

Synthetic scheme

[A] = Acetyl acetone.

[B] = Product (1, 4-dihydropyridine derivatives) - B1, B2, B3, and B4 etc.

Characterization of the synthesized compounds

Compound B1

1 [5-acetyl-4 (4-chloro phenyl)-2, 6-dimethyl-1, 4-dihydroxypyridine-3-yl] ethan-1-one. Molecular formula: $C_{17}H_{18}ClNO_2$,Molecular weight: 303.102, m .p: 180-184 ^{0}C, yield: 85.2%. IR (KBr) cm^{-1}: 3334 (-NH str), 3078 (Ar-C-H), 2924 (C-H Aliph-C-H str), 1695 (C=O), 1582 (C=C), C-C (773), 1089 (C-N), 789 (C-Cl). ^{1}H-NMR ($CDCl_3$): δ 2.02 (s, 3H, CH_3), 2.9 (s, 3H, CH_3), 2.24 (s, 3H, $COCH_3$), 2.44 (s, 3H, $COCH_3$), 4.80 (s, 1H, CH), 7.18-8.82 (m, 7.18- 8.82-m, 4H, Ar-CH), 10.14 (s, 1H, NH). MS: m/z value-303.783. $(M)^+$ Ion peak.

Compound B2

1 [5-acetyl-4 (4-fluro phenyl)-2,6-dimethyl-1, 4-dihydroxypyridine-3-yl] ethan-1-one. Molecular formula: $C_{17}H_{18}FNO_2$, Molecular weight: 287.132, m .p: 175-179 ^{0}C, yield: 73.4%. IR (KBr) cm^{-1}: 3333 (-NH str), 3079 (Ar-C-H), 2923 (C-H Aliph-C-H str), 1695 (C=O), 1580 (C=C), C-C (775), 1088 (C-N), 688 (C-Cl). ^{1}H-NMR ($CDCl_3$): δ 2.01 (s, 3H, CH_3), 2.7 (s, 3H, CH_3), 2.23 (s, 3H, $COCH_3$), 2.42 (s, 3H, $COCH_3$), 4.81 (s, 1H, CH), 7.19-8.83 (m, 7.19- 8.84-m, 4H, Ar-CH), 10.13 (s, 1H, NH). MS: m/z value-287.32. (M)+ Ion peak.

Compound B3

1 [5-acetyl-4 (4-bromo phenyl)-2, 6-dimethyl-1, 4-dihydroxypyridine-3-yl] ethan-1-one. Molecular formula: $C_{17}H_{18}BrNO_2$, Molecular weight:347.052, m.p: 179-183 ^{0}C, yield: 69.9%. IR (KBr) cm^{-1}: 3334 (-NH str), 3070 (Ar-C-H), 2926 (C-H Aliph-C-H str), 1698 (C=O), 1581 (C=C), C-C (773), 1087 (C-N), 682 (C-Cl). ^{1}H-NMR ($CDCl_3$): δ 2.03 (s, 3H, CH_3), 2.9 (s, 3H, CH_3), 2.22 (s, 3H, $COCH_3$), 2.44 (s, 3H, $COCH_3$), 4.80 (s, 1H, CH), 7.19-8.84 (m, 7.19- 8.84-m, 4H, Ar-CH), 10.11 (s, 1H, NH). MS: m/z value-348.234 (M+1) Ion peak.

Compound B4

1 [5-acetyl-4 (4-nitro phenyl)-2, 6-dimethyl-1, 4-dihydroxypyridine-3-yl] ethan-1-one. Molecular formula: $C_{17}H_{18}N_2O_4$, Molecular weight: 314.12, m.p: 145-147 ^{0}C, yield: 78.2%. IR (KBr) cm^{-1}: 3333 (-NH str), 3078 (Ar-C-H), 2925 (C-H Aliph-C-H str), 1696 (C=O), 1582

(C=C), C-C (771), 1088 (C-N), 1342 (NO_2 grp). ^{1}H-NMR ($CDCl_3$): δ 2.01 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.24 (s, 3H, $COCH_3$), 2.44 (s, 3H, $COCH_3$), 4.81 (s, 1H, CH), 7.19-8.83 (m, 7.19-8.83-m, 4H, Ar-CH), 10.15 (s, 1H, NH). MS: m/z value-314.33 (M)+ Ion peak.

Evaluation of *in vitro* Anticancer activity by MTT assay[14-20]

Cell culture

Human small cell lung DMS 114 cancer cell line and Human colon cancer cell line HCC 2998 were provided by National Centre for Cell Science (NCCS), Pune and were grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week.

Cell treatment

The monolayer cells were detached and single cell suspensions were made using trypsin-ethylenediaminetetraacetic acid (EDTA). A hemocytometer was used to count the viable cells and the cell suspension was diluted with a medium containing 5% FBS in order to obtain final density of $1x10^5$ cells/ml. 96-well plates at plating density of 10,000 cells/well were seeded with one hundred micro litres per well of cell suspension and incubated for cell attachment at 37° C, 5% CO2, 95% air and 100% relative humidity. The cells were treated with serial concentrations of the test samples after 24 hr. Serial dilution method was used for preparing test samples of fixed concentration. Cells were initially dissolved in dimethylsulfoxide (DMSO) and further diluted with serum free medium to obtain twice the desired final maximum test concentration. The required final drug concentrations of 25 µg/ml was obtained by adding aliquots of 100 µl of drug dilutions to the appropriate wells already containing 100 µl of medium. After addition of the drug the plates were incubated for an additional 48 hr at 37° C, 5% CO2, 95% air and 100% relative humidity. The medium without samples served as control and triplicate was maintained for all concentrations.

MTT Assay

After 48 hrs of incubation, to each well 15 µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added and incubated at 37° C for 4 hrs. The medium with MTT was flicked off and the formed formazan crystals were solubilized in 100µl of DMSO. Using micro plate reader the absorbances were measured at 570 nm. The % cell inhibition was determined using the following formula:

$$\% \text{ Cell Inhibition} = 100 - [\text{Abs (sample)}/\text{Abs (control)}] \times 100$$

RESULT AND DISCUSSIONS

Chemistry

The synthesis of target compounds 1-[5-acetyl-4 (4-substituted phenyl)-2,6-dimethyl-1,4-dihydroxypyridine-3-yl]-ethan-1-one were carried out by reacting acetyl acetone with various aromatic aldehydes in the presence of ammonium acetate. The synthesized compounds were characterized by IR, NMR, and Mass spectroscopy and proposed the structure by spectral data. The purity of the synthesized compounds was ascertained by TLC and spectral analysis.

Biological screening

These synthesized compounds (B1-B4) were screened for their *in vitro* anticancer activity. Anticancer activity was carried out by MTT assay. A preliminary screening against both Human small cell lung DMS 114 cancer cell line and Human colon cancer cell line HCC 2998 displayed that the compounds, B1-B4 (at concentration 25µg/ml) as well as standard drugs doxorubicin and F-FU at concentration 10µg/ml were able to inhibit the proliferation of more than 50% cells (Figure: 1, 2, 3 and 4). It was appeared that compounds B1-B4 displayed anticancer activities with IC_{50} values below 100 µg/ml against these both cancer cell lines. In the USNCI screening program a compound is generally considered to have *in vitro* anticancer activity, if the IC_{50} value following incubation between 48 hrs and 72 hrs is less than 4 µg/ml or 10 µM[12]. In the present study IC_{50} values below 4 µg/ml were displayed by the compound B1 (IC_{50} of 4.0 µg/ml), B2 (IC_{50} of 3.4 µg/ml), B3 (IC_{50} of 3.2 µg/ml) and B4 (IC_{50} of 3.1 µg/ml) against Human small cell lung DMS 114 cancer cell line and B1(IC_{50} of 3.5 µg/ml), B2 (IC_{50} of 3.2 µg/ml), B3 (IC_{50} of 2.9 µg/ml) and B4 (IC_{50} of 2.9 µg/ml) against Human colon cancer cell line HCC 2998. The IC_{50} values of standard drugs doxorubicin and 5-FU were found to be 1. 2 µg/ ml and 1.1 µg/ml.

Table 1: For Percentage Cell Growth Inhibition (%) of Synthesized Compounds against DMS 114 (Human small cell lung) by MTT Assay

Synthesized compounds	Concentration of the drugs	Absorbance of the drug samples	Inhibition of Cell Growth (%)
B1	25 µg/ml	1.39	79.9
B2	25 µg/ml	1.41	84.3
B3	25 µg/ml	1.31	90.1
B4	25 µg/ml	1.29	92.5
Doxorubicin	10 µg/ml	1.22	96.2
Control		2.410	0

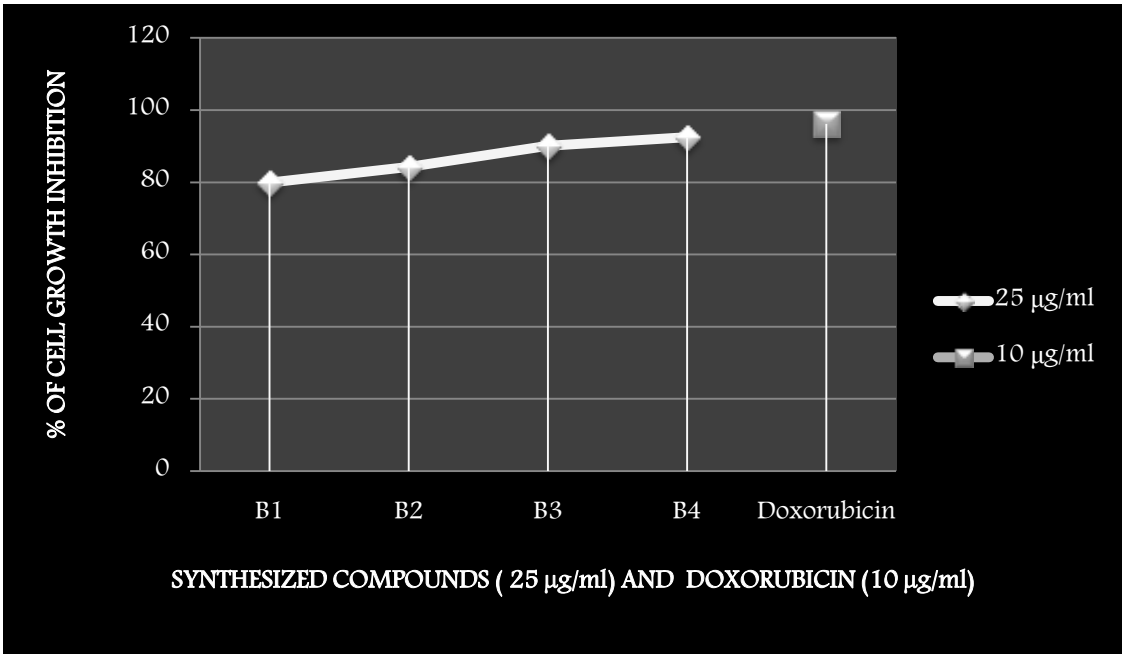


Figure 1: Inhibitory Percentage (%) of the Synthesized Compounds at 25 µg/ml and Standard drug doxorubicin (at 10µg/ml) on DMS 114 (Human small cell lung) Cancer Cell line

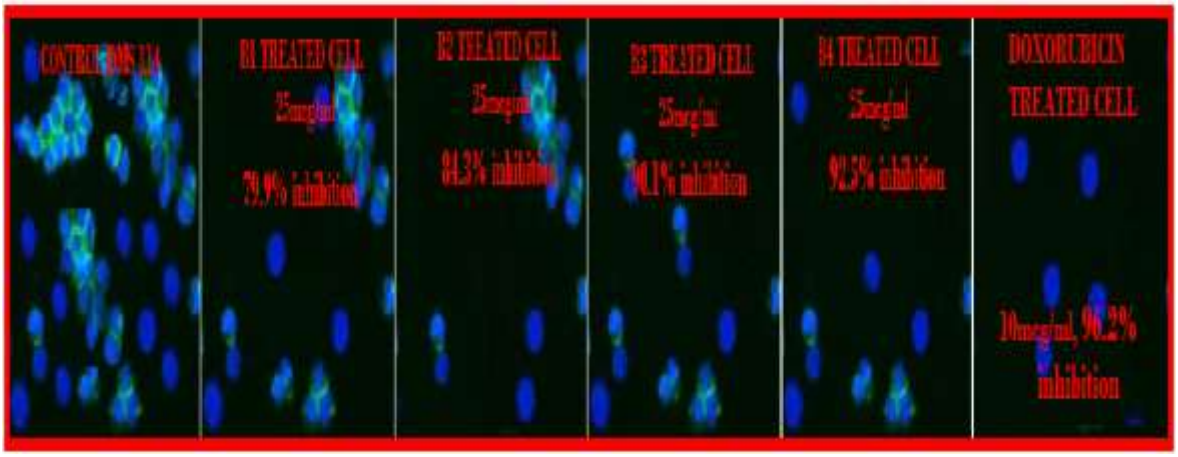


Figure 2: Inhibition of DMS 114 cancer cell by B1-B4 Compounds and std drug

Table 2: For Percentage Cell Growth Inhibition (%) of Synthesized Compounds against Human Colon Cancer cell HCC 2998 by MTT Assay

Synthesized compounds	Concentration of the drugs	Absorbance of the drug samples	Inhibition of Cell Growth (%)
B1	25 µg/ml	1.35	87.3
B2	25 µg/ml	1.33	90.1
B3	25 µg/ml	1.29	92.2
B4	25 µg/ml	1.25	94.4
5-FU	10 µg/ml	1.22	97.2
Control		2.410	0

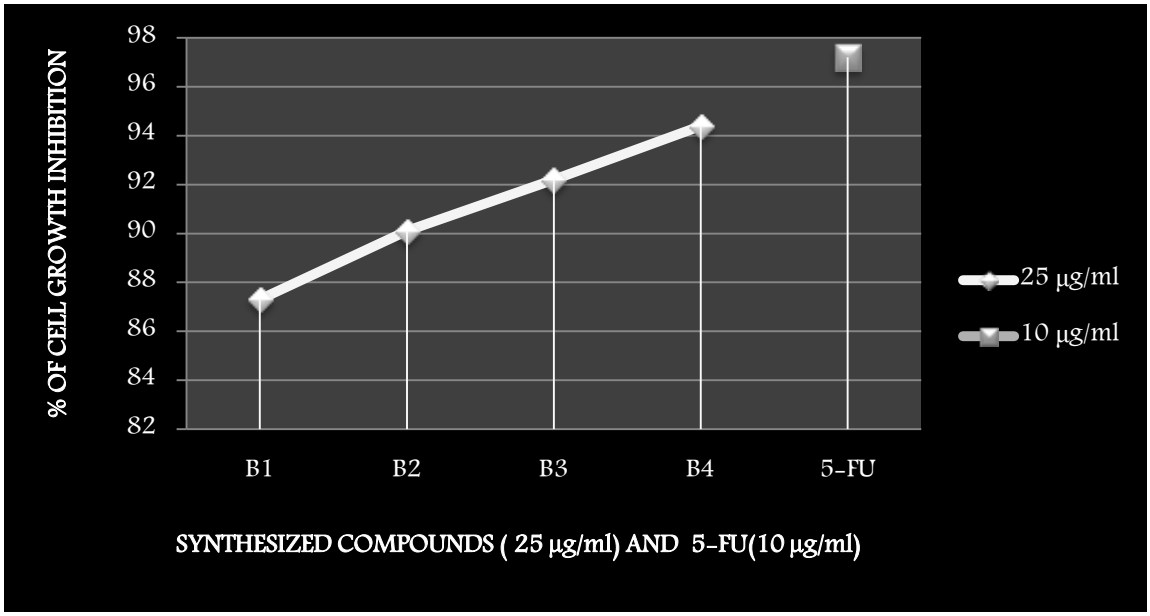


Figure 3: Inhibitory Percentage (%) of the Synthesized Compounds at 25 µg/ml and Standard drug 5-FU (at 10µg/ml) on HCC 2998 (Human Colon Cancer cell) Cancer Cell line

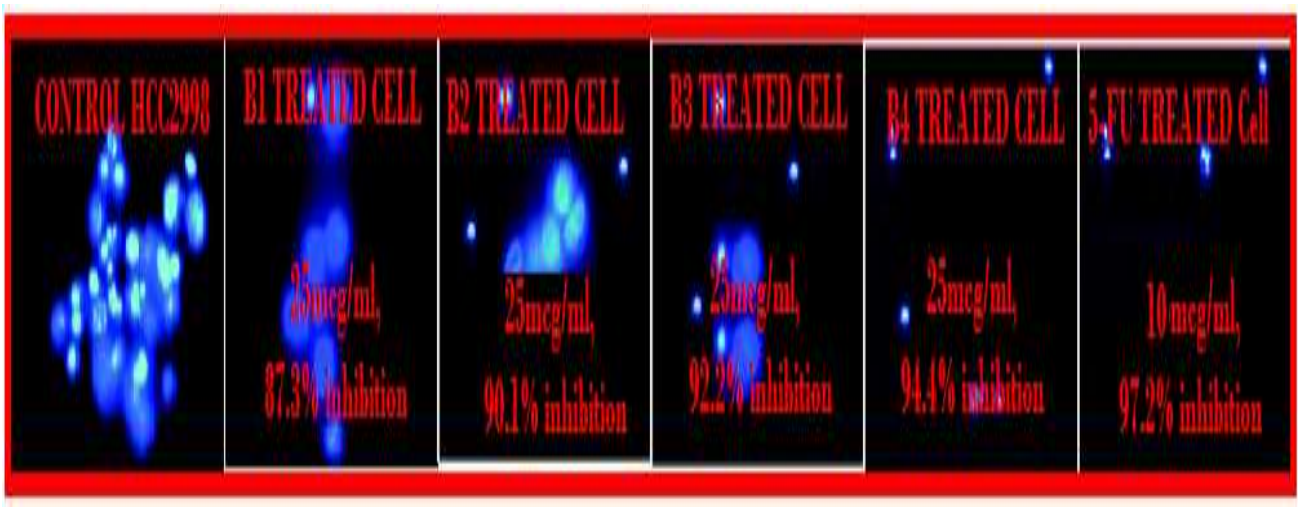


Figure 4: Inhibition of HCC 2998 cancer cell by B1-B4 Compounds and std drug

CONCLUSION

In conclusion, we report here a series of novel 1-[5-acetyl-4 (4-substituted phenyl)-2,6-dimethyl-1,4-dihydroxypyridine-3-yl]-ethan-1-one compounds (B1-B4) were prepared by reacting acetyl acetone with various aromatic aldehydes in the presence of ammonium acetate and their ability to kill tumor cells *in vitro*. The Anticancer activity of the compounds B1-B4 against both Human small cell lung DMS 114 cancer cell line and Human colon cancer cell line HCC 2998 can be considered very good with regards to the USNCI standard.

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